

Grain fever syndrome induced by inhalation of airborne grain dust

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To study the clinical and physiologic manifestations of the grain fever syndrome and the potentially pathogenic role of complement activation, 12 subjects (six grain workers and six healthy non-grain workers) underwent inhalation provocations with airborne grain dust. The clinical response was characterized by facial warmth, headache, malaise, myalgias, feverish sensation, chilliness, throat and tracheal burning sensation, chest tightness, dyspnea, cough, and expectoration. Fever developed in four grain workers and two controls. Leukocytosis, ranging between 11,700 and 24,300 leukocytes/mm³ with left shift, developed in five grain workers and five controls. There was no evidence of complement activation by the classical or alternate pathway. None of the subjects had serum precipitins to grain dust. The pulmonary response was characterized by a decrease in FEV₁, FVC, MMF, $\dot{V}_{max_{50}}$, and $\dot{V}_{max_{75}}$, with significant rise in pulmonary resistance and consistent change in dynamic compliance but without changes in static compliance or diffusing capacity. Hence, grain dust inhalation induced diffuse airways obstruction without detectable parenchymal reaction. The airways response to high concentrations of grain dust inhalation were unrelated to the presence of immediate skin hypersensitivity. Although we cannot exclude the etiopathogenetic role of an immunologic reaction to grain dust, our data do not support the hypothesis that the grain fever syndrome is a precipitin-mediated allergic pneumonitis. More likely, the manifestations of grain fever probably reflect the host reaction to grain dust bacterial endotoxins and/or nonallergic mediator release by grain or grain dust constituents. (J ALLERGY CLIN IMMUNOL 69:435, 1982.)

Recent epidemiologic studies¹⁻³ have shown that exposure to grain dust may induce acute conjunctival, nasal, respiratory, and systemic symptoms. Grain fever was reported by 6% to 30% of grain handlers.^{1, 2, 4} Recently, 32% (99 out of 310) of grain handlers that we studied using a standard questionnaire reported one or more episodes of a short-term, flulike syndrome developing during and/or after occupational exposure to high concentrations of grain dust. Although some workers reported only an isolated episode after their first heavy exposure, 16 workers related over a hundred episodes in their work life. In another study, we recognized that facial warmth, fever, or "chilly" sensations felt by workers during

or after a work shift were seldom associated with fever, i.e., body temperature above 37.8° C. Grain dust contains a wide variety of organic constituents, e.g., grain, fungi, bacteria,⁴⁻⁶ and endotoxin⁷ that could be potentially responsible for the grain fever syndrome. Because of its clinical resemblance to hypersensitivity pneumonitis, grain fever has been hypothesized to be a type III allergic reaction⁸ to fungi contained in grain dust. Physiologic or radiologic evidence of pneumonitis with grain fever has not been demonstrated. However, like the extracts of organisms associated with hypersensitivity pneumonitis,⁹ airborne grain dust also activates complement by the alternate pathway in in vitro studies.⁷

The purpose of this study was to delineate the time course and characteristics of the clinical and physiologic manifestations of grain fever syndrome and the potential pathogenic role of complement activation.

METHODS

Subjects

We studied six grain handlers with histories of recurring episodes of grain fever and six asymptomatic adult non-smokers who were not employed handling grain (controls).

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Abbreviations used

FEV ₁ :	Forced expiratory volume in one second
FVC:	Forced vital capacity
MMF:	Maximum midexpiratory flow rate
$\dot{V}_{\max_{50}}$:	Maximum instantaneous flow at 50% below total lung capacity
$\dot{V}_{\max_{75}}$:	Maximum instantaneous flow at 75% below total lung capacity
C _{stat} :	Static compliance
C _{dyn} :	Dynamic compliance
R _L :	Pulmonary resistance
FRC:	Functional residual capacity
D _L CO:	Diffusing capacity
PNU:	Protein nitrogen unit

The grain handlers were recruited from the Superior-Duluth grain elevator working population of 310 previously studied by us. The six controls were selected from Madison community residents who volunteered for the study in response to a newspaper advertisement. Each control subject in the study was a healthy asymptomatic nonsmoking male adult without spirometric evidence of airways obstruction and with normal physical examination and chest radiograph. Individuals who admitted long-term use of mood-altering drugs, e.g., cannabis or alcohol, or past histories of psychologic disturbances were excluded. Informed consent was obtained from each individual according to the guidelines of the University of Wisconsin.

The characteristics of these 12 individuals are summarized in Table I. The six grain handlers complained of respiratory symptoms and eye irritation during occupational exposure to grain dust. They had worked in the grain industry for 6 to 30 yr. Three of the grain handlers had histories of chronic productive cough of longer duration than 2 yr, two of them were smokers and one was a nonsmoker. One of the controls had occasional chest tightness on heavy exercise, another had a history of hay fever, and a third had had occasional wheezing with colds in childhood. None of the controls were current smokers, but two had smoked until a year before the study.

Pulmonary function tests

FEV₁, FVC, MMF, $\dot{V}_{\max_{50}}$, and $\dot{V}_{\max_{75}}$, were measured by standard techniques¹⁰ using an Ohio 840 rolling bar spirometer. The FVC time curves were displayed on a 7046A Hewlett Packard XY recorder and measured. Flow volume curves were displayed on a Tetronix 4111 storage oscilloscope, tape recorded using a Hewlett-Packard 3960 instrumentation recorder at a speed of 3.75 ips, and replayed at 15/16 ips onto an HP 7046A XYY recorder to measure $\dot{V}_{\max_{50}}$ and $\dot{V}_{\max_{75}}$ from the curve with the largest FEV₁ plus FVC.

Diffusing capacity for CO was measured by the single-breath method.^{10, 11} Predicted values were obtained from published information.¹¹⁻¹³ C_{stat}, C_{dyn}, and R_L were measured in nine of the 12 subjects with the esophageal balloon

technique previously described.¹⁴ Three subjects refused the esophageal balloon. FRC was measured in a pressure-type body plethysmograph. Methacholine inhalation tests were done by the recommended standard method,¹⁵ with dilution increments of 0.6, 1.25, 2.5, 5.0, 10.0, and 25.0 mg/ml. The provocation concentration causing a 20% fall of FEV₁ was calculated from the last two points of the log-dose response curve.

Preparation of saline extracts

Saline extracts of pulverized insects, mites, barley, and airborne grain dust were prepared by the method of Flaherty et al.¹⁶ and were lyophilized. The protein content of grain extracts was determined by the Micro-Kjeldahl technique.¹⁷ The results were expressed in PNU.

Growth conditions for insects and mites

Stock cultures of weevils, beetles, and mites were grown on the following media: (1) whole wheat (adult granary and rice weevils), (2) whole wheat 5% w/w brewer's yeast (adult confused flour beetle), (3) Purina Lab Chow with 5% w/w brewer's yeast (black carpet beetle), (4) eight parts Purina Lab Chow, three parts wheat germ, three parts dry milk, one part brewer's yeast, and one part meat and bone meal (dermestid beetle), and (5) three parts brewer's yeast and one part wheat germ (mold mite, house mite, and grain mite).

All cultures were incubated at 27° ± 1° C and 60% relative humidity with a 16:8 light:darkness ratio for 2 to 4 wk. The insects and mites were removed from the growth by passage through laboratory sieves. After microscopic examination to verify removal of the growth medium, all insects and mites were frozen and stored at -40° C.

Preparation of immediate prick-test reagents

The following skin-test reagents were prepared from airborne grain dusts by diluting the extracts to 100,000 PNU in sterile Coca's nonpyrogenic buffer (Endocrine Laboratory, Madison, Wisc.) containing 50% w/v glycerine: durum wheat, spring wheat, barley, corn, rye, oats, and sunflower seeds. Weevil mixtures (grain and rice weevils), beetle mixtures (confused flour, carpet, and dermestid beetles), and mite mixtures (mold, house, and grain mites) were prepared in the Coca's buffer glycine mixture at a concentration of 10 mg/ml. Commercially available giant/small ragweed mix, timothy grass, eastern oak, mixed feathers, cat epithelium, rat epithelium, *Alternaria herbarus*, and *Alternaria tenuis* extracts were purchased from Greer Laboratories, Lenoir, N.C. The same source supplied extracts of *Aspergillus fumigatus*, mixed *Penicillium* species, *Cladosporium*, and *Mucor* (1:20 w/v).

Immediate skin tests

Skin testing for detection of immediate cutaneous hypersensitivity to common aeroallergens, insects, mites, and airborne grain dust was done by the prick method. A wheal of 3 mm or greater at 20 min was considered a positive reaction.

TABLE I. Characteristics of subjects tested

Subject No.	Age (yr)	LOE (yr)	Current smoker	FEV ₁ *	FVC*	FEV ₁ /FVC (%)	MMF*	$\dot{V}_{max_{50}}$ *	$\dot{V}_{max_{75}}$ *	D _L CO*	Methacholine PC ₂₀ (mg/ml)†
Grain workers											
1	48	28	Yes	84	90	70	89	73	48	104	—
2	47	12	Yes	85	92	70	86	47	50	108	10
3	30	6	No	91	87	84	137	111	110	78	—
4	27	7	Yes	110	107	84	153	98	117	109	—
5	57	30	No	67	85	61	56	25	29	97	0.75
6	29	8	No	91	88	81	106	73	79	119	—
Mean	39.7	15.2	—	88.0	90.5	75.0	104.5	71.2	70.5	102.5	
SD	12.6	10.9	—	13.9	5.7	9.4	35.6	31.7	33.9	14.0	
Controls											
7	29	0	No	95	96	85	103	69	69	153	1.56
8	27	0	No	108	106	82	152	97	69	104	25
9	25	0	No	107	85	94	153	133	176	90	—
10	26	0	No	93.5	75	98	198	100	142	114	—
11	27	0	No	100	94	84	139	94	120	135	—
12	33	0	No	106	100	81	142	107	120	97	—
Mean	27.8	—	—	101.5	92.7	87.3	147.8	100.0	115.2	115.5	
SD	2.8	—	—	6.3	11.1	7.0	30.6	20.7	46.6	24.1	
Total											
Mean	33.8	—	—	94.8	91.6	81.2	126.2	85.6	94.1	109.0	
SD	10.7	—	—	12.5	8.5	10.2	38.9	29.6	43.6	20.0	

LOE = Length of employment in grain industry; PC₂₀ = provocation concentration causing a 20% fall in FEV₁.

*Data expressed as percent of predicted value.

†Methacholine test negative (—) if FEV₁ did not decrease >20% with 25 mg/ml dose.

Preparation of extracts for inhalation challenge

Twenty-four hours prior to challenge, lyophilized barley extract was resuspended in sterile nonpyrogenic Coca's buffer with 0.03% normal human albumin to a concentration of 100,000 PNU/ml. The mixture was filtered through a 0.22 micron Millipore filter (Millipore, Bedford, Mass.) into sterile containers. To confirm the sterility of the extracts, aliquots were inoculated onto three Sabouraud and three nutrient agar plates. The plates (one Sabouraud and one nutrient agar plate) were incubated at 4°C, at ambient conditions, and at 37°C.

Precipitating antibody determinations

Lyophilized saline extracts of airborne grain dusts, insects, mites, *Aspergillus* species, *Penicillium* species, thermophilic bacterial species, *Cladosporium*, and *Mucor* were resuspended in 0.1M borate citrate buffer at 10 mg/ml. The resuspended extracts were used in micro-Ouchterlony tests as described by Flaherty et al.¹⁶

Serum complement determinations

Total plasma C3 levels were ascertained by radial immunodiffusion¹⁸ using commercially available kits (Calbiochem/Behring, La Jolla, Calif.). Total C3 was considered to be significantly changed when the value changed more than

42 mg/dl. This value represented two standard deviations from the mean C3 values of the entire test group during baseline studies. Activation of the classic and/or alternate complement pathways was determined by immunoelectrophoresis with anti-C3 (anti- β 1A/ β 1C) and anti-C3 proactivator sera.^{19, 20} The latter antisera were generously donated by Dr. O. Goetze (Scripps Institute, La Jolla, Calif.).

Protocol of procedure for inhalation challenge

During a control day and at regular intervals for 24 to 48 hr after the inhalation challenge, we studied symptoms, body temperature, blood leukocyte counts, differential counts, FEV₁/FVC, MMF, $\dot{V}_{max_{50}}$, $\dot{V}_{max_{75}}$, D_LCO, and complement changes (total C3) and activation of complement by the classic and alternate pathways. On the first or control day, no inhalation material was given, but measurements were taken at regular intervals. On the second day, the subjects were exposed to airborne grain dust in an environmental chamber, where they stirred up the dust, as often as needed to simulate the environment that workers consider most likely to provoke an episode of grain fever. The exposure was terminated whenever the environment became intolerable to the subject or 120 min of actual exposure time was reached.

We considered that a significant postchallenge response

TABLE II. Results of selected parameters

Subject No.	Skin tests		Respirable dust level (mg/m ³)	Temperature (C°)		WBC (n × 1000)	
	CAA	Grain dust		Before challenge	After challenge	Before challenge	After challenge
Grain workers							
1	—	—	86.7	36.8	38.0*	7.7	14.3*
2	—	+	44	36.7	37.2	6.4	11.7*
3	—	—	86.7	36.6	39.0*	5.8	24.3*
4	—	—	23	36.8	38.4*	6.2	21.0*
5	+	+	23	36.3	36.9	5.3	8.6
6	+	+	38.6	36.8	38.9*	8.5	17.0*
Controls							
7	+	—	164	36.7	36.4	7.1	8.8
8	—	—	143	36.7	37.8*	5.7	24.3*
9	+	—	90.1	37.0	37.2	9.9	19.0*
10	+	+	164	36.8	36.9	6.8	14.6*
11	+	—	143	36.9	38.4*	5.5	21.2*
12	—	—	90.1	36.7	37.4	7.4	22.0*

CAA = common allergens (+ = atopy—two or more positive reactions to ragweed, grass, tree, *Alternaria*, cat hair, or feathers); airborne grain dust extract, + = wheal ≥ 3 mm; WBC = leukocyte count.

*Considered significantly changed from baseline.

†Similar decrement measured on control day.

in the experimental parameters had occurred when: (1) the blood leukocyte count rose above 10,500/mm³ (leukocytosis) and increased by at least 3000 from baseline, (2) oral temperature rose to or over 37.8° C (fever) from a normal baseline temperature, (3) C3 complement level changed more than 42 mg above or below the baseline value, (4) FEV₁, FVC, and D_LCO decreased more than 20% from baseline values and no changes greater than 10% from baseline were observed during control day, and (5) MMF, V_{max}₅₀, and V_{max}₇₅ decreased by more than 25% from baseline values.

On the third day, the subjects were tested at regular intervals without a new bronchial provocation test if they were still symptomatic, if leukocytosis persisted, or if FEV₁ had not returned to prechallenge baseline levels ± 10%. If the subject recovered on the third or fourth day, he was tested with saline extracts of barley at increasing doses administered with a dosimeter-powered DeVilbiss nebulizer until a 20% or greater decrement in FEV₁ occurred or a dose of 100,000 PNU/ml was reached. To establish a safe, initial dose for inhalation challenge with saline extract of barley, intradermal skin tests with this antigen were performed. The dilution at which an 8 mm wheat reaction was obtained was the initial challenge dilution. No skin tests were done with a dose greater than 1000 PNU.

Airborne dust concentration

Respirable dust concentrations were determined with 37 ml diameter acrylic 0.8 μm pore-size filters (DM 800 Gelman) in filter cassettes, which were placed into 10 ml nylon-cyclone assemblies attached to personal sampling

pumps equipped with pulsation flow dampers (Model GMSA). A flow rate of 1.7 ± 0.1 L/min was used. The pumps were interrupted when the subjects were removed from the chamber for testing. The time-weighted average respirable dust concentration was calculated by the actual time of exposure, which varied from 60 to 120 min, depending on the subjects' tolerance to the dust.

Microbiologic analysis

The microflora of the airborne grain dust was studied using pure culture methods.⁶ The airborne dust contained *Aspergillus* species, *Penicillium* species, and bacteria. The predominant *Aspergillus* species was *A. glaucus*, and *P. cyclopium* was the most commonly encountered *Penicillium* species. Other species identified were *A. flavus*, *A. nidulans*, *P. viridicatum*, *A. fumigatus*, and *P. versicolor*. These fungi had been previously identified in over 200 airborne dust samples collected at the Superior-Duluth terminal elevators.⁶

RESULTS

The pulmonary function studies done on these subjects before the challenge are presented in Table I. Pre-existing airway obstruction defined as an FEV₁/FVC less than 70% was observed in one grain worker.

Methacholine challenge

Methacholine bronchial hyperreactivity was observed in two grain workers and two controls (Table I).

Largest postchallenge percent change from baseline					
FEV ₁	FVC	MMF	V _{max} ₅₀	V _{max} ₇₅	D _L CO
-3	-6	4	-25*	-6	+14
-35*	-73*	-55*	-40*	-12	-5
-12	-3	-31*	-29*	-41*	+16
-27*	-16	-43*	-29*	-51*	-17†
-27*	-29*	-55*	-36*	-50*	-21†
-25*	-15	-40*	-44*	-38*	+17
-23*	-11	-36*	-37*	-35*	+7
-37*	-6	-33*	-37*	-35*	-2
-27*	-13*	-58*	-54*	-53*	+14
-11	-11	-25*	-29*	-21	-13
-23*	-22*	-18	-10	-8	-6
50*	-39*	-70*	-67*	26*	+15

Skin tests

Two of the grain workers and four controls had positive prick skin tests to two or more common allergens and were considered atopic individuals. Three grain workers and one control had positive prick skin tests to airborne grain dust antigen (Table II). No skin reactivity to barley was observed in the test or control group. Subjects 2, 6, and 7 had positive skin tests to *A. fumigatus*.

Precipitating antibodies

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

Airborne grain dust challenge

Respirable dust concentrations, time averaged for each individual, are presented in Table II. The mean respirable dust concentration was 84 mg/m³. Although all subjects were asked to produce airborne dust in a similar manner, the controls were exposed to a higher mean concentration (132 ± 34 mg/m³) than that for grain workers (50 ± 29 mg/m³).

Symptoms

All subjects became symptomatic during exposure. In some instances the symptoms lasted longer than 24 hr, but none persisted longer than 36 hr. The most common complaints were "flu-like" symptoms, including malaise, myalgias, tiredness, feverish feel-

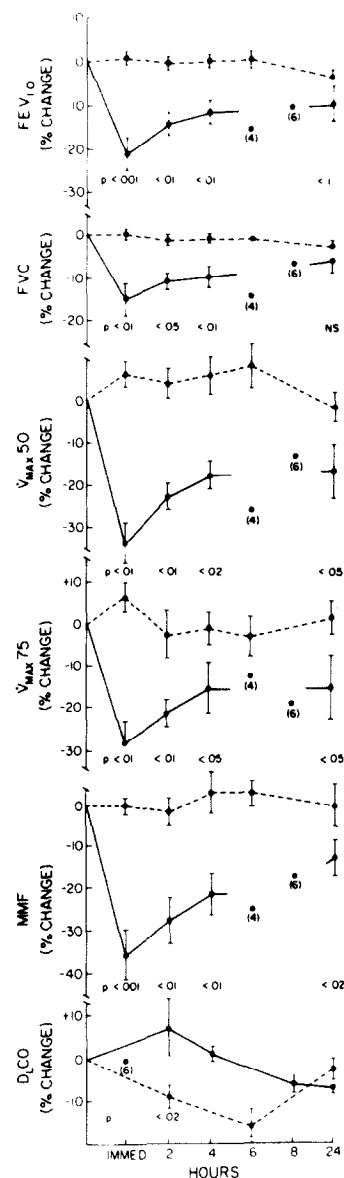


FIG. 1. Mean $\pm$ 1 SE percent change in lung functions from baseline during the control day (----) and the grain dust exposure day (—) for the 12 subjects unless specified otherwise by numbers in parentheses. Statistical significance of the change from baseline was determined by paired t test.

ing, chills, and flushed face, which were observed in all grain workers and in three controls. These symptoms varied in intensity from particularly marked in seven of the 12 subjects to very mild in two subjects. Seven subjects had headaches, and none complained of eye irritation. Nasal symptoms (4/12), throat burning (5/12), cough (12/12), wheezing or chest tightness (9/12), and shortness of breath (9/12) also occurred. The subjects with the most severe symptoms (Nos. 2, 8, and 12) had chest radiographs taken 6

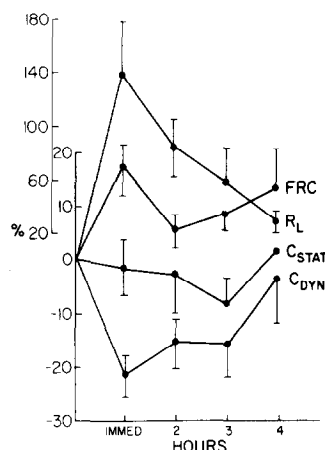


FIG. 2. Mean $\pm$ 1 SE percent change from baseline immediately (IMMED.) and 2, 3, and 4 hr after exposure to airborne grain dust (nine subjects).

hr after challenge, with the results unchanged from baseline.

Pulmonary function

Airway obstruction as indicated by a 20% or greater decline in FEV_1 developed in four of six grain workers and five of six controls (Table II). Evidence of airflow obstruction by changes in MMF, $\dot{V}_{max_{50}}$, and $\dot{V}_{max_{75}}$ greater than 25% from baseline were observed in all but one of the subjects. The maximum individual decline in FEV_1 was detected in most cases (nine subjects) immediately after the 60 to 120 min exposure to airborne grain dust. In two subjects, the maximum change in FEV_1 occurred within 4 to 6 hr and in another subject 24 hr after exposure. Two control subjects showed a marked decrease in FEV_1 28 hr after an initial improvement. The D_LCO decreased by 17% and 21% in two grain workers, but they had similar decreases on their control day. One control subject showed a decrease in D_LCO greater than 20% 28 hr after exposure.

The mean values immediately and 4 hr after exposure for FEV_1 , FVC, MMF, $\dot{V}_{max_{50}}$, and $\dot{V}_{max_{75}}$ (Fig. 1) were significantly lower ($p < 0.001$) than baseline values by *t* test analysis. There was no significant difference between grain workers and controls. Mean FEV_1 and FVC returned to baseline value in 24 hr. The mean MMF, $\dot{V}_{max_{50}}$, or $\dot{V}_{max_{75}}$, however, were still significantly different 24 hr after exposure due to a persistent reduction in mean value for the control subjects. The mean for grain workers almost reached the baseline in 24 hr. The mean FEV_1 , FVC, MMF, $\dot{V}_{max_{50}}$, and $\dot{V}_{max_{75}}$ for the 12 subjects were significantly lower within the first 4 hr after grain exposure than those values at corresponding times during the control nonexposure day (Fig. 1).

The mean D_LCO was lower during control day than after grain exposure.

Immediately after exposure there was a significant increase in R_L ($p < 0.01$) and consistent changes in C_{dyn} ($p < 0.02$), without significant changes in FRC or C_{stat} (Fig. 2), indicating airway obstruction with uneven distribution of ventilation time constants rather than parenchymal injury.

We analyzed the relationship between atopy or skin reactivity to grain dust and the development of airway reaction to exposure to dust utilizing chi-square analysis and it was not significant.

Four of the nine subjects who developed airway obstruction by the FEV_1 criteria were nonatopic and five were atopic. Airway obstruction developed in six of eight negative skin reactors to airborne grain dust and in three of four skin reactors to the airborne dust.

Blood leukocyte counts

Ten subjects (5/6 controls and 5/6 grain workers) developed leukocytosis, ranging from 11,700 to 24,300 white blood cells/mm³ (Table II), with relative increases in neutrophils without eosinophilia. Compared with baseline, the average increase in the leukocytes was 11,000/mm³. In one additional subject the white count increased by 3300 but remained below 9000. The maximum leukocyte change was seen between 4 and 8 hr in most subjects and in 24 hr in one subject. The mean postexposure leukocyte counts for the 12 subjects were significantly higher ($p < 0.001$) than the mean baseline values by *t* test analysis.

Body temperature

Body temperature rose above 37.8° C in six subjects (5/6 grain workers and 1/6 controls) 2 to 4 hr after exposure. In the six grain workers the mean postexposure temperature was significantly different ($p < 0.05$) when compared with the mean baseline temperature.

Serum complement levels

Baseline C3 levels were within normal limits (Table III) and insignificant changes in total C3 complements levels were found after exposure, except in one subject in whom an increase of >42 mg was seen 4 hr after exposure. No activation of complement by the alternate or classic pathway was demonstrable in any of the subjects.

Barley extract challenge

One subject developed "flulike" symptoms with hyperthermia and leukocytosis. Five subjects developed leukocytosis without symptoms. Lung functions did not change significantly after the challenge.

TABLE III. Total serum C3 complement levels after exposure to grain dust

Subject No.	Baseline* (mg/dl)	Immediate (mg/dl)	2 to 4 hr (mg/dl)	6 to 8 hr (mg/dl)	24 hr (mg/dl)
1	106	106	72	118	118
2	124	110	124	102	102
3	72	68	142 [†]	58	ND
4	102	102	112	92	106
5	110	110	106	92	106
6	76	76	72	76	ND
7	88	98	110	ND	ND
8	102	88	80	84	88
9	128	132	124	110	124
10	142	118	110	ND	132
11	88	88	102	92	84
12	92	116	92	ND	ND
Mean	102.5	101.0	103.8	91.6	107.5
SD	21.1	18.4	21.6	17.9	16.7

ND = not done.

*Normal range 55 to 120 mg/dl.

†Significant change from baseline.

DISCUSSION

We reproduced the grain fever syndrome in grain handlers and in non-grain handlers by exposure to high concentrations of grain dust. We noted that facial warmth and headache and the associated respiratory symptoms, i.e., throat and tracheal burning sensation, chest tightness, dyspnea, cough, and expectoration, developed mostly during the exposure to dust, whereas other systemic symptoms such as malaise, myalgias, chilliness and hyperthermia, and leukocytosis developed or reached their maximum intensity several hours after exposure. The sensations of fever, warmth, or chilliness were not always associated with oral hyperthermia ($>37.8^{\circ}\text{C}$). The symptoms improved overnight, but leukocytosis and/or airway obstruction persisted longer than 24 hr in half of the 12 subjects. That the spirometric changes were reflecting airway narrowing and not diffuse parenchymal inflammatory reaction was supported by the increase in R_L , frequency dependence of compliance, and no change in C_{stat} after dust exposure.

Warren et al.²¹ induced grain fever in six of 12 grain workers and two of five control subjects by inhalation of crude grain extracts. We found, as they did, no changes in C3 levels and no correlation between grain fever and the presence of precipitins to grain dust or fungi. Our results differ from theirs in that we found no correlation between the immediate skin hypersensitivity and the airways response to grain dust inhalation and in that our controls developed airways constriction as well as grain fever. These discrepancies may be due to differences in the experimental subject selection, in the antigenic solutions used for

skin testing, and/or in the source, composition, and concentration of the inhaled airborne dust and in the method used for inhalation challenge. In a previous study we found no correlation between sensitivity on skin testing and on bronchial provocation with extracts of durum wheat and of airborne durum wheat dust.²²

Chan-Yeung et al.²³ found asthmatic reactions without grain fever in only six of 22 grain workers challenged with crude grain extracts for 30 min and in none of the control subjects. The reactors to grain dust had pre-existing airway obstruction and high bronchial sensitivity to methacholine but no demonstrable specific skin hyperreactivity to grain dust. The much higher incidence of postchallenge asthmatic reactions and grain fever in our subjects (Table II), regardless of their methacholine sensitivity or pre-existing airway function, is most likely a result of the more prolonged and severe exposure to airborne grain dust in our experiments and, perhaps, to the differences in the contents of the dust. The high dust concentrations achieved during our experiment have been found in Canadian country grain elevators¹ (e.g., weighing hopper area, 406 mg/m³) as well as in American terminal grain elevators²³ (e.g., cleaner operator, main floor, 205 mg/m³). Nowadays, such high concentrations should occur only when dust control systems are not operating adequately.*

Particles of the grain (e.g., barley endosperm,

*Occupational Safety and Health Administration (OSHA) Citation. OSHA area office files, Milwaukee, WI, and Minneapolis, MN, May 1974.

germ or bran) are among the constituents of airborne grain dust that can be potential inducers of grain fever. With saline extracts of barley, a constituent of airborne dust, we reproduced the grain fever syndrome with hyperthermia and leukocytosis in one subject, and five subjects developed asymptomatic leukocytosis, indicating a subclinical host reaction to barley extract. This suggests that in some individuals, barley particles from the endosperm, germ, or bran contained in airborne grain dust may be responsible for the grain fever syndrome.

Grain dust also contains a wide variety of fungi, bacteria, and mite and insect particles,¹⁻⁵ including several species of *Aspergillus*, *Penicillium*, *Mucor*, *Alternaria*, *Cladosporium*, and thermophilic bacteria that are known to be capable of producing a variety of pulmonary immunologic reactions that include types I and III allergic reactions and hypersensitivity pneumonitis.⁸ The clinical correlates of these reactions include asthma, allergic bronchopulmonary aspergillosis,²⁴ and "farmer's lung" disease.²⁵ Except in isolated individuals, neither the role these organisms play nor the immunologic mechanism involved in the pulmonary reaction to grain has been identified. In those few instances, the constituent of dust responsible for the pulmonary reaction has been identified to be the debris of a grain weevil (*Sitophilus granarius*), a grain mite (*Glycophagus destructor*),²⁶ a specific fungus,²⁷ or a specific component of grain.²⁸

It has been suggested that grain fever is a type III allergic reaction similar to that seen in various forms of hypersensitivity pneumonitis.⁸ Despite the clinical similarities, grain fever, unlike allergic alveolitis caused by inhalation of organic materials, tends not to recur unless the exposure to grain dust is again massive. Grain fever has not been found to be related to the presence of precipitins to grain, grain dust, or fungi, and in none of the reported studies has hypersensitivity pneumonitis or its sequelae, chronic interstitial fibrosis, of the type seen in "farmer's lung" been established with certainty.

Our data do not support the hypothesis that grain fever is a hypersensitivity pneumonitis, since none of the subjects had precipitins to grain dust, complement activation was not detected, and changes in D_LCO or C_{stat} were not observed. The lack of reduction of D_LCO does not exclude the possibility of patchy, rather than diffuse, inflammatory parenchymal reaction. More importantly, the reduction in D_LCO from interstitial disease could be masked by a spuriously elevated D_LCO in the presence of coexistent airflow obstruction. Furthermore, the lack of evidence of complement activation in the subjects' sera does not exclude the probability of local activation of comple-

ment in the lungs. The fact that grain fever is often reported on either the first day of work and/or after exposure to high concentrations of dust and that during our experimental exposure both grain handlers and subjects not previously exposed to grain dust developed systemic symptoms and leukocytosis implies that grain fever is a nonallergic reaction to grain dust. The reaction to high concentrations of grain dust does resemble the systemic reactions that follow massive exposure to fungal spores such as mycotoxicosis.²⁹ Whether massive exposures to moldy grain dust actually occur in the Superior-Duluth terminal elevators is not known.

Pernis et al.³⁰ have suggested that febrile reactions after inhalation of vegetable dusts, e.g., mill fever, are caused by the inhalation of bacterial endotoxins, including its histamine-liberating properties. Recently, it has been demonstrated that airborne grain dust contains bacteria⁶ and endotoxin.⁷ Therefore it is possible that the systemic manifestations of grain fever are due to the host reaction to bacterial endotoxin. It is also possible that grain dust initiates nonallergic release of pharmacologically active substances from the lung after interaction between components of grain dust and lung cells. The airways response that may or may not be associated with grain fever might have a similar pathogenesis. However, the asthmatic reactions in response to the low levels of grain dust currently found in the grain elevators with dust control systems, e.g., less than 15 mg/m³ can be due in some individuals to an immediate hypersensitivity reaction to a component of grain dust.

To elucidate the immune and nonimmune mechanisms by which grain dust induces airway constriction, further investigation is needed.

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