Ozone Enhancement of Platinum Asthma in a Primate Model[1-5]

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SUMMARY Three groups of adult male cynomolgus monkeys (*Macaca fascicularis*) were exposed to either 200 μg/m³ ammonium hexachloroplatinate [$(NH_4)_2PtCl_6$], 200 μg $(NH_4)_2PtCl_6$ concurrently with 1 ppm ozone (O_3), or to 1 ppm O_3 only. The animals were exposed by inhalation for 6 h per day, 5 days per week for 12 wk. The experimental design included methacholine preexposure and Na_2PtCl_6 bronchoprovocation challenge evaluations, Na_2PtCl_6 threshold skin tests, and sera for analyses of antibodies. Two weeks after the 12-wk exposures, these same indices were reevaluated. Baseline pulmonary function was not significantly affected by the exposure regimens; however, the combination of exposure to O_3 and $(NH_4)_2PtCl_6$ significantly reduced the concentration of platinum (Pt) salt and methacholine necessary to increase average pulmonary flow resistance (RL) 200% (EC_{200} RL). Ozone or Pt exposure alone had no significant effect on these parameters. Platinum and methacholine EC_{200} RL values were highly correlated for both Pt-exposed groups after exposure. These data indicated that combined O_3 and Pt exposure significantly increased specific (Pt) and nonspecific (methacholine) bronchial hyperreactivity more often than did exposure to either O_3 or the Pt salt alone. Combined O_3 plus Pt exposure also significantly increases the incidence of positive Pt skin tests when compared with the other exposure groups. Similar to the human experience, radioallergosorbent testing (RAST) for Pt-specific antibodies was not as sensitive as direct skin testing in identifying allergic persons.

AM REV RESPIR DIS 1986; 134:719-725

Introduction

The potent pulmonary and skin-sensitizing properties of the soluble sodium and ammonium salts of chloroplatinate are well documented (1-8). Upper respiratory, asthmatic symptoms, and positive platinum (Pt) skin tests have been reported to occur in 20 to 100% of workers exposed to the Pt salts, mainly during the refining and purification of Pt group metals. Passive transfer of allergic sensitivity has been shown in humans (9) and in monkeys (9, 10), and Pt-specific IgE antibodies have been detected by the radioallergosorbent test (RAST) (10, 11). On the basis of this evidence, it appears that most soluble Pt salt hypersensitivity reactions are due to Type 1, immediate onset, IgE-immune mechanisms with a possible reaginic IgG_4 component. Positive Pt skin tests have been shown to persist in precious metal refinery workers for periods exceeding 4 yr, even in the absence of continued Pt exposure (10). Preliminary evidence also suggests that Pt bronchial hyperreactivity, once induced, is persistent for long periods of time in the absence of continued exposure (7).

There is a variable relationship between the duration of Pt exposure, the onset of respiratory symptoms, and positive Pt skin tests (1). Cold air hyperreactivity occurs in skin test negative and positive Pt refinery workers (12). Follow-up studies of these same workers suggest that pulmonary hyperreactivity (as evidenced by cold air challenge) precedes a conversion to positive Pt skin tests in some workers.

Refining of Pt metal from Pt-rich ores or metal concentrates is done by a rigorous chemical process involving sequential solubilization and precipitation. The relative inertness of Pt and related precious metals necessitates the use of concentrated acids and mixtures of acids to solubilize them. Because of this, workers employed in Pt refineries have potential exposure to Pt salt and metal aerosols, acidic mists, and/or irritant gases. These findings suggest that Pt refinery workers develop asthma and allergic sensitization at high prevalence rates because of concurrent exposures to irritant gases and aerosols in their work environment. Airway damage from these irritant exposures in combination with exposure to a compound with allergic-sensitizing properties, such as Pt, could explain these high prevalence rates.

The objectives of the present experiments were to test the hypotheses that combined inhalation exposure to Pt and ozone (a gas of known pulmonary irritant/inflammatory properties) would increase Pt salt and methacholine pulmonary hyperreactivity and the incidence of positive Pt skin tests compared with Pt or ozone exposure alone. These hypotheses were investigated in cynomolgus monkeys because of their physiologic, immunologic, and phylogenetic similarities to humans.

Methods

Animals

Twenty-three adult male cynomolgus monkeys (*Macaca fascicularis*) (Charles River Research Primate Corp., Port Washington, NY) were randomly selected from the NIOSH animal colony (weight, 4.5 ± 0.1 kg, mean ± SEM). The monkeys were fed standard chow daily (Monkey Chow Jumbo®; Ralston Purina Co., St. Louis, MO) and fresh fruit (oranges, apples, and bananas) once weekly. Water was provided ad libitum. The monkeys were maintained on a 12-h photoperiod (lights on, 7 A.M.; lights off, 7 P.M.). The animals were in excellent health, and routine screenings for tuberculosis and fecal parasites were negative.

(Received in original form August 8, 1985 and in revised form April 4, 1986)

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[2] Presented in part at the Annual Meeting of the American Association of Immunology, St. Louis, Missouri, 1984.

[3] This research was conducted in facilities fully accredited by the American Association for the Accreditation of Laboratory Animal Care.

[4] Mention of a product or company name does not constitute endorsement by NIOSH.

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Systematic Randomization and Exposure Groups

Because of the variable bronchoconstrictive effects of Pt in naive monkeys (13), exposure groups were initially prepared with equivalent pulmonary reactivity to Na_2PtCl_6. This was accomplished by systematic randomization based on individual increases in average pulmonary flow resistance (RL) to serially increasing bronchoprovocation challenge concentrations of Na_2PtCl_6 (14). The 3 exposure groups were: Group 1 (n = 7), exposed to 1 ppm ozone (O_3); Group 2 (n = 8), exposed to 200 μg/m³ ammonium hexachloroplatinate [$(NH_4)_2PtCl_6$]; Group 3 (n = 8), exposed to 200 μg $(NH_4)_2PtCl_6$ = 1 ppm O_3. Platinum concentrations are reported in μg/m³ Pt, based on the Pt content of $(NH_4)_2PtCl_6$. Ozone concentrations are on a ppm by volume basis.

Design of Study

The monkeys were exposed by inhalation for 6 h per day, 5 days per week for 12 wk. The experimental design included preexposure methacholine and Na_2PtCl_6 bronchoprovocation challenge evaluations, Na_2PtCl_6 threshold skin tests, and serum acquisition for immunologic evaluations. Two weeks after the 12-wk exposure regimens, these same parameters were reevaluated.

Atmospheric Generation and Analyses

Chamber aerosols were produced by compressed air nebulization (Bird Inc., Palm Springs, CA) of freshly prepared distilled water solutions of $(NH_4)_2PtCl_6$, 99.999%, (Lot no. 10697; Polysciences, Warren, PA). The $(NH_4)_2PtCl_6$ aerosols were introduced at the tangential airfeed manifolds of 2 "walkin-airlock" chambers designed by us and constructed by Charles A. Spengler and Associates (Cincinnati, OH) (figure 1). These chambers are 2-m (on dimension, length, width and height) stainless steel and glass cubes with a common middle wall. Both are fitted with top and bottom pyramidal cones and mesh floors. The chambers were operated at 10 to 15 air changes per hour (110 to 165 m³/h) at −0.25 cm H_2O. The chamber air supply was dehumidified, rehumidified, filtered, and conditioned (temperature, 23 ± 1° C; humidity, 50 ± 10%). The animals were exposed and housed in stainless steel cages (2.5-cm mesh) equipped with automatic watering systems. Prior to a daily exposure, uneaten food was removed from the cages and the water supply was cut off. All animal servicing was performed from an airlock enclosure attached to the chambers. The animals' chamber positions were systematically rotated biweekly to ensure comparable exposures. All chamber exhaust air was water-scrubbed and entrained prior to environmental discharge.

Ozone-only exposures (Group 1) were conducted in a 5-m³ stainless steel chamber (14) operated at the same air flow and other conditions as described above. Ozone was generated from oxygen and a commercially available ozonator (T-816 ozonator; Wellsbach, Philadelphia, PA). The animals were housed and serviced in the housing rooms of the NIOSH animal facility and transported daily to the chamber for exposure.

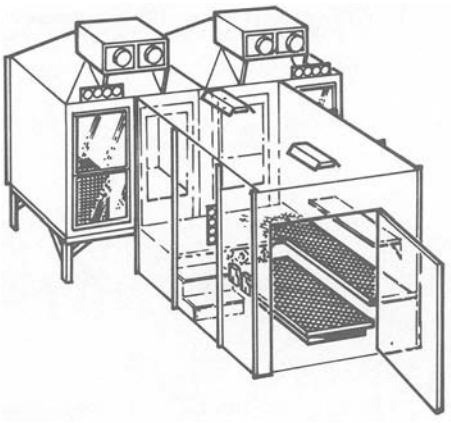

Fig. 1. Diagram of walkin-airlock chambers.

The Pt aerosol concentrations were monitored for the complete 6-h daily exposure periods by sampling chamber air through 2 series-connected Greenburg-Smith water-filled impingers (Fisher Scientific, Inc., Cincinnati, OH) placed near the monkeys' breathing zone. The impinger fluids were analyzed by ultraviolet spectroscopy (15). To detect any short-term excursions in aerosol concentration, the Pt aerosols were continuously monitored by light-scattering photometry (RAM-1; GCA Environmental Instruments, Bedford, MA). The chamber ozone concentrations were evaluated on-line with an ozone monitor (Meloy Laboratories, Springfield, VA). This instrument was calibrated daily with its own integral ozone source. The validity of this calibration was routinely assessed by generating known amounts of ozone by photochemical reaction with oxygen (Stable Ozone Generator; Ultraviolet Products, San Gabriel, CA). In addition, chamber ozone concentrations were intermittently evaluated by impingement and analysis by potassium iodide titration (16). Chamber particulates were sized by scanning electron microscopy–image analysis after collection on polycarbonate membrane filters (15).

Atmospheric Analyses

The chamber concentrations of $(NH_4)_2PtCl_6$ measured were: Group 2, 177 ± 8 μg/m³; Group 3, 208 ± 5 μg/m³ (mean ± SEM). The mean (± SEM) O_3 concentrations were: Group 1, 1.05 ± 0.01 ppm; Group 3, 1.07 ± 0.01 ppm. Representative particle size measurements were: Group 2: mass median aerodynamic diameter (MMAD), 0.94 μm; standard geometric deviation (σg), 2.03; Group 3: MMAD, 1.07 μm; σg 1.89.

Tranquilization and Serum Collection

For all physiologic and immunologic testing, the monkeys were tranquilized intramuscularly with a mixture of 70 mg/ml ketamine hydrochloride (Ketaset®: Bristol Laboratories, Syracuse, NY) and 6 mg/ml xylazine (Rompun®: Bayvet Division of Cutter Laboratories, Shawnee, KN) at 0.15 mL/kg body weight (17). Blood was withdrawn by femoral venipuncture before and after exposure. The blood was allowed to clot at room temperature, and the serum was separated by centrifugation and stored at −20° C until use.

Skin Testing and Passive Cutaneous Anaphylaxis

Skin testing was performed on tranquilized animals before and after the 12-wk exposure regimens. Areas of the chest and thorax were shaved, and 5 ml of a 0.5% solution of Evans blue dye was injected intravenously into the saphenous vein. Fifteen minutes later, 100 μl serial dilutions of 1×10^{-3} to 1×10^{-7} g/ml solutions of Na_2PtCl_6 in saline (0.9% NaCl) were injected intracutaneously in each animal. The injection sites were observed at 30 min for cutaneous blueing reactions. Vehicle and positive control skin testing was performed in all cases with saline and 0.1% histamine phosphate (Eli Lilly, Indianapolis, IN) in PBS (0.02 M phosphate buffer at pH 7.4 containing 0.9% NaCl). The undiluted histamine diphosphate solution was equivalent to 1 mg histamine base/ml.

Passive cutaneous anaphylaxis (PCA) evaluations were performed with undiluted serum. The serum samples were stored at −20° C until use. Samples (100 μl) were injected intracutaneously in randomly selected shaved naive cynomolgus monkeys obtained from the NIOSH animal colony. Twenty-four hours later, 5 ml of a 0.5% solution of Evans blue dye was injected intravenously into the saphenous vein. After an equilibration period of 15 min, 50-μl aliquots of 1×10^{-5} g/ml Na_2PtCl_6 were injected intracutaneously into the serum-pretreated sites. Thirty minutes later, the injection sites were observed for cutaneous blueing. Histamine injections as described above were used as positive controls, whereas saline and injections of 50 μl of 1×10^{-5} g/ml Na_2PtCl_6 served as negative controls.

Immunologic Tests

Immunologic assays were performed on serum collected before the initiation of exposures and immediately after the 12-wk exposure regimens. Total serum IgE concentrations were determined by radioimmunoassay with a commercially available kit (Kallestad Laboratories, Austin, TX). Total serum IgG values were determined using an immunochemistry analyzer (Beckman Immunochemistry Analyser: Beckman Instruments, Fullerton, CA). These assays were considered positive when the mean binding of duplicate determinations was greater than the mean results plus 2 SD of all preexposure serum samples. Radioallergosorbent (RAST) testing for Pt-specific IgE antibodies was performed using a Pt-protein conjugate (10) prepared with malic dehydrogenase (Sigma Chemical Co., St. Louis, MO). This conjugate was coupled to methylcellulose discs by cyanogen bromide treatment, and the RAST

analysis was performed as previously described (18).

Pulmonary Function Testing

For pulmonary function testing, each tranquilized monkey was transorally intubated with a cuffed endotracheal tube (Rusch, Inc., New York, NY.) of maximum diameter (20 to 22 Fr). An esophageal balloon was placed into the lower third of the esophagus and adjusted to demonstrate the most negative end-tidal transpulmonary pressures. The esophageal and mouth pressures were measured by a PM 131TC differential pressure transducer (Statham Instruments, Hato Rey, PR). Flow at the mouth was measured by observing differential pressures generated across a pneumotachygraph (Fleisch no. 0; Dynasciences Medical Products, Blue Bell, PA) and was electrically transduced (PM-5 transducer: Statham). Volume was obtained by electrical integration of air flow with a variable time constant. Measures were made with the monkeys in a supine position inside a variable pressure plethysmograph-respirator (19). Forced breathing maneuvers were produced by rapid external pressure changes producing adequate (> 35 cm H_2O intrapleural) driving pressures required for maximal flow and volume maneuvers.

Pulmonary mechanics (20) were obtained from simultaneous volume, flow, and transpulmonary pressure tracings displayed on a 12-channel photographic recorder (Model DR-12; Electronics for Medicine, White Plains, NY). Forced maneuvers were also followed with this recording system. The pulmonary function parameters studied were average RL, dynamic compliance, peak expiratory flow rate, forced vital capacity (FVC), forced expiratory volume in 0.5 second/FVC ($FEV_{0.5}$/FVC), and forced expiratory flows at 50 and 75% of VC normalized for FVC (FEF_{50}/FVC and FEF_{75}/FVC). Respiratory rates were measured by direct visualization and a stopwatch. All data sampling, storage, and calculations were performed by computer (DS-10; Tenet Information Services, Salt Lake City, UT). A minimum of 18 replicate breaths were analyzed for the calculation of RL and dynamic compliance. Flow-volume parameters were determined from 1 maximal expiratory flow-volume maneuver.

Bronchoprovocation Challenges

Bronchoprovocation challenges were performed with Na_2PtCl_6 rather than with $(NH_4)_2PtCl_6$ because of the latter's limited solubility in physiologic solutions. Other investigators (5) have shown similar bronchoconstrictive effects on challenge with both Na_2PtCl_6 and $(NH_4)_2PtCl_6$ in Pt-allergic subjects. Na_2PtCl_6 (99.99%; Pfaltz and Bauer, Stamford, CT) and methacholine (acetyl-β-methacholine chloride; Sigma) aerosols were generated for bronchoprovocation challenges using a micronebulizer (output = 0.065 ml/min) and positive-pressure ventilator/respirator (Bird) operated as previously described (13, 15, 21). Freshly prepared solutions of Na_2PtCl_6 in saline and methacholine in PBS were aerosolized using this system. The mass median aerodynamic diameters (MMAD) of the bronchoprovocation aerosols were determined using a particle size analyzer (APS 33 Aerodynamic Particle Sizer; TSI, Inc., St. Paul, MN). All Na_2PtCl_6 and methacholine aerosols had MMAD of 1.0 to 1.5 μm with σg of 1.7 to 2.0. Challenges were performed for 1 min (15 breaths) in the following sequences: PBS, 0.1, 0.5, 1.05, and 6.25 mg methacholine/ml or saline, 0.5, 2.5, 25 and 50 mg Na_2PtCl_6/ml. Increasing bronchoprovocation challenge concentrations were administered at 10-min intervals. Reactivity to bronchoprovocation challenge was calculated by determining the concentration of Na_2PtCl_6 necessary to increase average $EC_{200}RL$. This was accomplished by calculating percent baseline RL at each challenge concentration using the following relationship: Percent RL = RL after challenge − RL after saline/RL after saline ×100.

Preliminary studies had shown that 5 sequential saline or PBS challenges at 10-min intervals had no effect on baseline pulmonary function. The $EC_{200}RL$ values were calculated from these data by microcomputer spreadsheet (Lotus 1-2-3; Lotus Development Corp., Cambridge, MA) by linear interpolation and a log-concentration effect model. Because of the induction of marked sensitivity in some animals after exposure, the challenge protocols were stopped after the lowest challenge concentrations in order to ensure the animals' safety. These animals were assigned an $EC_{200}RL$ equal to the lowest challenge concentration of the agents (0.5 mg/ml Na_2PtCl_6 or 0.1 mg/ml methacholine). Alternatively, some animals had not reached an $EC_{200}RL$ after completing the challenge protocols. These animals were assigned an $EC_{200}RL$ equal to the highest challenge concentrations (50 mg/ml Na_2PtCl_6 and 6.25 mg/ml methacholine).

Statistical Analyses

All hypotheses tests were performed using nonparametric methods (22). To determine if the 12-wk exposures had any chronic effect, postexposure pulmonary function and saline challenge pulmonary function results were compared by a Kruskal-Wallis ANOVA. Postexposure Na_2PtCl_6 or methacholine reactivity was analyzed using Wilcoxon's signed-rank tests (two-tailed). The significance of positive Pt skin test conversions was investigated using contingency table analysis of the skin test results from all 3 exposure groups (3 × 2 chi-square). Spearman's rank correlation (r_S) was used to evaluate the association between methacholine and Pt $EC_{200}RL$ values. Results of all statistical tests were considered significant at a Type 1 error of $p = 0.05$ or less.

Results

Animal Weights and Observations

No significant differences in body weights were observed for the 3 groups after exposure, and growth would be considered normal for monkeys cared for under the experimental conditions of this study. The animals appeared to tolerate the exposure regimens with no overt ill effects at anytime during the study.

TABLE 1

POSITIVE Pt SKIN TEST CONVERSIONS IN EXPOSED MONKEYS

Exposure Group	Positive Tests* (*n*)	Negative Tests (*n*)
Ozone	0	7
Pt	1	7
Pt + O_3	4†	4

* Skin test positive at 10^{-6} g/ml or less Na_2PtCl_6.
† Significantly different at a Type 1 error of $p < 0.05$.

Skin Testing and Passive Cutaneous Anaphylaxis

Prior to exposure, all animals had negative skin test reactions to 100-μl intracutaneous injections of 10^{-6} g/ml Na_2PtCl_6 (absolute amount of Pt salt injected equals 0.1 μg). Non-Pt-exposed human volunteers have been shown to be nonreactive to skin prick testing with Pt at 10^{-3} g/ml (absolute amount of Pt equals 1 to 5 μg), whereas Pt-sensitive subjects yield positive skin tests at this amount (10). Four monkeys in Group 3 (Monkeys Q, S, U, and V) developed postexposure positive skin sensitivity at the 10^{-6} g/ml Na_2PtCl_6 concentration (absolute amount of Pt injected equals 0.1 μg). One monkey in Group 2 (Monkey O) developed positive skin sensitivity at the 10^{-7} g/ml skin testing concentration (absolute amount of Pt injected equals 0.01 μg). These data agree well with human skin prick testing results (10), considering that less test agent is necessary to observe positive reactions with intracutaneous skin tests than with skin prick tests (23). The increased prevalence of positive Pt skin tests in Group 3 (4 of 8) was significantly different ($p < 0.05$) (table 1) than that observed in the Pt-only exposed group (1 of 8) or the O_3-exposed group (0 of 7). Passive transfer of Na_2PtCl_6 skin sensitivity (monkey to monkey) was performed with serum from all Pt skin test positive animals. A positive PCA result was observed with serum from Monkey O.

Immunologic Studies

Preexposure and postexposure values for total IgE, IgG, and Pt-specific IgE (RAST) are shown in table 2. There were no significant differences between groups for any of these immunologic parame-

TABLE 2

IMMUNOGLOBULIN PROFILES OF EXPOSED MONKEYS*

Ig	Before	After
O_3-exposed group		
IgE, ng/ml	41.6 ± 3.4	37.9 ± 3.4
IgG, mg/dl	1,161 ± 88	1,300 ± 218
RAST†	4.2 ± 0.2	4.1 ± 0.2
Pt-exposed group		
IgE, ng/ml	40.1 ± 2.1	40.1 ± 3.5
IgG, mg/dl	1,246 ± 51	1,303 ± 69
RAST†	4.2 ± 0.2	4.1 ± 0.1
Pt + O_3-exposed group		
IgE, ng/ml	40.4 ± 1.6	35.5 ± 3.7
IgG, mg/dl	1,303 ± 56	1,397 ± 199
RAST†	4.4 ± 0.3	4.1 ± 0.3

* Mean ± SEM. No significant differences ($p > 0.05$) between groups for any immunoglobulin class at any time period.
† Percent binding ^{125}I goat antihuman IgE.

ters, either before or after exposure. Individual animals did show elevated postexposure values (results greater than the preexposure mean value for all 23 monkeys plus 2 SD). Monkey O in Group 2 and Monkey S in Group 3 had elevated total serum IgE values of 55.2 and 54.1 ng/ml, respectively. Monkey V in Group 3 had an elevated IgG of 2,720 mg/dl. Two animals in Group 2 (Monkeys N and O) and 1 animal in Group 3 (Monkey P) had positive RAST activity (6.1, 5.5, and 6.2%, respectively). These values are within the range of RAST values observed in humans skin-test sensitive to the Pt salts (10).

Postexposure Baseline Pulmonary Function

After the 12-wk exposure regimen, the 3 groups of monkeys were evaluated twice for their saline challenge pulmonary function values (once before the Pt challenge regimen and again before the methacholine challenges). In both cases, the groups of monkeys had pulmonary function results equivalent to saline challenge values found 12 wk earlier, and there were no significant differences between groups at either testing period (table 3).

Bronchoprovocation Challenge Results

Exposure to the Pt salt or O_3 independently had no significant effects on postexposure Pt or methacholine reactivity. The combination of exposure to Pt and O_3 had significant effects on postexposure Pt and methacholine pulmonary reactivity. Some animals exhibited extremely elevated RL values (as much as 180 cm H_2O/L/s) and hemoptysis after challenge with the most dilute solutions. The group exposed to the combination of Pt and O_3 had significantly lowered Pt $EC_{200}RL$ values (12.58 ± 6.11 mg/ml Na_2PtCl_6, mean ± SEM) (table 4). Significant $EC_{200}RL$ changes were not observed for the other 2 groups. Methacholine $EC_{200}RL$ values were also significantly lowered in the combined exposure group (1.23 ± 0.72, mean ± SEM) (table 5). Methacholine $EC_{200}RL$ values were not significantly affected by exposure in the other 2 groups.

Platinum and methacholine $EC_{200}RL$ values were significantly correlated after exposure ($r_S = 0.86$, $p < 0.01$ for the Pt+O_3-exposed group and $r_S = 0.85$, $p < 0.01$ for the group exposed to Pt alone). These same correlations were not significant before exposure (Pt+O_3-exposed group, $r_S = 0.26$; Pt-exposed group, $r_S = 0.52$). The O_3 only exposed group had nonsignificant correlation before ($r_S = 0.61$) and after exposure ($r_S = -0.59$). Respiratory rates were not significantly changed in any group after challenge with either Pt or methacholine.

Discussion

The results of this study show that combined inhalation exposure of 200 μg/m³ $(NH_4)_2PtCl_6$ and 1 ppm O_3 caused Pt salt and methacholine bronchial hyperreactivity in monkeys. Platinum pulmonary hyperreactivity was reversible, as the monkeys had normal pulmonary function before and returned to normal after Pt postexposure challenges. The prevalence of positive Pt skin tests was also significantly increased in the group of monkeys exposed to the combination of Pt and O_3. Exposure to the Pt salt or O_3 alone had no significant effects on nonchallenged pulmonary function, Pt, or methacholine bronchial reactivity. Methacholine $EC_{200}RL$ values were significantly correlated with Pt $EC_{200}RL$ values after exposure for both Pt-exposed groups, whereas these same correlations were nonsignificant before the onset of the daily inhalation exposures.

Other investigators have shown that repeated O_3 exposure increases the incidence or severity or both of allergic airway disease in animals. Matsumura (24) demonstrated that repeated exposure to greater than 2 ppm O_3 increased the prevalence of fatal anaphylaxis in guinea pigs sensitized by inhalation of egg and bovine serum albumins. No significant increase in anaphylaxis was reported from exposure to 1 ppm O_3 in conjunction with the albumins. Tests of pulmonary function and bronchial hyperreactivity to nonspecific stimuli were not included in that study. It is possible that significant nonfatal bronchial hyperreactivity was present in the guinea pigs exposed to less than 2 ppm O_3.

Direct skin testing is the most sensitive indicator of Pt allergy in humans (10), and it appears that skin testing is

TABLE 3

PREEXPOSURE AND POSTEXPOSURE BASELINE PULMONARY FUNCTION*

Parameter Units	Postexposure	Preexposure
O_3-exposed group		
RL, cm H_2O/L/s	7.86 ± 0.93	8.04 ± 0.63
CLdyn, ml/cm H_2O	29.7 ± 3.2	24.6 ± 4.3
FVC, ml	409 ± 28	412 ± 20
PEFR, ml/s	1,504 ± 101	1,331 ± 183
$FEV_{0.5}$/FVC, %	85 ± 3	81 ± 6
FEF_{50}/FVC, FVC/s	3.1 ± 0.4	2.7 ± 0.8
FEF_{75}/FVC, FVC/s	1.2 ± 0.4	1.1 ± 0.5
Pt-exposed group		
RL, cm H_2O/L/s	7.23 ± 0.55	8.43 ± 0.63
CLdyn, ml/cm H_2O	28.0 ± 3.0	22.9 ± 6.8
FVC, ml	402 ± 25	389 ± 31
PEFR, ml/s	1,528 ± 71	1,409 ± 141
$FEV_{0.5}$/FVC, %	91 ± 2	88 ± 7
FEF_{50}/FVC, FVC/s	3.4 ± 0.5	3.6 ± 0.5
FEF_{75}/FVC, FVC/s	2.0 ± 0.4	1.9 ± 0.9
Pt + O_3-exposed group		
RL, cm H_2O/L/s	8.26 ± 0.82	10.90 ± 1.53
CLdyn, ml/cm H_2O	20.5 ± 2.5	17.5 ± 9.6
FVC, ml	378 ± 18	384 ± 22
PEFR, ml/s	1,381 ± 72	1,219 ± 116
$FEV_{0.5}$/FVC, %	86 ± 1	83 ± 4
FEF_{50}/FVC, FVC/s	3.3 ± 0.4	3.4 ± 0.7
FEF_{75}/FVC, FVC/s	1.1 ± 0.1	1.0 ± 0.2

* After saline challenge. No significant differences ($p > 0.05$) were observed between groups before or after exposure for any pulmonary function variable. Mean values ± SEM. See text for details of exposures.

TABLE 4

AIRWAY RESPONSES TO Pt CHALLENGE IN EXPOSED MONKEYS

	Before		After		
Monkey	RL*	$EC_{200}RL$†	RL*	$EC_{200}RL$†	Difference‡
O_3-exposed group					
A	5.98	17.15	5.14	6.70	-10.45
B	9.62	50.00	4.43	50.00	0.00
C	9.97	12.18	6.72	12.13	-0.05
D	10.56	2.13	9.25	3.90	1.77
E	8.49	8.10	7.32	11.29	3.19
F	8.28	50.00	6.34	50.00	0.00
G	6.22	1.22	8.92	11.83	10.61
Mean	8.45	20.10	6.87	20.83	0.72
SEM	0.69	8.01	0.68	7.63	2.35
Pt-exposed group					
H	8.82	50.00	7.63	50.00	0.00
I	6.69	4.64	7.41	26.93	22.29
J	9.24	50.00	8.75	43.70	-6.30
K	6.41	30.39	8.15	7.82	-22.57
L	7.75	50.00	6.75	50.00	0.00
M	8.57	3.45	6.80	0.50	-2.95
N	12.54	4.56	10.81	0.50	-4.06
O	8.05	9.44	6.13	2.65	-6.79
Mean	8.51	25.31	7.80	22.76	-2.55
SEM	0.68	7.86	0.52	8.00	4.37
Pt + O_3-exposed group					
P	8.72	6.38	6.97	1.38	-5.00
Q	6.63	37.23	7.65	25.92	-11.31
R	11.05	46.15	11.82	10.80	-35.35
S	19.84	8.12	12.09	0.50	-7.62
T	9.68	4.95	11.64	2.64	-2.31
U	9.90	50.00	8.68	6.29	-43.71
V	8.36	3.89	8.07	3.08	-0.81
W	6.66	50.00	9.62	50.00	0.00
Mean	10.11	25.84	9.57	12.58§	-13.26
SEM	1.50	7.72	0.72	6.11	5.93

* cm H_2O/L/s.
† mg/ml Na_2PtCl_6 yielding a 200% increase in RL.
‡ Difference in EC_{200} values postexposure minus preexposure.
§ Significantly different at a Type 1 error of $p < 0.02$.

the most sensitive indicator of Pt allergy in monkeys. In our experience of skin testing more than 75 cynomolgus monkeys with Pt, we had never observed positive dermal sensitivity with solutions containing less than 10^{-5} g/ml Pt. Four of 8 of the monkeys exposed to the combination of Pt and O_3 developed dermal Pt hypersensitivity at the 10^{-6} g/ml Pt concentration, a tenfold reduction from their preexposure sensitivity. One of the monkeys exposed to the Pt salt alone had dermal sensitivity at the 10^{-7} g/ml Pt concentration. The dermal sensitivities of these monkeys occurred with absolute amounts of Pt, which would only yield positive skin tests in humans allergically sensitized to the Pt salts (10).

It was not surprising that RAST results for Pt-specific IgE antibodies were not significantly elevated in the animals exposed to the combination of Pt and O_3, as we have shown that Pt RAST testing is not sensitive enough for the individual diagnosis of allergy with serum from Pt-exposed humans (10). However, RAST testing does appear to be a useful epidemiologic tool for the detection of Pt-specific IgE antibodies in large groups of exposed workers. In addition, monkey IgE does not totally cross-react with antihuman IgE (25); this would be expected to lower the sensitivity of the RAST test using monkey serum and human reagents. Three monkey sera did contain elevated concentrations of RAST-detectable, Pt-specific IgE antibodies. Serum from the most sensitive skin test positive monkey (10^{-7} g/ml Pt) could support monkey to monkey PCA. Serum from the other skin test positive monkeys yielded negative PCA results. Again, this finding is not surprising as PCA is known to be 25 to 100 times less sensitive than direct skin testing (26).

From compilation of our work and the work of others, a progression of clinical signs and symptoms in humans exposed to the soluble Pt salts can be developed. Upon inital exposure for a period of weeks to a few months, some workers will complain of "respiratory" symptoms and irritation of the upper respiratory tract (1), and some will have positive Pt skin tests. Cold air hyperreactivity is positive in some skin test negative and skin test positive workers (12). With continued Pt exposure, skin test negative persons will convert to skin test positive. Positive Pt salt bronchoprovocation challenge results are observed in skin test positive persons (5). This information suggests that there is an initial period of tolerable respiratory irritation from exposure to the precious metal refinery environment. With continuing exposure, some workers become allergically sensitized to the Pt salts with increased intolerance to even minute amounts of Pt exposure (6). This information agrees well with the data observed in the monkeys of the present experiments. Methacholine and Pt $EC_{200}RL$ values were highly correlated after exposure in both groups of Pt-exposed monkeys; however, only the group exposed to the combination of Pt and O_3 had significant increases in Pt and methacholine pulmonary reactivity and significantly positive Pt skin tests. It is intriguing to speculate that O_3 exposure decreased the time necessary for the onset of allergic sensitization and positive specific and nonspecific airway hyperreactivity. However, exposure to the Pt salt alone for extended periods of time may have also led to allergic sensitization (the high correlation between methacholine and Pt bronchial hyperreactivity in the group of monkeys exposed to the Pt salt alone suggests that some airway damage had taken place, and individual monkeys did show postexposure reductions in Pt $EC_{200}RL$ values.

The combination of exposure to an irritant/inflammatory agent, such as O_3, could accelerate the production of specific IgE antibodies by increasing the interaction between allergen (or hapten) and the antibody-generating system. A tenable mechanism is that inflammation, epithelial damage, cell recruitment and modifications of cellular tight junctions caused by O_3 exposure (27–29) yields a greater probability of Pt penetration into the pulmonary epithelium and subepithelial tissue. This could lead to increased protein binding sites for the inhaled Pt, increased systemic absorption of the Pt salts or *in vivo* protein adducts, enhanced inflammatory response to the repeated Pt insult, unmasking of irritant receptors, and/or increased availability of submucosal mast cells or larger numbers of intraluminal mast cells. These factors ei-

TABLE 5

AIRWAY RESPONSES TO METHACHOLINE CHALLENGE IN EXPOSED MONKEYS

	Before		After		
Monkey	RL*	$EC_{200}RL$†	RL*	$EC_{200}RL$†	Difference‡
O_3-exposed group					
A	6.96	1.10	5.46	3.03	1.93
B	6.43	2.05	6.39	1.05	-1.00
C	10.62	0.62	6.02	0.57	-0.05
D	8.52	1.06	11.54	5.87	4.81
E	7.91	0.70	9.85	0.61	-0.08
F	6.14	1.07	5.95	0.85	-0.22
G	9.72	0.80	9.82	1.55	0.75
Mean	8.04	1.06	7.86	1.93	0.88
SEM	0.64	0.18	0.93	0.73	0.74
Pt-exposed group					
H	5.82	1.14	8.62	6.25	5.11
I	8.78	0.97	6.02	3.49	2.52
J	10.04	6.25	10.03	6.25	0.00
K	7.13	6.25	5.63	0.19	-6.06
L	8.00	2.34	7.10	6.25	3.91
M	6.77	0.68	7.52	1.26	0.57
N	10.65	3.06	7.40	0.13	-2.93
O	10.23	0.82	5.51	0.75	-0.07
Mean	8.43	2.69	7.23	3.07	0.38
SEM	0.63	0.83	0.55	1.00	1.28
Pt + O_3-exposed group					
P	12.98	4.62	5.77	0.52	-4.10
Q	6.68	0.83	4.66	0.75	-0.08
R	7.59	0.62	9.84	0.59	-0.03
S	20.26	0.82	9.6	0.11	-0.71
T	7.9	0.78	10.85	0.22	-0.56
U	10.46	1.37	7.04	0.78	-0.59
V	10.23	0.99	7.76	0.63	-0.63
W	11.07	6.25	10.53	6.25	0.00
Mean	10.90	2.04	8.26	1.23§	-0.84
SEM	1.53	0.76	0.82	0.72	0.48

* cm H_2O/L/s.
† mg/ml methacholine yielding a 200% increase in RL.
‡ Difference in EC_{200} values postexposure minus preexposure.
§ Significantly different at a Type 1 error of $p < 0.02$.

ther alone or in combination could enhance the development of pulmonary hyperreactivity and allergic sensitization. Local pulmonary allergic sensitization may have occurred in skin test negative monkeys who still demonstrated Pt pulmonary sensitivity. It is not known if skin test negative status in apparently allergically sensitized persons reflects low concentrations of circulating IgE antibodies because of incomplete saturation of high IgE affinity pulmonary mast and other pulmonary antibody-binding cells. After saturation of the pulmonary system, it is logical to assume that continued production of antibody would raise circulating IgE titers, yielding positive skin tests.

The present study supports previous reports from our laboratory (13, 15, 30) concerning the usefulness of cynomolgus monkeys as models for studies of acquired pulmonary hyperreactivity, allergic sensitization, and asthma. We have demonstrated that combined exposure to concentrations of Pt and O_3, which alone have no sensitizing properties, yield significant allergic Pt dermal hypersensitivity and Pt pulmonary hyperreactivity. Significant methacholine hyperreactivity was also observed in the group of animals exposed to the combination of Pt and O_3, and methacholine hyperreactivity was significantly correlated to Pt hyperreactivity. These results are consistent with the classic immunologic and physiologic findings in humans with Type 1, allergically mediated, immediate-onset asthma.

Acknowledgment

The writers wish to acknowledge the expert assistance of John C. Clark for the conduct of monkey pulmonary function evaluations, and Dr. Joan Gallagher of the University of Cincinnati for performing some of the immunochemical tests. In addition, they wish to express their appreciation to Jack Franchi, Terry Boyle, and Amir Khan of the Inhalation Toxicology Methods Section, NIOSH, for their capable assistance. And they would also like to thank Dr. Jack Pepys, Professor Emeritus, University of London, for helpful discussions during the planning phases for some of these experiments.

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