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Pulmonary Hyperreactivity in Cynomolgus Monkeys (*Macaca fasicularis*) from Nose-Only Inhalation Exposure to Disodium Hexachloroplatinate, Na_2PtCl_6[1]

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Pulmonary Hyperreactivity in Cynomolgus Monkeys (*Macaca fasicularis*) from Nose-Only Inhalation Exposure to Disodium Hexachloroplatinate, Na_2PtCl_6. BIAGINI, R. E., MOORMAN, W. J., SMITH, R. J., LEWIS, T. R., AND BERNSTEIN, I. L. (1983). *Toxicol. Appl. Pharmacol.* **69**, 377–384. The pulmonary and dermal effects of exposure to Na_2PtCl_6 were investigated in cynomolgus monkeys (*Macaca fasicularis*) exposed by the nose-only inhalation and percutaneous routes. Separate inhalation exposures were performed in monkeys at 200 $\mu g/m^3$ and 2 mg/m^3 (4 hr/day, biweekly for 12 weeks), while another group of monkeys was percutaneously exposed biweekly by an open patch method. After a 2-week refractory period, serial Na_2PtCl_6 bronchoprovocation challenges and intradermal Na_2PtCl_6 sensitivity evaluations were performed. Na_2PtCl_6 bronchoprovocation in naive control monkeys yielded significant impairments in post-challenge pulmonary mechanics and ventilatory function. These results indicate a pharmacologic or irritant-mediated bronchoconstriction mechanism for acute exposure to this compound. When the post-challenge pulmonary function of animals exposed for the 12-week exposure regimen (across treatments) was compared to pulmonary deficits observed in control animals upon challenge, significantly greater pulmonary deficits were seen in animals exposed at the 200 $\mu g/m^3$ concentration. Exposure at this concentration yielded significant changes in post-challenge average pulmonary flow resistance (R_L) and forced expiratory volume in 0.5 sec corrected for vital capacity ($FEV_{0.5}/FVC$) when compared to control monkey responses. Animals exposed by the percutaneous route or at 2 mg/m^3 showed no significant post-challenge pulmonary deficits when compared to control animals. Intradermal Na_2PtCl_6 sensitivity was found not to be exposure related in the conditions of this experiment.

There have been numerous reports in the literature concerning the incidence of pulmonary and dermal hypersensitivity in workers exposed to the soluble salts of platinum (Karasek and Karasek, 1911; Hunter *et al.*, 1945; Roberts, 1951; Freedman and Krupey, 1968; Parrot *et al.*, 1969; Pepys *et al.*, 1972, 1979; Kolpakov *et al.*, 1975; Cleare *et al.*, 1976; Dalley *et al.*, 1980; Hughes, 1980). Passive transfer of sensitivity has been shown in man (Pepys *et al.*, 1979) and monkeys (Pepys *et al.*, 1979; Biagini *et al.*, 1982), and serum antibodies have been periodically detected by the radioallergosorbent test (RAST) with platinum–human serum albumin (HSA) conjugates (Pepys *et al.*, 1979; Cromwell *et al.*, 1979).

A recent report (Dalley *et al.*, 1980) suggests that the atopic status of platinum refinery workers is not significant in disease devel-

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opment. Also, there is a variable relationship between the duration of platinum exposure, the onset of disease, and the conversion to positive platinum skin prick tests. In some cases, pulmonary disease status may precede conversion to positive skin prick test results by over a year, suggesting either an initial pulmonary pharmacologic mechanism or other factors which predispose some workers to peripheral immunity at different incidence rates.

Investigations in guinea pigs, rats, and dogs have shown increased bronchomotility and histamine release *in vitro* and *in vivo* after treatment with Na_2PtCl_6 (Parrot *et al.*, 1969; Saindelle and Ruff, 1969).

Soviet researchers have also documented the histamine releasing capabilities of the soluble platinum complexes (Tomilets *et al.*, 1980) and have reported both immediate- and delayed-type hypersensitivity reactions in guinea pigs treated with salts of platinum (Tomilets *et al.*, 1979).

The purposes of the present paper were to study the acute pharmacologic pulmonary and dermal sequelae of Na_2PtCl_6 exposure in cynomolgus monkeys and to determine if nose-only inhalation at two airborne concentrations and percutaneous application in small groups of cynomolgus monkeys could yield hyperreactive and/or hypersensitive pulmonary responses upon subsequent post-exposure Na_2PtCl_6 bronchoprovocation challenge. Another purpose of this experiment was to determine if the cynomolgus monkey would be a suitable model for further studies of the pulmonary, pharmacologic, and immunologic aspects of occupational asthma.

METHODS

Animals. Male cynomolgus monkeys (*Macaca fasicularis*—Primate Imports Corp., Long Island, N.Y.)[2] were randomly assigned to control and exposure groups. Nominally the groups were: *Group 1*—biweekly, 4 hr/day nose-only aerosol exposure to 200 $\mu g/m^3$ Na_2PtCl_6 for 12 weeks; *Group 2*—biweekly, 4 hr/day nose-only aerosol exposure to 2 mg/m^3 Na_2PtCl_6 for 12 weeks. When compared on the basis of monkey to human minute volume ratio, these concentrations result in an equivalent exposure of 3 to 4 and 30 to 40 times, respectively, of that to which a worker would be exposed in 1 week at the present TLV of 2 $\mu g/m^3$ (ACGIH, 1980). *Group 3* consisted of biweekly percutaneous exposure to 1 ml of 20 mg/ml Na_2PtCl_6 for 12 weeks and *Group 4* were unexposed controls. The mean group animal weights and number of animals per group were: *Group 1*—5.0 ± 0.5 kg ($\bar{X}$ ± SD), four animals; *Group 2*—4.6 ± 0.5 kg, four animals; *Group 3*—5.3 ± 1.0 kg, four animals; and *Group 4*—5.4 ± 1.1 kg, eight animals. The monkeys were fed standard chow daily (Monkey Chow Jumbo, Ralston Purina Co., St. Louis, Mo.)[2] and fresh fruit (oranges, apples, and bananas) once weekly. Water was provided *ad libitum* except during exposures. Monkeys were maintained on a 12-hr photoperiod (7AM to 7PM).

Atmospheric generation and analyses. Na_2PtCl_6, 99.99% pure, (Pfaltz and Bauer, Inc., Division of Aceto Chemical Co., Stamford, Conn.) was used in these studies. Aerosols were generated by nebulization of freshly prepared distilled water solutions of Na_2PtCl_6 (Bird dual jet nebulizer, Bird Inc., Palm Springs, Calif.) at 20 psi and 12 liters per minute airflow. The output of this system was about 50 ml per hour. The aerosols were introduced at the tangential airfeed manifold of a custom-designed rodent nose-only exposure chamber (Charles A. Spengler Co., Cincinnati, Ohio) modified for primate exposures. The exposure cylinder was operated at 70.8 liters per minute airflow (humidity, 50 ± 10%; temperature, 23 ± 1°C) at a positive differential pressure of 0.08 cm water with respect to the outer airlock chamber (cylinder, −0.254 cm water; airlock, −0.334 cm water). The chamber platinum aerosol concentrations were monitored for the complete 4-hr exposure periods by pulling 28.3 liters per minute of chamber air through two series connected 500 ml Greenburg–Smith impingers containing 225 and 150 ml water, respectively. After the 4-hr exposure period, the impinger contents were adjusted to 250 ml, respectively, and the ultraviolet absorbance at 262 nm was evaluated with a dual beam spectrophotometer (Beckman Model DB-G grating spectrophotometer, Beckman Instruments, Fullerton, Calif.). $PtCl_6^{2-}$ concentrations were calculated by a molar extinction coefficient of 24,500. Platinum concentrations were calculated from the spectrophotometric measurements based on the platinum content of the Na_2PtCl_6. The particle sizes of the platinum aerosols were determined after collection on 25-mm 0.1-μm-pore-size polycarbonate membrane filters (Nucleopore Corp., Pleasanton, Calif.). After collection, the filters were planchet mounted and coated with gold (sputter coater; Polaron Equipment Ltd., Waterford, Hertfordshire, U.K.). The coated sample was placed in a scanning electron microscope (JEOL, Peabody, Mass.), and the particle size was evaluated with an image analysis system (LeMont B-10 Image Analysis System, State College, Pa.)

Nose-only exposures. Animals to be exposed by the nose-only route were lightly anesthetized by an intra-

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

muscular injection of 25 mg ketamine hydrochloride (Ketaset, Bristol Labs, Syracuse, N.Y.). After the onset of anesthesia, the hands and feet were bound with "strapping tape" and the animal affixed to a Plexiglas restraint board (see Fig. 1). All animals were allowed to recover from anesthesia before the onset of exposures. Control monkeys were treated with the same anesthesia regimen as the exposed groups, but were not bound or placed in the exposure chamber.

Percutaneous exposures. An area approximately 7 × 7 cm was shaved on the intrascapular region of each ketamine-anesthetized animal. The shaved region was swabbed with 70% isopropyl alcohol, and the area was lightly abraded with the back of a scapel blade. Applied to the open patch area was 1 ml of a solution of 20 mg/ml Na_2PtCl_6 in distilled water.

Intradermal skin testing. Skin testing was performed in anesthetized animals before and after the 12-week exposure regimens. Areas of the chest and thorax were shaved, and 5 ml of a 0.5% solution of Evans blue dye was injected iv (saphenous vein). After 15 min, 100-μl serial dilutions of 1×10^{-7} to 1×10^{-3} g/ml solutions of Na_2PtCl_6 in saline were injected intradermally in each animal. After 30 min, injection sites were observed for markedly greater cutaneous bluing reactions than were seen in control injection sites. Vehicle control skin testing was performed in all cases with saline.

Bronchoprovocation challenges and pulmonary function evaluations. Bronchoprovocation challenges were performed 2 weeks after the termination of platinum exposures. Na_2PtCl_6 aerosols were generated for brochoprovocation challenges with a micronebulizer (output—0.065 ml/min) and a positive pressure ventilator respirator (Bird Mark 7, Bird Inc.) set to cycle at 20 cm water end-inspiratory pressure. Challenges were performed for 1 min (15 breaths) in the following sequence (cumulative dosing regime): saline, 0.1, 0.5, 2.5, 12.5, and 62.5 mg/ml solutions of Na_2PtCl_6. Ten minutes elapsed between challenges. Animals to be challenged were anesthetized with a mixture (Banknieder *et al.*, 1978) of 70 mg/ml ketamine hydrochloride and 6 mg/ml xylazine (Ketaset, Bristol Labs, Syracuse, N.Y.; Rompun, Bayvet Division of Cutter Labs, Shawnee, Kans.) at 0.15 ml/kg body weight. After the induction of anesthesia, an esophogeal ballon (3 × 0.7 cm) attached to polypropylene tubing (Sovereign polypropylene catheter, No. 5 French, Sherwood Medical Industries, St. Louis, Mo.) was placed in the lower third of the esophagus. The polypropylene tubing was connected to the dynamic side of a pressure transducer (PM 131 TC, Statham Labs, Inc., Hato Rey, Puerto Rico), while the static side of the transducer was connected to a vertical tap on the endotracheal tube adapter (mouth pressure). A 21 French endotracheal tube was inserted in the trachea (sublaryngeal) with the aid of a laryngoscope. The cuff of the endotracheal tube was inflated and excessive length of the distal end was trimmed even with the end of the mouth. The animal was then placed into a variable pressure (driving pressure ± 75 cm H_2O) plethysmographic chamber (supine position), and pulmonary function parameters were evaluated (Moorman *et al.*, 1975) after bronchoprovocation with saline and each serial challenge of Na_2PtCl_6. Flows were determined by observing differential pressures generated across a pneumotachograph (Fleisch No. 0, Dynasciences Medical Products, Blue Bell, Pa.) and were electrically transduced (PM-5 transducer, Statham Labs Inc., Hato Rey, Puerto Rico). Volume was obtained by electrical integration of airflow with a variable time constant. Pulmonary mechanics 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, N.Y.). Forced maneuvers were also followed with this recording system. The pulmonary function parameters evaluated in this study were average pulmonary flow resistance (R_L), dynamics compliance ($C_{L\ dyn}$), peak expiratory flow rate (PEFR), forced vital capacity (FVC), forced expiratory volume in 0.5 second/FVC ($FEV_{0.5}$/FVC), and forced expiratory flows at 50, 25, and 10% of vital capacity/FVC (FEF_{50}/FVC, FEF_{25}/FVC, and FEF_{10}/FVC). All data sampling, storage, and calculations were performed by computer (Healthgarde CPT-5, Healthgarde Inc., Salt Lake City, Utah). A minimum of 2 to 3 replicate, six-breath analyses were performed for the evaluation of R_L and $C_{L\ dyn}$. Flow–volume parameters are the results of one forced vital maneuver after saline or a serial Na_2PtCl_6 challenge.

Data reporting. Reactivity to Na_2PtCl_6 bronchoprovocation challenge was determined by the effect on each pulmonary function parameter after serial challenges with saline and 0.1, 0.5, 2.5, 12.5, and 62.5 mg/ml solutions of Na_2PtCl_6 in saline. Data are reported as percentage change in a pulmonary function parameter based on individual post-saline bronchoprovocation challenge pulmonary function results.

Statistical analyses. All hypothesis tests were performed by distribution-free (nonparametric) methods. Each variable was analyzed individually. The existence of pharmacologic bronchoconstriction effects in control monkeys were investigated by testing the percentage change (at the 62.5 mg/ml nebulizer concentration) in a pulmonary function variable from post-saline baseline values by one-tailed signed rank tests. The existence of hyperreactive responses attributed to exposure were analyzed by comparing percentage changes from baseline at the 62.5 mg/ml nebulizer concentration across groups for each pulmonary function variable with one-tailed Dunn's multiple comparisons procedures. Post-saline challenge group homogeneity was investigated by the Kruskal–Wallis test.

RESULTS

Animal Observations

The monkeys in this study tolerated the restraint, exposures, and testings with no obvious adverse effects. Physiologic measurements (blood pressure, heart rate, respiration rate, and body temperature) were performed

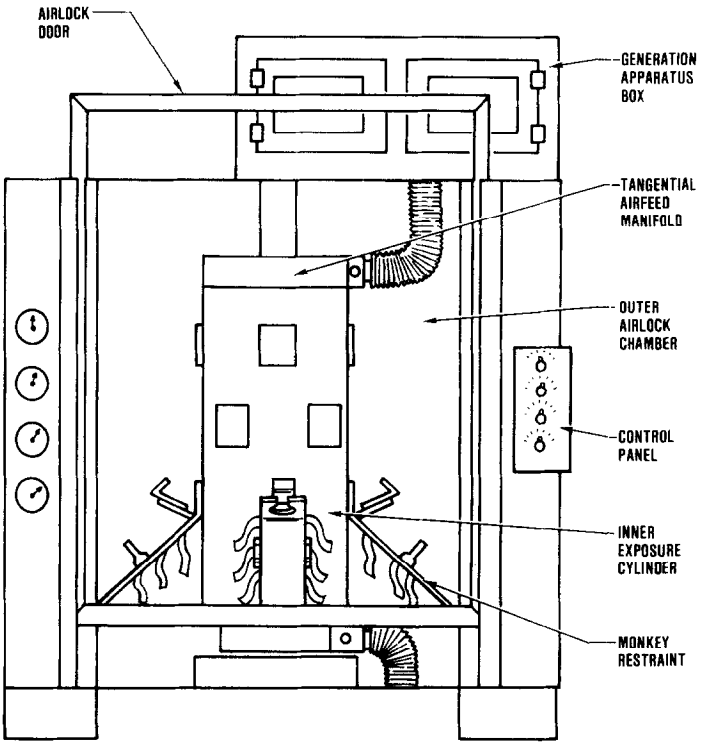

FIG. 1. Schematic diagram of primate-modified rodent nose-only inhalation chamber.

on four restrained control animals, and no adverse effects on these parameters were observed. It is possible that sedative effects during recovery from the dissociative anesthesia regime utilized to accomplish restraint minimized excitement in the monkeys. These findings are in agreement with the reports of others (Alarie *et al.*, 1970) indicating awake cynomolgus monkeys similarly restrained (face masks and restraint chairs) showed minimum excitement.

One accidental monkey death was observed in the 200 $\mu g/m^3$ exposure group (aspiration of regurgitated food while anesthetized).

Atmospheric Generation and Analyses

The mean chamber concentrations of Na_2PtCl_6 observed for the targeted 200 $\mu g/m^3$ and 2 mg/m^3 (based on Pt) exposures were 216 ± 31 $\mu g/m^3$ and 1.94 ± 0.43 mg/m^3 ($\bar{X}$ ± SD), respectively. Particulate sizing measurements revealed mass median aerodynamic diameters (MMADs) of 1.61 and 1.27 μm, respectively, for the two aerosols. Size distributions of the two exposure aerosols were also similar with standard geometric deviations (SDGs) of 1.93 and 2.09, respectively. The bronchoprovocation aerosol (62.5 mg/ml Na_2PtCl_6) had a MMAD of 1.02 micrometers with a SDG of 2.57. MMADs were calculated from density adjusted transformations (Hatch–Choate) of SEM-image analyses count diameters and SDGs (linear plots).

Dermal Testing

Results of direct skin testing with Na_2PtCl_6 before and after exposure showed no differences in dermal sensitivities. Each animal gave the same pre- and post-exposure positive dermal bluing reactions (12 of 19 animals were positive at dilutions of 10^{-5} g/ml Na_2PtCl_6, while 6 of 19 were positive at dilutions of 10^{-4} g/ml).

Post-platinum Bronchoprovocation Challenge Effect on Pulmonary Function

The Na_2PtCl_6 exposure regimens used in the present study had no effect on post-exposure baseline pulmonary function. The mean values for the pulmonary function variables evaluated (Table 1) were homogeneous ($p > 0.05$) for all groups after bronchoprovocation challenge with saline. However, serial bronchoprovocation challenges with increasing concentrations of the platinum salt showed marked effects on the pulmonary function in all exposed and control animals.

From the data given in Table 2, it can be seen that Na_2PtCl_6 bronchoprovocation challenge has dramatic effects on pulmonary mechanics and ventilatory performance in control (naive, previously unexposed to Na_2PtCl_6) animals, presumably due to a pharmacologic mechanism. Significant reductions in FVC ($p < 0.05$), PEFR ($p < 0.05$), $FEV_{0.5}/FVC$ ($p < 0.005$), FEF_{50}/FVC ($p < 0.01$), and FEF_{25}/FVC ($p < 0.005$) and a significant increase in R_L ($p < 0.005$) were observed after the 62.5 mg/ml Na_2PtCl_6 challenge concentration when these pulmonary function results are compared to post-saline bronchoprovocation values.

One-tailed Dunn's multiple comparisons of exposed groups vs controls were performed (62.5 mg/ml nebulizer concentration) for each variable with a Type I error level of 0.05. The percutaneous and 2 mg/m^3 exposure groups were not detectably different from controls for any pulmonary function variable tested by this procedure (i.e., control, 2 mg/m^3, and percutaneous exposure groups all had similar pharmacologic responses to increasing bronchoprovocation concentrations of Na_2PtCl_6). However, a significant increase in R_L and decrease in $FEV_{0.5}/FVC$ were observed in the 200 μg/m^3 exposure group when their data were compared to control responses (Table 2).

Statistical tests of the data for the lower challenge concentrations were not performed. Qualitative inspection of the data did not reveal any striking differences between groups, with the exception of 2 of 3 animals in the 200 μg/m^3 exposure that showed large increases in resistance of 1524 and 828% over baseline at the 12.5 mg/ml challenge concentration. Group mean R_L concentration–response results are given graphically in Fig. 2.

TABLE 1

MEAN GROUP PULMONARY FUNCTION AFTER SALINE BRONCHOPROVOCATION CHALLENGE[a]

Parameter	Units	Treatment group			
		Control ($N = 8$)[b]	Percutaneous ($N = 4$)	200 μg/m^3 ($N = 3$)	2 mg/m^3 ($N = 4$)
R_L[c]	cm H_2O/l/sec	6.4 ± 2.7[d]	7.3 ± 1.8	7.5 ± 1.7	10.0 ± 5.0
$C_{L\,dyn}$	ml/cm H_2O	20.5 ± 4.7	28.5 ± 5.0	16.0 ± 8.5	26.9 ± 8.4
FVC	ml	483 ± 109	332 ± 219	422 ± 102	396 ± 78
PEFR	ml/sec	1747 ± 405	1936 ± 336	1687 ± 267	1389 ± 184
$FEV_{0.5}/FVC$	%	82 ± 6	86 ± 6	88 ± 3	86 ± 6
FEF_{50}/FVC	FVC/sec	3.53 ± 0.81	3.49 ± 1.21	3.50 ± 0.36	3.27 ± 0.57
FEF_{25}/FVC	FVC/sec	1.23 ± 0.33	1.61 ± 0.73	1.24 ± 0.34	1.26 ± 0.37
FEF_{10}/FVC	FVC/sec	0.19 ± 0.06	0.42 ± 0.20	0.34 ± 0.14	0.28 ± 0.21

[a] No significant differences between groups ($p > 0.05$) for each pulmonary function variable.
[b] N = number of animals per group.
[c] Abbreviations for pulmonary function variables are given in text.
[d] Mean ± SD.

TABLE 2

PULMONARY REACTIVITY TO Na_2PtCl_6 BRONCHOPROVOCATION CHALLENGE

Parameter	Treatment group			
	Control (N = 8)[a]	Percutaneous (N = 4)	200 $\mu g/m^3$ (N = 3)	2 mg/m^3 (N = 4)
R_L[b]	447 ± 201[c,d]	666 ± 298	1057 ± 144[e]	489 ± 330
$C_{L\ dyn}$	70 ± 30	45 ± 31	58 ± 44	44 ± 28
FVC	90 ± 7[d]	91 ± 5	88 ± 5	96 ± 6
PEFR	84 ± 9[f]	82 ± 9	72 ± 7	91 ± 5
$FEV_{0.5}/FVC$	80 ± 5[d]	78 ± 6	69 ± 3[g]	86 ± 9
FEF_{50}/FVC	60 ± 8[h]	75 ± 23	41 ± 4	89 ± 15
FEF_{25}/FVC	53 ± 11[d]	58 ± 20	50 ± 18	77 ± 22
FEF_{10}/FVC	75 ± 31	86 ± 17	46 ± 5	102 ± 3

[a] N = number of animals/group.
[b] Abbreviations for pulmonary function variables are given in text, see Table 1 for units.
[c] Percentage of post-saline bronchoprovocation value observed at 62.5 mg/ml challenge concentration. Mean ± SD.
[d] Significantly different from post-saline bronchoprovocation value, $p < 0.005$.
[e] Significantly different from control group mean response, $p < 0.001$.
[f] Significantly different from post-saline bronchoprovocation value, $p < 0.05$.
[g] Significantly different from control group mean response, $p < 0.05$.
[h] Significantly different from post-saline bronchoprovocation value, $p < 0.001$.

DISCUSSION

Data in this report indicate that Na_2PtCl_6 bronchoprovocation challenge in naive monkeys results in significant pulmonary function changes. The pattern of post-challenge pulmonary function deficits observed in the present study suggests bronchoconstriction in both small and large airways. This bronchoconstrictor effect is thought to be the result of Na_2PtCl_6 induced release of histamine or other mediators through pharmacologic or irritant mechanisms. This result would be in agreement with Na_2PtCl_6 induced bronchocon-

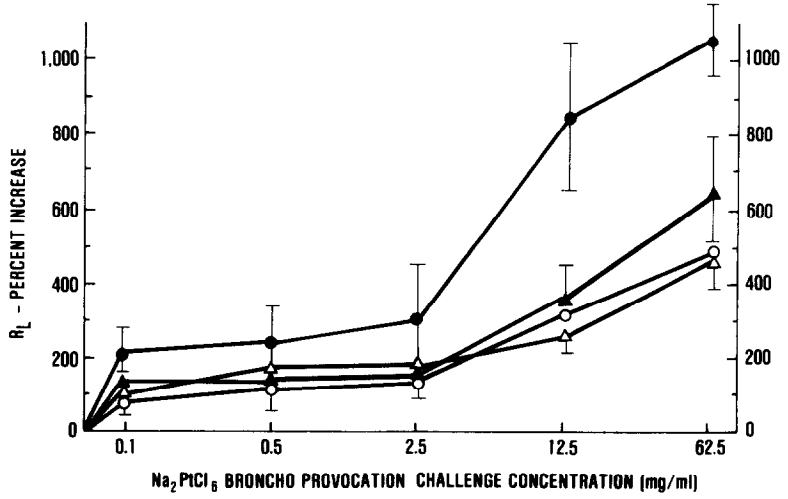

FIG. 2. Effect of serial Na_2PtCl_6 bronchoprovocation challenge on average pulmonary flow resistance (R_L ± SEM); △—control; ●—200 $\mu g/m^3$; ▲—percutaneous exposure; and ○—2 mg/m^3.

striction and anaphylactic-type shock due to histamine release shown by other investigators with different animal species (Saindelle and Ruff, 1969). Results showing increased vascular permeability, as demonstrated by dye exudation, on intradermal injections of Na_2PtCl_6 in nonexposed control monkeys also suggest a pharmacologic histamine or other mediator release-type mechanism in skin. Positive platinum skin prick test results in humans at higher prick testing concentrations have been reported (Cleare *et al.*, 1976).

When exposure-related pulmonary hyperreactivity to Na_2PtCl_6 bronchoprovocation challenge is evaluated, it was found that biweekly exposure at 200 $\mu g/m^3$ for 12 weeks yielded significant impairment in R_L (increased) and $FEV_{0.5}/FVC$ (decreased). It is interesting to note that dermal hypersensitivity to Na_2PtCl_6 was not observed in the presence of significant post-challenge pulmonary function impairment. Because of this finding, it is difficult to interpret if the observed pulmonary hyperreactivity is due to a superpharmacologic, irritant, local immune, or combination mechanisms. This finding is in agreement with data for platinum sensitive refinery workers, who reportedly have pulmonary disease status, in some cases for over a year, prior to converting to positive platinum skin prick test reactions (Dalley *et al.*, 1980). The concept of occupational asthma in the absence of a demonstrated peripheral immune response is not unique, as other investigators have reported of hyperreactive, hypersensitive bronchospasms in exposed workers from many industries, presumably from reflex, pharmacologic, or inflammatory mechanisms (Gandevia, 1970; Brooks, 1977). The finding of the present report suggesting that percutaneous applications of Na_2PtCl_6 alone do not produce post-challenge pulmonary deficits was not surprising. However, the absence of post-challenge pulmonary hyperreactivity or hypersensitivity in animals exposed at 2 mg/m^3 was perplexing. These data suggest a possible pulmonary tolerance mechanism, tachyphylaxis, or delay in the development of pulmonary symptoms at higher sensitization concentrations. Alternatively, a delayed (4 to 8 hr post-challenge) deficit in pulmonary function may have occurred which was not able to be evaluated by the protocols of the present study. Delayed-onset-only pulmonary responses have been reported in platinum sensitive workers on challenge (Pepys *et al.*, 1972).

Collectively these results suggest that the cynomolgus monkey may be a useful model for the study of occupational asthma. We have demonstrated through pulmonary function testing after unconjugated antigen provocative challenges in small groups of sensitized monkeys, pulmonary mechanical and ventilatory deficits consistent with human platinum asthma (Pepys *et al.*, 1972). Sample sizes in the present investigation were small, because the judicious use of primates for research purposes is necessary due to their limited availability and expense. Accordingly, the conservative use of these animals is a necessity. Previous studies with small groups of macaques (O'Neil *et al.*, 1971; Michoud *et al.*, 1978) have shown these animals to be valuable in elucidating the effects of bronchoactive agents and allergens, and suggest them to yield pulmonary and immune responses accurately analogous to human asthma. In general, challenge in pretreated cynomolgus monkeys in the present investigation gave comparable pulmonary function responses to those observed in asthmatic subjects on induced bronchoconstriction to a variety of environmental antigens (Olive and Hyatt, 1972).

In conclusion, we have demonstrated pharmacologic bronchoconstriction and dermal sensitivity in naive cynomolgus monkeys from acute exposure to Na_2PtCl_6. In addition, we have demonstrated statistically significant post-exposure hyperreactivity to Na_2PtCl_6 bronchoprovocation challenge in monkeys exposed at 200 $\mu g/m^3$ Na_2PtCl_6, biweekly, 4 hr/day for 12 weeks. Data were also presented suggesting that R_L and $FEV_{0.5}/FVC$ are sensitive indicators of Na_2PtCl_6 hyperreactivity in cynomolgus monkeys. Exposure to Na_2PtCl_6 by the percutaneous route alone at 20 mg twice weekly does not seem effective

in producing significant immediate-onset challenge hyperreactivity, as is the case with nose-only exposures at 2 mg/m^3 Na_2PtCl_6. Data were presented showing the cynomolgus monkey to be a useful model for the study of immediate pulmonary hypersensitivity and acute pulmonary pharmacological evaluations.

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