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Research Contract Final Report to State

of California Air Resources Board

Title of Contract: AIRWAY HYPERIRRITABILITY INDUCED BY OZONE

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I. ABSTRACT

A study of 14 healthy adult subjects was undertaken to determine whether brief exposure to 0.5-0.6 ppm of ozone would increase bronchial reactivity to inhaled irritants, as reflected by the rise in airway resistance provoked by aerosol challenge with weak solutions of histamine or methacholine. An additional study of 9 subjects with non-asthmatic allergic diseases was also undertaken to determine the effects on ozone on bronchial reactivity in atopic subjects. Subjects from both the non-atopic and atopic groups were exposed to ozone on several occasions to determine whether tolerance develops to the sensitizing effects of ozone on bronchial responsiveness.

The results indicate that exposure to 0.6 ppm of ozone for 2 hours increases bronchial reactivity to histamine in both normal and atopic subjects and that the increase in responsiveness is blocked by pretreatment with atropine, suggesting that postganglionic cholinergic mechanisms are involved. Bronchial reactivity returned to control levels in 1-7 days in most subjects, but tolerance to repeated exposures to ozone was not observed.

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The investigators wish to acknowledge the helpful guidance provided by Dr. Robert Frank of the University of Washington and the assistance of Drs. Lu-Yuan Lee and Claude Dumont in technical aspects of the project.

VI. CONCLUSIONS

The projects completed in this contract permit the following conclusions:

1. A 2-hour exposure to 0.5-0.6 ppm of ozone increases the bronchial response to inhalation of histamine and methacholine aerosol in normal subjects.

2. Identical exposure to ozone increases bronchial sensitivity to histamine in subjects with non-asthmatic atopic disease, many of whom have pre-existing bronchial hyper-reactivity.

3. The increase in bronchial reactivity is blocked by pre-treatment with atropine sulfate aerosol, indicating that it is mediated by cholinergic, post-ganglionic pathways.

4. Tolerance to the effect of ozone on bronchial reactivity is not apparent after two or three exposures (preliminary studies).

VII. RECOMMENDATIONS

The finding that brief exposure to ozone increases the degree of bronchospasm provoked by pharmacologic agents suggests that ozone may also increase the response of normal subjects to other inhaled materials, such as sulfur dioxide, and the response of atopic subjects to specific allergens. Future studies on ozone should include the following:

1. Study of the effect of ozone on airway responsiveness to inhaled SO_2 in healthy subjects and of the role of cholinergic pathways in any change noted in sensitivity to SO_2.

2. Study of the minimum concentration of ozone required to increase airway responsiveness in healthy subjects.

3. Study of the effect of the concentration of ozone vs. the cumulative dose of ozone on airway responsiveness to histamine in healthy subjects.

4. Study of the effect of repeated exposures to ozone at various intervals on bronchial reactivity.

5. Study of the effect of ozone on the airway responsiveness of asthmatics to both non-specific (e.g. histamine) and specific (e.g. antigen) stimuli.

6. Additional study of the mechanisms of ozone-induced increases in bronchial reactivity by investigating the effects of pre-treatment with drugs acting at different sites on the cholinergic pathway (e.g. hexamethonium, physostigmine, acetylcholine, bethanecol).

VIII. DISCLAIMER

The statements and conclusions in this report are those of the contractor and not necessarily those of the California Air Resources Board. The mention of commercial products, their source, or their use in connection with material reported herein is not to be construed as either an actual or implied endorsement of such products.

IX. BODY OF REPORT

Introduction:

The purpose of this project was to determine whether brief exposure to ozone, a common atmospheric pollutant in California cities, alters bronchial sensitivity to inhaled irritants. The airways of patients with asthma and of some patients with chronic bronchitis have long been known to be abnormally sensitive to inhalation of a wide variety of pharmacologically and antigenically unrelated materials (1). This abnormal sensitivity or "bronchial hyper-reactivity" can be quantitated by measuring the rise in airway resistance or fall in FEV_1 after inhalation of aerosols of solutions of histamine or methacholine. Doses of these compounds which cause little or no change in the lungs of normal subjects cause intense, often symptomatic bronchoconstriction in patients with asthma. Bronchial hyper-reactivity is considered so characteristic of asthma as to be included in definitions of the disease (2), but neither its mechanism of nor its significance are known. Several possible mechanisms have been proposed. One explanation is that the exaggerated bronchoconstriction is simply a reflection of the greater mass of bronchial smooth muscle known to be present in asthmatics (3,4). Another theory holds that asthmatics have narrowed airways prior to inhalation of irritating materials, and that the same shortening of smooth muscles has a greater effect on narrowing an already constricted lumen than it has on a lumen of normal initial diameter (5). A third theory is that the bronchial smooth muscle of an asthmatic is inherently more reactive because of a fundamental difference in the biochemical regulation of the contractile mechanism or because of a loss of modulating sympathetic activity (6).

An additional possible mechanism for the production of bronchial hyper-reactivity was suggested by our observation that both spontaneous viral upper respiratory infections and induced infections with attenuated influenza virus temporarily increase bronchial reactivity in normal human subjects (7,8). Because the increase in responsiveness can be blocked by pre-treatment with atropine sulfate, we have proposed that post-ganglionic cholinergic mechanisms are involved and that there is an increase in the reflex component of the response to histamine, possibly due to sensitization of irritant receptors resulting in some way from the epithelial damage of viral respiratory tract infections. Because low concentrations of ozone also cause epithelial damage, we studied the effects of ozone exposure on bronchial reactivity in dogs. We found that exposure to concentrations of ozone as low as 0.5 ppm for 2 hours increases airway responsiveness both to inhaled histamine and to inhaled prostaglandin $F_{2\alpha}$ in dogs (9). These studies suggest that concentrations of ozone so low as to have minimal effect on pulmonary function may sensitize the airways to mediators that are released in acute, antigen-induced attacks of asthma.

The studies we have completed during the past year were designed first to determine whether similar exposure to ozone increases bronchial reactivity to histamine in normal human subjects, and, if so, whether pre-treatment with an anticholinergic drug blocks the induced hyper-reactivity. We then undertook a study of the effects of ozone exposure on bronchial reactivity in subjects with atopic diseases, many of whom have pre-existing hyper-reactivity. We further investigated whether the increase in sensitivity to histamine was associated with an increased sensitivity to methacholine aerosol and whether repeated exposures to ozone result in a progressive diminution of bronchial reactivity, which would suggest that tolerance to this effect of ozone may develop.

The studies were conducted in three different phases. The first phase was devoted simply to determining whether ozone exposure affects bronchial reactivity in normal human volunteers. The next project was devoted to comparing changes in the responsiveness to histamine to changes in methacholine sensitivity, and to studying the effects of repeated exposure to ozone. The third project investigated the effects of ozone on subjects with non-asthmatic atopic diseases. These projects will be reported sequentially. The first project involved modification of an exposure chamber, calibration of an ozone meter, and other technical procedures which apply to all subsequent projects. These will be detailed only in the description of the first project.

Project #1: Bronchial hyper-irritability in healthy subjects after exposure to ozone.

Design, Construction, Material and Methods: The subjects were eight healthy, non-smoking adult volunteers, five women and three men, aged 22-30 yrs, who were informed of the risks of the experimental protocol and who signed consent forms approved by the Academic Senate Committee on Human Experimentation of the University of California. The subjects gave no history of atopy, asthma,

or chronic lung disease. None had had symptoms suggesting a viral upper respiratory infection within the month before they entered the study. All had normal results from screening tests of pulmonary function--spirometry, single-breath carbon monoxide diffusing capacity, single-breath oxygen distribution, and flow-volume curve (10) and a rise in airway resistance (Raw) to no more than 2.50 cm H_2O/LPS after inhaling 10 breaths of 1.6% histamine aerosol.

Bronchial reactivity to histamine was assessed before and after ozone exposure. For pre- and post-ozone baselines, Raw and thoracic gas volume were measured three times at 30-second intervals before inhalation of histamine and were averaged. For the responses, Raw and thoracic gas volume were measured every 30 seconds for five minutes after inhalation of 10 breaths of 1.6% histamine, and the three highest consecutive measurements were averaged. Baseline and response measurements were made before ozone exposure and immediately, one day, and one, two and three weeks after ozone exposure.

Airway resistance and thoracic gas volume were assessed with a constant-volume plethysmograph (11,12). Airflow was measured with a heated Fleisch No. 2 pneumotachograph and a Validyne DP 45 differential transducer. Mouth and box pressures were measured with Validyne pressure transducers (DP 7 and DP 45, respectively). The electrical output of these transducers entered Validyne CD 19 carrier demodulators, was amplified by a Tektronix 5A18N dual-trace amplifier, and was displayed as tracing on a Tektronix D15 single-beam storage oscilloscope. The slopes of the tracings were read directly from a protractor mounted to the oscilloscope face. One tracing for each pre- and post-histamine measurement was photographed with a Tektronix C-5 oscilloscope camera.

A 1.6% solution of histamine was prepared daily from a stock solution of 3.2% histamine and was buffered with an equal amount of sodium bicarbonate solution to a pH of 7.0. All solutions were delivered as aerosols from a DeVilbiss No. 40 glass nebulizer equipped with a dose-metering device (13). This device consisted of a breath-activated solenoid valve and a timing circuit in series with a compressed air source at 20 p.s.i. The solenoid valve was set to remain open for 0.8 seconds from the onset of inspiration, during which time air was allowed to flow through the nebulizer. Starting at functional residual capacity, the aerosol was delivered throughout the course of a sub-maximal inspiration so that the subject avoided inhaling to total lung capacity. The particles delivered by the nebulizer have been reported to have a mass mean diameter of 6.3 μm (14).

Each subject was exposed to ozone (0.6 ppm) for two hours in a 12 x 10 x 8 foot chamber painted with Varni-Lite, an oxidant-resistant material (Varni-Lite Corp., Santa Clara, Ca.) (Fig. 1). The subject was seated and wore a nose clip. Ozone was added to the room via a duct that enters midway between the ceiling and floor along one wall. The ozone was drawn across the chamber from this duct by an exhaust fan above the ceiling and was vented to the outside. Ozone was generated by passing 100% oxygen into a commercial ozonator (Ozone Research and Equipment Inc., Model 03V1) and was diluted with filtered clean air as it was drawn into the exposure chamber at a concentration and rate controlled by the exhaust fan to maintain an atmosphere of 0.5-0.6 ppm by volume. The concentration of ozone in the chamber was monitored by a chemi-luminescent ozone and analyzer (Monitor Lab., Inc., Model 8410). The analyzer

was calibrated manually by the potassium iodide method (15). All tubing exposed to ozone was made of Teflon to avoid decomposition of ozone.

To investigate the mechanism of the increase in bronchial response to histamine caused by ozone, we studied the effects of isoproterenol and atropine sulfate aerosols. Five subjects were treated with five or ten inhalations of a 0.5% solution of isoproterenol for relief of symptoms associated with histamine-induced bronchoconstriction. In four subjects selected because their bronchial reactivity was markedly increased immediately after exposure to ozone, bronchial reactivity to histamine was assessed after atropine sulfate premedication. For this study, atropine sulfate (0.1-0.2 mg/kg) was inhaled from a 10 mg/ml solution one-half hour before inhalation of histamine aerosol. The effect of atropine sulfate on the response to histamine was determined both six hours after exposure to ozone, when bronchial responsiveness was greatest, and one week after exposure, when responsiveness had returned toward control level (Fig. 3).

The statistical significance of group and individual measurements of bronchial reacitivity was determined with "Student's" t-tests for paired and unpaired data (16), respectively.

Results: In every subject the bronchial response to inhalation of histamine aerosol immediately after exposure to ozone was greater than the response before exposure to ozone (e.g., Fig. 2). Before exposure, inhalation of histamine increased the mean Raw from 1.2 ± 0.1 to 1.8 ± 0.2 cm H_2O/LPS (± S.E.) in the eight subjects studied, yielding a mean increase of 0.6 ± 0.2 cm H_2O/LPS. Immediately after the two hour exposure to ozone, histamine increased the mean Raw from 1.4 ± 0.1 to 3.3 ± 0.4 cm H_2O/LPS, yielding a mean increase of 1.9 ± 0.4 cm H_2O/LPS (Fig. 3). The mean baseline value for Raw after exposure to ozone did not differ significantly from the mean baseline value before exposure ($P>0.05$), but the mean increase in Raw produced by histamine after ozone exposure was significantly greater than the mean increase produced before ozone exposure ($P<0.05$). This increase in responsiveness to histamine was associated with symptoms of bronchial irritation: no chest discomfort, dyspnea, or coughing was reported after the control inhalation of histamine aerosol. After exposure to ozone, four subjects reported minimal substernal discomfort. On subsequent inhalation of histamine, five subjects complained of the appearance of worsening of substernal discomfort, four complained of cough, and four of dyspnea and chest tightness. Treatment with isoproterenol reversed all symptoms and returned Raw to its baseline value.

The greatest increase in bronchial reactivity occurred immediately after exposure to ozone; reactivity returned gradually to control values from 7-21 days after exposure. Although the mean increases in Raw at 1 and 7 days (1.1 ± 0.3 and 0.9 ± 0.3 cm H_2O/LPS, respectively) were not significantly greater than the control response for the group as a whole, two of our subjects appeared to have significantly greater responses to histamine for one week or longer after ozone exposure.

Atropine sulfate blocked the increase in bronchial reactivity to histamine caused by ozone exposure. When the four subjects who showed the greatest reactivity inhaled atropine sulfate six hours after being exposed to ozone, the mean baseline Raw was 0.7 ± 0.3 cm H_2O/LPS; after histamine inhalation Raw rose only slightly, to 0.8 ± 0.5 cm H_2O/LPS (Fig. 4). When we tested Raw on the next day, the hyper-reactivity to histamine returned, as indicated by the rise of resistance. These findings show that the effect of stropine was not due to a spontaneous diminution in bronchial reactivity.

The effect of atropine sulfate premedication was also assessed more than six weeks after ozone exposure, when airway responsiveness to histamine for these four subjects was presumed to have returned to the pre-ozone level. At this time Raw rose from 0.6 ± 0.4 to 0.8 ± 0.3 cm H_2O/LPS after inhalation of histamine. These results are very similar to the results of the atropine premedication study done six hours after ozone exposure, when airway reactivity was markedly increased (Fig. 4).

Comments: This study demonstrates that brief exposure of healthy subjects to ozone (0.5-0.6 ppm by volume) has little effect on baseline airway resistance but significantly increases bronchial reactivity to histamine aerosol. For two of the eight subjects, the increase in reactivity persisted for more than one week after a single two-hour exposure to ozone.

Project #2: Effects of ozone exposure on bronchial sensitivity to histamine and methacholine in normal human subjects.

Design, Construction, Material and Methods: The subjects were seven healthy, non-smoking adult volunteers, four men and three women, aged 21-35 yrs, who were informed of the risks of the experimental protocol and who signed consent forms approved by the Academic Senate Committee on Human Experimentation of the University of California. The subjects gave no personal or family history of atopy, asthma, or lung disease and all had less than two positive reactions to prick skin tests with mixes of seven allergens common to Northern California. None had had symptoms suggesting a viral upper respiratory infection within the month before they entered the study. All had normal results from screening tests of pulmonary function--spirometry, single-breath carbon monoxide diffusing capacity, single-breath oxygen distribution, and flow-volume curve.

Bronchial reactivity to histamine was assessed before and after a sham exposure and after ozone exposure, while reactivity to methacholine was assessed after sham and ozone exposures. For the pre- and post-sham and pre- and post-ozone baselines, airway resistance (Raw) and thoracic gas volume (TGV) were measured in a 900 L body plethysmograph (Electronics for Medicine, White Plains, N.Y.) five times at 30-second intervals before inhalations of histamine and methacholine, and values of specific airway resistance (SRaw) were calculated (Raw x TGV) and averaged. For the responses, SRaw was measured every 30 seconds for five minutes after inhalation of 10 breaths of 1.6% histamine, and the three highest consecutive measurements were averaged. Specific airway resistance after inhalation of 10 breaths of 1.0% methacholine was measured for 10 minutes and averaged in the same way. Baseline and response measurements were made before and after sham exposure and immediately and one day after ozone exposure. All subjects were followed at one to two week intervals until responses returned to control values.

To further investigate the mechanism of the increase in bronchial response to histamine by ozone exposure, we also studied the effect of atropine sulfate aerosol. For this study, atropine sulfate (0.1 mg/kg) was inhaled from a 10 mg/ml solution one-half hour before inhalation of histamine aerosol. The effect of atropine sulfate on the response to histamine was determined two hours after both sham and ozone exposure in six of the subjects.

We also investigated the possibility that repeated exposures might induce tolerance to ozone's effects on bronchial reactivity. Three subjects were re-exposed to ozone 1 to 4 weeks after their first exposure, and one subject was re-exposed a third time, 4 weeks after the second exposure. Bronchial sensitivity to histamine and methacholine were measured after these exposures as they had been after the first exposure.

A solution of histamine (1.6%) was prepared daily from a stock supply and was buffered to a pH of 7.0. Methacholine (1.0% solution) was made daily from a stock supply of methacholine chloride (Merck, Sharpe, Dome) dissolved in normal saline. All solutions were delivered as aerosols from a DeVilbiss No. 40 glass nebulizer equipped with a dose-metering device, as described in Project #1.

Each subject underwent a sham and an ozone exposure on separate days for two hours in the chamber described in Project #1. The subject wore a nose clip and alternated 15-minute periods of rest with exercise on a bicycle ergometer at a workload that doubled resting minute ventilation. Temperature and relative humidity were monitored during all exposures. Ozone was generated and introduced into the chamber as in the first project. The concentration was maintained at 0.5-0.6 ppm by volume and was monitored by an ultraviolet ozone analyzer (Dasibi, Model No. 1003 AH). The analyzer was calibrated manually by the potassium iodide method (15).

The statistical significance of group measurements of bronchial reactivity was determined with "Student's" t-tests for paired data (16).

Results: (see Table I) No significant difference in baseline SRaw or in the bronchial response to inhalation of histmine aerosol was present after sham exposure when compared to control values. In every subject the bronchial response to inhalation of histamine aerosol after exposure to ozone was greater than the response after sham exposure. After sham exposure, inhalation of histamine increased the mean SRaw from 5.27 ± 0.72 to 8.69 ± 1.44 liter x cm H_2O/LPS ($\pm$ S.E.) in the eight subjects studied, yielding a mean increase of 3.42 ± 0.65 liter x cm H_2O/LPS. Immediately after the two-hour exposure to ozone, histamine increased the mean SRaw from 5.84 ± 0.61 to 12.47 ± 2.00, yielding a mean increase of 6.58 ± 1.43 liter x cm H2O/LPS (Fig. 5). The mean baseline value for SRaw after exposure to ozone did not differ significantly from the mean baseline value following sham exposure ($P>0.5$), but the mean increase in SRaw produced by histamine after ozone exposure was significantly greater than the mean increase produced after sham exposure ($P<0.05$). This increase in responsiveness to histamine was associated with symptoms of bronchial irritation in six subjects.

Atropine sulfate blocked the increase in bronchial reactivity caused by ozone exposure. When five subjects were given atropine premedication after sham exposure, histamine inhalation caused a rise in SRaw from 3.27 ± 0.38 to 4.31 ± 0.65, an increase of 1.09 ± 0.33 liter x cm H_2O/LPS. After ozone exposure, the mean response to histamine after atropine pre-treatment was a rise in SRaw from 3.58 ± 0.53 to 5.16 ± 0.63 yielding an increase of 1.58 ± 0.40 liter x cm H_2O/LPS (Fig. 6).

The bronchial response to the inhalation of methacholine aerosol was also greater after exposure to ozone than after sham exposure in all subjects. After sham exposure, inhalation of methacholine increased mean SRaw by 3.62 ± 0.61, but after exposure to ozone, mean SRaw increased by 8.94 ± 2.12 liter x cm H_2O/LPS (Fig. 7). The mean baseline value for SRaw after exposure to ozone did not differ significantly from the mean baseline value following sham exposure ($P>0.5$), but the mean increase in SRaw produced by methacholine after ozone exposure was significantly greater than the mean increase produced after sham exposure ($P<0.01$).

The mean rise in SRaw provoked by histamine inhalation 24 hours after ozone exposure was 3.91 ± 0.78 liter x cm H_2O/LPS. While this was greater than the mean change of 3.42 ± 0.65 after sham exposure, the difference was not significant. In four subjects, however, the response to histamine was greater than control for one week or more.

In each of three subjects exposed to ozone on more than one occassion, the bronchial response to histamine was increased after each exposure, indicating that tolerance to this effect of ozone did not occur. One subject was exposed to 0.4 ppm of ozone after the increase in bronchial reactivity caused by the first exposure had returned to control levels. Although the level of ozone was 33% lower, the increase in reactivity was greater than that caused by the higher level (Fig. 8). In the subject re-exposed to ozone a third time, bronchial reactivity to histamine increased as it had after the previous exposures.

Comments: This study confirms the finding of the first project; that brief exposure of healthy subjects to ozone (0.5-0.6 ppm by volume) has little effect on baseline airway caliber but significantly increases bronchial reactivity to histamine aerosol. The increase in reactivity is not specific to histamine alone, for the response to methacholine aerosol is also increased. The effects of atropine pre-treatment suggest that the increase in bronchial reactivity is mediated by cholinergic postganglionic pathways, and the reproducibility of increased reactivity after repeated exposures suggests that tolerance to this effect of ozone does not readily develop.

Project #3: Effects of ozone exposure on bronchial reactivity in subjects with atopic disease.

Design, Construction, Materials and Methods: Nine non-smoking adult volunteers with atopic diseases, six men and three women, aged 24-35 yrs, consented to participate in the study after they were informed of the risks of the experimental protocol. All signed consent forms approved by the

Academic Senate Committee on Human Experimentation of the University of California. The subjects were classified as atopic on the basis of a personal history of hay fever, eczema, or childhood asthma and all had two or more positive cutaneous responses to skin testing with extracts of 7 antigens common to Northern California. Pulmonary function was normal in all subjects as assessed by spirometry, single-breath diffusing capacity, flow-volume curve, single-breath oxygen test, and plethysmographic values for airway resistance and thoracic gas volume. As in the previous projects, none had a history of symptoms of a recent viral upper respiratory infection and none were using antihistamiic or bronchodilator drugs at the time of the study.

Bronchial reactivity was assessed in each subject by measuring the rise in SRaw provoked by inhalation of histamine aerosol prepared in the same manner and delivered from the same DeVilbiss nebulizer and dose-metering device as in the previous projects. Because many atopic subjects are hyper-reactive to histamine, a dose-response curve to serially increasing concentrations of histamine aerosol (2-16 mg/ml, 10 breaths at each concentration) was constructed for each subject in the baseline state, immediately after sham exposure, and immediately and 24 hours after ozone exposure. The effect of pre-treatment with 0.1 mg/kg of aerosolized atropine sulfate on the response to histamine was assessed in five subjects after both sham and ozone exposures. As in Project #2, the response to histamine aerosol was expressed as the risk in specific resistance (Raw x TGV) measured in the body plethysmograph.

In subjects demonstrating an increase in bronchial reactivity after exposure to ozone, the response to histamine was measured at 1-2 week intervals until it returned to control levels. Nine subjects were then re-exposed to ozone, to investigate the possibility that tolerance develops to this effect of ozone.

The exposure chamber, the level of ozone, its generation and monitoring, and the use of intermittent light exercise during exposure were identical to the methods described in Project #2.

The results were analyzed by comparing the responses to the highest cumulative dose of histamine inhaled after both sham and ozone exposure. Statistical significance of group measurements was determined with "Student's" t-test for paired data (16).

Results:(see Table I) In these atopic subjects, sham exposure had no effect on the bronchial response to histamine aerosol. The mean increase in specific resistance (SRaw) on the initial visit was 5.50 ± 1.22 liter x cm H_2O/LPS (mean $\pm$ S.E.); after sham exposure the mean increase in SRaw was 6.20 ± 1.42 These changes do not differ ($P>0.50$). After exposure to ozone, however, the mean rise in SRaw caused by histamine challenge was 13.10 ± 3.08 liter x cm H_2O/LPS. This is significantly greater than both the initial response and the response after sham exposure ($P<0.05$). This increase in responsiveness could not be attributed to changes in baseline airway caliber, for the mean values for SRaw after sham exposure (3.94 ± 0.60) and after ozone exposure (4.34 ± 0.51 liter x cm H_2O/LPS) did not differ significantly. Five of the subjects spontaneously complained of substernal discomfort, pain on deep inspiration, or a desire to cough after exposure to ozone, but the occurrence of these symptoms did not correlate with the increase in bronchial reactivity to histamine.

The increased sensitivity to the bronchoconstrictor effects of histamine was blocked by pre-treatment with atropine sulfate aerosol in the subjects in whom it was tested with atropine pre-treatment. The mean increase in SRaw provoked by histamine after sham exposure was 1.06 ± 0.38; after ozone exposure it was 1.42 ± 0.75 liter x cm H_2O/LPS. These mean responses do not differ significantly ($P>0.05$, see Fig. 9).

The increase in bronchial reactivity subsided within 24 hours, for the mean rise in SRaw provoked by histamine challenge one day after ozone exposure was 6.61 ± 1.77 liter x cm H_2O/LPS. While greater than the control response, it is not significantly so ($P>0.05$). In four subjects the response to histamine was greater than the control response for one week or more.

If one defines as significant a greater than 50% increase in the change in SRaw provoked by histamine challenge, then a single two hours exposure to 0.5-0.6 ppm of ozone increased bronchial reactivity significantly in five of nine atopic subjects. A similar increase was caused in six of seven normal subjects.

Comment: This study demonstrates that atopic individuals, many of whom already have underlying bronchial hyper-reactivity, further increase their response to histamine inhalation after two hour exposure to 0.5-0.6 ppm of ozone. As in the normal subjects, the increase in reactivity appears to be mediated by post-ganglionic cholinergic pathways, for it is blocked by pre-treatment with atropine sulfate aerosol. That ozone exposure significantly increased bronchial reactivity in seven of twelve atopic subjects and in seven of eight normal subjects suggests that atopy does not increase the inducibility of bronchial hyper-reactivity by exposure to ozone. Such comparisons must be made with caution, however, for the doses of histamine given to the normal and the atopic subjects were not identical, and a different portion of the dose-response curve relating histamine and changes in airway caliber may have been examined in the two groups.

Discussion:

These studies demonstrate that two hours of exposure to 0.5-0.6 ppm of ozone significantly increases bronchial reactivity to histamine and methacholine in normal subjects, and increases bronchial reactivity to histamine in subjects with non-asthmatic atopic diseases, even if they have pre-existing bronchial hyper-reactivity.

This hyper-reactivity to inhalation of histamine after exposure to ozone appears to have been caused by constriction of bronchial smooth muscle rather than by edema or mucus formation because of its rapid appearance and its prompt reversal after inhalation of isoproterenol aerosol. Histamine stimulates contraction of smooth muscle in the airways by both direct (17) and indirect (18) pathways, either of which might be potentiated by exposure to ozone. For example, an increase in the permeability of the airway mucosa could directly increase bronchial reactivity by permitting a greater amount of histamine to penetrate to the muscle. However, in our study the increased airway response to histamine after ozone exposure appeared to be caused by an indirect mechanism. In all subjects in whom ozone exposure induced striking bronchial hyper-reactivity,

pre-treatment with atropine sulfate at the time of greatest reactivity inhibited the response to histamine. In fact, the response to histamine after atropine inhalation was not affected by ozone exposure. These findings indicate that ozone increases bronchial reactivity by a mechanism involving cholinergic, post-ganglionic pathways, as has been demonstrated in anesthetized dogs (9).

There are several possible mechanisms of ozone's effect on the cholinergic pathway, each operating at a different site. One possible mechanism is that a decrease in plasma acetylcholinesterase immediately after exposure to ozone (19) could exaggerate the response to any stimulus of cholinergic activity. This is an unlikely mechanism for the effect we found because several of our subjects had increased bronchial reactivity for more than one week after a single ozone exposure. We would not expect hyper-reactivity to persist so long if ozone exerted its effect by transient lowering of plasma acetylcholinesterase levels.

Another possible mechanism is that ozone potentiates the effect of efferent impulses normally travelling along the vagus nerve by releasing mediators such as serotonin or prostaglandins. Thus, in vagotomized dogs, doses of serotonin that do not increase baseline bronchomotor tone markedly augment the bronchoconstriction caused by electrical vagal stimulation (20).

It is possible that ozone increases bronchial reactivity by acting on the sensory portion of the cholinergic reflex arc. There are three types of pulmonary vagal sensory receptors: rapidly adapting, slowly adapting, and c-fiber endings. Any one of these sensory endings could be responsible for the increased bronchomotor response to histamine after ozone. Although our study is not definitive, we think that the rapidly adapting sensory receptors are likely to be involved in ozone-induced hyperirritability. These receptors line the airways and extend between epithelial cells in the mucosa (21). Their stimulation by gentle mechanical irritation of the airway surface (22) or by inhalation of dust (23), cigarette smoke (24), sulfur dioxide (25), or histamine aerosol (26) increases afferent vagal activity. This increased afferent vagal activity may cause reflex bronchoconstriction (27,23).

Studies of airway tissue have shown that ozone exposure causes desquamation and degeneration of bronchial epithelial cells (28,29). Therefore, ozone might increase bronchial reactivity by increasing the accessibility or the sensitivity of the afferent nerve endings responsible for initiating reflex bronchoconstriction to inhaled irritants.

Several observations from other studies support this proposed mechanism. Spontaneous viral upper respiratory infections (7) and experimental inoculation of airways with influenza virus (8) also increase bronchial reactivity. The pathologic changes in the airway after influenza infection are similar to those caused by ozone--desquamation and degeneration of the bronchial epithelium, although the biochemical changes induced by ozone, such as a decrease in plasma acetylcholinesterase, have not been shown to occur with viral infections. Another study (30) showed that exposure to ozone caused rapid, shallow breathing during exercise in otherwise healthy volunteers, an effect that occurs during stimulation of rapidly adapting airway receptors (31). Three other studies of ozone have reported coughing, chest discomfort on taking a deep breath, a sense of tracheal irritation, and, occasionally, dyspnea (32), symptoms that can all be ascribed to activity of rapidly adapting receptors.

This mechanism may also be the cause of the ozone effect noted in two other studies. Prior exposure to ozone increased the sensitivity of guinea pigs to histamine given by injection or by aerosol (32) and increased the proportion of guinea pigs that developed an anaphylactic sensitization to egg albumin delivered repeatedly into the respiratory tract (33). Both observations are consistent with the concept that ozone sensitizes irritant receptors, for histamine has been shown to stimulate discharge of rapidly adapting receptors (27,28), and both anaphylaxis and antigen-induced bronchoconstriction are affected by vagal pathways. (34). It has been proposed, in fact, that biochemical mediators of asthma and anaphylaxis act not just by their local effect, but also by stimulating afferent receptors in the airways, thereby triggering reflex bronchoconstriction (35).

Although the hypothesis that ozone increases bronchial reactivity by sensitizing rapidly adapting receptors in the airways is plausible, our finding that ozone increases the bronchial response to methacholine as well as to histamine suggests that other mechanisms may be operative. In their study of rapidly adapting receptors in dogs, Sampson and Vidruk found that histamine acts as a direct and potent stimulus of receptor activity, whereas acetylcholine acts only indirectly, stimulating receptor activity only in proportion to its effects on bronchial smooth muscle (36). If this is the case in humans, ozone's potentiation of receptor activity should be reflected by a greater change in sensitivity to histamine than to methacholine. Our findings did not show this to be the case.

Also against this proposed mechanism for ozone's effect is our finding that there was no correlation between the degree of symptoms of substernal discomfort - often suggested as being due to irritant receptor stimulation - and an increase in bronchial reactivity to histamine.

An increase in sensitivity to both methacholine and histmine would be predicted if bronchial smooth muscle were somehow made more sensitive to cholinergic stimulation, either because of a reduction in local acetylcholinesterase or because of an increase in the number or sensitivity of cholinergic receptors on the muscle. While this would account for the change in bronchial responsiveness to methacholine and to the reflex component of the response to histamine, it would not account for the changes in the pattern of breathing on exercise seen in dogs (37) and humans (30) after exposure to ozone. Those latter observations strongly suggest that the activity of some different receptor has been altered by ozone. The responsible receptors need not be the rapidly adapting or irritant receptors but instead may be the slowly adapting stretch receptors, which lie within bronchial smooth muscle, or the non-myelinated fiber endings recently described as being present in the bronchial mucosa (38) and which are similar to receptors serving a nociceptive function in other organs.

Additional studies in human subjects, in animals, and in isolated muscle preparation will be required to determine the mechanism whereby ozone induces bronchial hyper-reactivity. Whatever the mechanism, these three studies have demonstrated that brief exposure to realistic concentrations of a common atmospheric pollutant does increase the sensitivity of human airways to non-specific stimuli. The significance of this change is unknown. Bronchial hyper-reactivity is characteristic of asthma (39), and some workers have proposed that occupational asthma results from the induction of hyper-reactivity by the dusts or fumes

of the work place (40). By magnifying the response to materials delivered into the airways, an increase in bronchial reactivity would be expected to worsen the response to inhalation of dusts, cold air, or sulfur dioxide (1) and to worsen the response to the release of the contents of mast cells lining the airways. The common clinical observation that asthmatic symptoms are aggravated by viral upper respiratory infections, by exposure to occupational fumes, and by worsening air pollution may be explained by all of these events acting to increase bronchial reactivity.

SCHEMATIC DIAGRAM OF EXPOSURE CHAMBER

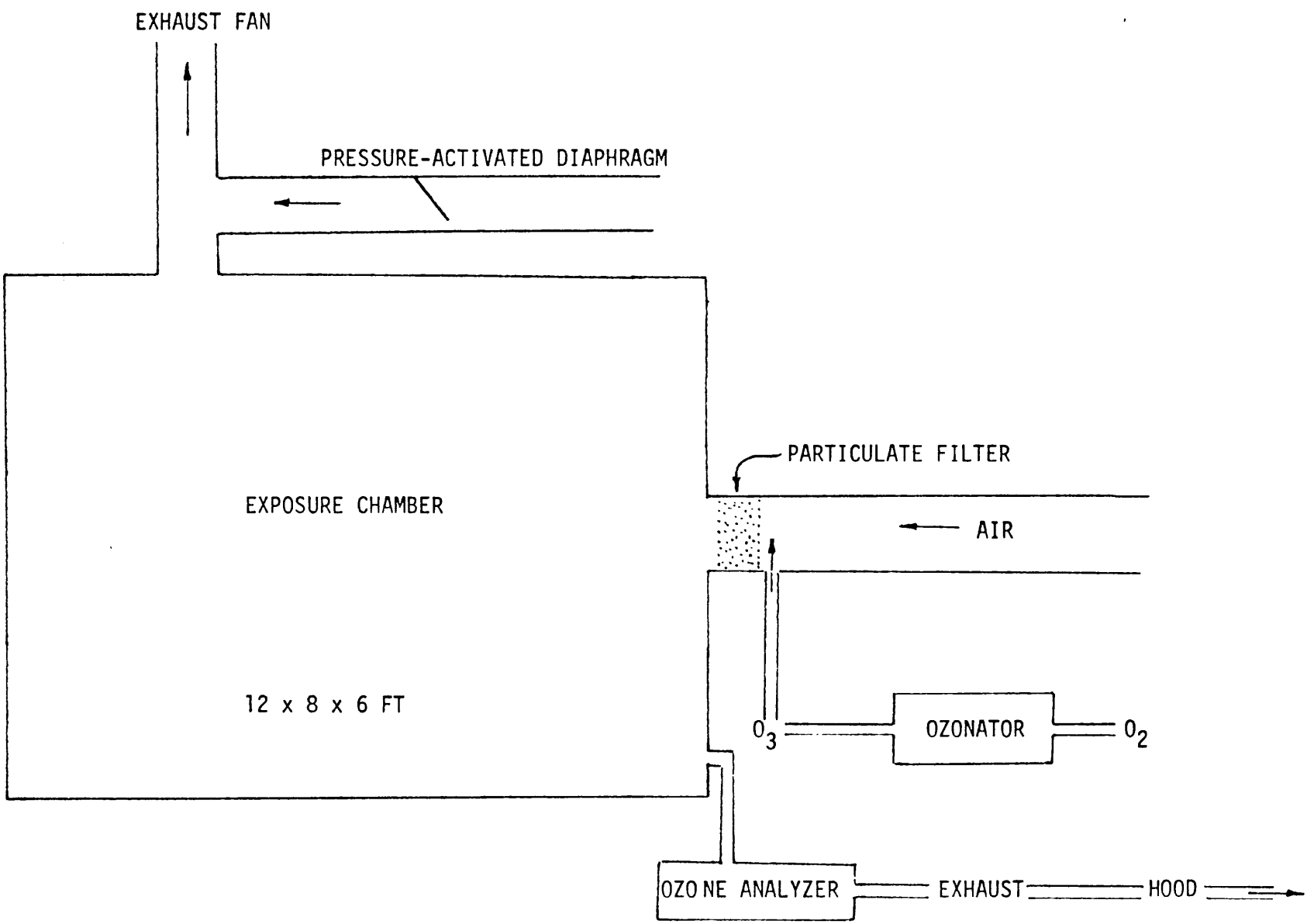

Figure 1: Schematic diagram of exposure chamber showing the duct which carries filtered air and ozone into the chamber, commercial ozonator, ozone analyzer, and exhaust fan above the ceiling and pressure-activated diaphragm which allows control of chamber turn-over time.

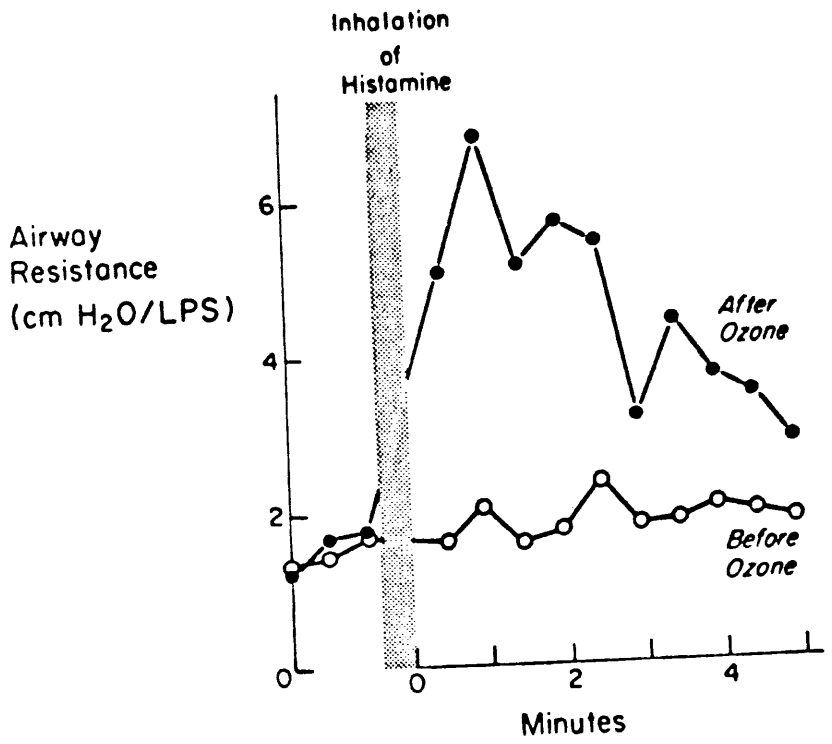

Figure 2: Increase in bronchial reactivity to histamine diphosphate in one subject immediately after exposure to ozone. Airway resistance was measured before and after the subject inhaled 10 breaths of 1.6% histamine (shaded bar) before (0) and after (0) exposure to ozone (0.6 ppm for 2 hours). Each point represents one measurement of airway resistance.

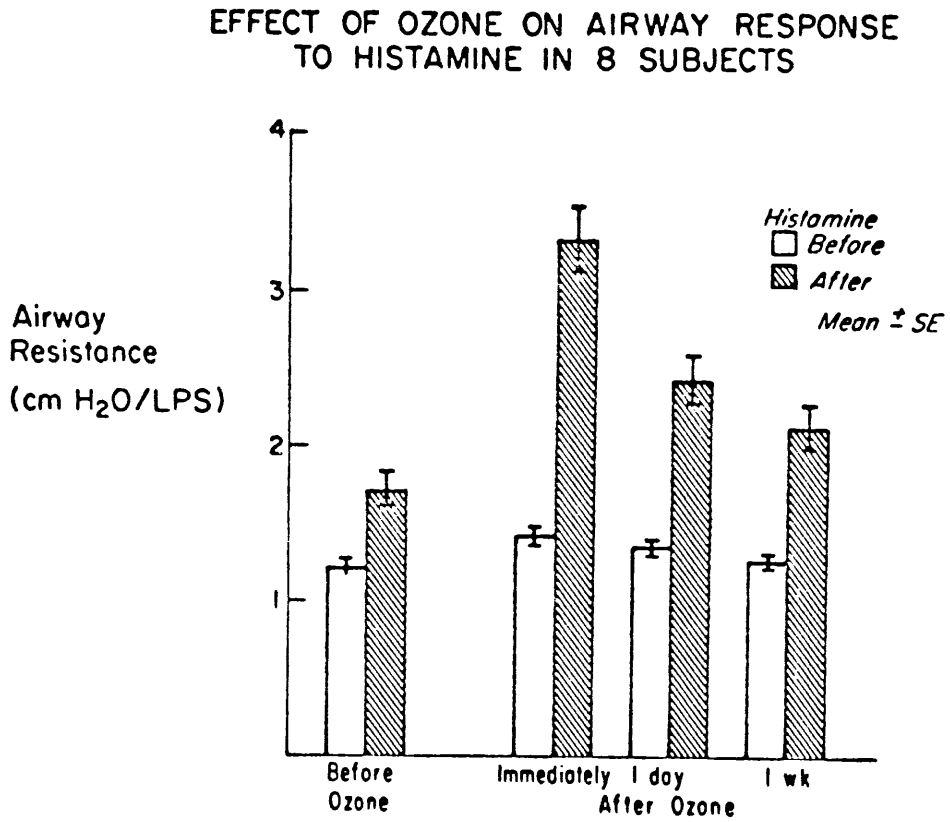

Figure 3: Effect of ozone on airway response to histamine in the group of 8 subjects.

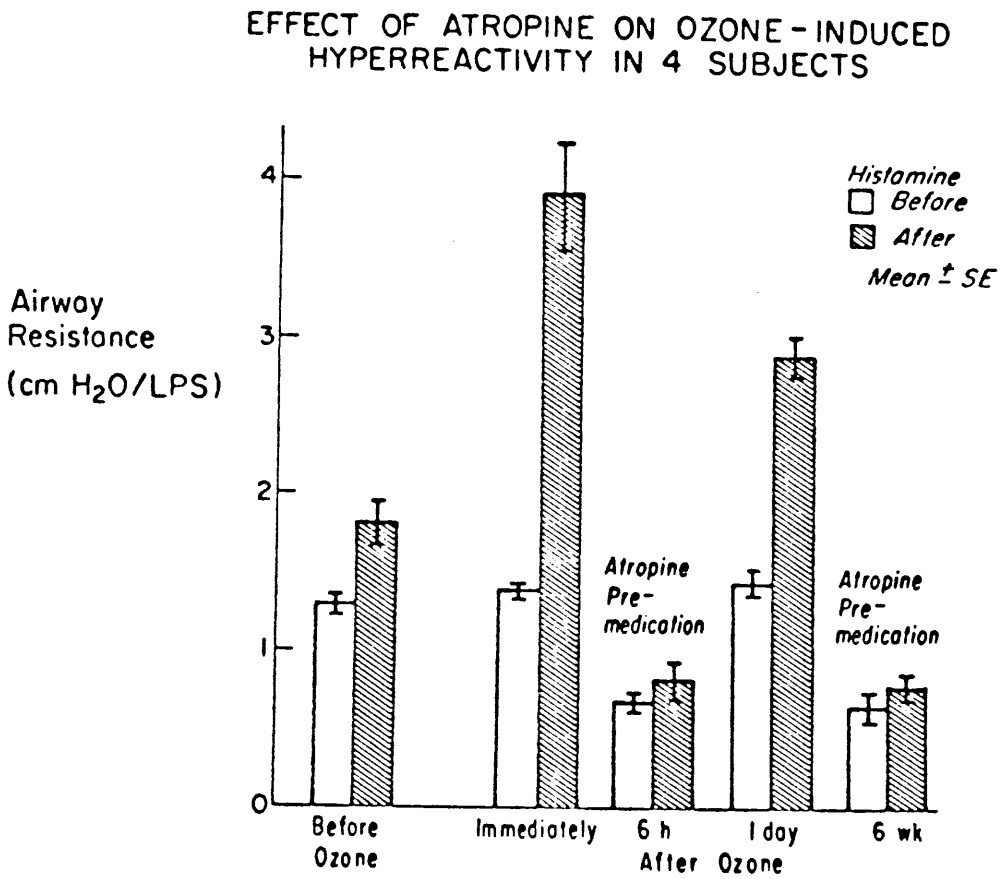

Figure 4: Effect of atropine sulfate aerosol on ozone-induced increase in bronchial reactivity to histamine. For the atropine pre-medication studies, atropine sulfate (0.1 = 0.2 mg/kg) was inhaled from a 10 mg/ml solution one-half hour before the histamine challenges.

EFFECT OF OZONE ON AIRWAY RESPONSE TO HISTAMINE

IN 7 NON-ATOPIC SUBJECTS

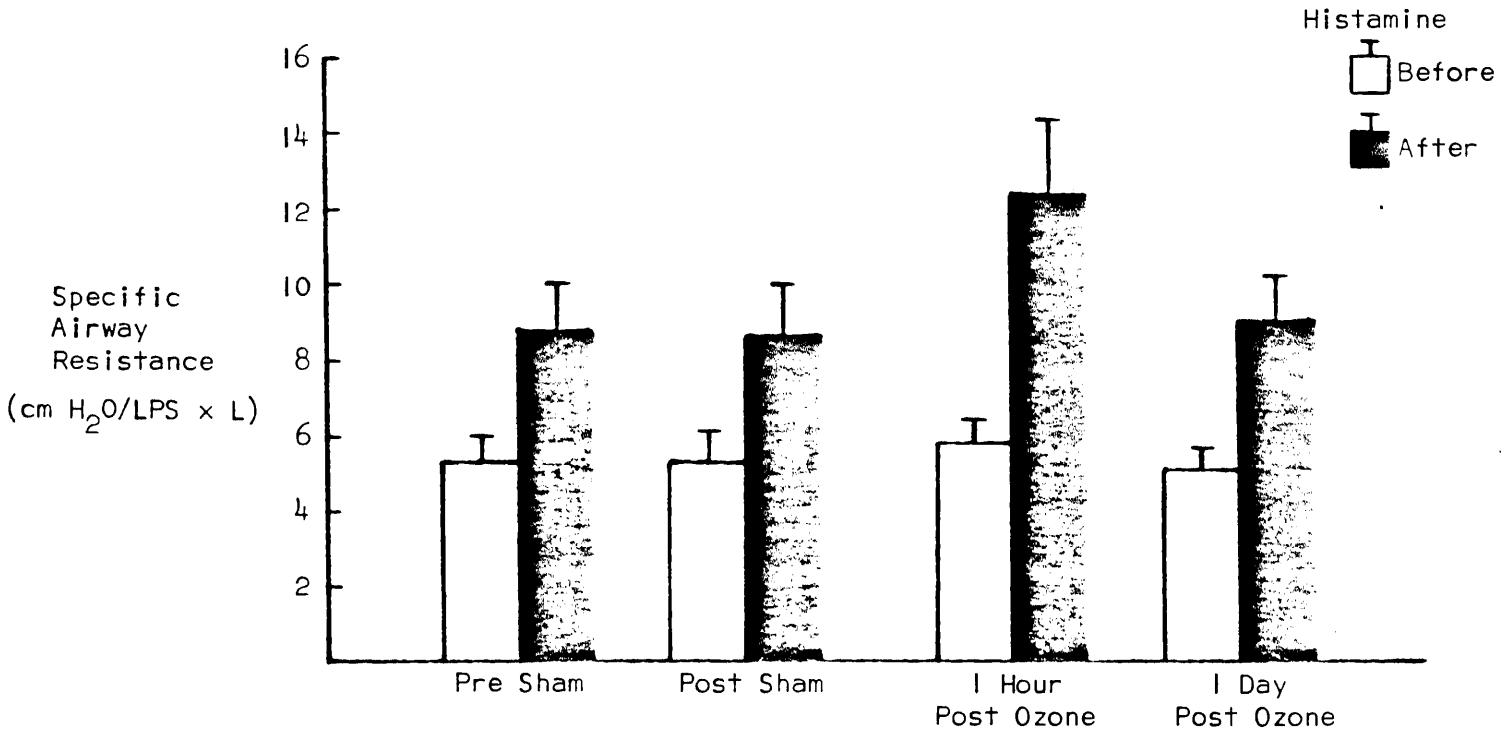

Figure 5. Effect of ozone (0.6 ppm; 2 hours) on the bronchomotor response to inhaled histamine aerosol (16 mg/ml; 10 breaths) in 7 non-atopic subjects. Open bars represent the averaged specific airway resistance measurements before inhalation of histamine aerosol. Darkened bars represent the averaged measurements after histamine. All data reported as mean ± S.E. Sham exposure had no effect, but ozone exposure transiently increased the response to inhaled histamine.

EFFECT OF ATROPINE ON OZONE-INDUCED HYPERREACTIVITY

IN 5 NON-ATOPIC SUBJECTS

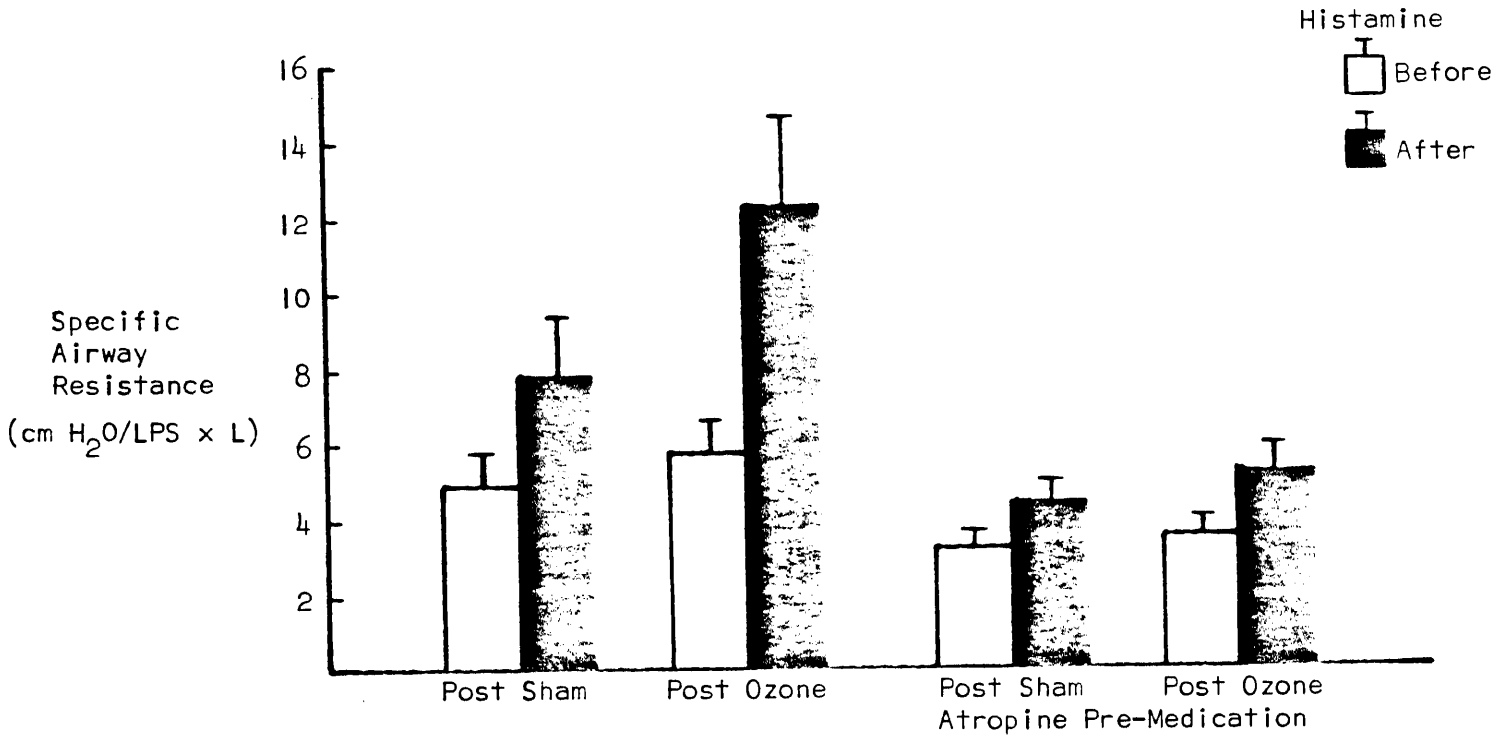

Figure 6. Effect of atropine sulfate aerosol on the ozone-induced increase in bronchial reactivity to histamine in 5 non-atopic subjects. For the atropine pre-medication studies, atropine sulfate (0.1 mg/kg) was inhaled one-half hour before the histamine challenges. All data reported as mean ± S.E. Atropine prevented the increased response to histamine after ozone.

EFFECT OF OZONE ON AIRWAY RESPONSE TO METHACHOLINE

IN 7 NON-ATOPIC SUBJECTS

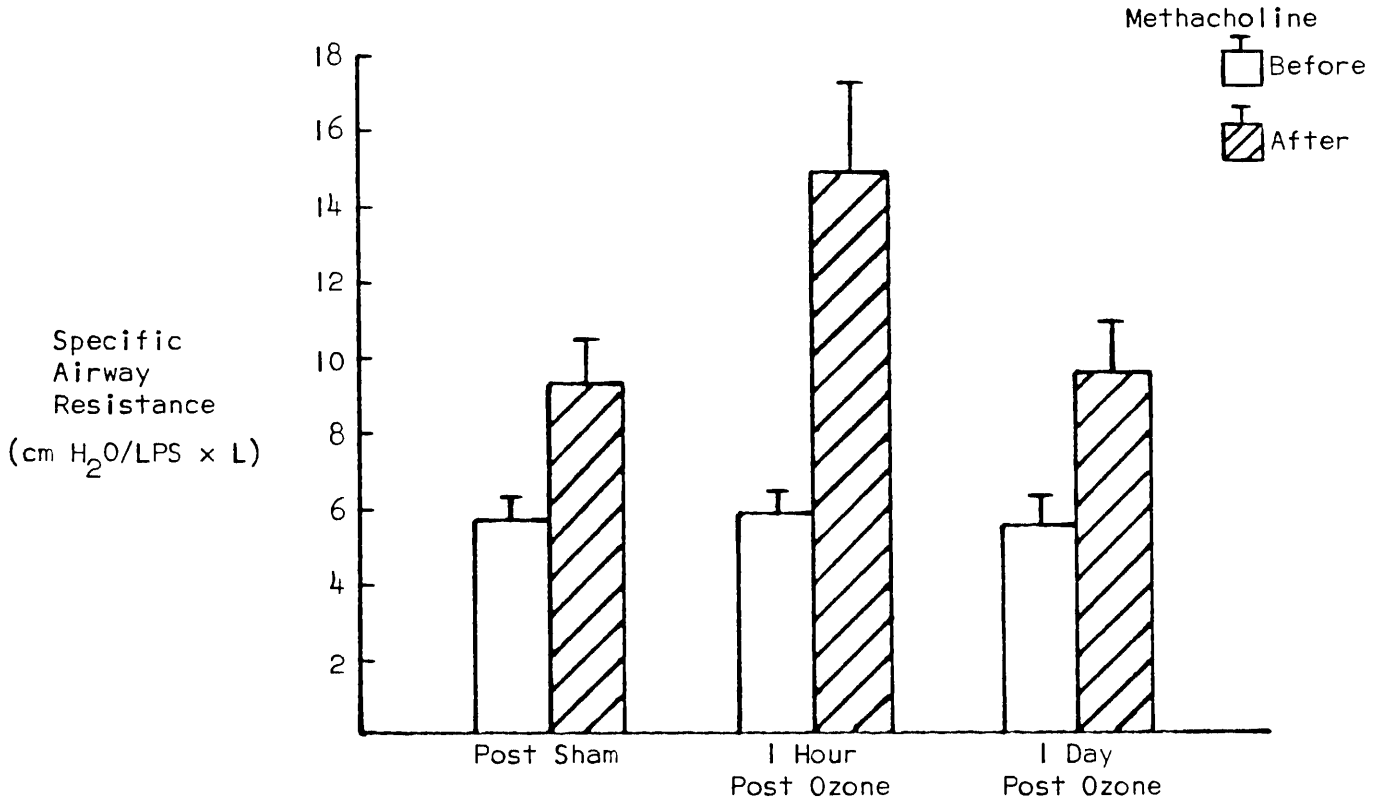

Figure 7. Effect of ozone (0.6 ppm; 2 hours) on the bronchomotor response to inhaled methacholine aerosol (10 mg/ml; 10 breaths) in 7 non-atopic subjects. Open bars represent the averaged specific airway resistance measurements before inhalation of methacholine aerosol. Cross-hatched bars represent the averaged measurements after methacholine. All data reported as mean ± S.E. Ozone exposure transiently increased the response to inhaled methacholine.

EFFECT OF REPEATED EXPOSURES TO OZONE ON BRONCHIAL REACTIVITY IN ONE SUBJECT

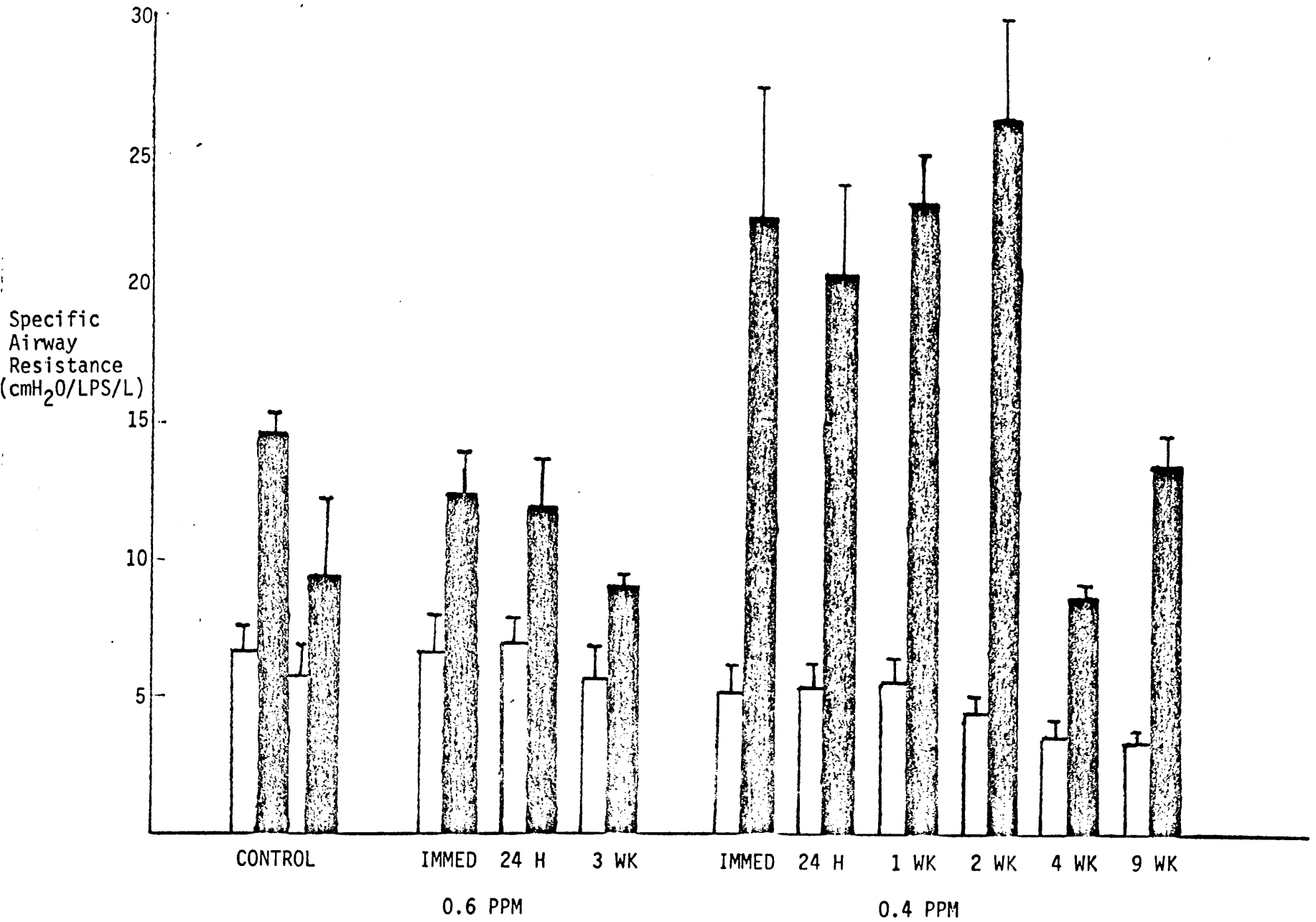

Figure 8: Effect of two ozone exposures (0.6 and 0.4 ppm) on the bronchomotor response to inhaled histamine aerosol (1.6% solution, 10 breaths) in one subject. All data reported as mean ± SD. No tolerance to ozone's effect was seen with the second exposure given 3 weeks after the first. Open bars represent baseline specific resistance (SRaw) and darkened bars represent the SRaw after histamine inhalation.

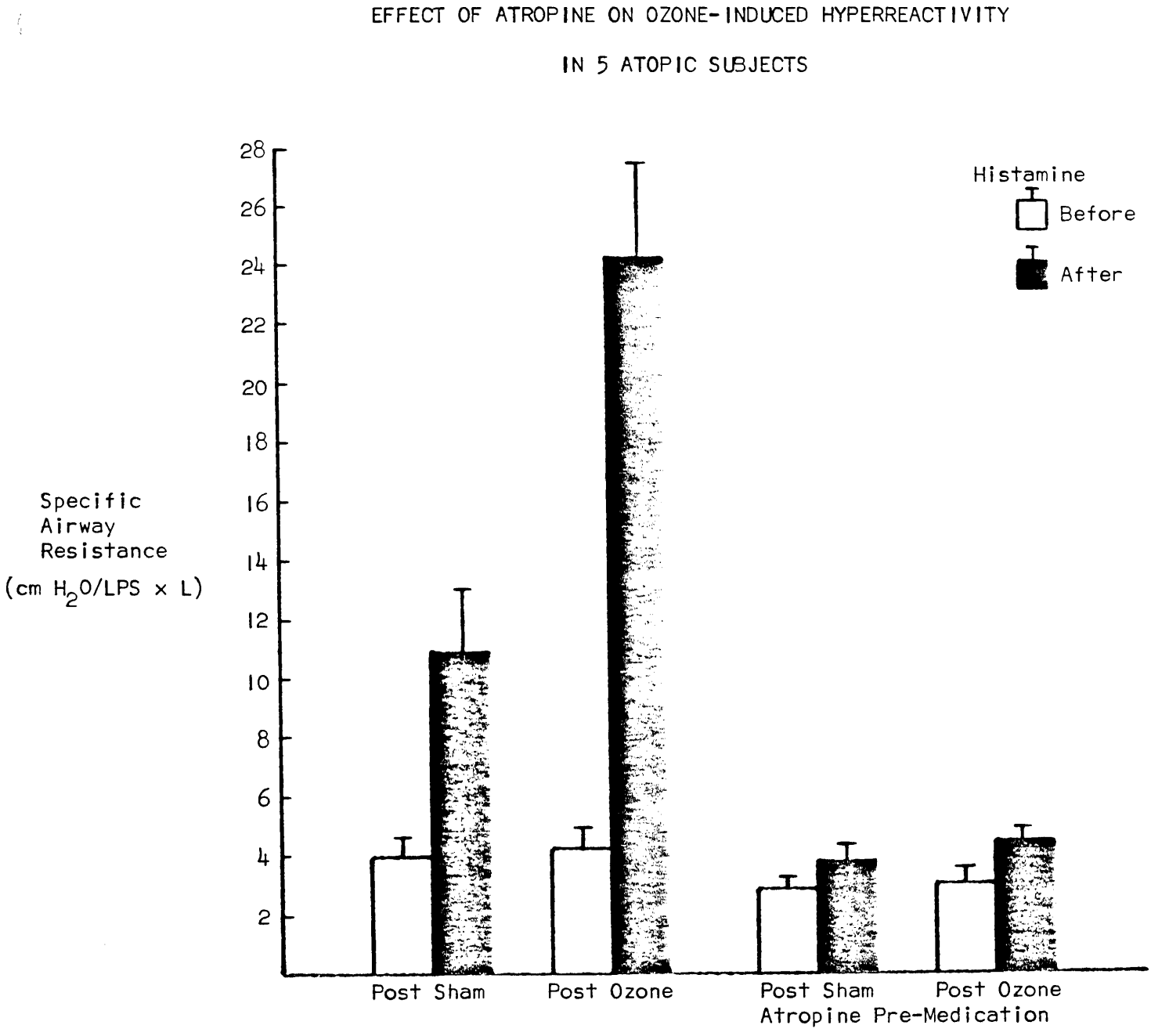

Figure 9. Effect of atropine sulfate aerosol on ozone-induced increase in bronchial reactivity to histamine in 5 atopic subjects. For the atropine pre-medication studies, atropine sulfate (0.1 mg/kg) was inhaled one-half hour before the histamine challenges. All data reported as mean ± S.E. Atropine prevented the increased response to histamine after ozone.

TABLE I

EFFECT OF OZONE ON BRONCHIAL RESPONSIVENESS
IN NON-ATOPIC AND ATOPIC SUBJECTS

Project #2: Non-atopic Subjects

Agent/No. Subjects	Pre-Sham Baseline	Pre-Sham Response	Post-Sham Baseline	Post-Sham Response	1 Hr Post O_3 Baseline	1 Hr Post O_3 Response	24 Hr Post O_3 Baseline	24 Hr Post O_3 Response
Histamine	5.37*	8.79	5.27	8.69	5.84	12.41	5.18	9.09
N=27	±0.65	±1.31	±0.81	±1.44	±0.61	±2.00	±0.60	±1.27
Atropine/Histamine			3.22	4.31	3.58	5.16		
N=5			±0.38	±0.65	±0.53	±0.63		
Methacholine			5.58	9.20	5.89	14.83	5.48	9.55
N=7			±0.75	±1.29	±0.60	±2.45	±0.96	±1.37

Project #3: Atopic Subjects

Agent/No. Subjects	Pre-Sham Baseline	Pre-Sham Response	Post-Sham Baseline	Post-Sham Response	1 Hr Post O_3 Baseline	1 Hr Post O_3 Response	24 Hr Post O_3 Baseline	24 Hr Post O_3 Response
Histamine	3.76	9.26	3.94	10.13	4.34	17.44	4.14	10.75
N=9	±0.60	±1.24	±0.60	±1.57	±0.51	±3.25	±0.47	±1.53
Atropine/Histamine			2.81	3.87	3.08	4.49		
N=5			±0.39	±0.45	±0.64	±0.47		

*All data in cm H_2O/LPS x L; mean ± S.E.

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XI. List of Inventions Reported and Publications:

1. Golden, J.A., Nadel, J.A. and Boushey, H.A.: Bronchial hyperirritability in healthy subjects after exposure to ozone. Am. Rev. Resp. Dis. (in press) 1978.

2. Golden, J.A., Boushey, H.A. and Nadel, J.A.: Bronchial hyperirritability after ozone in healthy subjects. The Physiologist 20:35, 1977 (abstract).

XII. Glossary of unfamiliar Terms, Abbreviations, and Symbols:

ppm = parts per million

R_{aw} = airway resistance

TGV = thoracic gas volume

SR_{aw} = specific airway resistance (R_{aw} x TGV)

cm H_2O/LPS = Units of airway resistance, expressed as driving pressure (in centimeters of water) per unit flow (in liters per second)