

Induction of type I hypersensitivity in guinea pigs after inhalation of phthalic anhydride

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Guinea pigs were exposed through inhalation to phthalic anhydride (PA) dust at 0.5, 1.0, and 5.0 mg/m³, 3 hours/day for 5 consecutive days. Inhalation challenge with aerosolized phthalic anhydride–guinea pig serum albumin (PA-GPSA) conjugate elicited immediate-onset respiratory reactions in animals exposed to all three levels of dust. Inhalation challenge of a subgroup of animals with phthalic anhydride dust did not elicit an immediate response, as measured by changes in respiratory frequency and plethysmograph pressure. Serologic studies showed that these animals had allergic IgG1a antibody to PA-GPSA. There was a dose-dependent increase in specific IgG antibody activity, as measured by ELISA. Animals exposed to and challenged with 5.0 mg/m³ PA dust had significant numbers of hemorrhagic lung foci. Those animals with the greatest number of foci had high IgG antibody activity to PA, as measured by ELISA. This study showed that exposure to levels of PA dust as low as 0.5 mg/m³, below the current threshold limit value of 6.0 mg/m³, can sensitize animals to produce allergic antibody. (J ALLERGY CLIN IMMUNOL 1994;94:747-56.)

Key words: Phthalic anhydride, sensitization, guinea pigs

Phthalic anhydride (PA) is a highly reactive, low-molecular-weight chemical used in the production of alkyd and epoxy resins. Like other anhydrides—namely, trimellitic anhydride (TMA) and tetrachlorophthalic anhydride—PA can covalently modify proteins and as such act as a hapten and induce an immune response.¹⁻⁴ Some individuals occupationally exposed to PA have an IgE-mediated hypersensitivity response associated with symptoms of rhinitis, conjunctivitis, and asthma on reexposure to PA.⁵⁻⁸ The first case report of asthma and allergy associated with PA was made in 1939 by Kern.⁹ Diagnosis of occupational allergic asthma to PA has been made by means of specific bronchial provocation testing with PA dust,⁷ serologic study, and skin testing performed with a PA–human serum albumin con-

Abbreviations used

GPSA: Guinea pig serum albumin
PA: Phthalic anhydride
PBS: Phosphate-buffered saline
PCA: Passive cutaneous anaphylaxis
TMA: Trimellitic anhydride

jugate. The recommended threshold limit value for exposure to PA is 6.0 mg/m³.¹⁰ According to Wernfors⁷ and Nielsen et al.,⁸ this threshold limit value may not be appropriate since sensitized workers experience symptoms at this level. Very little information is available for establishing an exposure level that can minimize allergic sensitization to PA.

The purpose of this study was to evaluate the allergic response to inhaled PA dust in a guinea pig model developed for evaluating the immunogenicity and allergenicity of low-molecular-weight chemicals to determine the minimal sensitizing concentration of PA dust. In a previous study it was shown that exposure of guinea pigs to a dust ranging in daily concentration from 0.5 to 6.0 mg/m³ could allergically sensitize these animals.¹¹ However, determination of a minimally sensitizing level or a “no-effect” level could not be made. In this article we show that animals exposed to low

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levels of PA dust produce both allergic and non-allergic antibodies specific to PA and experience immediate-onset respiratory symptoms when exposed to a PA-GPSA conjugate.

METHODS

Animals

Female Hartley smooth-haired guinea pigs (Charles River Labs, Portage, Mich.) weighing 350 to 400 gm were used throughout the study. Animals were allowed to acclimate in the test facilities for 2 weeks before being placed in the study. Water and food (Purina Guinea Pig Chow, Purina Mills, St. Louis, Mo.) were given *ad libitum*, except during inhalation exposures. For certain experiments, animals were treated at the IIT Research Institute, of Chicago, Ill., and transported (within 1 day) by climate-controlled truck to the Miami Valley Laboratories, of Cincinnati, Ohio, for further evaluation. All procedures used on animals were approved by the institutional animal care and use committees.

Preparation of chemical-protein conjugate

Guinea pig serum albumin (GPSA; fraction V, Sigma Chemical Co., St. Louis, Mo.) was used as the protein for conjugation. A 100:1 molar ratio of PA (Aldrich Chemical, Milwaukee, Wis.) to GPSA was used as the starting solution. Briefly, 1.5×10^{-3} mol/L of PA was added to a rapidly stirring protein solution (10 mg/ml) in 9% sodium carbonate solution (Fisher Chemical, Fair Lawn, NJ). After 30 minutes, the PA-GPSA protein solution was centrifuged at $1000 \times g$ for 30 minutes. The supernatant was collected and passed through a Sephadex G-25 column (Pharmacia, Sweden). The eluate was collected and dialyzed against phosphate-buffered saline (PBS) solution (Sigma) at 4° C for 48 hours. The recovered protein concentration was measured by using a protein assay kit (Bio-Rad Laboratories, Richmond, Calif.). The molar ratio of PA to GPSA was determined by measuring free amino groups on the protein by substitution with 2,4,6-trinitrobenzene sulphonic acid (Sigma).¹² The final molar ratio of PA:GPSA was 20:1.

Generation and analysis of PA dust atmospheres

PA dust was micronized to respirable-sized particles (5 μ m) by the Sturtevant Mill Company (Boston, Mass.). Dust atmospheres were generated with a dry materials feeder (Model 310; AccuRate, Whitewater, Wis.). Dust was picked up by a rotating helix and carried to the generator exhaust. A Transvector jet (Vortec Corp., Cincinnati, Ohio) blew the dust into a mixing chamber, where a second Transvector jet drew the aerosol into tygon lines that fed into 1 m³ stainless steel exposure chambers (Unifab Corp., Kalamazoo, Mich.). Chamber airflow varied between 305 and 375 L/min to generate the targeted levels of 0.5, 1.0, and 5.0

mg/m³ PA dust. PA chamber concentrations were determined by drawing a known volume of air from the exposure chamber across an open-faced glass fiber filter (Gelman Sciences, Ann Arbor, Mich.), eluting the PA into acetonitrile (Alltech Associates, Deerfield, Ill.) and measuring the optical density at 228 nm. The amount of PA extracted from the filter pads was calculated from a standard curve; chamber PA concentrations were calculated from the amount of PA extracted off the pads. The PA concentration in each chamber was measured at least twice during each exposure. The particle size distribution was determined once at each concentration during the study by using a continuous aerosol monitor (PCAM; PPM Inc., Knoxville, Tenn.).

Inhalation exposure and challenge

Two groups of eight guinea pigs each were exposed to 0.5 or 1.0 mg/m³ PA dust in 1 m³ stainless steel chambers, 3 hours/day for 5 consecutive days at the IIT Research Institute. One group of 16 guinea pigs was exposed to filtered air, and another group of 16 guinea pigs was exposed to 5.0 mg/m³ PA dust for the same amount of time. One day after the last exposure, eight animals from each exposure group were shipped to the Miami Valley Laboratory, in Cincinnati. Eight air control animals and eight animals exposed to 5.0 mg/m³ PA dust remained at the IIT Research Institute. Two weeks after the last air or dust exposure, guinea pigs were challenged with PA dust or PA-GPSA conjugate. Air control animals and animals exposed to 5.0 mg/m³ PA dust were challenged with 5.0 mg/m³ PA dust at the IIT Research Institute. Briefly, animals were placed in head-out body plethysmographs with their heads extending into a 0.5 m³ exposure chamber. Changes in plethysmograph pressure due to the animals' respiration were monitored with a pressure transducer (model MP45-14-871; Validyne Corporation, Northridge, Calif.) connected to a chart recorder and a computer. Changes in pressure and respiratory rate were monitored for each animal. Guinea pigs were allowed to acclimate to the chamber; respiratory data were collected 30 minutes before the dust challenge, during the 30 minutes of dust challenge, and up to 60 minutes after the challenge. Percent changes in pressure and respiratory rate were calculated from the baseline. A significant immediate-onset respiratory reaction to PA dust challenge was defined as an increase in pressure or rate greater than three standard deviations from the mean change in the same parameters of air control animals exposed to PA dust. Air control animals and animals exposed to 0.5, 1.0, and 5.0 mg/m³ PA dust were challenged with 2.0 mg/m³ aerosolized PA-GPSA conjugate at the Miami Valley Laboratory. (Because of unexpected technical difficulties, no challenge with GPSA aerosol was performed.) Briefly, guinea pigs were housed in whole-body glass plethysmographs connected to an ultra-low-range differential pressure transducer (Model DP103; Validyne Corp.). Changes in plethysmograph pressure

TABLE I. Characterization of PA dust exposure concentrations

Target	Concentration (mg/m ³)		
	0.5	1.0	5.0
Analytical concentration			
Mean $\pm$ SD	0.55 $\pm$ 0.06	1.27 $\pm$ 0.13	5.57 $\pm$ 0.75
Min/max	0.34/0.74	0.88/1.72	3.69/7.56
TWA	0.56	1.21	5.02
MMAD	3.12 $\pm$ 2.02	3.26 $\pm$ 1.96	3.91 $\pm$ 2.08
% Respirable ($\leq 10 \mu\text{m}$)	95.1	95.2	90.0

TWA, Time-weighted average exposure concentration; MMAD, mass median aerodynamic diameter $\pm$ geometric standard deviation.

and respiratory rate from individual animals were continuously monitored on a chart recorder (ZF-8000; Astro-Med, W. Warwick, R.I.) interfaced with an IBM PC/AT computer. Animals were allowed to acclimate to the plethysmographs. Respiratory data were collected 30 minutes before the challenge, during 30 minutes of conjugate challenge, and up to 60 minutes after the challenge. Percent changes in pressure and respiratory rate were calculated from the baseline. A significant immediate-onset respiratory reaction to conjugate challenge was defined as an increase in rate or pressure greater than three standard deviations from the mean change in the same parameters of air-exposed animals challenged with the conjugate.

Serum collection

Sera were obtained from ether-anesthetized animals by retroorbital bleeding, aliquoted into 0.25 ml volumes, and stored frozen at -20°C . Sera were collected from all study animals before the initial PA dust exposure (baseline) and 18 to 24 hours before respiratory challenge with PA dust or PA-GPSA conjugate.

Passive cutaneous anaphylaxis testing

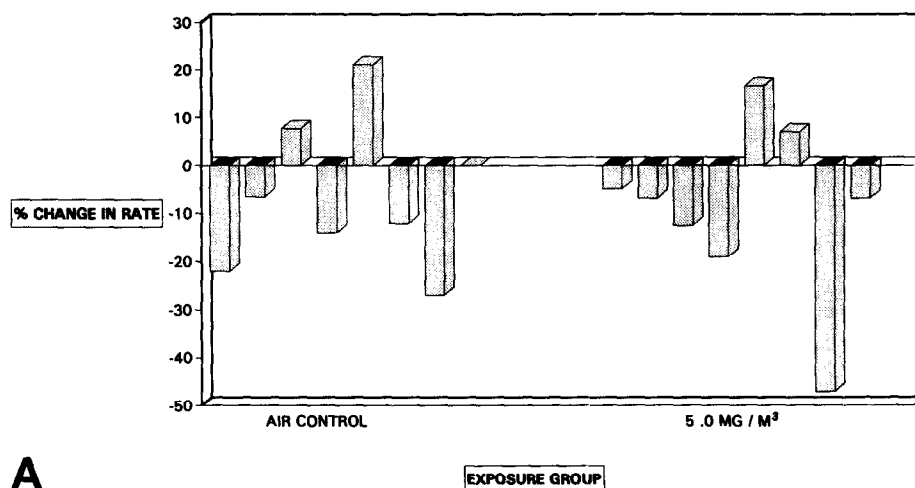
Naive guinea pigs were shaved and depilated dorso-laterally with Neet depilatory (Whitehall Labs) 24 hours before passive cutaneous anaphylaxis (PCA) testing for circulating allergic antibody (IgG1a and IgE). Animals were intradermally injected with 100 μl volumes of sera (collected from air control and PA-exposed animals) diluted $\frac{1}{4}$ to $\frac{1}{132}$ in physiologic saline solution. Four hours or 4 days later, ether-anesthetized animals were intracardially injected with 1.0 ml of 500 $\mu\text{g/ml}$ PA-GPSA or GPSA in a 1% Evans Blue Dye (Sigma) isotonic solution. IgG1a antibody titers (evaluated at 4 hours) and IgE antibody titers (evaluated at 4 days) were defined as the reciprocal of the highest dilution of serum showing visible bluing 30 minutes after intracardial injection. Confirmation of the presence of IgE antibody was made by testing untreated and heat-treated (56°C for 30 minutes) sera in the 4-day PCA test.

ELISA

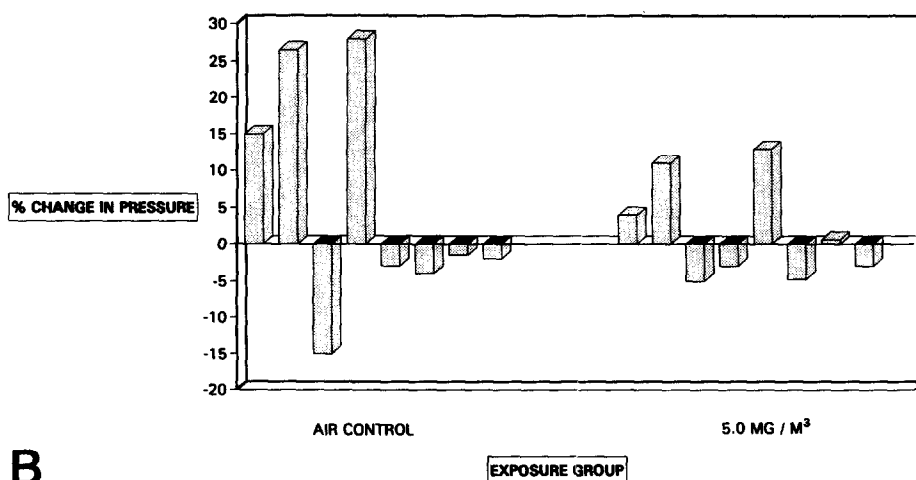
Guinea pig sera were evaluated for IgG antibody to PA-GPSA or GPSA alone by an indirect ELISA. Immunol II U-bottom 96-well plates (Dynatech Labs, Alexandria, Va.) were coated overnight at 4°C with either 100 $\mu\text{l/well}$ of 500 $\mu\text{g/ml}$ PA-GPSA or GPSA in carbonate buffer. The plates were washed 4 times with PBS solution containing 0.05% Tween 20 (PBS-Tween) and post-coated with 100 μl 10% Blotto (Carnation skim milk in PBS-Tween) for 1 hour at room temperature to block protein-binding sites in the wells. The plates were washed four times with PBS-Tween, and 100 μl guinea pig sera (diluted 1/100) were added to duplicate wells and incubated for 2 hours at room temperature. After incubation, unbound antibody was washed from the wells and 100 μl of a 1/700 dilution of alkaline phosphatase-conjugated rabbit anti-guinea pig IgG antisera (ICN Immunobiologics, Lisle, Ill.) in PBS-Tween was added to each well and incubated for 1 hour at room temperature. Excess antibody was removed by washing and 100 μl of substrate, 1 mg/ml p-nitrophenyl phosphate (Sigma) prepared in diethylaniline buffer (Aldrich), was added to each well. The plates were incubated for 30 minutes at room temperature, and the optical density (OD) of each well was measured with a MR-600 ELISA reader (Dynatech). Positive IgG antibody activity to PA-GPSA for each serum sample was defined as an OD reading that was greater than the mean plus two standard deviations of the OD of control wells (sera from filtered-air control animals). The mean OD readings for duplicate control wells ranged from 0.025 to 0.07 at a $\frac{1}{100}$ dilution of serum. (OD readings of GPSA-coated wells were comparable to OD readings of filtered air control animals.)

Gross pathology and histopathology

Twenty-four hours after the PA dust challenge and the PA-GPSA aerosol challenge, air control and PA-exposed animals were anesthetized with sodium pentobarbital and exsanguinated through the femoral artery. A gross examination of the major organs was performed on each animal. The lungs were removed and



A



B

FIG. 1. **A**, Percent change in respiratory rate of animals exposed to air or 5.0 mg/m³ PA dust and challenged with 5.0 mg/m³ PA dust. Changes in rate of the PA-exposed animals were not different from changes in rate of air-exposed animals challenged with 5.0 mg/m³ PA dust. **B**, Percent change in plethysmograph pressure from animals exposed to air or 5.0 mg/m³ PA dust and challenged with 5.0 mg/m³ PA dust. Changes in plethysmograph pressure from the PA-exposed animals were no greater than the changes in plethysmograph pressure from the air-exposed animals challenged with 5.0 mg/m³ PA dust.

trimmed of excess adherent tissue. The number of hemorrhagic foci on each lung lobe was counted. The lungs were inflated with 10% neutral buffered formalin, embedded with paraffin, sectioned to 5 μ m thickness, and stained with hematoxylin and eosin for examination by light microscopy. Representative animals from the PA-exposed/PA dust-challenge group, air exposed/PA dust-challenge group, and air-exposed/air-challenge group were selected for microscopic examination of the lungs.

Statistics

Statistical analysis included both normal distribution and distribution-free techniques. Since Bartlett's test

for homogeneity of variance was significant, comparisons with the air control group were based on the least significant difference criterion. For ELISA data, linear regression analysis was performed by using PA concentrations as the independent variable. All statistical tests were conducted at a 5%, two-sided risk level.^{13, 14} A minimum significance level of $p < 0.05$ was used for all comparisons.

RESULTS

Characterization of PA dust

Table I shows the mean concentration, time-weighted average concentration, and particle size

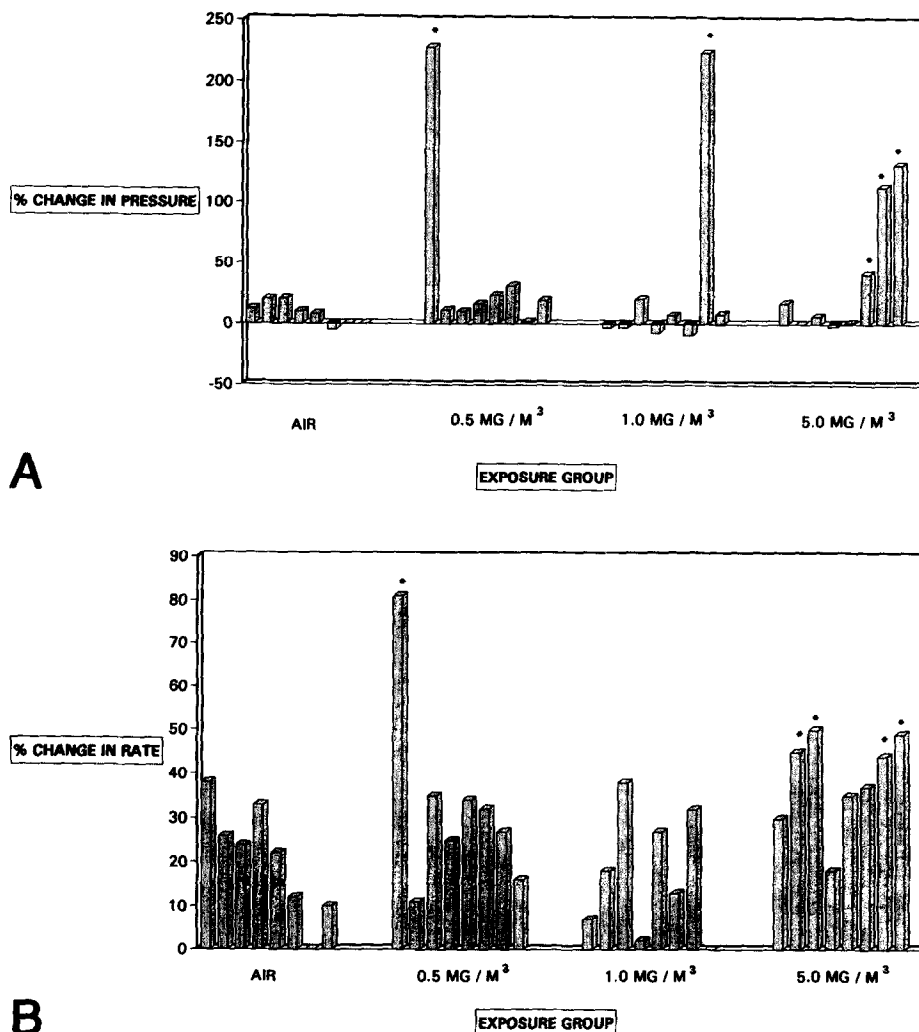


FIG. 2. A, Percent change in plethysmograph pressure from animals exposed to PA dust and challenged with 2.0 mg/m³ PA-GPSA conjugate. The *asterisks* denote those animals with respiratory responses that resulted in significant increases in chamber plethysmograph pressure. **B,** Percent change in respiratory rate of animals exposed to PA dust and challenged with 2.0 mg/m³ PA-GPSA conjugate. The *asterisks* denote those animals with sustained increases in respiratory rate that were significantly greater than changes in respiratory rates of air-exposed animals challenged with PA-GPSA conjugate.

distribution of PA dust during the exposure period. The variation around the target dust concentration was minimal from day to day. The particle size distribution of the dust at the three exposure levels ranged from 90% to 95% respirable ($\leq 10 \mu\text{m}$), with the mass median aerodynamic diameter ranging from 3.12 to 3.91 μg . (Consistent particle size distribution for all three exposure concentrations allowed for consistent comparisons among all three exposure groups.) Thus the time-weighted concentrations of PA, corrected for respirability for the five exposures, were 0.52, 1.21, and 5.02 mg/m³, respectively. No

PA was detected in the filtered-air control chamber at any time during the study.

Inhalation challenge with PA dust

Guinea pigs exposed to air or 5.0 mg/m³ PA dust during the induction period were challenged by inhalation with 5.0 mg/m³ PA dust. The time-weighted average concentration during the challenge was 5.53 mg/m³ PA dust. Fig. 1 shows the change in respiratory rate and plethysmograph pressure during the challenge period for air control animals and animals exposed to PA dust. The changes in respiratory rate were not significantly

TABLE II. Mean IgG antibody levels in sera from animals exposed to PA dust

PA dust exposure level (mg/m ³)	Mean O.D. ($\pm$ SE) at 1/100 serum dilution
Air control	0.048 $\pm$ 0.008
0.5	0.230 $\pm$ 0.071*
1.0	0.298 $\pm$ 0.024*
5.0	0.692 $\pm$ 0.106†
5.0 (IITRI)	0.530 $\pm$ 0.147†

*Significantly greater than air control, at $p < 0.05$ †Significantly greater than air control and 0.5 and 1.0 mg/m³ groups, at $p < 0.05$ **TABLE III.** Percent of animals with IgG1a and IgE allergic antibody to PA-GPSA; analysis by PCA test

Group	IgG1a	IgE
0.5 mg/m ³	3/8 (38%)	0/8 (0%)
1.0 mg/m ³	1/8 (13%)	0/8 (0%)
5.0 mg/m ³	5/8 (63%)	0/8 (0%)
5.0 mg/m ³ * (IITRI)	1/8 (13%)	ND

ND, Not determined.

*Animals receiving the PA dust challenge

greater than the changes in respiratory rate measured in air control animals challenged with PA dust. The decrease noted in plethysmograph pressure changes was not different from those measurements taken from air control animals exposed to the same concentration of PA dust.

Inhalation challenge with PA-GPSA conjugate

Guinea pigs exposed to filtered air and 0.5, 1.0, and 5.0 mg/m³ PA dust during the induction period were challenged by inhalation with 2.0 mg/m³ of aerosolized PA-GPSA conjugate. Fig. 2 shows the change in plethysmograph pressure and respiratory rate during the challenge period. One animal in the 0.5 mg/m³ group and four animals in the 5 mg/m³ group experienced significant and sustained increases in respiratory rate on challenge, as compared with the air control animals. The same animal in the 0.5 mg/m³ group, one animal in the 1 mg/m³ group, and three animals (two with significant increases in rate) in the 5.0 mg/m³ group experienced sustained respiratory reactions that resulted in significant increases in plethysmograph pressure, as compared with the air control animals.

ELISA

The levels of IgG antibody are shown in Table II. Significant levels of antibody were detected in sera from animals exposed to 0.5, 1.0, and 5.0 mg/m³ PA dust. Comparable levels of IgG antibody specific to PA-GPSA may be seen between the 0.5 mg/m³ exposure group and the 1.0 mg/m³ exposure group. A significant increase in IgG antibody was observed at the 5.0 mg/m³ exposure group. There was no difference in IgG antibody activity in both groups of animals exposed to 5.0 mg/m³ PA dust. Linear regression analysis showed a highly significant dose-response relationship ($p < 0.001$) for IgG antibody.

PCA

Table III shows the number of animals with allergic IgG1a antibody specific to PA-GPSA, as measured in the PCA test. Thirty-eight percent (3 of 8) of the animals in the 0.5 mg/m³ group had measurable circulating IgG1a antibody in serum. Of these three animals, one had a significant respiratory reaction on inhalation challenge with conjugate. One of eight animals (13%) in the 1.0 mg/m³ exposure group had allergic antibody; this same animal had significant respiratory reactivity on conjugate challenge. Sixty-three percent (5 of 8) of the animals in the 5.0 mg/m³ exposure group had allergic antibody. All five animals experienced respiratory reactivity on conjugate challenge. Only 1 of 8 (13%) of the animals retained at the IIT Research Institute had IgG1a antibody to PA-GPSA. None of the study animals had detectable IgE antibody to PA-GPSA.

Histopathology and antibody titers

The lungs of all animals exposed and challenged with 5.0 mg/m³ PA dust were examined for hemorrhagic lung foci (Table IV). Foci were observed in 8 of 8 animals in the PA dust-exposed and challenged group, with 3 of 8 having 189 foci or more. One or two lung foci were noted in 5 of 8 filtered air control/PA dust-challenged guinea pigs. Fig. 3, A, shows a section of lung from an air control animal challenged with 5.0 mg/m³ PA dust. No indication of hemorrhage or inflammation was noted. Fig. 3, B, shows a section of lung from a PA-sensitized animal challenged with 5.0 mg/m³ PA dust. Alveolar hemorrhage, with accumulation of red blood cells, and a few alveolar macrophages were observed. Minimal type II cell hyperplasia was also noted. Table IV lists the number of lung foci, along with the level of IgG antibody activity, for the air control/PA dust-challenged group and the 5.0 mg/m³ PA dust-

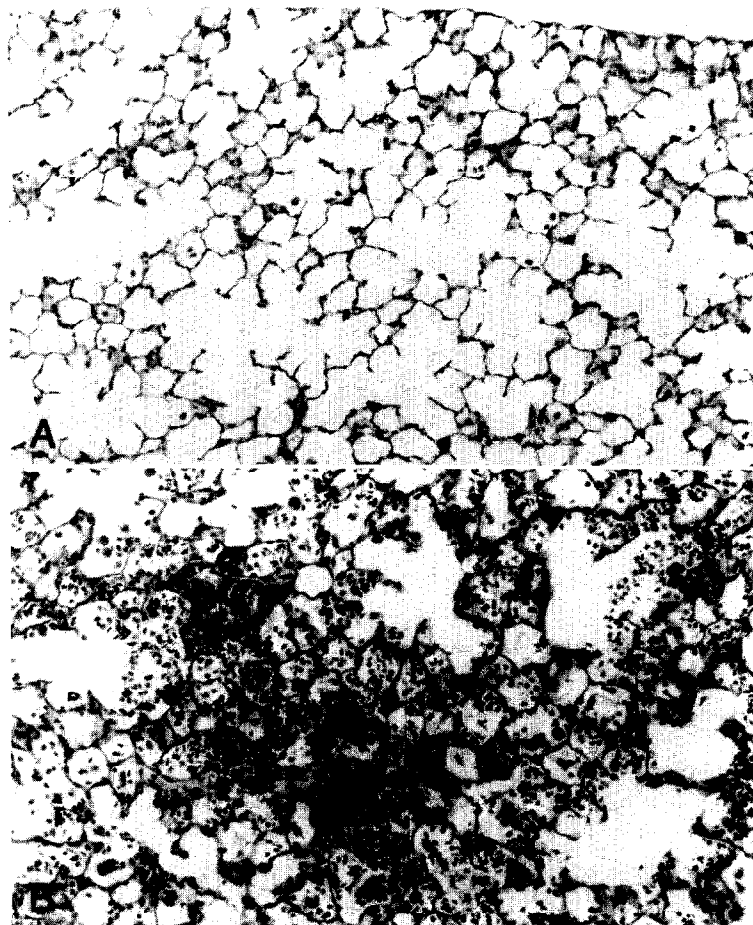


FIG. 3. **A.** Microphotograph of a section of lung from an air control animal challenged with 5.0 mg/m³ PA dust (original magnification = $\times 160$). **B.** Microphotograph of a section of lung from an animal exposed to 5.0 mg/m³ PA dust and challenged with 5.0 mg/m³ PA dust (original magnification = $\times 160$). Accumulation of red blood cells into the alveolar spaces was noted, along with a mild infiltration of inflammatory cells.

TABLE IV. Hemorrhagic lung foci and IgG antibody level in animals challenged with 5.0 mg/m³ PA dust

Filtered air control			5.0 mg/m ³ PA dust exposed		
An No.	Foci	IgG (OD = 1/100)*	An No.	Foci†	IgG (OD = 1/100)
33	0	0.075	41	11	0.425
34	1	0.042	42	6	0.315
35	0	0.081	43	1	0.165
36	1	0.100	44	365	1.102
37	2	0.065	45	14	0.551
38	1	0.054	46	2	0.135
39	1	0.088	47	331	1.240
40	0	0.061	48	189	0.312
Mean	1	0.071	Mean	115	0.530
SE	1	0.007	SE	157	0.147

*IgG antibody activity was defined as the optical density at a 1/100 dilution of serum.

†Significant number of lung foci was defined as greater than 10.

exposed and challenged group. Significant IgG antibody activity was associated with the presence of lung foci; 2 of the 3 animals with the greatest number of hemorrhagic foci had the highest level of IgG antibody activity. The mean number of foci and the mean IgG antibody level were significantly greater in the dust-exposed/challenged animals, compared with the air control/dust-challenged animals. No foci were observed on the lungs from animals challenged with PA-GPSA aerosol. Therefore no microscopic examination of the lungs of these animals was performed.

DISCUSSION

The purpose of this study was to examine the immunologic dose-response to inhaled PA dust in a guinea pig model designed to evaluate low-molecular-weight chemicals as respiratory allergens. Previous work clearly showed that PA was allergenic in the guinea pig.¹¹ However, due to technical problems in generating stable dust levels on a daily basis, it was difficult to accurately determine a minimum sensitizing concentration of PA dust. The data from this experiment clearly showed that PA was immunogenic and allergenic in guinea pigs exposed by inhalation to concentrations as low as 0.5 mg/m³. This sensitizing concentration is approximately tenfold lower than the recommended threshold limit value for PA (6.0 mg/m³).

Animals exposed to 0.5, 1.0, or 5.0 mg/m³ PA dust produced IgG1a (short latency, homocytotropic) allergic antibody to PA-GPSA. Allergic IgE antibody was not detected in the sera from these animals. A greater number of animals at the 5.0 mg/m³ exposure level had circulating IgG1a allergic antibody to PA-GPSA compared with animals exposed to the two lower concentrations of PA dust. Similarly, greater IgG antibody activity was detected in sera from animals exposed to 5.0 mg/m³ PA dust, when compared with the IgG antibody activity found in sera from animals exposed to 0.5 or 1.0 mg/m³ PA. A significant dose-response relationship was detected by linear regression analysis of the ELISA data. Exposure of guinea pigs to a low range of dust concentrations (0.05 to 0.2 mg/m³ PA dust over 5 days) did not induce detectable antibody as measured by serologic study and by skin testing.¹¹ Therefore a comparison of the antibody data generated in this study with the antibody data generated from the earlier experiment indicates that 0.5 mg/m³ is at or near the minimal concentration of PA dust

needed to immunize and allergically sensitize guinea pigs.

Respiratory reactivity was elicited by inhalation challenge with PA-GPSA in animals at all three dust-exposure levels. (Because of technical problems, a GPSA aerosol control challenge was not performed. We do recommend that this challenge be included in future studies.¹¹) With the exception of two animals in the 5.0 mg/m³ exposure group, responding animals experienced transient increases in respiratory rate followed by sustained diaphragmatic contractions that resulted in a significant increase in plethysmograph pressure. We did not observe any relationship between sensitizing PA dust concentration and the kinetics of the onset of the response or the severity of the response. We did observe that a greater number of animals in the 5.0 mg/m³ exposure group had respiratory reactivity on conjugate challenge. These immediate-onset respiratory reactions occurred in those animals that had IgG1a antibody to PA-GPSA. Not all animals with IgG1a antibody to PA-GPSA experienced immediate-onset respiratory reactions on conjugate challenge. This observation is consistent with previously reported data for toluene diisocyanate,^{11, 15} TMA,¹⁶ and PA.¹¹ These data suggest that, for the guinea pig, the presence of antigen-specific IgG1a antibody is one prerequisite for eliciting immediate-onset respiratory reactions. Other factors not measured in this study (e.g., bronchial smooth muscle "tone," other antibody isotypes) may also play a role in determining whether an animal will have a respiratory reaction on antigen challenge.

Those animals challenged with PA dust, rather than PA-GPSA conjugate, did not experience an immediate-onset respiratory reactions. This was not totally unexpected since only 1 of the 8 animals had detectable levels of circulating IgG1a antibody to PA-GPSA. In addition, the exposure conditions for challenge with PA dust may not have been optimal for elicitation of immediate-onset respiratory responses.¹¹ All eight animals did possess IgG antibody to PA-GPSA. We have no explanation as to why these eight animals did not produce detectable levels of IgG1a antibody, whereas their eight counterparts sent to the Ohio location did produce detectable levels of circulating IgG1a antibody. All 16 animals were exposed to the PA dust in the same exposure chamber; sera were collected at the same time points; and the PCA analysis was done at one location. The selection of which animals were to remain at the

IIT Research Institute for the dust challenge and which animals were to be shipped to the Miami Valley Laboratories, in Cincinnati, for the conjugate challenge was random.

PA is structurally similar to TMA. TMA has been associated with a pulmonary disease anemia syndrome that is characterized by anemia and hemoptysis.¹⁷ This rare syndrome is characterized by lung damage that is typified by hemorrhage. This syndrome developed in some workers who were exposed to high levels of TMA fumes when the chemical was sprayed on heated metal surfaces. Information generated in a rat model supports the role of an immune, IgG antibody-mediated reaction being responsible for the TMA-induced lung injury.¹⁸⁻²⁰ Although PA has not been associated with this type of lung injury in exposed human beings (perhaps due to differences in exposure conditions), we were interested in determining whether the lung injury noted in the TMA rat model could be duplicated by PA in the guinea pig. Although we evaluated only eight PA-sensitized animals and eight air control animals, we did find a significantly greater number of hemorrhagic foci associated with alveolar hemorrhage in lungs from the PA-sensitized animals versus the air control animals. This lung pathologic condition was associated with higher IgG antibody activity as measured in the ELISA. This limited evaluation in the guinea pig is consistent with the observed lung injury induced by TMA in the rat. A recent study showed that guinea pigs, sensitized through intramuscular injection with a TMA-protein conjugate, developed significant hemorrhagic lung foci after inhalation challenge with very high levels (130 mg/m³) of TMA dust.²¹ The lung foci noted in guinea pigs in our study were induced after inhalation exposure (induction and elicitation) to much lower levels of PA dust. These data raise some provocative questions regarding lung injury associated with anhydride exposure. No lung foci were observed in the animals challenged with PA-GPSA, suggesting the need for free anhydride to elicit this response. Because the filtered-air control animals did not respond to the PA dust challenge with lung foci and because similar observations were made in the rat model with TMA exposure, one can conclude that the lung foci are not caused by direct toxicity of anhydride. The association of this injury with the presence of specific IgG antibody to the anhydride leads to a tantalizing link of a potential immune mechanism. Other questions regarding

this lung injury include the following: Is this lung injury limited to anhydride exposure? It is associated with other anhydrides, or is it limited to a "class" of structurally similar anhydrides? Future studies designed to examine the immune response to inhaled anhydrides should answer some of these questions, as well as help put into perspective the relevance of this lung injury for human exposure to anhydrides.

Epidemiologic studies have been done on anhydride-exposed factory workers in Sweden. In one study the time-weighted average PA dust level ranged from 1.5 to 17.4 mg/m³, with a daily time-weighted average of 6.6 mg/m³ (monitored by personal air samplers).⁸ An earlier study of the same two factories plus two others showed that the level of PA dust ranged from 0.3 to 23 mg/m³, depending on the sampling location.⁷ Approximately 28% of evaluated workers had work-associated asthma, and 27% of the asthmatic workers had IgE allergic antibody to PA (PA was one of several allergenic chemicals present in the factories). Two of these asthmatic workers had significant drops in pulmonary function when challenged with PA dust at levels near or below the recommended threshold limit value of 6.0 mg/m³. Allergic antibodies were associated with allergic symptoms in these workers. The response of workers to PA exposure is similar to that which occurs with occupational exposure to TMA, in which allergic symptoms (asthma, rhinitis) are associated with increased levels of serum antibody specific to TMA-human serum albumin.^{22, 23} In the guinea pig study, animals were sensitized to PA dust at concentrations that were well within the range of dust concentrations measured in the Swedish factories. Symptoms in the guinea pig (elicited by PA-GPSA challenge) were associated with the presence of specific IgG1a allergic antibody to PA-GPSA. Therefore certain similarities between the human immune response to PA and the guinea pig immune response to PA exist, indicating that the guinea pig is an appropriate model to use when evaluating the sensitizing potential of inhaled PA.

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