STUDY OF THE PREVALENCE OF CHRONIC, NON-SPECIFIC LUNG DISEASE AND RELATED HEALTH PROBLEMS IN THE GRAIN HANDLING INDUSTRY

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Study of the Prevalence of Chronic, Non-specific Lung Disease

Section 1 - Body of Report

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BACKGROUND INFORMATION

"... Hence, whenever it is necessary to sift wheat and barley or other Kinds of grain to be ground in the mill, or to measure it when corn-merchants convey it hither and thither, the men who sift and measure are so plagued by this kind of dust that when the work is finished they heap a thousand curses on their calling. The throat, lung, and eyes are keenly aware of serious damage; the throat is choked and dried up with dust, the pulmonary passages become coated with crust formed by the dust, and the result is a dry and obstinate cough; the eyes are much inflamed and watery; and almost all who make a living by sifting and measuring are short of breath and cachectic and rarely reach old age; in fact they are liable to lapse into orthopnea and finally dropsy. The dust moreover is so irritating that it excites intense itching over the whole body, of the sort that it is sometimes observed in nettle rash."

Thus, did Rammazini describe the health hazards of cereal grain workers in 1713[1]. Although pulmonary symptoms associated with exposure to grain dust have been known for centuries, the mechanism by which grain dust exerts its harmful effect is unknown. New insights into the nature and extent of the health problems created by grain dust have been provided by several epidemiologic[1-8] and clinical studies[9,11,12,14-23] of grain workers. The consequences of symptomatic, recurrent, long-term exposure, however, have not been established with certainty.

During exposure to grain dust up to 75 % of grain workers frequently experienced symptoms of cough, expectoration, wheezing, chest tightness, eye and nasal irritation[2-7]. From 6 to 33 % of grain workers also experienced one or more episodes of "grain fever" characterized by malaise, chills and fever occurring during or several hours following exposure[2-6]. With the exception of coughing and wheezing, which occurred significantly more frequently among smokers, these effects were independent of age, length of employment and smoking habits[6].

Symptoms of chronic respiratory disease were also common among grain workers[2-8]. These symptoms included persistent cough[27-40%], phlegm[35-53%], wheezing[14-31%], or dyspnea on effort[16-46%]. Approximately one-third of the grain workers had chronic bronchitis or evidence[2-8] of airways obstruction as detected by spirometry. The MMF, $FEF_{25-75\%}$ were the most common abnormal individual tests of lung function, occurring in almost one-half of the workers who smoked and a quarter of the workers who had never smoked. Decreases in FEV_1 and FEV_1/FVC were found in approximately one-fourth of the workers who smoked and infrequently in workers who had never smoked. In all the studies reported, cigarette smoking was the predominant host factor in grain workers with obstructive lung disease. Moreover, the chronic bronchitis and chronic airways obstruction found in grain workers closely resembled that encountered in cigarette smokers. Because of the general lack of appropriately matched comparison populations in the reported epidemiologic studies, it is difficult to assess the contribution of grain dust to the obstructive lung disease

seen in grain workers. The studies of Becklake[5], and more recent information by Dosman[9], and Broder[24] suggest that the effects of dust and smoking are additive, if not synergistic.

Grain dust is a complex mixture of husk particles, cellulose hairs and spikes, starch granules, spores of fungi, insect debris, pollens, rat hair, and approximately 5 % mineral particles[10]. The mean particle size of the airborne dusts may be less than 5m. Particles of this size can cause small airways and alveolar reactions, as well as upper airways injury.

In workers exposed to wheat dust a reduction in ventilatory capacity was observed within 30 minutes of starting work[11]. In addition, Warren, Cherniak and Tse[12] reported immediate and late asthmatic reactions in some subjects exposed to an inhalation challenge with grain dust extract. More recently, Chan-Yeung has confirmed these results and has further shown that disodium cromoglycate given before the challenge inhibited the immediate bronchial reaction; beclomethasone dipropionate failed to prevent the immediate bronchial reaction, but inhibited the late asthmatic reaction. Half of the workers studied had a marked degree of bronchial reactivity to methacholine. Chan-Yeung's findings[34] suggest that grain dust asthma may have an allergic basis. Results from several surveys[2,6] have shown that wheezing and abnormal lung function were more prevalent among atopic workers and workers with positive immediate skin tests to grain dusts. Thus, as with cigarette smoking, allergy and exposure to grain dust may operate as independent or interdependent factors in the development of respiratory disorders in grain workers. As in the case of grain workers[6], a survey of the general population in Arizona[13] revealed a significant correlation between wheezing in adults and cutaneous reactivity to a variety of common allergens, suggesting that atopic status might predispose the individual to the development of chronic obstructive airways disease[13]. Conversely, Gerard[8] demonstrated that non-atopic grain buyers who were nonsmokers from non-atopic families showed no increase in bronchial reactivity to extracts of cereal grains, their common fungal contaminants or histamine and are not likely to develop lung disease as a result of grain handling. However, evidence suggests that grain handlers are a self-selected group. The most sensitive individuals are likely to seek other employment early because of pulmonary symptoms.

Grain dust and its contaminants contain many allergens that are potent sensitizers in man[10,14-21]. Reactions to grain dust components have been described during many phases in the handling of grain, e.g., harvesting[22], local storage[17,19], grain elevators[4,6,12], and processed material[18,20,21]. In isolated instances, the agent in grain dust that was responsible for the reaction observed in individual workers appeared to be debris of a grain weevil (Sitophilus granarius)[15,16], a grain mite (Glycophagus destructor)[17], a specific fungus[14,19] or a specific component of grain (Appendix 23). In general, the role of these agents in the respiratory disease of grain workers is unknown.

Grain dust contains a wide variety of fungi and bacteria[25-27], including several species of Aspergillus, Penicillium, Mucor,

Pullalaria and Thermophillic bacteria. These microbial agents can induce a variety of immunological reactions in the lung including a Type I (allergic) and a Type III (immune complex) reactions[14]. The clinical correlates of these reactions include asthma, allergic bronchopulmonary aspergillosis[28] and Farmer's Lung[29]. Except in isolated individuals, the immunological mechanism evoked by grain dust has not been identified. It was suggested[8] that grain fever is a Type III (immune complex) reaction similar to that seen in various forms of hypersensitivity pneumonitis. In the reported studies, hypersensitivity pneumonitis or its sequelae, chronic diffuse interstitial fibrosis of the type seen in Farmer's Lung, was not established with certainty. Also, doPico found no correlation between a history of grain fever and the presence of serum precipitins to fungi, grain or grain dust[6]. Whether or not hypersensitivity pneumonitis or its sequelae cause workers to leave the industry is unknown.

Emmanuel has described mycotoxicosis[30], a condition occurring in farmers exposed to massive concentrations of fungal spores. This syndrome resembles grain fever since there is no evidence of a Type III (immune complex) reaction and no chronic respiratory sequelae. Although the immunological mechanisms active in mycotoxicosis and grain fever are unknown, it was reported that airborne grain dusts activate complement by the alternative pathway, and that endotoxin can be recovered from all dust samples. Airborne grain dusts might be expected to elicit respiratory pathophysiology by a dose-dependent inflammatory response produced as a result of endotoxin or direct activation of the complement alternative pathway.

It is possible that grain dust may cause acute and chronic respiratory abnormalities by a direct irritant effect. Irritant receptors have been identified in the mucosa of bronchial airways[31]. Stimulation of these receptors in experimental animals with impulses going through the vagal pathways, led to hyperpnea and bronchoconstriction[32]. Vagal stimulation has also been shown to cause increased secretion of the bronchial mucous glands. It is thus conceivable that chronic non-specific stimulation of the bronchial irritant receptors by grain dust may lead to pathologic changes in the bronchial airways and mucous glands which are the basis for the chronic respiratory symptoms and abnormalities present in the majority of grain workers. The picture of chronic cough and phlegm, obstructive airways disease, episodes of tightness in the chest, fever and bronchial reactivity to grain dust extracts are similar to byssinosis and mill fever. Other similarities to byssinosis were observed such as airflow limitation in nonsmoking grain workers which appeared to be detectable at the level of the small airways[4,5]. This suggests that the target for inhaled grain dust may be similar to inhaled cigarette smoke or cotton dusts. The MZ phenotype with intermediate $alpha_1$-antitrypsin deficiency did not seem to be a significant host factor for the chronic obstructive lung disease found in grain workers[8].

No information is available concerning cumulative dust exposures or dose-response relationships in any of the reported studies. Dust concentrations inside grain elevators vary greatly. Available measurements of dust[2,5-7] varied from 10 mg/m3 in some of the modern

elevators in Vancouver[7] to 600 mg/m3 in some elevators in Montreal[5]. The reason for the wide range of dust concentrations is unclear. However, the terminal elevators in Vancouver generally handle grain that has been partly cleaned during its transport from the prairies. There may also be qualitative differences in the types of grain handled by each elevator. These quantitative and qualitative differences probably account for the generally lower prevalence of respiratory disease and grain fever reported by Chan-Yeung[7,34].

Population at risk. The exact number of workers exposed to grain dust is unknown since so many occupations are involved including farmers, grain elevator operators in small and large terminal elevators and workers in flour, feed and seed mills. The total population at risk in Canada was estimated at 100,000[34]. In the United States there are an estimated 500,000 grain elevator workers. The proportion of the more than 2 million farmers at risk is unknown. However, Wan and Wright[35] analyzed disability data from a survey conducted by the Bureau of Census and reported that U.S. farmers and farm managers had the highest prevalence of disabling respiratory diseases of any occupational group--a rate of 21.8/100,000.

Grain dust has also been identified as a community air pollutant capable of causing epidemics of asthma[36,37].

STUDY I. HEALTH STATUS OF A CROSS SECTION OF GRAIN HANDLERS WITHIN A SINGLE GEOGRAPHIC AREA.

The health status of grain handlers was evaluated by comparing the prevalence and characteristics of clinical, physiological, immunological, radiological, serological blood and urine parameters of 310 grain handlers with 239 city services workers (named controls) from the same geographic area.

1. MATERIALS AND METHODS

Population

1a. Grain Handlers

The 310 grain handlers that were studied represented 78% of the 397 total available working and acceptable workers (Table 1) from eight elevator companies, of Wisconsin and Minnesota State grain inspectors, and Wisconsin and of Minnesota longshoremen. Grain handlers from the elevator companies were members of Local 118 of the Grain Millers Association, the longshoremen were members of the International Longshore Association (ILA) and the state inspectors were members of the American Federation of State, County and Municipal Employees (AFSCME).

Workers were notified and asked to participate in the study by fliers, posted notices, union stewards, and general meetings with investigators. The purpose of the study was explained verbally and in writing. The management of each company was notified, the studies were explained to them and all agreed to permit their workers to participate without loss of personal income.

Companies absorbed the cost of temporary decreased manpower. Overall, the elevator operations and productivity did not appear to be significantly altered due to proper scheduling and coverage. For the cross sectional study, subjects were considered acceptable to participate if they were year round workers (defined as 9-12 months per year for grain handlers or 8 months per year for longshoremen); had worked for longer than one year; and were working at the time of the survey. The longshoremen accepted were those identified by the shiploading manpower companies as grain shiploaders exclusively. Workers on sick leave were studied, when possible, but their data was not included in the comparisons of group data analysis. Workers who refused to participate (N=51) on the first contact were contacted at least once more. Approximately 50% of the workers who initially refused, agreed to participate on the second contact. Thirty-nine workers agreed to participate but later, for reasons beyond their control, did not. One woman was studied but the data were not included in the group analysis.

1b. City Workers (Control Workers)

For the comparison population (called control population), subjects were recruited from outside workers of the cities of Superior and Duluth and from the Power and Light Company of Duluth, Minnesota. The arrangements were made with the cooperation of the mayors of these cities, city management officials, management of the Power and Light Company, and union (AFSCME) representatives.

Eligible workers were those whose work day was spent on outdoor functions at least 50% of the time. The job classifications included: engineers and bridge operators, street maintenance, water and gas (maintenance, meter readers, etc.), parks and zoo maintenance, sewage and sanitation maintenance and operations, building maintenance, airport mechanics and operators. Three hundred and eighty-six of 478 eligible city workers (Table 2) were contacted and informed of the nature of the study by fliers, general meetings, posters, or by department supervisors. Initial refusals were recontacted by supervisors and/or project coordinators. Two hundred and thirty-nine consented to participate and were studied (Table 2). The differences between the total eligible (N=478) and non-contacted (N=92) were explained by sex, vacation or sick leave, departments or divisions where management preferred services not be disturbed, failure to contact. The differences between those contacted (N=386) and not tested (N=147) were mostly due to refusals. The 239 city workers represent 62% of the contacted workers. This lower participation among city workers as compared to grain handlers may be explained by lack of motivation or other factors. Age, height, weight and smoking habit information were obtained from the non-participant workers. Their mean age was 44 % 12 years, height 172.3 % 6.5 cm, weight 83.3 % 13 kg, 49% smokers, 31% ex-smokers and 20% nonsmokers.

The characteristics of the test and control populations are presented in Table 3. All city workers and 99% of the grain workers were white males. Among grain workers there were one black, one hispanic and two American Indians. Table 3b shows the level of education in both groups. The distribution of smoking habit, height

and weight by age groups are presented in Tables 4a and 4b. The characteristics of the smoking habits are presented in Table 5. Past histories of occupational exposure to pulmonary irritants are shown in Table 6. Because the Superior-Duluth city service workers are exposed to environmental city contamination with small amounts of grain dust that could sensitize them, it was decided to study the skin reactivity to aero-allergens and grains in 100 city service workers from a city where no grain dust exposure is known to occur, i.e., Madison, WI. One-hundred and three male volunteers were studied from the Madison Gas and Electric Company. The mean age of this group was 38 % 10 years.

History

The history was obtained by a standard self-administered questionnaire (Appendix IV), reviewed for completeness by two trained interviewers who also assisted in the completion of the questionnaire when required. Additional histories were obtained by the physicians who reviewed the questionnaire and obtained a detailed history of grain fever and any other relevant health information.

Work history was filed using a job coding (Appendix III) which classified jobs by the type of hazard, site where job was performed and descriptive job title.

It became immediately apparent that not enough information could be obtained on grain fever because of the structure of questions Q46 and Q47. The answers to these two questions were therefore not entered in the workers files. Instead, an interpretation of the answers to questions 46 and 47 was made by the examining physician, with additional information as explained in Appendix V.

Examination

Physical examinations were performed by one of three physicians following a standard procedure for heart and lung auscultation and liver palpation (Appendix VII).

Pulmonary Function Studies

Pulmonary function studies were performed using standard equipment and following acceptable clinical procedures as described in Appendix VIII. Included were FEV_1, FVC, MMF, $Vmax_{50}$, $Vmax_{75}$, CV N2/L, D_{LCO}, V50He02 and VisoV. These tests were considered abnormal when: $FEV_1/FVC<70\%$; FEV_1 and D_{LCO} <80% of predicted; MMF, $Vmax_{50}$ and

$Vmax_{75}$<1.65 SD; and N2/L and CV <1.65 SD. (1.65 SD was chosen since the abnormality on these tests is undirectional.)

Immunologic Evaluation

a) Antigen preparation (Appendix X).

b) Immediate skin reactivity to common allergens: fungal antigens, mites, insects, grain extracts, airborne grain dust extracts and settled dust extracts were done by a prick test using commercial antigens or antigens prepared in our laboratory as explained in Appendix X and Appendix XI. These were considered positive if a wheal of 3 mm or greater developed 20 minutes following the prick.

c) Delayed hypersensitivity to PPD, mumps, Candida, Streptokinase-Streptodornase and Trichophyton was determined using commercially available antigens injected intradermally (.02 cc). Intradermal skin tests were considered positive when: PPD, 5TU was 10 mm or greater induration; SKSD, Trichophyton and Candida were 5 mm or greater induration and mumps 15 mm or greater erythema.

d) Serum precipitating antibodies were analyzed by techniques described in Appendix XII.

e) Immunoglobulins IgE, IgG, IgA and IgM were determined by techniques described in Appendix XIII.

Hemoglobulin, hematocrit, urinalysis and blood chemistries

Methods for the determination of pseudocholinesterase, serum SGPT, serum creatinine and gamma GT are described in Appendix IX and for $Alpha_1$-antitrypsin levels in Appendix XIII.

Chest Roentgenograms

Roentgenographic examinations of the chest complied with the specifications published in the Federal Registry, Vol. 28, No. 144, July 27, 1973. The posterior-anterior views were taken at Memorial Hospital, Radiology Department, Superior, Wisconsin. At the end of each testing day one of the principal investigators (GdP) reviewed all of the radiographs for quality and abnormalities that would require re-examination or recommendation to the patient of the need for further medical attention, e.g., bilaterial hilar adenopathy in subject #719. Workers with severe kyphoscoliosis or cardiomegaly would have been excluded in the group pulmonary function analysis. The PA chest roentgenographs were later read and interpreted independently by two physicians (a radiologist (M.E.P.) and a pulmonologist (H.D.). There was 95% agreement in the readings. The disagreements were on minor issues of questionable clinical or physiological significance, e.g., whether a single nodule was calcified or not, etc. When disagreements occurred, the roentgenographs were re-examined and the final readings agreed to by the two readers and a principal co-investigator (GdP.). The reading form for the chest roentgenograms is contained in Appendix XVI.

The levels of circulating immunoglobulins (G,A,M,E) were determined by the standard timed Mancini technique (Appendix IX) using frozen serum samples from 307 grain workers and 237 city services workers.

The reproducibility of the immunodiffusion system was insured by the following protocol: The same lot of immunodiffusion plates was used to test both grain workers and controls for IgG, IgA, IgM and IgE levels. Accuracy control (internal standard) was included on each plate and a three point protein reference curve was included on every third plate.

Quality control data indicated a 2.5% variation in the values of the internal control from plate to plate. Plots of the squared diameter of the precipitin rings (ordinate) obtained from the protein references against their respective concentrations (abscissa) on linear graph paper yielded an intercept ordinate of 11 % 2.5 mm3.

2. RESULTS

2a SYMPTOMS (ANALYSIS OF QUESTIONNAIRE)

The analysis of symptoms or symptom complexes was made with the following objectives in mind:

1) To determine if the prevalence of acute and chronic respiratory symptoms; eye, nose, throat, skin and joint symptoms; diseases or conditions diagnosed by a physician; and family histories of certain diseases among grain handlers were different than expected for people residing in the same geographic area with similar labor backgrounds, age, sex, smoking habits and socioeconomic status.

2) To determine the relative importance of the effects of cigarette smoking and grain handling on the prevalence of respiratory symptoms.

3) To determine if the prevalence of symptoms in grain handlers was related to job classification, place or length of employment.

4) To determine the prevalence and characteristics of the symptom complexes presented by grain handlers on exposure to grain dust and to pesticides.

5) To determine the relationship, if any, of acute and chronic symptoms with lung function or immunologic parameters of the individual (see Section I-2a b c Correlations).

Definitions

Determination of chronic bronchitis followed the currently accepted definition of chronic expectoration for two or more years. Using this definition the diagnosis of chronic bronchitis can be made from the answers to the questionnaire. The combination of answers that may represent this definition, however, has not been standardized We used primarily question 14E-- qreater than two years--as an indication of chronic bronchitis. In addition, we have used other combinations that may define the presence of chronic bronchitis and these are presented in Table 7.

Occupational asthma may be defined as wheezing and/or chest tightness when exposed to the working environment or the result of or aggravated by, exposure to the work environment. One may add to the definition: the association of cough and/or dyspnea also brought on or aggravated by exposure to the work environment, and/or the relief or improvement of these symptoms when away from work or when on vacation. Answers to questions that may be used to categorize four definitions of occupational asthma are explained in Table 8. Dyspnea on exertion. Grade 1: when hurrying on level ground or walking up a slight hill. Grade 2: when walking on level ground with people of own age. Grade 3: having to stop walking when walking on level ground at others pace. Grade 4: having to stop walking when walking at own pace.

Objective 1. (refer to Tables 9-11 and Appendix VI)

Overall respiratory symptoms and symptom complexes, as well as

symptoms of eye, nasal and throat irritations on exposure to the working environment were higher among grain handlers than controls (Table 9 and Appendix VI). Personal histories of pulmonary, cardiovascular, kidney and liver disease, diabetes or dermatitis diagnosed by a doctor and a family history of pulmonary disease were similar in the two occupational groups (Tables 10 and 11).

Usual cough and expectoration (Q 13 and 14).

There were significant differences between grain workers and controls ($P \leq .001$) in the prevalence of cough and expectoration: first thing in the morning, at other times during the day, four to six times a day at least four days a week (Q 13 & 14 a,b,c), at least three months of the year and for greater than two years (Q13 & 14 d,e). Of the 194 that had some type of cough, 175 had it for more than two years. There were 116 who did not have "usual" cough. Two hundred and sixty-six of the 310 grain workers had some type of expectoration; 151 had it for more than two years. The prevalence of chronic bronchitis as defined in Table 8 was significantly higher among the grain workers than the controls. The prevalence of chronic bronchitis I was 46% in grain handlers and 18% among city workers. The incidence of chronic bronchitis was 35% in nonsmoking grain workers.

Cough and/or expectoration in relation to work (Q 15-18)

The cough or expectoration was worse on work days in a greater percentage of the grain workers (73%) than in controls (18%) ($P < .001$). Seventy-nine % of the controls noted no difference in cough or expectoration between work days and weekends. Only 27% of the grain workers noticed no difference between work days and weekends (Q 15).

Eighty-two % of grain workers felt their cough and/or phlegm was better on vacation (Q 16), whereas only 28% of controls reported improvements in cough or phlegm ($P < .01$).

Cough and/or expectoration brought on or aggravated by exposure to grain dust, other dusts, gases or fumes at work (Q 18) was also significantly higher among the grain workers than controls. The aggravation of cough and/or expectoration by barn dust was also higher among the grain workers than controls ($P < .001$). There was no difference in the prevalence of symptoms aggravated by house dust, weather or other factors.

Wheezing and/or chest tightness (Q 21-36).

The prevalence of wheezing and/or chest tightness (Q 21a) was higher ($P < .001$) in the grain workers (65%) than among controls (42%). These significant differences were also apparent in all smoking categories between grain workers and controls and between smokers and nonsmokers in both occupational categories. Note that 57% of the nonsmoker grain workers complained of wheezing and/or chest tightness.

The controls appeared to have a greater prevalence of "only wheezing" and "mainly wheezing" (Q 22) than the grain workers, who had a greater prevalence of "both wheezing and/or chest tightness." However, the prevalence of "only chest tightness" or "mainly chest tightness" was not different between the two occupational groups.

The onset of wheezing during 0-15 years of age was greater among controls than among grain workers. Among the other age groups the onset of wheezing was not significantly different (Q 23).

Wheezing and/or chest tightness related to work exposure.
The prevalence of wheezing at work while performing their job (Q 25) was higher among grain workers (82.5%) than among controls (50%) ($P < .001$). The average frequency of wheezing and/or chest tightness during work appeared to be higher among the grain workers than controls. The prevalence of wheezing "at least once a day" and "a few times a month" was significantly higher ($P < .001$) among the grain workers than controls. The prevalence of wheezing "a few times a week" was significantly higher among controls (26% vs. 21%) ($P < .05$). The prevalence of wheezing and/or chest tightness occurring "a few times a year" or "ever" was not different between the two occupational groups. It would appear that smokers in both groups (grain and controls) were more likely to have more frequent wheezing than the nonsmokers. That is, they were more likely to have it "daily" rather than "a few times a month." There was only a small percentage (5% of the grain workers and 14% of the controls) that had wheezing only "once."

Among the grain workers who had wheezing at work, 60% reported wheezing was usually worse any day of the week at work, 15% reported wheezing the first day of the work week and 25% claimed no difference between the first day or any day of the week. None of the grain workers felt worse on the weekend. Among the controls, 76% answered that the day of the week made no difference, 20% claimed that wheezing was worse any day at work and 4% reported wheezing on the first day of work. None of the controls answered that wheezing was worse on weekends (Q 27).

The prevalence of wheezing and chest tightness that was better while on vacation or off work was significantly different and higher in grain workers (88%) than controls (20%) ($P < .001$). Most of the controls (78%) felt that their wheezing remained the same on vacation or when not working ($P < .001$). There were two controls who felt worse on vacation.

The prevalence of occupational asthma, wheezing and/or chest tightness brought on or made worse by exposure to grain dust, other dusts, fumes or gases at work was significantly higher among grain workers than controls ($P < .001$). The differences were also significant between grain workers and controls in the three smoking categories. Significant differences were also observed when other combinations of questions were used to indicate occupational asthma (Table 8b).

Nocturnal dyspnea (Q 33)

The prevalence of wheezing (Q 33a) that awakened a subject from sleep was higher in grain workers (20%) than controls (10%). There was no difference in the prevalence of this symptom among the three smoking categories of each occupational group (Q 33a). The frequency of individual episodes of nocturnal wheezing (Q 33b) was not different between grain workers and controls.

Wheezing and/or chest tightness relation to time of the year (Q 34)

The prevalence of wheezing with no specific relation to the time of the year was similar in grain workers and controls. Controls who noticed seasonal variation reported the predominance of wheezing in January (28/40 or 70%). In the grain workers, the highest prevalence was found during January (32%) followed by April (16%), May (11%), June (11%), August and September.

Wheezing with dyspnea (Q 36)

The prevalence of attacks of wheezing with shortness of breath was higher among grain workers than controls ($P < 0.001$) (Q 36).

Dyspnea (Q 37-42)

The prevalence of ever having shortness of breath (Q 37), Grade 1 (Q 38) or Grade 2 (Q 39) dyspnea was significantly higher in grain workers than controls ($P < 0.001$, $P < 0.01$, $P < 0.05$, respectively). There were no differences for dyspnea on exertion for Grade 3 (Q 40) and Grade 4 (Q 41). The number of years they had shortness of breath was not different between the two occupational groups (Q 42).

Dyspnea while performing work (Q 43-44)

The prevalence of shortness of breath while performing work (Q 43) was higher in grain workers (36%) than controls (11%).

Chest illnesses (Q 63)

The frequency of chest illnesses and their interference with normal activities was similar in grain workers and controls (Q 63a, b).

Disease or conditions diagnosed by a doctor (Q 64-67)

Except for the prevalence of allergic rhinitis, which was higher in the controls than grain workers, the prevalence for the diseases or conditions indicated in Table 10 was not different between the groups.

Family history (immediate blood relatives) (Q 74)

The prevalence of lung diseases shown in Table 11 in blood relatives of the grain workers and controls were not different.

Objective 2

Role of cigarette smoking.

A. Analysis of prevalence of symptoms by smoking categories.

Considering that the proportion of smokers, ex-smokers and nonsmokers was similar in grain workers and controls, the significantly higher prevalence of respiratory symptoms in grain handlers must be due to a significant effect of grain handling independent of smoking. To confirm this assertion and further evaluate the effects and possible interaction of cigarette smoking on symptom prevalence, we compared and analyzed the prevalence of symptoms or symptom complexes between grain workers and controls for each smoking category and between smokers and ex-smokers, ex-smokers and non-smokers, and non-smokers and smokers for each occupational group. (Table 9 and Appendix XI).

The significant differences were analyzed by chi-square analysis. Overall the symptoms were more prevalent among grain workers than controls in every smoking category (Table 9). For example (Table 12), the prevalence of chronic bronchitis among grain workers who smoke was higher than controls who smoke (57% vs. 30%) ($P < .001$), and higher among nonsmoking grain workers than nonsmoking controls (35% vs. 10%) ($P < .001$). Actually the prevalence of chronic bronchitis in nonsmoking grain workers was higher (35%) than in controls (30%) who smoked.

The prevalence of "occupational asthma I" was also higher among grain workers who smoke (67%) than smoking controls (13%) and higher in nonsmoking grain workers (50%) than nonsmoking controls (11%). The prevalence of symptoms among nonsmoking grain workers was higher than among controls who smoke.

In grain workers, the prevalence of chronic cough and expectoration, chronic bronchitis, wheezing and/or chest tightness was higher in smokers than nonsmokers or ex-smokers, but there were no differences in symptoms between nonsmokers or ex-smokers. The prevalence of nocturnal wheezing, dyspnea on exertion and "chest illness" was not significantly different between smoking categories (Table 12).

In grain handlers the prevalence of wheezing and/or chest tightness and cough and/or expectoration on exposure to the work environment ("occupational asthma II") was significantly higher among smokers than nonsmokers and ex-smokers. The prevalence of dyspnea at work and grain fever was not different in smoking categories.

2b. A quantitative analysis of the relative effects of smoking and grain handling on symptom prevalence.

In order to quantitate the effects of smoking during grain dust exposures we analyzed the data using a log-linear model. We found that the effects of smoking and grain handling were both highly statistically significant and independent. Factors, or quantities, by which grain handling or smoking increased the odds (risk) of having a specific symptom or symptom complex are presented in Table 13. Overall, the effects of grain handling were greater than the effects of smoking. For example, the grain handler had four and a half times greater risk of having chronic bronchitis than a non-grain handler regardless of the smoking habit. Smoking, independent of grain handling, increased the odds of having chronic bronchitis by a factor of three. Grain handling also increased the odds of having occupational asthma II by a factor of five to 10, regardless of the smoking habit and smoking by a factor of three regardless of grain handling.

Objective 3

To study the effects of job categories and length and place of employment on symptom prevalence among grain handlers, we used logistic

regression analysis adjusting for age, smoking habit and length of employment. Prevalence by smoking habits have been presented in Table 9 and by age groups in Table 14. Only eye and nasal symptoms were related to age. We found that wheezing, dyspnea, nasal symptoms on exposure to grain dust and usual cough first thing in the morning were positively related to length of employment (Table 15). Eye symptoms on exposure, chronic bronchitis as previously defined and grain fever were not related to length of employment (Table 15). In Table 15, the percent of prevalence was not adjusted for age or smoking. The P value indicates the significance of the relation between length of employment and prevalence of symptoms obtained from the used in the log-linear model adjusting for age and smoking. The job categories used in the analysis are presented in Table 16. Overall there were no significant differences in the prevalence of symptoms among the various job categories adjusting for age, smoking and length of employment (Table 17). Table 18 shows those symptoms in which there were significant differences in prevalence between jobs ranked using the z values from the regression analysis. We found the highest prevalence among weighers and longshoremen and the lowest among inspectors.

The prevalence of wheezing and dyspnea on exposure was significantly different among elevator companies, but other symptoms were not different (Table 19). We ranked the relative prevalence of symptoms adjusting for age, smoking, and length of employment among the companies from one to eight (Table 20). One corresponded to the company with the lowest prevalence; eight to the highest. The score value resulted from adding all the rank values for each symptom. Companies 1, 7, 5 and 4 appeared to have the highest prevalence, whereas 2 and 8 had the lowest. When we analyzed all other symptoms or symptom complexes and ranked them, we obtained similar results. The overall ranking, considering symptoms which were found to be significantly different or not found to be significantly different among the companies is indicated in parenthesis. The relatively more symptomatic populations appeared to be in companies 1, 7, 5 and 4 who had fewer symptoms than in companies 8 and 2.

Objective 4

The characteristics of the symptoms and symptom complexes (Table 9, 8, and Appendix VI) developed by the grain workers on exposure to grain dust were as follows:

Respiratory symptoms on exposure

Cough and/or expectoration brought on or aggravated by exposure to grain dust was present (Q 18a) in 200 of 310 grain workers or 65%, and it was significantly higher among smokers (75%) than nonsmokers (52%); the symptoms were equally prevalent among ex-smokers and nonsmokers. The grain dusts that were most likely to bring on or aggravate cough and/or expectoration were durum wheat (55%) and barley (48%). Next were spring wheat (25%), rye (27%) and oat (21%). Least likely were corn (4%), soybean (5%), sunflower and others (1.6%). The frequency of cough and/or expectoration on exposure to grain dust, regardless of the smoking category, was daily (77%), a few times a week (18%) and a few times a month or a few times a year (3.2%).

The frequency of wheezing at work was determined in grain workers.

Most subjects (59%) reported that wheezing occurred once a day, a few times a week or a few times each month; another 16% reported that wheezing occurred once a year.

Wheezing/or chest tightness brought on or made worse by exposure to grain dust was reported by 59% of the grain workers. Durum wheat and barley were reported to be the most common inducer of symptoms followed by spring wheat, rye and oats. The onset of wheezing and/or chest tightness was reported to occur: during work (70.5%), after work (11.5%) or during and after work (16%). Only three workers claimed wheezing before going to work. Of those who felt that wheezing started or got worse during work, 19% of 156 reported that it started immediately and 81% of 156 reported that wheezing started a few hours later. In the latter group, the workers reported that wheezing developed: 2 hours after starting work (42%), 4 hours after starting work (16%), the first hour of a work shift (14%) and 3 hours after starting work (14%). Only 6% of the workers felt the symptom develop during the fifth or sixth hour of work.

Some grain workers did report that wheezing and/or chest tightness occurred after the work shift. The symptom was likely to occur during the first hour after work (34%) or in the second hour (20%). However, some individuals had reactions 5, 6, 8, 9, 10 or more hours later.

Shortness of breath during or after exposure to grain dust was claimed by 49% of the grain workers. The prevalence of this symptom was higher among smokers than nonsmokers. The dusts that were most likely to bring on this symptom were durum wheat, barley, spring wheat, rye and oats. The dusts least likely to induce shortness of breath were soybean, corn, linseed, sunflower and beets. The workers reported that shortness of breath occurred: during work (82%), either during or after work (12%) or after work (6%). Of the subjects who reported shortness of breath at work, the onset occurred: 5 hours after starting work (66%), within 2 hours after starting work (50%) or immediately after starting work (33%). The few workers who developed shortness of this breath after work reportedly noticed it between one and three hours after work.

Grain fever syndrome (Table 21)

A detailed history was obtained on the 121 workers who reported fever and/or chills on exposure to grain dust. We concluded that a syndrome compatible with grain fever was present in 99 of the grain workers who complained of fever on exposure (82%). Although the history was questionable, an additional 16 workers may have had grain fever. In the remaining 6 workers, we could not exclude the possibility that the symptoms of grain fever were evoked by an upper respiratory tract viral infection or other infectious processes.

The prevalence of grain fever was similar in the three smoking categories. All subjects included in the grain fever group (N=115) had episodes of "a flu-like" syndrome with the sensation of fever, chills or chilliness, myalgia, arthralgias, malaise, warmth in the face with or without respiratory symptoms. Most of the workers (73%) recalled no associated respiratory symptoms with the grain fever and a smaller proportion of workers recalled cough, wheezing, or dyspnea associated

with the grain fever. In subjects who developed respiratory symptoms, the symptoms developed during or after work and improved in a few hours or by the next working day. These episodes were usually associated with heavy exposure to grain dust on that day. In 96 of the 115 workers, the number of grain fever episodes was ascertained. Forty-two percent of the workers had fewer than 10 episodes, whereas a small number of workers (16%) reported numerous episodes (Table 21). Workers reported that the grain fever usually occurred during work (32%), after work (35%), or during and after work (33%). Most of the workers (83%) indicated that grain fever occurred on any day of the week, whereas 17% indicated that grain fever occurred the first day at work after a weekend or vacation. When grain fever occurred on the first day of work, the symptoms were usually worse.

Eye, nose and throat symptoms on exposure at work (Q 48a, b, c - Appendix XI)

Grain workers (98%) reported symptoms of eye irritation on exposure to grain dust. After exposure, grain workers also reported a stuffy nose (99%) or a sore throat (52%). Durum wheat and barley were the most likely inducers of these symptoms, followed by rye, spring wheat and oats.

Skin pruritus (Q49 - Appendix XI)

On exposure to grain dust, pruritis (itching of skin) was reported by 63% of the grain workers. The most common inducer of skin pruritis was barley, followed by wheat, oats and rye dust.

Health problems caused by pesticide exposure at work (Table 22)

One hundred and sixty-eight of the 294 grain workers who reported being exposed to pesticides at one time or another during their work life, reported health problems associated with pesticide exposure (Table 22). The most common symptoms were: headache (37%), dizziness (28%), weakness (21%), nausea (21%) and trouble breathing (16%). Blurred vision, stomach pains, diarrhea, fainting and cramps occurred in fewer than 5% of the workers. Nineteen of the 167 (11%) who answered Q 61 and Q 62 had to seek medical attention, and twenty-eight (17%) could not continue regular work assignments on the day of exposure. Exposure to phostoxin, carbon tetrachloride, malathion and methyl bromide were reported by the workers. There were, however, many instances in which the workers could not identify the pesticide. 72% had fewer than 10 symptomatic exposures to pesticides. A small number (16%) reported 20 to 100 symptomatic exposures to pesticides.

2a. RESULTS OF PHYSICAL EXAMINATION (Tables 23 and 24)

Physical examination revealed no chest configuration differences between grain workers and controls (Table 23). There was no significant difference between grain workers and controls in auscultation of the heart or in the presence of hepatomegaly. However, the liver was more frequently palpable in the grain workers than controls.

Auscultation of the chest detected rhonchi or wheezes diffuse or localized, in 43% of the grain workers and 16% of the city workers ($p < .005$). The differences were also significant in each smoking category (Table 24). Once again, one should notice that 35% of the nonsmokers among the grain handlers had expiratory wheezes.

There was no difference in the incidence of abnormal diastolic blood pressures in grain workers and controls. Thirty-nine (13%) grain workers and 25 (10%) controls had diastolic pressures higher than 90 mmHg; 4 (1%) grain workers and 6 (3%) controls had diastolic pressures above 100 mmHg. Systolic pressures above 150 mmHg were found in 8% of grain workers and 13% of controls.

CONCLUSIONS

1) Clinical findings

Grain handlers had a higher prevalence of respiratory symptoms and signs (rhonchi) than comparable non-grain handling city service workers from the same geographic area (Table 7-9, 12, 24) whether or not they smoked. The effects of grain handling on prevalence of respiratory symptoms were highly significant, independent and usually greater than those of smoking (Table 13). The prevalence of work related respiratory symptoms adjusted for age and smoking habit was also positively related to place (Tables 19, 20) and length of employment (Table 15). The data suggested variable environmental working conditions among elevators and perhaps an accumulative respiratory effect due to recurring exposures to grain dust.

Grain workers suffer from:

a) acute and chronic airways reactions (occupational asthma and chronic bronchitis) induced by exposure to grain dust with varying degrees of cough, expectoration, wheezing and/or chest tightness and shortness of breath. Durum wheat and barley grain dust were the most common inducers of symptoms. During the work shift, wheezing and/or chest tightness occurred immediately after starting work or within two hours. In late reactors, wheezing occurred within two hours after leaving work. Very late reactions were not reported.

Wheezing and dyspnea on exposure were related to length of employment. This may indicate either increased sensitization to the allergens present in the environment or the bronchial mucosa being rendered more hyperactive by the recurrent non-specific inflammatory reactions of the airways by grain dust. The place of employment was found to affect the prevalence of symptoms. The highest prevalence of symptoms were found in companies 1, 7, 5 and 4 and the lowest in companies 2 and 8.

b) A grain fever syndrome (Table 21) is characterized by a short-term febrile illness (flu-like syndrome) that may be associated with respiratory symptoms. It usually occurs during work or shortly after work. It is related to exposure to high concentrations of dust any day of the work week and not necessarily the first day at work or the first day of the week. There was, however, a small percentage of workers who had a single episode of grain fever the very first time at work and not again. The workers stated that in the last three years, because of the improvement in the working conditions, grain fever occurred less frequently. Some workers had grain fever a few hours after work, compatible with allergic pneumonitis. However, none of these episodes were severe enough to require medical attention, and we lack radiographic proof of allergic pneumonitis. Furthermore, the symptoms tended not to recur unless very high concentrations of dust

were again present. Although we cannot deny that in some instances the grain fever syndrome may be a manifestation of allergic alveolitis, we have not found the typical history and radiographic changes of allergic alveolitis in these workers.

c) Acute recurrent conjunctivitis and rhinitis during exposure to grain dust occurred in most grain workers.

d) Skin pruritis occurred mostly on exposure to barley dust.

e) Pesticide exposure caused temporary disabling symptoms.

The long-term effects of recurrent symptomatic or asymptomatic exposures to pesticides are unknown. But we have encountered several former grain handlers with chronic neurological defects attributable to pesticide exposure.

Section I - Results 2b

2b. PULMONARY FUNCTION STUDIES

Lung function evaluations served the following purposes:

1) To determine if there was a difference in pulmonary function between grain handlers and people residing in the same geographic area with similar labor backgrounds, age, sex, smoking habits and socioeconomic status.

2) To determine the relative effects of cigarette smoking and grain handling on lung function.

3) To determine the prevalence of abnormal lung function and the patterns of dysfunction among grain handlers.

4) To determine if job category, place or length of employment had an effect on lung functions among grain handlers.

5) To determine the prevalence, if any, of abnormal lung function and patterns of dysfunction among grain handlers.

Objectives 1 and 2

The results of the pulmonary function studies by age and smoking groups are presented in Table 25 for the grain workers and Table 26 for the controls. The mean values for all lung functions (Table 27) were significantly different when grain handlers and city workers were compared by the unpaired t-test. There were no differences in the mean FEV_1 and FVC of workers tested either on the same day of exposure one or more than 2 days after the last exposure. MMF means were different ($P < .05$) between those tested the same day and those tested more than two days from the last exposure (Table 28).

The effects of grain handling, age, height and smoking habits on lung function were analyzed by multiple regression analyses (Table 29). Age had a significant effect on all lung functions except $Vmax_{50}$. The effects of grain handling were significant on all measures except CV. Smoking had an effect on all lung functions. The combined effects of grain handling and smoking were additive, but not synergistic when tested for interaction.

Objective 3

The prevalence of abnormal lung functions, except FVC, was higher

among grain workers than controls using chi-square analysis (Table 30). Airway obstruction, defined as FEV_1/FVC < 70%, was present in 16% of grain workers and 7% of control workers. If abnormal MMF, $Vmax_{50}$ and $Vmax_{75}$ are considered to indicate airways obstruction, then MMF detected airways obstruction as often as FEV_1/FVC, whereas, $Vmax_{50}$ and $Vmax_{75}$ detected a higher proportion of abnormalities in both grain workers and controls. Abnormality in the distribution of ventilation (CV, N2/L) may also reflect airways dysfunction but is affected by the parenchymal recoil status. Grain workers had a higher prevalence of abnormality in distribution of ventilation (CV, N2/L) than controls. N2/L, although it detected a higher percentage of workers with dysfunction than FEV_1/FVC, also detected a higher percentage of abnormalities among the controls. The prevalence of abnormal D_{LCO} was higher in controls than grain workers and higher in smoker controls than smoker grain workers. The percentage of abnormal function tests other than D_{LCO} in nonsmoker grain workers tended to be higher than that of nonsmoker controls, however, the differences were statistically significant only for MMF and $Vmax_{50}$. The prevalence of abnormal functions was consistently higher among smoking grain workers than non-workers and reached statistical significance for MMF, $Vmax_{50}$, $Vmax_{75}$, N2/L. More severe airways obstruction, indicated by an FEV_1/FVC < 60% was not more prevalent among grain handlers (4%) than among controls (3%) and there were only 3 grain and 3 control workers with FEV_1/FVC less than <50%.

To determine the relative importance of cigarette smoking and grain dust exposure on lung function, we analyzed the ratios of their regression coefficients from the regression analysis (Table 31). A ratio of 1 indicates that smoking and grain exposure had the same effect on lung function. Values greater than 1 indicate a greater effect of smoking. For example, smoking had a 44% greater effect on FEV_1 than grain handling. Smoking had a much greater effect on D_{lco} and CV whereas flows at low lung volumes were close to 1 or even lower. Hence, the effects of smoking were the same or greater than grain exposure for all lung functions except $Vmax_{50}$. The reasons for the latter are not clear.

In addition, to further quantitate the effects of smoking and grain dust exposure, we analyzed these data using a log-linear model. Both grain handling and smoking significantly and independently increased the odds of having airways obstruction by two and one-half times (odds factor = 2.6 for grain handling and 2.7 for smoking). That is, a grain handler had two and a half times greater risk of having airways obstruction than a non-grain handler regardless of their smoking habit. Smoking, independent of grain handling, also increased the odds of airways obstruction 2.5 times.

Objective 4

Lastly, to study the effects of type, place and length of employment, we also used multiple regression analysis to adjust for age and smoking. We found no significant differences in lung functions between the six job categories, places of employment and length of employment (Tables 32 and 33).

CONCLUSIONS

Grain dust exposure had an adverse effect on lung function (Tables 25-27, 29-31). The effects of grain dust on airways function was highly significant, and the overall effect was the same or of a smaller magnitude than that of smoking. Although there were more grain workers with mild airways obstruction than controls, moderately severe or severe airways obstruction was equally prevalent in both. The effect of grain handling appeared to be on the airways and not on the parenchyma. However, the high prevalence of abnormal N_2/L which may reflect parenchymal injury needs further evaluation. There was no correlation between lung function and job category, place or length of employment (Tables 32 and 33).

Section I - Results 2c

2c. SKIN TESTS-IMMEDIATE HYPERSENSITIVITY

A. Analysis of Prevalence of Positive Reactions in the Grain Workers and Control from Duluth Metropolitan Area and Controls from the Madison, Wisconsin Area (Tables 34 and 35).

Common Allergens

The prevalence of positive skin tests to oak pollen or timothy grass in the grain handling population was lower than observed in the control population from the same geographic area. Moreover, the prevalence of 1 or more positive skin tests to common allergens was lower in the grain handling population (Tables 34 and 35) than in the Superior-Duluth city workers.

Superior-Duluth city workers had a higher prevalence of positive skin tests to oak pollen than Madison workers. Moreover, more of the subjects from the Superior-Duluth area had positive skin tests to 1 or more common allergens.

Conclusion

The lower prevalence of atopy in grain workers (1 or more positive skin tests) than city workers from the same geographic area suggests that the more "allergic" individuals tend to avoid the grain dust environment or leave the industry.

Fungal Antigens

There was a very low prevalence of positive skin tests reactions to fungal extracts. No differences were found between the occupational groups. However, the skin test reagents were not representative of the fungi and flora we found in the airborne dust of grain elevators (See Dr. Smalley's subcontract report).

Insects and Mites

There was a higher prevalence of skin test reactivity to mixed grain mites and mixed grain beetles in grain workers when compared to Duluth city workers. Skin test reactivity to grain beetles among the grain workers was higher than in the Madison workers.

Conclusion

As expected, a higher proportion of grain workers reacted to grain mites and insects commonly found to contaminate cereal grain. The prevalence of reactivity is similar to that we found in 1974 with common house insect extracts.

Grain Antigens

The prevalence of positive skin tests to whole grain antigens was low in the test and control populations. The prevalence of positive skin tests to small seeds was different when the city workers were compared to Madison workers or when the Madison workers were compared to grain workers. There was, however, no difference in skin test reactivity to small seeds when grain workers were compared to city workers.

Conclusions

The low prevalence of positive reactions may be due to low antigenicity of grain, extracts tested at sub-optimal concentrations, or the loss of antigenic components during extraction procedure.

Airborne Dust

The prevalence of positive skin tests to durum and spring wheat dusts was higher in the grain workers when compared to the city workers and the Madison workers. The city workers had a higher prevalence of positive reactions to durum wheat, corn, rye, oats and sunflower seeds when compared to Madison workers. The prevalence of the skin reactivity to barley was not different in the grain workers and city workers. However, the grain workers had a higher prevalence of skin test reactivity to barley than the Madison workers. When considering the prevalence of skin test reactions to one or more of the dust antigens, there was no difference between grain workers and city workers. There was, however, a significant difference between Duluth-Superior city workers and Madison workers. Similar differences were observed when the grain workers were compared to Madison workers.

Conclusions

The increased frequency in skin test reactivity to wheat dust extracts reflects the higher exposure of grain workers to wheat dusts. City workers, however, also seem to be exposed to environmental contamination with several types of grain dusts based upon comparisons with the Madison workers.

Settled Dust

The prevalence of skin test reactivity to settled dust was similar in grain worker and Duluth/Superior city workers but both were significantly higher than Madison workers.

B. Analysis of the Intensity and Degree of Skin Hypersensitivity in the Three Occupational Groups using the Total Sum of Wheal Reactions (Fig. 1).

Figure 1 presents the distribution of sums of all wheal reactions for each group of antigens. The mean wheal reaction for each group is indicated with a horizontal bar. The mean wheal reaction for common atopic allergens in control workers was greater than in grain workers. There were no significant differences in mean wheal reactions to the other antigens between the three occupational groups.

C. Prevalence of Grain Dust and Insect-Mite Reactivity among Atopic Grain Workers and Controls (Table 37).

The prevalence of skin reactions to grain dusts and insects or

mites was significantly higher among atopic individuals (grain or control) than among non-atopic individuals.

Conclusions

Not surprisingly, atopic workers are more likely to become sensitized to antigens extracted from grain dust and the insects or mites which are commonly found in cereal grains.

2a, b, c RELATIONSHIPS BETWEEN SYMPTOMS, PULMONARY FUNCTIONS AND SKIN REACTIVITY.

2a, b. RELATIONSHIPS BETWEEN SYMPTOMS AND PULMONARY FUNCTION.

The relationships were analyzed by: i) multiple regression analyses using the lung function value as the dependent variable and selected independent variables: symptoms, symptoms complexes, age, height, and smoking habit; and ii) comparisons of abnormal lung function in symptomatic and asymptomatic grain workers.

i) Results of Multiple Regression Analysis

The relationships between acute and chronic symptoms or symptom complexes are presented in Table 38. There was a significant negative relationship between symptoms on exposure to grain dust and tests of ventilatory function (FEV_1/FVC, MMF, $Vmax_{50}$, $Vmax_{75}$). There was also a significant and negative relationship between chronic cough first thing in the morning and the FEV_1/FVC and MMF. Chronic bronchitis phlegm the first thing in the morning, wheezing at night, grain fever, and chest illness did not correlate with pulmonary functions as tested.

Among controls, there was a negative relationship between FEV^1/FVC and chronic bronchitis, wheezing at night and dyspnea on exertion.

ii) Results of Relationship between Symptoms and Abnormal Pulmonary Functions. The Prevalence of Abnormal Lung Function in Grain Workers with and without Selected Symptoms (Table 39).

A higher proportion of workers with chronic bronchitis had airways obstruction as measured by FEV_1, FVC, MMF and $Vmax_{50}$. A higher proportion of workers with respiratory symptoms (cough, wheezing or dyspnea) on exposure to grain dust had abnormal FEV_1/FVC, MMF, $Vmax_{50}$ and $Vmax_{75}$ N2/L and DL_{LCO}. There was no correlation between the history of grain fever or wheezing at night and abnormal pulmonary function. Workers with dyspnea on exertion had a higher prevalence of abnormal FEV_1/FVC, MMF, $Vmax_{50}$, FVC and D_{LCO}.

Conclusions

Clinico-physiological correlation. Grain workers with symptoms on exposure to dust had lower values of ventilatory function than workers without symptoms on exposure regardless of smoking habits (Table 38, 39). This suggests that such symptomatic workers are at a higher risk of developing airways dysfunction and possibly non-specific bronchial hypereactivity. The prevalence of chronic bronchitis with airways obstruction was higher in grain workers than controls, regardless of smoking habits. In addition, chronic bronchitis with airways

obstruction was related to length of employment. These findings suggest that chronic grain dust exposure may result in chronic obstructive pulmonary disease.

2b, c. RELATION BETWEEN PULMONARY FUNCTION AND SKIN TESTS.

This analysis was done by: i) multiple regression analysis adjusting for age, height and smoking habit (Tables 40a and 40b), and ii) comparing prevalence of abnormal lung function tests in positive and negative skin reactors (Table 41a and 41b).

i) Multiple Regression Analysis - Grain Workers.- (Table 40a).

There were negative relationships between the total mean wheal diameter for common allergens and grain dust antigens and FEV_1/FVC, FEV_1, FVC, MMF, $Vmax_{50}$ and $Vmax_{75}$. Using skin tests in the categorical way (positive or negative skin tests), there were also negative relationships between reactivity to common atopic allergens (CAA), airborne dust extract, durum wheat, fungi and settled dust extracts and FEV_1, MMF, $Vmax_{50}$ and $Vmax_{75}$ (Table 40a). We found no consistent relationship between reactivity to insects and mites, and barley or to one or more grain antigens and pulmonary function. There was no relationship between skin test reactivity and pulmonary function in the control group (Table 40b), except for vital capacity with total wheal for common allergens and total wheal for grain and grain dust antigens and with reactivity to barley, grain and settled dust antigens.

ii) The prevalence of abnormal lung function in positive and negative skin reactors is shown in Tables 41a-b. The prevalence of abnormal FEV_1/FVC, MMR, $Vmax_{50}$ and N2/L were not different between atopic and non-atopic grain workers or between reactors and non-reactors to insect and mite antigens. There was, however, a difference in the prevalence of abnormal lung functions in reactors and non-reactors to fungal antigens. An abnormal $Vmax_{50}$ was more prevalent in reactions to airborne grain dust. Among the controls (Table 41b), there were no significant differences between reactors and non-reactors.

Conclusions

The results of the regression analysis indicate that grain workers with atopy or skin reactivity to grain dust antigens are more likely to have lower lung function values than non-reactors to common allergens or grain dust antigens. The clinical significance of these findings is not clear since abnormal lung function is not more prevalent among atopic individuals or skin reactors.

2a, c. RELATIONSHIP BETWEEN SYMPTOMS AND SKIN REACTIVITY.

The relationship between the prevalence of symptoms on exposure to grain dust and chronic symptoms and skin reactivity to allergens are presented in Table 42.

The data demonstrated that: 1) dyspnea on exposure to grain dust was more frequent among those grain workers with positive skin reactivity to fungal antigens and to grain antigens, and 2) nasal symptoms on exposure to grain dust were more frequent among those workers with positive skin reactivity to grain antigens, barley and oats antigens. Overall, there were no significant correlations between

acute symptoms on exposure to grain dust, chronic symptoms, grain fever, and symptom complexes and skin reactivity to common allergens or specific allergens.

Section I

2c. SKIN TESTS - DELAYED HYPERSENSITIVITY (Table 43).

There was no difference in the prevalence of positive PPD tests in grain workers and controls. The prevalence of skin reactions to candida and mumps was higher in city workers. Conversely, the prevalence of positive skin tests to Trichophytin and SK/SD was higher in grain workers. Overall, the prevalence of positive tests (2 or more of positive tests) was not significantly different between groups.

2d. SERUM PRECIPITATING ANTIBODIES

The prevalence of precipitins are shown in Table 44. City workers had a greater frequency of precipitins to Trichoderma, T. vulgaris (Greer or Hollisteir strains), T. sacchari or to one or more extracts (#1-33). Conversely, grain workers had a greater frequency of precipitins to durum wheat and rye. The grain workers also had an increased frequency of precipitins to airborne dusts from durum wheat, barley, rye, oats and sunflower seeds.

Relationships between Serum Precipitins and Pulmonary Function.

To evaluate the relationship, we used the prevalence of abnormal functions:

FEV_1/FVC ratio < 70%, $Vmax_{50}$ < 1.65 SD, N2/L > 1.65 SD and D_{LCO} < 80% in subjects with positive or negative precipitins to one or more of the following:

1) Fungal, bacterial, and pigeon sera antigens I-33 (Table 44)
2) grain dust antigens labeled 42-52 (Table 44)
3) grain, grain dust, insects or mites 34-55 (Table 44)
4) Thermoactinomycetes #17-20 and #27-33 (Table 44)
5) Aspergillus fumigatus #5-10 (Table 44)

There was a significantly higher prevalence of airways obstruction (FEV_1/FVC < 70%) among grain handlers with precipitins to A. fumigatus. A higher prevalence of abnormal slope III or N2/L was observed in grain workers and controls with serum precipitins to one or more fungal antigens. There was no relationship between the presence of precipitins and abnormal $Vmax_{50}$, $Vmax_{75}$, MMF, and DL.

Relationship between Symptoms and Precipitins

The prevalence of symptoms on exposure or grain fever were not different in grain workers with or without precipitins as described above.

Conclusions

7) Serum precipitating antibodies. City workers had a greater prevalence of precipitins to Trichoderma, T. vulgaris, T. sacchari and to one or more fungi than grain workers (Table 44). Conversely, grain workers had a greater prevalence of precipitins to durum wheat, rye, and airborne dusts of wheat, barley, rye, oats and sunflower and to one of the settled dusts than controls. The larger prevalence of

precipitins to some grain dusts among grain workers was not surprising, yet they did not correlate with increased prevalence of symptoms or abnormal lung functions. Hence, the data infer that the respiratory reactions to grain dusts are not precipitin-mediated and that grain fever is not a manifestation of allergic alveolitis type III reaction. Serum precipitins reflect host response to antigens but not necessarily the presence of disease or abnormal pulmonary dysfunction. The reason for the greater prevalence of fungal precipitins among city workers is not clear.

Section I

2e RESULTS - BLOOD CHEMISTRIES, URINALYSIS, HEMOGLOBIN

The mean values for pseudocholinesterase , SGPT and creatinine were higher among grain workers (Table 45). The elevated values observed, however, were not high enough to indicate significant parenchymal liver disease. When the prevalence of abnormal values (larger than the highest range value for the laboratory) was considered, there was a difference only in GGT values.

Follow-up studies of abnormal liver function tests were attempted, but the returns from patients and physicians were not high. Hence, the data were inconclusive.

The presence of protein in urine was more frequent among grain workers when compared to controls.

Discussion - Liver Function Screening

SGPT

Alanine amino transferase (SGPT). The determination of SGPT is a sensitive indicator of minimal hepatocellular injury. Elevated levels may precede other evidence of viral hepatitis by several weeks. The levels may remain elevated following the return to normal of other laboratory parameters which are sensitive indicators of persistent hepatitis. Like other indices of necrosis, the transaminases are inferior to alkaline phosphatase and other cholestatic indicators in detecting infiltrative liver disease or cholestatic injury. The SGPT analysis contributes significantly to the differential diagnosis of hepatobiliary disease: as a general rule, levels greater than ten times the upper normal limits favor acute hepatic cellular injury, lesser elevations favor chronic cell injury, cholestasis or infiltrative liver disease. There are, however, a number of important exceptions. Alcoholic liver disease (severe, acute alcoholic hepatitis) is characterized by transaminase levels less than ten times normal. On the other hand, extremely high values (in excess of ten times normal) may occur in early cholestatic injury due to extra hepatic obstruction. Although the transaminases are sensitive indices of cell injury, the diagnostic accuracy of these determinations are limited by a lack of a specificity. SGPT is widely distributed in the body, but it is predominantly confined to the liver making it more specific than SGOT.

GGT

Gamma glutamyl transpeptidase (GGT) predominates in renal and hepatobiliary tract tissue and was shown by histochemical methods to be located in the endothelial cells of a variety of tissues. However,

serum GGT it is believed to originate in the hepatobiliary system. It is regarded as the most sensitive of the cholestatic indicators but has also been reported to be significantly elevated in virtually all hepatocellular conditions, especially in alcoholic liver disease and infiltrative hepatobiliary disease. Since GGT is absent from bone and placenta tissue, children, adolescents, pregnant patients and patients with bone disease show normal or only slightly elevated GGT values. A number of reports demonstrated that GGT was elevated in neurological disease, post-myocardial infarction, alcoholic patients without other evidence of liver disease and patients receiving enzyme-inducing drugs (e.g., anticonvulsants). This suggests that the elevated GGT levels should be interpreted with caution. As with SGPT, slightly elevated levels (i.e., slightly above the range of normal) are less organ specific than high abnormal values, but other organ parenchymal reactions cannot be excluded from consideration. For the purpose of screening for liver disease, the values did not reach levels (ï500) which were indicative of liver disease. Abnormal or elevated GGT values were found in 64 or (21%) of the grain workers and 31 (13%) of the controls. The elevated gamma GT rarely correlated with elevated SGPT or cholinesterase.

Cholinesterase may be an indicator of chronic liver disease but few abnormal values were found in this study. The frequency of abnormal values was not different in grain workers and controls.

CONCLUSION

We did not detect significant differences in the frequency of overt liver disease between grain workers and controls. We used three screening methods to detect liver disease. The questionnaire included a question: "has a physician ever diagnosed liver disease in the patient?" There was no significant difference in the answer to this question between grain workers and controls. Second, in the physical examination the presence of hepatomegaly was determined. There was no significant difference between the two groups, although the prevalence of a palpable liver was different between the two groups. Third, the serum enzymes (SGPT< GT and cholinesterase were not abnormal in the grain workers or controls).

Although the findings are inconclusive, we did find a higher number of abnormal values for GGT, a higher mean value of SSPT, and more palpable livers among grain workers. Since grain workers are occasionally exposed to hepatotoxic grain fumigants we recommend further prospective studies on the potential hepatotoxicity of grain fumigant exposure.

Renal function screening

There were few abnormal creatinine levels (Table 45) in grain workers and controls, but the mean value (t-test) was significantly higher among grain workers than controls (Table 46). The urinalysis revealed no differences in the frequency of blood, glucose and protein the urine when grain workers were compared to city workers. There was, however, a higher percentage of grain workers with a trace of protein in the urine.

CONCLUSION

11) Renal disease screening. The results of the renal function screening tests were inconclusive (Tables 45, 46). We would recommend further prospective studies on the potential renal morbidity of pesticide exposure.

Hemoglobin - hematocrit

The hemoglobin was evaluated: 1) to detect the presence of polycythemia or anemia and 2) for correction of diffusion values if necessary. We found no significant abnormalities in either occupational group.

RESULTS

2f RADIOLOGICAL FINDINGS

The chest roentgenogram changes found in grain workers and controls are shown in Table 47. The prevalence of abnormal findings was small and most changes, with a few exceptions, were of minor clinical significance. One control subject showed bilateral hilar adenopathies compatible with lymphoma or sarcoidosis. Another control had evidence of coronary bypass surgery and a third subject had a questionable paratracheal node (which in re-examination with other views could not be delineated). Among the grain workers there were three workers with small blebs, one with marked hyperinflation compatible with emphysema, one with a rib resection from a negative exploratory thoracotomy and a worker with bilateral calcified pleura thickening. The apical thickening and costophrenic angle pleural thickening was minimal. There were no cases with diffuse bilateral interstitial infiltration or fibrosis. Old healed rib fractures, degenerative changes of the thoracic spine, basilar bands of fibrosis or plate atelectasis were more commonly seen among grain workers.

Conclusion

Grain dust exposure does not appear to be associated with any specific roentgenographic abnormality.

2g. Immunoglobulin Levels

The levels of IgG and IgA observed in grain workers differed significantly from the city workers (Table 48), whereas the levels of IgM were similar in both groups. Since it was conceivable that differences in age, smoking habit, place of employment or length of employment introduced an inherent bias into the data, the test and control groups were further subdivided.

Table 49 shows the immunoglobulin levels when the test group (grain workers) and controls (city workers) were grouped on the basis of smoking habits. The levels of IgG were significantly higher in the grain workers when compared to city workers in each of the three smoking habit categories. Hence, the data suggest that the increased levels of IgG observed in the grain workers were not a reflection of smoking habit. Conversely, only ex-smoking and nonsmoking grain workers demonstrated elevated serum IgA levels when compared to control values. The data suggest that grain dust normally enhances the levels of serum IgA but that the response was blunted by smoking.

When the same data were grouped with respect to age, the following data were obtained: Grain workers between the ages of 31 to 50 years demonstrated elevated IgG levels (Table 50). There was no statistical difference in IgG levels when other age groups were considered. Statistical differences in IgA levels were only observed when grain workers between the ages of 41 to 50 years were compared to controls.

Since it was conceivable that length of employment influenced the data, the test and control immunoglobulin levels were subdivided with respect to length of employment (Table 51). Both IgG and IgA were elevated in the grain workers working in the elevators from 10.6 to 15.5 years. Increased levels of IgA were also observed in grain workers working fewer than 5.5 years in the industry. However, there was no relationship between IgA levels and place of employment. Increased levels of IgG (Table 52) were observed in workers in elevators 1 and 8.

The levels of circulating IgE were also ascertained in serum samples obtained from grain workers and controls using commercially available immunodiffusion plates. The lowest level of sensitivity of this system is 600 I.U. Only four of the 307 grain workers tested had IgE levels above 600 I.U. (1,000-4,000 I.U.) Similarly, only two of the 235 city workers tested had IgE levels above 600 I.U.

Data from the IgE determinations should be interpreted with caution. Recent data from our laboratories, using radioimmunoassays for determination of IgE levels, suggest that the level of IgE in normal serum is below 50 I.U./ml. Serum from highly allergic individuals contains between 300-600 I.U. and, rarely, levels above 900 I.U. Hence, the immunodiffusion method for determining IgE levels is not sensitive enough to detect increases in serum IgE occurring between 100-600 I.U.

CONCLUSION

Grain dust exposure enhances the levels of serum IgA and IgG, an effect which appears to be blunted by smoking (significantly in the case of IgA).

2h. ALPHA$_1$-ANTITRYPSIN (AAT) LEVELS

The levels of alpha$_1$-antitrypsin in serum samples from grain workers and controls were determined by the timed Mancini technique (Appendix XIII). The reproducibility of the system was insured through the use of the protocol outlined under the section on Materials and Methods.

There was no statistical difference when the AAT levels in grain workers (mean $\pm$ SD = 296 $\pm$ 5 mg/dl) were compared to controls (mean $\pm$ SD = 308 $\pm$ 6 mg/dl). When smoker grain workers were compared to smoker controls there was a significant decrease in the AAT levels observed in grain workers (Table 53). No significant differences were observed when other smoking categories were compared.

When the test group and controls were grouped by age, the AAT levels were significantly depressed in grain workers between the ages of 21 to 30 years and 41 to 50 years (Table 54a). Unlike the immunoglobulin levels, there was no relationship between length of employment and AAT levels (Table 54b).

Since it has been shown that subjects heterozygous for the AAT deficiency gene (Pi phenotype MX) have serum levels of AAT that are roughly 60% of the normal levels, we selected sera from the nine grain workers with AAT levels less than 60% of the normal values for phenotyping and trypsin inhibitory capacity (TIC) measurements. These studies were performed by Dr. Richard Talamo of Johns Hopkins University. None of the city workers exhibited intermediate AAT levels by the Mancini test.

The data show that three of the grain workers (#232, #239 and #240) had the heterozygous MZ phenotype and impaired TIC (Table 55). Another three subjects had the MZ phenotype and normal trypsin inhibitory capacity. The remaining three subjects had the MZ phenotype and normal TIC. Because of the small number of heterozygotes found in this study, we did not do statistical correlations with symptoms or lung function, but the review of these six subjects did not reveal any consistent abnormalities. Of the three subjects with MZ phenotype, one (#332) had a slightly decreased D_{LCO} and abnormal CV and N2/L but no evidence of airways obstruction. He had many years of chronic productive cough and wheezing. He also had elevated SGPT and GGT of unknown etiology. One of the three with MS (#52) had an FEV_1/FVC of 74% which may reflect some mild degree of airways obstruction at his age.

2i. MULTIPLE REGRESSION ANALYSIS OF THE EFFECT OF GRAIN DUST EXPOSURE ON IMMUNOGLOBULIN AND ALPHA$_1$-ANTITRYPSIN LEVELS (TABLE 56).

As will be seen in Table 57 grain dust exposure has a highly significant positive effect on the levels of IgA, and IgG ($p < 0.005$) but not on IgM. Length of employment and/or age is also positively related to levels of IgA and IgG with a significant relationship present for smoking ($p < 0.05$). Unfortunately it is not possible to separate the confounding effects of age and length of employment on immunoglobulin levels.

Conversely, grain dust exposure has a significant negative effect ($p < 0.025$) on the level of alpha$_1$-antitrypsin (AAT). Length of employment ($p < 0.0005$) and/or age ($p < 0.001$) and smoking ($p < 0.0005$) on the other hand show a highly significant positive relationship to the level of AAT. In this case age appeared to be a better predictor than length of employment.

Since grain dust exposure appeared to have a significant effect on the immune system which conceivably could in turn be related to the disease syndromes encountered in grain workers, we analyzed the relationship between chronic bronchitis, occupational asthma, grain fever and other symptoms, as well as skin test reactivity, tests of pulmonary function, and the level of immunoglobulins. Using an unpaired t test we found no statistically significant relationships between levels of immunoglobulins on AAT and skin test reactivity or symptoms. However, the levels of IgA, IgG and IgM were consistently higher in subjects who showed skin test reactivity to antigens from airborne grain dust, fungi, insects and mites. A similar trend was seen when comparing symptomatic with non-symptomatic workers for IgA and less consistently for IgG.

The results of a similar analysis of immunoglobulin levels in relation to abnormal tests of pulmonary function are seen in Table 57. Significant association was found between abnormal FVC, $Vmax_{50}$, DL and IgA and between abnormal FVC and IgG. Also a significant association was found between abnormal N2/L, DL and AAT. Once again subjects with abnormal pulmonary function had, with two exceptions, consistently higher levels of IgA and IgG.

CONCLUSIONS

Chronic exposure to grain dust appears to stimulate the immune system as reflected by its positive effect on serum IgA and IgG which increase with age and/or length of employment. The mechanism involved in producing these increases is unclear but may be an adjuvant effect. This may also explain the consistently higher levels of immunoglobulins in subjects who show skin reactivity to antigens found in grain dust. Evidence regarding any relationship of this effect on the immune system to the disease syndromes encountered in grain workers is conflicting. There is no relationship between immunoglobulin levels and acute or chronic symptoms of lung disease. On the other hand, workers with abnormal lung function have significantly higher levels of IgA and/or IgG. These findings merit further study.

Chronic exposure to grain dust appears to be associated with a decrease in the level of AAT. Smoking on the other hand is associated with a decrease in the level of IgA and IgG and an increase in AAT. The significance of the association between abnormal N2/L, DL and AAT is unclear. However, these tests may reflect an inflammatory reaction in the small airways (bronchiolitis) related to chronic grain dust exposure. These findings also merit further investigation.

STUDY II. WORK SHIFT STUDY

Materials and methods

Population

We studied 248 grain workers and 192 controls (city services workers). They represented 88% of the 283 grain workers and 80% of the 239 controls previously surveyed (see Study I). The 27 longshoremen were not asked to participate in the work shift study.

The characteristics of the test and control populations are presented in Table II-Ia and b. The following parameters were evaluated:

1) Symptoms during the shift and the workers' subjective appraisal of the dust exposure were obtained at the end of the work shift on a standard form.

2) Pulmonary functions studies before and after the shift included: FEV_1, FEVC, $Vmax_{50}$, $Vmax_{75}$. All of these values were obtained using a rolling bar Ohio 840 spirometer with the techniques described in Appendix VIII.

3) Blood studies before and after the shift included: a) leukocyte count and differential count, b) serum complement and complement activation measured studied as described in Appendix XIII and XIV.

4) Oral temperature were taken at 700-800 hours, 1100-1200 hours, 1500-1600 hours and 2000 hours.

5) Environmental studies: airborne total and respirable dust levels were measured on each of 209 grain workers and in a sample of 63 controls using personal dust samplers as described in Appendix XV.

6) Mycological studies: Airborne dust from personal samplers was analyzed for fungi as described in a separate report.

Medications taken by subjects during the day of study included: subject #9 - "Tedral," #7 - "Contac," #21 - "Robitusin," #242 - "Cough drops" and #233 - "Dristan."

RESULTS

Dust exposure. Of the 248 grain workers, 197 (79.4%) reported exposure to either wheat, barley or oats. Other exposures included sunflower seeds, corn and rye. Total dust levels in grain workers by elevator and job category are presented in Table II-2.

Symptoms during work.

Twenty-five % of the grain workers claimed they had worn a mask during at least part of that day. The incidence of respiratory symptoms (cough, expectoration, wheezing and dyspnea), nasal stuffiness and eye irritation (Table II-3) were higher in grain workers than controls.

The incidence of symptoms during work shifts in grain handlers by smoking categories and subjective appraisal of dust exposure is shown in Table II-4. Wheezing and/or chest tightness were more common among smokers than nonsmokers, whereas throat symptoms were more common among nonsmokers.

Grain workers who reported a normal or average exposure to dust during the work shift had a higher incidence of cough and phlegm when compared to workers exposed to less than average dust concentrations. Workers exposed to higher dust concentrations (more than average) had a higher incidence of dyspnea, wheezing, eye and nasal symptoms when compared to workers exposed to average or less than average dust concentrations. Most symptoms were more common among workers who reported a heavy exposure to dust some time during that day ($p < 0.05$). Incidence of respiratory symptoms by company and job category are shown on Table II-5.

Leukocyte count and serum complement

The leukocyte count and serum complement levels before and after work shifts are presented in Tables II-6 and II-7. The data show that the total white blood cell counts were not different when pre- and post-samples were compared (Table II-6). The differential white blood cell count suggests that there were slight shifts in leukocyte subpopulations during a work shift. Grain workers had slight increases in the percentage of segmental neutrophiles and decreases in lymphocytes when compared to controls.

There were no changes in the mean C3 levels during the work shift in either the grain working or control population (Table II-7). Moreover, there was no evidence of classical or alternate complement pathway activation. However, six of 191 controls demonstrated activation of the alternate complement pathway at pre- and post-shift intervals. This may represent faulty on-site specimen handling in Duluth/Superior.

Consideration was also given to individual changes in total C3 levels within the grain workers and city workers. Using the standard deviation from the pre-shift city workers (28 mg %) as the base, we considered a significant increase or decrease in C3 levels to be 2 standard deviations (56 mg %) from the population mean. Using this criterion to analyze the C3 data, seven of 248 grain workers decreased their C3 levels significantly as compared to three of 191 city workers. Conversely, nine of the grain workers increased C3 levels by more than 53 mg%. None of the city workers increased C3 levels by the same value.

There were no correlations between increases or decreases in complement levels and changes in pulmonary function tests, white blood counts or symptoms. Moreover, there was no relationship between changes in complement levels and activation of complement by either the classical or the alternative pathway.

Body temperature

Values are presented in Table II-8. There were no differences in body temperature (800, 1200, 1600 and 2000 hour values) between the grain workers and the controls.

Pulmonary function studies

The pulmonary function studies were performed before and after the work shift (Tables II-9 and II-10). Analysis of the pre- and post-shift values indicated that no significant acute effects on lung function occurred during the work shift (Table 10). On the other hand when the data were expressed as % difference in pre- and post-values (Table 9), the FVC, $Vmax_{50}$ and $Vmax_{75}$ in grain workers were significantly different from controls ($p < 0.05$). The difference was due to a slight increase in function in the controls and an average slight decrease or lesser increase in function in grain workers. Also the actual differences in pre- and post-shift $Vmax_{50}$ and $Vmax_{75}$ were slightly positive in controls and slightly negative in grain workers (Table II-9).

The changes in lung function, before and after a work shift, were also evaluated by multiple regression analyses using the actual pre-post-shift lung function difference or % differences as the dependent variable. The independent variables were grain handling, age, height, current smoking and ex-smoking. This analysis indicated that grain handling had a significant adverse or negative effect on pre-post-shift % differences in lung function independent of the effects of cigarette smoking (Table II-11).

To evaluate the possible clinical significance of changes in pulmonary function, we studied the incidence of post-shift reduction in

function of varying severity that might be considered an abnormal response to the environment (Table II-12a). Although the numbers of workers were small, there was a consistently higher number of grain workers with pre-post reductions in pulmonary functions when compared to city workers having no abnormal lung function changes. The characteristics of the 14 subjects with pre-post shift difference in FEV_1(>15%) are presented in Table 12b. The mean age (48.4 years) was higher than the population mean, and the mean length of employment was 15.8 years. Only 2 of the 14 were nonsmokers. Four had a history of chronic bronchitis and one subject had asthma. Nine of the 14 had complained of cough or wheezing on exposure. All subjects were exposed to wheat, barley and/or sunflower seeds. The total dust levels varied between .5 and 9.3 mg/m3. Ten of the 14 had pre-existing airways obstruction. Three were atopic and 5 had skin reactivity to grain dust antigens.

These data suggest that a decline in FEV_1 greater than 15% over a work shift can occur at average total dust levels lower than 10 mg/m3 in grain workers with pre-existing airways obstruction, regardless of smoking habits (smokers or ex-smokers).

Relationship between Symptoms during Work and Lung Function Changes

There was no difference (chi square analysis) in the % change of pre-post values when subjects with respiratory symptoms or fever were compared to subjects without symptoms (Table II-13).

Relationship between Symptoms of Pulmonary Function Changes during the Work Shift and Skin Hypersensitivity

The incidence of respiratory or nasal symptoms was not different between atopic (wheal reaction ī 3 mm to one or more common allergens) and non-atopic workers or between grain dust skin reactors and non-reactors.

Also using pre-post mean values for pulmonary function tests, there were no differences when atopic and non-atopic subjects or grain reactors and non-grain reactors were compared.

Relationship between Total Dust Level and Presence or Absence of Symptoms during Work Shift

Workers with respiratory symptoms (cough, expectoration, wheezing or dyspnea) during the work shift were exposed to a higher mean total dust level than workers who did not claim symptoms on the shift studied (Table II-14).

The incidence of symptoms by dust level categories is shown in Table II-15a. Few significant differences between grain workers and controls are seen at dust levels below 10 mg/m3. At levels above 10 mg/m3 there is a significantly higher incidence of cough, dyspnea, fever, eye and throat symptoms among grain workers. It should be noted, however, that relatively low dust levels were encountered during this study. Sixty-seven % of the measured values were below 2 mg/m3 and 86% were below 5 mg/M3. Only 7% of the values were between 5-10 mg/m3 and 7% were above 10 mg/M3. Compared with the conditions that existed prior to 1974, this is a remarkable achievement on the part of the grain companies to control grain dust in their elevators. During

this study only 4% of the measured dust levels exceeded the current nuisance dust standard of 15 mg/m3. On the other hand the relatively small number of workers exposed to grain dust levels above 5 mg/m3 limited our ability to establish a clear cut dose-response relationship. Since control workers could be considered as having zero grain dust exposure, we repeated the analysis using the entire cohort of city workers (N=192). The results are shown in Table 15b. As will be seen in this table, grain workers experience a significant excess of cough and expectoration even at dust levels 5 mg/m3.

Relationship between Total Dust Level and Workers' Subjective Estimation of Dust Exposure

There was a significant relationship between total dust level and workers' subjective estimation of dust levels (Table 11-14).

Relationship between Total Dust Level and Pre-Post Shift Differences in Lung Function Tests

Relationships were studied by multiple regression analyses using pulmonary function (FEV_1, FVC, $Vmax_{50}$ or $Vmax_{75}$) as the dependent variable and dust level, age, height, smoking and ex-smoking habit as independent variables. In the grain workers (Table 11-16) there was a significant negative relation between dust level and pre-post shift % changes in FVC, $Vmax_{50}$ and $Vmax_{75}$ ($P < 0.05$) adjusted for the effects of age, height and smoking habit (Table 11-16). In controls there was no relationship between the dust level and changes in pulmonary function using any test.

The negative effect of grain dust on tests of airways flow and vital capacity appears to be dose related.

Relationship between Dust Levels and Pre-Post Shift Difference in Leukocyte Count and C3 Complement Level

By regression analysis, pre-post shift changes in leukocyte count or in C3 complement level were considered dependent variables and dust level, age and smoking as independent variables. In the controls there was no relationship found between total dust levels and changes in leukocytes or C3 complement levels. In grain workers there was a positive relationship between total dust level and the pre-post shift difference in leukocyte count ($P < 0.05$), but no relation between complement level changes and dust levels. Grain dust exposure thus appears to induce a leukocyte response that is dose related.

Conclusions

Exposure to grain dust during a work shift has a dose related acute adverse effect on the worker. The effects, which are largely on the respiratory system, are seen at relatively low dust concentrations. When compared with city workers, grain workers show a significant excess of cough and expectoration during a work shift at dust concentrations below 5 mg/m3. In addition, the susceptible workers (i.e., those with pre-existing airways obstruction) can experience significant declines in ventilatory function at dust levels below 10 mg/m3. Because of the small proportion of workers (14%) who were exposed to dust concentrations above 5 mg/m3 during this study, it was difficult to establish an exact dose-response relationship between dust concentrations and ventilatory function. There seems to be little

doubt, however, that dust concentrations below the current nuisance dust standard of 15 mg/m3 can have an adverse acute effect on ventilatory function. The grain companies are to be congratulated on the remarkable decrease in dust levels that has been achieved since 1974. During this study 86% of the dust measurements were below 5 mg/m3 and 93% below 10 mg/m3. Further studies should include peak values of dust concentrations as well as time-weighted averages, since workers' symptoms and changes in lung function may be related more to peak concentrations than average concentrations of dust during an 8-hour shift.

STUDY III PROSPECTIVE 3 YEAR FOLLOW-UP (1974-1977)

The purpose was to determine changes in pulmonary function in grain elevator workers who had been previously studied.

Materials and Methods

We studied 172 of the 293 year-round grain workers who were studied in 1974. One hundred and twenty-one subjects were not included in the study or analysis because of the following reasons:

1) Thirty-two subjects were working in the elevators but refused or could not participate.
2) Thirteen subjects were laid off and unavailable.
3) Twelve subjects had retired at ages 62 to 65 except one who had retired earlier because of a stroke residual.
4) Thirteen subjects were on vacation, 8 were on sick leave, 8 had changed jobs and moved away from the area and 3 had been transferred to management.
5) Three had died (2 heart attacks and 1 car accident).
6) The status of 29 workers was unknown.

The characteristics of the population studied are shown in Table III-1.

Pulmonary Function Studies (see appendix VIII)

Pulmonary function studies included forced expiratory volume in 1 sec (FEV_1), forced vital capacity (FVC), and mean forced expiratory flow during the middle half of the FVC (MMF), all recorded on a 13.5 liter Collins spirometer. The FEV_1 and FEF 25-75% were measured from the largest of three acceptable FVC tracings, and all volumes were corrected to BTPS. The instantaneous maximal expiratory flows after exhalation of 50 and 75 % of the FVC ($Vmax_{50}$ and $Vmax_{75}$, respectively) were measured using a rolling bar spirometer and were displayed on an X-Y recorder. The average of 3 reproducible maximal expiratory flow volume curves was used.

Diffusing capacity of the lung for CO (D_{LCO}) was measured by the single-breath method of Ogilvie and associates[14].

Predicted values for FEV_1, FVC, and MMF, $Vmax_{50}$, $Vmax_{75}$ were obtained from the data of Knudson et al.[17]; D_{LCO} from Ogilvie and co-workers[14]. These methods were used in the 1974 study.

The changes in pulmonary function over 3 years were evaluated independently and also by the status of their smoking habit including: smoker who remained smoker; smoker who became ex-smoker; ex-smoker who

remained ex-smoker; nonsmoker who remained nonsmoker. Three ex-smokers who resumed smoking and 4 nonsmokers, who became smokers and then quit, were excluded from analysis.

Other information on these subjects was obtained as described under Material and Methods - Study I.

The current status of their respiratory symptoms was obtained on a standard form. Changes in smoking habit were obtained from the standard questionnaire.

Results

Symptoms. The symptoms reported by workers in 1977 are shown in Table III-2. Most workers (72-84%) reported that their respiratory symptoms remained about the same, but 9-25% were better or had symptoms less often. A small percentage felt their symptoms were worse (Table III-3).

Pulmonary function changes. There were no significant changes in FEV_1 and FVC, but there were significant changes in MMF, $Vmax_{50}$ and $Vmax_{75}$, both actual and when corrected for age by using the changes in % predicted values (Table III-4).

Similar results were detected in the different smoking categories (Tables III-5-8).

The yearly mean decrement (Table III-9) in FEV^1 and FVC was similar to that expected from published data (Knudson, et al.), but the yearly mean decrement in FFM, $Vmax_{50}$ and $Vmax_{75}$ was greater than expected.

There were no differences between the 3 year changes in atopic and non-atopic individuals, between skin reactors to grain dust and non-reactors, between those with chronic bronchitis and those without, or between workers with and without occupational asthma I (Table III-10).

CONCLUSION

This study is seriously faulted by the poor level of participation (59%) of workers previously studied in 1974. However several tests of lung function (MMF, $Vmax_{50}$ and $Vmax_{75}$) showed a yearly mean decrement that was greater than expected, which is probably indicative of the chronic effect of grain dust on ventilatory function.

STUDY IV BRONCHIAL CHALLENGE STUDY

IDENTIFICATION OF GRAIN DUST CONSTITUENTS WHICH CAN INDUCE PULMONARY REACTION

This study was undertaken to identify the constituent of grain dust responsible for grain handlers' symptoms and to determine a site of action in the lung. Host factors which may influence or contribute to this process were also assessed.

Material and Methods

Subjects: The subjects for the study were 11 grain handlers from northern Wisconsin and Minnesota who had respiratory symptoms on

exposure to durum wheat dust at work. Symptoms included cough, wheezing, chest tightness and expectoration. The workers were all men with a mean age of 38 and an age range of 27-59 years.

Skin tests: Subjects were tested for atopic diathesis by prick test using six common allergens: ragweed, feathers, oak, cat, Alternaria and timothy grass. The subject was considered atopic if he developed a 3 mm or greater wheal at 20 minutes to three or more of these allergens. Intradermal tests were used to detect immediate skin test reactivity to extracts of durum wheat, airborne durum wheat dust, molds, grain mites, grain weevils or grain beetles. These extracts were prepared from material collected from the workers' environment. Subjects were termed positive skin reactors if an 8 mm or greater wheal was raised at 10 minutes to an injection of 1000 PNU per ml or less of extract.

Precipitating antibody: serum precipitating antibodies against durum wheat, airborne durum wheat dust, molds and insects were measured by the immunodiffusion method of Ouchterloney.

Spirometry: Spirometry was performed on an Ohio 840 rolling bar spirometer. The FEV_1 and MMF were measured from the largest of two acceptable FVC tracings, and all volumes were corrected to BTPS. The instantaneous maximal expiratory flows after exhalation of 50 and 75% of FVC ($Vmax_{50}$ and $Vmax_{75}$) were measured from 2 reproducible maximal expiratory efforts which were displayed on an X-Y recorder and then averaged. Diffusing capacity of the lungs for CO (D_{LCO}) was measured by the single breath method of Ogilvie and associates. Before entering the study, each subject was tested for pre-existing airways obstruction. Subjects 40 years of age or under were termed obstructed if the FVC_1/FVC % was less than 70%, and subjects over 40 years of age were termed obstructed if the FEV_1/FVC % was less than 75%.

Preparation of challenge material: The preparation of extracts used for skin tests and bronchial challenge is described in Appendix X. Twenty-four hours prior to the challenge, the lyophilized extracts were resuspended in sterile, non-pyrogenic coca buffer with 3.0% human serum albumin to effect a final concentration of 100,000 PNU/ml. The resuspended extracts were filtered through a millipore filter (pore size .22mm), placed in sterile needle vials and tested for sterility on nutrient agar and Sabouraud's agar plates incubated at room temperature and 37°C. If the plates showed no growth after 24 hours, the resuspended extracts were diluted into additional needle vials using sterile coca buffer with 3.0% NSA to effect final concentrations of 100,000 PNU/ml, 50,000 PNU/ml, 10,000, 5,000, 1,000, 50 and 1.0 PNU/ml.

Bronchial challenge: Subjects were tested on 4 or 5 consecutive days. Each day a challenge was performed using a different extract: durum wheat, durum wheat dust, grain mites or grain insects. A Rosenthal dosimeter powering a #42 Devilbis nebulizer was used to administer the extracts. Five vital capacity inspirations were taken slowly by the subject and then held for 5 seconds at each concentration of extract. This maneuver was repeated at gradually increasing concentrations of extracts until either a drop in FEV_1 of at least 20% was noted or the maximum concentration (100,000 PNU/ml) of antigen

was given. Pulmonary function testing was performed before administration of antigen, 10 minutes after administration of antigen at each concentration, and at frequent intervals thereafter up to 24 hours.

Temperature: During each bronchial provocation, oral temperature was measured hourly.

Laboratory tests: Blood samples were drawn for white blood cell counts and for complement (C3) measurements at 20 minutes, 4, 8 and 24 hours. Complement was measured by the method described in Appendix XII.

Methachoiine provocation: Non-specific bronchial reactivity was measured using Methacholine inhalation by the method recommended by Chi and co-workers. Using the dosimeter technique, Methacholine was administered in increasing concentrations from 2.5 mg/ml to a maximum concentration of 25 mg/ml. The test was terminated when $\geq$ 20% decrease from the baseline FEV_1 was noted, or if no response was elicited, with the maximum concentration. The results were expressed as the concentration of Methachoiine producing a 20% decrease in FEV_1 (Pc20) calculated from a dose-response curve.

Challenge after sodium cromoglycate: One capsule of sodium cromoglycate was administered, via a spinhaler, 10 minutes prior to challenge.

Results

Bronchial provocation challenge: Five of the 11 subjects showed a decrease in FEV_1 ($\geq$ 20%) in response to bronchial provocation with extracts of durum wheat (IV-Fig. 1). These 5 were termed airways reactors. The other 6 showed no significant diminution in FEV_1 when challenged with these extracts and were termed non-reactors (IV-Fig. 2).

Type of response: One subject responded to extracts from both durum wheat and airborne durum wheat dust. In this subject the airways response occurred within 10-20 minutes (IV-Fig. 1). The other 4 subjects showed only late responses to durum wheat extract.

Methacholine response: Methacholine inhalation produced a positive test in 4 of the 5 airways reactors and in two of the 6 non-reactors (Table IV-1). However, the Pc 20 was lower in the positive airways reactors.

Contribution of airways obstruction: Pre-existing airways obstruction was present in 4 of the 5 airways reactors and 3 of the 6 non-reactors (Table IV-1). However, the obstruction was more severe in the reactors. Four of the 5 airways reactors were ex-smokers and one subject was a smoker. Of the 6 non-reactors, the 2 smokers were obstructed and 1 of the 2 ex-smokers was obstructed. The 4 airways reactors who had pre-existing airways obstruction responded to Methacholine inhalation while the non-reactors with pre-existing airways obstruction showed no response.

Effect of sodium cromoglycate: Four of the 5 airways reactors were pre-treated with sodium cromoglycate and challenged with extract of

durum wheat. The n airways response was blocked by pre-treatment in all four subjects (Fig. IV-3).

Skin test: One of the 5 airways reactors and 2 of the 6 non-reactors were atopic. There was no correlation between positive skin tests (CAA, insects, mites, durum wheat, durum wheat dust, and aspergillus species) and a positive bronchial challenge (Table IV-2).

Precipitating antibody: One of the 5 airways reactors had serum precipitating antibodies directed to the durum wheat extract and positive bronchial response to durum wheat. Five subjects showed serum precipitins against extracts which did not induce a bronchial response.

Diffusing capacity: There was no significant change seen in the D_{LCO}.

Blood tests: There was no change in leucocyte counts or levels of serum complement in airways or non-airways reactors after challenge.

Small airways measurement: If a decrement of 35% in MMF, $Vmax_{50}$ and $Vmax_{75}$ is used as the criterion of detecting airways obstruction, then these tests were no more sensitive than FEV_1 in detecting the acute airways response induced by durum wheat. However, in some instances $Vmax_{75}$ revealed an airways response which was not reflected in the FEV_1, which suggested a small airways reaction (Fig. IV-4).

Extracts of durum wheat induced an airways response in grain handlers. This response was not duplicated by extracts of A. fumigatus or grain insects, grain mites or grain weevils. The airways response was not related to either the atopic status of the individual or the presence of precipiting antibodies in the serum.

CONCLUSION

Durum wheat induces an airways response in grain handlers. The effect of inhaled durum wheat appeared to be on the large airways without parenchymal or systemic reactions and without complement consumption. This response can be inhibited by sodium cromoglycate.

Study V - Grain Fever Syndrome

Purpose

This study was designed to delineate the clinical, physiological and immunological events which occurred during episodes of grain fever. It also intended to answer several questions:

1) Does "grain fever" develop during and/or after exposure to grain dust? Can saline extracts of barley induce grain fever?

2) What individuals are more likely to develop "grain fever," grain handlers previously exposed to grain dust or individuals not exposed to grain dust (controls)? Atopic individuals? Skin reactors to grain dust extract of grain, i.e., barley, fungal mite or insect antigens? Bronchial hyperreactors to methacholine? Individuals with pre-existing airways obstruction? Individuals with precipitating antibodies to airborne grain dust or precipitins to fungal antigens?

3) Does fever develop in all subjects with symptoms described as "grain fever?"

4) Do respiratory symptoms occur with "grain fever?"

5) Is there an airways response? Are there parenchymal reactions?

6) Is the complement system activated during these reactions? By the alternate or classical pathway?

7) Is there a blood leukocyte response?

Materials and Methods

Subjects

We studied 6 grain handlers with history of recurrent episodes of "grain fever" detected during the health survey and 6 asymptomatic healthy adults without occupational history of grain handling. The characteristics of these 12 individuals are summarized on Table V-1. The 6 grain handlers complained of respiratory symptoms and eye irritation during exposure to high concentrations of grain dust. Three subjects had a history of productive cough for more than 2 years, 3 were smokers and 3 were nonsmokers. All had worked in the grain industry for 1 to 30 years with a mean of 13 years. The controls were unemployed, nonsmoking men. One of the controls had some chest tightness on heavy exercise, a second a history of hay fever and a third a history of some wheezing with colds in childhood.

Inhalation Challenge

During the inhalation challenge we studied symptoms, body temperature, blood leukocyte count and differential counts, FEV_1/FVC, MMF, $Vmax_{50}$, $Vmax_{75}$, DLCO and complement changes (total C3) and activation of classical or alternate complement pathway. The Methacholine inhalation tests were administered as recommended by the Asthma and Allergy Disease Center Report for standardization of bronchial challenge procedures. The Pc20 (the provocation concentration which will cause a fall in FEV_1 of 20%) was calculated from the last two points on the log dose-response curve. On the first day, or control day, no inhalation material was given, but measurements were taken at regular intervals. On the second day, baseline values were obtained, and the subjects were exposed to airborne grain with dust in an environmental chamber. The subjects manually created dust aerosols similar to levels which provoked episodes of grain fever. Each subject was tested at frequent intervals for 24-48 hours to provoke an episode of grain fever.

Respirable dust concentrations were determined using a 37 millimeter diameter acrylic filter with an 0.8 micron pore size (DA 800, Gelman). All filters were pre-weighed to the nearest .001 milligram. Prepared filter cassettes with cellulose backup pads were then capped and securely placed into a 10 milliliter nylon cyclone assembly, attached by .75 meter long tygon tubing to personal sampling pumps equipped with pulsation- flow dampers (Model G, MSA). Pumps were periodically monitored over the exposure time to insure a flow-rate of 1.7 liters per minute + .1 liter per minute. Air sampling was stopped when the subjects were removed from the chamber for testing. Based on the actual exposure time, the time-weighted average respirable dust levels were calculated considering the actual time of exposure, which varied between 60 minutes and 120 minutes. Any change in the parameters measured was compared to a baseline established earlier. On

the third day, if the subject was still symptomatic and/or leukocytosis persisted and/or FEV_1 had not returned to the pre-challenge baseline level ± 10%, the subject was tested at regular intervals. Eight of the 12 subjects were observed for 48 hours. If the subject returned to baseline levels, he was tested, on the third day, with saline extract of barley (Appendix X). Each subject was challenged with increased concentrations of barley using a dosimeter-powered Deviblis nebulizer until a decrease of 20% in FEV_1 occurred or a dose of 100,000 PNU/ml was reached. To establish a safe initial dose for inhalation challenge with barley extract, intradermal skin tests were performed. The dilution at which an 8 mm wheal reaction was obtained was used as the initial challenge dilution. No skin test was done with a dose greater than 1000 PNU.

Skin testing (See appendix XI)
Precipitating antibodies (See appendix XII)
Complement (See appendix XIII & XIV)
Grain dust concentration (See appendix XV)

Results

A significant change in experimental parameters was considered to be:

1) Blood leukocyte counts (WBC) above 10,500 per mm3 (leukocytosis).
2) Increased oral temperature 37.8 C (fever).
3) C3 complement levels more than 38 mg % from baseline.
4) A decrease $\geq$ 20% in FEV_1, FVC and DLCO when compared to baseline values. No change greater than 10% was observed during the control day.
5) A decrease $\geq$ 35% in MMF, $Vmax_{50}$ and $Vmax_{75}$ when compared to baseline values.

Precipitins

None of the 12 subjects had precipitins to grain dust or fungal antigens.

Skin tests:

Two of the grain workers and 4 controls had positive prick skin tests to 2 or more common allergens and were considered atopic individuals. Three grain workers and 1 control had positive prick skin test to airborne grain dust antigen. No skin reactivity to barley was observed in the test or control group.

Methocholine Challenge

Methocholine bronchial hyperreactivity was observed in 2 grain workers and 2 controls. Pre-existing airways obstruction defined as an FEV_1/FVC less than 70% was observed in 1 grain worker. The pulmonary function studies done on these subjects before the challenge are presented in Table V-1.

Airborne grain dust challenge

Respirable dust concentrations, time averaged for each individual, are presented in Table V-1. The mean respirable dust concentration was 84 mg/m^3.

Symptoms

All subjects became symptomatic during exposure. In some instances, the symptoms lasted longer than 24 hours but no greater than

36 hours. The most common complaints were "flu-like" symptoms: malaise, myalgias, tiredness, feverish feeling, chills and flushed face. These symptoms were observed in all grain workers and in 3 of the controls. These symptoms varied in intensity and were particularly marked in 7 of the 12 subjects. The symptoms were very mild in 2 of the 12 subjects. Seven of the 12 workers had headaches; none complained of eye irritation. The following symptoms were also observed: nasal symptoms (4/12), throat burning (5/12), cough (12/12), wheezing or chest tightness (9/12) and shortness of breath (9/12). Using baseline comparisons, 10/12 subjects (5/6 controls and 5/6 grain workers) developed leukocytosis (>11,700 per mm3: range 11,000-24,300). The average increase in the leukocytes was 11,000 per mm3. In one additional subject the white count increased by 3,300 but remained below 9,000. Body temperature rose above 37.8C in 6 of the 12 subjects (5/6 grain workers and 1/6 controls), 2 to 40 hours after exposure.

Pulmonary Function

Airways obstruction (decline in FEV_1 = 20%) developed in 4 of 6 grain workers and 5 of the 6 controls (Table V-1). Diffusing capacity also decreased in two controls within 24 hours after exposure. T-test analysis of the changes in FEV_1 between the baseline and immediate post-exposure values revealed a significant ($P < 0.05$) change for grain workers and controls (Fig. V-1). After challenge, the maximum changes in FEV_1 occurred: 1 hour (1), 2 hours (4), 4 hours (4), 6 hours (1), 8 hours (1) and 24 hours (1) later. Two of the controls showed a marked decrease in FEV_1 after an improvement from the initial decrease at the 28th hour. The changes in FVC for the group by t-test analysis was also significant in the grain workers, the controls and both groups together (Fig. V-2). For the MMF the paired t-test showed significant change for the 12 subjects analyzed together ($p < 0.001$) (Fig. V-3) and for the controls ($p < 0.01$), but it was not significant for the grain workers alone. $Vmax_{50}$ showed significant differences between baseline and control and the grain workers ($p < 0.01$), the controls ($p < 0.02$) and in both groups ($p < 0.001$) (Fig. V-4).

The changes in leukocyte count by t-test on the group was also significant for the grain workers, controls and both groups together ($P<0.02$), (Table V-1).

The maximum leukocyte change was seen between 4 and 8 hours on most subjects and in 24 hours on 1 subject.

The changes in temperature in the grain workers was significant ($P < 0.05$) by the t-test but was not significant for the controls or grain workers plus controls (Table V-1).

We analyzed the relationship between atopy and the development of airways reaction to dust exposure utilizing x^2 analysis and it was not significant. Of the 6 atopic individuals, 5 developed a significant change in FEV_1 or airways obstruction (83%) and 1 (17%) did not. Of the non-atopic individuals 4 of 6 (57%) developed airways obstruction; but 2 of the 6 (33%) did not.

In those individuals with positive skin tests to airborne dust, 3

of 4 (75%) developed airways obstruction, and 1 of the 4 did not. Of those with negative skin tests, 6 of 8 developed airways obstruction (75%); 2 of the 8 did not. Airways obstruction developed in 9 of 12 subjects, 4 of which were non-atopic while 5 were atopic. Airways obstruction developed in 6 of 9 negative skin reactors to airborne grain dust; 3 of 9 reactors were reactors to the airborne dust.

Barley Challenge

Inhalation of barley extracts reproduced grain fever syndrome in 1 of 10. Six of 10 had leukocytosis with left shift, but 1 had fever over 37.8 C. One of 10 had a 20% or greater decline in FEV_1. One of 10 had an increasing C3 level of 44.

Conclusions

Inhalation of high concentrations of airborne dust for 1 to 3 hours induces the grain fever syndrome lasting 24 to 36 hours. This syndrome is characterized by systemic reaction, facial warmth, headache, chills, malaise, myalgias, leukocytosis, left shift fever and is commonly associated with respiratory symptoms, throat and tracheal burning, chest tightness, dyspnea, cough, expectoration and airways obstruction. There is no evidence of parenchymal reaction.

These reactions occur in both grain workers and controls and are independent of previous exposure, atopic status, bronchial hypersensitivity, pre-existing airways obstruction or the presence of precipitins.

Our data do not support the hypothesis that grain fever is a type III allergic reaction since none had precipitins to grain dust, complement was not activated and changes in DLCO were not observed. This data may suggest that grain fever is due to bacterial endotoxin or non-specific release of pharmacologically active substances from the lung after interactions between components of grain dust and lung cells.

Study I. Health Status of a Cross-section of Grain Handlers in the Twin Ports of Duluth and Superior.

The health status of grain handlers was evaluated by comparing the prevalence of clinical, physiological, immunological, radiological, serological, blood and urine parameters in 310 grain workers (test group) and 239 city service workers (controls) from the same geographic area. The control group was matched to the test group with respect to sex, age, height, weight and smoking habit.

All subjects were studied according to the following protocol:

1) a self-administered questionnaire reviewed for completeness by trained interviewers;

2) a physical examination performed by physician;

3) pulmonary function tests including FEV_1, FVC, MMF, $Vmax_{50}$, $Vmax_{75}$, CV, N2/L, DLCO;

4) a chest roentgenograph, postero-anterior view;

5) skin prick tests for detection of immediate hypersensitivity to common allergens, fungal antigens, grain mites, grain insects, grain, airborne grain dust and settled grain dust;

6) intradermal skin tests for delayed hypersensitivity to PPD, mumps, Candida albicans, Streptokinase-Streptodornase (SK/SD) and

Trichophyton;
7) detection of serum precipitating antibodies directed toward: fungal antigens, bacterial antigens, pigeon sera, grain, airborne grain dust and settled grain dust;
8) the levels of circulating immunoglobulins (G,A,M,E);
9) blood hemoglobin and hematocrit;
10) urinalysis for protein, glucose and blood;
11) serum creatinine;
12) serum alanine aminotransferase (SGPT), gamma glutamyltranspeptidase (GGT) and pseudocholinesterase;
13) $alpha_1$-antitrypsin levels.

Results and Conclusions from this Study

1) Clinical findings. Grain handlers had a higher prevalence of respiratory symptoms and signs (ronchi) than comparable non-grain handling city service workers from the same geographic area (Table 7-9, 12, 24) whether or not they smoked. The effects of grain handling on prevalence of respiratory symptoms were highly significant, independent and usually greater than those of smoking (Table 13). The prevalence of work related respiratory symptoms adjusted for age and smoking habit was also positively related to place (Tables 19, 20) and length of employment (Table 15). The data suggested variable environmental working conditions among elevators and perhaps an accumulative respiratory effect due to recurring exposures to grain dust.

Grain workers suffer from:

a) acute and chronic airways reactions (occupational asthma and chronic bronchitis) induced by exposure to grain dust with varying degrees of: cough, expectoration, wheezing and/or chest tightness and shortness of breath. Durum wheat and barley grain dust were the most common inducers of symptoms. During the work shift, wheezing and/or chest tightness occurred immediately after starting work or within 2 hours. In late reactors, wheezing occurred within 2 hours after leaving work. Very late reactions were not reported.

Wheezing and dyspnea on exposure were related to length of employment. This may indicate either increased sensitization to the allergens present in the environment or the bronchial mucosa being rendered more hyperreactive by the recurrent non-specific inflammatory reactions of the airways by grain dust. The place of employment was found to affect the prevalence of symptoms. The highest prevalence of symptoms were found in 4 companies and the lowest in 2 companies.

b) A grain fever syndrome (Table 21), characterized by a short-term febrile illness (flu-like syndrome) that may be associated with respiratory symptoms. It usually occurs during work or shortly after work. It is related to exposure to high concentrations of dust any day of the work week and not necessarily the first day at work or the first day of the week. There was, however, a small percentage of workers who had a single episode of grain fever the first time at work and not again. The workers stated that in the last 3 years, because of the improvement in the working conditions, grain fever occurred less frequently. Some workers had grain fever a few hours after work, compatible with allergic pneumonitis. However, none of these episodes were severe enough to require medical attention, and we lack

radiographic proof of allergic pneumonitis. Furthermore, the symptoms tended not to recur unless very high concentrations of dust were again present. Although we cannot deny that in some instances the grain fever syndrome may be a manifestation of allergic alveolitis, we have not found the typical history and radiographic changes of allergic alveolitis in these workers.

c) Acute recurrent conjunctivitis and rhinitis during exposure to grain dust occurred in most grain workers.

d) Skin pruritus occurred mostly on exposure to barley dust.

e) Pesticide exposure caused temporary disabling symptoms

The long-term effects of recurrent symptomatic or asymptomatic exposures to pesticides are unknown, but we have encountered several former grain handlers with chronic neurological defects attributable to pesticide exposure.

2) Pulmonary function status. We concluded that grain dust exposure had an adverse effect on lung function (Tables 25-27, 29-31). The effect of grain dust on lung function was highly significant, and the overall effect was the same or of smaller magnitude than that of smoking. Although there were more grain workers with mild airways obstruction than controls, moderately severe or severe airways obstruction was equally prevalent in both. The effect of grain handling appeared to be mostly on the airways. The high prevalence of abnormal W N2/L, indicating abnormal distribution of ventilation time constants, needs further evaluation. Simple, reproducible spirometric measurements were sufficiently sensitive to detect the effects of grain dust exposure on lung function in the cross-sectional study. Other tests offer little or no advantage, but the potential usefulness in longitudinal studies needs to be further evaluated. There was no correlation between lung function and job category, place or length of employment (Tables 32, 33)[8].

3) Clinico-physiological correlation. Grain workers with symptoms on exposure to dust had lower values of ventilatory function than workers without symptoms on exposure, regardless of smoking habits (Tables 38, 39). This suggests that symptomatic exposure to grain dust results in lower ventilatory function and conceivably leads to non-specific bronchial hyperreactivity. It is also possible that grain workers have a pre-existing lower ventilatory function due to undiagnosed mild or non-symptomatic asthma or non-specific bronchial hyperreactivity, and exposure to grain dusts aggravates this condition. The prevalence of chronic bronchitis with airways obstruction was higher in grain workers than controls, regardless of smoking habits. In addition, chronic bronchitis with airways obstruction was related to length of employment. These findings suggest that chronic grain dust exposure may result in chronic obstructive pulmonary disease.

4) Skin hypersensitivity (allergic). Atopy was more prevalent among controls than grain workers (Table 35). The lower prevalence of atopy in grain workers may imply that the more "allergic" individuals tend to avoid the grain dust environment or leave the industry. This hypothesis could be tested in future longitudinal studies and a cross-sectional study of the "non-survival" population of grain workers.

The higher prevalence of positive skin test reactivity to insects

and mites in grain workers suggested that the antigens used were more specific for the grain workers because the extracts were prepared with the grain insects and grain mites commonly found in elevators. Hence, grain workers would be more likely to be sensitized. The low prevalence of positive reactions to grain antigens may be due to a low allergenicity of the grain extracts; too low a concentration or loss of the antigenic component of grain during saline extraction. According to previous studies, however, the saline extractable fraction seemed to be the most allergenic of the wheat fractions.

The high prevalence of positive skin tests to airborne grain dusts observed in grain workers suggests that a greater proportion were sensitized to grain dust. Since some city service workers also had positive skin tests to airborne dust, the data suggest that air in the Duluth-Superior area was contaminated with dust from the grain elevators.

The prevalence of skin test reactions to grain dust and insect/mites was significantly higher (by x^2) in atopic grain workers and in atopic control workers than in non-atopic individuals. The data imply that atopic individuals are more likely to become sensitized to grain dust or insect-mite airborne particles than are non-atopic individuals (Table 37).

5) Skin hypersensitivity-symptoms correlation. Overall, there were no significant correlations between symptoms on exposure, chronic symptoms, grain fever, or symptom complexes and skin reactivity to common allergens or specific allergens. The exceptions were: a) dyspnea on exertion was more prevalent among grain workers with positive skin reactivity to fungal antigens and to grain antigens. b) Nasal symptoms on exposure to grain dust were more prevalent among grain workers with positive skin reactivity to grains, barley and oats (Table 42).

6) Skin hypersensitivity-pulmonary function correlation. Grain workers with atopy or skin reactivity to grain dust were more likely to have lower airways function values. The clinical significance of these findings is not clear since abnormal lung function was not more prevalent among atopic individuals or skin reactors to specific grain extracts (Tables 40, 41).

7) Serum precipitating antibodies. City workers had a greater prevalence of precipitins to Trichoderma, T, vulgaris, T. sacchari and to one or more fungi than grain workers (Table 44). Conversely, grain workers had a greater prevalence of precipitins to: durum wheat, rye and airborne dusts of wheat, barley, rye, oats and sunflower to one of the settled dusts than controls. The larger prevalence of precipitins to some grain dusts among grain workers was not surprising, yet they did not correlate with increased prevalence of symptoms or abnormal lung functions. Hence, the data imply that the respiratory reactions to grain dusts are not precipitin-mediated, and that grain fever is not a manifestation of allergic alveolitis type III reaction. Serum precipitins reflect host response to antigens but not necessarily the presence of disease or abnormal pulmonary dysfunction. The reason for the greater prevalence of fungal precipitins among city workers is not clear.

8) Alpha$_1$-Antitrypsin level (AAT). The levels of AAT in grain workers and controls were similar. Pi phenotyping of grain workers with AAT levels less than 60% of the normal values detected 3 MZ's with impaired trypsin inhibitory capacity (TIC) and 3 MZ's with normal TIC. These subjects showed no consistent abnormality in lung function (Tables 53, 55).

9) Chest roentgenograms. The prevalence of abnormal chest roentgenogram findings was small and most changes were of minor clinical significance with a few exceptions (Table 47). There were no cases with diffuse bilateral interstitial infiltration or fibrosis.

10) Liver disease screening (SGPT, GGT, Cholinesterase). We did not detect differences in the frequency of overt liver disease between grain workers and controls (Table 45). Certain findings make us recommend that future prospective morbidity studies include evaluation of liver disease prevalence. In the questionnaire, the grain workers reported exposure to hepatotoxic pesticides. The liver was palpable in a significant number of grain workers. Moreover, the mean values for SGPT were elevated in grain workers. Grain workers had a greater number of abnormal values for GT.

11) Renal disease screening. The results of the renal function screening tests were inconclusive (Tables 45, 46). We would recommend further prospective studies on the potential renal morbidity of pesticide exposure.

12) Immunoglobulins. The levels of IgG and IgA observed in grain workers differed significantly from the city workers, whereas the levels of IgM were similar in both groups (Tables 48, 52). The data suggest that grain dust normally enhances the levels of serum IgA, but that the response was blunted by smoking. Elevated IgG and IgA levels were observed only in grain workers working in the elevators from 10.6 to 15.5 years. Increased levels of IgA were also observed in grain workers working in the industry fewer than 5.5 years.

The place of employment influenced the level of circulating IgG. (Increased levels of IgG in elevators 1 and 8)

Only 4 of the 307 grain workers and 2 of 235 city workers tested had IgE levels above 600 I.U. Data from the IgE determinations should be interpreted with caution. Using radioimmunoassays for determination of IgE, the level of IgE in normal serum is below 50 I.U./ml. Serum from highly allergic individuals contains between 300-600 I.U. Levels above 900 I.U. are rarely observed. Hence, the immunodiffusion method for determining IgE levels is not sensitive enough to detect increases in serum IgE occurring between 100-600 I.U.

Study II - Work Shift Study

Two hundred and forty-eight (248) grain workers and 192 controls (city service workers) were studied. The following parameters were evaluated: 1) symptoms, 2) pulmonary functions (FEV_1, FVC, $Vmax_{50}$ and $Vmax_{75}$) before and after the shift, 3) leukocyte count and differential, 4) serum complement C3 level and complement activation

before and after the shift, 5) oral temperature at 7-800 hrs, 1200 hrs, 15-1600 hrs and 2000 hrs, 6) total and respirable time average dust levels by personal sampler, 7) mycological studies described Whidden et al. "Microbial Flora and Fauna of Respirable Grain Dust from Grain Elevators."

Results and Conclusions from this Study

Exposure to grain dust during an 8 hour work shift appeared to have an adverse, dose-related, acute effect on the workers. This adverse effect is suggested by the following:

1) Grain workers reported more symptoms during work than city service workers of similar age, height and smoking habit (Table II - 3).
2) Grain workers were exposed to a higher concentration of dust than city service workers (Table II - 2).
3) The incidence of respiratory symptoms was positively related to the workers' subjective estimates of dust levels and to time-weighted average total dust concentration (Tables II - 4, 14).
4) Grain workers' subjective estimation of dust level correlated with the measured total dust concentrations (Table II - 14).

The incidence of respiratory symptoms was higher among grain workers exposed to mean total airborne dust (time-weighted average concentration) of 13.9 mg/m3 when compared to grain workers exposed to 4 mg/m3 or less. In the latter group of grain workers the incidence of symptoms was similar to that found among controls.

The negative effect of grain dust exposure on lung function also tends to support the hypothesis that grain dust exposure has an adverse effect on the workers.

The negative effect of grain dust exposure on lung function was suggested by:
1) In a small number of subjects, a greater proportion of grain workers had a significant decrease in FEV_1 (-15%), $Vmax_{50}$ and $Vmax_{75}$ (-25%) (Table II - 12a).
2) There was a negative correlation between $Vmax_{50}$ and $Vmax_{75}$ and total dust level. The higher the dust level the more negative the change in function value (Table II - 11).
3) There was a significant difference between the pre-post-shift lung function changes observed in grain workers and controls. The mean control group values tended to increase slightly during the day, whereas grain workers showed slightly negative changes (Table II-9).

Overall, the acute effects of the dust concentrations found in this study on the lung function did not seem to be of clinical significance since there was no correlation between the presence of symptoms and pre-post-shift changes in function. This small, negative, acute effect may or may not have long-term effects such as a greater than expected yearly loss of function.

At the total dust concentrations these workers were exposed to we found no consistent systemic reaction, i.e., oral temperature, leukocyte count or serum complement level or activation of complement (Tables II - 6-8).

Study III. Prospective 3 Year Follow-up Study

The pulmonary function parameters (FEV_1, FVC, $Vmax_{50}$, $Vmax_{75}$ and DLCO) studied in 1974 were compared with the values obtained in cross-sectional study performed in 1977.

Results and Conclusions

No definite conclusions as to the chronic effects of recurrent grain dust exposure can be derived from this short-term follow-up study using tests of airways flow and diffusing capacity. The non-working grain handlers (left the industry, retired, laid-off) were not included in the evaluation. The results would be affected by those who had to stop working because of respiratory disability and were lost to follow-up.

The mean decline in FEV_1 or FVC was no greater than that expected for aging alone. The results did show, however, a significant mean decline in other tests of airways flow--i.e., MMF, $Vmax_{50}$ and $Vmax_{75}$--which was greater than expected for age alone in any smoking category (Tables III-4-10). Although nonsmokers showed a greater mean decline in flows at low lung volumes than smokers, a very small proportion of nonsmokers showed a decline in function greater than expected. This significant mean function (MMF, $Vmax_{50}$ and $Vmax_{75}$) decline in nonsmokers suggests a grain dust effect independent of age. However, since the majority of those who showed a greater than expected (age related) decline in these functions were smokers, cigarette smoking probably has a greater adverse long-term effect on these functions than grain dust exposure.

Study IV: Pulmonary Reaction to Grain Dust Constituent - Pilot Study in the Identification of Etiologic Agents

The pulmonary and systemic response to extracts of durum wheat, durum wheat airborne dust and insects or mites was studied by inhalation provocation tests on 11 grain workers with symptoms on exposure to grain dust.

Conclusions

Durum wheat, a constituent of grain dust, induced an airways response in grain handlers (Figures IV-1,2). This response was inhibited by sodium cromoglycate (Figure IV-3). The effect of inhaled durum wheat appeared to be on the large airways without parenchymal or systemic reaction and without complement activation. The bronchial reaction was not always related to the atopic status or acquired skin hyperreactivity to grain or grain dust antigens. Our data suggest that a type III allergic reaction does not play a role in the bronchial response to durum wheat extract. Moreover, the data imply a nonimmunologic release of mediators, e.g., histamine, or type I immediate IgE mediated allergic reactions are responsible for the reaction.

Study V: Grain Fever Syndrome

Clinical, physiological and immunological parameters were evaluated in 12 subjects (6 grain workers with grain fever and 6 nonsmoking, asymptomatic controls) after exposure in a chamber to high concentration (>15 mg/m3) of grain dust to reproduce an environment.

Workers considered this concentration of dust similar to that most likely to provoke an episode of grain fever at work. Results after challenge were compared to baseline and to control day results.

Conclusions

Inhalation of high concentrations of airborne grain dust for one to three hours induced the grain fever syndrome manifestation lasting 24 to 36 hours. The syndrome was characterized by systemic reactions: facial warmth, headache, chills, malaise, myalgias, leukocytosis, left shift fever and by respiratory reaction: throat and tracheal burning, chest tightness, dyspnea, cough, expectoration and airways obstruction. There was no evidence of parenchymal reaction.

The reactions occurred in both grain workers and controls and were independent of previous exposure, atopic status, bronchial hypersensitivity, pre-existing airways obstruction or the presence of precipitins.

Our data do not support the hypothesis that grain fever is a type III allergic reaction. None of the test subjects had precipitins to grain dust, complement was not activated and changes in DLCO were not observed. This data may suggest that grain fever is due to non-specific release of pharmacologically active substances from the lung after interactions between components of grain dust and lung cells.

Health Effects of Grain Dust Exposure Summary

Grain dust exposure can induce acute symptomatic reactions of the skin, conjunctiva, upper and lower airways (asthma) and systemic febrile reaction (grain fever syndrome). Grain fever could be induced by inhalation of high concentrations of respirable airborne dust (>20 mg/m3) and was temporarily disabling. Respiratory symptoms were more likely to occur among workers exposed to a total dust time-weighted average concentration of 13.9 mg/m3. They also occurred among workers exposed to a mean total dust time average concentration of 4 mg/m3 and less, but overall the incidence of symptoms in that group of grain workers was similar to that in controls.

Grain dust exposure can induce chronic expectoration (chronic bronchitis) and dyspnea on exertion. The effect of grain dust exposure on symptom prevalence was of greater or the same magnitude as the effect of smoking. The acute physiologic pulmonary changes were significant (e.g., pre-post work shift FEV_1 > 20%) at high (time-weighted average) respirable dust concentrations (> 20 mg) but are infrequent at time average total dust levels below the current TLV of 15 mg/m3.

Recurrent daily exposure to low concentrations (assuming that time-weighted average dust concentrations at the elevator was TLV of 15 mg/m3 or lower) may result in lower ventilatory function than expected for men of the same geographic area not exposed to grain dust. The adverse effect of grain dust exposure on lung function was of equal magnitude or smaller than that of cigarette smoking. The physiologic changes, however, were of small magnitude and may have no significant long-term effects on the worker's sense of well-being, working performance or longevity. On certain occasions, the acute symptoms on

exposure at weighted average total dust levels below accepted TLV nuisance dust (15 mg/m3) appeared to affect workers' work performance and sense of well-being during and after work, thus affecting their quality of life.

It is reasonable to suspect that acute symptoms on exposure are more related to peak airborne dust concentrations rather than to time-weighted average levels. It is also conceivable that significant transient decreases in ventilatory function may occur during the work shift when high peak airborne dust concentrations occur. Hence, further studies are needed to determine the relationship between peak dust levels and the biological response. In addition, prospective studies are needed to determine the long-term effect of the : 1) recurrent adverse acute pulmonary adverse effects of grain dust exposure, 2) the grain fever episodes, and 3) the symptomatic pesticide exposure.

There was no evidence to suggest that grain dust can cause hypersensitivity pneumonitis.

The effects of grain handling and smoking on the lungs were statistically significant, independent and additive. Although allergic predisposition must play an important pathogenetic role in some individuals, acute asthmatic responses can be elicited regardless of the atopic status of the individual.

A component of grain dust, durum wheat, has been identified as an etiologic agent. The mechanism by which durum wheat or grain dust induces asthma is not known. The prevention of asthma to grain dust by pre-treatment with sodium cromolyn suggests an allergic or pharmacologically mediated histamine release. Further studies are needed to identify the fractions of d. wheat and other components of grain dust (e.g., barley, oats, rye, insects, mites or fungi) capable of inducing asthmatic reactions and their mechanism of action.

HEALTH EFFECTS OF GRAIN DUST EXPOSURE

Acute inflammatory reactions

+ skin irritation with pruritus
+ Conjunctiva
I Upper airways: nasal passages, larynx, trachea
I Lower airways: (asthma) + large airways
o Alveoli (no evidence of alveolitis) Small airways (probable) but possible under certain conditions

Acute systemic reaction (inflammatory? toxic?)

+ Grain fever syndrome

Chronic respiratory effects

+ Chronic bronchitis without airways obstruction
? Hyperreactive airways (probable)
? Chronic bronchitis with airways obstruction (probable)
? Loss of lung function greater than expected for age

Toxic effects of pesticides

+ Acute neurological and gastrointestinal
? Chronic neurological disease from recurrent exposures (probable)
o Hepatotoxicity or nephrotoxicity

Other health effects: Unknown

+ There is evidence to support the cause-effect relationship with grain dust exposure
? Cause-effect still debatable and more information is needed. In some instances the cause-effect is highly probable.
o No evidence of cause-effect or even that it ever occurred following grain dust exposure.

Based on the results of the studies described above we conclude that grain dust exposure has an adverse effect on grain handlers.

We can conclude with certainty that: a) grain dust exposure can induce acute reactions of the exposed mucosa of acute clinical significance as indicated by Studies I, II, IV and V, and b) exposure to high dust concentrations have a definite negative physiologic effect on the airways and systemic response (leukocytosis, fever) as demonstrated by Study V.

At low dust concentrations (Study II) there is a negative physiologic effect which, except for a few instances, is of small magnitude. The prognostic significance or long-term effect of this small negative effect is not clear. The prospective pulmonary function data (Study III) would suggest that this effect may result in a yearly loss of airways flow greater than expected for age. However, this may only be significantly abnormal in smokers.

Our data (Study II) lend support to the workers' subjective correlation of acute respiratory response to dust level, since there was a relationship between prevalence of symptoms on exposure and lung function changes and total dust levels. The recurrent exposure to grain dust also appears to have definite chronic clinic effects related to length of exposure, but the chronic functional effects are less clear-cut. The higher prevalence of abnormal airways flow in grain workers and the greater than expected yearly loss of airways flow suggest a chronic negative functional effect. Whether or not this effect leads to early disability or decreased performance or affects an individual's sense of well-being cannot be answered as yet. The long-term effects of the acute pulmonary recurrent negative effects of grain dust exposure; grain fever episodes; and symptomatic pesticide exposure will have to be answered by prospective studies.

We found no acute or chronic evidence that suggests that grain dust exposure can cause hypersensitivity pneumonitis. The results of these studies allow us to make some conclusions about host factors that affect the response to grain dust and about the mechanism of action of grain dust.

Cigarette smoking: Cigarette smoking has an additive effect on grain handling in regard to the prevalence of acute and chronic respiratory symptoms, pulmonary function status and prevalence of pulmonary function abnormalities. Although smokers are more likely to be affected by chronic grain dust exposure, acute symptoms on exposure can occur independently from smoking habit.

Allergic sensitization (as detected by immediate reaction to common or specific allergens): Overall, in this working (survival)

population of grain workers, there was no relation between prevalence of symptoms or abnormal lung function and the atopic status of individuals. In addition, positive bronchial provocation challenge occurred regardless of the atopic status of individuals. There is evidence, however, that allergy may play a role since there was a negative relation between lung function and skin test reactivity in Study I. The bronchial reaction seen in a subject in Study IV can be interpreted as being an immediate IgE type I allergic reaction in a "very atopic" individual. Hence, the lack of overall relation between skin sensitivity and respiratory response should not exclude the probability that in certain individuals the bronchial reaction is mediated via an allergic mechanism. Also, it is likely that very atopic individuals with hyperreactive airways are unlikely to remain in this industry for long. This is suggested by the lower than expected prevalence of atopy to common allergens among the grain workers.

Bronchial hyperreactivity: Bronchial hyperreactivity is likely to predispose to bronchial reaction to grain dust, but Studies IV and V demonstrate that responses to durum wheat or high concentrations of airborne grain dust can occur in subjects without pre-existing hyperreactivity. Further studies on non-specific bronchial hyperreactive airways of the biologic response to airborne dust are needed. To determine if bronchial hyperreactivity is acquired or pre-existing; if it reverses once exposure is discontinued; and to establish the usefulness of Mecholyl test to detect high risk individuals before or during employment.

Pre-existing airways obstruction: Based on Study IV we conclude that airways response to grain is more likely to occur in subjects with severe airways obstruction but according to Study V high concentrations of grain dust can induce bronchial reaction even if there is no pre-existing airways obstruction.

Alpha$_1$-Antitrypsin activity: No apparent relationship was found between level of alpha$_1$-antitrypsin and pulmonary response to grain dust.

Mechanism of action: We have shown that durum wheat saline extract, durum wheat airborne dust saline extract and airborne grain dust from elevators can induce the bronchial reaction by mediator release, since their reaction can be blocked by sodium cromoglycate. An allergic IgE mediated type I reaction was apparent in one subject. We found no evidence that a type III precipitin-mediated reaction or complement activation plays a significant pathogenetic role (Studies II, IV and V).

Further studies are required to identify: the components of grain dust responsible for the pulmonary reaction, their mechanism and site of action; the role of mediator release and complement activation in the pathogenesis of the airways reaction; and the role of host factors which can modify the responses to grain dust, i.e., non-specific bronchial hyperreactivity and allergic predisposition or sensitization.

Section 2 - References
#210-76-0175

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Section 3 - Tables

Study I

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Study I

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TABLE 1.

GRAIN HANDLERS POPULATION

	Working Population			Not Working				
	WORKING TOTAL	STUDIED N	%	REF	CC	VAC	SL	TOTAL
ELEVATOR 1	37	35	95	2	0	2	1	40
ELEVATOR 2	23	22	96	1	0	1	0	40
ELEVATOR 3	17	12	71	1	4	1	1	19
ELEVATOR 4	81	49	60	15	17	4	4	89
ELEVATOR 5	18	17	94	1	0	2	2	22
ELEVATOR 6	21	17	80	4	0	1	2	24
ELEVATOR 7	44	39	89	3	2	4	1	49
ELEVATOR 8	37	24	65	10	3	1	2	40
ELEVATOR OPERATORS	278	215	77	37	26	16	13	307
STATE INSPECTORS	79	68	85	10	5	5	0	88
LONGSHOREMEN	40	27	68	4	8	0	0	39
TOTALS:	397	310	78	51	39	21	13	434

Working = At time of survey study.
CC = Unable to leave work to be tested, or not properly notified of examination time.
SL = Sick leave - 11 studied.
VAC = On vacation.
REF = Refused.

TABLE 2.

COMPARISON (CONTROL) POPULATION
CITY SERVICES WORKERS

	(E) Eligible	(C) Contacted	Studied		
			n	% of C	% of E
Duluth City	322	273	137	50%	43%
Duluth Power & Light Co.	92	59	59	100%	64%
Superior City	64	54	43	90%	67%
Total	478	386	239	81%	50%

TABLE 3a

CHARACTERISTICS OF THE POPULATIONS

	GRAIN HANDLERS N = 310	P	CONTROLS N = 239
Mean age (years)	40.8 ± 12.4	(NS)	40.8 ± 11.5
Mean weight (kg)	84.1 ± 14.1	(NS)	85.5 ± 13.6
Mean height (cm)	176.8 ± 7.0	(NS)	176.0 ± 6.9
Current smokers	49%	(NS)	44%
Ex-smokers	30%	(NS)	29%
Nonsmokers	21%	(NS)	26%

TABLE 3b.

LEVEL OF EDUCATION

Highest Grade:	GRAIN WORKERS % of 310	CONTROLS % of
0-3	0	0
4-8	15%	6%
9-12	76%	85%
>13	10%	10%
Live or work on farm	11%	1%

TABLE 4

DISTRIBUTION OF SMOKING HABITS, HEIGHT, WEIGHT, AGE GROUP
GRAIN WORKERS

Age (years)	Smokers				Ex-Smokers				Nonsmokers				All Groups		
	(no.)	(%)*	Height (cm)	Weight (kg)	(no.)	(%)*	Height (cm)	Weight (kg)	(no.)	(%)*	Height (cm)	Weight (kg)	(no.)	Height (cm)	Weight (kg)
20-29	47	53	177.1	78.3	19	22	178.1	82.2	22	25	179.4	83.5	88	177.9	80.4
30-39	35	67	178.5	84.8	9	17	177.3	81.5	8	15	176.2	83.4	52	177.9	84.0
40-49	42	52	175.9	83.7	20	25	177.0	87.7	19	23	175.3	89.5	81	176.0	86.1
50-64	29	33	174.8	78.2	44	49	176.3	86.3	16	18	176.0	84.5	89	175.7	83.3
20-64	153	49	176.7	81.2	92	30	176.9	85.3	65	21	176.9	85.5	310	176.8	83.3

*Percent of smokers, ex-smokers, or nonsmokers for each group.

TABLE 4b

DISTRIBUTION OF SMOKING HABITS, HEIGHT, WEIGHT, AGE GROUP
CONTROLS

Age (years)	Smokers				Ex-Smokers				Nonsmokers				All Groups		
	(no.)	(%)*	Height (cm)	Weight (kg)	(no)	(%)*	Height (cm)	Weight (kg)	(no.)	(%)*	Height (cm)	Weight (kg)	(no.)	Height (cm)	Weight (kg)
20-29	26	52	176.8	81.7	7	14	177.3	81.0	17	34	179.9	85.6	50	177.9	82.9
30-39	35	45	176.8	83.9	22	29	176.2	84.2	20	26	175.8	83.1	77	176.4	83.8
40-49	29	56	174.5	84.5	13	25	179.4	98.2	10	19	175.1	91.4	52	175.8	89.2
50-64	16	28	174.6	79.7	26	45	174.6	86.7	16	28	172.4	83.3	58	174.0	83.8
20-64	106	45	175.8	82.9	68	29	176.3	87.5	63	27	175.9	85.1	237	176.0	84.8

*Percent of smokers, ex-smokers, nonsmokers for each group

TABLE 5

SMOKING HABITS OF SMOKERS AND EX-SMOKERS

	GRAIN				CONTROL			
	SMOKER #153		EX-SMOKER #92		SMOKER #106		EX-SMOKER #70	
#CIGARETTES/ONLY	n	%	n	%	n	%	n	%
< 14	15	10	14	15	15	14	8	11
15 - 25	78	51	43	47	45	43	32	46
> 25	60	39	35	38	46	43	30	43
# PACK/YEARS								
0 - 14.9	43	28	38	41	38	36	26	37
15 - 30	51	34	27	29	29	27	25	36
30.5 - 200	58	38	27	29	39	37	19	27
SMOKE/YEARS								
< 14.5	57	38	38	41	37	35	31	44
14.6 - 25.5	37	24	20	22	39	37	25	36
> 25.6	58	38	34	37	30	28	14	20
INHALED SMOKE	147	97			102	96		
AGE STARTED								
0 - 20	134	91	83	90	98	93	61	87
21 - 301	13	9	8	9	8	7	9	13
> 30	0	0	1	1	0	0	0	0

TABLE 6

SELECTED OCCUPATIONAL PAST HISTORIES IN GRAIN WORKERS AND CONTROLS

Job (code)	GRAIN WORKERS (310)				CONTROLS (239)			
	N	%	LOE x yrs.	LOE Range	N	%	LOE x yrs.	LOE Range
(10) Ore loaders or Cement Mixing Operation	12	4	9.3	2-18	13	5	2.8	.25-10
(11) Metal work foundries	28	9	4	.5-14	25	10	3.5	.33-24
(12) Coke gas plant	3	1	9	5-11	1	0	.5	.5
(16) Farming	16	5	12.4	1-36	4	2	2.8	1-5
(20) Welding	12	4	.4.5	.3-14	17	7	4.3	.5-27
(22) Feed mill, grain mill Brewery	8	3	6.7	1-19	3	1	1.7	1-2
(7,9,6) Equipment operator, longshoremen, grain inspector					11	4	3	.25-7
Workers who had past history	77	25			55	23		

LOE = Length of employment

TABLE 7

PREVALENCE OF CHRONIC COUGH AND EXPECTORATION THAT MAY INDICATE PRESENCE OF CHRONIC BRONCHITIS

	Grain Workers		Controls	
	n	%	n	%
Chronic Bronchitis:				
I 14E > 2 Yrs	151	49	43	18
II Yes to 14C+14E > 2 Yrs	119	38	27	11
III 13 > 2 Yrs	132	43	37	14
IV Yes to 14D+14E > 2 Yrs	132	43	36	15
V Yes to 13A or B or C	193	62	67	28
VI Yes to 14A or B or C	165	53	51	21
VII Yes to 13A	109	35	37	15
VIII Yes to 14A	116	37	36	15
IX Yes to V or VI	194	63	67	14

COUGH AND PHLEGM:

13a. Do you usually cough first thing in the morning? (Exclude clearing throat.) 1. Yes 2. No

b. Do you usually cough at other times during the day or night? 1. Yes 2. No

c. Do you cough as much as 4-6 times a day for 4 or more days out of the weeks? 1. Yes 2. No

IF YES TO EITHER 13a, b OR c, ANSWER d AND e:

d. Do you cough on most days for as much as 3 months of the year? 1. Yes 2. No (x)

e. For how many years have you had this cough? _____ Years (x)

TABLE 7 (continued)

14a. Do you usually bring up phelgm from the chest first thing in the morning? (Not from the back of your nose. Count swallowed phlegm from the chest.) 1. Yes 2. No

b. Do you usually bring up phlegm from the chest at other times during the day or night? 1. Yes 2. No

c. Do you bring up phlegm like this as much as twice a day, 4 or more days out of the week? 1. Yes 2. No

IF YES.TO EITHER 14a, b OR c, ANSWER d and e:

d. Do you bring up phlegm from the chest on most days for as much as 3 months of the year? 1. Yes 2. No (x)

e. For how many years have you raised phlegm from the chest? _____ Years (x)

TABLE 8

PREVALENCE OF WHEEZING AND/OR CHEST TIGHTNESS (W &/OR CT) THAT MAY INDICATE THE PRESENCE OF OCCUPATIONAL ASTHMA*

	Grain Workers n=310		Controls n=239	
	n	%	n	%
Occupational Asthma if:				
I W &/or CT brought on or aggravated by exposure to dusts, gases or fumes at work (Yes to Q 28A or B or C)	183	59	31	13
II I and cough aggravated by or brought on by exposure to dusts, gases or fumes at work (Yes to Q 28A or B or C and Q 18A or B or C)	145	47	20	8
III II and dyspnea while performing job (Yes to Q 28A or B or C and Q 18A or B or C)	81	26	0	0
IV I and wheezing better when off work or vacation (Yes to Q 28A or B or C and Q 35A)	167	54	13	5
V IV and episodes of wheezing with dyspnea (Yes to Q 28A or B or C and Q 35A and Q 36)	72	23	2	1
Grain Handlers Only				
VI W &/or CT while at work (Yes to Q 25A)	165	53	-	-
VII W &/or CT brought on or aggravated by grain dust (Yes to Q 28A)	183	59	-	-
VIII W &/or CT and cough on exposure (Yes to Q 28A and Q 18A)	144	46	-	-
IX VIII and dyspnea on exposure (Yes to Q 28A and Q 18A and Q 44A)	102	33	-	-
X W &/or CT on exposure and wheezing better when off work or on vacation (Yes to Q 35A and Q 40)	165	53	-	-

See Questionnaire (Appendix IV) for text of questions.
Q = question.

TABLE 9.
PREVALENCE OF RESPIRATORY SYMPTOMS AND SYMPTOM COMPLEXES IN ALL GRAIN WORKERS AND CONTROLS AND BY SMOKING CATEGORIES.

		TOTAL		P1	SMOKER		P2	EXSMOKER		P3	NONSMOKER		P4
		N	%		N	%		N	%		N	%	
TOTAL	G	310	100		153			92			65		
	C	239	100		106			70			63		
CHRONIC BRONCHITIS I (Q14 > 2 YRS)	G	151	49	<.001	88	57	<.001	.05	40	43	<.001	NS	23	35	<.001	.005
	C	43	18		32	30		.001	5	7		NS	6	10		.005
CHRONIC BRONCHITIS II (Q14C > 2 YRS)	G	119	38	<.001	48	44	<.001	.100	30	33	<.001	NS	21	32	<.001	.100
	C	27	11		21	20		.010	4	6		NS	2	3		.005
COUGH FIRST THING IN A.M. (Q13A)	G	109	35	<.001	75	49	.005	.001	19	21	.001	NS	15	23	.05	.001
	C	35	15		31	29		.001	1	1		.10	5	8		.005
PHLEGM FIRST THING IN A.M. (Q14A)	G	116	37	<.001	71	46	.005	.005	26	28	.001	NS	19	29	.005	.05
	C	36	15		28	26		.001	3	4		NS	5	8		.005
DYSPNEA ON EXERTION GRADE I (Q38)	G	118	38	<.01	65	42	.05	NS	29	32	NS	NS	24	37	.005	NS
	C	64	27		32	30		NS	25	36		.001	7	11		.005
DYSPNEA ON EXERTION GRADE II (Q39)	G	23	7	<.05	11	7	NS	NS	9	10	NS	NS	3	5	NS	NS
	C	8	3		3	3		NS	4	6		NS	1	2		NS
HX OF WHEEZING AND/OR CHEST TIGHTNESS (Q21A)	G	200	65	<.001	110	72	.001	.05	53	58	.05	NS	37	57	.05	.05
	C	101	42		53	50		NS	29	41		NS	19	30		.05
WHEEZING AT NIGHT (Q33A)	G	61	20	.005	34	22	.05	NS	16	17	NS	NS	11	17	NS	NS
	C	24	10		12	11		NS	8	11		NS	4	6		NS
EPISODES OF WHEEZING AND DYSPNEA (Q36)	G	76	25	.001	48	31	.001	NS	20	22	.05	NS	8	12	NS	.005
	C	17	7		7	7			7	10		NS	3	5		NS

TABLE 9 (cont.)

		TOTAL N	%	P1	SMOKER N	%	P1	P2	EXSMOKER N	%	P1	P3	NONSMOKER N	%	P1	P4
TOTAL	G	310	100		153				92				65			
	C	239	100		106				70				63			
CHEST ILLNESS*	C	123	40		60	39		NS	36	39		NS	27	42		NS
(63B, C or D)				NS			NS				NS				.05	
	C	78	33		42	40		NS	23	33		NS	13	21		.05
SYMPTOMS RELATED TO WORK EXPOSURE																
COUGH AND/OR	G	201	65		115	75		.005	52	57		NS	34	52		.001
EXPECTORATION				.001			.001				.001				.001	
(18A or B or C)	C	41	17		28	26		.05	9	13		NS	4	6		.005
DYSPNEA AT WORK	G	113	37		61	40		NS	35	38		NS	17	26		.1
(Q43)				.001			.001				.001				.01	
	C	26	11		13	12		NS	8	11		NS	5	8		NS
"OCCUPATIONAL	G	183	59		104	67		.01	45	50		NS	33	50		.05
ASTHMA" I				.001			.001				.001				.001	
	C	31	12		14	13		NS	10	14		NS	7	11		NS
"OCCUPATIONAL	G	145	46		89	58		.005	35	38		NS	21	32		.001
ASTHMA" II				.001			.001				.001				.001	
	C	20	8		12	11		NS	5	7		NS	3	4		NS
"OCCUPATIONAL	G	81	26		48	31		NS	22	24		NS	11	17		.05
ASTHMA" III				.001			.001				.001				.005	
	C	0	0		0	0			0	0			0	0		
"OCCUPATIONAL	G	167	54		95	62		.05	45	49		NS	27	42		.01
ASTHMA" IV				.001			.001				.001				.001	
	C	13	5		8	8		NS	3	3		NS	2	3		NS
GRAIN EXPOSURE ONLY - TOTAL		310			153				92				65			
COUGH (18A)	G	200	65		114	75		.005	52	57		NS	34	52		.005
WHEEZING (28A)	G	183	59		104	68		.01	46	50		NS	33	50		.05
DYSPNEA (44A)	G	151	49		84	55		NS	47	51		.05	20	31		.005

*Cannot do usual activities because of chest illness more then twice in last 3 years.

Table 9 (cont.)

		TOTAL N	%	P1	SMOKER N	%	P1	P2	EXSMOKER N	%	P1	P3	NONSMOKER N	%	P1	P4
EYEBURNING (48A)	G	242	78		123	80		NS	69	75		NS	50	77		NS
NASAL STUFFINESS (48B)	G	246	79		126	82		NS	70	76		NS	50	77		NS
SORE THROAT (48C)	G	161	52		88	58		NS	43	47		NS	30	46		NS
GRAIN FEVER	G	99	32		47	31		NS	31	33		NS	21	32		NS

For definition of "Occupational Asthma" see Table 8 (Refer to Questionnaire - Appendix IV).
P - Significance of the difference in prevalence (χ^2 analysis). P1 grain workers vs. controls: P2 smokers vs. ex-smokers; P3 ex-smokers vs. nonsmokers; P4 nonsmokers vs. smokers. NS - Not significant P > .05.

TABLE 10

MEDICAL HISTORY*

	Grain Workers N = 310		Controls N = 239	
	N	%	N	%
"Has your doctor ever told you you had...?"				
Bronchitis	53	17	36	15
Emphysema	6	2	2	1
Pleuresy	28	10	18	8
Tuberculosis - Lung	6	2	1	.5
Cancer of the Lung	0	0	0	0
Chest surgery	4	1	3	1
Chest injury	11	4	10	4
Sinus "trouble"	72	23	74	31
Farmer's Lung	0	0	0	0
Pneumonia or bronchopneumonia	68	22	60	25
Bronchial asthma	12	4	4	2
Heart "trouble"	20	6	15	6
High blood pressure	49	16	33	14
Allergic rhinitis - Hay fever	20	6	29	12**
Kidney "trouble"	24	8	15	6
Liver "trouble" or jaundice	17	5	13	5
Diabetes	6	2	11	5
"Have you ever suffered from...?"				
Eczema in childhood	13	4	10	4
Skin rashes	92	30	72	30
Painful or swollen joints	96	31	72	30

*Number and proportion of grain workers and controls who answered yes to questions 64, 65a, 66a, 67, 68, 69, 70a and 71a.
**P.05

TABLE 11

FAMILY HISTORY (IMMEDIATE - BLOOD RELATIVES)

	Grain Workers		Controls	
	N = 310		N = 239	
	N	%	N	%
Chronic bronchitis	15	5	9	4
Emphysema	16	5	18	8
Asthma	33	11	25	10
Hay fever	20	6	27	11
Cystic fibrosis	1	.5	0	0
Cancer of the lung	12	4	17	8
Farmer's Lung Disease	0	0	1	.5
Other lung diseases	11	4	8	3

TABLE 12

PREVALENCE OF SELECTED SYMPTOMS OR SYMPTOM COMPLEXES
IN GRAIN WORKERS AND CONTROLS AND BY SMOKING HABIT

	Chronic Bronchitis		Occupational Asthma I		Dyspnea at Work		Nocturnal Asthma	
	%	P	%	P	%	P	%	P
Controls - nonsmokers (63)	10		11		8		6	
	]	<.005		NS		NS		NS
Controls - smokers (106)	30		13		12		11	
	]	NS		<.001		<.05		NS
Grain worker nonsmokers (65)	35		50		26		17	
	]	<.005		< .05		.1		NS
Grain worker smokers (153)	57		67		40		22	

( ) Number of subjects per category.

TABLE 13

FACTORS BY WHICH GRAIN EXPOSURE OR SMOKING INCREASE THE ODDS* (RISK) OF CHRONIC BRONCHITIS OR ASTHMA

	GRAIN HANDLING	SMOKING
Chronic Bronchitis (I)	4.4	2.9
Chronic Bronchitis (II)	4.9	2.3
Occupational "Asthma" (I)	4.6	1.9
Occupational "Asthma" (II)	9.9	2.8
Nocturnal Asthma	2.2	1.8

*See text for explanation.

TABLE 14

PREVALENCE OF SYMPTOMS IN GRAIN HANDLERS BY RANGES OF AGE

Age	29.9 N=88		30-39.9 N=52		40-49.9 N=81		50 N=89		P*
	N	%	N	%	N	%	N	%	
Chronic Bronchitis (I) (135)	31	35	18	35	43	53	43	48	NS
Cough on Exposure (200)	55	63	35	67	52	64	58	65	NS
Occupational Asthma VII (183)	47	53	31	60	51	63	54	61	NS
Dyspnea on Exposure (151)	35	40	27	52	41	51	48	54	NS
Eye Sx on Exposure (242)	78	89	44	85	60	74	60	67	.05
Nasal Sx on Exposure (246)	76	86	42	81	64	79	64	72	.05
Grain Fever (99)	22	25	16	31	29	36	32	36	NS
Cough in A.M. (109)	31	35	13	25	30	37	35	39	NS
Expectoration in A.M. (116)	30	34	14	27	34	42	38	43	NS
Wheezing at Night (50)	10	11	5	10	18	22	17	19	NS
Chronic Bronchitis + $FEV_1/FVC70\%$ pred. (31)	2	2	1	2	11	14	17	19	.05
Occupational Asthma X (69)	14	16	13	25	21	26	21	24	NS
Occupational Asthma IX (97)	21	24	17	33	28	35	31	35	NS
Chest Illness† (123)	39	44	19	37	34	42	31	35	NS

( ) Number of subjects with the symptom.

†Cannot do usual activities because of chest illness more than twice in last 3 years.

*Significance of the differences in symptom prevalence between age groups adjusting for smoking and length of employment by regression logistic analysis.

TABLE 15

PREVALENCE OF SYMPTOMS BY LENGTH OF EMPLOYMENT CATEGORIES

Length of Employment in Years	5.5 N=93*		5.6-10.5 N=77*		10.6-15.5 N=32*		15.6-20.5 N=45*		2 20.6-25.5 N=22*		25.6-30.5 N=30*		30.6 N=11*		P†
	N	%	N	%	N	%	N	%	N	%	N	%	N	%	
Chronic Bronchitis (I) (135)**	27	29	34	44	22	69	16	36	12	55	16	53	8	73	NS
Cough on Exposure (200)**	55	59	50	65	25	78	26	58	15	68	19	63	10	91	NS
Occupational Asthma VII (183)**	41	44	45	58	26	81	31	69	15	68	17	57	8	73	.02
Dyspnea on Exposure (151)**	30	32	37	48	23	72	24	53	12	55	17	57	8	73	.003
Eye Sx on Exposure (242)**	73	78	65	84	27	94	36	80	14	64	17	57	10	91	.01
Nasal Sx on Exposure (246)**	71	76	61	79	28	88	33	73	19	86	25	83	9	82	NS
Grain Fever (99)**	21	23	20	26	16	50	22	49	7	32	10	33	3	27	NS
Cough in A.M. (109)**	24	26	30	39	12	38	15	33	8	36	14	47	6	55	NS
Expectoration in A.M. (116)**	21	23	29	38	13	41	20	44	10	45	14	47	9	82	.001
Wheezing at Night (50)**	9	10	11	14	8	25	5	11	7	32	5	17	5	45	NS
Chronic Bronchitis + $FEV_1/FVC70\%$ (31)**	3	03	8	10	1	3	6	13	5	23	5	17	3	27	.02

TABLE 15 (cont.)

PREVALENCE OF SYMPTOMS BY LENGTH OF EMPLOYMENT CATEGORIES (CONTINUED)

Length of Employment	5.5 N=93*		5.6-10.5 N=77*		10.6-15.5 N=32*		15.6-20.5 N=45*		20.6-25.5 N=22*		25.6-30.5 N=30*		30.6 N=11*		P†
	N	%	N	S	N	%	N	%	N	%	N	%	N	%	
Occupational Asthma X (69)**	13	14	14	18	11	34	8	18	9	41	11	37	3	27	.02
Occupational Asthma IX (97)**	17	18	26	34	14	47	12	27	10	45	12	40	5	45	NS
Chest Illness (123)**	34	37	31	40	17	53	16	36	8	36	15	50	2	18	NS

*Number of workers in category
**Number of workers with symptom.
†Significance of the differences in relative prevalence between length of employment categories adjusted for age and smoking by logistic regression analysis.

TABLE 16

GRAIN WORKERS - JOB CATEGORIES

CODE	N
06 Inspectors	(94)
02 Annex Men	
03 Laborers	(90)
04 Transport	
05 Maintenance	(41)
07 Operators Outdoors	(31)
09 Longshoremen	(29)
01 Weighers	(20)
08 In House Firemen*	(5)

*Included in all analysis except when using job categories because of small number.

PREVALENCE OF SYMPTOMS BY JOB CATEGORIES

Job	# (305)	06 Inspector (94)		02 Indoor (90)		05 Maint (41)		07 Outdoor (31)		09 Longshore (29)		01 Weigher (20)		P*
		n	%	n	%	n	%	n	%	n	%	n	%	
Chronic Bronchitis (I)	135	32	34	39	43	21	51	16	52	14	48	2	60	NS
Cough on Exposure	200	53	56	59	66	29	71	23	74	20	69	15	75	NS
Occupational Asthma VII	183	47	50	50	56	26	63	19	61	22	76	16	80	NS
Dyspnea on Exposure	151	38	40	45	50	21	51	15	48	17	59	13	65	NS
Eye Sx on Exposure	242	69	72	69	77	33	80	25	81	27	93	15	75	NS
Nasal Sx on Exposure	246	78	83	66	73	32	78	24	77	26	90	17	85	.03
Grain Fever	99	30	32	20	22	13	32	11	35	18	62	6	30	NS
Cough in A.M.	109	32	34	30	33	17	41	10	32	9	31	10	50	NS
Expectoration in A.M.	116	31	33	32	36	18	44	11	35	14	48	9	45	NS
Wheezing at Night	50	14	15	8	9	9	22	4	13	7	24	7	35	NS
Chronic Bronchitis + $FEV_1/FVC70\%$ pred.	31	9	10	7	8	6	15	4	13	2	7	3	15	NS
Occupational Asthma X	69	13	14	20	22	13	32	7	23	4	14	10	50	.05
Occupational Asthma IX	97	17	18	31	34	16	39	10	32	12	41	10	50	NS
Chest Illness	123	29	31	42	47	22	54	10	32	6	21	12	60	.01

*P significance of differences in relative prevelence between job categories adjusted for age, smoking and LOE.

TABLE 18

JOBS RANKED BY RELATIVE PREVALENCE OF SYMPTOMS*

	Inspectors 06	Indoor 02-04	Maint. 05	Outdoor 07	Longshoremen 09	Weighers 01
Eye Symptoms	1	2	5	3	6	4
"Occupational Asthma VII"	1	3	5	4	2	6
Chest Illness†	2	4	5	3	1	6
Score	4	9	15	10	9	16
Ranking	1	3	5	4	3	6

*Prevalence significantly different among jobs, adjusting for age, smoking and LOE.
†Chest illness affecting usual activities.
Highest rank value = highest relative prevalence of symptoms. Jobs are ranked using the z value from the regression analysis.

TABLE 19

PREVALENCE OF SYMPTOMS BY ELEVATOR COMPANY

		I N=35	II N=22	III N-12	IV N=49	V N=17	VI N=17	VII N=39	VIII N=24	P*
hronic Bronchitis (I)	N	20	8		4	22	10	5	18	11
	%	57	36	33	45	59	29	46	46	NS
ough on Exposure	N	25	13	7	32	13	12	27	10	
	%	71	59	58	65	76	71	69	42	NS
heezing on Exposure	N	27	9	4	31	13	10	26	7	
ccupational Asthma VII%	%	77	41	33	63	76	59	67	29	.01
yspnea on Exposure	N	22	10	2	30	6	8	24	7	
	%	63	45	17	61	35	47	62	29	.02
ye Symptoms on Exposure	N	29	17	7	36	15	16	33	15	
	%	83	77	58	73	88	94	85	63	NS
ose Symptoms on Exposure	N	31	14	8	37	13	13	34	16	
	%	89	64	67	76	76	76	87	67	NS
rain Fever	N	9	1	5	15	4	4	16	6	
	%	26	5	42	31	24	24	41	25	.05
ough in a.m.	N	14	7	3	20	11	8	9	4	
	%	40	32	25	41	65	47	23	17	NS
hlegm in a.m.	N	17	61	4	14	9	6	13	7	
	%	49	27	33	29	53	35	33	29	NS
heezing at Night	N	14	2	1	5	3	1	6	3	
	%	40	9	8	10	18	6	15	13	NS
hronic bronchitis + FEV_1/	N	6	1	1	6	1	1	2	3	
VC 70%	%	17	5	8	12	6	6	5	13	NS
ccupational Asthma X	N	14	3	1	14	6	4	11	4	
	%	40	14	8	29	35	24	28	17	NS
ccupational Asthma IX	N	15	5	1	21	6	5	16	4	
	%	43	23	8	43	35	29	41	17	.05
hest Illness (C-D-E)	N	15	6	5	19	10	5	13	3	
	%	43	27	42	39	59	29	33	13	NS
hest Illness (C-D)	N	18	8	2	26	8	7	20	6	
	%	51	36	17	53	47	41	51	25	NS

P* - significance of differences in relative prevalence of symptom analyzed by regression nalysis adjusting for age, smoking and LOE.

TABLE 20

RANKING OF COMPANIES BY RELATIVE PREVALENCE OF SYMPTOMS
ADJUSTED FOR AGE, SMOKING AND LENGTH OF EMPLOYMENT

	I	II	III	IV	V	VI	VII	VIII	
Grain Fever	2	1	7	6	4	3	8	5	P<.05
Occupational Asthma VII	8	2	3	5	6	4	7	1	P<.01
Dyspnea on Exposure	6	4	1	7	3	5	8	2	P<.02
Subtotal Score	16	7	11	18	13	12	25	8	
Rating	(6)	(1)	(3)	(7)	(5)	(4)	(8)	(2)	
Cough on Exposure	7	2	3	5	6	4	8	1	
Eye Sx on Exposure	6	4	1	3	7	8	5	2	
Nose Sx on Exposure	7	1	2	4	6	5	8	3	
Chronic Bronchitis	5	4	8	1	7	3	2	6	
Early Cough	5	2	3	6	8	7	4	1	
Early Phlegm	7	1	5	2	8	4	6	3	
Wheezing at Night	8	2	3	4	6	1	7	5	
Wheezing and SOB	8	1	2	7	5	4	6	3	
Chest Illness*	6	3	5	7	8	2	4	1	
Chest Illness†	6	3	1	7	5	4	8	2	
CB and AO**	8	2	1	7	3	4	5	6	
Subtotal Score	73	25	34	53	69	46	63	33	
Rating	(8)	(1)	(3)	(5)	(7)	(4)	(6)	(2)	
Total Score	89	32	45	71	82	58	88	41	
Overall Rating	(8)	(1)	(3)	(5)	(6)	(4)	(7)	(2)	

*Much to a great deal.
**Chronic bronchitis + airways obstruction
†Unable to do usual activity two or more times in last 3 years.
Rating highest value = highest adjusted prevalence. Companies are ranked by the z value from the regression analysis for each symptom.

TABLE 21

GRAIN FEVER SYNDROME (GRAIN HANDLERS)
N=115 (16 questionable)

Number of episodes in work cycle:	N	% of 96 (19 no answer)
0-9	40	42
10-19	22	23
20-99	18	19
100-300	16	17
Fever and/or "chills" noticed:	**N**	**% of 115**
During work	37	32
After work	40	35
Either during or after work	38	33
Mostly noticed on:	**N**	**% of 115**
First day of work	15	13
Any day of the week	95	83
Any day but worse on first day	5	4
Associated respiratory symptoms:		
Nose	84	73
Cough	19	17
Wheezing	15	13
Dyspnea	7	6

Table 22

PESTICIDE EXPOSURE AND RELATED
HEALTH PROBLEMS IN GRAIN HANDLERS (N=310)

		N	%	
Exposed (ever)		294	95	
Health problems from above symptoms:		168	54	
	a. Weakness	65	21	
	b. Fainted	7	2	
	c. Dizziness	88	28	
	d. Headache	116	37	
	e. Convulsions	0	0	
	f. Trouble breathing	51	16	
	g. Nausea	65	21	
	h. Stomach pain	12	4	
	i. Diarrhea	12	4	
	j. Cramps	5	2	
	k. Blurred vision	14	5	
	l. Jaundice	0	0	
	m. Other	17	5	
Unable to do regular job because of symptoms:		28	17	*(of 167 that answere
Had to be taken to doctor:		19	11	*(of 167 that answere
Number of episodes:	0-5		51	(of 164 that answered
	6-10		21	
	11-20		4	
	21-50		9	
	51-100		5	
	100-300		2	
Type of pesticide:				(of 168 that answered
	Do not know	40	30	
	Carbon Tet.	103	61	
	Malathion	52	31	
	Methyl Bromide	51	30	
	Phostoxin	84	50	
	Other	1	1	

TABLE 23

PHYSICAL EXAMINATION

	GRAIN WORKERS N=310		CONTROLS N=238	
Chest Configuration	N	%	N	%
Normal	309	100	233	98
Kyphoscoliosis+	1		2	1
Pectum Excavatum	0		3	1
Auscultation Posterior Chest				
Normal	178	57	201	85
Ronchi (See table)	131	43**	37	16
Rales Bilateral	100	32**	30	13
Unilateral	31	10**	7	3
Cardiac Auscultation				
Normal	278	90	216	91
Murmur	15	5	14	6
Arrhythmia	6	2	3	1
Other	10	3	5	2
Abdomen	N=305		N=238	
Not palpable	171	56	184	77
Palpable*	132	43**	51	21
Hepatomegaly	2	1	3	1
(span 14 cm)				

\+ reported as mild
* On deep inspiration at rib costal margin, mid-clavicular line.
** P<.005 - Significance of the differences between grain workers and controls by χ^2 analysis.

TABLE 24

PREVALENCE OF RONCHI

	Total		Smoker		Ex-Smoker		Nonsmoker	
	N	%	N	%	N	%	N	%
Bilateral								
Grain Workers	100	32 *	55	36 *	28	17 *	17	26 *
Controls	30	13	20	19	7	10	3	5
Localized								
Grain Workers	31	10 *	18	12 *	8	9	6	9 *
Controls	7	3	3	3	4	6	0	0
Both								
Grain Workers	131	43 *	73	48 *	36	26 *	23	35 *
Controls	37	16	23	22	11	16	3	5

*P<.005 Significance of differences in prevalence of ronchi between grain workers and city workers.

TABLE 25
RESULTS OF PULMONARY FUNCTION STUDIES IN GRAIN-SMOKERS, EX-SMOKERS AND NONSMOKERS, BY AGE GROUP

Test	Smoking Group	Age Years: 20-29 N	mean	SD	30-39 N	mean	SD	40-49 N	mean	SD	50-62 N	mean	SD	All Age Groups N	mean	SD
FEV_1	Smoker	(47)	4484	606	(35)	4202	657	(42)	3393	677	(29)	3052	590	(153)	3849	853
	Ex-smoker	(19)	4587	376	(9)	4175	963	(20)	3825	523	(44)	3190	782	(92)	3713	877
	Nonsmoker	(22)	4726	592	(8)	4239	358	(19)	3799	531	(16)	3443	750	(65)	4079	782
	All	(88)	4567	564	(52)	4203	672	(81)	3595	638	(89)	3191	723	(310)	3857	853
FVC	Smoker		5437	750		5244	797		4565	726		4188	728		4917	892
	Ex-smoker		5549	461		5469	1240		4790	585		4330	702		4793	866
	Nonsmoker		5694	796		5032	625		4731	734		4881	901		5033	920
	All		5525	711		5250	857		4660	695		4311	747		4904	891
FEV_1/ FVC%	Smoker		82	5		80	6		73	9		72	9		77	8
	Ex-smoker		82	5		76	10		79	6		72	11		76	10
	Nonsmoker		83	6		84	9		80	7		76	7		81	7
	All		82	5		80	8		76	8		73	10		78	9
MMF	Smoker		276.9	61.4		250.4	68.5		171.8	74.0		142.1	54.5		216.4	85.0
	Ex-smoker		285.0	50.1		230.1	79.0		233.2	82.5		158.7	77.7		207.9	89.0
	Nonsmoker		299.6	69.4		288.7	91.7		220.2	51.0		185.9	71.0		247.1	82.0
	All		284.3	61.4		252.7	74.5		198.3	76.1		158.2	70.6		220.3	86.5
$Vmax_{50}$	Smoker		4.94	1.25		4.35	1.28		3.18	1.53		2.86	1.31		3.93	1.59
	Ex-Smoker		5.28	1.45		4.08	1.51		4.16	1.48		3.08	1.47		3.87	1.68
	Nonsmoker		5.17	1.63		4.69	1.63		4.12	:88		3.52	1.39		4.40	1.50
	All		5.07	1.39		4.35	1.36		3.64	1.46		3.09	1.41		4.01	1.61
$Vmax_{75}$	Smoker		1.98	.60		1.49	.52		.99	.45		.75	.30		1.36	.69
	Ex-smoker		2.11	.73		1.51	.53		1.23	.45		.87	.43		1.27	.70
	Nonsmoker		2.26	.94		2.06	.90		1.40	.29		1.05	.49		1.69	.84
	All		2.08	.72		1.58	.61		1.15	.45		.86	.42		1.40	.74

TABLE 25 (cont.)

RESULTS OF PULMONARY FUNCTION STUDIES IN GRAIN WORKERS-SMOKERS, EX-SMOKERS AND NONSMOKERS, BY AGE GROUP

Test	Smoking Group	Age, Years: 20-29 N	20-29 mean	20-29 SD	30-39 N	30-39 mean	30-39 SD	40-49 N	40-49 mean	40-49 SD	50-62 N	50-62 mean	50-62 SD	All Age Groups N	All Age Groups mean	All Age Groups SD
CV	Smoker	(45)	13	5	(32)	15	5	(41)	19	5	(29)	21	6	(147)	17	6
	Ex-smoker	(19)	13	7	(9)	17	4	(20)	19	5	(39)	21	7	(87)	18	7
	Nonsmoker	(22)	10	4	(7)	16	7	(18)	18	5	(15)	21	5	(62)	16	7
	All	(86)	13	5	(48)	15	5	(79)	19	5	(83)	21	6	(296)	17	6
$\Delta N_2/L$	Smoker	(45)	1.32	.83	(33)	1.39	.84	(41)	2.16	1.15	(29)	3.43	2.11	(148)	1.98	1.48
	Ex-smoker	(19)	.96	.55	(9)	1.26	.91	(20)	1.33	.65	(39)	2.54	1.90	(87)	1.79	1.52
	Nonsmoker	(22)	.86	.28	(8)	.85	.54	(19)	1.17	.57	(15)	1.67	.82	(64)	1.14	.64
	All	(86)	1.13	.70	(50)	1.28	.68	(80)	1.72	1.03	(83)	2.70	1.92	(299)	1.75	1.40
DL_{CO}	Smoker	(40)	35.6	5.6	(32)	34.4	5.9	(40)	30.8	5.0	(28)	27.9	6.0	(140)	32.4	6.3
	Ex-smoker	(17)	39.3	4.3	(9)	37.4	4.7	(19)	36.1	5.4	(44)	30.6	5.4	(89)	34.2	6.2
	Nonsmoker	(14)	41.1	6.7	(7)	38.0	5.0	(17)	35.2	3.4	(16)	33.8	6.0	(54)	36.7	6.0
	All	(71)	37.6	5.9	(48)	35.5	5,7	(76)	33.1	5.3	(88)	30.3	6.0	(283)	33.8	6.4

( ) parenthesis - if not stated N = N of above function

TABLE 26

RESULTS OF PULMONARY FUNCTION STUDIES IN CONTROLS-SMOKERS, EX-SMOKERS AND NONSMOKERS, BY AGE GROUP

Test	Smoking Group	Age, Years: 20-29 N	20-29 mean	20-29 SD	30-39 N	30-39 mean	30-39 SD	40-49 N	40-49 mean	40-49 SD	50-62 N	50-62 mean	50-62 SD	All Age Groups N	All Age Groups mean	All Age Groups SD
FEV_1	Smoker	(26)	4566	648	(35)	4282	572	(29)	3721	654	(16)	3209	778	(106)	4036	792
	Ex-smoker	(7)	4408	420	(22)	4310	477	(13)	4055	461	(26)	3356	710	(68)	3907	717
	Nonsmoker	(17)	4635	717	(20)	4404	570	(10)	4058	602	(16)	3605	348	(63)	4208	689
	All	(50)	4567	640	(77)	4232	541	(52)	3869	614	(58)	3384	659	(237)	4045	750
FVC	Smoker		5507	794		5245	684		4667	720		4547	643		5045	801
	Ex-smoker		5020	511		5298	541		5133	493		4477	792		4924	725
	Nonsmoker		5580	917		5360	644		4929	759		4512	497		5136	816
	All		5464	814		5290	629		4834	696		4506	671		5034	745
FEV_1/ FVC%	Smoker		82	6		81	5		79	7		70	13		79	8
	Ex-smoker		88	4		81	6		78	5		74	11		78	9
	Nonsmoker		82	5		82	3		81	5		79	5		81	4
	All		83	5		81	5		79	6		74	10		79	8
MMF	Smoker		288	64.5		274.8	70.7		223.5	72.8		160.1	90.1		246.7	84.6
	Ex-smoker		317.7	50.2		272.4	82.2		235.7	60.2		180.5	81		234.9	88.1
	Nonsmoker		289.1	72.2		270.9	53.4		276.4	66.5		222.6	52.0		264.4	64.6
	All		292.5	65.1		273	69.4		236.7	70.4		186.5	79.4		248	81.3
$Vmax_{50}$	Smoker		5.49	1.35		5.26	1.55		4.47	1.42	(16)	3.21	1.69	(106)	4.79	1.66
	Ex-smoker		6.36	1.02		5.12	1.52		4.35	1.34	(25)	3.54	1.50	(67)	4.51	1.68
	Nonsmoker		5.22	1.55		5.08	.98		5.06	.95	(15)	4.48	1.29	(62)	4.97	1.24
	All		5.52	1.40		5.17	1.40		4.55	1.32	(56)	3.70	1.56	(235)	4.76	1.57
$Vmax_{75}$	Smoker		2.25	.71		1.93	.69		1.44	.65	(16)	3.21	1.69	(106)	1.71	.79
	Ex-smoker		2.61	.62		1.90	.63		1.42	.38	(25)	1.00	.43	(67)	1.55	.73
	Nonsmoker		2.19	.75		2.01	.57		1.61	.53	(15)	1.25	.46	(62)	1.81	.69
	All		2.28	.71		1.94	.64		1.47	.56	(56)	1.02	.44	(235)	1.69	.75

TABLE 26 (cont.)

RESULTS OF PULMONARY FUNCTION STUDIES IN CONTROLS-SMOKERS, EX-SMOKERS AND NONSMOKERS, BY AGE GROUP

Test	Smoking Group	Age, Years: 20-29 N	20-29 mean	20-29 SD	30-39 N	30-39 mean	30-39 SD	40-49 N	40-49 mean	40-49 SD	50-62 N	50-62 mean	50-62 SD	All Age Groups N	All Age Groups mean	All Age Groups SD
CV	Smoker	(25)	13	5	(35)	14	3	(28)	17	4	(14)	22	6	(102)	16	5
	Ex-smoker	(7)	11	3	(22)	14	3	(13)	18	4	(23)	22	5	(65)	18	6
	Nonsmoker	(16)	10	3	(18)	15	4	(10)	17	3	(14)	19	4	(58)	15	6
	All	(48)	12	4	(75)	14	3	(51)	18	3	(51)	21	5	(225)	16	5
$\Delta N_2/L$	Smoker		1.32	.55		1.21	.60		1.87	.96	(15)	3.38	2.32	(103)	1.73	1.31
	Ex-smoker		1.12	.46		1.11	.43		1.23	.48	(23)	1.69	.76	(65)	1.34	.63
	Nonsmoker		.84	.31		1.00	.31		1.19	.64	(14)	1.44	.71	(58)	1.10	.54
	All		1.13	.51		1.13	.49		1.57	.86	(52)	2.11	1.59	(226)	1.46	1.02
DL_{CO}	Smoker	(25)	32.7	6.2	(30)	30.4	6.2	(20)	31.1	7.3	(13)	21.6	6.7	(88)	29.9	7.4
	Ex-smoker	(5)	30.7	5.5	(21)	32.8	6.3	(11)	32.9	5.2	(21)	27.9	5.7	(58)	30.9	6.1
	Nonsmoker	(16)	39.8	9.1	(20)	32.5	6.7	(7)	37.0	5.9	(14)	27.9	7.0	(57)	34.0	8.6
	All	(46)	35.0	8.0	(71)	31.7	6.4	(38)	32.7	6.7	(48)	26.2	6.9	(203)	31.3	5.6

TABLE 27.

RESULTS OF PULMONARY FUNCTION STUDIES
IN GRAIN HANDLERS AND CONTROLS

	GRAIN HANDLERS			CONTROLS			
	N	$\bar{X}$	±1SE	N	$\bar{X}$	±SE	P*
FEV_1 ml	(310)	3857	49	(237)	4045	49	.005
FVC ml	(310)	4904	51	(237)	5034	51	.05
FEV_1/FVC %	(310)	77.6	.5	(237)	79.4	.8	.01
MMF /min	(310)	220	5	(237)	248	5	.005
$Vmax_{50}$ /sec	(310)	4.01	.09	(235)	4.76	.10	.005
$Vmax_{75}$ /sec	(310)	1.40	.04	(235)	1.69	.05	.005
CV %	(296)	17.1	.4	(225)	16.0	.36	.025
ΔN_2/	(299)	1.75	.08	(226)	1.46	.07	.005
D_L ml CO/m/Torr	(283)	33.8	.4	(203)	31.3	.5	.005

*Significance of the difference by unpaired t-test.

TABLE 28

Pulmonary Function Test Values
in Relation to Last Exposure to Grain Dust

	FEV_1		FVC		MMF	
Last Exposure	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD
Same day of testing	3902 ±	813	4938 ±	866	225.7 ±	85
Day before testing	3711 ±	948	4827 ±	975	200.7 ±	88
> 2 days before testing	3842 ±	891	4768 ±	877	227.6 ±	86*

*Significantly different from MMF mean of "same day tested" by unpaired t-test.

TABLE 29

MULTIPLE REGRESSIONS OF RESULTS OF PULMONARY FUNCTION TESTS WITH SMOKING AND GRAIN HANDLING HISTORY, HEIGHT AND AGE OF GRAIN WORKERS AND CONTROLS. RELATIVE CONTRIBUTIONS (b) OF THE INDEPENDENT VARIABLES AND SIGNIFICANCE OF THE CONTRIBUTION(p)

TEST	GRAIN HANDLING		SMOKING		PREVIOUS SMOKING		AGE		HEIGHT	
	b	p	b	p	b	p	b	p	b	p
FEV_1	-172	.0002	-248	.0000	-119	.03	-41	.0000	103	.0000
FVC	-139	.009	-137	.04	- 55	NS	-33	.0000	140	.0000
$FEV_1\%_{FVC}$	- 1.3	.04	- 3.2	.0001	- 2.0	.025	- 3.3	.0000	- .13	NS
MMF	- 24.1	.0000	- 29.5	.0001	- 13	NS	- 4.0	.0000	4.4	.0000
$Vmax^{50}$	- 7.2	.0000	- 35	.02	- .10	NS	- 0.07	.0000	.06	.004
$Vmax^{75}$	- .25	.0000	- .27	.0000	- .13	.03	- .04	.0000	.03	.003
CV	.62	NS	1.4	.01	1.4	.01	.30	.0000	.06	NS
$\Delta N_2/L$	.23	.02	.79	.0000	.25	.05	.05	.0000	- .06	.0005
D_LCO	3.13	.0000	- 4.58	.0000	- 1.77	.02	- .23	.0000	.88	.0000

TABLE 30

Prevalence of Abnormal Lung Functions

		ALL			SMOKERS			EX-SMOKERS			NONSMOKERS				
		N	%	p^1	N	%	p^1	N	%	p^1	N	%	p^1	p^2	p^3
FEV_1/FVC % <70	G (310)	51	16		(153)	28	18	(92)	17	18	(65)	6	9	<.1	NS
				<.001			<.05			NS			NS		
	C (237)	16	7		(106)	9	8	(68)	6	9	(63)	1	2	<.1	NS
MMF<1.65SD	G (310)	60	19		(153)	33	22	(92)	23	25	(65)	4	6	<.01	NS
Of Predicted				<.001			<.005			<.001			<.05		
	C (237)	12	5		(106)	9	8	(68)	3	4	(63)	0	0	<.05	<.05
$V_{max}50$ <1.65SD	G (310)	130	42		(153)	68	44	(92)	43	47	(65)	19	29	<.05	NS
of Predicted				<.001			<.001			<.05			<.01		
	C (235)	46	19		(106)	21	20	(67)	19	25	(62)	6	3	<.1	NS
$V_{max}75$ <1.65SD	G (310)	153	49		(153)	83	54	(92)	51	55	(65)	19	29	<.001	NS
of Predicted				<.001			<.001			<.05			NS		
	C (235)	68	29		(106)	31	29	(68)	25	37	(63)	12	19	NS	NS
CV<1.65SD	G (296)	43	15		(147)	20	14	(87)	17	20	(62)	6	10	NS	NS
				<.001			NS			<.01			NS		
	C (225)	12	5		(102)	7	7	(65)	3	5	(58)	2	3	NS	NS
$\Delta N_2/L$>1.65SD	G (299)	101	34		(148)	67	45	(87)	24	28	(64)	10	16	<.001	<.01
of Predicted				<.01			NS			NS			NS		
	C (226)	51	23		(103)	35	34	(65)	12	18	(58)	4	7	<.001	<.05
FVC <80%	G (310)	17	5		(153)	10	7	(92)	5	5	(65)	2	3	NS	NS
of Predicted				<.1			NS			NS			<.05		
	C (237)	6	3		(106)	4	4	(68)	2	3	(63)	0	0	NS	NS
D_LCO<80 %	G (283)	22	8		(140)	14	10	(89)	6	7	(54)	2	4	NS	NS
of Predicted				<.005			<.01			NS			NS		
	C (203)	33	16		(88)	20	23	(58)	9	16	(57)	4	7	<.05	NS

() Total tested with each test on each category. P^1 Grain vs. Control Workers.
P^2 Smokers vs. Nonsmokers. P^3 Smokers vs. Ex-smokers. Values $\leq$.05 are considered not significant.

TABLE 31

RATIOS* OF THE EFFECT OF SMOKING TO THE EFFECT OF GRAIN HANDLING FROM MULTIPLE REGRESSION ANALYSIS

FEV_1	1.44
FVC	.99
FEV_1/FVC	2.46
MMF	1.22
$Vmax_{50}$	.49
$Vmax_{75}$	1.08
D_LCO	1.46
CV	2.26
$\Delta N_2/L$	3.43

*Ratio of the regression coefficient for smoking and grain handling from the multiple regression analysis that included ex-smoking, age and height as the other independent variables.

TABLE 32

PULMONARY FUNCTIONS ON GRAIN WORKERS BY JOB CATEGORIES

	(06) N=94		(02, 03, 04) N=90		(05) N=41		(07) N=31		(09) N=29		(01) N=20	
	Mean	1 SD	Mean	1 SD	Mean	1 SD	Mean	1 SD	Mean	1 SD	Mean	1 SD
FEV_1 ml	4017	763.5	3393	960.8	3631	726.0	3734	835.6	3703	851.4	3328	596.8
% Predicted	99.5	12.7	99.7	19.7	97.8	16.4	98.2	15.7	96.5	15.6	94.3	18.8
FVC ml	5054	783.4	5081	1010.7	4663	816.0	4714	912.0	4714	778.6	4324	514.5
% Predicted	101.6	11.9	103.1	16.6	100.3	15.1	99.7	13.9	99.8	9.6	97.6	14.2
FEV_1/FVC %	78.6	8.06	77.6	9.11	76.9	9.99	78.2	7.76	75.8	9.59	76.2	9.59
MMF l/min.	299.6	86.3	277.8	87.8	206.9	83.7	209.8	76.7	216.4	98.4	190.0	74.4
% Predicted	76.1	25.3	76.3	27.9	73.6	28.2	73.3	24.2	74.8	29.9	70.7	28.3
V_{max}^{50} l/sec	4.17	1.60	4.01	1.60	3.90	1.56	3.58	1.44	4.02	1.67	3.65	1.38
% Predicted	67.2	25.6	65.2	26.1	64.3	25.8	59.5	23.8	64.5	26.3	61.6	23.8
V_{max}^{75} l/sec	1.58	.85	1.48	.78	1.23	.52	1.20	.67	1.37	.70	1.16	.57
% Predicted	48.2	25.4	45.6	22.3	40.1	17.6	36.8	19.7	42.7	20.6	39.5	20.3
CV %	16.3	6.08	16.1	6.55	18.5	6.76	18.6	6.82	17.9	6.12	19.2	4.93
% Predicted	116.4	35.9	119.9	40.9	114.8	55.8	119.4	32.6	109.6	36.7	114.1	26.0
ΔN_2/l	1.53	.97	1.69	1.50	1.96	1.80	1.65	.98	2.05	1.90	2.35	1.40
% Predicted	137.5	80.7	150.9	120.1	165.1	142.8	142.6	78.7	168.5	143.1	195.8	110.8
D_LCO	35.5	5.8	34.1	6.2	32.2	5.4	32.1	8.2	33.9	6.7	31.5	6.0
% Predicted	113.6	18.9	111.0	19.7	104.0	16.1	105.6	25.4	104.5	20.6	101.8	15.8

TABLE 33

PULMONARY FUNCTION ON GRAIN WORKERS BY LENGTH OF EMPLOYMENT

	5.5		5.6 - 10.5		10.6 - 15.5		15.6 - 20.5		20.6 - 25.5		25.6 - 30.5		30.6	
	Mean	1 SD	Mean	1 SD	Mean	1 SD	Mean	1 SD	Mean	1 SD	Mean	1 SD	Mean	1 SD
FEV_1	4340	794	3946	800	3585	842	3406	709	3520	747	3555	743	3267	587
% Predicted	103.2	12.7	100.2	18.1	94.1	15.4	92.3	17.8	94.6	15.0	98.3	18.6	95.0	13.6
FVC	5319	883	4905	860	4711	963	4565	808	4619	729	4767	746	4287	607
% Predicted	103.8	12.9	100.4	15.7	99.0	13.5	98.2	13.9	99.2	9.8	104.7	16.0	98.3	9.3
FEV_1 % FVC	81.1	6.33	79.5	9.0	75.0	8.6	73.6	9.8	75.1	9.6	73.5	7.9	75.0	8.3
MMF	261.8	79.9	238.0	80.2	193.1	84.5	172.9	70.4	198.1	88.8	180.2	78.7	172.7	76.1
% Predicted	84.7	23.1	80.1	25.7	67.3	27.6	62.4	25.7	70.8	28.4	65.9	27.3	66.2	29.3
V_{max}^{50}	4.63	1.49	4.28	1.50	3.37	1.57	3.32	1.30	3.48	1.24	3.45	1.63	3.18	1.60
% Predicted	74.6	23.0	69.8	25.7	55.1	25.6	56.0	24.2	56.0	17.9	55.8	25.4	51.5	22.2
V_{max}^{50}	1.85	.84	1.56	.71	1.04	.51	1.08	.43	1.09	.46	1.00	.41	1.01	.65
% Predicted	55.4	24.0	49.0	22.8	32.3	15.6	35.4	15.8	33.5	13.7	32.4	14.3	34.6	21.8
CV	14.15	6.03	16.02	6.17	18.5	6.82	18.9	5.08	19.5	4.62	21.9	6.23	21.3	2.60
% Predicted	121.2	50.2	118.4	38.1	114.0	34.6	111.0	31.7	112.2	25.3	117.1	34.9	107.7	16.7
Slope	1.27	.72	1.53	1.18	1.95	1.14	2.48	2.14	2.00	1.51	2.07	1.66	2.59	1.58
% Predicted	122.1	65.1	139.1	99.4	164.8	92.5	202.9	165.0	168.3	119.4	164.8	125.2	215.0	129.8
D_LCO	36.5	6.17	33.5	6.27	31.5	7.23	32.5	5.37	34.7	4.70	31.7	6.88	31.3	4.94
% Predicted	119.2	19.0	109.0	18.7	98.7	20.0	103.9	18.6	109.4	12.5	100.0	20.0	99.5	14.2

TABLES 34/35

PREVALENCE OF ERYTHEMA REACTION 5 mm OR GREATER

		Grain Workers n=305			Controls n=235			MDN Workers n=103		
		N	%	p[1]	N	%	p[2]	N	%	p[3]
COMMON ALLERGENS		1:20 w/v								
	-	286	93.8	NS	214	91.1	NS	92	89.3	NS
Ragweed	+	19	6.2		21	8.9		11	10.7	
	-	281	92.1	.001	195	83.0	NS	90	87.4	NS
Timothy Grass	+	24	7.9		40	17.0		13	12.6	
	-	294	96.4	.01	216	91.9	NS	94	91.3	.01
Feathers	+	11	3.6		19	8.1		9	8.7	
	-	291	95.4	.005	210	89.4	NS	94	91.3	NS
Oak	+	14	4.6		25	10.6		9	8.7	
	-	293	96.1	.001	206	87.7	.05	97	94.2	NS
Cat	+	12	3.9		29	12.3		6	5.8	
	-	301	98.7	.005	222	94.5	NS	98	95.1	
Rat	+	4	1.3		13	5.5		5	4.9	.01
To one or more										
FUNGAL ANTIGENS		1:20 w/v								
	-	292	95.7	NS	219	93.2	NS	94	91.3	.10
A. Fumigatus	+	13	4.3		16	6.8		9	8.7	
	-	297	97.4	.1	221	94.0	NS	95	92.2	.05
Penicillium	+	8	2.6		14	6.0		8	7.8	
	-	303	99.3	.05	228	97.0	NS	101	98.1	NS
Mucor Sp.	+	2	.7		7	3.0		2	1.9	
	-	297	97.4	NS	228	97.0	NS	101	98.1	NS
Cladosporium Sp.	+	8	2.6		7	3.0		2	1.9	
	-	298	97.7	NS	231	98.3	NS	100	97.1	NS
Alternaria Sp.	+	7	2.3		4	1.7		3	2.9	
	-	302	99.0	NS	232	98.7	NS	102	99.0	NS
Rust	+	3	1.0		3	1.3		1	1.0	
	-	303	99.3	NS	230	97.9	NS	100	97.1	NS
Smut	+	2	.7		5	2.1		3	2.9	
To one or more										

TABLE 34/35

PREVALENCE OF WHEAL REACTION 3 mm OR GREATER

		Grain Workers n=305			Controls n=235			MDN Workers n=103		
		N	%	p[1]	N	%	p[2]	N	%	p[3]
COMMON ALLERGENS										
	-	286	93.8	NS	214	91.1	NS	94	91.3	NS
Ragweed	+	19	6.2		21	8.9		9	8.7	
	-	286	93.8		195	83.8		91	88.3	-.1
Timothy Grass	+	19	6.2	.001	40	17.0		12	11.7	
	-	288	94.4	NS	216	91.9	NS	96	93.2	NS
Feathers	+	17	5.6		19	8.1		7	6.8	
	-	292	95.7	.01	211	89.8	.05	100	97.1	NS
	+	13	4.3		24	10.2		3	2.9	
	-	287	94.1	.1	211	89.8	NS	97	94.2	NS
	+	18	5.9		24	10.2		6	5.8	
	-	298	97.7	NS	224	95.3	NS	99	96.1	NS
	+	7	2.3		11	4.7		4	3.9	
To one or more		46	15.1	.05	51	21.7	.05	13	12.6	NS
FUNGAL ANTIGENS										
	-	288	94.4	NS	221	94.0	NS	96	93.2	NS
A. Fumigatus	+	17	5.6		14	6.0		7	6.8	
	-	294	96.4	NS	221	94.0	NS	97	94.2	NS
Penicillium	+	11	3.6		14	6.0		6	5.8	
	-	300	98.4	NS	228	97.0	NS	102	99.0	NS
Mucor Sp.	+	5	1.6		7	3.0		1	1.0	
	-	299	98.0	NS	229	97.4	NS	103	100.0	NS
Cladosporium Sp.	+	6	2.0		6	2.6		0		
	-	295	96.7	NS	231	98.3	NS	100	97.1	NS
Alternaria Sp.	+	10	3.3		4	1.7		3	2.9	
	-	295	96.7	NS	232	98.7	NS	101	98.1	NS
Rust	+	10	3.3		3	1.3		2	1.9	
	-	297	97.4	NS	231	98.3	NS	101	98.1	NS
Smut	+	8	2.6		4	1.7		2	1.9	
To one or more		35	11.5	NS	29	12.3	NS	12	11.7	NS

TABLE 34/35

PREVALENCE OF ERYTHEMA REACTION 5 mm OR GREATER

		Grain Workers n=305			Controls n=235			MDN Workers n=103		
		N	%	p[1]	N	%	p[2]	N	%	p[3]
AIRBORNE DUST		100,000 PNU/ml								
Wheat Durum	-	247	81.0	NS	199	84.7	.005	99	96.1	.001
	+	58	19.0		36	15.3		4	3.9	
Wheat Spring	-	280	91.8	NS	222	94.5	NS	101	98.1	.05
	+	25	8.2		13	5.5		2	1.9	
Barley	-	280	91.8	NS	216	91.9	NS	98	95.1	NS
	+	25	8.2		19	8.1		5	4.9	
Corn	-	286	93.8	NS	209	88.9	.05	100	97.1	NS
	+	19	6.2		26	11.1		3	2.9	
Rye	-	290	95.1	.005	208	88.5	.1	98	95.1	NS
	+	15	4.9		27	11.5		5	4.9	
Oats	-	286	93.8	.05	209	88.9	NS	96	93.2	NS
	+	19	6.2		26	11.1		7	6.8	
Sunflower	-	283	92.8	NS	215	91.5	NS	97	94.2	NS
	+	22	7.2		20	8.5		6	5.8	
To one or more										
Settled Dust		100,000 PNU/ml								
Dust I	-	276	90.8	NS	208	88.5	.1	98	95.1	NS
	+	28	9.2		27	11.5		5	4.9	
Dust II	-	271	89.1	NS	208	88.5	NS	96	93.2	NS
	+	33	10.9		27	11.5		7	6.8	
Dust III	-	281	92.7	.05	203	86.4	NS	94	91.3	NS
	+	22	7.3		32	13.6		9	8.7	
To one or more										

Significance of the difference between grain and city workers χ^2.
Significance of the difference between city and MDN workes χ^2.
Significance of the difference between grain and MDN workers χ^2.

TABLE 34/35

PREVALENCE OF WHEAL REACTION 3 mm OR GREATER

		Grain Workers n=305			Controls n=235			MDN Workers n=103		
		N	%	P[1]	N	%	P[2]	N	%	P[3]
AIRBORNE DUST										
Wheat Durum	-	240	78.7	.01	205	87.2	.05	99	96.1	.001
	+	65	21.3		30	12.8		4	3.9	
Wheat Spring	-	276	90.5	.001	230	97.9	NS	102	99.0	.005
	+	29	9.5		5	2.1		1	1.0	
Barley	-	278	91.1	NS	221	94.0	NS	100	97.1	.05
	+	27	8.9		14	6.0		3	2.9	
Corn	-	284	93.1	NS	217	92.3	.05	101	98.1	.1
	+	21	6.9		18	7.7		2	1.9	
Rye	-	288	94.4	.1	213	90.6	NS	100	97.1	NS
	+	17	5.6		22	9.4		3	2.9	
Oats	-	287	94.1	NS	213	90.6	.05	100	97.1	NS
	+	18	5.9		22	9.4		3	2.9	
Sunflower	-	288	94.4	NS	216	91.9	.05	101	98.1	NS
	+	17	5.6		19	8.1		2	1.9	
To one or more		81	26.6	.1	48	20.4	.005	8	7.8	.001
Settled Dust										
Dust I	-	276	90.8	NS	211	89.8	.05	100	97.1	.05
	+	28	9.2		24	10.2		3	2.9	
Dust II	-	268	88.2	NS	213	90.6	NS	96	93.2	NS
	+	36	11.8		22	9.4		7	6.8	
Dust III	-	278	91.7	NS	211	89.8	.1	99	96.1	.1
	+	25	8.3		24	10.2		4	3.9	
To one or more		46	15.1	NS	36	15.3	.1	8	7.8	.05

Significance of the difference between grain and city workers χ^2.
Significance of the difference between city and MDN workes χ^2.
Significance of the difference between grain and MDN workers χ^2.

TABLE 34/35

PREVALENCE OF ERYTHEMA REACTION 5 mm OR GREATER

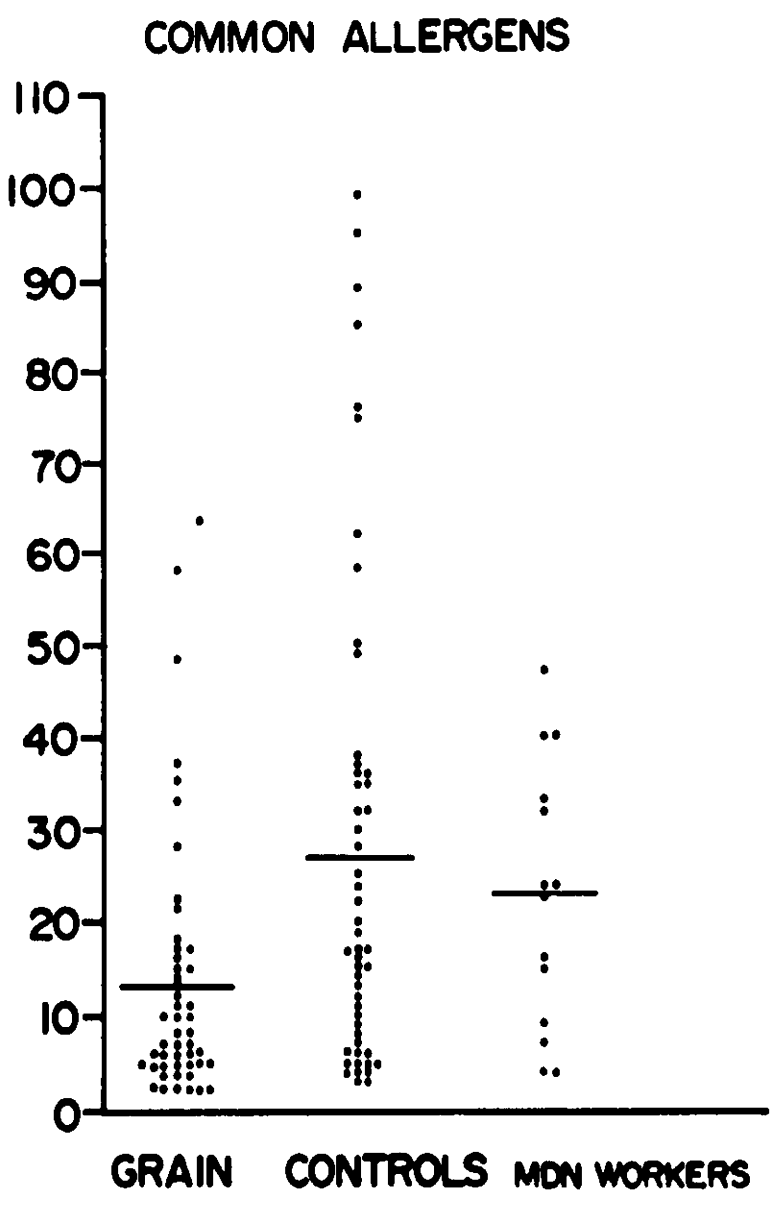
		Grain Workers n=305			Controls n=235			MDN Workers n=103		
		N	%	p[1]	N	%	p[2]	N	%	p[3]
INSECTS-MITES	10 mg/ml									
Mites Mixed	-	264	86.6	NS	209	88.9	NS	90	87.4	NS
	+	41	13.4		26	11.1		13	12.6	
Beetles Mixed	-	264	86.6	NS	211	89.8	NS	95	92.2	NS
	+	41	13.4		24	10.2		8	7.8	
Weevils	-	267	87.5	NS	209	88.9	NS	89	86.4	NS
	+	38	12.5		26	11.1		14	13.6	
To one or more										
Grain	100,000 PNU/ml									
Wheat Durum	-	296	97.0	NS	229	97.4	NS	99	96.1	NS
	+	9	3.0		6	2.6		4	3.9	
Wheat Spring	-	300	98.4	.1	225	95.7	NS	100	97.1	NS
	+	5	1.6		10	4.3		3	2.9	
Barley	-	298	97.7	NS	227	96.6	NS	99	96.1	NS
	+	7	2.3		8	3.4		4	3.9	
Corn	-	296	97.0	NS	225	95.7	NS	99	96.1	NS
	+	9	3.0		10	4.3		4	3.9	
Rye	-	303	99.3	.005	223	94.9	NS	101	98.1	NS
	+	2	.7		12	5.1		2	1.9	
Oats	-	301	98.7	NS	228	97.0	NS	102	99.0	NS
	+	4	1.3		7	3.0		1	1.0	
Sunflower	-	296	97.0	NS	228	97.0	NS	98	95.1	NS
	+	9	3.0		7	3.0		5	4.9	
Small Seeds	-	289	94.8	NS	219	93.2	NS	100	97.1	NS
	+	16	5.2		16	6.8		3	2.9	
Soybean	-	305	100.0	NS	234	99.6	NS	103	100.0	
	+	0			1	.4		0		
To one or more										

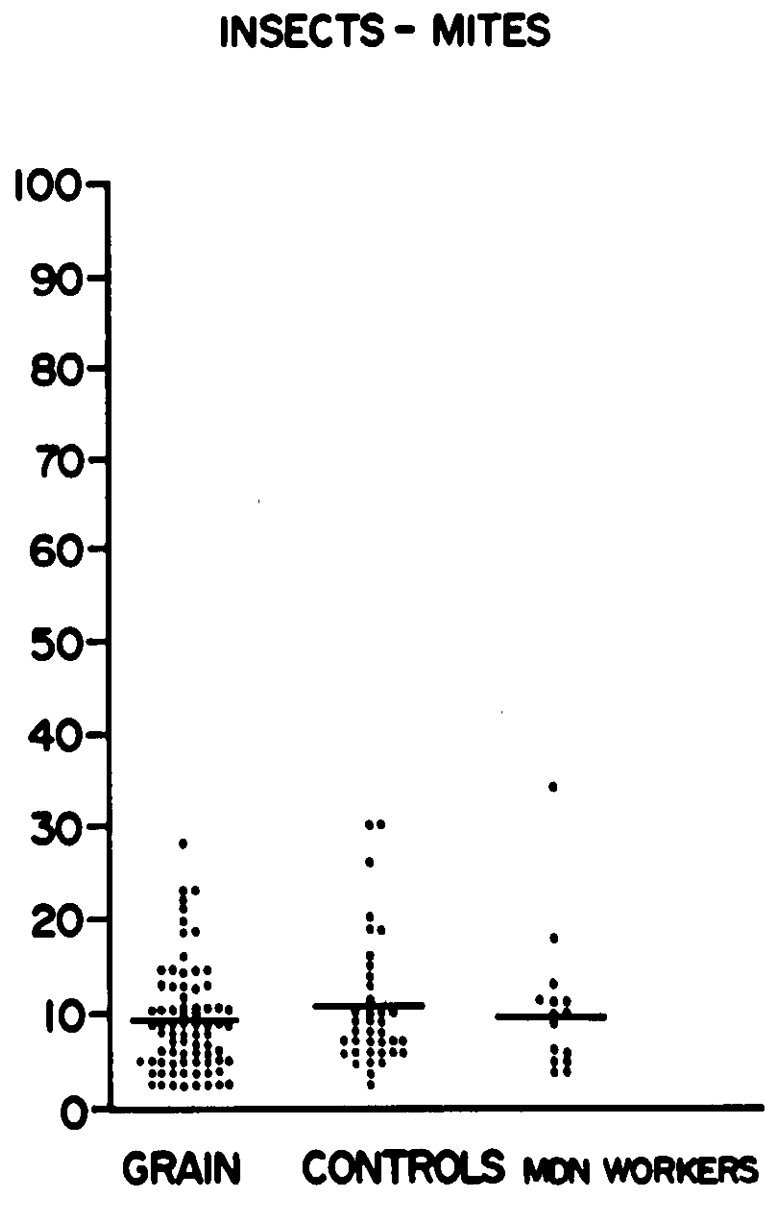
TABLE 34/35

PREVALENCE OF WHEAL REACTION 3 mm OR GREATER

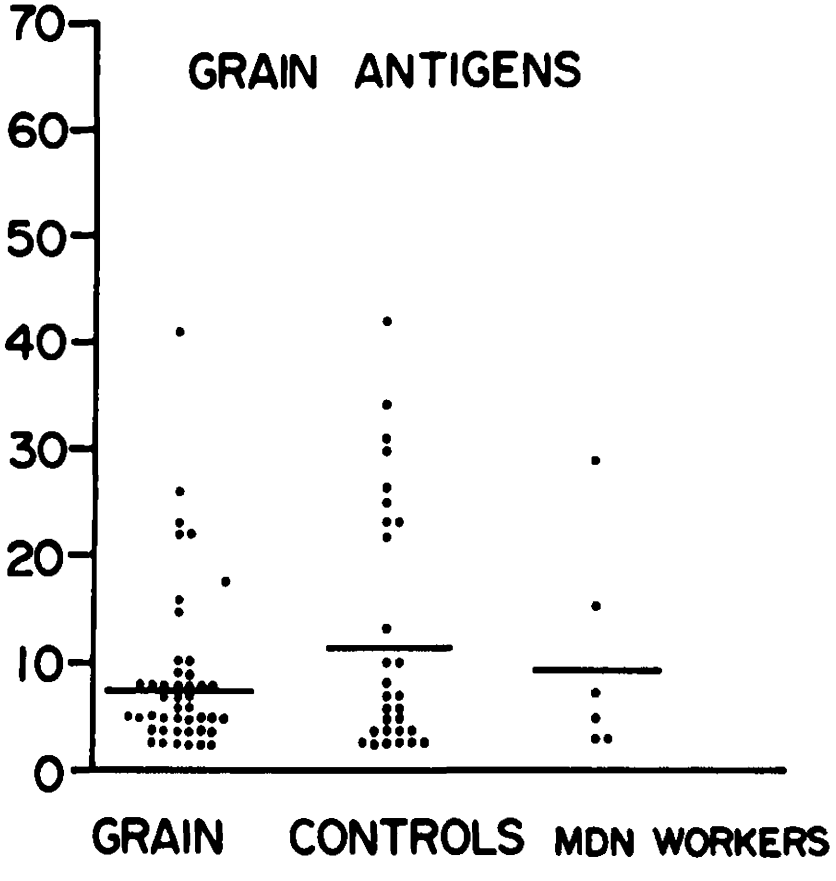
		Grain Workers n=305			Controls n=235			MDN Workers n=103		
		N	%	p[1]	N	%	p[2]	N	%	p[3]
INSECTS-MITES	10 mg/ml									
Mites Mixed	-	259	84.9	.05	213	90.6	NS	95	92.2	.1
	+	45	15.1		22	9.4		8	7.8	
Beetles Mixed	-	260	85.2		215	91.5	NS	96	93.2	.05
	+	45	14.8	.05	20	8.5		7	6.8	
Weevils	-	266	87.2	NS	210	89.4	NS	93	90.3	NS
	+	39	12.8		25	10.6		10	9.7	
To one or more		69	22.6	.05	36	15.3	NS	15	14.6	.1
Grain										
Wheat Durum	-	296	97.0	NS	231	98.3	NS	101	98.1	NS
	+	9	3.0		4	1.7		2	1.9	
Wheat Spring	-	299	98.0	NS	228	97.0	NS	102	99.0	NS
	+	6	2.0		7	3.0		1	1.0	
Barley	-	300	98.4	NS	228	97.0	.1	103	100.0	NS
	+	5	1.6		7	3.0		0		
Corn	-	297	97.4	NS	228	97.0	NS	101	98.1	NS
	+	8	2.6		7	3.0		2	1.9	
Rye	-	300	98.4	NS	227	96.6	NS	102	99.0	NS
	+	5	1.6		8	3.4		1	1.0	
Oats	-	300	98.4	NS	229	97.4	NS	103	100.0	NS
	+	5	1.6		6	2.6		0		
Sunflower	-	293	96.1	NS	228	97.0	NS	100	97.1	NS
	+	12	3.9		7	3.0		3	2.9	
Small Seeds	-	285	93.4	NS	221	94.0	.05	102	99.0	.0?
	+	20	6.6		14	6.0		1	1.0	
Soybean	-	305	100.0		235	100.0		103	100.0	
	+	0			0			0		
To one or more		38	12.4	NS	28	11.9	.1	6	5.8	.1

INDIVIDUAL SUM OF WHEAL REACTIONS





INDIVIDUAL SUM OF WHEAL REACTIONS



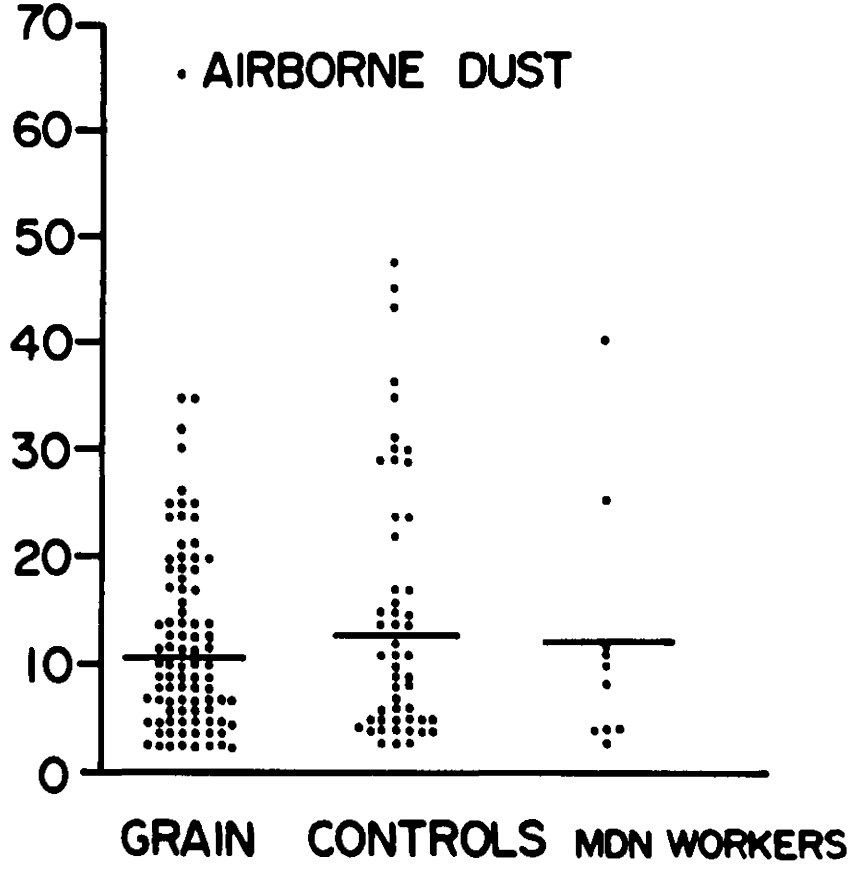


TABLE 37

PREVALENCE OF GRAIN DUST AND INSECT-MITE REACTIONS
AMONG ATOPIC GRAIN OR CONTROL WORKERS

		Grain Dust Reactors		Insect-Mite Reactors	
		N	%	N	%
Atopic Grain Workers	(46)	37**	80	31**	67
Non-atopic	(259)	44	17	38	15
Atopic Controls	(51)	24**	47	13*	25
Non-atopic	(184)	24	13	23	12

*P = .05 by χ^2 for the difference between atopic and non-atopic.
**P = .001

TABLE 38. REGRESSION COEFFICIENTS (b) AND T RATIOS (t) OF THE SIGNIFICANT* RELATIONS BETWEEN ACUTE AND CHRONIC SYMPTOMS AND LUNG FUNCTION ADJUSTED FOR AGE, HEIGHT, SMOKING HABIT MULTIPLE REGRESSION ANALYSIS+

		FEV_1/FVC	FEV_1	FVC	MMF	$V_{MAX}50$	$V_{MAX}75$	CV	$\Delta N_2/L$	DL
GRAIN WORKERS df (n-1)	=	310	310	310	310	310	310	296	299	283
Chronic Bronchitis	b									
	t									
Cough First Thing	b	-2.51			-16.70					
in A.M.	t	-2.7			- 2.0					
Phlegm First Thing	b									
in A.M.	t									
Dyspnea on Exertion	b	-1.85	-178.8	-130.0						
	t	-2.0	-2.7	-1.7						
Wheezing at Night	b									
	t									
Cough on Exposure	b	-3.07	-157.8		-19.11	-0.487	- .140			
	t	- 3.4	- 2.4		-2.4	-3.0	-2.2			
Occupational	b	-1.63	-130.8			-0.305	-0.167		0.272	
Asthma I	t	- 1.8	- 2.0			-1.9	-2.7		+1.9	
Dyspnea on Exposure	b		-144.9			-0.264	-0.109			
	t		- 2.3			-1.7	-1.8			
Occupational	b	-1.79	-116.5			-0.275	-0.120		0.281	
Asthma II	t	- 2.0	- 1.8			-1.7	-1.9		+2.0	
Occupational	b		-172.9	-164.7					0.331	
Asthma IV	t		- 2.2	- 1.9					+1.9	
Grain Fever	b									
	t									
Chest Illness	b									
	t									
CONTROLS df (n-1)	=	237	237	237	237	235	235	225	226	203
Chronic Bronchitis	b	-3.50		- 25.7					0.550	
	t	-3.10		- 2.2					+3.53	
Dyspnea on Exertion	b	-1.99							0.275	
	t	-1.99							+2.02	
Wheezing at Night	b	-3.72			-0.631					
	t	-2.63			-2.10					
Occupational Asthma II	b									
	t									

*P < .05 when t > 1.66 using one tail area of t distribution and > 200 degrees of freedom (P < .05 when t > 1.98 using two tail)

+Multiple regression analysis using lung function test value as the dependent variable and symptom, age, height, smoking and nonsmoking as independent variables.

TABLE 39

PREVALENCE OF ABNORMAL LUNG FUNCTION IN GRAIN WORKERS WITH AND WITHOUT SYMPTOMS

Abnormal Function	Cough on Exposure					Wheezing on Exposure					Dyspnea on Exposure					Grain Fever				
	Yes		No		P*	Yes		No		P*	Yes		No		P*	Yes		No		P*
	#	%	#	%		#	%	#	%		#	%	#	%		#	%	#	%	
	N=200		N=100			N=183		N=127			N=151		N=159			N=99		N=211		
FEV_1FVC <70% (310)	41	20	10	9	<.01	37	20	14	11	<.05	31	21	20	13	<.1	20	20	31	15	NS
FVC <80% (310)	12	6	5	5	NS	13	7	4	3	NS	14	9	3	2	<.005	5	5	12	6	NS
MMF <1.65 SD (310)	47	29	13	12	<.05	45	25	15	12	<.01	37	25	23	14	<.05	22	22	38	18	NS
$\dot{V}_{Max}50$ <1.65 SD (310)	92	46	38	35	<.1	89	49	41	32	<.005	76	50	54	34	<.005	44	44	86	41	NS
$\dot{V}_{MAX}75$ <1.65 SD (296)	105	53	48	44	NS	107	58	46	36	<.001	89	59	64	40	<.005	53	54	100	47	NS
	N=200		N=96			N=183		N=113			N=151		N=145			N=99		N=197		
CV <1.65 SD (296)	30	15	13	14	NS	32	17	11	11	NS	28	19	15	10	<.05	13	13	30	15	NS
	N=200		N=96			N=183		N=110			N=151		N=148			N=99		N=209		
$\Delta N_2/L$ <1.65 SD (299)	77	39	23	23	<.01	67	37	33	28	NS	60	40	40	27	<.05	35	35	65	33	NS
	N=184		N=99			N=169		N=114			N=143		N=140			N=92		N=191		
D_LCO < 80% (283)	19	10	3	3	<.05	17	10	5	4	<.1	14	10	8	6	NS	10	11	12	6	NS

TABLE 39 (CONT'D.)

Abnormal Function	Chronic Bronchitis Yes #	%	N=135	No #	%	N=175	P*	Cough First Thing in A.M. Yes #	%	N=183	No #	%	N=127	P*	Wheezing at Night Yes #	%	N=151	No #	%	N=159	P*	Dyspnea on Exertion Yes #	%	N=99	No #	%	N=211	P*
FEV_1FVC <70% (310)	31	23		20	11		<.01	25	23		26	13		<.05	9	15		42	17		NS	30	25		21	11		<.001
FVC <80% (310)	5	4		12	7		NS	6	6		11	5		NS	5	8		12	5		NS	12	10		5	3		<.005
MMF <1.65 SD (310)	36	27		24	14		<.005	31	28		29	14		<.005	13	21		47	19		NS	33	28		27	14		<.005
V_{Max}^{50} <1.65 SD (310)	68	50		62	35		<.01	49	45		81	40		NS	32	52		98	39		<.1	56	47		74	39		NS
V_{MAX}^{75} <1.65 SD (296)	75	36		78	45		<.1	56	51		97	48		NS	36	59		117	47		<.1	66	56		87	45		<.1
			N=135			N=161				N=109			N=187				N=61			N=235				N=118			N=178	
CV <1.65 SD (296)	18	13		25	16		NS	15	14		28	15		NS	12	20		31	13		NS	14	12		29	16		NS
			N=135			N=164				N=109			N=190				N=61			N=238				N=118			N=181	
$\Delta N_2/L$ <1.65 SD (299)	48	36		52	32		NS	46	42		54	28		<.05	22	36		78	33		NS	47	40		53	29		NS
			N=125			N=158				N=105			N=178				N=57			N=226				N=108			N=175	
D_LCO 80% (283)	13	10		9	6		NS	13	12		9	6		.05	6	11		17	7		NS	15	14		7	4		.005

*Significance of difference in prevalence of abnormal lung functions in workers with and without symptoms.
NS = Not significant.

TABLE 40a

REGRESSION COEFFICIENTS (b) AND Â T RATIOS (t) FOR THE SIGNIFICANT* RELATIONS OF SKIN REACTIVITY TO COMMON OR SPECIFIC ANTIGENS AND PULMONARY FUNCTION ADJUSTED FOR AGE, HEIGHT, SMOKING HABIT USING MULTIPLE REGRESSION ANALYSIS† (GRAIN WORKERS)

		Total Wheal CAA††	Total Wheal Grain††	Durum Wheat	Barley	CAA**	Airborne Dust	Grain	Insects Mites	Fungi	Settled Dust
FEV_1/ FVC	b	- .064	- .049						-1.83	- 3.77	- 2.44
	t	-1.95	-1.73						-1.73	- 2.74	- 1.98
FEV_1	b	-6.08	-5.24	-183.7		-170.3	-161.6			-268.5	-240.5
	t	2.61	-2.57	- 2.40		- 1.92	- 2.25			2.71	2.73
FVC	t	-1.75	-1.77	- 1.94		- 1.76	- 1.96				
MMF	b	- .67	- .549			- 17.9	- 14.49			- 30.1	- 23.3
	t	-2.39	-2.26			- 1.69	- 1.69			- 2.55	- 2.21
$V_{MAX}50$	b	- .014	- .010	- .404			- .364			- .703	- .412
	t	-2.49	-2.10	- 2.16			- 2.08			- 2.92	- 1.91
$V_{MAX}75$	b	- .005	- .004	- 1.89			- 1.71			- .213	- .178
	t	-2.23	-2.26	- 2.59			- 2.49			- 2.23	- 2.09
$\Delta N_2/L$	b										
	t										
DL	b									- 1.87	- 1.57
	t									- 1.98	- 1.83

*P < .05 when t > 1.66 using one tail area of t distribution and > 200 degrees of freedom (P < .05 when t > 1.98 using two tail)

† Multiple regression analysis using lung function test values as the dependant variable and skin reactivity, age, height, and smoking as independent variables.

** Common allergens.

†† Individual sum of wheal reactions to common allergens (CAA) or to grain antigens (grain).

TABLE 40b

REGRESSION COEFFICIENTS (b) AND Δ T RATIOS (t) FOR THE SIGNIFICANT* RELATIONS OF SKIN REACTIVITY TO COMMON OR SPECIFIC ANTIGENS AND PULMONARY FUNCTION ADJUSTED FOR AGE, HEIGHT, SMOKING HABIT USING MULTIPLE REGRESSION ANALYSIS† (CONTROLS)

		Total Wheal CAA††	Total Wheal Grain††	Durum Wheat	Barley	CAA**	Airborne Dust	Grain	Insects Mites	Fungi	Settled Dust
FEV_1/ FVC	b										
	t										
FEV_1	b				244.0						
	t				1.88						
FVC	b	3.52	5.73		3.60		110.9	.198		186.0	212.0
	t	2.12	2.49		2.51			1.67			2.03
MMF	b						-19.6				-26.3
							- 1.78				- 2.11
V_{MAX}^{50}	b										
	t										
V_{MAX}^{75}	b										
	t										
$\Delta N_2/L$	b										
	t										
DL	b							2.12			
	t							1.70			

*P < .05 when t > 1.66 using one tail area of t distribution and 200 degrees of freedom (P < .05 when t 1.98 using two tail)

† Multiple regression analysis using lung function test values as the dependent variable and skin reactivity, age, height, and smoking as independent variables.

** Common allergens.

†† Individual sum of wheal reactions to common allergens (CAA) or to grain antigens (grain).

TABLE 41a

PREVALENCE OF ABNORMAL FUNCTION IN POSITIVE AND NEGATIVE SKIN REACTIONS

GRAIN WORKERS (305)

		Abnormal FEV_1/FVC								Abnormal MMF < 1.65 SD								Abnormal V50 < 1.65 SD								Abnormal $\Delta N_2/L$ > 1.65 SD							
			ALL		S		EX		NS		ALL		S		EX		NS		ALL		S		EX		NS		ALL		S		EX		NS	
	N	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%
Common Allergens	+ 46	8	17	3	7	5	11	0	-	9	20	3	7	6	13	0	-	20	43	6	13	7	15	7	15								
			NS								NS								NS								NS					
	-259	42	16	24	9	12	5	6	2	49	19	28	11	17	6	4	2	108	42	60	23	36	14	12	5	79	31	54	21	17	7	8	3
Airborne Dust	+ 81	12	15	6	7	6	7	0	-	14	17	4	5	10	12	0	-	34	42	16	20	14	17	4	5	35	43	23	28	10	12	2	2
			NS								NS								<.001								NS					
		40	18	22	10	12	5	6	3	44	20	27	12	13	6	4	2	40	18	19	8	17	8	4	2	82	37	51	23	23	10	8	4
Insects Mites	+ 69	14	20	8	12	6	9	0	-	14	20	7	10	7	10	0	-	30	43	16	23	10	14	4	6	23	33	16	23	5	7	2	3
			NS								NS								NS								NS					
	-236	36	15	19	8	11	5	6	3	44	19	24	10	16	7	4	2	98	42	50	21	33	14	15	6	74	31	47	20	19	8	8	3
Fungi	+ 35	11	31	4	11	7	20	0	-	16	46	6	17	10	29	0	-	24	69	11	31	11	33	2	6	17	49	9	26	7	20	1	3
			<.05								<.001								<.001								<.05					
	-270	39	14	23	9	10	4	6	2	42	16	25	9	13	5	4	2	104	39	55	20	32	12	17	6	80	30	54	20	17	6	9	3

+Positive to one or more antigens of the group of antigens.
%Percentage of skin reactions or nonreactions with the abnormal function for each smoking category.
S = Smoker; EX = Ex-smoker; NS = Nonsmoker.

TABLE 41b

PREVALENCE OF ABNORMAL FUNCTION IN POSITIVE AND NEGATIVE SKIN REACTIONS

CONTROL (235)

		Abnormal FEV_1/FVC								Abnormal MMF < 1.65 SD								Abnormal V50 < 1.65 SD								Abnormal $\Delta N_2/L$ > 1.65 SD							
			ALL		S		EX		NS		ALL		S		EX		NS		ALL		S		EX		NS		ALL		S		EX		NS	
	N	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%
Common Allergens	+ 51	1	2	1	2	0	-	0	-	2	4	2	4	0	-	0	-	9	18	5	10	3	6	1	2	3	6	9	18	3	6	1	2
			NS								NS								NS								NS					
	-184	15	8	8	4	6	3	1	1	10	5	7	4	3	2	0	-	37	20	16	9	16	9	5	3	38	21	26	14	9	5	3	2
Airborne Dust	+ 48	3	06	0	-	2	4	1	2	1	2	0	-	6	13	2	4	2	4	2	4	14	29	6	13	5	10	3	6				
			NS								NS								NS								NS					
	-187	17	9	9	5	8	4	0	-	11	6	9	5	2	1	0	-	40	21	19	10	17	9	4	2	54	29	37	20	14	7	3	2
Insects Mites	+ 36	2	6	0	-	2	6	0	-	1	3	0	-	1	3	0	-	3	8	0	-	3	8	0	-	11	31	8	22	3	8	0	-
			NS								NS								<.1								NS					
	-199	36	15	19	8	11	5	6	3	44	19	24	10	16	7	4	2	98	42	50	21	33	14	15	6	74	31	47	20	19	8	8	3
Fungi	+ 29	2	7	1	3	1	21	0	-	2	7	2	7	0	-	0	-	8	28	4	14	2	7	2	7	7	24	5	17	1	3	1	3
			NS								NS								NS								NS					
	-206	14	7	8	4	5	2	1	0	10	5	7	3	3	1	0	-	38	18	17	8	17	8	4	2	44	21	30	15	11	5	3	1

+Positive to one or more antigens of the group of antigens.
%Percentage of skin reactions or nonreactions with the abnormal function for each smoking category.
S = Smoker; EX = Ex-smoker; NS = Nonsmoker.

TABLE 42

PREVALENCE OF SYMPTOMS IN POSITIVE AND NEGATIVE SKIN REACTORS - (GRAIN WORKERS)

	Skin Reaction	CAA		ABD		I-M		Fungi		Grain		SD		D Wh		Barley		Oats		Rye	
	+	46		+81		+69		+35		+38		+46		+65		+27		+12		+17	
Total No.	-	259		-224		-236		-270		-267		-259		-240		-278		-287		-288	
		n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Chronic Bronchitis	+	21	46	39	48	32	46														
	-	127	49	109	49	116	49														
Cough on Exposure	+	32	70	55	68	45	65	27	77	27	71	30	65	43	66	20	74	11	61	14	82
	-	165	64	142	63	152	64	170	63	170	64	167	64	154	64	177	64	186	65	183	64
Occupational	+	30	65	55	68	45	65	26	74	27	71	31	67	44	68	19	70	9	50	12	71
Asthma I		150	58	125	56	135	57	154	57	153	57	149	58	136	57	161	58	171	60	168	58
Dyspnea on Exposure	+	27	59	44	54	34	49	24	69	25	66	28	61	37	57	16	59	10	56	10	59
	-	121	47	104	46	114	48	124	46*	123	46*	120	46	111	46	132	47	138	48	138	48
Nasal Sx on Exposure	+	38	83	66	81	56	81	30	86	37	97	40	87	57	88	27	100	18	100	17	100
	-	199	77	171	76	181	77	213	79	206	77*	203	78	186	78	216	78*	225	78*	226	78
Grain Fever	+	19	41	25	31	21	30														
	-	80	31	74	33	78	33														
Cough First Thing	+	16	35	29	36	29	42														
in A.M.	-	90	35	77	34	77	33														
Wheezing at Night	+	11	24	17	21	15	22														
	-	50	19	44	20	46	19														
Occupational	+	12	26	20	25	13	19														
Asthma IV	-	56	22	48	21	55	23														
Occupational	+	25	54	44	54	37	54														
Asthma II	-	118	46	99	44	106	45														

*Significant ($P < .05$) by χ^2; CAA = Common Allergens; ABD = Airborne Grain Dust; I-M = Insects and Mites; SD = Settled Dust; D WH = Durum Wheat

TABLE 43

PREVALENCE OF POSITIVE DELAYED HYPERSENSITIVITY SKIN TESTS

Dose Injected	Grain Workers (N=232) n	%	Controls (N=156) n	%	X^2	Source	Lot No.	Stock Concentration	Working Solution* (ml)	Skin Test Antigen Concentration (0.1 ml)
PPD (Tinne Tests)	34	14	15	10	NS					
SK/SD (4 u/lu) (Varidase)	164	71	89	57	<.01	Lederle	500-283	20000 U SK	40 U SK	4 U
Mumps skin test antigen (0.1 ml)	104	45	95	61	<.005	Lilly	OH544A	1.0 ml	-	0.1 ml
Trichophyton 1:1000 w/v	90	39	28	18	<.001	Hollister-Steir	H9701702	1:10 w/v	1:10	1:1000
Candida (Monilia) Albions Antigen (10 PNU)	28	12	50	32	<.001	Hollister-Steir	6I08934	10,000 PNU	100 PNU	10 PNU
To 2 or More	134	58	104	67	NS					

*All dilutions prepared in sterile Coca's non-allergenic buffer.

Tests were considered positive when induration was: >5.0 mm for SK/SD, Trichophyton and Candida; ≥10 mm for PPD or Erythema, ≥15 mm for mumps and after 48 hours of .1 cc of solution injected intradermally.

TABLE 44

PREVALENCE OF PRECIPITATING ANTIBODIES

		GRAIN WORKERS (310)		CONTROLS (236)	
		N	%	N	%
1.	Aerobasidium	2	.6	0	0
2.	Alternaria	0	0	0	0
3.	Aspergillus clavatus	1	.3	0	0
4.	Aspergillus falvus	0	0	0	0
5.	Aspergillus fumigatus (1)	5	1.6	0	0
6.	Aspergillus fumigatus (5)	1	.3	8	3.4
7.	Aspergillus fumigatus (6)	0	0	1	.4
8.	Aspergillus fumigatus (8)	0	0	0	0
9.	Aspergillus fumigatus (9)	0	0	0	0
10.	Aspergillus fumigatus (1022)	1	.3	1	.4
	All Aspergillus fumigatus 5-10	0	0	0	0
	One or more Aspergillus fumigatus 5-10	7	2.2	10	4.0
11.	Aspergillus niger	1	.3	1	.4
12.	Candida Albicans	2	.6	2	.8
13.	Cephalosporium	1	.3	3	1.3
14.	Fusarium	3	.9	5	2.1
15.	Hormodendrum	5	1.6	5	2.1
16.	House dust	4	1.2	6	2.5
17.	Micropolyspora faeni (Greer)	3	.9	4	1.7
18.	Micropolyspora faeni (Marsh)	0	0	0	0
19.	Micropolyspora faeni (UW)	4	1.2	2	.8

TABLE 44 (Cont'd.)

		GRAIN WORKERS (312)		CONTROLS (236)		
		N	%	N	%	
20.	Moldy hay	87	27.8	78	33.1	
	All micropolyspora faeni and hay 17-20	0	0	0	0	
	One or more micropolyspora faeni or hay 17-20	89	29.0	82	34.0	
21.	Mucor	0	0	2	.8	
22.	Penicillium casei	1	.3	1	.4	
23.	Penicillium rubrum	7	2.2	5	2.1	
24.	Phoma	3	.9	9	0	
25.	Pigeon sera	0	0	2	.8	
26.	Trichoderma	3	.9	14	5.9	<.001
27.	Thermoactinomycetes candidus (Kosky)	5	1.6	0	0	
28.	Thermoactinomycetes candidus (UW)	4	1.2	0	0	
29.	Thermoactinomycetes vulgaris (Grear)	1	.3	34	14.4	<.001
30.	Thermoactinomycetes vulgaris (H/S)	7	2.2	25	10.6	<.001
31.	Thermoactinomycates vulgaris (Marsh)	6	1.9	4	1.7	
	All Thermoactinomycetes vulgaris	0	0	1	0	
	One or more Thermoactinomycetes vulgaris	11	3.5	38	16.0	<.001
32.	Thermoactinomycetes sacchari	2	.6	45	19.1	.001
33.	Thermoactinomycetes viridans	15	4.8	6	2.5	
	One or more of above 1-33	115	37.0	133	56.0	<.00
34.	Wheat Durum	11	3.5	2	.8	<.05
35.	Wheat Spring	14	4.4	0	0	
36.	Barley	10	3.2	3	1.3	
37.	Corn	14	4.4	15	6.4	
38.	Rye	11	3.5	2	.8	<.05
39.	Oats	10	3.2	6	2.5	

TABLE 44 (Cont'd.)

	GRAIN WORKERS (312)		CONTROLS (236)		
	N	%	N	%	
40. Sunflower seeds	3	.9	7	3.0	
41. Small seeds	21	6.7	11	4.7	
One or more of above 34-41	38	12.0	28	12.0	
42. Wheat durum dust	25	8.0	2	.8	<.001
43. Wheat spring dust	21	6.7	0	0	
44. Barley dust	14	4.4	3	1.3	<.05
45. Corn dust	21	6.7	15	6.4	
46. Rye dust	17	5.4	2	.8	<.005
47. Oats dust	25	8.0	6	2.5	<.01
48. Sunflower seed dust	7	2.2	0	0	
49. Soybean dust	0	0	0	0	
50. Settled dust I	94	30.1	59	25.0	
51. Settled dust II	21	6.7	12	5.1	
52. Settled dust III	30	9.6	11	4.7	<.05
One or more 42-52	115	37.0	70	29.0	<.1
53. Mites mixed	4	1.2	1	.4	
54. Beetles mixed	3	.9	0	0	
55. Weevils mixed	0	0	2	.5	
One or more 53-55	7	2.2	3	1.0	
One or more 34-55	123	40.0	80	33.0	

TABLE 45

BLOOD CHEMISTRIES

		G = Grain				C = Control Workers			
		N	Mean	SD	Unpair t	N	Abnormal %	P	Grain Workers Values 2 SD of Controls
Cholinesterase (4-16 µ/l)	G	308	10.9	3.68	-2.21*	7	2	NS	5
	C	234	11.6	2.99		10	4		
GGT (Upper breath) (30 µ/l)	G	205	21.7	13.1	1.15	64	21	.05 -	6
	C	232	20.0	20.8		31	13		
Creatinine (0.4-19 ml/l)	G	305	1.20	.22	8.92*	3	1	NS	59
	C	232	1.05	.14		0	--		
SGPT (10-50 µ/l)	G	302	25.3	15.2	8.23*	8	3	NS	30
	C	234	15.6	10.9		5	2		
HGB GM%	G	306	16.1	1.14	.931				
	C	192	16.0	1.03					
HCT%	G	306	47.5	2.84	6.54*				
	C	192	45.8	2.53					

*=P < .01
( ) in parentheses normal range for our laboratory.

TABLE 46

URINALYSIS

	Grain Worker N=303		Control Worker N=231	
	N	%	N	%
Blood	6	2	5	2
Glucose TR	4	1	1	
>2+	0	0	5	2
Protein TR	29	10	8	3.5
>1+	10	3	6	3.

TABLE 47

RADIOLOGICAL FINDINGS IN GRAIN WORKERS AND CONTROLS

	Grain Workers (293)		Controls (236)		P
	N	%	N	%	
Thorax					
Normal	264	90	218	93	
Rib fracture (old)	12	4	5	2	NS
Degenerative spine changes	16	5	5	2	<.1
Scoliosis (mild)	2	1	5	2	
Other			3*	1	
Heart					
Normal	292	100	236	100	
Questionably enlarged	1	0			
Enlarged					
Lungs					
Normal	279	95	225	95	
Nodule(s) calcified	2	1	6	3	
Nodule(s) non-calcified	4	1	2	1	
Mass (>2.5 cm)	0	0	2+	1	
Reticulonodular pattern	0	0	0	0	
Band atelectasis or fibrosis	5	2	1	0	
Blebs	3	1	1		
Hyperinflation	1	0	0	0	
Pleura					
Normal	280	95	228	96	
Apical thickening	2	1	3	1	
Costophrenic angles-unilateral	9	3	4	2	NS
-bilateral	4**	1	1**	0	
Post thoracotomy changes	1+	0	2++	1	

*One rib fibrodysplasia, one pectus excavatus, one mild old fracture of clavicle.
+One bilateral hilar mass, probably lymphoma, one paratracheal node.
**One grain worker and one control with calcification of pleura.
++One grain worker and one control rib resection from thoracotomy, one control mid-sternal sutures from mid-sternotomy for coronary bypass.

TABLE 48

LEVEL OF IMMUNOGLOBULINS (G,A,M)* IN GRAIN WORKERS AND CONTROLS

	Number Tested	IgG mg/dl	IgA mg/dl	IgM mg/dl
Grain Workers	307	1587 ± 27	266 ± 6	156 ± 4
Controls	235	1436 ± 23**	238 ± 6**	151 ± 5

*Results expressed as Mean ± Standard Error of the Mean
**Statistically significant ($p < 0.05$) between grain workers and controls with Students t test.

TABLE 49

LEVELS OF IMMUNOGLOBULINS (G,A,M)* IN SMOKING, EX-SMOKING AND NONSMOKING GRAIN WORKERS AND CONTROLS

	Smokers		Ex-smokers		Nonsmokers	
	Grain Workers (N=180)	Controls (N=105)	Grain Workers (N=92)	Controls (N=67)	Grain Workers (N=65)	Controls (N=63)
IgG mg/dl	1514±36	1384±36**	1687±35**	1427±35**	1631±62	1532±45**
IgA mg/dl	246±8	236±10	288±12	238±11**	280±15	241±12**
IgM	148±7	151±7	163±8	143±8	167±9	160±9

*Results expressed as the Mean ± Standard error of the Mean
**Statistically significant ($p < 0.05$) with Students T test using comparisons of means between grain workers and controls in each smoking category.

TABLE 50

THE LEVELS OF IMMUNOGLOBULINS (G,A,M)* IN AGE GROUPED GRAIN WORKERS AND CONTROLS

	≤20 Years		21-30 Years		31-40 Years		41-50 Years		51-60 Years		61-70 Years	
	Grain Workers (N=2)	Controls (N=2)	Grain Workers (N=97)	Controls (N=58)	Grain Workers (N=44)	Controls (N=72)	Grain Workers (N=50)	Controls (N=48)	Grain Workers (N=71)	Controls (N=44)	Grain Workers (N=12)	Controls (N=11)
IgG mg/dl	1531±722	1591±298	1475±42	1445±44	1533±77	1377±35**	1695±54	1401±59**	1658±55	1538±55	1579±03	1496±96
IgA mg/dl	182±118	164±14	235±12	213±11	251±15	234±11	305±13	241+15**	271±10	264±14	283±26	290±14
IgM mg/dl	170±86	133±28	170±08	164±10	143±10	138±07	147±08	163±14	158±10	149±09	149±20	135±16

*Results expressed as the Mean ± Standard Error of the Mean.
**Statistically significant ($P < 0.05$) using Students t-test comparing the mean values of grain workers and controls in each age group.

TABLE 51

THE LEVELS OF IMMUNOGLOBULINS (G,A,M)* BY LENGTH OF EMPLOYMENT IN AGE GROUPED GRAIN WORKERS AND CONTROLS

	5.5 Years		5.6-10.5 Years		10.6-15.5 Years		15.6-20.5 Years	
	Grain Workers (N=92)	Controls (N=76)	Grain Workers (N=77)	Controls (N=49)	Grain Workers (N=32)	Controls (N=37)	Grain Workers (N=45)	Controls (N=36)
IgG mg/dl	1535±49	1428±40	1537±51	1419±49	1780±103	1289±52**	1559±61	1512±59
IgA mg/dl	248±12	214±10**	257±14	248±14	298± 17	244±14**	274±15	237±17
IgM mg/dl	171± 9	157±10	157±10	154± 9	154± 11	155±12	156±10	156±11

	20.6-25.5 Years		25.6-30.5 Years		30.6-35.5 Years		> 35.6 Years	
	Grain Workers (N=22)	Controls (N=17)	Grain Workers (N=30)	Controls (N=9)	Grain Workers (N=9)	Controls (N=7)	Grain Workers (N=2)	Controls (N=3)
IgG mg/dl	1578±81	1459±70	1748±91	1654±190	1546±158	1690±92	1673±242	1421±134
IgA mg/dl	279±23	298±22	295±20	228± 41	250± 27	264±27	284± 17	268± 90
IgM mg/dl	135±14	121± 1	136±11	132± 22	123± 23	135±14	198± 34	108± 34

*Results expressed as Mean ± Standard Error of the Mean.
**Statistically different ($P < 0.05$) using Students t-test of comparisons between mean levels of grain workers and controls in each age group or length of employment group.

TABLE 52

IMMUNOGLOBULIN (G,A,M)* LEVELS GROUPED BY PLACE OF EMPLOYMENT

	Grain Workers (N=307)	Elevator 1 (N=35)	Elevator 2 (N=21)	Elevator 3 (N=12)	Elevator 4 (N=49)	Elevator 5 (N=17)	Elevator 6 (N=16)	Elevator 7 (N=39)	Elevator 8 (N=24)
IgG mg/dl	1587±27	1737±95**	1612±101	1288±05	1560±56	1566±157	1556±30	1632±94	1714±122**
IgA mg/dl	266±06	266+16	273±17	301±04	264±16	278±39	286±30	264±21	281±25
IgM mg/dl	156±04	147±13	186±20	162±24	158±09	143±15	133±15	160±80	153±22

*Results expressed as mean ± standard error of the mean.
**Statistically significant ($P < 0.05$) of the difference between mean level of the grain workers and the mean level for each elevator company.

TABLE 53

ALPHA$_1$-ANTITRYPSIN (AAT) LEVELS* IN SMOKERS, EX-SMOKERS AND NONSMOKERS

	Smokers		Ex-smokers		Nonsmokers		All	
	Grain Workers (N=150)	Controls (N=105)	Grain Workers (N=92)	Controls (N=67)	Grain Workers (N=65)	Controls (N=67)	Grain Workers (307)	Controls (234)
AAT mg/dl	306 ± 7**	329 ± 7	284 ± 8	302 ± 10	292 ± 10	281 ± 12	296 ± 5	308 ± 6

*Results expressed as Mean ± Standard Error of the Mean.
**Statistically significant ($P < 0.05$) using Students t-test.

TABLE 54a

LEVELS OF ALPHA$_1$-ANTITRYPSIN* IN AGE GROUPED GRAIN WORKERS AND CONTROLS

20 Years		21-30 Years		31-40 Years		41-50 Years		51-60 Years		61-65 Years	
Grain Workers (N=2)	Controls (N=2)	Grain Workers (N=97)	Controls (N=58)	Grain Workers (N=44)	Controls (N=72)	Grain Workers (N=80)	Controls (N=48)	Grain Workers (N=71)	Controls (N=44)	Grain Workers (N=12)	Controls (N=11)
253±49	345±23	270±08	302+9**	297±13	288±10	306±8**	342±17	315±10	312±12	331±32	317±16

*Results expressed as the Mean ± Standard Error of the Mean.
**Statistically significant ($P < 0.05$) using Students t-test.

TABLE 54b

LEVELS OF ALPHA$_1$-ANTITRYPSIN* IN GRAIN WORKERS AND CONTROLS GROUPED ACCORDING TO LENGTH OF EMPLOYMENT

5.5 Years		5.6-10.5 Years		10.6-15.5 Years		15.6-20.5 Years	
Grain Workers (N=92)	Controls (N=76)	Grain Workers (N=17)	Controls (N=49)	Grain Workers (N=32)	Controls (N=37)	Grain Workers (N=45)	Controls (N=36)
284±8**	306±8	288±10	288±12	298±15	304±14	305±12	314±12

20.6-25.5 Years		25.6-30.5 Years		30.6-35.5 Years		35.6 Years	
Grain Workers (N=22)	Controls (N=17)	Grain Workers (N=30)	Controls (N=9)	Grain Workers (N=9)	Controls (N=7)	Grain Workers (N=2)	Controls (N=3)
314±14	343±35	305±14	353±20	353±40	326±54	417±33	322±54

Results expressed as Mean ± Standard Error of the Mean.
Statistically significant ($P < 0.05$) using Students t-test.

TABLE 55

THE TRYPSIN INHIBITORY CAPACITY AND ALPHA$_1$-ANTITRYPSIN (AAT) PHENOTYPE OF SUBJECTS WITH INTERMEDIATE LEVELS OF AAT

Subject No.	T.I.C.*	%**	Pi Type	Age (Yrs)	Smoking
18	1.40	118.6	M		
52	.92	77.9	MS	27	Ex
98	1.30	110.0	M		
133	1.36	115.2	M		
143	1.27	107.9	MS	30	S
210	.94	79.6	MS	21	NS
232	.63	55.5	MZ	36	NS
239	.72	61.5	MZ	31	S
240	.81	68.6	MZ	28	Ex

*Trypsin inhibitory capacity expressed as mg of trypsin inhibited per ml of serum.

**Percentage of normal standard pool.

TABLE 56

IMMUNOGLOBULIN (G,A,M) AND ALPHA$_1$-ANTITRYPSIN (AAT) LEVELS IN GRAIN WORKERS WITH (+) OR WITHOUT (-) ABNORMAL PULMONARY FUNCTION

Abnormal Condition		N	IgA X	IgA ±SD	IgA P	IgG X	IgG ±SD	IgG P	IgM X	IgM ±SD	IgM P	AAT X	AAT ±SD	AAT P
FEV_1/FVC	+	51.0	286.2	96.7		1540.4	458.4		144.2	76.1		306.4	77.8	
<70%	-	25.9	262.1	113.7	NS	1596.8	469.7	NS	158.6	78.9	NS	294.4	83.7	NS
FVC	+	17.0	331.4	138.1		1815.1	540.3		151.3	76.5		331.5	83.1	
<80%	-	293.0	262.2	108.6	<.025	1574.3	460.5	<.05	156.6	78.7	NS	294.3	82.4	NS
$V_{max}50$	+	130.0	285.0	108.8		1608.8	515.7		150.4	74.9		306.0	74.9	
<1.65	-	180.0	252.3	111.4	<.025	1572.3	430.2	NS	160.6	80.9	NS	289.3	80.3	NS
MMF	+	60.0	290.2	104.1		1641.6	510.2		147.3	76.0		315.0	81.6	
<1.65	-	250.0	260.3	112.4	NS	1574.8	457.0	NS	158.4	79.1	NS	292.0	82.6	NS
$V_{max}75$	+	153.0	277.8	114.1		1621.3	520.1		156.8	84.9		303.8	84.8	
<1.65	-	157.0	254.5	107.6	NS	1554.6	408.6	NS	155.8	71.9	NS	289.1	80.3	NS
CV	+	43.0	283.3	132.7		1534.2	434.8		158.3	70.0		289.7	83.0	
<1.65	-	267.0	263.2	107.4	NS	1596.3	472.9	NS	156.0	79.9	NS	297.4	82.8	NS
ΔN2/L	+	100.0	260.7	105.0		1616.0	142.2		142.2	86.5		321.1	92.4	
<1.65	-	210.0	268.5	114.4	NS	1574.2	468.5	<.05	163.0	73.7	<.05	284.6	75.2	<.005
DL	+	22.0	333.8	120.8		1655.1	547.8		137.9	74.0		343.0	72.9	
<80%	-	288.0	260.8	109.1	<.01	1582.4	461.5	NS	157.7	78.8	NS	292.8	82.5	<.01

TABLE 57

REGRESSION COEFFICIENTS (b) T RATIOS (t) AND SIGNIFICANCE (p) IN MULTIPLE REGRESSION OF IMMUNOGLOBULIN (A,G,M) AND ALPHA$_1$-ANTITRYPSIN (AAT) LEVELS ON GRAIN DUST EXPOSURE, SMOKING HABIT, AGE AND/OR LENGTH OF EMPLOYMENT (LOE)

		Age	Smoking	Ex-Smoking	Grain	LOE	Smoking	Ex-Smoking	Grain
	(b)	1.237	- 20.8	2.2	28.63	1.735	-19.8	-5.4	28.34
IgA	(t)	2.5	- 1.86	.18	3.20	4.63	- 1.8	- .44	3.22
	(p)	.01	.05	NS	.005	.0001	.05	NS	.005
	(b)	4.55	-132.8	-46.0	157.6	3.99	-134.5	-27.5	NS
IgG	(t)	2.99	- 2.97	- .93	4.4	2.01	- 2.99	- .56	157.6
	(p)	.005	.005	.005	.0001	.025	.005	NS	.0001
	(b)	- .394	- 15.07	- 7.48	6.30	-1.150	- 15.67	- 7.79	7.07
IgM	(t)	-1.41	- 1.84	- .82	.96	-3.19	- 1.93	- .87	1.09
	(p)	.0001	.05	NS	NS	.0001	.005	.05	NS
	(b)	1.586	32.37	- 2.96	-15.83	1.578	31.94	3.21	-15.99
AAT	(t)	5.26	3.65	- .30	- 2.23	3.97	3.56	.33	-2.23
	(p)	<.0001	<.0001	<.0005	<.025	<.0001	<.0005	<.0005	<.025

SECTION 3 - TABLES

STUDY II

#210-76-0175

STUDY II

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TABLE II-1a

DISTRIBUTION OF SMOKING HABITS, HEIGHT AND WEIGHT BY AGE GROUP
SHIFT STUDY-GRAIN WORKERS

Age (Years)	Smokers (no.)	(%)	Height (cm)	Weight (kg)	Ex-smokers (no.)	(%)	Height (cm)	Weight (kg)	Nonsmokers (no.)	(%)	Height (cm)	Weight (kg)	All Groups (no.)	Height (cm)	Weight (kg)
20-29	36	50	178.5	78.8	16	22	178.2	81.6	20	28	179	82	72	178.5	80.4
30-39	27	63	178.9	84.3	9	21	177.3	81.5	7	16	177	82	43	178.3	83.3
40-49	33	51	175.0	82.4	17	26	176.6	87.7	15	23	175	90	65	175.5	85.5
50-64	22	32	174.6	77.8	34	50	176.8	87.9	12	18	173	81	68	175.4	83.4
20-64	118	48	176.9	80.9	76	31	177.1	85.8	54	22	176.3	84	248	176.8	83.1

TABLE II-1b

DISTRIBUTION OF SMOKING HABITS, HEIGHT AND WEIGHT BY AGE GROUP
SHIFT STUDY-CONTROLS

Age (Years)	Smokers (no.)	(%)	Height (cm)	Weight (kg)	Ex-smokers (no.)	(%)	Height (cm)	Weight (kg)	Nonsmokers (no.)	(%)	Height (cm)	Weight (kg)	All Groups (no.)	Height (cm)	Weight (kg)
20-29	22	52	176.9	81.3	6	14	178.4	94.1	14	33	179.6	84.3	42	178.0	82.7
30-39	26	43	176.4	83.3	17	28	176.5	85.3	18	30	175.2	82.7	61	176.0	83.7
40-49	25	60	175.1	84.7	11	26	179.4	95.6	6	14	177.8	94.3	42	176.6	88.0
50-64	14	30	174.6	76.8	19	40	175.3	87.7	14	30	172.6	84.1	47	174.3	83.4
20-64	87	45	175.9	82.1	53	28	176.9	88.2	52	27	166.0	84.9	192	176.2	84.5

TABLE II-2

MEAN TOTAL DUST LEVELS AND RESPIRABLE DUST LEVELS
JOB CATEGORY, COMPANY AND WEEK

	Total Dust Level			Total Dust Level				
	N	x	±1SD	Range	n ≥10 mg/m^3	% ≥10 mg/m^3	n ≥15 mg/m^3	% ≥15 mg/m^3
GRAIN WORKERS								
All Samples	209	3.29	6.95	.03-54.98	15	7.2	9	4.3
Company*								
1	36	2.81	5.39	.196-30.18	2	5.6	1	2.8
2	17	3.17	5.25	.03-20.16	2	11.8	1	5.9
3	5	9.48	16.59	.73-38.95	1	20.0	1	20.0
4	46	3.78	7.95	.14-39.12	4	8.7	3	6.5
5	17	1.72	2.18	.27- 8.29	0	0	0	0
6	18	4.95	12.52	.22-54.08	2	11.1	1	5.6
7	32	4.24	5.81	.23-32.62	3	9.4	1	3.1
8	32	1.70	3.52	.18-20.19	1	3.1	1	3.1
9†	6	.45	.30	.20- 1.03	0	0	0	0
Job Category								
01	21	1.55	1.33	.27- 5.47	0	0	0	0
02	9	11.75	12.72	.79-38.95	4	44.4	3	33.3
03	35	4.27	7.96	.18-36.08	4	11.4	2	5.7
04	14	2.98	2.89	.22-10.30	1	7.1	0	0
05	43	4.18	9.85	.196-54.08	2	4.7	2	4.7
06	56	1.24	1.24	.03- 6.72	0	0	0	0
07	25	4.10	7.13	.14-30.18	4	16.0	2	8.0
08	6	1.05	1.20	.41- 3.47	0	0	0	0
02,03,04	58	5.12	8.42	.18-38.95	9	15.5	5	8.6
Week								
10/10	37	4.38	8.76	.14-39.12	4	10.8	3	8.1
10/17	22	2.73	4.65	.03-20.16	2	9.0	1	4.5
10/24	41	1.64	3.27	.18-20.19	1	2.4	1	2.4
10/31	24	4.87	10.87	.23-54.08	3	12.5	1	4.1
11/7	20	4.63	7.03	.43-32.62	2	10.0	1	5.0
11/14	43	2.60	4.97	.20-30.18	2	4.6	1	2.3
11/27	22	3.48	8.19	.27-38.95	1	4.5	1	4.5
CONTROLS								
All Samples	65	.60	.56	.09- 2.56	0	0	0	0

*Company where elevator operator or state inspector worked the day tested.
†Other - not specified on dust level report from NIOSH.

TABLE II-3

INCIDENCE OF SYMPTOMS DURING SHIFT STUDY
IN GRAIN WORKERS AND CONTROLS

	Grain Workers (248)		Control (192)		
	#	%	#	%	P
Cough	119	48	61	32	<.001
Expectoration	93	38	36	19	<.001
Wheezing*	30	12	17	9	<.001
Dyspnea	29	12	10	5	<.050
Fever	13	5	6	3	N.S.
Eye Sx†	29	12	10	5	<.050
Stuffy Nose	91	37	48	25	<.010
Throat Sx**	15	6	13	7	N.S.
One or More Sx	163	66	81	42	<.001

*Wheezing and/or chest tightness
†Eyes burning, watering or itching
**Throat sore or burning

TABLE II-4

INCIDENCE OF SYMPTOMS DURING WORK SHIFT IN GRAIN HANDLERS BY SMOKING CATEGORIES AND SUBJECTIVE APPRAISAL OF DUST EXPOSURE

Symptom	All (248)		Smoker (118)		Exsmoker (76)		Nonsmoker (54)		Subjective Appraisal of Dust Exposure[1]							
									Average (76)		Less Than Average (158)		More Than Average (14)		Exposure Heavy Yes (48)		No (200)	
	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%
Cough	119	47.9	66	55.9	28	36.8	25	46.2	44	57.8†	64	40.5	11	78.5	30	62.5**	89	44.5
Expectoration	93	37.5	51	43.2	26	34.2	16	29.6	32	42.1	54	34.1	7	50.0	30	62.5**	63	31.5
Wheezing and/or Chest Tightness	30	12.0	17	14.4	11	14.4	2•	3.7	10	13.1	15	9.4	5	35.7*	14	29.1**	16	8.0
SOB	29	11.6	19	16.1	6	7.8	4	7.4	8	10.5	16	10.1	5	35.7*	15	31.2**	14	7.0
Fever	13	5.2	5	4.2	5	6.5	3	5.5	8	10.5†	4	2.5	1	7.1	4	8.3	9	4.5
Eye Symptoms	29	11.6	14	11.8	7	9.2	8•	14.8	9	11.8	13	8.2	7	50.0*	14	29.1**	15	7.5
Stuffy Nose	91	36.6	42	35.5	23	30.2	26	48.1	27	35.5	55	34.8	9	64.2*	23	47.9	68	34.0
Throat Symptoms	15	6.0	6	5.0	1	1.3	8	14.8	7	9.2	8	5.0	0	---	4	8.3	11	5.5
One or More Symptoms	163	65.7																

*Significantly different incidence between average and more than average $P < .05$.
†Significantly different prevalence between average and less than average $P < .05$.
**P value < .05 - Significant difference between "yes" and "no."
•P < .05 - Significant difference between nonsmokers and smokers.

Total dust level $\bar{x} \pm 1$ SD = 14 ± 12 for "more than average"
4 ± 8.6 for "average"
1.8 ± 4 for "less than average"

[1]Obtained by post shift answers to "In your opinion, the amount of dust you were exposed to today was a) average, b) less than average, c) more than average." "Were you exposed to heavy dust at any time today?" 1) Yes 2) No

TABLE II-5

SHIFT STUDY

INCIDENCE OF RESPIRATORY SYMPTOMS* BY JOB CATEGORY
AND PLACE OF EMPLOYMENT

Job		COMPANY 1	2	3	4	5	6	7	8	9-10	Total with Symptoms		% Studied of Study
	N	N=34	N=21	N=6	N=44	N=17	N=14	N=31	N=19	N=62	N	%	
01	19	5	0	0	2	1	1	1	1	0	11	58	95
02-03-04	73	3	4	1	18	4	4	7	2	0	43	59	81
05	37	6	3	1	4	3	1	3	3	0	24	65	90
06	83	3	1	1	5	0	0	1	2	36	49	56	94
07	25	2	1	0	4	4	1	4	1	0	17	68	81
08	5	1	0	0	0	0	0	0	0	0	1	20	
Total in Shift Study	248	20	9	3	33	11	7	16	9	36	145	58	
% with Symptoms		59	43	50	75	71	50	52	47	58			
% Studied of Study I		97	95	50	90	100	82	79	79	91			

* Cough on expectoration or wheezing or dyspnea during the shift.
** % of the workers studied in Study I that participated in Study II.

TABLE II - 6

LEUKOCYTE COUNT AND DIFFERENTIAL COUNTS
BEFORE AND AFTER A WORK SHIFT
IN GRAIN WORKERS (G) AND CONTROLS (C)

		Pre-Shift		Post-Shift		Difference Pre-Post Shift	
		$\bar{x}$	±SD	$\bar{x}$	±SD	$\bar{x}$	±SD
Total number per mm^3	G	6.9	1.7	7.8	1.5	.91	1.2
	C	6.8	1.7	7.8	1.8	.99	1.15
Neutrophils Segmented % Total	G	53.5	9.9	59.1	8.1	6.4	9.9
	G	55.9	9.2	58.5	9.8	2.6	9.2
Neutrophils Bands % Total	G	.75	1.13	.37	.98	- .42	1.44
	C	1.43	1.74	.39	.90	-1.06	1.87
Eosinophils % Total	G	2.9	2.4	2.4	2.2	- .62	2.6
	C	2.9	2.1	1.8	1.9	-1.06	2.5
Basophils % Total	G	.49	.73	.02	.16	- .50	.74
	C	.43	.67	.01	.07	- .41	.67
Lymphocytes % Total	G	41.5	9.7	37.6	8.3	- 4.5	9.7
	C	38.7	8.8	39.2	9.8	.46	9.2
Monocytes % Total	G	.75	1.10	.21	.88	- .55	1.32
	C	.67	.91	.13	.42	- .54	1.05

The range for leukocyte counts from grain workers was: pre-shift 4.2-14.7 and post-shift 4.7-14.1. The range of leukocyte counts from control city workers was: pre-shift 3.9-12.2 and post-shift 3.2-14.5.

TABLE II - 7

COMPLEMENT LEVELS ON GRAIN WORKERS AND CONTROLS

	Grain Workers (N=248)		Controls (N=191)	
	Pre-shift	Post-shift	Pre-shift	Post-shift
Total C3* (B1A/B1C) mg/%	106 ± 29	104 ± 33	101 ± 28	98 ± 23
Range mg/%	66 - 266	58 - 294	54 - 256	51 - 167
Activation Classical Pathway	0	0	0	0
Activation Alternate Pathway	0	0	0	0

*Results expressed as the Mean ± 1 SD.

TABLE II - 8

BODY TEMPERATURE DURING DAY OF SHIFT STUDY

	800 Hrs	1200 Hrs	1600 Hrs	2000 Hrs
Grain Workers				
$\bar{x} \pm 1$ SD	(245) 97.7 ± .9	(244) 98.3 ± 1.1	(245) 98.2 ± .9	(212) 98.3 ± .8
Range	96.0 - 99.8	96.0 - 104.2	96.0 - 100.0	96.0 - 102.2
Controls				
$\bar{x} \pm 1$ SD	(191) 97.9 ± .9	(167) 98.6 ± .8	(191) 98.5 ± .8	(167) 98.6 ± .7
Range	96.0 - 100.4	96.2 - 100.2	96.4 - 100.6	96.8 - 100.0

TABLE II - 9

PULMONARY FUNCTION BEFORE AND AFTER WORK SHIFT
IN GRAIN WORKERS (G) n=241 AND CONTROLS (C) n=191

		Pre			Post		Pre-Post Difference			Pre-Post % Difference		
		x ± SD		P	x ± SD		x ± SD		P*	x ± SD		P*
FEV_1 ml	G	3474	828	NS	3466	868	-8.0	271	NS	-.25	9.31	NS
	C	3874	746	<.05	3911	718	36.3	236		1.41	8.25	
FVC ml	G	4725	917	<.02	4679	948	-46.3	280	NS	-.95	6.45	<.05
	C	4830	776	NS	4827	730	-2.8	265		.25	5.80	
$Vmax^{50}$ L/sec	G	3.70	1.47	NS	3.65	1.52	-.06	.65	<.05	-1.05	17.7	<.01
	C	4.54	1.60	NS	4.60	1.55	.06	.40		3.62	17.5	
$Vmax^{75}$ L/sec	G	1.34	.66	NS	1.32	.65	-.03	.31	<.001	.15	23.4	<.001
	C	1.66	.72	<.001	1.74	.72	.08	.24		8.0	22.5	

P* Significance of pre-post differences in grain workers versus city workers.
P Significance of pre versus post values in grain workers and in controls by paired t tests.

TABLE II - 10

PULMONARY FUNCTION CHANGES DURING WORK SHIFT IN GRAIN WORKERS (G) AND CONTROL WORKERS (C) BY SMOKING CATEGORY

		Smoker				Ex-smoker				Nonsmoker			
		Pre mean ±1SD	Post mean ±1SD	Diff mean ±1SD	P^1	Pre mean ±1SD	Post mean ±1SD	Diff mean ±1SD	P^2	Pre mean ±1SD	Post mean ±1SD	Diff mean ±1SD	P^3
FEV_1 ml	G	3473 ±859	3481 ±865	-.92 ±280		3395 ±819	3352 ±897	-43.4 ±253		3576 ±773	3601 ±815	31.3 ±271	
	C	3795 ±846	3835 ±809	40.1 ±289		3837 ±601	3870 ±592	32.7 ±206		4040 ±678	4003 ±860	33.4 ±153	
FVC ml	G	4732 ±943	4695 ±978	-48.1 301		4623 ±883	4582 ±949	-41.5 ±278		4835 ±920	4771 ±879	-47.6 ±234	
	C	4804 ±813	4807 ±801	3.5 ±169		4782 ±693	4783 ±603	.74 ±396		4919 ±790	4812 ±991	-17.1 ±235	
FEV_1/ FVC	G	73.2 ±9.7	73.8 ±9.4			73.2 ±9.9	72.6 ±10.5			74.2 ±9.3	75.3 ±8.7		
	C	78.6 ±9.7	79.5 ±9.0			80.6 ±8.2	80.9 ±6.2			82.2 ±5.8	81.6 ±12.7		
$Vmax^{50}$ L/sec	G	3.61 ±1.51	3.59 ±1.53	-.03 ±.66		3.60 ±1.42	3.54 ±1.54	-.05 ±.56		4.05 ±1.41	3.99 ±1.51	-.08 ±.79	
	C	4.46 ±1.64	4.43 ±1.62	-.03 ±.44	<.02	4.43 ±1.77	4.62 ±1.71	.19 ±.59		4.77 ±1.32	4.77 ±1.35	.09 ±.48	
$Vmax^{75}$ L/sec	G	1.31 ±.63	1.26 ±.57	-.06 ±.28		1.26 ±.59	1.25 ±.66	-.01 ±.32		1.54 ±.77	1.55 ±.73	.02 ±.35	
	C	1.65 ±.78	1.67 ±.74	.02 ±.21	<.01	1.55 ±.68	1.68 ±.71	.13 ±.22		1.78 ±.64	1.88 ±.71	.13 ±.29	<.02

By unpaired t-test: P^1 smokers vs. ex-smokers; P^2 ex-smokers vs. nonsmokers; P^3 smokers vs. nonsmokers. Blanks: no significance

TABLE II - 11

MULTIPLE REGRESSION ANALYSIS USING PRE-POST-SHIFT PERCENT DIFFERENCE IN LUNG FUNCTION AS THE DEPENDENT VARIABLE AND GRAIN HANDLING, AGE HEIGHT, SMOKING AND EX-SMOKING AS INDEPENDENT VARIABLES

		Independent Variables				
		Grain Handling	Age	Height	Smoking	Previous Smoking
FEV_1	b	- .0162	- .00038	.0004	.0017	- .0124
	t	-1.88	-1.01	+.24	+ .16	-1.03
FVC	b	- .0118	- .00041	-.0004	- .0005	+ .0035
	t	-1.96*	-1.55	-.38	- .07	+ .41
$V_{MAX}50$	b	- .0486	-.00007	.00009	- .0104	+ .0142
	t	-2.84*	- .10	.29	- .49	.61
$V_{MAX}75$	b	- .0801	.0016	.0023	- .0653	- .0186
	t	-3.62*	-1.67	.56	-2.36*	- .01

b = regression coefficient
*P < .05 (two tail)

TABLE II - 12a

NUMBER OF WORKERS WITH PRE-POST SHIFT REDUCTIONS
IN FUNCTION OF VARYING DEGREES

		Grain Workers (241)	City Workers (191)
FEV_1	≥ 10%	26	0
	≥ 15%	14	1
	≥ 20%	4	0
	≥ 30%	2	0
FVC	≥ 20%	2	1
$V_{MAX}50$	≥ 25%	13	1
	≥ 35%	5	1
	≥ 50%	3	0
$V_{MAX}75$	≥ 25%	23	4
	≥ 35%	13	0
	≥ 50%	3	0

TABLE II 12b

CHARACTERISTICS OF THE 14 SUBJECTS WITH PRE-POST SHIFT FEV_1 % DIFFERENCE > 15%

					History				Sx During Shift			Exposed to on Shift	FEV_1/FVC Pre %	FEV_1 Pre	FEV_1 Post	FEV_1 %Δ	Dust Level Resp. Pre	Dust Level Resp. Post	Dust Level Total Pre	Dust Level Total Post	Skin Test CAA	Skin Test Grain Dust
No.	Age	LOE	Smoking	Pkg/Yr	Job Code	Chronic Bronchitis	Wheezing on Exp.	Cough on Exp.	Wheeze	Cough	SOB											
30	43	11	EX	25	03	0	X	X	X	X	0	Sunflower	91	3.55	2.95	-17	NA	NA	48	64	5.9	NA	NA
16	48	8	S	48	07	+	X	X	X	0	0	Wheat	55	2.45	2.05	-16	NA	NA	120	94	.8	--	--
28	51	25	EX	82	01	+	X	X	X	X	X	Wheat, Rye Barley	46	1.65	1.05	-34	6.2	6.5	88	80	1.4	--	--
45	59	7	EX	25	04	0	X	X	X	0	X	Wheat	55	2.52	1.70	-32	6.7	8.6	114	112	1.0	--	--
45	48	22	S	2	03	+	X	X	0	X	0	Wheat & Barley	60	2.15	1.80	-16	6.7	7.4	150	134	1.5	--	--
56	35	13	EX	18	04	Asthma	X	X	X	X	0	Sunflower	54	2.50	2.10	-16	5.8	8.6	70	70	2.1	+	+
70	39	14	S	28	05	0	0	X	0	X	0	Not Specified	63	3.35	2.65	-21	12.0	12.0	120	128	1.0	-	+
90	44	18	M	0	04	0	0	0	0	0	0	Sunflower	90	3.18	2.63	-17	5.9	4.4	98	98	1.6	--	--
15	43	2	EX	8	05	0	0	0	0	0	0	Wheat	89	3.09	2.53	-18	8.1	8.6	114	294	3.3	-	-
36	51	5	EX	41	02	+	X	X	0	0	0	Wheat & Barley	68	2.26	1.87	-17	5.2	5.9	90	94	2.9	-	+
21	49	29	M	0	06	0	0	0	0	0	0	Wheat & Sunflower	63	2.67	2.19	-18	5.4	5.8	87	91	NA	-	-
92	58	29	EX	31	03	0	0	0	0	0	0	Wheat & Sunflower	62	3.00	2.30	-23	6.2	7.4	95	90	9.3	-	+
100	51	20	S	30	03	0	X	X	0	X	0	Barley	64	2.10	1.70	-19	NA	NA	142	111	1.8	+	+
69	39	16	S	39	03	0	X	X	0	X	X	Not Specified	83	3.75	3.20	-15	8.5	10.0	70	74	.5	+	+

TABLE II - 13

CHANGE IN PULMONARY FUNCTION DURING WORK SHIFT IN GRAIN WORKERS WITH AND WITHOUT RESPIRATORY SYMPTOMS DURING WORK

	Respiratory Symptoms** Yes (N=122)	No (N=87)	P*
$FEV_{1.0}$ % Δ Pre-Post-Shift	- .5	.08	NS
FVC % Δ Pre-Post-Shift	-1.4	-.4	NS
$\dot{V}_{MAX}50$ % Δ Pre-Post-Shift	-1.6	4.9	NS
$\dot{V}_{MAX}75$ % Δ Pre-Post-Shift	- .67	6.0	NS

*By t-test.
**Cough and/or expectoration and/or wheezing and/or dyspnea.

TABLE II - 14

RELATIONSHIP OF TOTAL DUST LEVELS TO SYMPTOMS
DURING WORK AND SUBJECTIVE ESTIMATION OF DUST EXPOSURE

During Shift Symptom		N	Total Dust Level (mg/m^3) $\bar{X} \pm 1SD$		P
Respiratory Symptoms:					
	Yes	122	4.11	8.16	<.05
	No	87	2.14	4.54	
Fever:	Yes	9	2.30	3.10	NS
	No	200	3.33	7.07	
Eye or Nasal Symptom:					
	Yes	82	3.72	7.45	NS
	No	127	3.01	6.62	
Subjective Estimation of Dust Exposure:					
Less than Average		134	1.84	3.92	<.01
Average		62	4.21	8.62	<.001
More than Average		13	13.87	11.78	
Heavy at Any Time That Day:					
	Yes	42	10.08	12.92	<.001
	No	167	1.58	2.15	

P = significance by unpaired t-test.

TABLE II - 15a

PROPORTION OF WORKERS AND CONTROLS WITH RESPIRATORY SYMPTOMS BY TOTAL DUST LEVEL EXPOSURE CATEGORY

Dust Levels Range mg/m^3	A Cough (98) N	A Cough (98) %	B Expectoration (81) N	B Expectoration (81) %	C Wheezing (27) N	C Wheezing (27) %	D Dyspnea (25) N	D Dyspnea (25) %	A-D One or More (122) N	A-D One or More (122) %	E Fever (9) N	E Fever (9) %	F Eye (22) N	F Eye (22) %	G Nose (76) N	G Nose (76) %	H Throat (10) N	H Throat (10) %	A-H One or More (137) N	A-H One or More (137) %
Grain Workers (209)																				
0-5** (179)	80	45	63	35	22	12	17	9	100	56	8	4	10	6	64	36	7	4	104	58
P	NS		<.1		NS		NS		NS		NS		NS		<.05		NS		NS	
5-10 (15)	6	40	8	53	1	7	1	7	10	67	0	-	5	33	5	33	1	7	11	73
P	NS		<.05		NS		NS		NS		NS		<.05		NS		NS		NS	
10-15 (6)	4	67	4	67	2	33	3	50	4	67	1	17	3	50	3	50	2	23	4	66
	NS		<.05		NS		<.005		NS		<.01		<.005		NS		<.05		NS	
>15 (9)	8	89	6	67	2	22	4	44	8	89	0	-	4	44	4	44	0	-	8	89
	<.01		<.01		NS		<.005		<.05		NS		<.005		NS		NS		<.05	
Controls (63)																				
0-5*	25	40	15	24	7	11	5	8	29	46	1	2	6	10	14	22	5	8	32	51

*60 out of 63 had dust levels from 0 to 2 mg/m^3
**141 out of 179 had dust levels from 0-2 mg/m^3
P = significance of the difference between grain workers to controls

TABLE II - 15b

PROPORTION OF WORKERS AND CONTROLS WITH RESPIRATORY SYMPTOMS BY TOTAL DUST LEVEL EXPOSURE CATEGORY

Dust Levels Range mg/m^3	A Cough (98) N	%	B Expectoration (81) N	%	C Wheezing (27) N	%	D Dyspnea (25) N	%	A-D One or More (122) N	%	E Fever (9) N	%	F Eye (22) N	%	G Nose (76) N	%	H Throat (10) N	%	A-H One or More (137) N	%
Grain Workers (209)																				
0-5** (179)	80	45	63	55	22	12	17	9	100	56	8	4	10	6	64	36	7	4	104	58
P	<.05		<.001		NS		NS		<.001		NS		NS		<.05		NS		<.005	
5-10 (15)	6	40	8	53	1	7	1	7	10	67	0	-	5	33	5	33	1	7	11	73
P	<.1		<.005		NS		NS		<.05		NS		<.001		NS		NS		<.05	
10-15 (6)	4	67	4	67	2	33	3	50	4	67	1	17	3	50	3	50	2	23	4	66
	<.1		<.005		<.05		<.001		NS		<.1		<.001		NS		<.05		NS	
>15 (9)	8	89	6	67	2	22	4	44	8	89	0	-	4	44	4	44	0	-	8	89
	<.001		<.001		NS		<.001		<.005		NS		<.001		NS		NS		<.01	
Controls (192)																				
0-5*	61	32	36	19	17	9	10	5	71	37	6	3	10	5	48	25	13	7	81	42

*60 out of 63 had dust levels from 0 to 2 mg/m^3
**141 out of 179 had dust levels from 0-2 mg/m^3
P = significance of the difference between grain workers to controls

TABLE II - 16

MULTIPLE REGRESSION ANALYSIS USING PRE-POST SHIFT PERCENT DIFFERENCE IN LUNG FUNCTION AS THE DEPENDENT VARIABLE AND TIMED WEIGHTED TOTAL DUST CONCENTRATION, AGE, HEIGHT, SMOKING AND EX-SMOKING AS INDEPENDENT VARIABLES

Grain Workers		Independent Variables				
		Total dust mg/m^3	Age	Height	Ex-smoking	Smoking
FEV	b	.096	.096	- .27	-2.07	-.45
% Δ Pre/Post Shift	t	1.03	-1.73	-1.07	-1.11	-.26
FVC	b	- .153	- .063	- .155	-1.72	-2.0
% Δ Pre/Post Shift	t	2.3*	-1.59	- .86	-1.28	-1.62
$\dot{V}_{max}50$	b	- .42	- .148	- .277	.41	.82
% Δ Pre/Post Shift	t	-2.43*	-1.44	- .59	.12	.26
$V_{max}75$	b	- .537	- .037	- .618	1.44	-3.62
% Δ Pre/Post Shift	t	-2.36*	-.28	-1.0	.32	- .86

b = regression coefficient
t = t ratio
* = b .05 (using two tail analysis)

SECTION 3 - TABLES

STUDY III

#210-76-0175

STUDY III

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TABLE III - 1

DISTRIBUTION OF SMOKING HABITS, HEIGHT AND WEIGHT BY AGE GROUP (FOLLOW-UP STUDY)

Age (Years)	Smokers - 1974-1977 (no.)	(%)	(cm)	(kg)	Ex-smokers - 1974-1977 (no.)	(%)	(cm)	(kg)	Nonsmokers - 1974-1977 (no.)	(%)	(cm)	(kg)
20-29	19	56	177.4	80.6	5	15	182.0	81.4	2	6	175.3	74.9
30-39	23	68	180.2	85.8	2	6	181.9	90.7	3	6	177.0	81.6
40-49	25	48	176.3	82.8	10	19	178.0	89.6	9	17	176.1	92.1
50-64	19	37	172.7	76.0	14	27	176.9	95.7	10	19	173.0	81.5
20-64	86	50	176.8	81.6	31	18	178.4	91.1	24	14	174.8	84.9

Age (Years)	Smokers -1974 Ex-smokers -1977 (no.)	(%)	(cm)	(kg)	Ex-smokers - 1974 Smokers - 1977 (no.)	(%)	(cm)	(kg)	Nonsmokers - 1974 Ex-smokers - 1977 (no.)	(%)	(cm)	(kg)	All Groups (no.)	(cm)	(kg)
20-29	7	21	176.4	81.6	0	0	0	0	1	3	172.7	81.0	34	177.6	80.6
30-39	5	15	175.3	77.3	1	3	178.4	86.0	0	0	0	0	34	179.2	84.4
40-49	6	12	175.9	78.6	1	2	177.8	73.4	1	2	172.7	83.3	52	176.4	85.1
50-64	6	12	170.4	76.1	1	2	175.3	75.6	2	4	185.1	95.9	52	174.1	83.1
20-64	24	14	174.4	78.6	3	2	177.1	78.3	4	2	178.9	89.0	172	177.0	83.0

TABLE III-2

SYMPTOMS REPORTED IN 1977
BY THE 177 GRAIN WORKERS FOLLOWED SINCE 1974

	N	%
Cough in a.m.	66	37
Chronic Bronchitis	82	46
Cough on Exposure	119	67
Wheezing on Exposure	113	64
Dyspnea on Exposure	98	55
Grain Fever	53	30
Eye symptom on exposure	139	79
Nasal symptom on exposure	140	79

TABLE III - 4

PULMONARY FUNCTION
ALL GRAIN WORKERS (FOLLOW-UP STUDY)

	N	1974 $\bar{x} \pm 1$ SD	1977 $\bar{x} \pm 1$ SD	1974-1977 Difference $\bar{x} \pm 1$ SD	P
FEV_1 ml	177	3829 ± 846	3801 ± 860	- 29 ± 323	NS
% Predicted		98 ± 18	99 ± 18	.1 ± 8.6	NS
FVC ml	177	4903 ± 914	4838 ± 913	- 64 ± 429	<.02
% Predicted		101 ± 15	101 ± 15	- .2 ± 9.8	NS
MMF L/min	165	252 ± 81	228 ± 74	- 24 ± 39	<.001
% Predicted		87 ± 27	78 ± 23	-9.4 ± 14.4	<.001
$\dot{V}_{Max\ 50}$ L/sec	170	4.5 ± 1.6	4.0 ± 1.5	- .5 ± 1.0	<.001
% Predicted		73 ± 26	65 ± 24	-7.8 ± 16.6	<.001
$\dot{V}_{Max\ 75}$ L/sec	122	1.8 ± .6	1.6 ± .5	- .2 ± .4	<.001
% Predicted		56 ± 18	49 ± 17	-6.8 ± 12.1	<.001
$D_L$ml/CO/min /mmHg	168	32.0 ± 6.1	33.3 ± 6.2	1.3 ± 4.2	<.001
% Predicted		104 ± 19	107 ± 19	2.9 ± 14.1	<.01

TABLE III - 5

PULMONARY FUNCTION
GRAIN WORKERS - SMOKERS 1974-1977 (FOLLOW-UP STUDY)

	N	1974 $\bar{x} \pm 1$ SD	1977 $\bar{x} \pm 1$ SD	1974-1977 Difference $\bar{x} \pm 1$ SD	P
FEV_1 ml	87	3905 ± 956	3808 ± 953	33 ± 305	NS
% Predicted		95 ± 19	96 ± 18	.8 ± 8.3	NS
FVC ml	87	4887 ± 1000	4870 ± 979	- 16 ± 429	NS
% Predicted		99 ± 16	100 ± 16	.6 ± 10.3	NS
MMF L/min	80	249 ± 83	227 ± 81	- 22 ± 38	<.001
% Predicted		84 ± 27	76 ± 23	-9.1 ± 14.3	<.001
$\dot{V}_{Max\ 50}$ L/sec	84	4.3 ± 1.6	3.9 ± 1.6	- .4 ± 1.0	<.01
% Predicted		69 ± 25	63 ± 24	-6.3 ± 16.9	<.01
$\dot{V}_{Max\ 75}$ L/sec	56	1.8 ± .6	1.6 ± .3	- .2 ± .4	<.001
% Predicted		55 ± 18	49 ± 18	-5.9 ± 12.9	<.001
$D_L$ml/CO/min /mmHg	83	30.8 ± 5.4	31.7± 6.0	.9 ± 3.4	<.02
% Predicted		102 ± 16	103 ± 17	1.4 ± 11.4	NS

TABLE III - 6

PULMONARY FUNCTION
GRAIN WORKERS - SMOKERS 1974-1977 (FOLLOW-UP STUDY)

	N	1974 $\bar{x} \pm 1$ SD	1977 $\bar{x} \pm 1$ SD	1974-1977 Difference $\bar{x} \pm 1$ SD	P
FEV_1 ml	32	3878 ± 791	3793 ± 815	85 ± 316	NS
% Predicted		99 ± 19	99 ± 19	- 1.8 ± 7.9	NS
FVC ml	32	5075 ± 862	4891 ± 886	- 184 ± 342	<.005
% Predicted		105 ± 15	102 ± 14	- 3.0 ± 7.1	<.01
MMF L/min	29	247 ± 68	224 ± 68	-22.7 ± 33.1	<.001
% Predicted		86 ± 25	78 ± 25	- 9.1 ± 11.7	<.010
$\dot{V}_{Max\ 50}$ L/sec	30	4.6 ± 1.6	3.9 ± 1.4	- .8 ± 1.1	<.01
% Predicted		75 ± 27	63 ± 24	-11.5 ± 17.0	<.001
$\dot{V}_{Max\ 75}$ L/sec	22	1.8 ± .7	1.4 ± .5	- .4 ± .5	<.001
% Predicted		57 ± 22	44 ± 17	-12.6 ± 15.6	<.01
$D_L$ml/CO/min /mmHg	31	35.7 ± 7.3	36 ± 6	+ .5 ± 5.9	NS
% Predicted		110 ± 24	110 ± 19	.1 ± 20.0	NS

TABLE III - 7

PULMONARY FUNCTION
GRAIN WORKERS - SMOKERS 1974-1977 (FOLLOW-UP STUDY)

	N	1974 $\bar{x} \pm 1$ SD	1977 $\bar{x} \pm 1$ SD	1974-1977 Difference $\bar{x} \pm 1$ SD	P
FEV_1 ml	26	3784 ± 702	3784 ± 650	+ .3 ± 399	NS
% Predicted		102 ± 14	105 ± 15	1.5 ± 10.5	NS
FVC ml	26	4697 ± 883	4700 ± 805	3.6 ± 529.3	NS
% Predicted		101 ± 16	104 ± 16	2.4 ± 11.7	NS
MMF L/min	26	261 ± 94	224 ± 68	- 37 ± 43	<.001
% Predicted		94 ± 30.1	82 ± 22	-11.8 ± 14.9	<.001
$\dot{V}_{Max\ 50}$ L/sec	25	4.8 ± 1.5	4.1 ± 1.3	- .7 ± .9	<.01
% Predicted		80 ± 26	69 ± 22	-11.3 ± 16.7	<.01
$\dot{V}_{Max\ 75}$ L/sec	21	1.7 ± .6	1.5 ± .6	- .1 ± .2	<.02
% Predicted		55 ± 20	51 ± 19	- 3.9 ± 7.3	<.05
$D_L$ml/CO/min /mmHg	24	33.5 ± 5.5	36 ± 5	2.6 ± 4.2	<.01
% Predicted		109 ± 20	116 ± 20	7.2 ± 13	<.02

TABLE III - 8

PULMONARY FUNCTION
CRAIN WORKERS - SMOKERS 1974, EX-SMOKER-1977 (FOLLOW-UP STUDY)

	N	1974 $\bar{x} \pm 1$ SD	1977 $\bar{x} \pm 1$ SD	1974-1977 Difference $\bar{x} \pm 1$ SD	P
FEV_1 ml	24	3904 ± 752	3875 ± 891	-28.8 ± 313	NS
% Predicted		101 ± 15	102 ± 19	- .1 ± 9.0	NS
FVC ml	24	4974 ± 798	4920 ± 932	-54.3 ± 399	NS
% Predicted		105 ± 13	104 ± 15	- .3 ± 9.1	NS
MMF L/min	22	264 ± 77	242 ± 69	- 22 ± 45	<.05
% Predicted		91.8 ± 28	84 ± 20	- 9.8 ± 18.5	<.05
$\dot{V}_{Max\ 50}$ L/sec	23	4.5 ± 1.3	4.3 ± 1.4	- .3 ± .7	NS
% Predicted		75 ± 23	70 ± 23	- 4.4 ± 15	NS
$\dot{V}_{Max\ 75}$ L/sec	18	1.8 ± .5	1.6 ± .5	- .2 ± .3	<.01
% Predicted		58 ± 15	51 ± 14	- 6.1 ± 9.0	<.01
$D_L$ml/CO/min /mmHg	22	30.5 ± 5.8	33 ± 6	+ 2.8 ± 4.3	<.01
% Predicted		103 ± 19	112 ± 18	+ 8.5 ± 15	<.02

TABLE III - 9

YEARLY DECREMENTS IN LUNG FUNCTIONS TESTED
IN ALL GRAIN WORKERS AND BY SMOKING CATEGORIES

Yearly Decrement:	Expected*	Actual All x ± 1SD	Smoker-Smoker x ± 1SD	Ex-smoker Ex-smoker x ± 1SD	Nonsmoker Nonsmoker x ± 1SD	Smoker Ex-smoker x ± 1SD
FEV_1, L	- .029	- .029 ± .323	- .033 ± .305	- .085 ± .316	+.003 ± .399	-.028 ± .313
FVC, L	- .025	- .064 ± .429	.016 ± .429	- .184 ± .342	+ 3.6 ± .529	-.054 ± .399
MMF, L/min	-1.86	- 24 ± 39	- 22 ± 38	- 22.7 ± 33.1	- 37 ± 43	- 22 ± 45
$\dot{V}max^{50}$, L/sec	- .015	- .5 ± 1.0	- .4 ± 1.0	- .8 ± 1.1	- .7 ± .9	- .3 ± .7
$\dot{V}max^{75}$, L/sec	- .012	- .2 ± .4	- .02 ± .4	- .4 ± .5	- .1 ± .2	- .2 ± .3
D_LCO	- .166	1.3 ± 4.2	.9 ± 3.4	.5 ± 5.9	+ 2.6 ± 4.2	+ 2.8 ± 4.3

*Expected yearly mean decrement in men (>25 years old) from Knudson et al.

TABLE III - 10

PROSPECTIVE STUDY
PULMONARY FUNCTION CHANGES IN SKIN REACTORS AND NON-REACTORS
AND IN WORKERS WITH OR WITHOUT CHRONIC BRONCHITIS OR OCCUPATIONAL ASTHMA

	All Workers	Common Antigens		Airborne Grain Dust		Chronic Bronchitis		Occupational Asthma I	
	Mean (±1 SD)	Mean (±1 SD)		Mean (±1 SD)		Mean (±1 SD)		Mean (±1 SD)	
	n=177	n=26 (+)	n=139 (-)	n=49 (+)	n=116 (-)	n=63 (+)	n=114 (-)	n=108 (+)	n=69 (-)
FEV_1 % Pred	97.7	97.7	97.8	97.3	97.9	93.7	99.9	94.5	102.8
1974	±17.7	±14.6	±18.2	±12.7	±19.5	±18.8	±16.7	±19.5	±12.9
1977	98.7	96.7	98.9	96.9	99.3	95.9	100.2	95.4	103.7
	±18.1	±16.1	±18.6	±12.5	±20.1	±18.7	±17.6	±19.7	±13.8
Diff	.07	-1.42	.34	-1.22	.60	1.52	-.73	.30	-.28
	±8.6	±6.8	±8.4	±6.7	±8.8	±9.2	±8.3	±9.1	±8.0
MMF % Pred	86.9	85.6	87.4	81.7	89.5	81.9	89.4	83.4	91.9
1974	±27.2	±29.9	±27.1	±22.7	±29.1	±26.6	±27.2	±27.7	±25.8
1977	78.4	77.5	78.7	75.3	79.9	74.0	80.6	76.0	81.8
	±23.3	±23.6	±23.4	±21.7	±24.0	±24.0	±22.8	±24.2	±21.8
Diff	-9.4	-8.2	-9.8	-7.5	-10.5	-8.4	-9.9	-8.1	-11.4
	±14.4	±13.1	±14.5	±10.3	±15.7	±11.6	±15.7	±14.2	±14.6
$\dot{V}_{Max}50$ % Pred	72.8	68.9	73.5	70.1	74.0	68.8	74.9	68.8	79.1
1974	±26.0	±24.2	±26.4	±23.3	±27.2	±29.3	±24.0	±26.9	±23.4
1977	65.0	65.4	65.0	61.7	66.5	62.0	66.5	61.7	70.2
	±23.7	±23.8	±23.8	±21.9	±24.5	±25.5	±22.7	±24.7	±21.2
Diff	-7.8	-3.5	-8.6	-8.4	-7.4	-6.8	-8.4	-7.1	-8.9
	±16.6	±16.4	±16.7	±12.5	±18.3	±18.3	±15.8	±18.4	±13.5
$\dot{V}_{Max}75$% Pred	55.5	52.3	56.0	55.2	55.5	50.8	57.6	54.1	57.3
1974	±18.4	±21.1	±18.1	±15.4	±19.8	±18.4	±18.1	±18.1	±18.8
1977	48.7	49.3	48.6	48.1	49.0	45.2	50.2	47.1	50.7
	±17.0	±20.4	±16.4	±12.6	±18.5	±14.7	±17.8	±17.I	±16.9
Diff	-6.8	-3.1	-7.4	-7.2	-6.5	-5.6	-7.3	-7.0	-6.6
	±12.1	±9.7	±12.8	±12.6	±12.4	±11.4	±12.5	±13.7	±9.9

No significant changes in lung function (1974-1977) were found between positive or negative skin reactors or workers with or without chronic bronchitis or occupational asthma by unpaired t-test.

SECTION 3 - TABLES

STUDY IV

#210-75-0175

STUDY IV

TABLE OF CONTENTS

TABLE IV - 1

CHARACTERISTICS OF GRAIN HANDLERS TESTED

Subject	Age	Job Years	Cigarette pack-yrs	Smoking Status	Pulmonary Function Tests Pre-challenge Percent Predicted FEV_1	FVC	FEV_1/FVC	$V_{Max\ 50}$	$V_{Max\ 75}$	D_LCO	PC_{20}
Reactors											
1	59	7	25.5	Ex	66	106	49	23	31	105	2.5
2	51	25	32.5	Ex	50	83	48	19	29	187	2.5
3	35	13	18.0	Ex	43	71	49	16	16	122	2.5
4	48	18	28.9	S	75	111	54	33	33	124	2.5
5	28	7.5	19.5	Ex	104	114	74	72	54	103	Neg
x	44	14	35.0		68	97	55	33	33	128	
Non-reactors											
6	33	10	18.0	S	94	107	70	54	52	78	Neg
7	34	9	21.2	S	102	117	70	61	46	95	Neg
8	30	5	8.7	Ex	102	96	78	97	88	97	Neg
9	27	3	13.5	Ex	90	96	75	67	66	95	9.6
10	46	23	0	NS	94	101	75	63	59	139	16.0
11	28	8	0	NS	101	119	69	68	67	82	Neg
x	33	10	10.0		97	106	73	68	63	98	

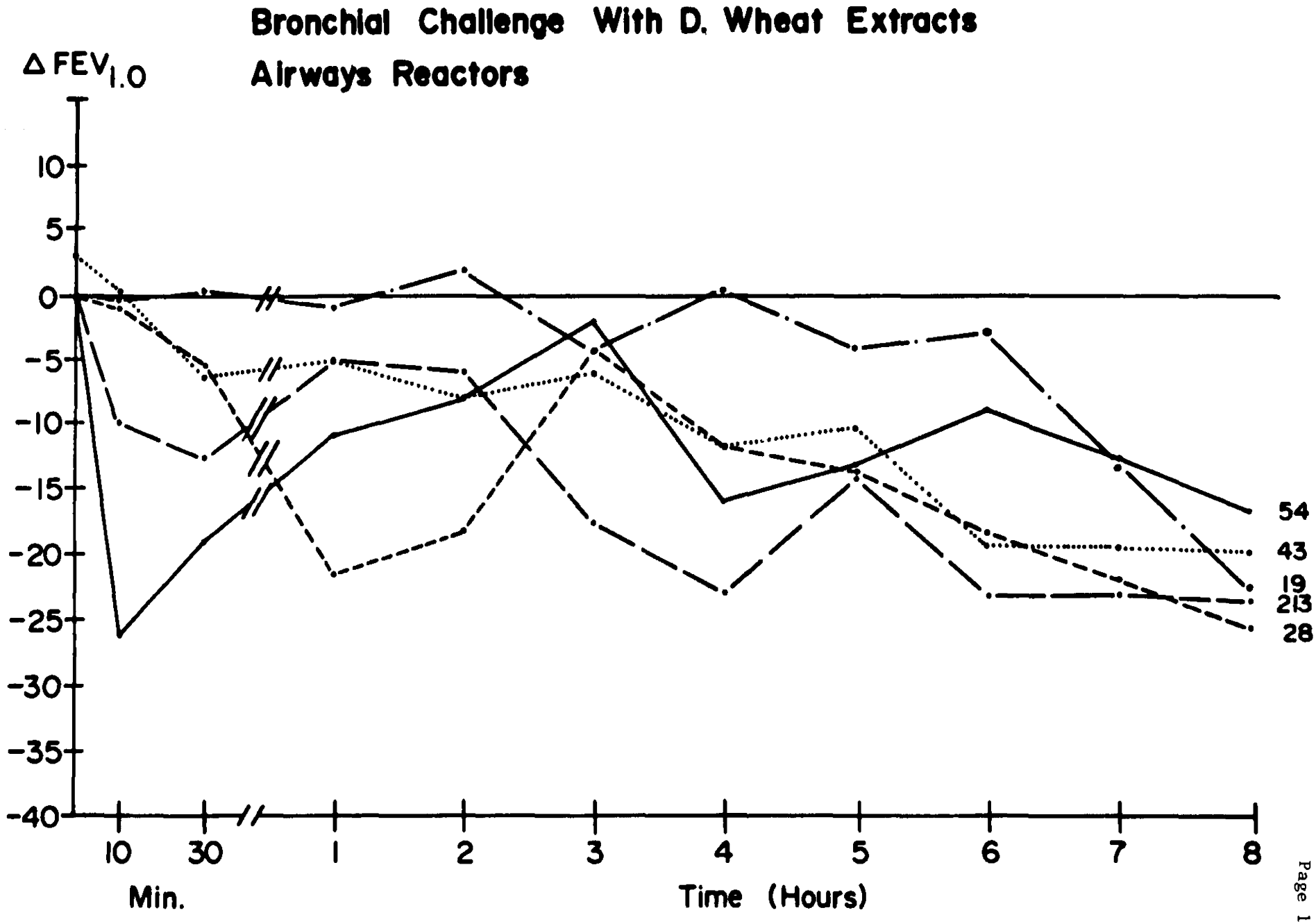
S = Smoker; Ex= Ex-smoker; NS = Nonsmoker.
PC_{20} = provocation concentration of Methacholine (mg/ml) which caused at least a 20% decrease in baseline FEV_1.

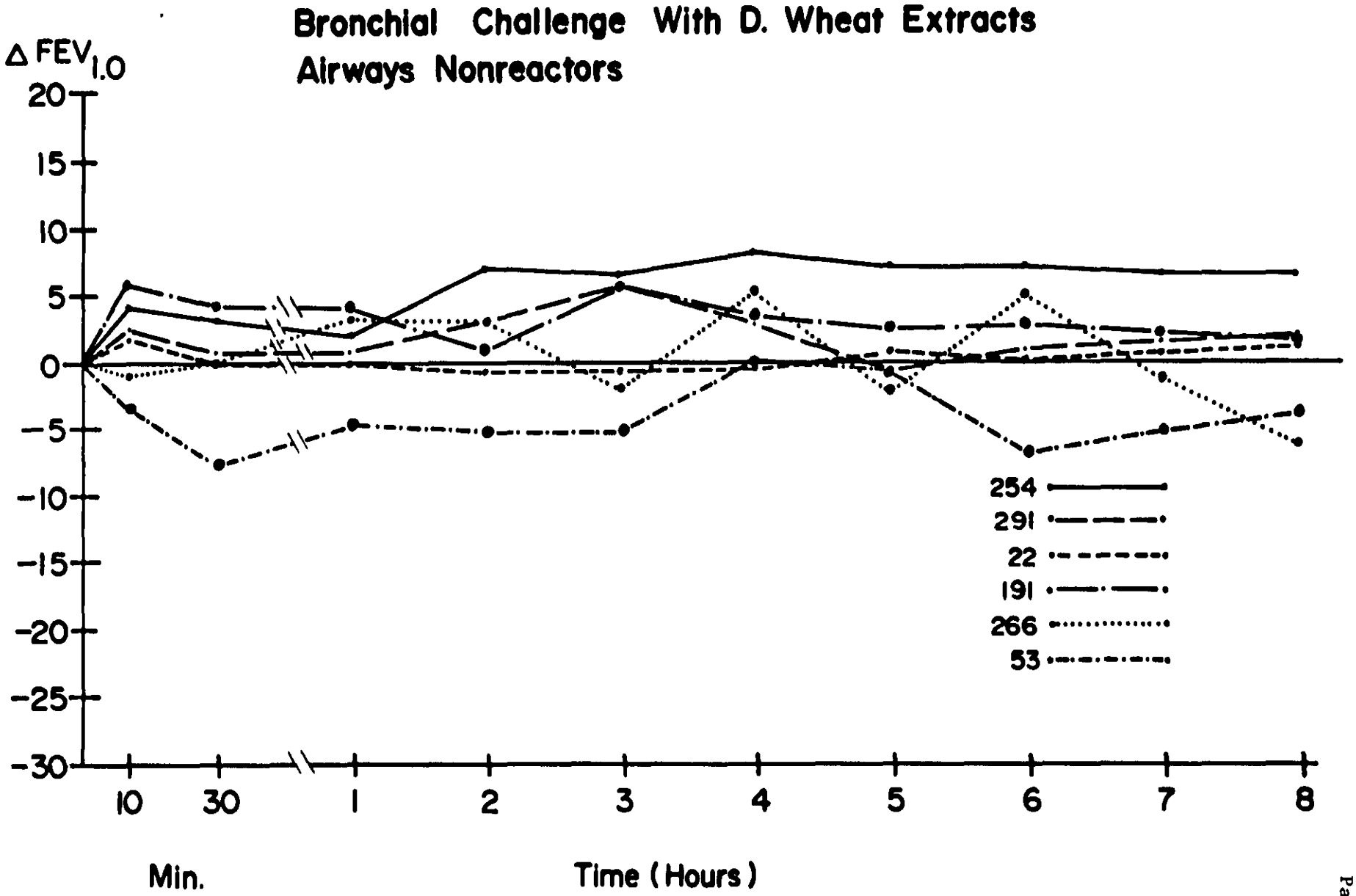
TABLE IV - 2

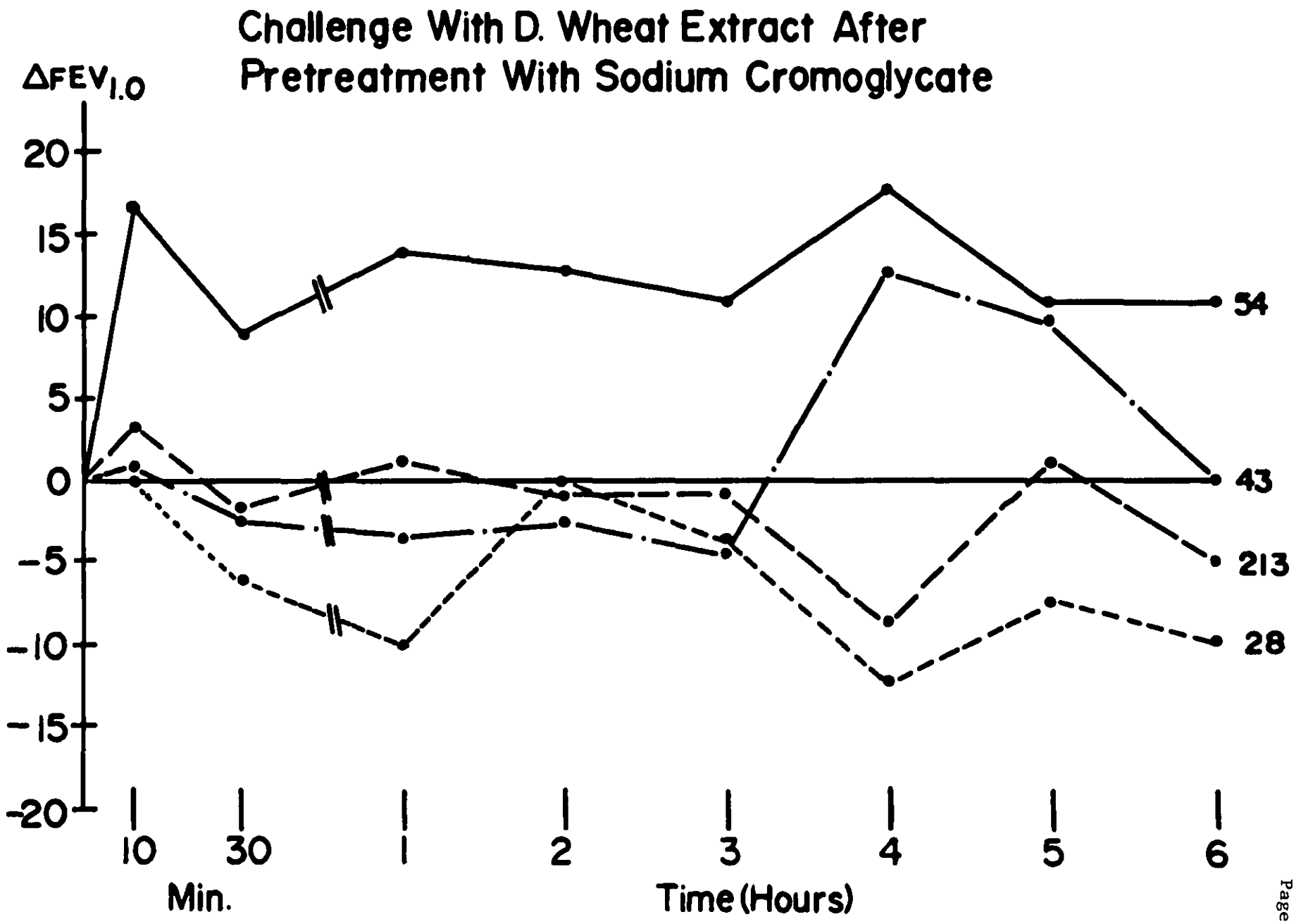
RELATIONSHIP BETWEEN SKIN REACTIVITY TO COMMON AND SPECIFIC ALLERGENS AND BRONCHIAL REACTIVITY TO SPECIFIC ALLERGENS

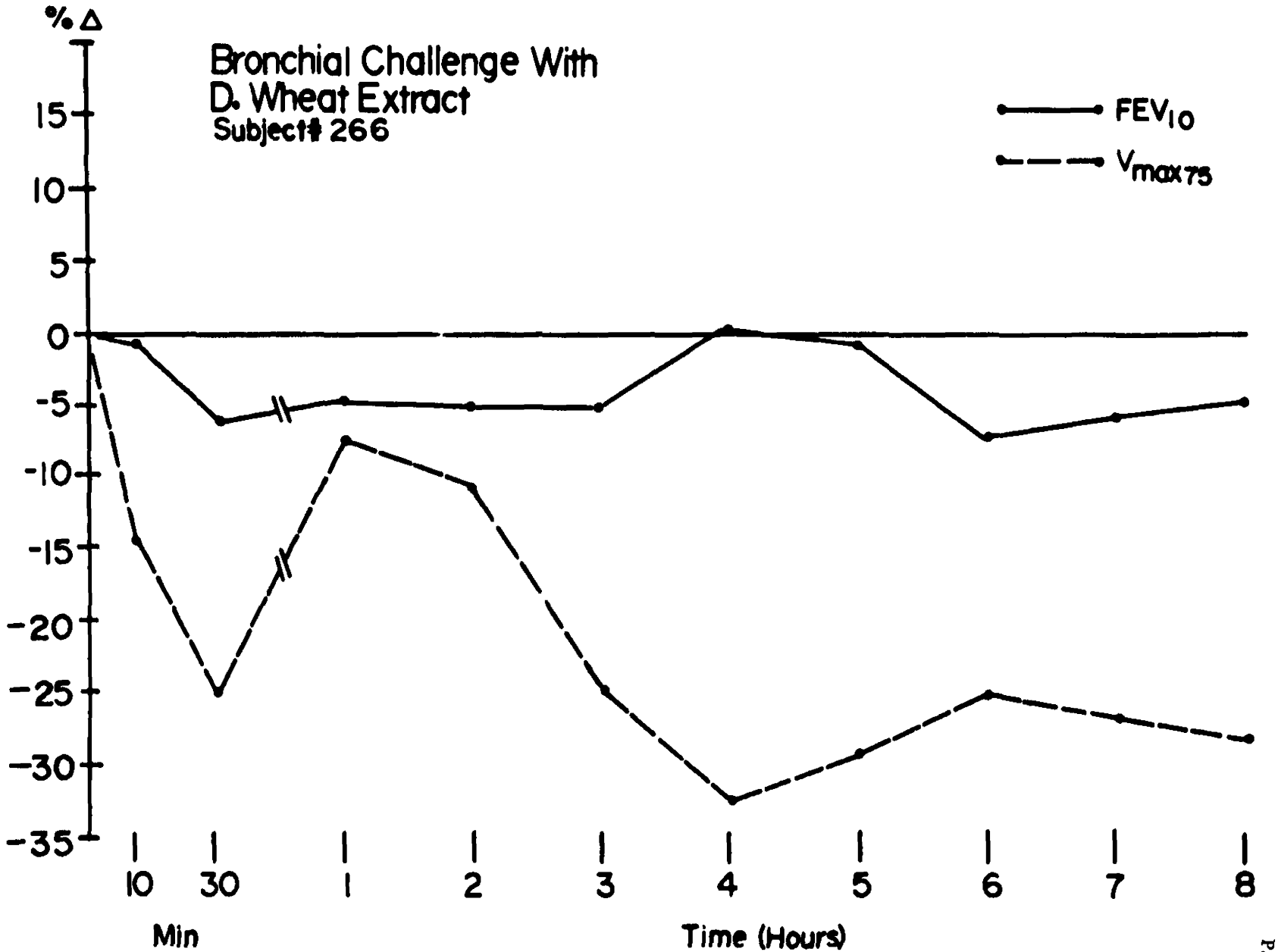
Subject	Skin Tests							Bronchial Challenge		
	Prick Test			Intradermal						
	CAA	Insect	Mite	AF	Insect or Mite	DW	ADW	Insect or Mite	DW	ADW
1	-	-	-	-	-	-	-	-	+	-
2	-	-	-	-	-	-	-	-	+	-
3	+	+	+	+	-	+	+	-	+	+
4	-	+	+	-	-	+	+	-	+	-
5	-	+	+	-	+	+	+	-	+	-
6	+	+	+	-	+	-	+	-	-	-
7	-	+	+	-	+	-	+	-	-	-
8	+	-	-	-	-	+	+	-	-	-
9	-	+	+	-	+	-	-	-	-	-
10	-	-	-	-	-	-	+	-	-	-
11	-	-	-	-	-	-	-	-	-	-

CAA - Common Allergens
AF - Aspergillus Fumigatus
DW - Durum Wheat
ADW - Airborne Durum Wheat Dust


Bronchial Challenge With D. Wheat Extracts
Airways Reactors
Δ FEV1.0
10
5
0
-5
-10
-15
-20
-25
-30
-35
-40
10
30
1
2
3
4
5
6
7
8
Min.
Time (Hours)
54
43
19
213
28


Bronchial Challenge With D. Wheat Extracts
Airways Nonreactors
Δ FEV1.0
20
15
10
5
0
-5
-10
-15
-20
-25
-30
254
291
22
191
266
53
10
30
1
2
3
4
5
6
7
8
Min.
Time (Hours)


Challenge With D. Wheat Extract After
Pretreatment With Sodium Cromoglycate
ΔFEV1.0
20
15
10
5
0
-5
-10
-15
-20
54
43
213
28
10
30
1
2
3
4
5
6
Min.
Time (Hours)


% Δ
Bronchial Challenge With
D. Wheat Extract
Subject# 266
FEV10
Vmax75
15
10
5
0
-5
-10
-15
-20
-25
-30
-35
10
30
1
2
3
4
5
6
7
8
Min
Time (Hours)

SECTION 3 - TABLES

STUDY V

#210-76-0175

STUDY V

TABLE OF CONTENTS

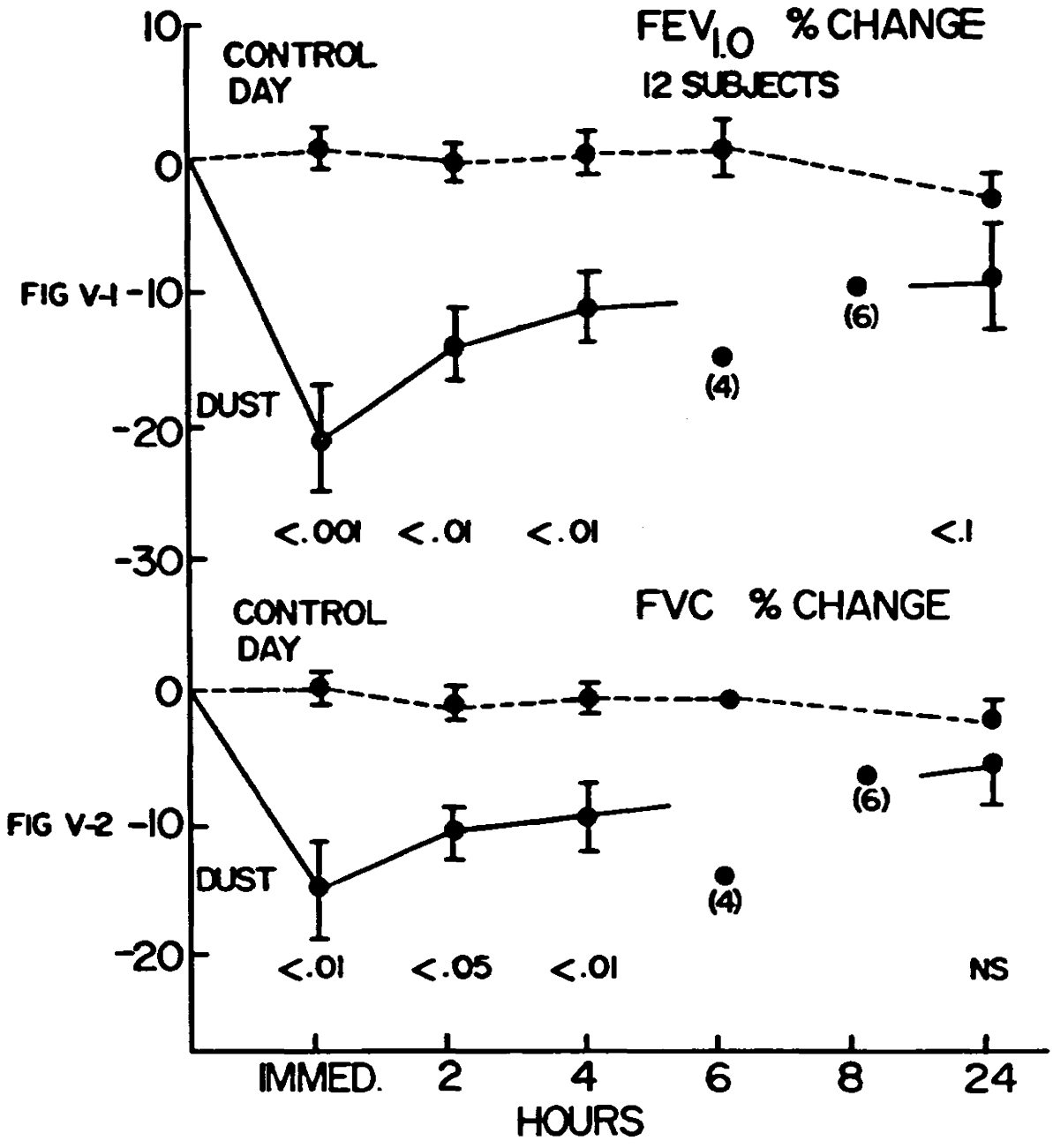
TABLE V - 1

BASELINE AND POST-CHALLENGE WITH GRAIN DUST RESULTS OF SELECTED PARAMETERS

Subj. No.	Age	Current Smoker	Skin Prick Test CAA	Skin Prick Test Grain Dust	Base FEV_1/FVC %	Respir. Mecholyl† P_c20 mg/ml	Dust Level mg/m^3	Largest Percent Change Within 24 Hours Pre-challenge Baseline $FEV_{1.0}$	FVC	MMF	$V_{Max}50$	$V_{Max}75$	DLCO	Maximum Value Post-Challenge Temp C^o	WBC Mx1000	PMNs %	C3
81	48	Yes	-	-	70	-	86.7	- 3	- 6	- 4	-75	- 6	+44	38.0*	14.3*	79	-
82	47	Yes	-	+	70	10	44	-35*	-73*	-55*	-40*	-12	- 5	37.2	11.7*	70	-
56	30	No	-	-	83	-	86.7	-12	- 3	-31	-29	-41*	+16	39.0*	24.3*	82	+
84	27	Yes	-	-	84	-	23	-27*	-16	-43*	-29	-51*	-17X	38.4*	21.0*	81	-
23	57	No	+	+	61	.75	23	-27*	-29*	-55*	-36*	-50*	-21X	36.9	8.6	66	-
32	29	No	+	+	81	-	38.6	-25*	-15	-40*	-44*	-38*	+17	38.9*	17.6*	86	-
84	29	No	+	-	85	1.56	164	-23*	-11	-36*	-37*	-35*	+ 7	36.4	8.8	77	-
83	27	No	-	-	82	25	143	-37*	- 6	-33	-37*	-35*	- 2	37.8*	24.3*	78	-
81	25	No	+	-	94	-	90.1	-27*	-13*	-58*	-54*	-53*	+14	37.2	19.0*	83	-
85	26	No	+	+	98	-	164	-11	-11	-25	-29	-21	-13	36.9	14.6*	80	-
82	27	No	+	-	84	-	143	-23*	-22*	-18	-10	- 8	- 6	38.4*	21.2*	87	-
80	33	No	-	-	88	-	90.1	-50*	-39*	-70*	-67*	-26	+15	37.4	22.0*	83	-
Total X	33.8																

Footnotes: CAA = common allergens, + = atopy = 2 or more positive reactions to ragweed, grass, tree alternaria, cat hair or feathers; Airborne grain dust extract, + = wheal 3mm; Temp = temperature; WBC = leukocyte count; PMN's = neutrophils; C3 = C3 level total serum complement. † Mecholyl test negative (-) if no FEV_1 decrease 20% with 25 mg/ml dose. X similar decrement measured on control day. *=considered significantly changed from baseline.

MEAN± ISE % CHANGE FROM BASELINE ON CONTROL DAY AND AFTER EXPOSURE TO AIRBORNE GRAIN DUST



MEAN ± 1SE % CHANGE FROM BASELINE ON CONTROL DAY AND AFTER EXPOSURE TO AIRBORNE GRAIN DUST

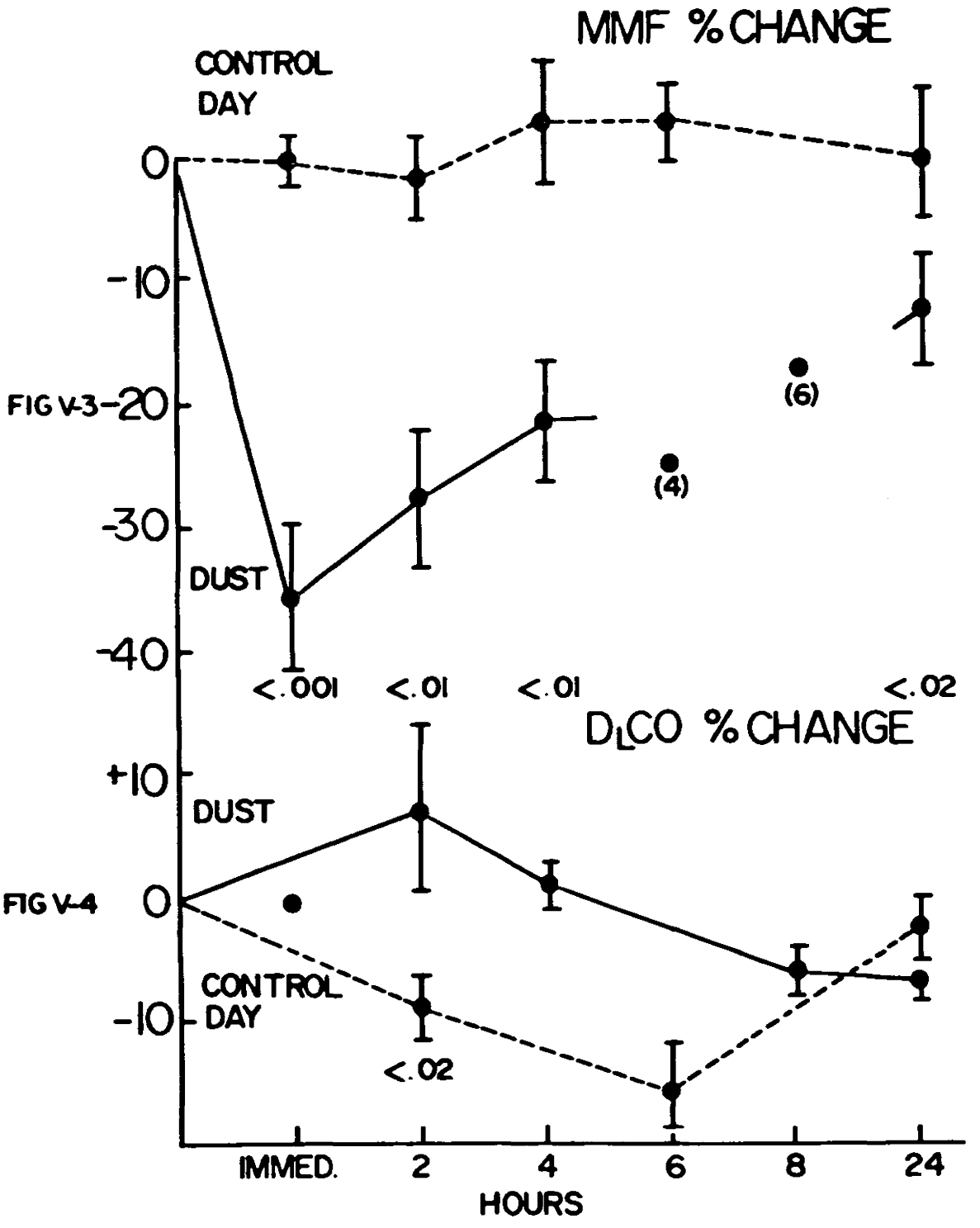
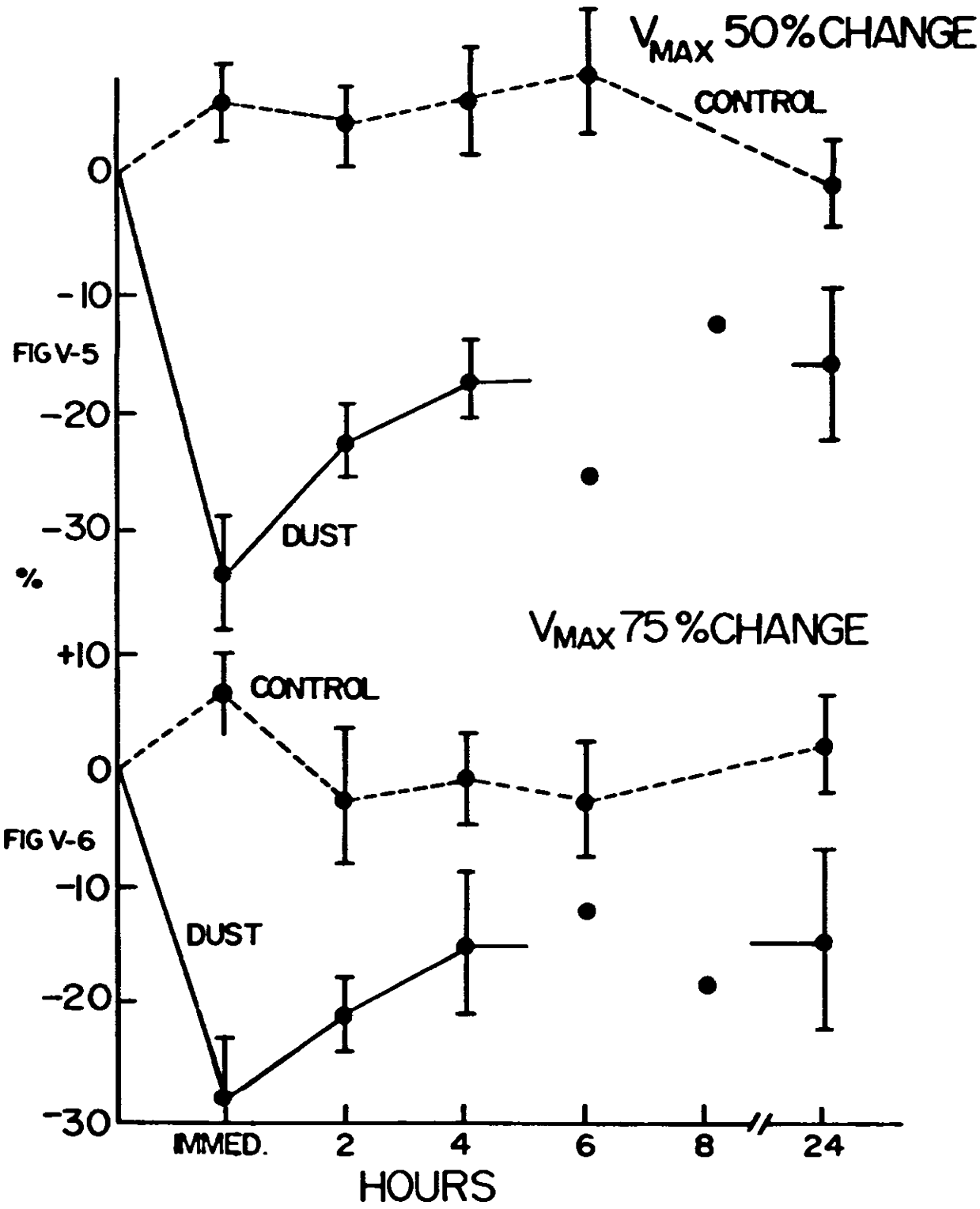


FIG V-3

FIG V-4

MEAN± ISE % CHANGE FROM BASELINE ON CONTROL DAY AND AFTER EXPOSURE TO AIRBORNE GRAIN DUST



APPENDIX I

Privacy Act of 1974 - Comments

Field Operations Manual
NIOSH Contract No. 210-76-0175

APPENDIX I
Privacy Act of 1974 - Comments

The study we have performed as a non-government organization under contract with a Federal agency adhered to the Privacy Act of 1974, which requires that the government: 1) maintain no secret files on individuals; 2) inform people at the time it is collecting information about them why this information is needed, and how it will be used; 3) assure that personal information is used only for the reasons given, or seek the person's permission when another purpose for its use is considered necessary or desirable; 4) allow people to see the records kept on them; and 5) provide people with the opportunity to correct inaccuracies in their records.

In this study all completed forms, computer output, back up tapes and related documents will be maintained in locked cabinets on secure premises. All subjects will be assigned a study number upon signature of the informed consent. Only in a single file will the subject's name, social security number and study number be present in an unscrambled form. In most files only the subject number will be used. In those cases where the subject's identification is needed for specific tests such as the subject's physical exam record, code work scrambled identifiers will be used. In certain processers these scrambled words are replaced by asterisks upon printing, unless a decoding command is used. Access to codes will be restricted to the data manager.

APPENDIX I
Privacy Act of 1974 - Comments

ALOSH will receive one sealed copy of the list of subjects by name, social security number and study number. All samples will be transferred by subject number and sample number with a sample number list identifying the source and details pertinent to that sample. At study completion, ALOSH will specify in writing which subject records are to have identifying data purged prior to transfer.

All subjects, prior to signing the consent form, were informed of their rights under the Privacy Act and that the Medical Ethics Code applied to all their medical information to protect their privacy. Medical information was released to the individual or with his written consent to his physician.

APPENDIX II

Research Participants' Document

Field Operations Manual
NIOSH Contract No. 210-76-0175

OMB No. 68-S77027
Exp. 7-79

NIOSH/UNIVERSITY OF WISCONSIN HUMAN SUBJECTS RESEARCH PARTICIPANT DOCUMENT

I. PROJECT DESCRIPTION

1. Project Title and Number: Disease Prevalence & Health Hazards of Grain Handlers; NIOSH Project VKCR21132.

2. Sponsor and/or contractor: National Institute for Occupational Safety and Health, Morgantown, West Virginia 26505 and University of Wisconsin, Madison, Wisconsin 53706.

3. Purpose and Benefits:

This study will be divided into two parts: Part A -- Determination of General Health Status and Biological Response to the Working Environment; and Part B -- Determination of Response to Specific Environmental Agents. The participant may limit his willingness to cooperate to either Part A or Part B or he may elect to participate in both of these studies.

Part A: Determination of General Health Status and Biological Response to the Working Environment

This study is designed to define the presence and extent of health hazards associated with occupational exposure to grain dusts. However, in order to determine the uniqueness of the effect of the exposure to grain dust it is necessary to evaluate what the health status is of other working people not exposed to grain dust. Therefore, to participate in this study you do not necessarily have to be associated with the grain industry.

During the course of the study, our examinations may identify diseases or conditions (which may or may not be related to grain handlers) which should have further medical attention or treatment. With your permission, we will notify you and your private physician of such findings so that they can be taken care of. This is one way in which you may personally benefit from the tests. In addition, all workers may benefit from the study if it is found that present precautions against occupational disease are inadequate, and that improved preventive measures should be taken.

Part B: Determination of Response to Specific Environmental Agents

This study is designed to identify the agent that may cause a lung reaction to grain dust in sensitive individuals. Grain dust contains not only grain particles but also particles of insects and molds to which some people may react. If the agent causing your problem can then be recognized, steps can be taken to prevent or reduce its effects on your health. However, in order to determine if only sensitive individuals will react, it is also necessary to do this study on people who have not shown reaction to grain dust. In this case the participant will not derive any direct benefit from the study.

II. CONSENT TO PARTICIPATE

I, _____, age _____, hereby voluntarily agree to cooperate in the above named study and to undergo the tests listed in Attachment A as follows:

(Indicate your willingness to cooperate in either Part A or Part B or both studies by writing "YES" or "NO" in the space provided.)

Part A: _____

Part B: _____

The studies have been discussed with me and I have been given a copy of the document. I understand that:

1. The procedures and tests to be followed are as stated in Attachment A with those procedures which are experimental so identified.

2. Attendant discomforts and risks are as noted in Attachment A and, except as noted, are minimal and provision has been made for any necessary medical care, and I have been told what to do if I have any reaction.

3. Benefits are as indicated in the Purpose and Benefits section in Part I.

4. If alternative procedures advantageous to me are available, they are specified in Attachment A; and if they become available during the project, the procedure most advantageous for me will be indicated and used or an explanation will be given to me as to use of any other procedure.

5. My inquiries will be answered by the examining personnel, by the Project Director, Dr. John Rankin, University of Wisconsin, Department of Preventive Medicine, Rm. 101, 504 N. Walnut Street, Madison, Wisconsin 53706, (608-263-2881); or by the Project Officer, Dr. Pervis C. Major, NIOSH, 944 Chestnut Ridge Road, Morgantown, West Virginia 26505 (304-291-4256).

6. I am free to terminate my consent and to discontinue participation in the project at any time without prejudice to myself.

7. My identity and my relationship to any information (1) disclosed by me in completing any project questionnaire, and (2) reported by me or derived from me during my participation in the above named project shall be kept confidential and will not be disclosed to others without my written consent except as required by law and except that such information will be used for statistical and research purposes in such a manner that no individual can be identified. I understand that if any information is found out concerning me that can endanger the health and safety of others, this information will be given to the proper authority.

8. If any of my medical records are required for purposes of this project, a separate written consent for release of the records will be requested from me.

9. There will be questions that I will be asked to answer, and my inquiries concerning the questions will be answered by the examining personnel, by Dr. John Rankin (608-263-2881), or by Dr. Pervis Major (304-291-4256).

10. A report of any significant information from the study that specifically concerns me, including medical information, will be furnished by the project officer or his designated representative to me or to my designated physician(s) upon completion of the study or earlier if appropriate.

SIGNATURE_____ DATE_____
(Subject)

SIGNATURE_____ DATE_____
(Parent or Guardian)

11. INVESTIGATOR_____
(Name, title and signature)

III. REQUEST AND AUTHORIZATION FOR RELEASE OF INFORMATION

I_____, hereby request and authorize the Project Director to inform the following physicians whose names and addresses I have entered below of any significant findings from the above named study concerning me. (Do not leave blank. Write "NO" where you do not wish to give a name and address.)

1. My personal physician(s): Dr._____

Street:_____

City:_____

2. Other physician: Dr._____

Street:_____

City:_____

SIGNATURE_____DATE_____

IV. The "Medical Health Surveillance of Grain Handlers" questionnaire is required under Part A of this study and it will constitute this Part IV as a separate attachment to be retained by the Project Director. A copy of the questionnaire is not retained by the participant.

OMB No. 68-S77027
Exp. 7-79

ATTACHMENT A

A. Project Title and Number: Disease Prevalence & Health Hazards of Grain Handlers; NIOSH Project VKCR21132.

B. Procedures and tests which involve human subjects in conduct of this project are as follows:

The examination will be divided into two parts: Part A -- Determination of General Health Status and Biological Response to the Working Environment; and Part B --Determination of Response to Specific Environmental Agents. The participant may limit his willingness to cooperate to either Part A or Part B or he may elect to participate in both of these studies.

PART A:

The procedures and tests that you will be asked to do are part of a physical examination which consists of: (1) Filling out a questionnaire which contains a series of questions which will be reviewed with you later by a trained interviewer. These questions will be about your work history, your use of tobacco, possible health problems, and your family history; (2) your height and weight will be measured and recorded; (3) x-rays of your chest will be made; (4) breathing tests will be made to determine if there is any increased resistance in your air passages; (5) blood and urine will be collected and analyzed; and (6) allergic skin tests will be done with common allergy testing agents (e.g., ragweed) and with specific agents related to the grain industry (e.g., wheat). Reactions to skin tests look like a hive and may give some local discomfort and itching. None of these tests is experimental, all are widely used during medical examinations and usually cause no discomfort to the health of participants.

Occasionally, the breathing tests may cause some temporary chest discomfort and coughing. Some pain, as you may feel with a pin prick, may be associated with the blood collection and skin tests.

One tube (20 ml) of blood will be drawn from an arm vein before and after a work shift. The blood will be analyzed for white cell counts, globulins, complement, enzymes, creatinine and precipitins against common molds and grains. A sample of your blood will be frozen and transferred to the National Institute for Occupational Safety and Health (NIOSH), Morgantown, West Virginia, where they may elect to perform additional blood tests or repeat those performed in this study.

Urine will be collected and analyzed for protein, sugar, and blood.

There will be no direct costs to you for these tests.

Qualified professional personnel and proper medical supplies will be available to treat any unforeseen reaction such as fainting. There are no alternative procedures to those noted above which will permit you to participate in the study. You may, of course, refuse to take any of them without incurring any penalty.

PART B:

This part of the study will be done in a hospital. Each individual will

be exposed to a spray-mist of a solution made from substances obtained from either grain, mite, or insects and/or molds to which he has shown to be allergic in the skin test. He will be tested before, immediately after the exposure, and at regular intervals over a 24-hour period.

The tests will include the assessment of symptoms, temperature, white blood count, and standard breathing.

The individual may develop fever, cough, wheezing and/or shortness of breath which may be rapidly improved by available standard medication. Some temporary chest discomfort and coughing may come with the breathing tests and some pain, as a pin prick, may be associated with the collection of the blood sample.

Although the tests used to evaluate the effects of the exposure are not experimental, the exposure to the material is experimental. However, to assure that no untoward reactions will occur, only those individuals who are commonly exposed to these materials at work and who have shown they have reacted to them (as indicated by the positive skin tests to these materials) will be selected as the test population. Only those individuals who are non-reactive (as indicated by negative skin tests to these materials) will be selected as controls.

C. Rights Under the Privacy Act of 1974 Title 5 United States Code, Section 552 (a) (e) (3).

The information required to be given to me under the Privacy Act of 1974 is as follows:

(1) Authority for collecting information is the Occupational Safety and Health Act 1970, Section 20 (29 USC 669).

(2) The principal purpose or purposes for which the requested information is intended to be used is for accurate assessment of the participants' general and occupational health status and is being solicited for specific epidemiological analysis and/or as stated in Section I, Item 3.

(3) The anticipated routine use which may be made of the solicited information is in developing criteria and programs for a safe and healthful place of employment or as published in the Federal Register, Vol. 41, No. 240, #0146.00, pp. 54223-54225, Monday, December 13, 1976.

(4) I do not have to furnish any information I do not wish to. Nothing happens to me as a result of my not providing information, whether all or in part of that requested, except that I may be terminated for the project.

APPENDIX III

Coding Date: Hazard/Site/Occupation

Field Operations Manual
NIOSH Contract No. 210-76-0175

[Deleted by NIOSH]

APPENDIX IV

Questionnaire: Grain Handlers

Field Operations Manual
NIOSH Contract No. 210-76-0175

EXPLANATION:

Questions 46 and 47 in the grain handlers' questionnaire were independently verified by clinically experienced physicians. This verification was facilitated through the use of a work sheet (see Appendix V), citing two headings (questions 46 and 47) which differ only in form and not substance from questions 46 and 47 as originally presented. It is important to note that the form adopted on the physician's work sheet is the form reported in the questionnaire analysis appendix.

ID #_____

ALL THE INFORMATION OBTAINED FROM THIS STUDY WILL BE KEPT CONFIDENTIAL.

Please answer the questions by circling the number of the best answer or by filling in a blank with a number or word. If uncertain or in doubt, circle No.

EXAMPLE: Do you live or work on a farm? 1. Yes 2. No

If you desire help in answering a question, please put a ( ) in front of the question number. You will be helped with these questions by a member of our personnel.

1. Name (Last) (First) (MI)

3. Phone Number
AREA CODE (___)
____-_____

4. Social Security #
*(optional, see below)
/ / /-/ / /-/ / / / /

2. Current Address (Number, street or rural route, city or town, county, state, zip code)

5a. Birthdate (mo, day, year)

5b. Age (last birthday)

6. Sex
1. / / Male 2. / / Female

7. Ethnic Group or Ancestry
1. / / White, not of Hispanic Origin
2. / / Black, not of Hispanic Origin
3. / / Hispanic
4. / / American Indian or Alaskan Native
5. / / Asian or Pacific Islander
6. / / Other: _____

8. Marital Status
1. / / Married 3. / / Divorced
2. / / Widowed 4. / / Never Married

9a. Height _____(cm)
/ / with shoes
/ / with boots
/ / bare feet

9b. Weight _____(kg)
/ / with clothing/ street shoes
/ / with clothing/ safety shoes
/ / in underwear

10. What was the highest grade of regular school you completed?
/___/

(For example: completion of high school is 12.)

11. Do you live or work on a farm?
1. Yes 2. No

*(Furnishing your Social Security number is voluntary. Your refusal to provide this number will not affect any right, benefit, or privilege to which you would be entitled if you did provide your Social Security number. Your Social Security number is being requested since it will permit use in future determinations in statistical research studies.)

ID # _____

12. List all jobs, occupations or type of work you have held or done through life and state approximate dates and lengths of time.

Company or Industry	Job Classification	Code	Type of Work of Task	Length of Time in Years	Years From	Years To	Average no. of Months Per Year	Location Name of City or Rural
1.					19__	19__		
2.					19__	19__		
3.					19__	19__		
4.					19__	19__		
5.					19__	19__		
6.					19__	19__		
7.					19__	19__		
8.					19__	19__		

ID #_____

(CHECK APPROPRIATE ANSWER AFTER EACH QUESTION. WHEN IN DOUBT, ANSWER NO.)

COUGH AND PHLEGM:

13a. Do you usually cough first thing in the morning? (Exclude clearing throat) — 1. Yes 2. No

b. Do you usually cough at other times during the day or night? — 1. Yes 2. No

c. Do you cough as much as 4-6 times a day for 4 or more days out of the week? — 1. Yes 2. No

IF YES TO EITHER 13a, b OR c, ANSWER d AND e:

d. Do you cough on most days for as much as 3 months of the year? — 1. Yes 2. No (x)

e. For how many years have you had this cough? _____ Years (x)

14a. Do you usually bring up phlegm from the chest first thing in the morning? (Not from the back of your nose. Count swallowed phlegm from the chest.) — 1. Yes 2. No

b. Do you usually bring up phlegm from the chest at other times during the day or night? — 1. Yes 2. No

c. Do you bring up phlegm like this as much as twice a day, 4 or more days out of the week? — 1. Yes 2. No

IF YES TO EITHER 14a, b OR c, ANSWER d AND e:

d. Do you bring up phlegm from the chest on most days for as much as 3 months of the year? — 1. Yes 2. No (x)

e. For how many years have you raised phlegm from the chest? _____ Years (x)

IF YOU NEVER HAD COUGH OR PHLEGM, GO TO Q 21.

15. When is your cough worse? — a. On workdays (x)
b. On weekends when not working
c. I notice no difference

16. Is your cough and/or phlegm better, the same or worse when on vacation or not working? — a. Better (x)
b. The same
c. Worse

ID # _____

17a. Is your cough and/or phlegm worse at different times of the year? 1. Yes 2. No (x) Go to Q 18

_____IF YES TO 17a, CIRCLE THE MONTHS IN WHICH YOU HAVE BEEN MOST TROUBLED._____

b. Jan 1 Feb 2 Mar 3 Apr 4 May 4 Jun 6 Jul 7 Aug 8 Sep 9 Oct 10 Nov 11 Dec 12 (x)

---

18. Is your cough and/or phlegm brought on by or made worse by exposure to: (xy)

a. Grain dust at work? 1. Yes 2. No (x)

b. Other dusts at work? 1. Yes 2. No

c. Gases or fumes at work? 1. Yes 2. No

d. House dust or fumes in the home? 1. Yes 2. No

e. Barn dusts, silage or hay? 1. Yes 2. No

f. Weather changes? 1. Yes 2. No

g. Other 1. Yes 2. No

_____
(Specify)

_____IF YES TO GRAIN DUST AT WORK, ANSWER Q 19:_____
(Otherwise, Go to Q21a.)

19. In your opinion, <u>which grain dusts</u> are most likely to bring on <u>cough and/or phlegm</u>, or make it worse? (x)
(May circle more than one.)

a. Durum wheat
b. Spring wheat
c. Rye
d. Oats
e. Barley
f. Corn
g. Soybean
h. Linseed
i. Sunflower seed
j. Beets
k. Malt
l. Other _____
(Specify)

20. When you are working regularly, how frequently (on the average) have you experienced <u>cough and/or phlegm</u> during work? (x)

a. Usually at least once a day.
b. Only a few times each week.
c. Only a few times each month.
d. Only a few times each year.
e. Only a few times ever.
f. Only once.

ID #_____

WHEEZING AND/OR CHEST TIGHTNESS:

21a. Have you ever noticed any wheezing and/or tightness in your chest? 1. Yes 2. No (x)

Go to Q 37

_____IF YES TO 21a, ANSWER b AND c:_____

b. Do you get this only with colds? 1. Yes 2. No (x)

c. Do you get this even when you don't have a cold? 1. Yes 2. No (x)

IF YOU HAVE NEVER NOTICED WHEEZING AND/OR TIGHTNESS IN YOUR CHEST, SKIP Q 22 THROUGH 36 AND GO TO Q 37.

22. Which of these symptoms have you experienced: wheezing, chest tightness or both?
a. Only wheezing (x)
b. Only chest tightness
c. Mainly wheezing
d. Mainly chest tightness
e. Both wheezing and chest tightness

23. At what age did your wheezing and/or chest tightness first occur? _____ Years (x)

24. At what age did wheezing and/or chest tightness last occur? _____ Years (x)
(If you are still having these, put your present age.)

25. Do you have wheezing and/or chest tightness at work while you are performing your job? 1. Yes 2. No

Go to Q 28

26. When you are working regularly, how frequently (on the average) have you experienced wheezing and/or chest tightness during work?
a. Usually at least once a day. (x)
b. Only a few times each week.
c. Only a few times each month.
d. Only a few times each year.
e. Only a few times ever.
f. Only once.

27. Is your wheezing and/or chest tightness usually worse on:
a. First day back at work. (x)
b. Any day(s) at work.
c. Weekends, when not working.
d. Makes no difference

ID #_____

28. Is your wheezing and/or chest tightness brought on by or made worse by exposure to: (xy)

a. Grain dust at work?	1. Yes 2. No	(x)
b. Other dusts at work?	1. Yes 2. No	
c. Gases or fumes at work?	1. Yes 2. No	
d. House dust or fumes in the home?	1. Yes 2. No	
e. Barn dusts, silage or hay?	1. Yes 2. No	(x)
f. Moldy or musty barn dusts, silage or hay?	1. Yes 2. No	
g. Contacts with animals?	1. Yes 2. No	
h. Plants, pollens or weeds?	1. Yes 2. No	
i. Weather changes?	1. Yes 2. No	
j. Other exposures	1. Yes 2. No	

_____
(Specify)

____IF YES TO GRAIN DUST AT WORK, ANSWER Q 29 THROUGH 32: _____
(Otherwise, Go to Q 33a.)

29. In your opinion, which grain dusts are most likely to bring on wheezing and/or chest tightness or make it worse? (May circle more than one.) (x)

a. Durum wheat
b. Spring wheat
c. Rye
d. Oats
e. Barley
f. Corn
g. Soybean
h. Linseed
i. Sunflower seed
j. Beets
k. Malt
l. Other _____
(Specify)

30. When is your wheezing and/or chest tightness most likely to start or get worse? (Circle only one) (x)

a. Before work
b. During work
c. After work
d. Either during or after work

31. If it starts or gets worse during work, how soon after the beginning of the work shift does not happen? (x)

a. Right away
OR
b. _____hours after

32. If it starts or gets worse after work, how many hours after work does this happen?

_____hours after

ID # _____

33a. Does wheezing and/or chest tightness ever wake you up from your sleep? (x)
1. Yes 2. No

_____IF YES TO 33a, ANSWER b:_____

b. How often does this happen? (x)

A. Almost every night.
B. A few times each month.
C. A few times each year.
D. A few times ever.
E. Only once.
F. Never.

34a. Is your wheezing and/or chest tightness worse at different times of the year? (x)
1. Yes 2. No

Go to Q 35

_____IF YES TO 34a, CIRCLE THE MONTHS IN WHICH YOU ARE MOST TROUBLED BY WHEEZING AND/OR CHEST TIGHTNESS_____

b. Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	(x)
1	2	3	4	5	6	7	8	9	10	11	12	

---

35. Is your wheezing and/or chest tightness better, the same or worse when on vacation or not working? (x)

a. Better
b. The same
c. Worse

36. Have you ever had 2 or more attacks of wheezing that has made you feel short of breath? 1. Yes 2. No (x)

SHORTNESS OF BREATH:

37. Have you ever been troubled by shortness of breath? 1. Yes 2. No

38. Are you troubled by shortness of breath when hurrying on level ground or walking up a slight hill? 1. Yes 2. No

39. Do you get short of breath walking with other people of your own age on level ground? 1. Yes 2. No

40. Do you have to stop for breath while walking at your own pace on level ground? 1. Yes 2. No

41. Do you get short of breath dressing or walking about the house? 1. Yes 2. No

ID # _____

_____IF YES TO Q 38, 39, 40 OR 41, ANSWER Q 42:_____

42. For how long have you had this shortness of breath? _____ Years (x)

---

43. Do you get short of breath while at work, performing your job? 1. Yes 2. No

44a. Do you get short of breath during or after exposure to grain dust? 1. Yes 2. No (x)

Go to Q 45a

_____IF YES TO Q 44a, ANSWER b, c, d AND e:_____

b. In your opinion, which grain dusts are most likely to bring on shortness of breath or make it worse? (May circle more than one.) (x)

A. Durum wheat
B. Spring wheat
C. Rye
D. Oats
E. Barley
F. Corn
G. Soybean
H. Linseed
I. Sunflower seed
J. Beets
K. Malt
L. Other _____ (Specify)

c. When is your shortness of breath most likely to get worse? (Circle only one) (x)

A. During work
B. After work
C. Either during or after work

d. If it starts during work, how soon after the beginning of the work shift does this happen? (x)

A. Right away
OR
B. _____ hours after

e. If it starts after work, how many hours after work does this happen? _____ hours after (x)

IF IN YOUR WORK YOU ARE EXPOSED TO GRAIN DUST, PLEASE ANSWER THE NEXT WORK QUESTIONS, IF NOT, GO TO Q 50.

FEVER AND/OR CHILLS (SHIVERING):

45a. Have you ever had fever and/or chills during exposure, or after being exposed to grain dust? 1. Yes 2. No (x)

Go to Q 48

b. If yes to 45a, did you have:

A. Only fever?
B. Only chills?
C. Mostly fever?
D. Mostly chills?
E. Both fever and chills?

ID # _____

46. When have you noticed the fever and/or chills? (Circle only one.)
A. During work. (x)
B. After work.
C. Either during or after work.

_____IF IT STARTS AFTER WORK:_____

47a. About how many hours after work did this (these) happen? _____ hours after work (x)

b. About how many hours did this (these) last? _____ hours

c. How many times in your work life as a grain handler have you had fever and/or chills after work? _____ times

d. When have you experienced this fever and/or chills?
A. On first day back to work.
B. Any other day at work.
C. On either the first day back on any other day.

e. If on the first day back to work, how long had you been off work? _____ number of days

---

48. <u>During</u> exposure to grain dust have you ever had: (x)

a. Eyes burning, watering or itching? 1. Yes 2. No

b. Stuffy nose? 1. Yes 2. No

c. Throat sore or burning? 1. Yes 2. No

_____IF YES TO Q 48a, b OR c, ANSWER d:_____

d. In your opinion, which grain dusts are most likely to bring on these symptoms or make them worse? (May circle more than one.) (x)

A. Durum wheat
B. Spring wheat
C. Rye
D. Oats
E. Barley
F. Corn
G. Soybean
H. Linseed
I. Sunflower seed
J. Beets
K. Malt
L. Other _____ (Specify)

49a. During or immediately after exposure to grain dust, have you ever had <u>itching</u> on your skin? 1. Yes 2. No (x)

Go to Q 50

ID # _____

_____IF YES TO 49a, ANSWER b AND c:_____

b. How many times in a year is this likely to happen? _____ times (x)

c. In your opinion, which grain dusts are most likely to bring on the skin itching? (May circle more than one.) (x)

A. Durum wheat
B. Spring wheat
C. Rye
D. Oats
E. Barley
F. Corn
G. Soybean
H. Linseed
I. Sunflower seed
J. Beets
K. Malt
L. Other _____ (Specify)

TOBACCO SMOKING

50. Have you ever smoked cigarettes? (If you have smoked less than 20 packs of cigarettes in your lifetime, check No.) 1. Yes 2. No

Go to Q53a

51a. Do you now smoke cigarettes? (Answer "yes" if you currently smoke or if you stopped smoking within the last month.) 1. Yes 2. No

Go to Q 52a

_____IF YOU SMOKE REGULARLY NOW:_____

b. Do you inhale the cigarette smoke? 1. Yes 2. No (x)

c. How old were you when you began to smoke cigarettes? _____Age (x)

d. How many cigarettes do you usually smoke each day at the present time? (Please give best estimate: one pack contains 20 cigarettes.) _____cigarettes per day (x)

e. What is the usual number of cigarettes you have smoked per day since you began to smoke? (Please give best estimate: one pack contains 20 cigarettes.) _____cigarettes per day (x)

f. If there have been periods when you abstained from smoking, please enter total years of abstinence from smoking. (If less than one year, do not fill in.) _____ years (x)

IF YOU HAVE COMPLETED THIS SECTION, GO TO Q 53a.

ID # _____

52a. Did you used to smoke cigarettes? 1. Yes 2. No (x)

_____IF YOU DO NOT SMOKE CIGARETTES REGULARLY NOW, BUT USED TO SMOKE THEM: (If you have not smoked at least 20 packs of cigarettes in your lifetime, check here: /____/

b. how old were you when you began to smoke cigarettes? _____ Years (x)

c. How old were you when you stopped smoking cigarettes regularly? _____ Age (x)

d. What was the usual number of cigarettes you smoked per day? (Please give best estimate: one pack contains 20 cigarettes.) _____ cigarettes per day (x)

e. If there have been periods when you abstained from smoking, please enter total number of years of abstinence from smoking. (If less than one year, do not fill in.) _____ Years (x)

53a. Do you now smoke pipes or cigars? 1. Yes 2. No

Go to Q 54

b. Do you usually inhale when you smoke either pipes or cigars? 1. Yes 2. No (x)

PESTICIDES:

54. Have you ever been exposed to pesticides? 1. Yes 2. No

Go to Q 63a

55. During or immediately after exposure to pesticides, have you ever had any health problems or symptoms? 1. Yes 2. No (x)

Go to Q 63a

ID # _____

IF YES TO Q 55, ANSWER THE NEXT QUESTIONS

56. Where did this (these) exposures happen?

a. At work (x)
b. At home
c. On a farm
d. Other _____ (Specify)

57. What kind of health problems did you have?

a. Weakness
b. Fainted
c. Dizziness
d. Headache
e. Convulsions
f. Trouble breathing
g. Nausea and/or vomiting
h. Stomach pain
i. Diarrhea
j. Muscle twitching, cramps
k. Blurred vision
l. Jaundice
m. Other _____ (Specify)

58. How many days did these problems last?

_____ Days

59. How many times have you had these problems?

_____ Times

60. Have you ever been ill following the exposure to pesticides that you couldn't do your regular job?

1. Yes 2. No

61. Have you ever had to go or be taken to a doctor or hospital because of these problems?

1. Yes 2. No

62. What pesticides caused you to have symptoms?

a. Do not know
b. Carbon tet (weevilcide)
c. Malathion
d. Methyl bromide
e. Phostoxin
f. Other _____ (Specify)

ID # _____

THE NEXT SET OF QUESTIONS IS ABOUT ILLNESSES YOU HAVE HAD OR HAVE CURRENTLY. WHEN RECORDING AGE, WRITE IN THE YOUNGEST AGE AT WHICH THE ILLNESS OCCURRED.

63a. During the past 3 years, how much trouble have you had with illnesses such as chest colds, bronchitis or pneumonia?

A. None (x)
B. Little
C. Moderately
D. Much
E. A great deal

b. During the past 3 years, how often were you unable to do your usual activities because of illnesses such as chest colds, bronchitis or pneumonia?

A. None
B. One time
C. 2-5 times
D. More than 5 times

64. Has a doctor ever told you that you had any of the following:

		AGE
a. Bronchitis (or bronchial trouble)	1. Yes 2. No	____
b. Emphysema	1. Yes 2. No	____
c. Pleurisy	1. Yes 2. No	____
d. Tuberculosis of the lung	1. Yes 2. No	____
e. Cancer of the lung	1. Yes 2. No	____
f. Chest surgery (including heart surgery)	1. Yes 2. No	____
g. Chest injury	1. Yes 2. No	____
h. Sinus trouble	1. Yes 2. No	____
i. Farmer's Lung Disease	1. Yes 2. No	____

65a. Has a doctor ever said you had: Pneumonia or broncho-pneumonia? 1. Yes 2. No

Go to Q 66a

_____IF YES TO Q 65a, ANSWER b AND c:_____

b. How many times have you had pneumonia? _____ Times (x)

c. Your age (or ages) when this (these happened? ____, ____, ____, ____ Years (x)

66a. Has a doctor ever said you had bronchial asthma? 1. Yes 2. No

Go to Q 67

ID # _____

_____IF YES TO 66a, ANSWER b, c AND d:_____

b. How old were you when your asthma started? ____ Age started (x)

c. Do you still have asthma? 1. Yes 2. No (x)

d. If no, how old were you when your asthma stopped? ____ Age stopped (x)

---

67. Has a doctor ever told you that you had any of the following?

a. Heart trouble 1. Yes 2. No

b. High blood pressure 1. Yes 2. No

c. Allergic reaction in your nose, such as hay fever 1. Yes 2. No

d. Kidney trouble 1. Yes 2. Ho

e. Liver trouble or jaundice 1. Yes 2. No

f. Diabetes 1. Yes 2. No

68. Have you ever had a serious skin rash in infancy (eczema)? 1. Yes 2. Ho

69. Have you ever suffered from skin rashes? 1. Yes 2. No

Go to Q 71

70a. If yes, have you ever suffered from skin rashes lasting longer than 2 weeks? 1. Yes 2. No

Go to Q 71a

_____IF YES TO Q 70a, ANSWER b:_____

b. What area was involved? (x)

A. Face	F. Chest
B. Ears	G. Back
C. Scalp	H. Abdomen
D. Hands	I. Legs
E. Arms	J. Feet

71a. Have you ever suffered with painful or swollen joints? 1. Yes 2. No

Go to Q 72

ID # _____

_____IF YES TO Q 71a, ANSWER b AND c:_____

b. Which joints were involved? (x)

A. Fingers	E. Spine
B. Wrists	F. Hips
C. Elbows	G. Knees
D. Shoulders	H. Ankles

c. Were the joints swollen? 1. Yes 2. No (x)

---

72. Do you have frequent "chills" with fever, sweating and perhaps shaking? 1. Yes 2. No

73. Do you have swelling of both ankles? 1. Yes 2. No

74. Has any member of your <u>immediate family</u> (blood relative) had any of the following diseases?

		RELATIVE
a. Chronic bronchitis	1. Yes 2. No	_____
b. Emphysema	1. Yes 2. No	_____
c. Asthma	1. Yes 2. No	_____
d. Hay fever	1. Yes 2. No	_____
e. Cystic fibrosis	1. Yes 2. No	_____
f. Cancer of the lung	1. Yes 2. No	_____
g. Farmer's Lung Disease	1. Yes 2. No	_____
h. Other lung disease (Specify)	1. Yes 2. No	_____

75a. Have you ever had a chest x-ray in the past? 1. Yes 2. No

_____IF YES TO Q 75a, ANSWER b and c:_____

b. Where was the last chest x-ray taken?

_____ in _____ in 19 ____
(Hospital) (City)

<u>OR</u>

_____ in _____ in 19 ____
(Doctor's office) (City)

c. Have you ever been told you had an abnormal chest x-ray? 1. Yes 2. No

---

ID # _____

76. Are you taking any drugs or medications? (Prescribed or not) 1. Yes 2. No

If yes, please list the medications here:

_____

_____

_____

_____

77. When was the last time you were exposed to your working environment?

a. Today
b. Yesterday
c. 2 days ago
d. _____ days ago

_____ _____

(Date) (Signature)

OMB No. 68-S77027
Exp. 7/79

DEPARTMENT OF HEALTH, EDUCATION AND WELFARE

Public Health Service

Center for Disease Control

National Institute for Occupational Safety and Health

Appalachian Laboratory for Occupational Safety and Health

Division of Respiratory Disease Studies

Morgantown, West Virginia 26505

UNIVERSITY OF WISCONSIN CENTER FOR HEALTH SCIENCES
Lung Disease Center Research
504 N. Walnut Street
Madison, Wisconsin 53706

HEALTH STATUS OF GRAIN HANDLERS

Identification No.	Interviewer Code
Location	Date of Interview

APPENDIX V

Physician's Verification Work Sheet
Re: Questions 46 and 47

Field Operations Manual
NIOSH Contract No. 210-76-0175

APPENDIX V

Physician's Verification Work Sheet
Re: Questions 46 and 47

The following questions are designed to verify self-administered responses to questions 46 and 47 listed in Appendix IV - Questionnaire.

Physician's Name:_____ Date: _____

46a. Are the participant's signs and symptoms compatible with a diagnosis of: (Circle only one)

/___/ A: Grain fever

/___/ B: Questionable grain fever

/___/ C: Not grain fever

b. How many episodes of grain fever did the participant experience during his/her work life?

/___/ 0-9 /___/ 10-19 /___/ 20-99 /___/ 100-300

c. When did the participant notice the episode (fever and/or chills)? (Circle only one)

/___/ A: During work

/___/ B: After work

/___/ C: Either during or after work

d. When did the participant experience an episode (fever and/or chills)?

/___/ A: On first day back to work

/___/ B: Any day of the week

/___/ C: Any day of the week but predominantly (or worse) first day back to work

e. If episode was experienced on the first day back to work, how long had he/she been off work (days)?

/___/ 1-7 /___/ 8-30 /___/ 31-180 /___/ 181-300

47. Associated respiratory symptoms:

/___/ A: None or not registered

/___/ B: Cough and/or expectoration

/___/ C: Tightness and/or wheezing

/___/ D: Dyspnea

APPENDIX VI

Questionnaire Analysis

Field Operations Manual
NIOSH Contract No. 210-76-0175

APPENDIX VI

Questionnaire Analysis

Introductory Statement:

The answers to the individual questionnaires were entered into our computer file. Prevalence of symptoms or "conditions" were obtained by simple statistical analysis to determine the proportion of workers with the symptoms, e.g., cough in the morning or "condition," e.g., age, lived on a farm, had chest illness often. The significance of the differences in prevalence of symptoms or "conditions" between grain workers and controls was done using Chi-Square analysis. The questions analyzed by Chi-Square are indicated by # by the question number. This analysis compares the proportion of grain workers with or without the symptom with the proportion of controls with or without the symptom. More detailed analysis is described in the text of the report from page ___ to page ___ and Tables ____.

To simplify the table, key words and/or abbreviations were used to describe each question. For the proper interpretation and for understanding this table the reader should read the complete text of the question from the questionnaire (Appendix IV).

In addition, the following clarifications are provided:

Grain = Grain workers (see text for definition)

Control = City services workers (see text for definition)

Smoker = Current cigarette smoker = yes to Q 51a

Nonsmoker = Never smoked = no to Q 50
(See Questionnaire for details on smoking)

Ques = Question number

= Chi-Square analysis result reported here

Description = See text of each question on questionnaire (Appendix IV)

Code = Y = yes; N = no (see Questionnaire for details)

NR = Number of workers from which proportion (%) were obtained. NR varies depending on question, e.g., one may want to know what proportion of all workers (NR=300) interviewed had wheezing at work or how many of those workers with wheezing on exposure (NR=183) had it during work.

APPENDIX VI

CNT = Number of positive answers to question or number of workers that selected a particular choice of answers to multiple choice questions.

% = Refers to percent of NR

"C+E" = Indicates positive answer to questions C and E

"18ABC+28ABC" = Indicates positive answer to questions 18A or B or C and 28A or B or C.

+ or · by CNT/Total = Indicates P < .05 by Chi-Square analysis on questions tested marked # or those questions with no symbol by CNT number indicates no significant difference between grain and control workers.

QUESTIONNAIRE ANALYSIS - TOTAL AND ALL SMOKING HABITS

Q = chi-square done; U + = p<.05;9= p<.001; E Blank = not significant

S	DESCRIPTION	C O D E	GRAIN TOTAL NR.	GRAIN TOTAL CNT	GRAIN TOTAL %	GRAIN SMOKER NR.	GRAIN SMOKER CNT	GRAIN SMOKER %	GRAIN EX-SM NR.	GRAIN EX-SM CNT	GRAIN EX-SM %	GRAIN NONSM NR.	GRAIN NONSM CNT	GRAIN NONSM %	CONTROLS TOTAL NR.	CONTROLS TOTAL CNT	CONTROLS TOTAL %	CONTROLS SMOKER NR.	CONTROLS SMOKER CNT	CONTROLS SMOKER %	CONTROLS EX-SM NR.	CONTROLS EX-SM CNT	CONTROLS EX-SM %	CONTROLS NONSM NR.	CONTROLS NONSM CNT	CONTROLS NONSM %
# 11	Live/work on farm	Y	307	359	11	151	14	9	92	12	13	64	9	14	239	2	1	106	2	2	70	-	-	63	-	-
# 13	Cough:																									
	First thing in a.m.	A	310	109	35	153	75	49	92	19	21	65	15	23	239	37	15	106	31	29	70	1	1	63	5	8
	Other times	B	310	173	56	153	104	68	92	42	46	65	27	42	239	57	24	106	43	41	70	7	10	63	7	11
	x4-6/d > 4x/wk	C	310	132	43	153	75	49	92	34	37	65	23	35	239	38	16	106	28	26	70	5	7	63	5	8
	Most days/3 months	D	310	143	46	153	85	56	92	34	37	65	24	37	239	47	20	106	35	33	70	7	10	63	5	8
	Cough more than 2 yrs	E	310	175	56	153	104	68	92	45	49	65	26	40	239	58	25	106	44	42	70	7	10	63	7	11
# 13Z	Cough: Years																									
	0-2			187	29	16									64	18	28									
	3-5			187	55	29									64	19	30									
	6-10			187	50	27									64	13	20									
	11-20			187	29	16									64	9	14									
	21-51			187	24	13									64	5	8									
#	Phlegm:																									
14	First thing in a.m.	A	310	116	37	153	71	46	92	26	28	65	19	29	239	36	15	106	28	26	70	3	4	63	5	8
	Other times	B	310	137	44	153	77	50	92	36	39	65	24	37	239	30	13	106	22	21	70	5	7	63	3	5
	x4-6/d > 4x/wk	C	310	128	41	153	72	47	92	33	36	65	23	35	239	30	13	106	23	22	70	5	7	63	2	3
	Most days/3 months	D	310	140	45	153	84	55	92	33	36	65	23	35	239	39	16	106	30	28	70	6	9	63	3	5
	Cough more than 2 yrs	E	310	151	49	153	88	57	92	40	43	65	23	35	239	43	18	106	32	30	70	5	7	63	6	10
		C+E	310	119	38	153	68	44	92	30	33	65	21	32	239	27	11	106	21	20	70	4	6	63	2	3

QUES	DESCRIPTION	CODE	GRAIN TOTAL NR.	GRAIN TOTAL CNT	GRAIN TOTAL %	GRAIN SMOKER NR.	GRAIN SMOKER CNT	GRAIN SMOKER %	GRAIN EX-SM NR.	GRAIN EX-SM CNT	GRAIN EX-SM %	GRAIN NONSM NR.	GRAIN NONSM CNT	GRAIN NONSM %	CONTROLS TOTAL NR.	CONTROLS TOTAL CNT	CONTROLS TOTAL %	CONTROLS SMOKER NR.	CONTROLS SMOKER CNT	CONTROLS SMOKER %	CONTROLS EX-SM NR.	CONTROLS EX-SM CNT	CONTROLS EX-SM %	CONTROLS NONSM NR.	CONTROLS NONSM CNT	CONTROLS NONSM %
	Phlegm: Years																										
# 14E	0-2		159	24	15										50	15	30										
	3-5		159	39	25										50	13	26										
	6-10		159	36	23										50	15	30										
	11-20		159	39	25										50	5	10										
	21-50		159	21	13										50	2	4										
#	13>2 yrs + 14>2 yrs		310	194*	63	113	36		49	16		32	10		239	67	28	49	21		9	4		9	4		
# 15	Cough Worse:																										
	Workdays	A	192	140*	73	114	78	68	48	40	83	30	22	73	67	12	18	51	10	20	9	1	11	7	1	14	
	Weekends	B	192	1	1	114	1	1	48	0	0	30	0	0	67	2	2	51	2	4	9	0	0	7	0	0	
	No Difference	C	192	51*	27	114	35	31	48	8	17	30	8	27	67	53	79	51	39	77	9	8	89	7	6	86	
# 16	Cough and/or phlegm on vacation:																										
	Better	A	207	170*	82	118	95	81	54	47	87	35	28	80	78	22	28	56	15	27	12	3	25	10	4	40	
	Same	B	207	37*	18	118	23	20	54	7	13	35	7	20	78	56	72	56	41	73	12	9	75	10	6	60	
	Worse	C	207	0	0	118	0	0	54	0	0	35	0	0	78	0	0	56	0	0	12	0	0	10	0	0	
# 18	Cough and/or phlegm made worse by exposure to:																										
	Grain Dust	A	310	200*	65	153	114	75	92	52	57	65	34	52	239	1	.5	106	0	0	70	0	0	63	1	2	
	Other Dust	B	310	67*	22	153	38	25	92	13	14	65	16	25	239	25	10	106	18	17	70	4	6	63	3	5	

QUES	DESCRIPTION	CODE	GRAIN TOTAL NR.	GRAIN TOTAL CNT	GRAIN TOTAL %	GRAIN SMOKER NR.	GRAIN SMOKER CNT	GRAIN SMOKER %	GRAIN EX-SM NR.	GRAIN EX-SM CNT	GRAIN EX-SM %	GRAIN NONSM NR.	GRAIN NONSM CNT	GRAIN NONSM %	CONTROLS TOTAL NR.	CONTROLS TOTAL CNT	CONTROLS TOTAL %	CONTROLS SMOKER NR.	CONTROLS SMOKER CNT	CONTROLS SMOKER %	CONTROLS EX-SM NR.	CONTROLS EX-SM CNT	CONTROLS EX-SM %	CONTROLS NONSM NR.	CONTROLS NONSM CNT	CONTROLS NONSM %
18 CONTINUED																											
	Gases or Fumes	C	310	58	19	153	33	22	92	14	15	65	11	17	239	31	13	106	20	19	70	7	10	63	4	6
	House dust	D	310	14	5	153	7	5	92	3	3	65	4	6	239	6	3	106	4	4	70	1	1	63	1	2
	Barn dust	E	310	239	7	153	10	7	92	7	8	65	6	9	239	1	.5	106	1	1	70	0	0	63	0	0
	Weather	F	310	34	11	153	13	8	92	9	10	65	12	18	239	31	13	106	20	19	70	5	7	63	6	1
	Other	G	310	19	6	153	11	7	92	3	3	65	5	8	239	14	6	106	12	11	70	1	1	63	1	2
#	18 ABC + 28 ABC		310	1459	46	153	89	58	92	35	38	65	21	32	239	20	8	106	12	11	70	5	7	63	3	4

QUES	DESCRIPTION	CODE	GRAIN TOTAL NR.	GRAIN TOTAL CNT
19	Which grain dusts bring on cough and/or phlegm:			
	Durum wheat	A	200	172
	Spring wheat	B	200	77
	Rye	C	200	84
	Oats	D	200	65
	Barley	E	200	150
	Corn	F	200	13
	Soybean	G	200	17
	Linseed	H	200	3
	Sunflower	I	200	5
	Beets	J	200	1
	Malt	K	200	0
	Other	L	200	5

QUES	DESCRIPTION	CODE	GRAIN												CONTROLS											
			TOTAL			SMOKER			EX-SM			NONSM			TOTAL			SMOKER			EX-SM			NONSM		
			NR.	CNT	%	NR.	CNT	%	NR.	CNT	%	NR.	CNT	%	NR.	CNT	%	NR.	CNT	%	NR.	CNT	%	NR.	CNT	%
20	Cough and/or phlegm frequence:																									
	Once a day	A	199	153	77	113	83	74	52	42	81	34	28	82	1	1	100	0	0	0	0	0	0	1	1	10
	Few times week	B	199	35	18	113	22	20	52	9	17	34	4	12	1	0	0	0	0	0	0	0	0	1	0	0
	Few times month	C	199	5	3	113	5	4	52	0	0	34	0	0	1	0	0	0	0	0	0	0	0	1	0	0
	Few times year	D	199	4	2	113	3	3	52	1	2	34	0	0	1	0	0	0	0	0	0	0	0	1	0	0
	Few times ever	E	199	2	1	113	0	0	52	0	0	34	2	6	1	0	0	0	0	0	0	0	0	1	0	0
	Only once	F	199	0	-	113	0	0	52	0	0	34	0	0	1	0	0	0	0	0	0	0	0	1	0	0
# 21 A	Wheezing and/or chest Tightness:	Y	310	200*	65	153	110	72	92	53	58	65	37	57	239	101	42	106	53	50	70	29	41	63	19	30
# 21 B	Wheeze only With colds	Y	200	33*	17	110	19	17	63	7	13	37	7	19	101	46	46	53	22	42	29	13	45	19	11	58
# 22	Symptoms:																									
	Only wheezing	A	200	22+	11	110	11	10	53	7	13	37	4	11	100	20	20	53	11	21	28	2	7	19	7	37
	Only chest tightness	B	200	37	19	110	15	14	53	12	23	37	10	27	100	19	19	53	12	23	28	4	14	19	3	16
	Mainly wheezing	C	200	13+	7	110	10	9	53	2	4	37	1	3	100	14	14	53	8	15	28	4	14	19	2	11
	Mainly chest tightness	D	200	38	19	110	16	15	53	12	23	37	10	27	100	17	17	53	9	17	28	7	25	19	1	5
	Both wheeze/chest t.	E	200	90+	45	110	58	53	53	20	38	37	12	32	100	30	30	53	13	25	28	11	39	19	6	32

QUES	DESCRIPTION	CODE	GRAIN										CONTROLS									
			TOTAL			SMOKER			EX-SM			NONSM				TOTAL			SMOKER			EX-SM			NONSM		
			NR.	CNT	%	NR.	CNT	%	NR.	CNT	%	NR.	CNT	%	NR.	CNT	%	NR.	CNT	%	NR.	CNT	%
23-24	Total Years of wheezing																					
	0-5		200	85	43	110	44	40	53	18	34	37	23	62	100	47	47	53	31	59	28	10	36	19	6	32
	6-10		200	45	23	110	33	30	53	6	11	37	6	16	100	12	12	53	5	9	28	3	11	19	4	21
	11-15		200	34	17	110	16	15	53	12	23	37	6	16	100	11	11	53	7	13	28	3	11	19	1	5
	16-20		200	11	6	110	7	6	53	3	6	37	1	3	100	10	10	53	4	8	28	2	7	19	4	21
	21-25		200	9	5	110	4	4	53	4	8	37	1	3	100	5	5	53	2	4	28	2	7	19	1	5
	26-30		200	7	4	110	2	2	53	5	9	37	0	0	100	10	10	53	3	6	28	6	21	19	1	5
	31-70		200	9	5	110	4	4	53	5	9	37	0	0	100	5	5	53	1	2	28	2	7	19	2	11
23	Age wheezing first occurred:																					
	0-15		199	10+	5	110	5	5	52	5	10	37	0	0	98	17	17	52	4	8	27	5	19	19	8	42
	16-25		199	69	35	110	35	32	52	17	33	37	17	46	98	26	27	52	16	31	27	6	22	19	4	21
	26-35		199	53	27	110	37	34	52	11	21	37	5	14	98	25	26	52	14	27	27	9	33	19	2	11
	36-45		199	40	20	110	23	21	52	8	15	37	9	24	98	18	18	52	13	25	27	4	15	19	1	5
	46-55		199	23	12	110	9	8	52	10	19	37	4	11	98	11	11	52	5	10	27	2	7	19	4	21
	56-70		199	4	2	110	1	1	52	1	2	37	2	5	98	1	1	52	0	0	27	1	4	19	0	0

QUES	DESCRIPTION	CODE	GRAIN											CONTROLS									
			TOTAL			SMOKER			EX-SM			NONSM			TOTAL			SMOKER			EX-SM			NONSM	
			NR.	CNT	%	NR.	CNT	%	NR.	CNT	%	NR.	CNT	%	NR.	CNT	%	NR.	CNT	%	NR.	CNT	%	NR.	CNT	%
24	Age wheeze first occur:																								
	15-25		200	23	12	110	13	12	53	3	6	37	7	19	100	10	10	53	6	11	28	1	4	19	3	16
	26-35		200	52	26	110	30	27	53	12	23	37	10	27	100	35	35	53	18	34	28	9	32	19	8	42
	36-45		200	35	18	110	25	23	53	15	9	37	5	14	100	26	26	53	16	30	28	9	32	19	1	5
	46-55		200	60	30	110	34	31	53	16	30	37	10	27	100	18	18	59	9	17	28	3	11	19	6	32
	56-70		200	30	15	110	8	7	53	17	32	37	5	14	100	11	11	53	4	8	28	6	21	19	1	5
# 25	Wheezing at work	Y	310	165+	53	153	91	59	92	45	49	65	29	45	239	50	21	106	26	25	70	14	20	63	10	6
	OF Y21A	Y	200	165+	83	110	91	83	53	45	85	37	29	78	100	50	50	53	26	49	28	14	50	19	10	53
# 26	Wheezing frequence:																								
	Once a day	A	165	469	28	91	28	31	45	14	31	29	4	14	50	6	12	26	4	15	14	2	14	10	0	0
	Few times/week	B	165	34+	21	91	14	15	45	15	33	29	5	17	50	13	26	26	10	39	14	2	14	10	1	10
	Few times/month	C	165	509	30	91	27	30	45	9	20	29	14	48	50	11	22	26	5	19	14	2	14	10	4	40
	Few times/year	D	165	26	16	91	16	18	45	7	16	29	3	10	50	13	26	26	5	19	14	5	36	10	3	30
	Few times/year	E	165	9	6	91	6	7	45	0	0	29	3	10	50	6	12	26	1	4	14	3	21	10	2	20
	Only once	F	165	0	0	91	0	0	45	0	0	29	0	0	50	1	2	26	1	4	14	0	0	10	0	0
# 27	Wheezing worse:																								
	First day at work	A	165	259	15	91	14	15	45	8	18	29	3	10	50	2	4	26	2	8	14	0	0	10	0	0
	Any day at work	B	165	999	60	91	54	59	45	28	62	29	17	59	50	10	20	26	5	19	14	4	29	10	1	10
	Weekends	C	165	0	0	91	0	0	45	0	0	29	0	0	50	0	0	26	0	0	14	0	0	10	0	0
	Makes no difference	D	165	419	25	91	23	25	45	9	20	29	9	31	50	38	76	26	19	73	14	10	71	10	9	90
#	26 A or B or C or D		310	1569	50	153	85	56	92	45	49	65	26	45	23	43	18	106	24	23	70	11	16	63	8	13

QUES #	DESCRIPTION	CODE	GRAIN												CONTROLS									
			TOTAL			SMOKER			EX-SM			NONSM			TOTAL			SMOKER			EX-SM			NONSM		
			NR.	CNT	%	NR.	CNT	%	NR.	CNT	%	NR.	CNT	%	NR.	CNT	%	NR.	CNT	%	NR.	CNT	%	NR.	CNT	%
28	Wheezing made worse:																									
	Grain Dust	A	310	183*	59	153	104	68	92	46	50	65	46	50	239	5	2	106	2	2	70	0	0	63	3	5
	Other Dust	B	310	48	15	153	25	16	92	13	14	65	10	15	239	24	10	106	11	10	70	9	13	63	4	6
	Gases or fumes	C	310	53+	17	153	27	18	92	16	17	65	10	15	239	20	8	106	9	8	70	7	10	63	4	6
	House dust	D	310	9	3	153	5	3	92	2	2	65	2	3	239	12	5	106	2	2	70	7	10	63	3	5
	Barn dusts	E	310	14	5	153	4	3	92	5	5	65	5	8	239	5	2	106	2	2	70	2	3	63	1	2
	Moldy/musty barn	F	310	22+	7	153	8	5	92	7	8	65	7	11	239	6	3	106	3	3	70	2	3	63	1	2
	Contact with animals	G	310	4	1	153	1	1	92	2	2	65	1	2	239	5	2	106	2	2	70	2	3	63	1	2
	Plants	H	310	7	2	153	3	2	92	2	2	65	2	3	239	12	5	106	3	3	70	4	6	63	5	8
	Weather	I	310	34	11	153	14	9	92	13	14	65	7	11	239	37	15	106	17	16	70	13	19	63	7	11
	Other	J	310	11	4	153	4	3	92	3	3	65	4	6	239	14	6	106	8	8	70	6	9	63	0	0
	NA		310	124	40	153	49	32	92	44	48	65	31	48	239	179	75	106	78	74	70	48	69	63	53	8
29	Grain dusts bring on wheezing:																									
	Durum wheat	A	183	168	92																					
	Spring wheat	B	183	76	42																					
	Rye	C	183	82	45																					
	Oats	D	183	55	30																					
	Barley	E	183	129	75																					
	Corn	F	183	10	5																					
	Soybean	G	183	16	9																					

QUES	DESCRIPTION	CODE	GRAIN TOTAL NR.	GRAIN TOTAL CNT	GRAIN TOTAL %	GRAIN SMOKER NR.	GRAIN SMOKER CNT	GRAIN SMOKER %	GRAIN EX-SM NR.	GRAIN EX-SM CNT	GRAIN EX-SM %	GRAIN NONSM NR.	GRAIN NONSM CNT	GRAIN NONSM %	CONTROLS TOTAL NR.	CONTROLS TOTAL CNT	CONTROLS TOTAL %	CONTROLS SMOKER NR.	CONTROLS SMOKER CNT	CONTROLS SMOKER %	CONTROLS EX-SM NR.	CONTROLS EX-SM CNT	CONTROLS EX-SM %	CONTROLS NONSM NR.	CONTROLS NONSM CNT	CONTROLS NONSM %
29 Continued																											
	Linseed	H	183	2																							
	Sunflower	I	183	4																							
	Beets	J	183	2																							
	Malt	K	183	0																							
	Other	L	183	3																							
30 Wheezing most likely to start:																											
	Before work	A	183	3	2	104	2	2	46	1	2	33	0	0													
	During work	B	183	129	71	104	70	67	46	35	76	33	24	73													
	After work	C	183	21	12	104	12	12	46	4	9	33	5	15													
	Either during/after	D	183	30	16	104	20	19	46	6	13	33	4	12													
31 Wheezing during work																											
	Right away	A	156	30	19	88	16	18	41	6	15	27	8	30													
	Hours After	B	156	126	81	88	72	82	41	35	85	27	19	70													
31B# Hour wheeze start during work:																											
	1		127	18	14	73	9	12	35	5	14	19	4	21													
	2		127	53	42	73	30	41	35	16	46	19	7	37													
	3		127	18	14	73	10	14	35	6	17	19	2	11													

QUES	DESCRIPTION	CODE	GRAIN TOTAL NR.	GRAIN TOTAL CNT	GRAIN TOTAL %	GRAIN SMOKER NR.	GRAIN SMOKER CNT	GRAIN SMOKER %	GRAIN EX-SM NR.	GRAIN EX-SM CNT	GRAIN EX-SM %	GRAIN NONSM NR.	GRAIN NONSM CNT	GRAIN NONSM %	CONTROLS TOTAL NR.	CONTROLS TOTAL CNT	CONTROLS TOTAL %	CONTROLS SMOKER NR.	CONTROLS SMOKER CNT	CONTROLS SMOKER %	CONTROLS EX-SM NR.	CONTROLS EX-SM CNT	CONTROLS EX-SM %	CONTROLS NONSM NR.	CONTROLS NONSM CNT	CONTROLS NONSM %
31B	CONTINUED:																										
	4		127	20	16	73	13	18	35	3	9	19	4	21													
	5		127	8	6	73	4	6	35	4	11	19	0	0													
	6		127	10	8	73	7	10	35	1	3	19	2	11													
	7		127	0	0	73	0	0	35	0	0	19	0	0													
	8		127	0	0	73	0	0	35	0	0	19	0	0													
31B	Wheeze # hours before start:																										
	0-2		127	71	56	73	39	53	35	21	60	19	11	58													
	3-4		127	38	30	73	23	32	35	9	26	19	6	32													
	5-6		127	18	14	73	11	15	35	5	14	19	2	11													
	7-8		127	0	0	73	0	0	35	0	0	19	0	0													
	8-20		127	0	0	73	0	0		0	0	19	0	0													
32	Wheeze after work-# hrs																										
	1		35	12	34	21	8	38	6	2	33	8	2	25													
	2		35	7	20	21	3	14	6	2	33	8	2	25													
	3		35	2	6	21	0	0	6	0	0	8	2	25													
	4		35	4	11	21	4	19	6	0	0	8	0	0													
	5		35	1	3	21	1	5	6	0	0	8	0	0													
	6		35	5	14	21	2	10	6	2	33	8	1	13													
	7		35	0	0	21	0	0	6	0	0	8	0	0													

QUES	DESCRIPTION	CODE	GRAIN TOTAL NR.	GRAIN TOTAL CNT	GRAIN TOTAL %	GRAIN SMOKER NR.	GRAIN SMOKER CNT	GRAIN SMOKER %	GRAIN EX-SM NR.	GRAIN EX-SM CNT	GRAIN EX-SM %	GRAIN NONSM NR.	GRAIN NONSM CNT	GRAIN NONSM %	CONTROLS TOTAL NR.	CONTROLS TOTAL CNT	CONTROLS TOTAL %	CONTROLS SMOKER NR.	CONTROLS SMOKER CNT	CONTROLS SMOKER %	CONTROLS EX-SM NR.	CONTROLS EX-SM CNT	CONTROLS EX-SM %	CONTROLS NONSM NR.	CONTROLS NONSM CNT	CONTROLS NONSM %
32	CONTINUED:																										
	8		35	1	3	21	0	0	6	0	0	8	1	13													
	9		35	1	3	21	1	5	6	0	0	8	0	0													
	10		35	1	3	21	1	5	6	0	0	8	0	0													
	>10		35	1	3	21	1	5	6	0	0	8	0	0													
33A	Wheezing-wake up (See Below)	Y	20	61	30	111	34	31	52	16	31	38	11	29	101	24	24	53	12	23	29	8	28	19	4	21
33B	Wheezing wake up-how often:																										
	every night	A	60	6	10	33	4	12	16	2	13	11	0	0	24	3	13	12	1	8	8	0	0	4	2	50
	few times/month	B	60	21	35	33	11	33	16	5	31	11	5	45	24	8	33	12	4	33	8	4	50	4	0	0
	few times/year	C	60	23	38	33	11	33	16	6	38	11	6	55	24	8	33	12	5	42	8	2	25	4	1	25
	few times/ever	D	60	8	13	33	7	21	16	1	6	11	0	0	24	5	21	12	2	17	8	2	25	4	1	25
	only once	E	60	2	3	33	0	0	16	2	13	11	0	0	24	0	0	12	0	0	8	0	0	4	0	0
	never	F	60	0	0	33	0	0	16	0	0	11	0	0	24	0	0	12	0	0	8	0	0	4	0	0
34A	Wheeze worse time/year	Y	195	64	33	110	36	33	49	12	25	36	16	44	106	10	38	57	20	35	29	15	52	20	5	25
34B	Wheeze-months worse																										
	January	01	62	20	32	35	11	31	12	4	33	15	5	33	40	8	20	20	18	90	15	8	53	5	2	40
	February	02	62	1	2	35	0	0	12	0	0	15	1	7	40	1	3	20	1	5	15	0	0	5	0	0
	March	03	62	3	5	35	3	9	12	0	0	15	0	0	40	2	5	20	0	0	15	2	13	5	0	0
	April	04	62	10	16	35	6	17	12	2	17	15	2	13	40	2	5	20	0	0	15	2	13	5	0	0
33A	Wheezing - Wake-up	7	310	61	20	153	34	22	92	16	17	65	11	17	239	24	10	106	12	11	70	8	11	63	4	6

QUES	DESCRIPTION	CODE	GRAIN TOTAL NR.	GRAIN TOTAL CNT	GRAIN TOTAL %	GRAIN SMOKER NR.	GRAIN SMOKER CNT	GRAIN SMOKER %	GRAIN EX-SM NR.	GRAIN EX-SM CNT	GRAIN EX-SM %	GRAIN NONSM NR.	GRAIN NONSM CNT	GRAIN NONSM %	CONTROLS TOTAL NR.	CONTROLS TOTAL CNT	CONTROLS TOTAL %	CONTROLS SMOKER NR.	CONTROLS SMOKER CNT	CONTROLS SMOKER %	CONTROLS EX-SM NR.	CONTROLS EX-SM CNT	CONTROLS EX-SM %	CONTROLS NONSM NR.	CONTROLS NONSM CNT	CONTROLS NONSM %
34B	CONTINUED																										
	May	05	62	7	11	35	4	11	12	1	8	15	2	13	40	4	10	20	1	5	15	1	7	5	2	40
	June	06	62	7	11	35	5	14	12	1	8	15	1	7	40	0	0	20	0	0	15	0	0	5	0	0
	July	07	62	2	3	35	1	3	12	1	8	15	0	0	40	0	0	20	0	0	15	0	0	5	0	0
	August	08	62	5	8	35	1	3	12	2	17	15	1	13	40	0	0	20	0	0	15	0	0	5	0	0
	September	09	62	5	8	35	3	9	12	1	8	15	1	7	40	1	3	20	0	0	15	1	7	5	0	0
	October	10	62	1	2	35	0	0	12	0	0	15	1	7	40	2	5	20	0	0	15	1	7	5	0	0
	November	11	62	1	2	35	1	3	12	0	0	15	0	0	40	0	0	20	0	0	15	0	0	5	1	20
	December	12	62	0	0	35	0	0	12	0	0	15	0	0	40	0	0	20	0	0	15	0	0	5	0	0
35	Wheezing on vacation:																										
	Better	A	196	1729	88	109	98	90	51	46	90	36	28	78	101	20	20	53	13	25	29	4	14	19	3	16
	Same	B	196	249	12	109	11	10	51	5	10	36	8	22	101	79	78	53	38	72	29	25	86	19	16	84
	Worse	C	196	0	0	109	0	0	51	0	0	36	0	0	101	2	2	53	2	4	29	0	0	19	0	0
36	Wheeze 2 or more	Y	196	769	39	109	48	44	52	20	39	35	8	23	101	17	17	53	7	13	29	7	24	19	3	16
		Y	310	76	25	153	48	31	92	20	22	65	8	12	239	17	7	106	7	7	70	7	10	63	3	5

QUES	DESCRIPTION	CODE	GRAIN TOTAL NR.	GRAIN TOTAL CNT	GRAIN TOTAL %	GRAIN SMOKER NR.	GRAIN SMOKER CNT	GRAIN SMOKER %	GRAIN EX-SM NR.	GRAIN EX-SM CNT	GRAIN EX-SM %	GRAIN NONSM NR.	GRAIN NONSM CNT	GRAIN NONSM %	CONTROLS TOTAL NR.	CONTROLS TOTAL CNT	CONTROLS TOTAL %	CONTROLS SMOKER NR.	CONTROLS SMOKER CNT	CONTROLS SMOKER %	CONTROLS EX-SM NR.	CONTROLS EX-SM CNT	CONTROLS EX-SM %	CONTROLS NONSM NR.	CONTROLS NONSM CNT	CONTROLS NONSM %
	Shortness of breath:																										
37	SOB ever	37	310	175[9]	56	153	97	63	92	49	53	65	29	45	239	90	38	106	48	45	70	30	43	63	12	19
38	SOB slight hill	38	310	118[+]	38	153	65	42	92	29	32	65	24	37	239	64	27	106	32	30	70	25	36	63	7	11
39	SOB other people/ level ground	39	310	23[+]	7	153	11	7	92	9	10	65	3	5	239	8	3	106	3	3	70	4	6	63	1	2
40	SOB own pace/level	40	310	4	1	153	3	2	92	1	1	65	0	0	239	3	1	106	1	1	70	2	3	63	0	0
41	SOB dressing/walking about house	41	310	7	2	153	4	3	92	2	2	65	1	2	239	5	2	106	3	3	70	2	3	63	0	0
42	How many years had SOB																									
	0-5		145	77	53	84	43	51	32	12	38	29	22	76	71	42	59	36	19	53	26	14	54	9	9	10C
	6-10		145	43	30	84	30	36	32	10	31	29	3	10	71	14	20	36	9	25	26	5	19	9	0	0
	11-15		145	14	10	84	5	6	32	5	16	29	4	14	71	7	10	36	6	17	26	1	4	9	0	0
	16-20		145	8	6	84	5	6	32	3	9	29	0	0	71	7	10	36	2	6	26	5	19	9	0	0
	21-25		145	1	1	84	1	1	32	0	0	29	0	0	71	1	1	36	0	0	26	1	4	9	0	0
	26-50		145	2	1	84	0	0	32	2	6	29	0	0	71	0	0	36	0	0	26	0	0	9	0	0
43	SOB at work	Y	310	113	37+	153	61	40	92	35	38	65	17	26	239	26	11	106	13	12	70	8	11	63	5	8
44A	SOB to grain dust	Y	310	151	49	153	84	55	92	47	51	65	20	31												

QUES	DESCRIPTION	CODE	GRAIN TOTAL NR.	GRAIN TOTAL CNT	GRAIN TOTAL %	GRAIN SMOKER NR.	GRAIN SMOKER CNT	GRAIN SMOKER %	GRAIN EX-SM NR.	GRAIN EX-SM CNT	GRAIN EX-SM %	GRAIN NONSM NR.	GRAIN NONSM CNT	GRAIN NONSM %	CONTROLS TOTAL NR.	CONTROLS TOTAL CNT	CONTROLS TOTAL %	CONTROLS SMOKER NR.	CONTROLS SMOKER CNT	CONTROLS SMOKER %	CONTROLS EX-SM NR.	CONTROLS EX-SM CNT	CONTROLS EX-SM %	CONTROLS NONSM NR.	CONTROLS NONSM CNT	CONTROLS NONSM %
44B	SOB grain dust worse:																										
	Durum	A	151	136	90	84	77	92	47	42	89	20	17	85													
	Spring	B	151	71	47	84	45	54	47	18	38	20	8	40													
	Rye	C	151	69	46	84	34	40	47	25	53	20	10	50													
	Oats	D	151	47	31	84	27	32	47	12	26	20	8	40													
	Barley	E	151	99	66	84	50	60	47	33	70	20	16	80													
	Corn	F	151	5	3	84	4	5	47	1	2	20	0														
	Soybean	G	151	16	11	84	11	13	47	4	9	20	1	5													
	Linseed	H	151	1	1	84	0		47	1	2	20	0														
	Sunflower	I	151	4	3	84	1	1	47	1	2	20	2	10													
	Beets	J	151	0	0	84	0		47	0		20	0														
	Malt	K	151	0		84	0		47	0		20	0														
	Other	L		4	3	84			47	2	4	20	1	5													
44C	SOB when worse:																										
	During work	A	149	122	82																						
	After work	B	149	9	6																						
	Either	C	149	18	12																						
44D	SOB start during work:																										
	Right away	A	143	48	34																						
	Hours later	B	143	15	66																						

QUES	DESCRIPTION	CODE	GRAIN TOTAL NR.	GRAIN TOTAL CNT	GRAIN TOTAL %	GRAIN SMOKER NR.	GRAIN SMOKER CNT	GRAIN SMOKER %	GRAIN EX-SM NR.	GRAIN EX-SM CNT	GRAIN EX-SM %	GRAIN NONSM NR.	GRAIN NONSM CNT	GRAIN NONSM %	CONTROLS TOTAL NR.	CONTROLS TOTAL CNT	CONTROLS TOTAL %	CONTROLS SMOKER NR.	CONTROLS SMOKER CNT	CONTROLS SMOKER %	CONTROLS EX-SM NR.	CONTROLS EX-SM CNT	CONTROLS EX-SM %	CONTROLS NONSM NR.	CONTROLS NONSM CNT	CONTROLS NONSM %
44D	SOB start during work/ hours:																									
	0-1		95	19	20																					
	2-3		95	47	50																					
	4-5		95	24	25																					
	6-7		95	5	5																					
	8-9		95	0	0																					
44B	SOB start after work/ hours:																									
	0-1		20	7	35	10	3	30	4	3	75	6	1	17	2	1	50	1	0	0	0	0	0	1	0	
	2-3		20	11	55	10	6	60	4	1	25	6	4	67	2	1	50	1	1	100	0	0	0	1	0	
	4-5		20	1	5	10	1	10	4	0	0	6	0	0	2	0	0	1	0	0	0	0	0	1	0	
	6-7		20	1	5	10	0	0	4	0	0	6	1	17	2	0	0	1	0	0	0	0	0	1	0	
	8-9		20	0	0	10	0	0	4	0	0	6	0	0	2	0	0	1	0	0	0	0	0	1	0	
	10-11		20	0	0	10	0	0	4	0	0	6	0	0	2	0	0	1	0	0	0	0	0	1	0	
	12-13		20	0	0	10	0	0	4	0	0	6	0	0	2	0	0	1	0	0	0	0	0	1	0	
	14-20		20	0	0	10	0	0	4	0	0	6	0	0	2	0	0	1	0	0	0	0	0	1	0	
45A	Fever and/or chills	Y	310	121	39	153	60	39	92	40	44	65	21	32												
45B	Fever and/or chills, Which:	?	123	1	1																					
	Only fever	A	123	28	23																					
	Only chills	B	123	9	7																					
	Mostly fever	C	123	15	12																					
	Mostly chills	D	123	3	2																					
	Both fever & chills	E	123	67	55																					

QUES	DESCRIPTION	CODE	GRAIN TOTAL NR.	GRAIN TOTAL CNT	GRAIN TOTAL %	GRAIN SMOKER NR.	GRAIN SMOKER CNT	GRAIN SMOKER %	GRAIN EX-SM NR.	GRAIN EX-SM CNT	GRAIN EX-SM %	GRAIN NONSM NR.	GRAIN NONSM CNT	GRAIN NONSM %	CONTROLS TOTAL NR.	CONTROLS TOTAL CNT	CONTROLS TOTAL %	CONTROLS SMOKER NR.	CONTROLS SMOKER CNT	CONTROLS SMOKER %	CONTROLS EX-SM NR.	CONTROLS EX-SM CNT	CONTROLS EX-SM %	CONTROLS NONSM NR.	CONTROLS NONSM CNT	CONTROLS NONSM %
46A	Grain Fever?																										
	Grain Fever	A	310	99	32	153	47		31	92	31	33	65	21	32												
	Questionable G.F.	B	310	16	5																						
	Not G.F.	C	310	6	2																						
46B	Grain Fever Episodes																										
	0-9		96	40	42																						
	10-19		96	22	23																						
	20-99		96	18	19																						
	100-300		96	16	17																						
46C	Grain Fever Noticed:																										
	During work	A	115	37	32																						
	After work	B	115	40	35																						
	Either	C	115	38	33																						
46D	Grain Fever day:																										
	First day	A	115	15	13																						
	Any day	B	115	95	83																						
	Any day/worse 1st	C	115	5	4																						
46E	# Days off work before grain fever:																										
	1-7		16	2	13																						
	8-30		16	5	31																						
	31-180		16	3	19																						
	181-300		16	6	38																						

QUES	DESCRIPTION	CODE	GRAIN TOTAL NR.	GRAIN TOTAL CNT	GRAIN TOTAL %	GRAIN SMOKER NR.	GRAIN SMOKER CNT	GRAIN SMOKER %	GRAIN EX-SM NR.	GRAIN EX-SM CNT	GRAIN EX-SM %	GRAIN NONSM NR.	GRAIN NONSM CNT	GRAIN NONSM %	CONTROLS TOTAL NR.	CONTROLS TOTAL CNT	CONTROLS TOTAL %	CONTROLS SMOKER NR.	CONTROLS SMOKER CNT	CONTROLS SMOKER %	CONTROLS EX-SM NR.	CONTROLS EX-SM CNT	CONTROLS EX-SM %	CONTROLS NONSM NR.	CONTROLS NONSM CNT	CONTROLS NONSM %
47	Associated respiratory Symptoms:																										
	None		115	84	73																						
	Cough		115	19	17																						
	Wheeze		115	15	13																						
	Dyspnea		115	7	6																						
48	Sx during exposure to grain dust:																										
	Eyes burning	A	310	242	78																						
	Stuffy nose	B	310	246	79																						
	Throat sore	C	310	161	52																						
48D	Worse exposure dusts:																										
	Durum	A	281	234	83																						
	Spring	B	281	116	41																						
	Rye	C	281	136	48																						
	Oats	D	281	113	40																						
	Barley	E	281	213	76																						
	Corn	F	281	15	5																						
	Soybean	G	281	22	8																						
	Linseed	H	281	2	1																						
	Sunflower	I	281	10	4																						
	Beets	J	281	5	2																						
	Malt	K	281	0																							
	Other	L	281	9	3																						

QUES	DESCRIPTION	CODE	GRAIN TOTAL NR.	GRAIN TOTAL CNT	GRAIN TOTAL %	GRAIN SMOKER NR.	GRAIN SMOKER CNT	GRAIN SMOKER %	GRAIN EX-SM NR.	GRAIN EX-SM CNT	GRAIN EX-SM %	GRAIN NOWSM NR.	GRAIN NOWSM CNT	GRAIN NOWSM %	CONTROLS TOTAL NR.	CONTROLS TOTAL CNT	CONTROLS TOTAL %	CONTROLS SMOKER NR.	CONTROLS SMOKER CNT	CONTROLS SMOKER %	CONTROLS EX-SM NR.	CONTROLS EX-SM CNT	CONTROLS EX-SM %	CONTROLS NOWSM NR.	CONTROLS NOWSM CNT	CONTROLS NOWSM %
	Smoking questions summarized on Table 5.																									
54	Ever been exposed to pesticides	Y	310	294	95+										239	49	21									
55	Pesticide health problems	Y	294	168	57+										49	7	14									

QUES	DESCRIPTION	CODE	GRAIN										CONTROLS									
			TOTAL			SMOKER			EX-SM			NONSM			TOTAL			SMOKER			EX-SM			NONSM		
			NR.	CNT	%	NR.	CNT	%	NR.	CNT	%	NR.	CNT	%	NR.	CNT	%	NR.	CNT	%	NR.	CNT	%	NR.	CNT	%
56	Where did exposures happen?																									
	At work	A	310	168	54										239	6	3									
	Home	B	310	2	1										239	0	0									
	Farm	C	310	0	0										239	0	0									
	Other	D	310	0	0										239	1	.5									
57	What kind of health problems did you have?																									
	Weakness	A	310	65	21										239	4	2									
	Fainted	B	310	7	2										239	0	0									
	Dizziness	C	310	88	28										239	4	2									
	Headache	D	310	116	37										239	6	3									
	Convulsions	E	310	0	0										239	0	0									
	Trouble breathing	F	310	51	16										239	2	1									
	Nausea	G	310	65	21										239	3	1									
	Stomach pain	H	310	12	4										239	1	5									
	Diarrhea	I	310	12	4										239	0	0									
	Cramps	J	310	5	2										239	0	0									
	Blurred vision	K	310	14	5										239	0	0									
	Jaundice	L	310	0	0										239	0	0									
	Other	M	310	17	5										239	0	0									

QUES	DESCRIPTION	CODE	GRAIN TOTAL NR.	GRAIN TOTAL CNT	GRAIN TOTAL %	GRAIN SMOKER NR.	GRAIN SMOKER CNT	GRAIN SMOKER %	GRAIN EX-SM NR.	GRAIN EX-SM CNT	GRAIN EX-SM %	GRAIN NONSM NR.	GRAIN NONSM CNT	GRAIN NONSM %	CONTROLS TOTAL NR.	CONTROLS TOTAL CNT	CONTROLS TOTAL %	CONTROLS SMOKER NR.	CONTROLS SMOKER CNT	CONTROLS SMOKER %	CONTROLS EX-SM NR.	CONTROLS EX-SM CNT	CONTROLS EX-SM %	CONTROLS NONSM NR.	CONTROLS NONSM CNT	CONTROLS NONSM %
59	How many times pesticide problems?																										
	0-5		164	84	51										7	5	71										
	6-10		164	34	21										7	1	14										
	11-20		164	21	4										7	1	14										
	21-50		164	14	9										7	0	0										
	51-100		164	8	5										7	0	0										
	100-300		164	3	2										7	0	0										
60	Couldn't do regular job	Y	167	28	17										7	2	29										
61	Taken to Doctor?	Y	167	19	11										7	0	0										
62	What pesticides?																										
	Do not know	A	310	50	16										239	1	.5										
	Carbon tet	B	310	103	33										239	1	.5										
	Malathion	C	310	52	17										239	1	.5										
	Methyl Bromide	D	310	51	16										239	1	.5										
	Phostoxin	E	310	84	27										239	1	.5										
	Other	F	310	1	.5										239	3	1										

QUES	DESCRIPTION	CODE	GRAIN												CONTROLS									
			TOTAL			SMOKER			EX-SM			NONSM			TOTAL			SMOKER			EX-SM			NONSM		
			NR.	CNT	%	NR.	CNT	%	NR.	CNT	%	NR.	CNT	%	NR.	CNT	%	NR.	CNT	%	NR.	CNT	%	NR.	CNT	%
63A	How much trouble with dust illnesses?																							
	None	A	310	66	21	153	35	23	92	22	24	65	9	14	239	57	24	106	21	20	70	14	20	63	22	35
	Little	B	310	137	44	153	70	46	92	40	43	65	27	42	239	119	50	106	47	44	70	40	57	63	32	51
	Moderate	C	310	84	27	153	38	25	92	25	27	65	21	32	239	55	23	106	33	31	70	13	19	63	9	14
	Much	D	310	21	7	153	10	7	92	5	5	65	6	9	239	7	3	106	5	5	70	2	3	63	0	0
	Great Deal	E	310	2	1	153	1	1	92	0	0	65	1	2	219	1	.5	106	0	0	70	1	1	63	0	0
63B	Unable to do usual activities?																							
	None	A	309	145	47	152	77	51	92	39	42	65	29	45	239	132	55	106	53	50	70	37	53	63	42	67
	One Time	B	309	41	13	152	15	10	92	17	19	65	9	14	239	29	12	106	11	10	70	10	14	63	8	13
	2-5 Times	C	309	103	33	152	52	34	92	29	32	65	22	34	239	67	28	106	35	33	70	20	29	63	12	19
	> 5	D	309	20	7	152	8	5	92	7	7	65	5	8	239	11	5	106	7	7	70	3	4	63	1	2
64	Pulmonary problems																							
	Bronchitis	A	310	53	17	153	27	18	92	16	17	65	10	15	239	36	15	106	18	17	70	11	16	63	7	11
	Emphysema	B	310	6	2	153	4	3	92	2	2	65	0	0	239	2	1	106	0	0	70	2	3	63	0	0
	Pleuresy	C	310	28	10	153	17	11	92	8	9	65	3	5	239	18	8	106	10	9	70	5	7	63	3	5
	Tuberculosis	D	310	6	2	153	4	3	92	1	1	65	1	2	239	1	5	106	0	0	70	1	1	63	0	0
	Cancer	E	310	0	0	153	0	0	92	0	0	65	0	0	239	0	0	106	0	0	70	0	0	63	0	0
	Chest Surgery	F	310	4	1	153	2	1	92	1	1	65	1	2	239	3	1	106	0	0	70	3	4	63	0	0
	Chest Injury	G	310	11	4	153	6	4	92	2	2	65	3	5	239	10	4	106	6	6	70	2	3	63	2	3

64 continued . . .

QUES	DESCRIPTION	CODE	GRAIN TOTAL NR.	GRAIN TOTAL CNT	GRAIN TOTAL %	GRAIN SMOKER NR.	GRAIN SMOKER CNT	GRAIN SMOKER %	GRAIN EX-SM NR.	GRAIN EX-SM CNT	GRAIN EX-SM %	GRAIN NONSM NR.	GRAIN NONSM CNT	GRAIN NONSM %	CONTROLS TOTAL NR.	CONTROLS TOTAL CNT	CONTROLS TOTAL %	CONTROLS SMOKER NR.	CONTROLS SMOKER CNT	CONTROLS SMOKER %	CONTROLS EX-SM NR.	CONTROLS EX-SM CNT	CONTROLS EX-SM %	CONTROLS NONSM NR.	CONTROLS NONSM CNT	CONTROLS NONSM %
64	CONTINUED:																										
	Sinus trouble	H	310	72	23	153	34	22	92	26	28	65	12	18	239	74	31	106	37	35	70	27	39	63	10	16
	Farmer's lung	I	310	0	0	153	0	0	92	0	0	65	0	0	239	0	0	106	0	0	70	0	0	63	0	0
65A	Pneumonia	Y	310	68	22	153	29	19	92	24	26	65	24	37	239	60	25	106	32	30	70	18	26	63	10	16
65B	Pneumonia # times																									
	0-2		67	59	88	29	24	83	24	22	92	14	13	93	60	46	77	32	22	69	18	16	89	10	8	80
	3-5		67	7	10	29	4	14	24	.2	8	14	1	7	60	13	22	32	10	31	18	2	11	10	1	10
	6-10		67	1	2	29	1	3	24	0	0	14	0	0	60	1	2	32	0	0	18	0	0	10	1	10
	10-30		67	0	0	29	0	0	24	0	0	14	0	0	60	0	0	32	0	0	18	0	0	10	0	0
66A	Bronchial asthma	Y	310	12	4	153	3	2	92	8	9	65	1	2	239	4	2	106	0	0	70	2	3	63	2	3
66B	Age asthma started:																									
	0-10		11	7	64	3	2	67	7	4	57	1	1	100	4	2	50	0	0	0	2	0	0	2	2	100
	11-20		11	2	18	3	0	0	7	2	29	1	0	0	4	0	0	0	0	0	2	0	0	2	0	0
	21-30		11	0	0	3	0	0	7	0	0	1	0	0	4	2	50	0	0	0	2	2	100	2	0	0
	31-40		11	0	0	3	0	0	7	0	0	1	0	0	4	0	0	0	0	0	2	0	0	2	0	0
	41-50		11	2	18	3	1	33	7	1	14	1	0	0	4	0	0	0	0	0	2	0	0	2	0	0
	51-60		11	0	0	3	0	0	7	0	0	1	0	0	4	0	0	0	0	0	2	0	0	2	0	0
	61-70		11	0	0	3	0	0	7	0	0	1	0	0	4	0	0	0	0	0	2	0	0	2	0	0
66C	Still have asthma	Y	11	4	36	3	1	33	7	3	43	1	0	0	4	2	50	0	0	0	2	2	100	2	0	0

QUES	DESCRIPTION	CODE	GRAIN TOTAL NR.	GRAIN TOTAL CNT	GRAIN TOTAL %	GRAIN SMOKER NR.	GRAIN SMOKER CNT	GRAIN SMOKER %	GRAIN EX-SM NR.	GRAIN EX-SM CNT	GRAIN EX-SM %	GRAIN NONSM NR.	GRAIN NONSM CNT	GRAIN NONSM %	CONTROLS TOTAL NR.	CONTROLS TOTAL CNT	CONTROLS TOTAL %	CONTROLS SMOKER NR.	CONTROLS SMOKER CNT	CONTROLS SMOKER %	CONTROLS EX-SM NR.	CONTROLS EX-SM CNT	CONTROLS EX-SM %	CONTROLS NONSM NR.	CONTROLS NONSM CNT	CONTROLS NONSM %
66D	Age asthma stop:																									
	0-10		7	5	71	2	2	100	4	2	50	1	1	100	2	0	0	0	0	0	0	0	0	2	0	0
	11-20		7	1	14	2	0	0	4	1	25	1	0	0	2	2	100	0	0	0	0	0	0	2	2	100
	21-50		7	0	0	2	0	0	4	0	0	1	0	0	2	0	0	0	0	0	0	0	0	2	0	0
	51-70		7	1	14	2	0	0	4	1	25	1	0	0	2	0	0	0	0	0	0	0	0	2	0	0
67	Other health problems:																									
	Heart	A	310	20	6										239	15	6									
	Blood pressure	B	310	49	16										239	33	14									
	Allergic	C	310	20	6										239	29	12									
	Kidney	D	310	24	8										239	15	6									
	Liver	E	310	17	5										239	13	5									
	Diabetes	F	310	6	2										239	11	5									
68	Eczema	Y	307	13	4										239	10	4									
69	Skin rashes	Y	310	92	30										239	72	30									
70	Skin rashes > 2 wks	Y	92	56	61										72	42	58									
70B	Skin rash area:																									
	Face	A	310	12	4										239	11	5									
	Ears	B	310	6	2										239	6	3									
	Scalp	C	310	7	2										239	10	4									
	Hands	D	310	22	7										239	17	7									

CONTINUED ...

QUES	DESCRIPTION	COD	GRAIN TOTAL NR.	GRAIN TOTAL CNT	GRAIN TOTAL %	GRAIN SMOKER NR.	GRAIN SMOKER CNT	GRAIN SMOKER %	GRAIN EX-SM NR.	GRAIN EX-SM CNT	GRAIN EX-SM %	GRAIN NONSM NR.	GRAIN NONSM CNT	GRAIN NONSM %	CONTROLS TOTAL NR.	CONTROLS TOTAL CNT	CONTROLS TOTAL %	CONTROLS SMOKER NR.	CONTROLS SMOKER CNT	CONTROLS SMOKER %	CONTROLS EX-SM NR.	CONTROLS EX-SM CNT	CONTROLS EX-SM %	CONTROLS NONSM NR.	CONTROLS NONSM CNT	CONTROLS NONSM %
70B	CONTINUED:																									
	Arms	E	310	27	9										239	20	8									
	Chest	F	310	11	4										239	13	5									
	Back	G	310	16	5										239	13	5									
	Abdomen	H	310	15	5										239	13	5									
	Legs	I	310	32	10										239	22	9									
	Feet	J	310	16	5										239	11	5									
71A	Swollen Joints	Y	310	96	31										239	72	30									
71B	Which joints:																									
	Fingers	A	310	37	12										239	30	13									
	Wrists	B	310	25	8										239	11	5									
	Elbows	C	310	34	11										239	18	8									
	Shoulder	D	310	40	13										239	25	10									
	Spine	E	310	12	4										239	8	3									
	Hips	F	310	18	6										239	8	3									
	Knee	G	310	44	14										239	35	15									
	Ankle	H	310	24	8										239	11	5									
71C	Joints swollen	Y	95	48	51										70	37	53									
72	Frequent chills	Y	309	35	11										239	11	5									
73	Swelling both ankles	Y	307	10	3										237	4	2									

QUES	DESCRIPTION	CODE	GRAIN TOTAL NR.	GRAIN TOTAL CNT	GRAIN TOTAL %	GRAIN SMOKER NR.	GRAIN SMOKER CNT	GRAIN SMOKER %	GRAIN EX-SM NR.	GRAIN EX-SM CNT	GRAIN EX-SM %	GRAIN NONSM NR.	GRAIN NONSM CNT	GRAIN NONSM %	CONTROLS TOTAL NR.	CONTROLS TOTAL CNT	CONTROLS TOTAL %	CONTROLS SMOKER NR.	CONTROLS SMOKER CNT	CONTROLS SMOKER %	CONTROLS EX-SM NR.	CONTROLS EX-SM CNT	CONTROLS EX-SM %	CONTROLS NONSM NR.	CONTROLS NONSM CNT	CONTROLS NONSM %
74	Family diseases:																										
	Chronic bronchitis	A	310	15	5										239	9	4										
	Emphysema	B	310	16	5										239	18	8										
	Asthma	C	310	33	11										239	25	10										
	Hay fever	D	310	20	6										239	27	11										
	Cystic fibrosis	E	310	1	.5										239	0	0										
	Cancer, lung	F	310	12	4										239	17	8										
	Farmer's lung	G	310	0	0										239	1	.5										
	Other lung	H	310	11	4										239	8	3										
77D	Last exposed to work:																										
	0-3 Days		14	5	36	5	3	60	5	2	40	4	0	0	1	0	0	0	0	0	1	0	0	0	0	0	
	4-14 Days		14	6	43	5	2	40	5	2	40	4	2	50	1	1	0	0	0	0	1	1	100	0	0	0	
	28-30 Days		14	3	21	5	0	0	5	1	20	4	2	50	1	0	0	0	0	0	1	0	0	0	0	0	
	31-180		14	0	0	5	0	0	5	0	0	4	0	0	1	0	0	0	0	0	1	0	0	0	0	0	
77	Last exposed to work	A	310	218	70																						
		B	310	65	21																						
		C	310	25	8																						

APPENDIX VII

Physical Examination Protocol

Field Operations Manual
NIOSH Contract No. 210-76-0175

INSTRUCTIONS TO PHYSICIANS ON GRAIN HANDLERS' FIELD STUDY

The physician's assessment of the health status of the patient will include an interview during which the salient points of the questionnaire will be reviewed with particular emphasis on the manifestations related to the cardiovascular system to the symptoms related to exposure to grain dust and those symptoms related to exposure to detectable pesticides.

The questionnaire will be reviewed for completeness by one of two trained assistants before you see the worker, but you are required to recheck it to assure it is complete. Pay particular attention to the following:

- Check to see that the Consent Form is signed and obtain necessary permission for release of information from other physicians if necessary.

- Check work history, particularly whether the patient has worked in mining, farming, in a foundry, steel mill, with asbestos, in a shipyard, chemical plant, quarry, grain flour industry, welder, etc.

- Question 13e--be sure that the number of years that he/she has had the cough is entered.

- Is his/her cough better when non-exposed?

- Question 20--be sure that he/she understands that "only once" means only once in a lifetime and not once a day.

- Be sure that he/she enters the number of years on Questions 23 and 24.

I. Interview:

The interview should emphasize the clarification of complaints in the questionnaire, but answers to questions should not be modified. Questions 46 and 47 should be verified using Work Sheet provided (Appendix V).

The information you obtain from the patient should be written on the margins or at the end of the questionnaire. This anecdotal information will serve to clarify interpretation of historical, physical or laboratory findings in individuals if necessary. Analysis of symptoms felt in relation to his/her work should be done to rule out cardiac disease as the cause of the usual symptoms of cough, dyspnea, wheezing and chest tightness. Be sure to answer the following question:

In your opinion is his dyspnea from heart disease?

Yes ____ No ____ Not sure ____

- Obtain more details on pesticide exposure symptoms.

- On Question 64d--if he/she has had tuberculosis, did he/she receive treatment; did he/she receive a vaccine for tuberculosis?

- On Question 64e--specify age and treatment for the cancer.

- On Question 64f--specify reason for surgery, what was done, when.

- On Question 64i--how was Farmer's Lung disease diagnosed?

- If yes to Question 65a--pneumonia diagnosed by a doctor. Obtain detailed history for each pneumonia.

  Pneumonia #1: Did he/she have a chest x-ray, to be hospitalized, cough, phlegm, chest pain, wheezing, dyspnea, sore throat, earache, stuffy nose, muscle aches.

  Pneumonia #2: Did he/she have a chest x-ray, to be hospitalized, cough, phlegm, chest pain, wheezing, dyspnea, sore throat, earache, stuffy nose, muscle aches.

  Pneumonia #3: Did he/she have a chest x-ray, to be hospitalized, cough, phlegm, chest pain, wheezing, dyspnea, sore throat, earache, stuffy nose, muscle aches.

- Question 67a--type of heart disease or heart trouble. Therapy, if any.

- Questions 67d and 67e--what trouble, when, doctor's diagnosis, what doctor, what tests were done, was he/she hospitalized, where.

II. The physical examination will include:

a. The measurement of height, weight, blood pressure and heart rate, which will be performed by a technician.

b. The description of the chest configuration as outlined in the physical examination form.

c. Auscultation of the chest to be done in the upper and lower lung fields posteriorly while the patient breathes deeply through open mouth followed by auscultation during forced expiratory maneuvers. These will be reported as: 1) none, 2) obvious and common, or 3) on forced expiration only. If present, whether bilateral and diffuse or unilateral and localized. Rales will be reported as: 1) none, 2) bilateral, or 3) unilateral. The anterior or ventral chest will be auscultated over the four usual precordial areas, that is, the apex of the heart, the left sternal border, the aortic valve area and the pneumonic valve area. The findings will be reported as: 1) normal heart, 2) murmur and specify, 3) abnormal rhythm, or 4) other and specify. Also, specify whether in your opinion the murmur is "functional" rather than "organic" in origin. With the patient then lying down, palpate the liver at the mid-clavicular line. If palpable, the span should be measured by percussion and palpation. If the span is greater than 14 cm., hepatomegaly is present. Any other obvious physical findings should be reported under "Other Findings."

PHYSICAL EXAMINATION RECORD

STUDY ID # / / / / / / / / / / / /

BLOOD SAMPLE / /

URINE SAMPLE / /

WEIGHT / / / / / / Pounds

BLOOD PRESSURE / / / / / /-/ / / / / /
(SYSTOLIC) (DIASTOLIC)

HEIGHT / / / / / / Inches

PULSE RATE / / / / / / Beats/Min

RECORDER _____

CHEST CONFIGURATION (Check ONLY one) that pertain)

/ / 1. Normal

If NOT Normal:

/ / 2. Kyphoscoliosis

/ / 3. Scoliosis

/ / 4. Pectus Excavatum

/ / 5. Other

AUSCULTATION - POSTERIOR CHEST

Rhonchi and/or Wheezes (Check ONLY one)

/ / 1. None

Or obvious on deep but not forced expiration: (Check ONLY one)

/ / 2. Bilateral-diffuse

/ / 3. Unilateral-localized

Or if ONLY on forced expiration:

/ / 4. Bilateral-diffuse

/ / 5. Unilateral-localized

IV AUSCULTATION - ANTERIOR CHEST (Check ALL

/ / 1. Normal

If NOT Normal:

/ / 2. Murmur (Specify: _____

/ / 3. Abnormal rhythm

/ / 4. Other (Specify: _____

_____

V. ABDOMEN (Palpate liver at mid-clavicular line

If palpable, measure span. If greater than 14 cm. = hepatomegaly.)

Liver

/ / 1. Not palpable

If palpable, span = ___ ___ . ___cm

/ / 2. Hepatomegaly absent

/ / 3. Hepatomegaly present

PHYSICAL EXAMINATION RECORD (continued)

Rales (Check ONLY one)

/ / 1. None

/ / 2. Bilateral

/ / 3. Unilateral

OTHER FINDINGS: (ANY other abnormal physical findings)

APPENDIX VIII

Pulmonary Function Studies

Field Operations Manual
NIOSH Contract No. 210-76-0175

FORWARD

This manual contains a description and detailed procedures of the standardized techniques used in conducting the on-site pulmonary function tests specific to this study. Recorded data will be appropriately filed in data storage folders and subsequently measured and transcribed to computer input forms in Madison, Wisconsin, following completion of the field testing. Frequent on-site measurements and computations on a programmable calculator (TI SR-52 with PC 100 Printer) will be performed to spot check technical procedures and measurements.

Since prior to 1977 our epidemiological studies were supported by an NHLI Specialized Center for Lung Research Grant. Our laboratory had standardized pulmonary function testing in accordance with the recommendations of the NHLI, Division of Lung Disease, report of workshops on epidemiology of respiratory disease, October, 1972, and November, 1973. In addition, for measurements of FEV_1 and FVC we have considered the preliminary information available from the ATS Committee on Standardization of Spirometry.

TABLE OF CONTENTS

STANDARDS FOR PULMONARY FUNCTION TESTING

I. Spirometry, FEV_1, FVC, and MMF for Studies I & III

1. Instrumentation - Studies I & III (Rolling bar 840 will be used for Studies II, IV, & V.)

Standard equipment utilized for these procedures includes:

A. 13.5ℓ chain-linked, water-sealed, spirometer (W. E. Collins), with 3 speeds (32, 160, 1920 mm/min).

B. Mouthpieces - large rubber

C. Two-way bypass valve

D. Two 34" lengths of 1 1/2" ID corrugated wire wound flexible plastic tubing with 1 3/8" ID rubber coupling ends

E. Recording pens and ink

F. Noseclamp

With nose occluded, the subject is connected to the spirometer through the rubber mouthpiece attached to a large free breathing bypass valve and two lengths of corrugated tubing. Any CO_2 absorbent is removed from the system to minimize resistance to air flow.

2. Calibration

Weekly check for:

A. Deformities in spirometer bell

B. Leaks around water seal

C. Accuracy of kymograph drum speed

Forced Expiratory Volume (FEVt) - the volumes exhaled in 1.0 and 3.0 sec. total. (See Fig. 1)

3. Procedure

Record:

1. a. Date

b. Subject name, sex*

c. Ht. (cm)*

d. Wt. (kg)*

e. Age (yrs)*

f. Barometric pressure (mmHg)

g. Spirometer temperature (^{o}C)

*b-e optional if recorded elsewhere

2. Seat subject in a comfortable upright posture

3. Loosen any tight clothing

4. Explain test procedure

5. Install nose clip

6. Go onto mouthpiece, breath normally to room air

7. Fill drum approximately 2/3 full

Turn subject into spirometer (kymograph speed of 160 mm/min) and followng a few quick breaths request a maximal inspiration - (hold at TLC for ~1 second while switch is turned to 1920 ml/min paper speed); then request a maximal expiration (vigorously encourage subject to breathe out as hard and fast as possible).

Repeat steps 8 & 9 until at least three acceptable curves are obtained.

4. Criteria of Acceptability

Acceptability is based upon the technician's observation that the subject understood the instructions and performed the test with a smooth, continuous exhalation with apparent maximum effort and without: a) coughing; b) Valsalva maneuver; c) premature expiratory termination (in normals, before completion of the breath; in obstructed individuals this would be assumed if expiratory time was less that 5 seconds); d) a leak; e) an obstructed mouthpiece (e.g., tongue becoming positioned in front of mouthpiece); f) unsatisfactory start of expiration from TLC, characterized by excessive hesitation or false starts thus preventing accurate back extrapolation to time 0. (Extrapolated volume on the volume time tracing (spirogram) must be less than 10% of the FVC); g) excessive variablilty among the three acceptable curves i.e., exceeding $\pm$ 10% of the reading or 100 +mm.; whichever is greater, between the two best curves.

5. Measurements/Calculations

(a) FEV_t - volume of gas exhaled over a given time interval during the performance of a vital capacity maneuver. (See Fig. 1) FEV 1.0 is the volume expired in the first 1.0 sec. of the FEV_t. FEV 1.0 as well as FEV 2.0 and 3.0 are obtained from the spirogram by determining the time intervals on the spirometer chart paper. FEV and FEV_1 volumes are expressed as a % of total (i.e., FEV 1.0%), the values for which may be determined from different acceptable curves.

$$\text{FEV 1.0 (\%)} = \frac{FEV_1 \text{ (ml)} \times 100}{\text{FVC (ml)}}$$

The maximum FVC and the maximum FEV_1 (BTPS) will be computed following examination of data from all the acceptable curves even if the two values do not come from the same curve. From these values the $FEV_{1.0}$ (1%) will be calculated. In addition, the maximum FVC and FEV_1 from the maximum FVC as well as the ratio of this FEV_1/FVC X 100 will be recorded for purposes of the three year follow-up study.

Volumes expressed in absolute terms must be converted from ATPS to BTPS. $FEV_{BTPS} = FEV_{ATPS}$ X BTPS factor.

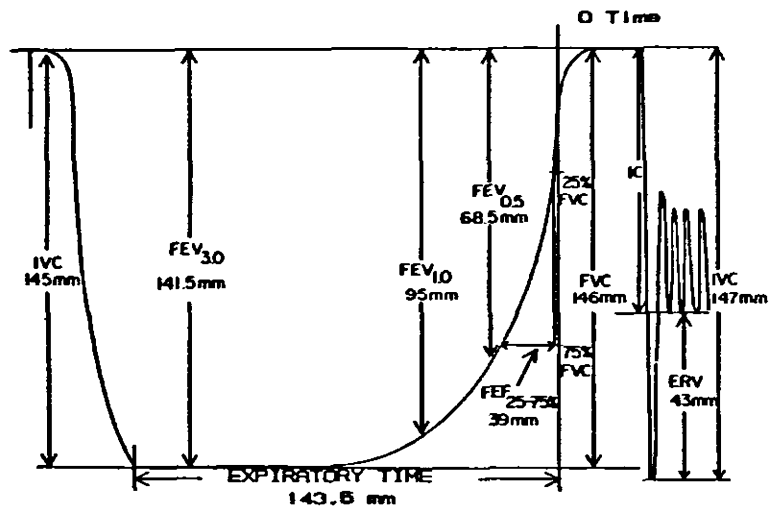


Fig. 1 Forced Expiratory Spirogram

(b) FEF 25-75% - the average flow rate during the middle two quarters of the volume segments of the forced expiratory spirogram (i.e., from 25-75% of the volume -- Fig. 2)

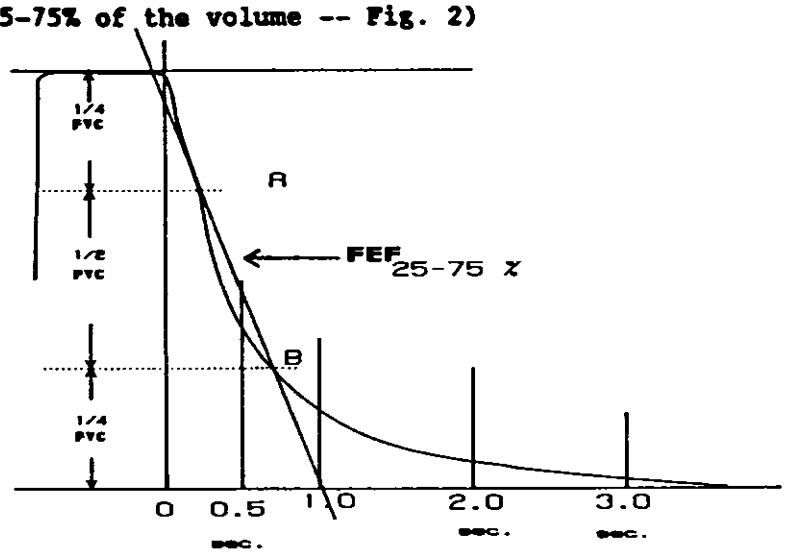


Fig. 2 Forced Expiratory Spirogram

"A" represents the intersection of the FEF with the line between the first and second quarter of the FVC volume (25%); "B" is the intersection of the FEF with the line between the third and fourth quarter of the FVC volume (75%). Points "A" and "B" are determined by measurement or with preset quadrant calipers from the point of commencement (0 time) of forced exhalation. Zero (0) time is determined by the back extrapolation method (Fig.3). Extrapolated volumes that exceed 10% of FVC are suboptimal and should be so noted for subsequent interpretations.

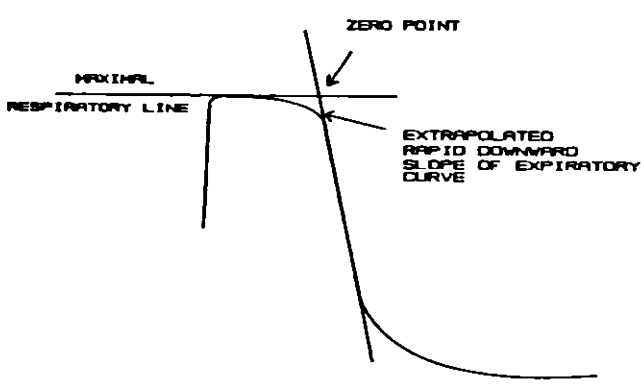


Fig. 3 Back Extrapolation Method of Establishing 0 Time Point

The slope of the line connecting "A" and "B" is the forced mid-expiratory flow (FEF 25-75%) which is determined by extending line "AB" until it crosses two time lines (i.e., 1 sec. apart). The distance between where the line crosses the two time lines represents the FEF 25-75% volume. Therefore:

$FEF\ 25\text{-}75\%_{ATPS}$=_____ℓ/sec x 60 = _____ℓ/min

$FEF\ 25\text{-}75\%_{BTPS}$=$FEF\ 25\text{-}75\%_{ATPS}$ x BTPS factor = _____

The $FEF_{25-75\%}$ will be calculated from the curve producing the largest sum of FVC and FEV_1. In addition, the MMF that corresponds with the largest FVC will be recorded for purposes of the 3 year follow-up study.

Prediction Values:

Absolute values of FEV_1, FVC, and MMF will be compared with the following prediction equations of Knudson, et al.

$FEV_{1.0}$<25 years old = .045 Age (yrs) + .046 Ht (cm) - 4.808

(men) <25 years old =-.027 Age (yrs) = .052 Ht (cm) - 4.203

FVC <25 years old = .078 Age + .05 Ht - 5.508

(men) >25 years old = -.029 Age (yrs) + .065 ~ .065 - 5.459

MMF >25 years old = .059 Ht (cm) - 5.334

(men) >25 years old = - .031 Age + .045 Ht (cm) - 1.864

For Studies II, IV, and V:

The instrumentation is described under "Flow-volume Curve." Forced expiratory volume-time will be obtained using an Ohio 840 Rolling Bar Spirometer. The FVC-time curve will be displayed on an HP 1046A X-Y-Y recorder and measured on the paper record as explained above.

References

Kanner, R.E. and A.H. Morris, eds., Spirometry, Ch. 1, "Clinical Pulmonary Function Testing," 1975.

Kory, R.C., J. Rankin, G.L. Snider, and J.F. Tomashefski. Clinical Spirometry (recommendations of the section on pulmonary function testing, Committee on Pulmonary Physiology, Am. Col. of Chest Phys.), Dis. of the Chest, (43):214-219, 1963.

Frayser, R. and J.C. Ross. Clinical Spirometry. Laboratory Procedure Manual, Dept. Medicine, Indiana Univ. School of Medicine,:11-19, 1966.

Knudson, R.S., Slatin, R.C., Lebowitx, M.D., Burrows, B. The maximal expiratory flow volume curve, normal standards, variability and effects of age. Am. Rev. Resp. Div. 113:587-600, 1976.

National Heart and Lung Institute, Report on Workshop on Standardization of Methods used in Epidemiologic Studies, 1973.

IIa. CLOSING VOLUME - NITROGEN METHOD

1. Instrumentation: Standard equipment utilized for this procedure includes:

A. Wedge Spirometer (Med. Science 570)

B. Nitralyser (Med. Science 505)

C. XYY recorder (Hewlett-Packard 7046A)

D. Two-way bypass valve

E. Tubing (corrugated) 24-30 " length 1-7/8 " ID

F. Mouthpiece - large rubber

G. O_2 cylinder (100% O_2), with pressure regulator

H. Recording paper (Hewlett-Packard 9270-1024)

I. Recording pens

J. Nose clamp

With nose occluded, the subject is connected to the Wedge spirometer through a large rubber mouthpiece, corrugated tubing and bypass valve. The output of the spirometer is monitored by a Nitralyser connected to one axis of the XY_1Y_2 recorder allowing for simultaneous recording of the flow volume and N_2 concentration curve.

2. Calibration:

A. Y_1Y_2 recorder: according to Ch. V, in Maintenance, Performance, Checks and Adjustments of the Operating and Service Manual for XY_1Y_2 Recorder 7046A.

B. Nitralyser: Weekly with concentrations ranging from 0-80% dry N_2 from calibrated tanks. (Monthly calibrations may prove adequate.)

C. Wedge Spirometer: Calibration of volume (ℓ) + N_2 concentration (%), is performed against a built-in electronic reference and displayed on each subject's record of CV tracings.

3. Procedure

A. Flush the Wedge Spirometer with 100% O_2 until N_2 concentration reads 0.

B. Install noseclip and have subject come onto the mouthpiece with the two-way valve turned to room air.

C. The subject takes a deep breath and exhales to residual volume(RV).

D. At RV the subject is turned into the spirometer and slowly inspires a vital capacity breath of pure O_2 and, without breath holding, slowly expires a second time to RV.

During inhalation of O_2 the Y_2 channel of the recorder is switched from .5 V/cm to 25 mv/cm. The subject is instructed to maintain expiratory flow rate (monitored from the Y_1 display channel) at 0.4 lps.

E. At the end of the second expiration to RV, the subject is turned into room air.

F. The operator urges the subject repeatedly at both extremes of vital capacity; during exhalation of the measurement, however, it must be only after the Phase IV deflection is apparent (See Fig. 2).

G. A minimum of 3 and maximum of 6 measurements are made; the number of measurements determined by visual acceptance of the curves.

H. Complete O_2 washout is not necessary between measurements. This necessitates accurate F_AN_2 measurements. A delay in repeating the test is advised if, during the preliminary air breathing phase, F_IN_2 and F_EN_2 differ by more than 5%.

4. <u>Criteria for Acceptability of Single Breath N_2 Closing Volume Curves</u>

The following criteria must be met for acceptability, failure to satisfy any one of these leads to rejection of the curve:

A. Mean expiratory flow after the first 500 ml is expired must equal or be less than 0.5 lps (the subject is instructed to aim for 0.4 lps).

B. Except for the first 500 ml of expiration during closing volume measurement, expiratory flow transients must not exceed 0.7 lps. Unacceptable flow transients are defined as deviation from the required flow which persists during expiration of more than 300 ml.

C. Difference between inspired and expired VC must be less than 5%.

D. Differences in VC between blows must not exceed 10%.

E. There must not be a step change in the expired N_2 concentration with continued cardiogenic oscillations after the step. The causes of such step changes are obscure but are probably not related to airway closure. If such curves are accepted the onset of Phase IV will frequently be read as the volume at which the step occurs.

5. Measurements/Calculations

Ideally on all subjects 3 acceptable tracings will be obtained. The mean of the 3 values of each measurement is taken as the final value. When only 2 readable tracings are obtained, the mean of the 2 values is used. When only one readable tracing is obtained, the subject is discarded from the series. Readers must keep a careful track of the number of individuals with 3, 2, 1, and 0 readable curves. Figure 4 depicts a sample closing volume tracing.

CLOSING VOLUME AND CLOSING CAPACITY

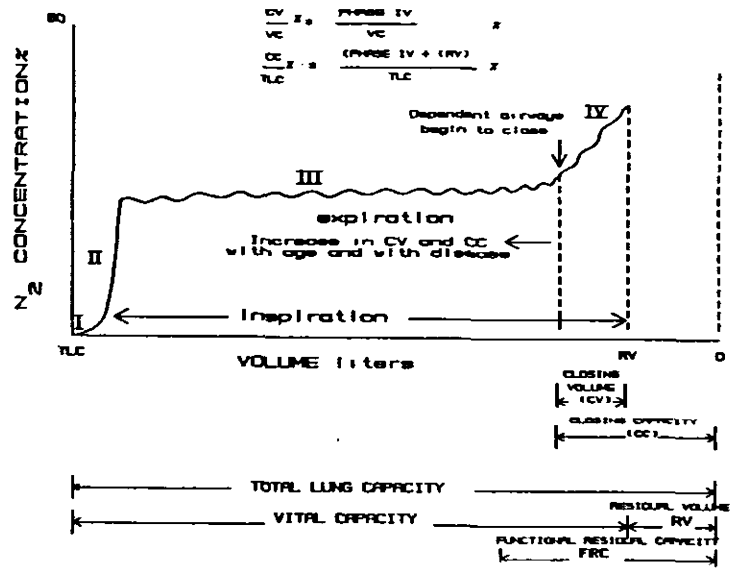


Fig. 4 - Characteristic changes in expired nitrogen concentration which occur during a vital capacity maneuver following an inhalation of 100% oxygen.

A. Closing Volume (CV)

The onset of phase IV should be determined by the best-fit line drawn by eye through the latter half of phase III. The point of final departure from this line is the onset of phase IV. In some subjects there is a sharp drop in N_2 concentration after the onset of phase IV. Occasionally this can intersect the line drawn through phase III. Under these circumstances the onset of phase IV is taken as the first definite point of departure of the N_2 tracing from the best-fit line. The closing volume is the volume from the onset at phase IV to residual volume (RV). CV is expresses as % of the expired vital capacity (VC).

B. Slope of phase III

The slope of phase III is determined by the best-fit line, between 70% VC and the onset of phase IV. The slope is reported as the angle formed by the line of best fit with the horizontal axis.

The analysis of these curves cannot always be made in a totally objective manner. On some curves in particular, the onset of phase IV is difficult to determine and when the same reader blindly reads such curves twice, there is not very good agreement between the two measurements. This appears to be due to differences between individuals, because when a subject generates such a curve, it is likely that all curves that he generated will be difficult to analyze. On the other hand, if a subject generates a curve which is easy to analyze, in all likelihood, all curves obtained from him will be easy to analyze. Obviously, curve readers will have to use good judgment and they may decide that some curves, although conforming to the criteria of acceptability, are unreadable and therefore must be rejected. It is impossible at present to establish a set of rigid rules governing such cases.

Prediction Values:

For purposes of comparison with general population studies, CV and Phase III slope values will be compared with the predictions of Buist.

Buist: Closing Volume:

CV/VC % = 0.318 Age (yrs) + 1.919 $\pm$ SEE 4.61 (MEN AND WOMEN)

Phase III Slope:

$\Delta N_2/L$ = 0.710 + 0.010 Age (yrs) $\pm$ SEE 0.43 (Men)

$\Delta N_2/L$ = 1.036 + 0.009 Age (yrs) $\pm$ SEE 0.57 (Women < 60)

$\Delta N_2/L$ = 1.777 + 0.058 Age (yrs) $\pm$ SEE 1.30 (Women > 60)

REFERENCES

Suggested Standardized Procedures for Closing Volume Determinations (Nitrogen Method), Division of Lung Diseases, National Heart and Lung Assoc. 1973

Buist, A.S. and B.B. Ross. Predicted values for closing volume using a modified single breath N_2 test. Am. Rev. Resp. Dis., 107:744-752, 1973.

Buist, A.S. and B.B. Ross. Quantitative analysis of the alveolar plateau in the diagnosis of early airway obstruction. Am. Rev. Resp. Dis., 108:1078-1087, 1973.

EXPIRATORY FLOW-VOLUME CURVE

IIb. 1. Instrumentation:

A. An Ohio 840 Rolling Bar Spirometer

B. Hewlett-Packard X-Y Recorder HP 7046A for volume-time curves.

C. Hewlett-Packard X-Y Recorder HP 7046A for flow volume curves. (slowing speed Y axis = 76 cm/sec and acceleration Y axis = 6350 cm/sec).

D. Large base flexible plastic tubing and large rubber mouthpiece. If during the performance of a forced vital capacity maneuver expired airflow is plotted against expired (or lung) volume, the resultant relationship has a characteristic configuration as depicted in Fig. 5. Expired flows increase readily to a peak and then decrease relatively linearly with decreasing lung volume. Obstructive and restrictive lung disease results in flow patterns different from the normal response (Fig. 5), providing a clear, graphical display of characteristic patterns of pulmonary disease.

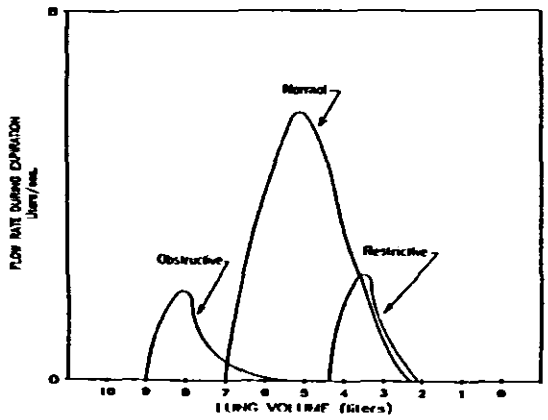


Fig 5 - Schematic representation of typical maximal expiratory flow volume curves from a normal subject and from patients with restrictive and obstructive lung disease. Airway obstruction results in low expired flows both in absolute terms and relative to the lung volume. In contrast, restrictive disease results in low flow in absolute terms, but normal or slightly high flows when corrected for lung volume.

With nose occluded, the subject is connected to the spirometer through a large rubber mouthpiece and corrugated tubing. Simultaneous monitoring of expired air flow against: a) lung volume and b) time are traced on the XY axis of individual recorders.

2. Calibration:

Initial calibration of volume is done using the 1 liter glass syringe previously checked against a 13 liter Collins spirometer. Flow rate is determined with a Fisher rotometer which has been previously calibrated against a Tissot spirometer with room air. Individual calibration will be done by the operator for each patient, using the function known on the Ohio 840 spirometer.

3. Procedure:

A. Set function knob at operate, BTPS at the appropriate setting, and piston variation knob at 5 liters.

B. With nose occluded, have subject go onto mouthpiece and breathe normally on room air.

C. Following 2-3 normal breaths, instruct subject to inspire maximally (TLC).

D. Lower pens of both recorders to contact surface and request a maximal effort of fast exhalation followed by a maximal inspiration.

E. Disconnect subject; remove nose clip; let subject relax and flush spirometer.

F. Repeat steps A-E until three acceptable curves have been obtained.

4. Criteria for Acceptability of Measured Data:

A. Inspired and expired volumes must check within 5% and duplicate curves within 5%.

B. Three acceptable curves are required.

5. Measurements/Calculations:

Measurement of maximum instantaneous flow over portions of the expiratory volume are determined by the linear distances (mm) of the excursion height, converted to volume (ℓ) with the appropriate conversion factor determined from the electrical calibration. FEV_1, FVC, and MMF will be measured as explained in "Spirometry" section. These values will be used in Studies II, IV, and V. Measurements of maximum instantaneous flows will be made on three acceptable vital capacity loops that do not vary by more than ± 5% (that is, not less than 5% of the largest EVC). Flow will be measured after a volume equal to 50% and to 75% of the EVC has been expired. $V_{max}50$ and $V_{max}75$ corresponding to: I) the largest EVC and, II) the mean V_{max} of the two or three acceptable curves (II) will be measured and recorded. V_{max} by method (I) will be used in studies I, IV, V, and by method (II) in studies II and III.

Prediction Values:

Values for VC and MEF flow measured with the rolling bar spirometer in these studies will be compared with the following predicted values of Knudson et al.

Vmax50 Table:

<25 years old 0.081 Age + 0.051 Ht - 4.975

>25 years old 0.015 Age + 0.069 Ht - 5.400

Vmax75 Table:

<25 years + .032 Ht - 2.455

>25 years old -0.012 Age + .044 Ht - 4.143

NOTE: Applies to Studies II, IV, and V - when Ohio 840 output was taped for later display and analysis. Regarding Instrumentation: add 1) 4 channel Hewlett-Packard tape recorder (multiple speed); 2) Tetronix storage oscilloscope.

3.D Add "Turn on tape recorder, identify patient and time of day," clear storage oscilloscope screen.

E.E Add "Turn off tape recorder."

Additions to Above:

Study I. FEV_1 and FVC measured from Ohio 840 records will be compared to those obtained on Collins spirometer and used for future prospective studies.

Study II. Add to above instrumentation procedures and measurement: During Study II flow and volume signals from Ohio 840 spirometer were also recorded on a Superscope 301A cassette recorder. The taped data were analyzed using a micro-processor based on an Intel 8080 system with 4 analog to digital channels and 4 digital to analog channels and a 2K circulating memory for each channel. The data was displayed on a two channel oscilloscope with cursor signal on the two channel. The operator used the cursor, under control of a basic program, to mark the curves to be analyzed.

Studies IV and V. Add to above technique description: Flow and volume from Ohio 840 spirometer was displayed on a Tetronix 411 storage oscilloscope and recorded on magnetic tape using a Hewlett-Packard 3960 instrumentation recorder at speed of 3.75 ms/sec. From the taped data played back at 15/16 ms/sec we obtained a paper record using a Hewlett-Packard X_Y_Y 7046A recorder to measure FVC, V_{50}, V_{75}, and ΔV_{50} $He\text{-}O_2$.

III. SINGLE BREATH CARBON MONOXIDE DIFFUSING CAPACITY

1. Instrumentation

Equipment required:

A. 30 liter test bag for gas containing approximately 0.2% CO, 10% He, 21% O_2, 69% N_2

B. A bag-in-box respirometer (W. E. Collins)

C. Infared CO analyzer, or gas chromatograph

D. Linear He analyzer (15% full-scale)

E. Solenoid valve for direction of expiratory volume into a 5 liter sample bag or to room air

F. Mouthpiece - large rubber

G. 9 liter Collins Spirometer

H. Gas Cylinder and regulator

I. Recording supplies - paper, pens, ink

J. Stopwatch

K. 5 liter rubber sample bags

The measurement of diffusion capacity is the rate at which CO disappears from the lung into the blood. The nature of this disappearance beginning at the initiation of inspiration is exponential with time, the slope of the curve being dependent upon the diffusivity of the CO molecule along the whole diffusion pathway from alveolus to hemoglobin molecule.

The bag-in-box system (See Fig. 6) is completely flushed and the bag filled with the test gas mixture from which a sample is analyzed. The subject is seated, nose occluded, and kymograph drum speed set at 160 mm/min. The subject inspires to TLC, holds breath, goes onto the mouthpiece, exhales to RV, then inspires to TLC as rapidly as possible and holds his breath for 8-12 secs. He then exhales rapidly to RV during which the first 800-1000 ml. of VC is expired to room air and the remainder directed to a collection bag for subsequent analysis. From the changes in gas concentration between inspired

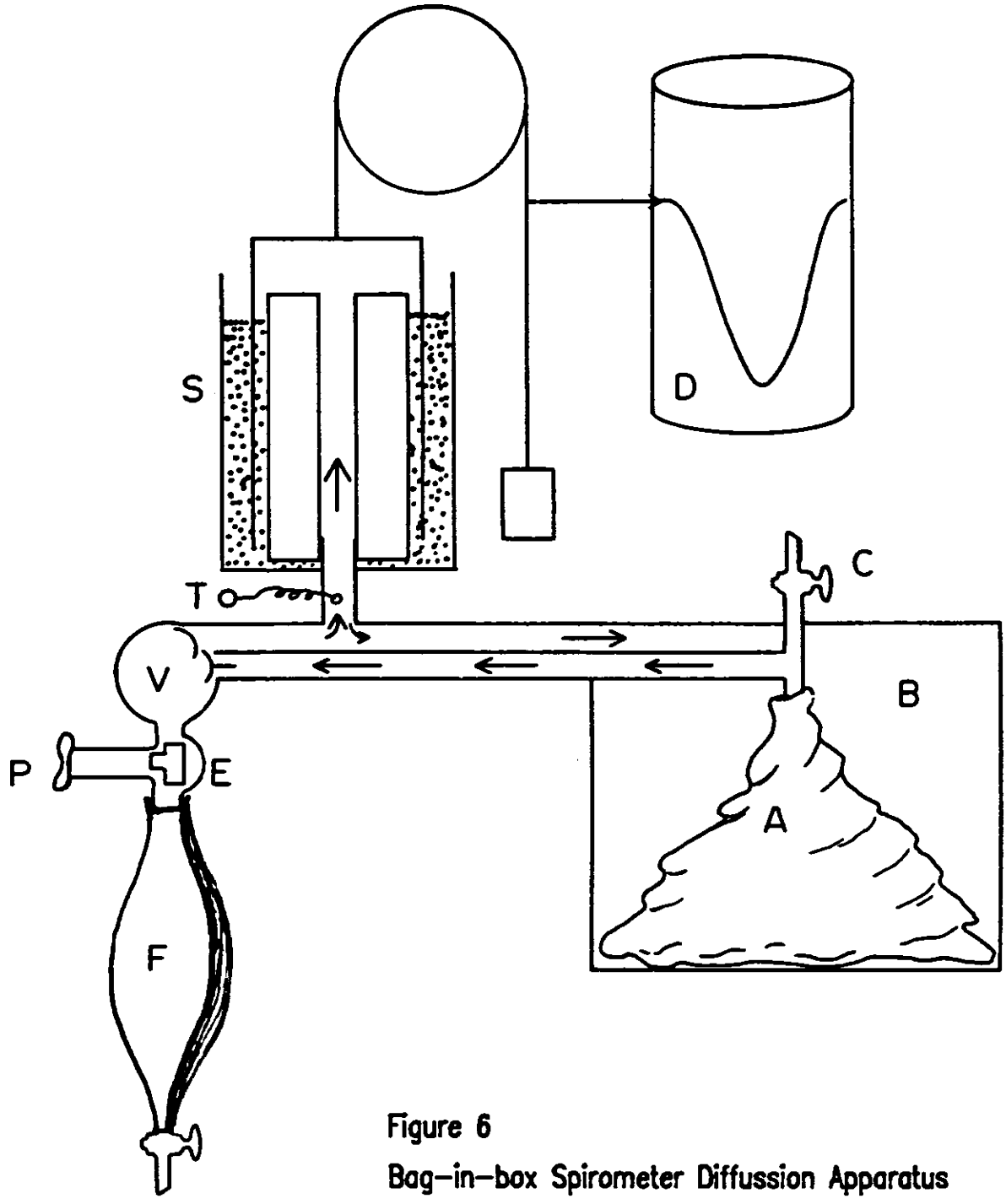


Figure 6
Bag–in–box Spirometer Diffussion Apparatus

A – 30 litre bag of test gas
B – rigid box
C – stopcock for filling A
V – solenoid valve permitting expiration into the spirometer (s) and inspiration from the bag (A) via flexable rubber tubing
E – large bore – 3 – way valve
P – mouthpiece
F – 5 litre sample bag
S – 8 litre recording soirometer
D – kymograph drum revolving 2.66 mm/sec.
T – thermometer

and expired gas and the associated spirometric tracing, the D_LCO and He dilution lung volumes may be calculated.

2. Calibration

(1) Testing for leaks:

Check Spirometer weekly for:

A. Deformities in spirometer bell

B. Leaks around water seal

C. Accuracy of kymograph drum speed

D. Leaks in the 30 liter test gas bag

E. Solenoid valve operation

(2) CO and He meters

Calculation of single breath D_L requires calculations of ratios of two measurements of He concentration and two measurements of CO concentration. Since only the ratios of He and CO are important, precise measurements of either He and CO concentration is not essential.

CO meter calibration and He meter linearity procedure:

A. Scales should read zero with no power, adjust mechanical zeros as required

B. Following several hours of warm-up, flush system with room air and zero both instruments

C. Introduce sample of test gas and adjust CO analyzer gain controls to approximately 90% of full scale deflection. Thus, a slightly higher CO% (e.g., 0.3) will not read greater than 100% full scale. Once set, do not alter gain control further.

Whenever a non-linear CO analyzer is used, it is necessary to carefully construct a calibration curve from which meter

deflections can be used to determine the actual concentration of CO in the test sample. Once the calibration curve is established, the gain adjustment of the reference gas should always be set at the identical reference value.

Alternately, if CO samples are analyzed using gas chromatography, then the recorded peak heights of reference gas curves are used to establish a proportionality for determination of test sample concentrations.

With 10% He, the He meter should read 8-12% with appropriate adjustment of the gain control as required.

K. Repeat zero and test gas checks; readjust zero and upper scale deflections as necessary. Verify further with test gases of different concentrations.

3. Procedure

(1) Check depletion level of CO_2 absorber system

(2) Flush system rebreathing circuit leaving test gas bag completely evacuated

(3) Fill the reservoir balloon (bag-in-box) with test gas

(4) Seat subject and occlude nose

(5) Set kymograph speed at 160 mm/min

(6) Have subject inspire to TLC, go onto mouthpiece, exhale to RV, then inspire to TLC as rapidly as possible. Hold breath at end of inspiration for approximately 10 secs then expire forceably to RV

(7) After discarding the initial "dead space" (800-1000 ml portion) of the expired volume into room air, activate the solenoid switch and collect the remainder of the expirate.

(8) Have subject come off mouthpiece and breath room air

(9) Clamp, remove alveolar sample balloon and analyze contents.

(10) Flush rebreathing circuit thoroughly

(11) Repeat steps (1-10) following a minimum of 5 minutes before retesting to insure complete washout of CO and He from lungs. Repeat test 3 times.

4. Criteria for acceptability for single breath D_L.

Acceptable tracings will have a sharp and rapid inspiration, level hold for 8-10 seconds from the beginning of inspiration to start of sample collection. Rapid expiration of the appropriate volume will show about the same vital capacity and expired gas values from trial to trial.

5. Measurements/Calculations:

$D_L CO$ is determined according to the following equation:

$$D_L CO \text{ (ml/min/mmHg)} = \frac{VA \times 60}{(PB - 47)_t} \times \ln \frac{F_A CO_9}{F_A CO_t}$$

where:

$$F_A CO_O = F_I CO * \frac{F_A He}{F_I He}$$

where: VISTPD = volume inspired corrected to STPD

VD = assumed dead space volume of .150 ℓ

$F_I He$ = % inspired He

FeHe = % expired He ($F_A He$)

60 = # secs in one min.

Bp-47 = Barometric pressure - water vapor pressure at body temperature of $37^{\circ}C$.

t = time in secs of breath held

F_ICO - % inspired CO

F_ACO_t = % expired CO

ln = natural log raised to power of expression in parenthesis

Prediction Values:

For purposes of comparison with general population studies, DL values will be compared with the following predictions of Ogilvie, et al. and Rankin, et al.

Ogilvie:

DL (ml/min/mm) = 18.85 surface area - 6.8

Rankin:

DL (ml/min/mm) = 2.0474 Ht (in.) - .166 Age (Yrs) - 102.62

REFERENCES

Kanner, R.E. and A.H. Morris, eds., Carbon Monoxide Diffusing Capacity, Ch. IV, "Clinical Pulmonary Function Testing," 1975.

Measurements and Concepts of Alveolar - Capillary Diffusion. Laboratory Operations Manual, Pulmonary Function Laboratory, University of Wisconsin, 1971.

Ogilvie, C.M., R.E. Forster, W.S. Blakemore and J.W. Morton. A standardized breath-holding technique for the clinical measurement of the diffusing capacity of the lung for carbon monoxide. J. Clin. Invest., 36:1-8, 1957.

Rankin, J., J.B.L. Gee and L.W. Chosy. The influence of age and smoking on pulmonary diffusing capacity in healthy subjects. Med. Thorax, 22 (3):282, 1965.

HELIUM-OXYGEN FLOW VOLUME TEST

1. Instrumentation

   A. An Ohio 840 Spirometer

   B. Esterling Angus X-Y Recorder

   C. Ohio Demand Valve

   D. Large bore 3-way stopcock

   E. Appropriate tubing and connectors

   Following completion of three flow volume loops, the subject inspires four slow VC breaths of an 80% Helium/20% O_2 mixture. At RV of the fourth breath the subject is turned into a spirometer containing the same 80% Helium/20% O_2 gas mixture, and instructed to inspire to TLC followed by a maximal forced expiration. Following this maneuver, the subject returns to the He/O_2 circuit for several more breaths and the process is repeated.

2. Calibration

   Initial calibration of volume is done using the 1 liter glass syringe previously checked against a 13 liter Collins spirometer. Flow rate is determined with a Fisher rotometer which has been previously calibrated against a Tissot spirometer both with air and with the 80% Helium/20% O_2 mixture. Individual calibration will be done by the operator for each patient, using the function knob on the Ohio 840 spirometer.

3. Procedure

   A. Set function knob at operate, BTPS knob at the appropriate temperature setting, and piston variation knob at 5 liters.

   B. Have subject perform three flow volume loops (see Section 11a).

   C. Flush spirometer and fill with 80% He, 20% C_2 mixture.

D. Turn 3-way stopclock connecting subject to the inspired gas mixture via the demand valve.

E. Request four slow deep breaths to TLC expiring to RV each time.

F. At the end of expiration, turn subject into spirometer with the 3-way stopcock.

G. Request a maximal inspiration (to TLC) followed by a maximal forced expiration (to RV).

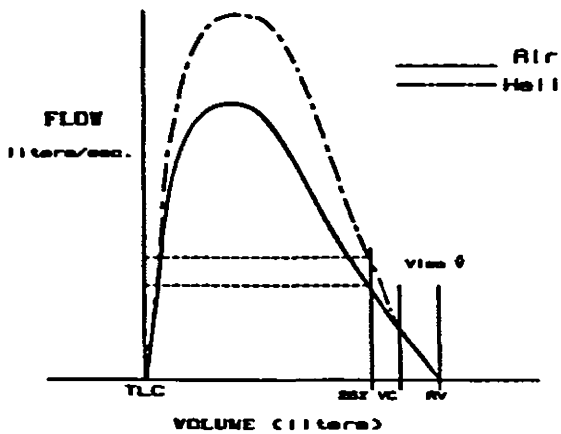
H. Return subject to He/O_2 mixture for several more breaths (while spirometer is rinsed) and repeat steps C-G.

4. Criteria of Acceptability

A minimum of three acceptable tracings are required. The EVC and IVC must correspond within 10% of each other. The agreement between the helium-oxygen curve and the curve on room air must be within 5% of each other in regard to EVC. If after a group of test runs there is lack of agreement in EVC, the loop that has the largest EVC, meeting the requirement of EVC/IVC being within 10% of each other, is chosen as the test run.

5. Measurements/Calculations:

The helium/oxygen curve is superimposed on the room air curve at RV (Fig. 7). The point of intersection of flow is found. The volume at which the flow rates were identical (isovolume point) on both $He + O_2$ air, that is, independent of density, is identified and expressed as a percent of VC. In addition, $V_{max\ 50}$ is measured both on room air and helium.



$$\Delta\dot{V}_{Max}\ He\ air = \frac{\dot{V}_{Max50}\ He - \dot{V}_{Max50}\ air}{\dot{V}_{Max}\ air} \times 100 = \quad \%$$

Ranges: $\dot{V}iso\ V$ range 3-40% VC air

$\Delta\dot{V}_{Max50}$ He = 0-120%

Fig. 7 Maximal expiratory flow-volume curves breathing air and air $He + O_2$. The response to $He + O_2$ is expressed as the percentage of increase in flow compared to flow in air at 25 percent of VC; i.e., $BC/AB \times 100 = \Delta\dot{V}_{25}$. The point where flows breathing air and $He + O_2$ are the same as identified and $Viso\ \dot{V}$ is measured from this point to RV.

REFERENCES

Hutcheon, M., P. Griffin, H. Levison, and N. Zamel. Volume of isoflow. A new test in detection of mild abnormalities of lung mechanics. Amer. Rev. Resp. Dis., 110:458, 1974.

APPENDIX IX

Hematology Procedure

Field Operations Manual

NIOSH Contract No. 210-76-0175

I. Hemoglobin Determination.

Quantitative estimation of hemoglobin is used as a routine test to detect the existence and/or degree of anemia. In the hemoglobin determinations red blood cells are lysed to release the hemoglobin fraction. The hemoglobin is then quantitatively converted to cyanmethemoglobin by the addition of Drabkin's Reagent.

Reagents.

A. Drabkin's Reagent

Sodium bicarbonate	1.0 gm
Potassium ferricyanide	0.2 gm
Potassium cyanide	0.005 gm

Dissolve in distilled water and dilute to one liter. Store in a dark bottle. The solution is stable for one month if protected from light and evaporation.

Caution: Cyanide salts and solutions are poisonous and should be handled carefully. Pipette solutions with a bulb. Mix solutions by swirling. If any of the compounds are spilled, clean them up quickly and carefully. When disposing of solutions in the sink, wash down generously with cold water.

Method for Cyanmethemoglobin Determination.

A. Calibration of Fisher hemoglobin detector.

Use the commercial standard (Hycel). The undiluted standard in this method represents 20 gm. percent of hemoglobin.

1. Prepare cuvettes as follows:

Volume of Standard	Volume of Drabkin's Reagent	Gm % Hgb
6 ml	2 ml	15
2 ml	6 ml	5

2. Set the Hi control knob on the machine at 15 with the 15 gm % standard and the Lo knob at 5 with the 5 gm % standard.

3. Read the Fisher artificial standards (in sealed tubes) against the hemoglobin standards.

4. Plainly label the Fisher standard tubes with the values thus obtained.

B. Hemoglobin Method.

1. Dispense 5 ml of Drabkin's Reagent in Fisher cuvettes.

2. Add 0.02 ml of whole blood to the solution (1-25- ml dilution).

3. Set the Hi and Lo knobs at the values indicated on the sealed artificial standards provided with the instrument.

4. Insert the cuvette with test sample and determine the hemoglobin in gm % on the direct reading scale.

II. Oxyhemoglobin Determination.

In this measurement hemoglobin is converted to oxyhemoglobin in the presence of dilute or weak alkali solutions. This determination measures active hemoglobin; hence, the values may be lower than cyanmethemoglobin in the same samples.

Reagents.

A. 0.04% Ammonium Hydroxide

Dilute 0.4 ml of concentrated NH_4OH to 1.0 liter using distilled water

Method for oxyhemoglobin determination.

1. Calibrate the detector as described previously.

2. Dispense 5.0 ml of 0.04% NH_4OH into cuvettes.

3. Add 0.02 ml of capillary or venous blood.

4. Mix well.

5. Read immediately or within 24 hours.

III. Hematocrit Determinations.

Method

1. Select capillary tubes approximately 7 cm long with a 1.0 mm internal diameter.

2. Using capillary action fill the tube with blood to within 1.0 cm of the end.

3. Plug one end with plasticine.

4. Centrifuge the tubes in a microhematocrit centrifuge at 5000 xg for 10 minutes.

5. Measure the length of the blood column, including the plasma.

Normal values.

Hemoglobin	Hematocrit
Adult females 12-16 gm %	42 ± 5
Adult males 14-18 gm %	47 ± 7

APPENDIX X

Antigen Preparation

Field Operations Manual
NIOSH Contract No. 210-76-0175

Rationale:

The presentation of a foreign substance to the immune system elicits the production of antibodies which are directed toward the foreign substance. The nature of the antibody produced, in part, determines the immunopathology of the disease. The production of antibodies of the IgE class would result in immediate allergic reactions upon subsequent challenge. Conversely, the production of antibodies of the IgG class and, occasionally of the IgM class, would favor the elicitation of a hypersensitivity pneumonitis reaction in the lung on re-exposure to the foreign substance.

It is possible to ascertain the presence of antigen specific IgE antibodies which evoke allergic reactions by intradermal skin testing (Appendix XI) and the presence of IgM or IgG antibodies directed toward specific antigens by precipitation (Appendix XII). The determination of both the allergic antibody and the precipitating antibodies depend heavily on the preparation of extracts from organic material. Extracts from organic material can be prepared from pulverized washed grains, grain dusts, pulverized grain insects and from culture filtrates of bacteria and fungi known to cause hypersensitivity pneumonitis.

Reagents:

1. The panel of organic material used for saline extraction of antigenic materials:

Intact Grains	Respirable Grain Dust	Insects and Mites
Durum wheat	Durum wheat	Adult granary
Spring wheat	Spring wheat	and rice weevils-mix
Barley	Barley	
Corn	Corn	Confused flour, Dermestid
Rye	Rye	and Black Carpet Beetle-mix
Oats	Oats	
Sunflower seeds	Sunflower	Mold, house and grain
Small seeds	Seeds	mite-mix
Soybeans		
Culture Filtrates		Culture Filtrates
Thermophilic bacteria		Fungi
Micropolyspora faeni-UW		A. fumigatus-1
Micropolyspora faeni-Marshfield		A. fumigatus-5
Micropolyspora faeni-Greer		A. fumigatus-6
		A. fumigatus-9
T. candidus-Medical College of Wisconsin		A. fumigatus-1022
T. candidus-UW		A. flavus
		A. niger

Culture Filtrates Thermophilic Bacteria	Culture Filtrates Fungi
T. sacchari	A. clavatus
	Aureobasidium
T. vulgarus-Marshfield	Alternaria species
T. vulgarus-Hollister/ Steir	c. albicans
	Cephalosporium
T. viridans	Fusarium
Settled dust	Hormodendrum
	Mucor
	Phoma
Settled dust I-Rafter Dust	Trichoderma
Settled dust II- Holding tank	P. casei
Settled dust III-Dump Station	P. rubrum
Hay and Dusts	Other antigen
Moldy Hay	Pigeon serum
House Dust	

B 0.85% Saline
C Whatman No. 1 filter paper
D Dialysis tubing
E Coca's non-allergenic buffer (Hollister-Steir Laboratory)
F Glycerine
G Sterile dropper vials - 2.0 ml (Greer Laboratories)
H 0.1 M borate citrate buffer pH 8.4 (Appendix XII).

Methods:

1. Preparation of saline extracts of grain, respirable grain dust, insects and mites.

   a. It is necessary to prepare a fine powder of intact grains and insects. Intact grains can be pulverized in a Ball mill, whereas insects and mites can be ground into a fine powder with a mortar and pestle. The growth conditions for insects and mites are shown in Table I.

   b. The powder is suspended in 0.85% saline in a 1:10 W/V ratio and incubated with constant agitation at 4°C for 24 hours.

   c. The mixture is allowed to settle for 2 hours at 4°C and the supernatant fluid removed.

   d. The supernatant fluid is filtered through a Buchner filter using Whatman No. 1 filter paper.

e. The effluent is placed in dialysis tubing and dialyzed against running, cold tap water for 24 hours.

f. After dialysis, the extract is concentrated to 20-50 ml. in the dialysis tubing by perevaporation.

g. The concentrated extract is placed in a new dialysis tubing and dialyzed against 0.85% saline at 4°C for 24 hours.

h. The extract is lyophilized by conventional methods.

i. The protein content of each extract is determined by Micro-Kjeldahl protein determinations (A10.1). Results are expressed as protein nitrogen units per mg. of lyophilized material.

j. Store lyophilized extracts in a -20°C freezer.

2. Preparation of saline extracts from culture filtrates of bacteria and fungi.

a. The broth cultures of each organism are grown as shown in Table II.

b. The broth culture is centrifuged at 1000 xg for 30 minutes at 4°C.

c. The supernatant fluid is carefully decanted into a beaker without disturbing the precipitate.

d. The supernatant fluid is filtered through Whatman No. 1 filter paper using a Buchner funnel.

e. The effluent is placed in dialysis tubing and dialyzed against cold, running tap water for 24 hours.

f. The remaining steps of the procedure are identical to those described for preparation of grain, respirable grain dusts and insects and mites.

3. Preparation of immediate skin test reagents.

a. Remove extracts of grain, grain dusts, and insects from the freezer.

b. Using sterile technique, prepare 50 ml of sterile Coca's buffer containing 50% glycerine v/v.

c. Using the PNU/mg determinations, weigh out 200,00 PNU of each lyophilized extract.

d. Add each extract to separate sterile dropper bottles. Label each bottle with the name of the extract and the date.

e. Using sterile technique, add 2.0 ml of the Coca's buffer with 50% glycerine. Note that the fluid concentration is 100,000 PNU/ml.

f. Solubilize the extracts by gentle agitation.

g. Store the reconstituted extracts at 4°C.

4. Preparation of extracts for determination of precipitating antibodies.

   a. Remove lyophilized extracts of grain, respirable grain dust, insects and the culture filtrates of bacteria and fungi from the freezer.

   b. Weigh out 15 mg of each of the extracts.

   c. Place extract in a small 2.0 ml screw-topped vial. Label the vial with the name of each extract and the date.

   d. Add 1.0 ml of the 0.1 ml borate citrate buffer pH 8.4 to each vial and replace the screw top. Note that the final concentration of antigen is 15 mg/ml.

   e. Store the reconstituted extracts in a refrigerator.

Normal or Reference Values:

The reference values, in terms of protein nitrogen units (PNU) for grain, respirable grain dusts, insects and settled dusts are shown below.

	Intact Grains		Respirable Dust	
	PNU/ MG	µg Protein/ µg of Solid	PNU/ mg	µg Protein/ µg of Solid
Durum Wheat	12000	760	5600	350
Spring Wheat	12600	790	5800	325
Barley	12600	790	4300	270
Corn	8200	515	3600	225
Rye	8000	500	5000	310
Oats	11300	710	5000	310
Sunflowers	12700	790	2900	180
Small Seeds	7000	435	---	---
	Insects			
Weevils-Mix	12900	810		
Beetles-Mix	9900	620		
	Settled Dust			
Settled Dust I	---	---	5300	300
Settled Dust II	---	---	4600	290
Settled Dust III	---	---	5100	32

Limitations of the Procedure:

Most organic antigens can be extracted into saline and this method has proven suitable for extraction of antigenic material from culture filtrates of bacteria and fungi known to cause hypersensitivity pneumonitis. One must be aware, however, that the metabolic antigens produced by bacteria and fungi differ quantitatively and qualitatively during growth. Therefore, careful consideration must be given to the dynamics of antigen production by each organism and the cultures must be harvested during peak antigen production. The quantitation of antigens can be achieved by two dimensional cross-immunoelectrophoresis using a human serum with known serological reactivity to the metabolic antigens (A10.2). Samples of culture fluids can be assayed at weekly intervals and the cultures harvested when there is an increase in the length of precipitin arcs or in the numbers of precipitin arcs. Since there is some batch to batch variation in serological reactivity of culture filtrates from bacteria and fungi, it is necessary to produce enough of each extract to test on control populations with the same batch of antigen extract.

There is also variation in serological reactivity within strains of the same species of bacteria or fungi and/or the immune response to these organisms is strain specific. These phenomena have been observed in precipitin analyses using extracts from A. fumigatus, Penicillium and the thermophillic actinomycetes. It is, therefore, necessary to include in the screening panel for precipitating antibodies several extracts of different strains of the same species of certain bacteria and fungi.

Extracts of the thermophillic bacteria and certain fungi were not included in the panel used to ascertain immediate skin test reactivity. Many of these extracts, particularly the thermophillic bacteria, evoke toxic skin reactions which render them useless in determining skin reactions. Moreover, these agents usually evoke hypersensitivity pneumonitis which lacks a true allergic immunological component. Hence, even if the extracts were suitable for use in immediate skin tests, one would expect negative skin tests with these extracts.

The use of saline extracts of grain and respirable grain dusts for immediate skin tests has a major limitation. Many of the proteins of grain are insoluble in saline, including the entire gluten complex consisting of both gliadin and glutenin. Approximately 80% of the endosperm protein is associated with the gluten complex (A10.3). The endosperm constitutes 70% of the total protein in the grain. Only the albumin and globulin fraction are extracted with saline. Hence, the saline extracts of grain used for skin tests measures the presence of atopy to albumin or globulins and not the atopic potential of other grain proteins.

REFERENCES
Appendix 10

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A10.2 Weeke, B: Crossed Immunoelectrophoresis, Scand. J. of Immunol., 1973, 2(Suppl 1):47.

A10.3 Nerxheimer, H: The hypersensitivity to flour of bakers apprentices. Acta. Allergol. (KbH), 1973, 28:42.

TABLE I. The Growth Conditions for Insects and Mites.

		Growth Media
Granary Weevils Adults	Sitophilus granarius	Whole Wheat
Rice Weevils Adults	Sitophilus Oryzae	Whole Wheat
Confused Flour Beetle Adults and Larvae	Tribolium confusion	Whole Wheat with 5% Brewers Yeast
Black Carpet Beetle Larvae	Attagenus megatoma	Purina Lab Chow with 5% Brewers yeast
Dermestid Beetle	Trogoderma glabrum	8 Purina Lab Chow* 3 Wheat Germ 3 Dry Milk 1 Brewers yeast 1 Meat & Bone Meal
Mold Mite	Tyrophagus putrescentiae	3 Brewers Yeast* 1 Wheat Germ
House Mite	Glyophagus domesticus	3 Brewers Yeast 1 Wheat Germ
Grain Mite	Acarus species	3 Brewers Yeast 1 Wheat Germ

*weight:weight ratio

All insects were maintained at 27 ± 1°C and 60% relative humidity with a 16:8 Light:Darkness photo ratio. All insects were screened from the growth medium and then examined microscopically for remaining media. When necessary, the insects were rescreened and further separated from remaining media. The insects were then promptly frozen and stored in a -40°C freezer.

TABLE II. Growth Conditions for Bacteria and Fungi.

	No. 1 liter Prescription Bottles	Growth Media	Amount of Media (ml)	Incubation Temp	Incubation Time Weeks
Asp. fumigatus 1*	48				
Asp. fumigatus 5*	48				
Asp. fumigatus 6*	48	Czapek-Dox	200	37°C	3
Asp. fumigatus 9*	48				
Asp. fumigatus 1022**	48				
Asp. flavus	48				
Asp. niger	48				
Asp. clavatus	48	Czapek-Dox	200	Room temp	4
Aerobasidium	48	Czapek-Dox	200	Room temp	5
Alternaria	48	Czapek-Dox	200	Room temp	5
C. albicans	48	Sabourauds Broth	200	37°C	5
Cephalosporium	48	Sabourauds Broth	200	Room temp	5
Fusarium	48	Czapek-Dox	200	Room temp	5
Hormodendrum	48	Czapek-Dox	200	Room temp	5
Mucor	48	Czapek-Dox	200	Room temp	5
Phoma	48				
Trichoderma	48	Czapek-Dox w/ 30 g/L dextrose	200	Room temp	5
Moldy Hay		Saline extract			
House Dust		Saline extract			
M. faeni-Marshifield					

TABLE II (continued)

M. faeni-U.W.		Trypticase Soy Broth***		56°C	1
M. faeni-Greer Labs					
T. sacchari		Trypticase Soy Broth***		56°	1
T. candidus-Med. Coll. Wis.		Trypticase Soy Broth***		56°	1
T. candidus-U.W.		Trypticase Soy Broth***		56°	1
T. iridans		Trypticase Soy Broth***		56°	1
T. vulgaris-Marshfield		Trypticase Soy Broth***		56°	1
T. vulgaris-Hollister Steir		Trypticase Soy Broth***		56°	1
T. vulgaris-Marshfield		Trypticase Soy Broth***		56°	1
Pen casei	48	Czapek-Dox	200	Room temp	5
Pen. rubrum	48	Czapek-Dox	200	Room temp	5
Pigeon Serum		---		---	-

*Isolated from sputum cultures

**American type culture collection

***Double dialysis technique of Edwards (Med. Lab. Technol., 28:172, 1971)

APPENDIX XI

Skin Testing Protocol

Field Operations Manual
NIOSH Contract No. 210-76-0175

RATIONALE

It is possible to reproduce both allergic and cell mediated immune reactions by introducing small amounts of antigenic material into the skin. Based on the time course of the reaction, the skin tests can be classified as immediate reactions which occur within 20 minutes and delayed reactions which are noted after 24-48 hours. Although both skin test reactions have similar morphologies, the immunological mechanisms which mediate the reactions are different. The immediate skin test reaction is due to the interaction of allergens with antibodies of the IgE class which are bound to mast cells and basophils in the skin. The allergen antibody complex intiates the release of histamine, SRS-A and other mediators from the mast cells and basophils. The pharmacologically active agents increase vasoconstriction and increase vascular permeability which results in a localized area of edema called a wheal which is surrounded by a less defined area of redness called an erythema. Since allergen specific IgE is only found in allergic individuals, the immediate skin test is a direct measure of the allergic or atopic status of the test subject. Moreover, in respiratory allergies, skin tests are often positive when respiratory challenge fails to reproduce the disease (A11.1).

The prick test is the method of choice for determining immediate skin test reactivity in large population studies. The method is reasonably safe with minimal systemic antigen absorption. Moreover, one can perform large numbers of tests without discomfort to the test subject. Unlike the intradermal tests, nonspecific reactions seldom occur with the prick test.

The delayed skin reaction is the result of the interaction between sensitized lymphocytes and antigen introduced into the skin. The sensitized

lymphocytes are, in effect, memory cells which remember previous exposure to selected bacterial, viral and fungal agents (i.e. tuberculosis, mumps, streptococcal infection, Candida and trichophyton infections). Upon introduction of these antigens into the skin, sensitized lymphocytes localize in the skin test area and release small molecular migration and metabolic activity of other cell types including the peripheral blood monocytes. Monocytes with increased metabolic activity are localized in the skin test area and initiate an acute inflammatory response. The inflammatory response results in localized swelling called induration surrounded by an area of erythema. Hence, the delayed skin test reaction to selected antigens is a measure of the functional status of the cell mediated immune system. It follows that the delayed skin reactivity reaction is useful in determining whether there is decreased delayed hypersensitivity if reactions are observed it would suggest that exposure to environmental agents caused an immuno-suppresive effect.

Principle:

The immediate skin tests measure the allergic sensitivity to common areo-allergens and possible occupation-related environmental allergens. Conversely, the delayed skin tests measure the status of the cell mediated immune system as measured by the capacity to mount an inflammatory response to microbial, viral and fungal antigens.

Reagents:

I. Immediate Skin Tests

a. Prick test reagents for immediate skin tests (Appendix VI)

Common* Allergens	Fungal* Extracts	Grain Insects
Giant/Small Ragweed	Aspergillus fumigatus	Adult grain & rice weevils - mix
Timothy Grass	Penicillium species-mix	
Mixed feathers	Aspergillus species mix	Adult confused flour, blank carpet and dermestid beetle - mix
Eastern Oak	Mucor	
Cat epithelium	Cladosporium werneckii	
Oak Rust	Alternaria herbarum	Mold, house & grain mite - mix
Grain smut		
Insect Grains	Airborne Grain Dust	Settled dust
Durum wheat	Durum wheat	Settled Dust I
Spring wheat	Spring wheat	Settled Dust II
Barley	Barley	Settled Dust III
Corn	Corn	
Rye	Rye	
Oats	Oats	
Sunflower seeds	Sunflower seeds	
Small seeds		
Soybeans		
Positive Control	Negative Control	
1.0% histamine in diluting fluid with 50% glycerine	Diluting fluid with 50% glycerine	

b. Sterile stainless steel needles

c. Alcohol impregnated pads

d. 2x2 gauze pads

e. Magic markers

f. Drug box with resuscitation equipment and adrenalin (1:1000 V/V)

*Purchased from Greer Laboratories, Lenoir, NC

II. Delayed Skin tests

a. Skin test panel for delayed skin testing

PPD - tine test

SK/SD - 4U/IU in - 0.1 ml

Mumps - 0.1 ml of stock

Candida - 10 PNU in 0.1 ml

Trichophyton - 1:1000 dilution in -0.1 ml using Δ 1:10 w/v stock solution

b. Tuberculin syringes with 27 gauge needles

c. Alcohol impregnated pads

d. 2x2 gauze pads

e. Corticosteroid impregnated tape

Methods:

A. Immediate skin tests

1. Subject removes shirt or blouse and lies face down in a horizontal position.
2. The back is cleansed with alcohol impregnated pads.
3. Using a magic marker, the numbers 1-10 are painted in two columns approximately 3" from and on either side of the spine. The numbers should begin near the shoulder and terminate near the waist. By placing extracts on either side of both columns, it is possible to test 40 different extracts.
4. The skin test reagents are placed in rack rows of 10. The initial sample in the first row should be the positive histamine control and the last sample in the test panel should be the negative control.
5. Using the column of numbers nearest the technician, a single drop of extract numbered 1-10 is placed on the back.

6. Using the opposite side of the same numerical column, a drop of extracts 11-20 are placed on the back, beginning at the shoulder and working downward.
7. With a sterile needle the skin is gently scratched beneath each drop. The needle is directed so that the skin is slightly raised as the needle punctures the skin. Clean the needle with a 2x2 gauze pad between tests.
8. Wipe the back clean of extracts 1-20 using 2x2 gauze pads.
9. Using the column of numbers farthest from the technician, place drops of extracts 21-30 on the back.
10. Using the opposite side of the same column of numbers, place a drop of extracts 31-40 on the back.
11. Repeat step 7.
12. Repeat step 8.
13. The subject is given a laboratory timer and asked to report back to the technician after 20 minutes.
14. The largest axis of the wheal and erthyema is determined using a ruler graduated in millimeters.

B. Delayed skin tests

1. Sterile 1.0 ml tuberculin syringes are loaded with 0.15 ml of each antigen. Bubbles are removed from the barrel by gentle agitation. The plunger is then pressed until only 0.1 ml remains in the syringe barrel and the needle.
2. Ask the test subject to roll up sleeves to the elbow.
3. Cleanse the forearms with alcohol impregnated pads and allow to dry.
4. Using the left forearm, inject intradermally 0.1 ml of three compounds (Candida, mumps, PPD) in alphabetical order beginning near the elbow joint.
5. Using the right forearm, inject 0.1 ml of the remaining two compounds (SK/SD and trichophyton) in alphabetical order, beginning near the elbow.

6. The test subject is asked to return in 48 hours.
7. The longest axis of the induration and erythema is determined using a ruler graduated in millimeters.

Normal or Reference Values:

In the course of the study, a positive immediate skin test reaction was considered to be a wheal ≥3.0 MM and/or erythema greater than 5.0 MM. Positive control histamine induced skin reactions were greater than 5.0 MM wheal.

Because of the variability in the potency of allergenic extracts, it is impossible to give reference value for the frequency of positive immediate skin tests in a population. Hence, for scientific validity the frequency of immediate skin tests in a test population should be compared to the frequency of skin tests in a similar-sized control population.

When the delayed skin tests were used in the study the induration and erythema was measured after 48 hours. The criteria for positive delayed skin reactions were:

Antigen	Induration	Erythema
Candida	≥ 5.0 MM	≥ 15.0 MM
PPD	≥ 10.0 MM	
SK/SD	≥ 5.0 MM	
Trichophyton	≥ 5.0 MM	

The frequency of delayed skin reactions to intermediate strength antigens used in the study has been defined in the general population (A11.2).

Antigen	%
Candida	39
Mumps	78
PPD	26
SK/SD	55
Trichophyton	28

Limitations:

The use of the prick test for skin testing has one disadvantage. The prick test is less sensitive than intracutaneous skin testing. Hence, allergy cannot be ruled out on the basis of a negative prick test (A11.3).

Conversely, persons with some skin conditions will respond to all skin test reagents. These individuals usually present with skin dermography of several types. These individuals can be identified by virtue of the fact that they will also have a positive response to the diluting fluid. Although the individuals should be excluded from the data pool, it is sometimes possible to demonstrate true allergic reactions which are greater than reactions observed with the diluting fluid (A11.3).

It is also possible that some individuals will not respond to the positive histamine control. This may be due to certain medications ingested by the subject or poor technique (A11.3). If the subject has taken medications which influence histamine action, he should be excluded from the data pool.

The delayed skin tests also have several theoretical and practical limitations. First, it is conceivable that the test population has not been exposed to the test antigen(s). Hence, one would observe a decreased frequency of positive reactions within the population. It is necessary, therefore, to determine the frequency of positive delayed skin test reactions in control population of similar size from the same geographic area. Second, certain immunopathological processes preclude demonstration of a positive delayed skin test. Hence, persons with atopic dermatitis should be excluded from the study. Third, persons receiving corticosteroid therapy, which depresses the inflammatory response, should also be excluded from the study. Fourth, a strong immediate response (20 minutes) at the reaction site may yield a false negative delayed reaction. Hence, subjects with a strong immediate response should be excluded from consideration in the analyses of the data (A11.2).

Trouble Shooting:

There are problems that arise with determination of skin test frequency within a population. The first problem is associated with the shelf life of immediate skin test reactions. The shelf life of reconstituted prick allergenic extracts is 18 months in 50% glycerine when stored at refrigerated temperatures. Therefore, for accurate determinations of immediate skin test reactivity in test and control populations, one must test both populations with the Same Lot of allergenic extracts within 18 months. It is not advisable to change lots of allergenic extracts during the course of the study. There is considerable variation in the allergenic potency of immediate skin test reagents when different lots of the same allergic extract are compared.

Although the prick test is considered to be a safe, rapid method for determining atopic status, one must be aware that systemic allergic reactions may occur in a small number of individuals. Therefore, a physician should be available in an emergency. Moreover, the skin testing facility should be equipped with a drug box containing the necessary resuscitation equipment and aqueous adrenalin (1:1000 V/V).

The serological reactivity of some delayed skin test reagents is not well standardized (i.e. SK/SD, Trichophyton and Candida). In this case, it is necessary to determine the concentration necessary to evoke delayed hypersensitivity reactions in number of normal controls prior to the start of the population study.

The lack of antigen standardization creates another problem. Some individuals will have massive delayed hypersensitivity reactions to low doses of SK/SD (4U/IU). These reactions include induration greater than 40 MM, sloughing of the epidermis and swelling of the entire forearm with associated joint pains. Although this problem is not serious, it does cause a great

degree of apprehension among other participants in the study. Should these accelerated reactions occur, a physician should be consulted and the reaction site covered with corticosteroid impregnated tape.

With respect to potency and shelf life, the same problems apply to the delayed skin test reagents that were outlined for the immediate skin test reagents. Care should be taken to insure that both the test and control population are tested with the same lot of antigen within the shelf life of the reagent.

There are problems associated with the determination of positive skin tests. To abrogate the variability in actually reading the positive skin tests, it is necessary to insure that the same individual(s) read the skin test during the course of the study. It is also necessary to make a prior criterion for a positive skin test, in terms of the size of the wheal or induration, for each antigen before initiating the study. Obviously, this criterion will depend on the sensitivity of the person reading the skin test.

Interpretation

Patterns of immediate skin test reactivity is difficult to interpret. In some industries, the more allergic individuals are forced out of the work environment. Hence, there is a survivor working population which will have overall patterns of immediate skin test reactivity which are lower than a control population from the same geographic area. Moreover, some individuals may have positive skin tests to specific allergens but no clinical episodes of asthma associated with exposure to the same allergens used in the skin tests.

The concept of a survival population is difficult to establish because of the limited number of individuals working in an industry. However, comparison of the skin test frequencies in: (a) control population; (b) currently working; and (c) non-working people who left the industry, should establish whether atopic sensitivity played a role in the decision to leave the industry, and whether occupation related allergenic activity was observed in the non-working group.

The relationship of positive skin tests to occupation-related allergens and occupation-related asthma must be confirmed. This can be achieved by demonstrating a strong correlation between a positive skin test to occupation-related allergens and a history of occupational asthma provoked by the same agent.

The presence of anergy to delayed skin test with a population can be assessed by several techniques as described by Spitler (All.2):

1. Positive responses to fewer than two of the five skin test antigens in an individual.
2. The total sum of induration to all five skin test antigens is less than 10 MM.

REFERENCES

All.1 Calldahl, H: Acta Allergol., 1952, 5:133.

All.2 Spitler, L: Delayed Skin Testing in Manual of Clinical Immunology, edited by NR Rose and H Friedman, American Society for Microbiology, Washington, D.C., 1976, p. 53.

All.3 Norman, PS, Lichtenstein, LM and Ishizaka, K: J. Allergy & Clin. Immunol., 1973, 52:210.

SKIN TEST - PRICK TEST

STUDY ID# /___/ /___/ /___/ /___/ /___/ /___/

COMMON ATOPIC ALLERGENS	DIAMETER (mm) ERYTHEMA	WHEAL
Ragweed		
Timothy grass		
Feathers		
Oak		
Cat		
Rat		
Other		

FUNGAL ANTIGENS	DIAMETER (mm) ERYTHEMA	WHEAL
Asper. fumigatus		
Penicillium sp.		
Mucor sp.		
Cladosporium sp.		
Alternaria sp.		
Rust		
Smut		
Other		

INSECTS	DIAMETER (mm) ERYTHEMA	WHEAL
Mites mixed		
Beetles mixed		
Weevils		
Other		

GRAIN	DIAMETER (mm) ERYTHEMA	WHEAL
Wheat durum		
Wheat spring		
Barley		
Corn		
Rye		
Oats		
Sunflower seed		
Small seeds mixed		
Soybean		
Other		

AIRBORNE DUST	DIAMETER (mm) ERYTHEMA	WHEAL
Wheat durum		
Wheat spring		
Barley		
Corn		
Rye		
Oats		
Sunflower seeds		
Other		

SETTLED DUST	DIAMETER (mm) ERYTHEMA	WHEAL
Dust I		
Dust II		
Dust III		
Other		

SKIN TEST - INTRADERMAL

DELAYED 48 HOURS

	PPD (Diameter mm)		MUMPS (Diameter mm)		CANDIDA (Diameter mm)		SK-SD (Diameter mm)		TRICHOPHYTON (Diameter mm)	
STUDY ID #	ERYTHEMA	WHEAL	ERYTHEMA	WHEAL	ERYTHEMA	WHEAL	ERYTHEMA	WHEAL	ERYTHEMA	WHEAL
/ / / / / /	____	____	____	____	____	____	____	____	____	____
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APPENDIX XII

Precipitating Antibody Determination

Field Operations Manual
NIOSH Contract No. 210-76-0175

RATIONALE

Exposure to a foreign antigen elicits the production of antibodies directed toward the antigen. If the antigen is a soluble molecule, the reaction between antibodies and antigen can be determined by precipitation reactions. The precipitated reaction is a two stage chemical which takes place in a liquid or gel matrix. The first stage the antibody reacts with antigenic determinants on the antigen. Since both the antigen and the antibody are charged molecules, the reaction is dependent on pH and ionic strength of the buffer used in the reaction. Hence, precipitation reactions are carried out in buffered media containing electrolytes. When the primary reaction has reached an equilibrium, a secondary reaction takes place. This reaction is possible since only one antigen combining site on the divalent antibody is reacted with antigen during the primary stage of antigen-antibody interaction. The second unbound antibody receptor now attaches to additional antigenic determinants on the antigen. This results in lattice formation between multiple antibodies and antigenic determinants. Visible precipitation then occurs because the lattice formation is large enough to be insoluble in the buffered media.

The precipitation reaction occurs only when there are optimal interactions between antigen and antibody. Hence, the reaction is dependent directly on the concentration of both antigen and antibody. If there is excess antibody relative to the amount of antigen in the system, visible precipitation will not be observed. Under this condition, the antibody will complex with two antigenic determinants. Because the antibody has complexed with two antigenic determinants, it is unable to react with additional antigenic determinants necessary for lattice formation. Conversely, if there is excess antigen relative to antibody in the system there will also be no visible

precipitation. The lack of visible preciptation is due to the fact that there are two antibodies to combine with all the receptor sites necessary for lattice formation.

Precipitation reactions in agarose gels are widely used in immunology to detect the presence of antibodies to soluble antigens. The reaction is identical to precipitation reactions in liquid media. Inwells are cut in solidified agarose. Antibody is placed in one well and antigen is placed in another well. During the incubation period, the antibodies and antigen diffuse toward each other and interact in a manner described previously. When optimal concentrations are achieved, lattice formation takes place and a precipitate forms that is visible through the agar as a white line.

Using immunodiffusion techniques careful consideration must be given to the size of the wells and the distance between wells. If proper sized wells are used, the optimal interactions between antigens and antibodies will not be achieved, and no visible precipitation will occur. Moreover, if the wells are too far apart for optimal interactions to occur, no visible precipitation will occur. Therefore, the immunodiffusion technique must be standardized from laboratory to laboratory to yield reproducible results.

The determination of precipitating antibodies directed against organic molecules by immunodiffusion techniques is often helpful in the diagnosis of certain lung diseases. Subjects with a history of hypersensitivity pneumonitis (HP) often have precipitating antibodies to the etiological agent in their serum (A12.1). Hence, the presence of precipitating antibodies to extracts of thermophillic actinomycetes is used to support a tentative diagnosis of HP. The presence of precipitins alone, without a clear clinical history of the disease, cannot be used to diagnose HP.

The determination of precipitating antibodies to occupation-related antigenic material is also useful determining the relative exposure of industrial

populations to agents which may cause HP. Again, the frequency of precipitins in the test population must be compared to frequency in control populations.

REAGENT

1. Panel of extracts used for determination of precipitating antibodies (Appendix X)
2. Pre-cleaned microscope slides
3. 0.1 M borate citrate buffer pH 8.4
   a. 6.19 g boric acid
   b. 9.54 g sodium tetraborate
   c. 4.38 g sodium chloride
   d. Dissolve in 1.0 liter of deionized, distilled water
   e. Add 1.0 g of citric acid
   f. Adjust the pH to 8.4
4. Agarose
5. 0.1% Agarose for precoating slides
   a. 0.1 g of agarose in 100 ml deionized distilled water
   b. Bring to a boil
   c. Cool to 56^{o}C in a water bath
   d. Add 0.5% glycerol and stir
6. 1.0% agarose for agar slides
   a. 0.5 g of agarose in 50 ml of 0.1 M borate citrate buffer, pH 8.4
7. Coplin jars
8. Immunoframes
9. Leveling table
10. Gel cutter
11. Humid chambers

METHODS

1. Pre-coating microscope slides
   a. Place 0.1% agarose suspension in a beaker and place in a water bath on a hot plate.
   b. Bring the agarose suspension to a boil.
   c. Pour the liquid agarose into a Coplin jar large enough to immerse microscope slide.
   d. Using a pair of tweezers, quickly immerse microslides in the Coplin jar. Be sure that the entire slide is beneath surface of the liquid.
   e. Immediately remove the microscope slides and place at a 45 degree angle.
   f. Let the slides air dry.
   g. Wrap the slides in groups of six in paper towels. Be sure that the wrapping process prevents slide to slide interaction.
   h. Secure the paper with a rubber band. Mark each pack with the date.
   i. Store at room temperature.
2. Preparation of gel diffusion plates
   a. Mark six slides with the numbers 1-6 using a diamond point pen.
   b. Place the six slides in an immunoframe on a leveling table. Be sure that the slides are in numerical sequence, and that the slides are level.
   c. Weigh out 0.5 g of agarose.
   d. Suspend the agarose in 50 ml of 0.1 M borate citrate buffer pH 8.4.
   e. Place the agarose suspension in a water bath on a hot plate.
   f. Bring the mixture to a boil.

g. Let the liquid cool to 56°C in a waterbath. Keep the molten agarose in the water bath.

h. Place 10 ml of the molten agar onto the three microscope slides on one side of the immunoframe.

i. Place another 10 ml onto three slides in the other side of the immunoframe.

j. Allow the agar to harden at room temperature for 15 minutes.

k. Place the agar-coated microscope slides in a humid chamber for 30 minutes.

l. Repeat steps a-k for a second set of slides.

3. Cutting the gel diffusion wells

a. Using an LKB gel cutter, prepare two well patterns per microscope slide. Each pattern should contain six peripheral (2.0 MM in diameter) wells separated from a central well (6.0 MM in diameter by 3.0 MM).

b. Remove one immunoframe with agarose-coated slides from the humid chamber.

c. Place the well cutter over the agarose-coated slides and press the plunger.

d. Repeat the process until two patterns have been cut in each of the six microscope slides.

e. Gently remove the agarose from the wells using a capillary pipette attached to a water aspirator. Make sure that the wells are straight and free of agarose fragments.

f. Place the microscope slides in the humid chamber.

4. Immunodiffusion analyses

   a. Remove the panel of extracts used to detect precipitating antibodies from the refrigerator. Allow to warm to room temperature.

   b. Place the extracts in groups of six in test tube rack and remove the caps.

   c. Place a capillary pipette with a rubber bulb in each of test extracts.

   d. Make a master list of the extracts as they appear in each group of six.

   e. Remove the agarose-coated slides from the humid chamber.

   f. Begin with the first pattern of microscope slide #1. Add approximately 10 µl of the first six extracts to wells 1-6. Be careful not to overfill the wells. If overfilling occurs, blot with disposable wiper.

   g. Repeat the procedure with the second well pattern on the first slide using the next set of six extracts.

   h. Repeat until all of the wells are filled in all six microscope slides.

   i. Place a sample of undiluted test serum into the center well of each pattern. Make sure the well is filled (approximately 50 µl). Do not overfill.

   j. Place the slides in a humid chamber.

   k. Examine for precipitin lines at 24 and 48 hours.

LIMITATIONS

Although the immunodiffusion method described previously is as sensitive as other immunodiffusion methods (e.g., gel template method) and more

sensitive than the counter immunoelectrophoresis method, immunodiffusion does have several limitations. First, it is less sensitive than other methods used to detect the presence of specific antibodies. However, the more sensitive methods do not readily lend themselves to large population studies using many different antigens and test sera. Secondly, the large antibody well used in the immunodiffusion method is not commercially available and must be manufactured by the individual investigator. Third, serum lipoproteins may precipitate around the antiserum wells and inhibit the visualization of precipitin lines. Fourth, some of the precipitin lines observed in immunodiffusion method may not be classical antigen-antibody interactions. Reactions between acid proteins of the antigen can form precipitin lines in gels (A12.2). Conversely, interactions between basic proteins in serum (i.e., lysozyme) and acidic proteins in antigen preparations can also initiate non-specific precipitation in gels. Reactions of C-reactive protein, a serum complement produced during an inflammatory response, and C-polysaccharide also produce non-specific precipitation in gels. C-polysaccharide is produced by several bacteria and some species of aspergillus (A12.3). It is also conceivable that certain antigenic extracts contain a protein similar to C-reactive protein. Hence, interaction between antibodies and substances analogous to C-reactive protein initiate precipitation. Some non-specific precipitation has also been demonstrated after non-specific interaction between non-antibody serum proteins and teichoic acids of bacterial cell wall (A12.4), and serum alpha macroglobulin and certain antigens. Non-specific precipitation should be suspected in large population studies if 40-50% of the test and control populations demonstrate serological reactivity to specific antigens.

Some types of non-specific precipitation can be prevented by use of reactant modifiers or changes in the buffer system, or the use of agarose as the supporting matrix. Non-specific precipitation of serum lipoproteins can be prevented by using 1.0 M glycine in the agar gels (A12.5). The interaction of acidic-basic proteins can be prevented by the absence of NaCl in the buffer (A12.6). The use of the clotting agent citrate may prevent the CA^{++} requiring interaction between C-reactive protein and C-polysaccharide. The citrate also prevents precipitation of serum lipoproteins in agar (A12.7, A12.8).

If changes in buffers or addition of reactant modifiers fail to alter the precipitin lines, one must use other immunological methods to demonstrate the nature of the immunological reaction. To determine whether α-2 macroglobulin-antigen interactions result in precipitin lines, immunoelectrophoresis techniques can be employed (A12.9). Wells are cut on either side of a trough and test sera is placed in one well and normal serum is placed in the other well. After electrophoresis, the antigen extract is placed in the trough and allowed to diffuse toward the electrophoresed serum components. If the reaction is due to reactions between α-2 macroglobulin and antigen, a line of precipitation will be observed in the α-2 region with both the test and control sera; no line will be observed in the antibody containing α region. Conversely, if the antigen is interacting with antibodies, the line of precipitation will be observed in the γ region. The specificity of the reaction may or may not be determined by the reaction. If the reaction is non-specific, lines of precipitation will be observed in the γ electrophoretic region of both the test and control sera. If the reaction is specific, a reaction will only be observed in the γ electrophoretic region of the test serum.

It is best, however, to actually demonstrate the antigen-antibody reaction observed in immunoelectrophoresis as the result of antibody binding to antigen via the F $(ab)_2$ portion of the molecule. This can be achieved by isolating the IgG fraction of test sera by salt fractionation (A12.10, A12.11). The isolated IgG is then treated with pepsin which digests the Fc portion of the antibody but has no effect on the F $(ab)_2$ portion of the antibody (A12.12, A12.13). Since C-reactive protein antibody interactions occur in the Fc portion of the antibody, pepsin digestion, in effect, will prevent non-specific interactions from occurring via the Fc receptor. After column chromatography, to separate the F $(ab)_2$ and Fc fragments, the F $(ab)_2$ fragments are concentrated and tested in the immunodiffusion system against the same antigen. The presence of a precipitin line proves that the reaction is the consequence of classical antigen-antibody reaction since the antibody preparation lacks other serum proteins which can give false positive reactions and the reaction can only occur via the F $(ab)_2$ portion of the antibody.

INTERPRETATIONS

1. Presence of a precipitin line:

   The demonstration of a precipitin line indicates the previous exposure to the antigen. The presence of a precipitin line to specific antigens should be correlated with clinical history, pulmonary function changes and/or x-ray changes to determine if there is an association with lung disease.

2. Negative precipitin lines:

   The lack of a precipitin line does not preclude exposure to the antigen. Two explanations for the lack of precipitin lines can be put forth. First, the test panel may not contain the proper antigen. Second, the precipitating antibodies are present in small concentrations which cannot be detected by the immunodiffusion method.

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APPENDICES

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APPENDIX XIII

Determination of Immunoglobulins, C3 and α1-Antitrypsin

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RATIONALE

Several constituents of serum can be measured in vitro by immunochemical techniques. The proteins include immunoglobulins (G, A, M) complement component (C3) and alpha$_1$-antitrypsin (AAT). The usual way to quantitate these proteins is radial immunodiffusion (RID). In this technique, heterologous antibody directed toward the protein is distributed evenly within a solidified matrix. Wells are then cut into the agar and the test protein (antigen) is placed in the wells. The antigen diffuses radially from the wells into the matrix containing antibodies. When optimal concentration of antigen and antibody are attained (Appendix XII) a visible precipitate, in the form of a ring, is observed. Since the antibody concentration is fixed in the reaction, the point of optimal concentration of antigen-antibody necessary for precipitation is dependent solely on the concentration of antigen in the system. Hence, the larger the diameter of the precipitin ring the higher the concentration of antigen.

Two different radial immunodiffusion methods can be used to quantitate serum protein. In the timed technique the diameter of the precipitin ring is determined as it is still expanding (A13.1). Theoretically, the timed method is feasible since, as more antigen diffuses from the well, the precipitin ring will dissolve in antigen excess and will reappear at a more distinct point. Since the timed method requires less incubation time, it can be used when quick results are needed. The second method used to quantitate serum proteins is the limit diffusion method. In this method, the diameter of the ring is determined at the conclusion of the reaction when the precipitin ring has stopped expanding (A13.2).

The relationship of the antigen concentration to the diameter of the ring is different when the timed and limit diffusion method are compared. In the

timed reaction, an approximate linear plot is observed when the log of the antigen concentration is plotted versus ring diameter. Conversely, in the limit diffusion technique, the relationship between the diameter squared $(D)^2$ and the antigen concentration is linear.

The limit diffusion method is the method of choice for quantitation of serum proteins. This method has been found to be highly accurate and is not influenced by environmental factors (i.e. temperature changes) which may alter the results of timed tests (A13.3).

The quantitation of serum proteins is useful for several reasons. Determination of levels of IgG, IgA, IgM and C3 can be used in assessing the immunological status of test populations in cases where immunosuppression is suspected. Conversely, $alpha_1$ antitrypsin is the major serum protein which inhibits trypsin activity in the lungs. Hence, the lack of AAT may predispose individuals to syndromes involving this trypsin-induced auto-digestion of the lungs.

REAGENTS

1. Commercially available (Calbiochem/Behring) Limit Diffusion Radial Immunodiffusion Plates for Determination of: IgG, IgA, IgM, C3, $alpha_1$ antitrypsin.
2. 0.85% saline
   a. 0.85 g of NaCl in 100 ml of deionized distilled water.
3. Test tubes
4. Microliter pipette
5. Scotch tape
6. Calibrated magnifier
7. Accuracy control for each protein
8. Internal standard
   a. Fresh frozen normal human serum

METHOD

1. RID Technique

   A. Remove the test samples and the internal control from the freezer. The method for obtaining samples is described in Appendix XIV.

   B. Allow the samples to reach room temperature.

   C. Make the appropriate dilutions of the test samples in 0.85% normal saline according to the manufacturer directions.

      1. IgG - 1:10
      2. IgA - undiluted
      3. IgM - undiluted
      4. C3 - 1:2
      5. AAT - 1:10

   D. Make a master list of the samples relating the sample to the plate and well number.

   E. Remove the RID plates, accuracy control and protein reference standards from the refrigerator. Carefully remove from the aluminum foil envelope as directed by the manufacturer.

   F. Remove the lids of the RID plates and allow them to stand open for 5 minutes at room temperature.

   G. To the first well on plates 1 and 3, add 5 µl of Standard Solution No. I.

   H. To the second well on plates 1 and 3 add 5 µl of Standard Solution No. II.

   I. To the third well on plates 1 and 3 add 5 µl of Standard Solution No. III.

   J. Add 5 µl of the accuracy control to well 4 on plates 1 and 3 and well number 1 on plate 2.

K. Add 5 µl of the internal control to well 5 on plates 1 and 3 and well number 2 on plate 3.

L. Place test serum samples (5 µl) in the remaining wells.

M. Replace the lid of the RID plate and replace in the aluminum foil envelope.

N. Seal the envelope with scotch tape to prevent loss of moisture.

2. Incubation at Room Temperature

A. IgG 50 hours

B. IgA 50 hours

C. IgM 80 hours

D. C3 48 hours

E. AAT 48 hours

3. Calibration Curves

A. Remove the plates from the foil packages.

B. Using a calibrated magnifier, measure the diameter of the precipitin rings for each standard protein solution and internal and accuracy control. The measurement must be accurate to 0.1 mm.

C. Determine the mean and standard deviation for each standard protein solution and the controls. The variability from plate to plate should be less than 0.5 mm.

D. Calculate the diameter squared for each mean value $(D)^2$.

E. Using linear graph paper plot the $(D)^2$ of the protein standards, I, II and III (ordinator) against the concentrations (abscissa). The plot should result in a straight line which intercepts the ordinate at 11 ± 3.4 mm^2. If the intercept value is greater than 14.5 mm^2 or less than 7.5 mm^2, the test must be repeated.

4. Determination of Internal Accuracy and Reproducibility

   A. Calculate the diameter squared $(D)^2$ of the accuracy control and the internal control.

   B. Determine the concentration of the controls from the protein reference calibration curve. The protein concentration of the accuracy control should be less than the standard deviation of the mean value supplied from the manufacturer. The internal control should not vary more than 2.5%.

5. Determination of Protein Test Sera

   A. Calculate the diameter squared $(D)^2$ for each test sample.

   B. Determine the protein concentration from the standard reference curve.

6. Conversion Formulae

   A. To convert mg/100 ml to I.U./ml:

      1. IgG $\frac{\text{mg/100 ml}}{100} \times 11.5 = \text{I.U./ml}$

      2. IgA $\frac{\text{mg/100 ml}}{100} \times 59.5 = \text{I.U./ml}$

      3. IgM $\frac{\text{mg/100 ml}}{100} \times 115 = \text{I.U./ml}$

REFERENCE OR NORMAL VALUES

	Mg/100 ml	International Unit/ml
IgG	800-1800	92-207
IgA	90-450	54-268
IgM Males	60-250	69-287
Females	70-280	80-322
C3	55-120	--
$Alpha_1$ antitrypsin	200-400	--

LIMITATIONS

There are several practical and theoretical limitations of the RID test. Both timed and end point diffusion plates are commercially available, but the end point diffusion method should be used. These plates should be prepared by the method of Mancini, Carbonara and Heremans (A13.2). When there is a question of reaction kinetics, the manufacturer should be consulted. Moreover, the RID method must be used as the manufacturer specifies. Radial immunodiffusion plates designed for timed diffusion cannot be used to determine end point diffusion.

There are also several technical factors which may influence the results of RID. First, it is conceivable that there may be batch to batch variation in the serological reactivity of antibody in the RID plates; therefore, one must use the same lots of plates to test both test and control populations. Second, there may be some plate to plate variation in antibody reactivity in RID plates. The use of an internal standard and accuracy control on every plate can be used to detect plate to plate variation. Usually, the diameter of the accuracy control and internal standard will be 2.0 $\pm$ 1.0%. Lastly, to insure the accuracy of the standard reference curve, a three point reference protein curve should be run on every third plate.

Several sources of error can be ascribed to the filling of the wells. If the wells are not completely filled with a constant amount of antigen or the antigen is spilled outside the wells, the results will be spurious. If the wells contain air bubbles, the results will be invalid.

The determination of serum proteins by immunodiffusion technique detects only the presence of the test protein and not the functional capacity. Hence, if functional abnormalities of the serum proteins are suggested, more

sophisticated immunological tests must be employed. For example, increases in a specific immunoglobulin may suggest a monoclonal gammopathy, but clinical interpretation would depend on total serum protein levels and serum electrophoresis patterns. Conversely, decreased immunoglobulin levels may suggest an immunosuppression or immunodeficiency, but clinical interpretation will depend on assessment of the antibody mediated immune system.

INTERPRETATION

The data are difficult to interpret because certain disease conditions and/or environmental stimuli may increase or decrease the levels of serum proteins. Moreover, the levels of serum proteins may be altered by increases in synthetic rate or decreases in the metabolic rate. It is conceivable, therefore, that decreases in complement C3 may be due to an increase in catabolism rather than consumption in an antigen-antibody reaction or an immune defect in synthesis.

The AAT levels in serum present a unique problem. Decreased levels of AAT may be due to a genetically determined partial or total inhibition of synthesis of AAT. Hence, it is necessary to determine the P_i phenotype and the trypsin inhibitory capacity of (TIC) serum samples with less than 60% of the normal mean value for AAT. Phenotyping and TIC determinations and beyond the scope of most laboratories and should be done only in regional reference centers. Using these techniques the presence of the MZ or ZZ phenotype with decreased trypsin inhibitory capacity may suggest a propensity to develop emphysema. The data should, however, be evaluated in conjunction with familial and clinical history and possible exposure to agents which induce emphysema.

The following table shows the effect of certain conditions on the levels of serum proteins.

Alpha 1 Antitrypsin

Increased	Decreased
Acute/chronic inflammatory disease	familial emphysema
Stress syndrome	familial infantile cirrhosis
Malignant tumors	severe hepatic damage
Pregnancy	nephrotic syndrome
Hematologic disorders	malnutrition

Complement (C3)

Increased	Decreased
Acute inflammatory response	acute glomerulonephritis
	membranoproliferate glomerulonephritis
	immune complex disease
	active systemic lupus erythematous
	inborn C3 defect

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APPENDIX XIV

Determination of Factor B Level and Activation

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Rationale:

The complement system is a group of nine blood proteins which interact in a cascading effect. The complement cascade can be initiated by two mechanisms. In the classical pathway, antigen-antibody complex initiate the reaction, and the complement proteins interact in a defined manner (C1,42356789). The second pathway, which does not require the presence of antibody-antigen complexes, is termed the alternate pathway. The interactions of complement proteins with certain microbial or viral antigens are the results of direct interaction of C3 with the antigen (C356789). As a consequence of complement interaction by either the classical or alternate pathway, soluble complement components are liberated which can initiate the release of histamine from mast cells, initiate chemotaxis of phagocytic cells and increase phagocytosis by phagocytic cells.

It is possible to demonstrate activation of the classical or alternate complement pathway by in vitro methods. These methods are predicated on the fact that the electrophoretic mobility of intact complement components and complement fragments differ in an agarose matrix. Since complement protein C3 is necessary for both the classical and alternate pathways, the demonstration of products of C3 unique to either the classical or alternate pathway can be used to measure complement activation.

Activation of intact C3 (1C) by the classical pathway liberates four major fragments: C3a, C3b, C3c ($_1$A) and C3d (α2d). The C3d (α2d) remains in the serum. Since the C3d (α2d) fragment has a slower electrophoretic mobility than intact C3 (1) it is possible to separate intact C3 (1C) from the C3d and (α2d) in an electrical field using immunoelectrophoresis or cross-immunoelectrophoresis.

Activation of C3 by the alternate pathway liberates different complement products. Complexes of C3b, C3 proactivator convertase (Factor D) and C3 proactivator (Factor B) initiate the cleavage of C3 proactivator (Factor B) into two profactors Ba and Bb (C3 activator). The Ba fragment is quickly metabolized whereas factor Bb (C3 activator) remains in serum. Under an electrical potential, intact C3 proactivator (Factor B) migrates in the B-2 region and the Bb fragment (C3 activator) migrates in the α region. Hence, intact C3 proactivator (Factor B) and presence of the Bb fragment (C3 activator) can be ascertained by immunoelectrophoresis.

Reagents:

1. 10 ml Vacutainer tubes (EDTA or serum separation tubes)
2. Vacutainer holders
3. Tourniquets
4. Multiple sample Vacutainer needles
5. Alcohol impregnated pads
6. Sterile 2 x 2 gauze pads
7. Agarose
8. 0.1% Agarose with glycerol
   a) 0.1g of Agarose in distilled water
   b) Bring to a boil
   c) Cool to 56^{o}C in a water bath
   d) Add 0.5% v/v glycerol and stir
9. Stock barbital buffer pH 8.6 (2x)
   a) 2.466g of barbituric acid
   b) 9.76g of sodium barbital
   c) Suspend to 1.0 liter
10. Working buffer for positive control (1x)--Buffer A
    a) Dilute 5.0 ml of stock buffer with 5.0 ml of deionized, distilled water.

11. 0.2M EDTA

    a) 76g of EDTA tetrasodium salt A

    b) 74.4g of EDTA disodium dihydrate

    c) Dissolve in 900 ml of deionized, distilled water

    d) Adjust the pH to 8.6

    e) Adjust concentration to 1.0 liter

12. Working buffer for agarose preparation and electrophoresis (1x)--Buffer B

    a) 500 ml of stock barbital buffer

    b) 400 ml of deionized distilled water

    c) 100 ml of 0.2m EDTA

13. Microscope slides 1 x 3 inch

14. Coplin jars

15. Immunoframes

16. Leveling table

17. Gel cutter and knife

18. Control sera

    a) Plasma samples recovered from blood drawn in heparin and stored at $-70^{o}C$

    b) Plasma samples recovered from blood drawn in EDTA and stored at $-70^{o}C$

19. Inulin

20. Antisera

    a) Anti-B1A/B1C

    b) Anti-C3 proactivator

21. Provials - 20 ml (Cooke Scientific)

Methods:

1. Preparation of blood samples.
   a. The arm is cleansed with alcohol impregnated pads.
   b. Venipuncture is performed without traumatizing the skin or vein using a Vacutainer and either 10.0 ml EDTA or serum separation tube.
   c. Plasma is obtained from EDTA tubes by centrifugation at room temperature for 10 minutes. Blood in serum separation tubes is allowed to clot for 5 minutes and centrifuged for 10 minutes at room temperature.
   d. Serum or plasma is recovered and aliquoted into 2.0 ml provials. Vials are labeled with subject's name or identification number.
   e. Samples are immediately stored at $-70^{o}C$.
2. Precoating of microscope slides.
   a. Place 0.1% agarose suspension in a beaker and place in a water bath on a hot plate.
   b. Bring the agarose suspension to a boil.
   c. Pour the liquid agarose into a Coplin jar large enough to immerse microscope slide.
   d. Using a pair of tweezers, quickly immerse microslides in the Coplin jar. Be sure that the entire slide is beneath surface of the liquid.
   e. Immediately remove the microscope slides and place at a 45 degree angle.
   f. Let the slides air dry.
   g. Wrap the slides in groups of six in paper towels. Be sure that the wrapping process prevents slide to slide interaction.
   h. Secure the paper with a rubber band. Mark each packet with the date.
   i. Store at room temperature.

3. Preparation of gel diffusion plates.

   a. Mark six slides with the numbers 2-6 using a diamond point pen.

   b. Place the six slides in an immunoframe on a leveling table. Be sure that the slides are in numerical sequence and that the slides are level.

   c. Weigh out 0.5g of agarose.

   d. Suspend the agarose in 50 ml of the working barbital buffer (Buffer B).

   e. Place the agarose suspension in a water bath on a hot plate.

   f. Bring the mixture to a boil.

   g. Let the liquid cool to 56^{o}C in a waterbath. Keep the molten agarose in the water bath.

   h. Place 10.1 ml of the molten agar onto three microscope slides on one side of the immunoframe.

   i. Place another 10 ml onto three slides on the other side of the immunoframe.

   j. Allow the agar to harden at room temperature for 15 minutes.

   k. Place the agar-coated microscope slides in a humid chamber for 30 minutes.

   l. Repeat steps a-k for a second set of slides.

4. Gel patterns.

   a. With a gel punch, cut two wells and a trough in the agar. The size of the wells will differ for each system and the correct size must be determined in each laboratory.

   b. Remove the plugs from the well with a capillary pipette by gentle suction using a water aspirator.

   c. Place the slides in a humid chamber.

5. Preparation of positive and negative controls.

   A. Positive Control.

      1. Weigh out 10 mg of inulin.

      2. Resuspend the inulin in 1.0 ml of working barbital buffer which lacks EDTA (Buffer A).

      3. Pipette 20 µl of the inulin suspension into a tube containing 20 µl of fresh normal human serum or normal human plasma drawn in heparin and stored at -70°C.

      4. Incubate for 30 minutes at 37°C.

      5. Centrifuge at 1000xg for 10 minutes at room temperature.

      6. Recover supernatant fluid for use as a positive control.

   B. Negative Control.

      1. Fresh normal serum drawn in EDTA or frozen serum drawn in EDTA and stored at -70°C.

6. Electrophoresis.

   a. Prepare 1.0 liter of 1 working barbital buffer (Buffer B).

   b. Cut filter paper strips (Reeve Angel No. 3) in strips approximately 2 inches wide and 3 inches long.

   c. Remove test serum samples from the freezer and thaw.

   d. Arrange the sera in groups of 10 samples and make a master list of the sample order.

   e. Remove the agarose-coated slides from the humid chamber.

   f. Add 4.0 µl of the positive control to the top well of slide number 1.

   g. Add 4.0 µl of the negative control to the bottom well of slide number.

   h. Add the test samples to the wells on each of the remaining slides.

i. Fill the electrophoresis chamber with the working barbital buffer solution (Buffer B).

j. Place the immunoframe in the electrophoresis chamber.

k. Place the filter paper strips on each end of the immunoframe. Let hang into the buffer vessels of the electrophoresis chamber.

7. Electrophoresis Conditions.

a. For determination of C3 activator, samples are electrophoresed at 250V. The current should be between 6-9m Amps with a running time of 75 minutes.

b. For determination of C3 activation, samples are electrophoresed at 50V for 3-6 hours. The current should be between 2-4m Amps.

8. Antiserum Placement.

a. Remove the immunoframe from the electrical field.

b. Remove the agarose from the trough with a gel knife.

c. Fill the trough with 80 µl of diluted anti-C3 proactivator or anti-C3 (B_1A/B_1C). The proper dilution of antiserum will vary depending on the source.

9. Incubation.

a. Return the immunoframes to the humid chamber and incubate at room temperature for 24-48 hours.

10. Results.

a. For determination of C3 activator, a positive reaction is denoted by a precipitin arc in the α region (Factor Bb) and a precipitin arc in the ß region (C3PA). A negative reaction is denoted by a single precipitin arc in the ß region.

b. For determination of C3 activation, a positive reaction is denoted by a double-humped precipitin arc in the ß region.

Limitations

The determinations of complement conversion products have several limitations. The validity of the system depends on the ability of the antisera to recognize the presence of unique antigens of complement products. In the determination of C3d (α2d) this presents a problem in that antiserum to C3d (α2d) is not readily available. Antiserum to intact C3 (B1A/B1C) must be used with the assumption that the antisera will recognize the D antigen which is present on intact C3, C3b and C3c (B1A). Although the presence of C3b and C3c (1a) in the immunoelectrophoretic system does denote complement activation, it is unlikely that C3b and C3c (1A) will be demonstrated because of rapid metabolism. Therefore, the antiserum must have the capacity to recognize the D antigen. Commercially available antisera to B1A/B1C differ significantly in their ability to recognize the D antigen, and is necessary to screen several lots of antisera from several companies in order to find one which will work in the system.

Antisera used to detect activation of the alternate pathway present little problem. Antiserum to Factor B (C3 proactivator) will react with intact Factor B and the Ba and Bb fragments due to the fact that the major antigenic determinants are the Ba and Bb molecules which are present on intact Factor B (C3 proactivator) and the fragments. The most reliable source of the anti-Factor B antiserum is, however, Dr. Otto Goetze of the Scripps Institute.

In determination of activation of C3 by the classical pathway, two methods are available. Conventional immunoelectrophoresis is suitable for large population studies if adequate separation can be achieved in the electrical field. The method is less sensitive, however, than the cross-immunoelectrophoresis which detects as little at 5% conversion (A14.1, A14.2). The choice of method, therefore, depends on the individual investigator.

The demonstration of complement conversion products may be affected by technical parameters. Complement can be activated in blood samples by interactions, between plasmin and C1 or a direct effect of plasmin on C3. Hence, it is suggested that blood be drawn in EDTA, which prevents complement interactions or in serum separation tubes which also inhibit complement activation. In addition, some lots of agarose will activate complement during electrophoresis. This phenomenon, however, can be prevented by adding small amounts of EDTA to the agaroe and the buffers used in electrophoresis.

The use of agar as the electrophoretic matrix is not recommended because of electro-osmotic effect; all charged proteins will be carried to the cathode thus altering the normal electrophoretic pattern. Agarose has little electro-osmosis and no affinity for acidic or basic proteins. Hence, proteins migrate more homogeneously with greater resolution.

Interpretations

1. Activation of the classical pathway.

   Demonstration of complement fragments liberated by the classical pathway suggests that an antigen-antibody reaction has taken place.

2. Activation of the alternate pathway.

   Demonstration of complement fragments liberated by the alternate pathway suggests that complement has been activated without antigen antibody interaction.

3. Lack of complement activation by either pathway.

   The failure to demonstrate complement activation does not preclude the possibility that complement has been activated. If the reaction takes place in other areas of the body (i.e. lungs) the conversion products may be diluted to a point where they are no longer detectable by immunochemical means.

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APPENDIX XV

Sampling/Measurement Protocol for Airborne Dust Levels

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NIOSH Contract No. 210-76-0175

APPENDIX XV

Sampling/Measurement Protocol.

for Airborne Dust Levels

Environmental Studies:

Airborne dust levels: These studies were performed by NIOSH Personnel. Method of collection of respirable and total personal dust samples were:

1) "Respirable, personal dust samples were collected utilizing an air sampling train consisting of a 10 mm nylon cyclone respirable dust sample assembly connected to a personal air pump by a 2 ft. length of 1/4" diameter tygon tubing. Each pump was calibrated to provide an air sampling flow rate of 1.7 ± 0.1 L/min. over a full work shift. The dust samples were collected in a two-piece filter cassette holder (supplied with the cyclone containing a 37 mm diameter pore size DM-800 Gelman filter supported on a cellulose back-up pad. The samplers were placed on each worker studied immediately after his pre-shift medical examination and removed just prior to his post-shift medical examination conducted by the University of Wisconsin laboratory for gravimetric analysis and mycological evaluation. All gravimetric analyses (including pre- and post-weights) and filter cassette assemblies were conducted by the University of Wisconsin, Department of Plant Pathology Laboratory personnel."

"Personal total dust samples were collected in the same manner as the respirable dust samples except the cyclones were not used and FWSB 5.0 um MSA filters were used instead of DM-800's. In addition, the Utah Biomedical Test Laboratory (UBTL) provided the two-piece filter cassette used and conducted pre- and post-weighing for analysis. Pump flow rates for all total dust samples were 2.0 ± 0.1 L/min."

"Sampling error is 0-5% for both respirable and total air sampling."

2) For Controls

b. "Respirable dust concentrations of Superior city workers were determined using a 37 millimeter diameter acrylic copolymer 0.8 micrometer pore size filter (DM-800, Gelman) desiccated and preweighed to the nearest 0.001 milligram, supported by a cellulose backup pad and sealed with cellulose bands into a two-piece 37 mm filter cassette. Prepared filter cassettes were uncapped and securely placed into a 10 mm nylon cyclone assembly attached by 0.75 m long tygon tubing to personal sampling pumps equipped with pulsation flow dampers (Model G, MSA). Sampling pumps were periodically monitored over the shift to insure a flow rate of 1.7 Lpm $\pm$ 0.1 Lpm. At the end of sampling, filter cassettes were removed from the cyclones, capped, taped and hand carried back to the laboratories for desiccation and re-weighing."

"Total dust sampling was conducted in a similar manner to respirable dust sampling except cyclones were not used, and the sampling pumps were calibrated at the flow rate of 2 Lpm $\pm$ 0.1 Lpm."

"Ten percent of the filters used in a sampling day were used as controls and treated identically to sampling filters except no air was drawn through the filters."

Notice of Related Work:

The mycological and entomological contaminations of grain and grain Dust were examined independently under a separate NIOSH contract (No. 210-77-0150) entitled "Combined Mycological/Entomological Evaluation of Grain dust Components"; University of Wisconsin-Contractor.

Appendix XVI

Chest Radiograph Reading Form
Field Operations Manual
NIOSH Contract No. 210-76-0175

CHEST ROENTGENOGRAM

Date:
Reader:

A. QUALITY
0. Not done (reason_____)
1. Adequate
2. Deficient, but acceptable
3. Inadequate

B. THORACIC CAGE (May be more than one)
1. Normal
2. Kyphoscoliosis
3. Abnormal rib fracture (specify)
a) old b) new
4. Abnormal degenerative arthritis of spine
5. Abnormal - other (specify_____)

C. HEART
1. Normal
2. Abnormal - enlarged
3. Abnormal - other (specify_____)

D. AORTA
1. Normal
2. Abnormal

E. LUNGS
1. Normal
2. Abnormal
3. Questionable abnormality

E-1 Type of Lesion (only one)

1. Increase in lung markings a) localized b) diffuse-bilateral
2. Reticulonodular pattern a) localized b) diffuse-bilateral
3. Reticulo-linear pattern a) localized b) diffuse-bilateral
4. Nodular (many rounded opacities less than 3mm)
a) localized-unilateral
b) diffuse-bilateral
IF localized, circle location: RU LU
RM or LM or R hilum area (RHA)
RL LL L hilum area (LHA)

IF yes to 1, 2, 3 or 4:
Severity of above lesions (degree of profusion of lesions)
0. Minimal or questionable
1. Definitely present, but few
2. Numerous opacities, but lung markings still present
3. Very numerous, obscuring vascular pattern

5. Nodule, non-calcified (3 mm - 2.5 cm)

   a) single _____

   b) more than one _____, circle location: RU, RM, RL, LU, LM, LL, RHA, LHA

6. Nodule calcified (3 mm - 2.5 cm)

   a) single _____

   b) more than one _____, circle location:RU, RM, RL, LU, LL, RHA, LHA

7. Mass 2.5 cm, circle location: RU, RM, RL, LU, LM, LL, RHA, LHA

F. PLEURA

1. Normal

2. Abnormal a) unilateral

   b) bilateral

   Describe _____

G. DIAPHRAGM

1. Normal

2. Abnormal - flat (hyperinflated lungs)

3. Abnormal - other (specify _____)

H. OTHER FINDINGS AND NARRATIVE: _____

_____

_____

_____

_____

APPENDIX XVII
Blood Chemistries

A Manual and Automated Procedure for Measuring Serum Cholinesterase Activity and Identifying Enzyme Variants

ALANINE AMINOTRANSFERASE (ALT)
GLUTAMATE-PYRUVATE TRANSAMINASE - (GPT)

GAMMA-GLUTAMYL=TRANSPEPTIDASE (GGTP)

CREATININE

ALANINE AMINOTRANSFERASE (ALT)

(Glutamate-pyruvate transaminase - GPT)

Principle

This procedure utilizes the Calbiochem Single Vial Reagent (S.V.R.) system. (Catalog number 869302)

In this reaction, α-ketoglutarate and L-alanine, in the presence of ALT, yield L-glutamate and pyruvate. The latter is reduced by lactate dehydrogenase (LDH) to L-lactate; simultaneously a mmolar equivalent of NADH is oxidized. The rate of change in absorbence at 340 nm is proportional to the activity of the ALT in the sample.

Specimen:

Serum. Hemolysis does not interfere but do not use specimen with appreciable hemolysis. GPT (ALT) activity is 7 times higher in RBC's than serum.

Reagents: (Note A)

1.	TES buffer	0.08 moles/L
2.	L-Alanine	0.56 moles/L
3.	α-ketoglutarate	2.0×10^{-2} moles/L
4.	NADH	2.0×10^{-4} moles/L
5.	LDH (animal)	720 IU/L
6.	pH	7.5

7. Non-reactive stabilizers

Reconstitute by adding 15 ml double distilled water to vial. One vial will be sufficient for 5 tests. Substrate is stable 72 hours when stored between 2° and $8^{\circ}C$.

If reagent shows initial absorbence reading of less than 1:1, or evidence of bacterial contamination, discard.

Procedure:

1. Set up Coleman 124D spectrophotometer; (see general instructions under Coleman 124D). Variable settings are as follows:

Lamp:	D_2
Wavelength:	340 nm
Mode:	ultraviolet
Scale:	0-1
Reference Cell:	dichromate (Note C)

2. Determine total number of assays. Each patient is done in duplicate and in separate runs. There must be at least one control per run. A "run" is comprised of 4 assays monitored sequentially at 15 second intervals.
3. Reconstitute appropriate number of GPT vials. Mix vials gently by inversion to dissolve but DON'T SHAKE. You will need 3 ml substrate per assay.
4. After solution is complete, pour all vials (if more than one is reconstituted) into a larger container and swirl to mix. This will eliminate vial-to-vial variation in the run.
5. Pipette 3 ml pooled substrate solution (step 4) into disposable cuvettes and place in a 30°C water bath for 5-8 minutes to bring to reaction temperature. Do not pre-incubate more than eight cuvettes at a time.
6. Add 0.200 ml of control or specimen (Eppendorf Pipet). Cap cuvet. Mix well by inversion, tap to remove bubbles. Remove cap. Wipe cuvet and place in holder #1 making sure clear sides of cuvet are in the light path.
7. Repeat step 6 until all four positions in the cell chamber are filled, inserting 2nd and 3rd and 4th cuvets in a counter-clockwise manner. (See notes B & C)
8. When the final cuvet is in place, activate cell programmer by switching from manual to auto mode. Switch recorder to "chart" position.

9. Allow recorder to chart change in absorbence for several minutes. Refer to section on calculations to determine patient and control results. (See note E & F)

Notes:

A. If bottle does not have a vacuum or shows evidence of moisture, do not use.

B. Remove cover only long enough to place cuvette in position in order to maintain 30°C in well.

C. Offset may have to be used to get some of the samples on the chart. If that still doesn't work, switch to 0-2 scale settings and see note in calculation section.

D. This test should be run only after one is familiar with kit information supplied by Calbiochem (Document No. L03426, 4/1/78).

E. If ΔA is greater than .390/min, the activity is greater than 1000 mU/ml and the sample should be diluted with saline and rerun.

F. Elevated levels of ALT (GPT) may be substantially reduce NADH before initial absorbence is recorded. If a sample gives an initial reading of 0.6 or less dilute with saline and rerun.

G. Dichromate solution: Use reagent #PD3 from Oxford Spectrocheck set. Dilute 1:100 as directed on vial. Cover reference cuvette with parafilm so that it may be reused.

Calculations:

$$\frac{\Delta A/\text{min} \times \text{total volume} \times 1000}{\text{mM absorptivity} \times \text{sample vol.} \times \text{light path} \times \text{min}} = \text{mU/ml}$$

mM absorptivity = 6.22 @ 340 nm for NADH

This reduces to:

$$\frac{\Delta A/min \times 3.2\ ml \times 1000}{6.22 \times 0.2\ ml \times 1 \times 1} = 2572 \times \Delta A/min = mU/ml$$

When reading from the chart let each square represent a unit of absorbence; then

$$\Delta A \times 2.572 = mU/ml$$

when a line is extrapolated to cover a 10 minute period.

Note: If using 0-2 scale because of lipemic specimens, use:

$$\Delta A \times 5.144 = mU/ml$$

(also use a 10 minute line)

Expected Values: (taken from kit literature)

Male: 1-25 mU/ml @ 30°C

Female: 2-24 mU/ml @ 30°C

References:

See kit insert, Calbiochem Doc. No. L03426.

GAMMA-GLUTAMYL-TRANSPEPTIDASE (GGTP)

Principle:

The assay is based on the transfer of the glutamyl group from L-γ-glutamyl-p-nitroanilide to glycyl-glycine in the presence of GGTP. The rate of p-nitroaniline formation measured at 405 nm is proportional to the GGTP concentration in the sample. (Sigma Technal Bulletin #415, 1/77).

Specimen:

Plasma: Blood is drawn into a tube containing either heparin or EDTA and centrifuged to obtain plasma. (See note D)

Serum: Blood is drawn into a plain tube and allowed to clot. The serum is separated from the clot as soon as possible. (See note D)

Storage: GGTP is stable in serum for at least 1 week at $4^{o}C$ and 2 months at $-18^{o}C$. A minimum of 1 ml is needed for analysis.

Instrument Settings:

Allow a 30 minute warm-up for instrumental system (Coleman 124D spectrophotometer and attachments).

1. Spectrophotometer:
    a. Wavelength: 405 nm
    b. Slit width: 1.0 nm
    c. Read absorbence on 0-1 scale.
    d. Place dichromate solution in reference. Set zero to keep readings on chart (1:100 dilution of stock #PD-3) (Note A).
    e. Tungsten lamp: on
    f. Mirror towards tungsten lamp
2. Recorder:
    a. Chart speed (20)
    b. Range (10)
    c. Power on Servo, then to chart when reading absorbence.

3. Cell Programmer:

   a. Power on

   b. Measurement period - 15 seconds

   c. Manual, initially, then to auto for readings

4. Scale Expander:

   a. All offset dials to 0

   b. High readings can be offset by turning appropriate knob to make readings stay on recorder.

5. Constant Temperature Circulating Water Bath:

   a. Set temperature 30.0°C (31°C on thermometer) (See Note E).

   b. Turn tap water on slowly.

6. Water Bath for Incubation

   Set at 30°C.

Reagent Composition:

GGTP substrate: (Sigma stock #445-5

L-γ-glutamyl-p-nitro anilide	70 µmol/L
Glycylglycine	600 µmol/L

A.M.P.D. buffer (Sigma stock #415-8)

2-amino-2-methyl-1, 3-propanediol	0.2 mol/L
pH 8.6	

Reagent Preparation:

Add 15.5 ml A.M.P.D. buffer to substrate vial. Shake vigorously for a few seconds and place in 37°C water bath 2-3 minutes until substrate is dissolved. Each vial contains enough substrate for 5 tests. Reagent is stable 2-3 hours at R.T.

Procedure:

1. Determine total number of assays: each patient is done in duplicate and at least one control in each run; a "run" consists of four assays being monitored sequentially at 15 second intervals.
2. Prepare the appropriate number of GGTP substrate vials. Pour all vials into a larger container and swirl to mix. This eliminates any vial-to-vial variation.
3. Pipet 3 ml of the pooled substrate into disposable square cuvettes and place in a 30°C water bath 5-8 minutes to bring to reaction temperature. Do not pre-incubate more than 8 vials at one time.
4. Add 200 µL sample to a cuvet with an Eppendorf pipet. Cap and invert several times to mix. Tap to remove any air bubbles. Remove cap, wipe cuvet and place in holder #1. (Clear sides of cuvet in light path.)
5. Repeat step 4 with 2nd, 3rd & 4th cuvettes, placing them in holders in a counter-clockwise manner. (Note B)
6. Switch cell programmer from manual to auto mode and immediately switch recorder to "chart" position.
7. Allow recorder to trace changes in absorbence for several minutes. Refer to calculations for how to determine patient and control results. (Note C)

Notes:

A. This is the same blank used in the ALT & AST procedures. If a sample is unusually lipemic or icteric, 200 µL of sample should be added to a cuvet containing the dichromate solution and this mixture should be used as a blank for that sample (to keep the readings on the chart).

B. Remove top from cuvet well only long enough to place each cuvet in its holder in order to maintain temperature in well.

C. If A is greater than 0.125/min, dilute the sample with saline and rerun.

D. Fluoride, oxalate and citrate have been found to inhibit GGTP activity. Falsely elevated levels occur in patients taking antiepileptic drugs, such as phenytoin and barbiturates.

E. The heating unit of the constant temperature water bath is usually set at 31°C depending on room temperature. The circulating water will cool as it warms the cell chamber. The temperature of the cell chamber can be checked periodically by placing tight fitting styrofoam material on the top of the cell chamber and then pushing a thermometer through this material.

Calculations:

$$\text{mU/ml} = \frac{\Delta A/\text{min} \times \text{total volume} \times \text{temp. correction factor}}{\text{micromolar extinction factor} \times \text{sample volume}}$$

$$\text{mU/ml} = \frac{\Delta A/\text{min} \times 3.2 \times 0.8}{.0099 \times 0.2}$$

$$\text{mU/ml} = \Delta A \times 1293$$

Note: When reading from chart, let each square represent a unit of absorbence. Extrapolate a line for a 5 minute reading, then ΔA (5 minutes) x 2586 = mU/ml.

Controls:

Controls consist of two levels of unassayed control material (Hyland Scan I & II). Control limits are ±2.0 standard deviations or other range as indicated in the current Scan Control Data Book. Unknowns are to be assayed in duplicate but not within the same run of four. There must be one control

in each run of four. If control values are not acceptable, check wavelength, slit width, cuvets, reaction temperature, pipetting, age of reagents, storage of reconstituted reagents, manual reading of reaction curves, and finally reconstitute new controls.

Normal Range:

Adults: up to 30 mU/ml

Reference:

Sigma Technical Bulletin No. 415, January, 1977.

CREATININE

Principle:

Creatinine reacts with picrate under alkaline conditions (Jaffe reaction) to give a yellow-red solution which is measured photometrically at 505 nm.

The determination is made on diluted urine or on protein-free filtrate (dialysate) of plasma or serum.

The method employed is a modification of the procedure of Folin and Wu taken from the text "Hawk's Physiological Chemistry."

Creatinine clearance is a sensitive measure of glomerular filtration rate. Relatively minor changes in serum creatinine are accompanied by changes in creatinine clearance which are more dramatic, especially in the early phase of kidney disease.

Specimen:

Creatinine may be determined in any biological fluid, but plasma, serum, amniotic fluid, and urine are the specimens most commonly employed. Plasma and serum are preferred to whole blood since considerable amounts of noncreatinine chromogens are present in red cells. If kept for a few days, specimens for creatinine are best stored at refrigerator temperatures; if kept for longer periods, they should be frozen. Aqueous solutions of creatine and creatinine very slowly approach a state of equilibrium with respect to each other. Creatinine is formed rather quickly from creatine in either alkaline or acid solutions.

When performing a creatinine clearance, a precisely timed urine specimen and serum sample is required. The blood is generally collected in the middle of the urine collection period. Submit a 50-100 ml aliquot of the well-mixed 24 hour urine collection with a record of the total volume.

Controls:

1. Hyland Scan I and II (serum)
2. 2 levels of frozen serum pools
3. 2 levels of frozen urine pools
4. Occasional assayed lyophilized urine material from Hyland

Reagents:

1. Saline, 9.0 gm NaCl 1000 ml double distilled water

   Add 0.5 ml Brij-35-mix

   Stable indefinitely at room temperature.

2. Sodium Hydroxide, 0.5 N

   20 gm/1000 ml double distilled water. Stable indefinitely at room temperature.

3. Saturated Picric Acid

   13 gm/1000 double distilled water. Stable indefinitely. (See note 1)

   a. To 13 gm of reagent grade picric acid in a one liter volume flask. Add distilled water to the mark.

   b. Allow the excess picric acid to remain in contact with the water and shake occasionally.

   c. Filter and store in a polyethylene bottle:

4. Stock creatinine standard (1 mg/ml)

   1000 gm/1000 ml 0.1 N HCl (Stable 1 year at room temperature)

5. Working creatinine standards:

   Dilute stock creatinine standard with 0.2 N HCl.

ml stock	Dilute to:	mg creatinine/100 ml
0.5	100 ml	0.5
1.0	"	1.0
2.0	"	2.0
3.0	"	3.0
4.0	"	4.0
5.0	"	5.0
7.0	"	7.0
10.0	"	10.0

Stable 3 months at room temperature.

Procedure:

Equipment Needed

Autoanalyzer I

1. Sampler II - (run at 60 per hour)
2. Proportioning Pump
3. Dialyzer (37°C - Type C Membrane)
4. 40 ft. time delay coil (Room temperature)
5. Colorimeter (505 mu - 15 mm tubular flow cell)
6. Recorder

(See attached flow diagram)

The sample stream segmented with air, is diluted with 0.9% sodium chloride. This combined stream enters the sample side of the dialyzer. The recipient stream consists of water segmented with air. (See notes 3, 4, & 5) After emerging from the dialyzer it is joined with a stream formed by a combination of saturated picric acid and 0.5 normal sodium hydroxide. The streams are mixed, sent through a time delay coil and then go into the colorimeter. The developed color is read at 505 nm using a 15 mm tubular flow cell.

-Warm up time of colorimeter - 20 min.

-Time to bring up reagents - 20 min.

-Set Baseline at 95% T

-Keep washline separate from picric acid and NaOH line.

-Serum samples should be mixed and centrifuged before being placed on sampler.

-Results that are higher than the 10 mg/dl standard should be diluted and repeated. Multiply result by appropriate dilution factor.

Plate format:

1. 0.5 mg/dl
2. 1.0 "
3. 2.0 "
4. 3.0 "
5. 4.0 "
6. 5.0 "
7. 7.0 "
8. 10.0 "
9. Water
10. S I
11. S II
12. Water
13. Frozen Pool #1
14. Frozen Pool #2
15. Water
16. Serum specimens
17. Water
18. Urine Pool #1
19. Urine Pool #2
20. Water
21. Urine specimens
22. Water

Notes:

1. Sigma Stock #925-40 (As a safety precaution, aqueous picric acid should be purchased, as the dry powder can be explosive)
2. A is obtained from a surface-area monogram. (p. 916, Hawk's Physiological Chemistry. See attached monogram).
3. For optimal bubble pattern and low noise use 0.5 ml of Brij-35 per liter of saline and distilled water recipient.

4. The noise with serum may sometimes be due to the formation of a precipitate. If this occurs, it is advisable to try a different lot of picric acid. It may also be helpful to clean the picric -sodium hydroxide lines and coils as well as the flow cell with 10% acetic acid.
5. When running the creatinine determination a check should be made of the noise. This can be done by continually aspirating a 5 mg/100 ml creatinine standard. The noise level should be no greater than $\pm$0.5 transmission line. If the noise level is greater, a check of the manifold and dialyzer should be made to insure that a good bubble pattern is being obtained. Noise is generally related to a poor bubble pattern which gives poor proportioning of reagents.

Calculations:

1. Serum or Plasma - these are read directly from a standard curve. Results are reported in mg/dl.
2. Urine results are reported in gm/24 hr vol. Samples are generally diluted 1:30 before analysis.

$$\text{mg/100 ml} \times 30 \times \frac{\text{aliquot vol}}{\text{24 hr vol}} \times \frac{1\ \text{gm}}{1000\ \text{mg}} = \text{gm/24 hr vol}$$

where mg/100 ml is the reading from standard curve and 30 is the dilution factor

3. Creatinine clearance is calculated as follows:

$$C = \frac{UV}{P} \times \frac{1.73}{A}$$

where U = mg creatinine/ml urine

V = ml urine/min

P = mg creatinine/ml serum

A = body surface area of individual being tested.

(See Note 2 or attached nomogram)

C = ml serum cleared/min/std surface area

Normal Ranges:

1. Serum or plasma:

Female	0.8-1.2 mg/dl
Male	0.9-1.4 mg/dl

2. Urine

Female	0.8-1.8 gm/24 hr vol
Male	1.0-2.0 gm/24 hr vol

3. Creatinine clearance

Female	75-115 ml/min
Male	85-125 ml/min

References:

1. Technicon Autoanalyzer Methodology Method File N-11B

2. Fundamentals of Clinical Chemistry, Norbert Tietz, 1976 p.996-998

3. Hawk's Physiological Chemistry, Edited by Bernard L. Oser, Fourteenth Edition 1965.

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