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DEMONSTRATION OF INFLAMMATORY CELL POPULATION CHANGES IN RAT LUNGS IN RESPONSE TO INTRATRACHEAL INSTILLATION OF SPRING WHEAT DUST USING LUNG ENZYMATIC DIGESTION AND CENTRIFUGAL ELUTRIATION

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Abstract—The inhalation of grain dust by grain workers is responsible for a large number of pulmonary pathophysiologies. These problems may be acute or chronic and may be mediated by the chronic activation of the immune system. Constant inflammatory states in the lung may eventually lead to tissue damage and respiratory deficit. This study was designed to measure the changes in the relative number of inflammatory cells in peripheral blood, bronchoalveolar spaces, and lung interstitium that occur in response to intratracheally instilled airborne spring wheat dust in rats. It was found that 6 h after instillation with dust, neutrophils were present in greater numbers in the blood and bronchoalveolar spaces than in lung interstitium. After 24 h, there appeared to be a larger number of neutrophils in the lung interstitium in dust-instilled animals than in saline-instilled controls. These results indicate that intratracheal instillation of grain dust initiates an acute inflammatory reaction, and that there is an initial influx of neutrophils into the air spaces of the lung followed by transit of these cells into the lung interstitium.

Key words: Inflammation, spring wheat dust, lung, rats, neutrophils, enzymatic digestion, centrifugal elutriation, occupational health, agriculture

Résumé—L'inhalation de la poussière de blé par les ouvriers agricoles est responsable d'un grand nombre de troubles respiratoires pulmonaires. L'activation chronique de système immunitaire peut aboutir à ces affections chroniques ou aiguës. Un état inflammatoire prolongé des poumons peut causer des lésions et un déficit respiratoire. Cette étude a été entreprise dans le but d'évaluer les modifications numériques subies par les leucocytes circulants et les cellules inflammatoires des espaces bronchoalvéolaires et du parenchyme pulmonaire de rats en réponse à l'introduction intratracheale poussière de blé de printemps dispersée dans l'air. Six heures après introduction le nombre des neutrophiles était accru dans le sang circulant et dans les espaces bronchoalvéolaires davantage que dans le parenchyme du poumon. Après 24 h, le nombre de neutrophiles dans poumons des rats traités à la poussière est superieur à celui du groupe de contrôle traité avec du sérum physiologique. Ces résultats indiquent que l'introduction de la poussière de blé dans la trachée déclanche une réaction inflammatoire aiguë, et qu'il y a un afflux initial des neutrophiles dans les espaces du poumon suivi par le transit de ces cellules dans le parenchyme du poumon.

Mots-clefs: L'inflammation, la poussière de blé de printemps, le poumon, les rats, les neutrophils, la digestion enzymatique, l'élutriation centrifuge, la maladie professionnelle, l'agriculture

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Abbreviations: HBSS: Hanks' Balanced Salt Solution; U: Units; v/v: volume per volume; w/v: weight per volume; IL-1: interleukin 1; PBS: phosphate buffered saline.

Mention of company names or products does not constitute endorsement by the National Institute for Occupational Safety and Health.

INTRODUCTION

For over 250 years, grain workers have been shown to exhibit health problems due to exposure to grain dust [1]. However, it was suggested only recently that grain dust should be classified as more than just a nuisance dust [2]. Grain dust has been found to cause a number of health problems including nose, throat and eye irritation [3, 4], allergy [5], asthma [6, 7], coughs and other respiratory symptoms [4, 8], grain fever [4, 9, 10], bronchitis [4, 7, 11], and chronic obstructive pulmonary disease [5, 12].

Grain dust is a heterogeneous mixture of grain products [13], microorganisms [14, 15] and chemicals [3]. Specifically, decomposition products of grain, silica, fungi and fungal toxins, lectins, insects, mites, animal hairs, excreta, and pesticides have been reported [7]. In addition, airborne exposure to grain dust can be marked. Concentrations of up to 57.5 mg/m^3 of respirable dust have been reported in grain elevators [16].

Among the mechanisms postulated to be involved in respiratory pathophysiology after inhalation of grain dust are the increased production of interleukin-1 (IL-1) and the activation of complement. IL-1 production has been found to be associated with exposure of alveolar macrophages to airborne grain dust [17, 18]. IL-1 is released from macrophages and mediates many of the signs and symptoms of acute inflammation [19]. Among the IL-1 effects are neutrophil release from bone marrow and other stores [20], and chemotaxis of neutrophils to a site of inflammation [21]. Activation of the complement cascade results in inflammatory events by the classical pathway and is also triggered by relatively non-specific stimuli (alternative pathway). Activation of complement generates the anaphylatoxin C5a which has chemotactic effects on polymorphonuclear leukocytes, and grain dust exposure has been shown to bring about the antibody-independent activation of both the alternative and classical complement pathways [22].

It is evident from the literature that the inflammatory cell response to inhaled grain dust has been studied incompletely. The purpose of this study was to monitor the response of inflammatory cells in the lung and peripheral blood during a short-term time-course. In addition, an attempt was made to correlate the findings to pathological changes in the lung.

MATERIALS AND METHODS

General protocol

Each set of animals was divided into two groups. One group received intratracheal instillation of saline, and the other was instilled with spring wheat dust suspension. Either 6 or 24 h after instillation, the animals were killed by an overdose of pentobarbital, blood samples were taken from each animal, and alveolar macrophages were collected by bronchoalveolar lavage. The lungs of the animals were then excised for either enzymatic digestion or snap freezing for sectioning as described below. Determinations of statistical significance were based on arcsine transformation [23].

Dust

Airborne spring wheat dust was collected from port grain terminals in the Superior-Duluth region of the United States as previously described [24] and stored at 4°C until used. Airborne spring wheat dust suspensions were prepared by making a suspension of 4 mg/ml in pyrogen-free saline (Travenol Laboratories, Inc., Deerfield, IL). The particles were dispersed immediately before instillation using a Sonifier Cell Disruptor (Model 350; Branson Scientific, Danbury, CT).

Animals

Specific pathogen-free Lewis strain rats were used. These animals were maintained in laminar flow hoods and sterilized laboratory chow and water were provided *ad libitum*.

Intratracheal instillation

Rats were anesthetized by intraperitoneal injection of 0.2 ml sodium pentobarbital (65 mg/ml; Butler Co., Columbus, OH). The rats were secured on a sloped board and the tongue of each animal was pulled aside to visualize the epiglottis. During inspiration, 0.25 ml of fluid was injected via a curved, blunt, 20 gauge feeding needle (Popper and Sons, Inc., New Hyde Park, NY). Isotonic, non-pyrogenic saline (Travenol Laboratories, Inc.) was instilled as a control solution.

Bronchoalveolar lavage

6 or 24 h after exposure rats were anesthetized with 1.0 ml sodium pentobarbital by intraperitoneal injection and pulmonary lavage performed as previously described [17]. Ten milliliters of Hanks' balanced salt solution (HBSS; GIBCO, Grand Island, NY) containing 100 U/ml of heparin (Chromalloy Pharmaceuticals, Inc., Oak Park, MI) and 1% v/v 0.075% sodium bicarbonate (M.A. Bioproducts, Walkersville, MD) were instilled into the lungs and recovered by aspiration. The procedure was repeated until approx 50 ml of fluid were collected from each animal. To isolate the alveolar macrophages, the lavage fluid was centrifuged at 400 ***g*** for 15–20 min, the cell pellet was resuspended in a small volume of medium M-199 (Whittaker M.A. Bioproducts, Walkersville, MD) and the cells were counted. Seventy-five microliters of the cell suspension were air dried onto clean microscope slides, stained with Wright–Giemsa stain (Cambridge Chemical Products, Inc., Ft Lauderdale, FL) according to manufacturer's recommendations, and examined by light microscopy.

Lung enzymatic digestion

Following exposure to spring wheat dust suspension or sterile saline and after bronchoalveolar lavage, the lungs were digested as described previously [25] but modified for the collection of lymphocytes. Briefly, rat lungs were exposed and perfused with injectable saline (Travenol Laboratories, Inc.). The left atrium and right ventricle were cut and intravenous tubing inserted (0.040 in. inner dia; Cole-Parmer, Chicago, IL) through the right ventricle into the pulmonary artery. Saline was infused until the lung blanched (i.e. blood was removed), and the lungs were lavaged as described above. After the last lavage step, the lungs were insufflated with either 10 ml of 42 U/mg elastase solution (Sigma Chemical Co., St Louis, MO) or 10 ml of 0.2 mg trypsin/100 ml phosphate buffer (Difco Laboratories, Detroit, MI) and emptied. Then the lungs were re-insufflated with 10 ml of the same enzyme, clamped closed, and incubated in a 37°C shaking water bath for 25 min. Following incubation, the lungs were cut with scissors into small pieces and the lung pieces chopped with a tissue chopper (Mickle Laboratory Engineering Co., Gamshall, England) set at 0.5 mm thickness. The tissue was chopped 4 times with the platform rotated 90° each time. Chopped lung tissue was then added to PBS containing 10% fetal bovine serum plus DNase I (0.006% w/v; United States Biochemical Corp., Cleveland, OH) to inhibit the elastase or 15 ml of soybean trypsin inhibitor (0.1 mg/ml phosphate buffer; Sigma Chemical Co.) to stop the trypsin reaction. The tissue was then

incubated at 37°C in a shaking water bath for 10 min. The suspension was filtered sequentially through coarse (103T), medium (330T), and fine (420T) nylon mesh. The resulting suspension was centrifuged at 500 ***g*** for 5 min and the pellet was resuspended in a small amount of phosphate buffer with DNase. The suspension was fractionated by elutriation (Model J-21 centrifuge with JE-6 rotor; Beckman Instruments, Inc., Irvine, CA) using phosphate buffer with 0.5% bovine serum albumin. With the rotor speed maintained at 2000 rpm, and the temperature at 4°C, an initial 150 ml fraction was obtained at a pump speed of 5 ml/min. The pump speed was then increased to 10 ml/min, and a second 150 ml fraction was collected. This fraction contained lymphocytes from healthy lungs and lymphocytes and neutrophils from inflamed lungs. After centrifugation at 500 ***g*** for 5 min, the cells contained in the second fraction were resuspended in 1 ml of medium M-199, dried onto clean microscope slides, stained with Wright–Giemsa stain, and examined by light microscopy.

Blood differential analysis

In a syringe containing 0.1 ml of heparin (1000 U/ml; Chromalloy Pharmaceuticals, Inc.), approx 1 ml of blood was obtained from the vena cava of each rat. The collected blood was smeared on clean microscope slides and stained with Wright–Giemsa stain according to manufacturer's recommendations. The cell types were determined by light microscopy using oil immersion (1000×) and tabulated after at least one hundred leukocytes were counted for each slide.

Preparation of lungs for sectioning

After the lungs were lavaged *in situ*, the lungs were removed with tracheas intact, and insufflated with a 1:1 mixture of Ames O.C.T. embedding compound (Miles Laboratories, Elkhart, IN) and non-pyrogenic isotonic saline (approx. 10 ml per animal). The lungs were placed in isopentane (J. T. Baker Chemical Co., Phillipsburg, NJ) which was immersed in liquid nitrogen. The rapidly frozen lungs were kept at −70°C until sectioning with a Frigocut Cryotome (American Optical Co., Buffalo, NY). The thicknesses of the sections were varied intentionally from 7 to 20 μm. The sections were placed on slides and fixed with 10% buffered formalin and stained according to Luna [26] with Harris hematoxylin and eosin.

Counterimmunoelectrophoresis

Sera from randomly selected control and experimental rats were tested for the presence of precipitating antibodies to airborne spring wheat dust extract as described previously [27].

Endotoxin analysis

To measure the extractable endotoxin in the spring wheat dust, 10 mg dust were extracted with 10 ml sterile, non-pyrogenic water. After rocking at room temperature for 60 min, the solution was filtered through a 0.45 μm pore size filter (Millex; Millipore Corporation, Bedford, MA). The filtrate was then assayed in duplicate for endotoxin using the quantitative chromogenic *Limulus* amebocyte lysate assay (Whittaker M.A. Bio-products, Inc.). Results were analyzed by linear regression analysis after computation of a standard curve.

Table 1. Blood differential analysis on dust-instilled and control rats for 6-h and 24-h exposure times

Treatment (*N*)	Cell type			
	Eosinophils (%)	Neutrophils (%)	Lymphocytes (%)	Monocytes (%)
6 h				
Saline (8)	0.5 ± 0.4	27.8 ± 2.3	69.7 ± 2.3	1.9 ± 0.4
Dust (9)	0.2 ± 0.2	43.6 ± 3.3[ab]	54.3 ± 3.2[a]	1.8 ± 0.4
24 h				
Saline (11)	0.8 ± 0.4	17.5 ± 1.6	79.4 ± 1.6	2.3 ± 0.5
Dust (15)	1.1 ± 0.4	23.9 ± 1.9[a]	72.8 ± 1.7[ab]	2.1 ± 0.4

[a]Significantly different for treatment ($P < 0.05$).
[b]Significantly different for time period ($P < 0.05$).
Data are means ± SE.

RESULTS

Initial experiments were performed to determine the elutriation conditions which gave the best cell yield. Lungs from 4 healthy, normal rats were digested with elastase as described in the Materials and Methods section. The resulting cell suspension was fractionated using the elutriator. With a constant rotor speed of 2000 rpm the flow rate was increased intentionally from about 3 ml/min to approx 10 ml/min. We found that the lower flow rate eluted membrane fragments and red blood cells, and not until a flow rate of 10 ml/min was achieved were significant numbers of nucleated cells eluted. Microscopic examination revealed that the 10 ml/min fraction contained predominantly lymphocytes (>80%) with neutrophils also being present in this fraction. Based upon these initial results, all subsequent studies were performed using 5 ml/min flow rate to remove cell debris and red blood cells, and 10 ml/min to collect the lymphocyte/neutrophil fraction for analysis. Slightly higher cell yields were seen with trypsin than with elastase, but the ratios of cells were not affected, and therefore the data were combined for analyses.

Results from the peripheral blood analysis are shown in Table 1. Significant increases in neutrophils were seen at both the 6 and 24 h time periods in the animals which received dust. The number of lymphocytes in each case was significantly higher in the saline-treated animals than in the dust-treated ones. In addition, there were significantly more neutrophils seen in the 6 h time period than in the 24 h time period for the dust-treated animals. Neutrophil counts were also higher in the saline-treated animals at 6 h after treatment than at 24 h. Lymphocytes were significantly lower at the 6 h time period for both dust and saline treatments.

Table 2 shows that there was a significantly higher number of granulocytes in the lung and airways surfaces, as determined by bronchoalveolar lavage, for the dust-treated animals at 6 h when compared to the controls. This was also seen at 24 h. The dust treatment granulocyte number was higher for 6 h than for 24 h. There was no difference in the saline-treated animals for the time period effect. Even though the percentage of macrophages was reduced in the exposed rats as compared to the controls, the total number of macrophages increased slightly for the dust-treated animals.

Examination of enzymatic digestion of the rat lungs showed a significantly higher number of neutrophils in the dust-exposed animals at 6 and 24 h than was seen for the saline-treated animals (Table 3). Lymphocytes showed a concomitant lower percentage at the 6 and 24 h time periods for the dust-exposed animals. There were significant differences between time periods for this treatment which showed an increased number of neutrophils in the lung interstitium for dust-treated animals at 24 h compared to the 6 h time point.

Table 2. Bronchoalveolar lavage analysis of dust-instilled and control rats for 6-h and 24-h exposure times

Treatment (*N*)	Cell type	
	Macrophages (%)	Granulocytes (%)
6 h		
Saline (8)	89.1 ± 4.2	10.9 ± 4.2
Dust (9)	46.4 ± 9.6[a]	52.6 ± 9.6[ab]
24 h		
Saline (15)	91.7 ± 2.5	8.3 ± 2.5
Dust (16)	79.0 ± 6.1[ab]	21.0 ± 6.1[a]

[a]Significantly different for treatment ($P < 0.05$).
[b]Significantly different for time period ($P < 0.05$).
Data are means ± SE.

Table 3. Lung enzymatic digestion analysis of dust-instilled and control rats for 6-h and 24-h exposure times

Treatment (*N*)	Cell type	
	Lymphocytes (%)	Neutrophils (%)
6 h		
Saline (6)	88.1 ± 2.0	11.7 ± 2.1
Dust (7)	80.5 ± 2.1	19.7 ± 2.2[a]
24 h		
Saline (6)	90.4 ± 2.5	9.6 ± 2.5
Dust (7)	55.7 ± 5.1[ab]	44.3 ± 5.1[ab]

[a]Significantly different for treatment ($P < 0.05$).
[b]Significantly different for time period ($P < 0.05$).
Data are means ± SE.

Histological examination of lung sections showed a large increase in the number of neutrophils in the alveoli in the dust-treated animals at the 6-h time period than seen in the saline-treated controls. Fewer neutrophils were seen in the alveoli of the dust-treated animals at the 24-h time period than at the 6-h time period.

Counterimmunoelectrophoresis of random serum samples from control and experimental rats showed no specific precipitating antibodies, although the positive control showed numerous lines of precipitation. This suggests that the animals were not sensitized prior to the experiment. Endotoxin analysis showed the amount of endotoxin present in the dust was 2.2 ng endotoxin/mg dust.

DISCUSSION

The data suggest a time-course for an inflammatory process subsequent to the instillation of spring wheat dust. Initially, neutrophils appear to have been recruited to the lung surface. Neutrophils were found also in increased numbers in the peripheral blood and lung interstitium. The relative numbers of the cells in this interstitial area were 4-fold higher at the 24-h post-exposure than at 6 h post-exposure. Thus, it appears that the influx of neutrophils in the lung interstitium increased more slowly with time than the influx of cells into the airways.

An initial response appears to have been generated against the grain dust which manifested itself primarily in the peripheral blood and lung surface. These cells may have originated from pre-existing leukocyte stores. A different response is seen at the 24-h time period. These inflammatory cells may be brought to the lung interstitium by slower-acting substances which might stimulate release of neutrophils from bone marrow rather than by simply attracting previously circulating cells. Alternatively this later influx could be a result of secondary secretion of chemotactic materials from dust-affected macrophages. Radiolabeling of neutrophils and tracing techniques could lead us to the actual source of the neutrophils.

Complement activation could explain the initial chemotaxis; grain dusts have been shown to activate complement [22]. With activation, the primary chemotactic peptides C3a and C5a would be released, and these peptides could cause the rapid influx of neutrophils into the lung. Interleukin-1 production might account for the slower response. Grain dusts have been shown to stimulate production of IL-1 [17, 18], and IL-1 stimulates the release of neutrophils from bone marrow and is chemotactic for them. The activity could account

for the continued accumulation of neutrophils, particularly in the lung interstitium. Histologically, the neutrophils invaded the alveolar spaces of the dust-treated animals. The histological sections showed more neutrophils present at 6-h after dust treatment than in the sections taken from the rats which were examined at 24 h after exposure.

From a technical standpoint, the results demonstrate the utility of enzymatic digestion and elutriation for studying pulmonary inflammation. This method provided relatively homogeneous cell populations. This technique has great potential for the study of lung cells and should provide useful information as the technique is expanded. Experimental variations such as using other enzymes, collagenase for example, and varying the pump or rotor speed may lead to increased sensitivity in defining cell populations.

As grain workers are exposed to periodic high levels of grain dust throughout their work day [7], the processes observed may result in the continued activation of inflammatory cells, as shown here. Because neutrophils exert destructive action partly by means of oxygen radicals [28] and secretion of lysosomal enzymes [29], it is likely that tissue damage may result. In addition, the presence of gram-negative bacterial endotoxins in the dust could act as priming agents which lead to enhanced responsiveness of neutrophils to other stimuli which are present in the heterogeneous grain dust [30]. Resulting tissue damage would then be increased.

In conclusion, intratracheal instillation of airborne grain dust results in inflammatory cell activation. The primary responding cell type was the neutrophil. Neutrophils were observed in increased relative numbers in the peripheral blood, bronchoalveolar spaces, and lung interstitium. There appeared to be a migration of neutrophils from the peripheral blood to bronchoalveolar spaces and later to the lung interstitium which increased with time of dust exposure. Finally, there is evidence of neutrophil infiltration of alveoli as determined by histological lung sections. These changes in pulmonary cell populations demonstrate, in part, the dynamic response of the lung to an insult of airborne grain dusts. The method of centrifugal elutriation was shown to be very effective for the study of pulmonary cell populations.

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REFERENCES

1. Ramazzini B. In *De Morbis Artificum* (Wright W. C., Transl.). Hafner, New York (1713).
2. Chan-Yeung M., Schulzer M., MacLean L., Dorken E., Tan F., Lam S., Enarson D. and Grzybowski S. A follow-up study of the grain elevator workers in the Port of Vancouver. *Arch. Envir. Hlth* **36,** 75–81 (1981).
3. NIOSH (National Institute for Occupational Safety and Health). Health Hazard Evaluation Determination Report No. 75-11-403. Port of Duluth-Superior Grain Elevators (1977).
4. doPico G. A., Reddan S., Flaherty D., Tsiatis A., Peters M. E., Rao P. and Rankin J. Respiratory abnormalities among grain handlers. *Am. Rev. Resp. Dis.* **115,** 915–927 (1977).
5. Broder I., Davies G., Hutcheon M., Leznoff A., Mintz S., Thomas P. and Corey P. Variables of pulmonary allergy and inflammation in grain elevator workers. *J. Occup. Med.* **25,** 43–47 (1983).
6. doPico G. A., Jacobs S., Flaherty D. and Rankin J. Pulmonary reaction to durum wheat: a constituent of grain dust. *Chest* **81,** 55–61 (1982).
7. Chan-Yeung M., Ashley M. J. and Grzybowski S. Grain dust and the lungs. *Can. Med. Ass. J.* **118,** 1271–1274 (1978).
8. Cotton D. J., Graham B. L., Li K-Y R., Froh F., Barnett G. D. and Dosman J. A. Effects of grain dust exposure and smoking on respiratory symptoms and lung function. *J. Occup. Med.* **25,** 131–141 (1983).
9. Tse K. S., Warren P., Janusz M., McCarthy D. S. and Cherniack R. M. Respiratory abnormalities in workers exposed to grain dust. *Arch. Envir. Hlth* **27,** 74–77 (1973).

10. doPico G. A., Flaherty D., Bhansali P. and Chavaje N. Grain fever syndrome induced by inhalation of airborne grain dust. *J. Allergy Clin. Immun.* **69,** 435–443 (1982).
11. Dosman J. A., Cotton D. J., Graham B. L., Li K-Y R., Froh F. and Barnett G. D. Chronic bronchitis and decreased forced expiratory flow rates in lifetime nonsmoking grain workers. *Am. Rev. Resp. Dis.* **121,** 11–16 (1980).
12. Warren P., Cherniack R. M. and Tse K. S. Hypersensitivity reaction to grain dust. *J. Allergy Clin. Immun.* **53,** 139–149 (1974).
13. Cotton D. J. and Dosman J. A. Grain dust and health. III. Environmental factors. *Ann. Intern. Med.* **89,** 420–421 (1978).
14. DeLucca II A. J., Godshall M. A. and Palmgren M. S. Gram-negative bacterial endotoxins in grain elevator dusts. *Am. Ind. Hyg. Ass. J.* **45,** 336–339 (1984).
15. Dutkiewicz J. Exposure to dust-borne bacteria in agriculture. I. Environmental studies. *Arch. Envir. Hlth* **33,** 250–259 (1978).
16. Farant J-P. and Moore C. F. Dust exposures in the Canadian grain industry. In *Occupational Disease Focus on Grain Dust and Health* (Edited by Dosman J. A. and Cotton D. J.), pp. 477–506. Academic Press, New York (1980).
17. Lewis D. M. and Mentnech M. S. Extracts of airborne grain dusts stimulate interleukin 1 (IL-1) production by alveolar macrophages. *Am. Rev. Resp. Dis.* **129,** A161 (1984).
18. Lewis D. M., Mentnech M. S. and Olenchock S. A. Effects of vegetable dust extracts on cellular immune responses. In *Proceedings of the Eighth Cotton Dust Research Conference* (Edited by Wakelyn P. J. and Jacobs R. R.), pp. 143–145. National Cotton Council, Memphis, Tenn. (1984).
19. Kampschmidt R. F. The numerous postulated biological manifestations of interleukin-1. *J. Leukocyte Biol.* **36,** 341–355 (1984).
20. Kampschmidt R. F. and Upchurch H. F. Neutrophil release after injections of endotoxin or leukocyte endogenous mediator into rats. *J. Reticuloendothel. Soc.* **28,** 191–201 (1980).
21. Sauder D. N., Mounessa N. L., Katz S. I., Dinarello C. A. and Gallin J. I. Chemotactic cytokines: the role of leukocyte pyrogen and epidermal cell thymocyte-activating factor in neutrophil chemotaxis. *J. Immun.* **132,** 828–832 (1984).
22. Olenchock S. A., Mull J. C. and Major P. C. Extracts of airborne grain dust activate alternative and classical complement pathways. *Ann. Allergy* **44,** 23–28 (1980).
23. Snedecor G. W. and Cochran W. C. *Statistical Methods*, pp. 327–329. The Iowa State University Press, Ames, Iowa (1967).
24. Peach III M. J., Olenchock S. A., Sorenson W. G. and Major P. C. Relevance of grain dust collection techniques to respiratory disease studies. In *Occupational PulmonaryDisease Focus on Grain Dust and Health* (Edited by Dosman J. A. and Cotton D. J.), pp. 507–512. Academic Press, New York (1980).
25. Mason R. J., Williams M. C., Greenleaf R. D. and Clements J. A. Isolation and properties of Type II alveolar cells from rat lung. *Am. Rev. Resp. Dis.* **115,** 1015–1026 (1977).
26. Luna L. G. *Manual for Histological Staining*, pp. 38–39. McGraw-Hill, New York (1968).
27. Olenchock S. A., Mentnech M. S., Mull J. C., Gladish M. E., Green F. H. Y. and Major P. C. Complement, polymorphonuclear leukocytes and platelets in acute experimental respiratory reactions to *Aspergillus. Comp. Immun. Microbiol. infect. Dis.* **2,** 113–124 (1979).
28. Curnutte J. T., Whitten D. M. and Babior B. M. Defective superoxide production by granulocytes from patients with chronic granulomatous disease. *N. Engl. J. Med.* **290,** 590–597 (1974).
29. Klebanoff S. J. and Hamon C. B. Role of myeloperoxidase-mediated antimicrobial systems in intact leukocytes. *J. Reticuloendothel. Soc.* **12,** 170–196 (1972).
30. Worthen A. S., Haslett C., Smedly L. A., Rees A. J., Gumbay R. S., Henson J. E. and Henson P. M. Lung vascular injury induced by chemotactic factors: enhancement by bacterial endotoxins. *Fed. Proc.* **45,** 7–12 (1986).