"PHYSIOLOGICAL EFFECTS OF AIR POLLUTANTS IN HUMANS SUBJECTED TO SECONDARY STRESS"

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FINAL REPORT

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ABSTRACT

Adult male volunteers were exposed to purified air or to ozone, alone or in combination with nitrogen dioxide and carbon monoxide, in an investigation of physiological effects of photochemical air pollution. Exposure conditions simulated those of a smoggy Southern California summer, including the secondary stresses of heat, exercise and repeated exposure. Pulmonary function, blood biochemistry, psychomotor performance capability, and symptoms experienced by the subjects were evaluated. Ozone exposures similar to those expected during pollution episodes produced significant decrement in pulmonary function, symptoms sufficient to restrict normal activity, and oxidative changes in erythrocytes. Psychomotor tracking ability and measures of attention were adversely affected by heat, but not by ozone exposure. Subjects with a history of cough, chest discomfort, or wheezing associated with allergy or exposure to air pollution, were more reactive than subjects without such a history. Addition of nitrogen dioxide and carbon monoxide to ozone in exposures did not produce additional detectable effects except for slight increases in carboxyhemoglobin levels and small variable decrements in psychomotor performance with carbon monoxide exposure. It is concluded that in sensitive subjects, exposures to photochemical oxidants at concentrations sometimes achieved in California urban areas may produce physiological dysfunction and inability to carry on normal activities.

This report was submitted in fulfillment of Contract No. ARB 2-372 by the Environmental Health Service/SCOR in Environmental Lung Disease, Rancho Los Amigos Hospital, under the sponsorship of the California Air Resources Board. Work was completed 30 June 1974.

CONCLUSIONS

The results show that exposures to ozone at 0.37 or 0.50 ppm for two hours or more with intermittent light exercise can have significant deleterious effects on health. Some subjects thus exposed not only developed measurable physiological and biochemical changes, but felt physically ill and were unable to perform their normal jobs during exposure and for several hours afterward. The most sensitive subjects tested experienced respiratory symptoms after a single two-hour exposure to 0.37 ppm ozone and developed measurable physiological changes after a second similar exposure the following day. The least sensitive subjects tested developed no respiratory symptoms or physiological changes even after five-hour exposures to 0.5 ppm ozone on two successive days; however, biochemical changes were observed even in these subjects. The more reactive subjects were generally those with history of asthma, allergy, or previous subjective adverse reactions to smog exposure. No additional effects of exposure were detected when 0.30 ppm nitrogen dioxide was added to ozone. Addition of 30 ppm carbon monoxide to the ozone-nitrogen dioxide mixtures produced no additional effects other than slight increases in blood carboxyhemoglobin levels and small decrements in psychomotor performance, which were not consistent in different subject groups.

Tentative inferences concerning threshold levels for ozone exposure may be drawn from the finding that the most sensitive of these subjects did not show significant changes when exposed to 0.25 ppm ozone for two hours. First approximation mean dose-response curves, generated by analysis of observed changes in stable physiological parameters plotted

as a function of ozone concentration, suggest a "zero-effect" level of 0.25 to 0.3 ppm. It must be emphasized, however, that these findings relate to relatively healthy, young to middle-aged adult men performing light exercise. Other groups such as children, older adults, pulmonary disease patients, or workers performing heavy exercise may be at risk at even lower ozone levels. In addition, the findings relate to ozone exposure against a background of highly purified air. Actual ambient exposures involve additional photochemical oxidants and other gaseous and particulate pollutants which may have additive or synergistic effects."

RECOMMENDATIONS

Public Health

The findings affirm the desirability of continued vigorous effort toward oxidant pollution abatement. They indicate that the present state episode criteria levels (stage 2, warning at 0.4 ppm oxidant and stage 3, emergency at 0.6 ppm) are not unreasonably stringent and should be maintained if public health is to be protected.

Future Research

The possible interaction of ozone with other pollutants such as sulfur dioxide, peroxyacyl nitrates, and particulates, in a health effects sense, requires further investigation preferably in controlled-exposure studies on healthy volunteers. The question whether pulmonary disease patients, the young, and the elderly are at increased risk from oxidant exposure must be answered. Accordingly, controlled-exposure studies on otherwise healthy volunteers with mild-to-moderate chronic pulmonary disease (asthma) should be undertaken. Such studies on children or patients with severe chronic pulmonary disease are needed; however, ethical considerations will impose definite limitations on deliberate exposure studies and the epidemiologic approach should also be used.

GLOSSARY OF TERMS AND ABBREVIATIONS USED IN THIS REPORT

AcChase	-	acetylcholinesterase
Cdyn	-	dynamic lung compliance
Cstat	-	static lung compliance
CC	-	closing capacity = CV + RV
CO	-	carbon monoxide
COHb	-	carboxyhemoglobin
CV	-	closing volume
dF	-	degrees of freedom for analysis of variance
DL_{co}	-	single breath CO diffusing capacity of the lungs
ΔN_2	-	increase in nitrogen concentration per liter expired
F	-	statistic for analysis of variance
FEV_1	-	forced expiratory volume, one second
FVC	-	forced vital capacity
GSH	-	reduced glutathione
GSSRase	-	glutathione reductase
G-6-PDH	-	glucose-6-phosphate dehydrogenase
H_2O_2	-	hydrogen peroxide
HR	-	heart rate
HRV	-	heart rate variability
l	-	liters
l/cm H_2O	-	lung compliance, liters per cm. of water pressure
LDH	-	lactate dehydrogenase
n/a	-	not available due to inadequate quality of data obtained
NADH	-	the cofactor nicotinamide adenine dinucleotide (reduced)

GLOSSARY (continued)

NADPH	-	nicotinamide adenine dinucleotide phosphate (reduced)
NS	-	no significant difference at .05 probability level
NO_2	-	nitrogen dioxide
NO_x	-	oxides of nitrogen
O	-	(exposure) - 0.50 ppm O_3
ON	-	(exposure) - 0.50 ppm O_3 + 0.30 ppm NO_2
ONC	-	(exposure) - 0.50 ppm O_3 + 0.30 ppm NO_2 + 30 ppm CO
O_3	-	ozone
P	-	probability of control-exposure difference being due to chance
ppm	-	parts per million, by volume
Raw	-	airway resistance, pressure-plethysmograph method
RV	-	residual volume
RBC	-	erythrocyte
S.D.	-	standard deviation
S.E.	-	standard error
-SH	-	reduced sulfydryl group
TGV	-	thoracic gas volume, pressure-plethysmograph method
$\dot{V}_{50}$	-	flow rate at 50% FVC, maximum expiratory flow-volume curve
$\dot{V}_{25}$	-	flow rate at 25% FVC, maximum expiratory flow-volume curve
$\dot{V}_{50}P$	-	flow rate at 50% FVC, partial expiratory flow-volume curve
$\dot{V}_{25}P$	-	flow rate at 25% FVC, partial expiratory flow-volume curve
$\dot{V}\ O_2$	-	oxygen consumption per minute

CHAPTER I

EXPERIMENTAL STUDIES ON HUMAN HEALTH EFFECTS OF AIR POLLUTANTS: I -- DESIGN CONSIDERATIONS

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EXPERIMENTAL STUDIES ON HUMAN HEALTH EFFECTS OF AIR POLLUTANTS:

I. DESIGN CONSIDERATIONS

SUMMARY

Because of the possible threat to public health posed by photochemical air pollution, a need exists for experimental studies of short-term respiratory effects of air pollutant exposure in humans. Such studies require rigorous control of the experimental air environment and exposure conditions to ensure that results are both reliable and relevant to public-health questions. In addition to biochemical and behavioral measures, a comprehensive battery of pulmonary tests is required to assure that effects at different levels of the respiratory tract are detected. We have developed a core protocol based on the foregoing principles. Findings from a series of studies using this protocol indicate that a wide range of sensitivity to photochemical pollutants exists and that more sensitive individuals develop significant symptoms, biochemical changes and respiratory function decrement under exposure conditions similar to those experienced during ambient pollution episodes.

EXPERIMENTAL STUDIES ON HUMAN HEALTH EFFECTS OF AIR POLLUTANTS:

I. DESIGN CONSIDERATIONS

Photochemical smog is a complex mixture of substances, including powerful oxidizing agents such as ozone (O_3), nitrogen dioxide (NO_2), and organic peroxides. It is formed by the action of atmospheric oxygen and sunlight on effluent gases, particularly hydrocarbons and nitric oxide (NO), emitted as a result of automotive and industrial fuel combustion.[1] The respiratory and other health effects of photochemical smog exposure on humans have not been well documented. The Los Angeles area is most often associated with such exposures, but they are by no means limited to this region. Significant photochemical oxidant concentrations have been reported in Canada[2] and in Europe,[3] and can probably occur in most areas with concentrations of automobile traffic or fuel-burning industry when sunlight is present and winds are too light to disperse effluent gases. Thus, photochemical smog and other air pollutants present a widespread potential public health problem. The appropriate government agencies have responded by setting air quality standards intended to protect the population

from dangerous levels of exposure. Most existing standards, however, are based on limited scientific information on the health effects of pollutants.[4] The standards are also frequently challenged legally because the economic and social costs of conforming may be high. It is thus apparent that there is a need for comprehensive experimental studies on the health effects of oxidant and other air pollutant exposure. Such studies must be controlled and documented as rigorously as possible to ensure reproducibility and to withstand legal challenges. Although numerous animal studies exist and others are in progress, they presently cannot be quantitatively related to human health. Epidemiologic studies can be useful but are limited by cost, dose-range available, presence of interfering pollutant substances and problems caused by many uncontrolled variables.

Human experimental studies of the health effects of oxidant air pollutants can be accomplished at fixed concentrations in the absence of interfering pollutants, under well-controlled environmental conditions and with a well characterized subject group of limited size. Studies of the health effects of well-specified ambient air

are also possible. A survey of the environmental control and monitoring technology used in previous experimental studies[4,5,6,7,8] indicates that significant limitations existed. Further studies are thus indicated, under highly controlled conditions, to repeat and extend previous work.

Bates and his co-workers, in a series of publications, have discussed some of the problems encountered in the design and execution of experimental studies on humans exposed to air pollutants, and have described an appropriate experimental protocol.[5,6,7,8] Despite some limitations in environmental assessment and control, they have documented impairment in pulmonary function in subjects exposed to ozone concentrations equal to or less than those encountered in severe photochemical smog episodes. This paper is intended to document the operational approach for a new series of studies, drawing heavily upon the experience of Bates and others, and using current technology to provide more rigorous experimental control and more comprehensive information than were previously obtainable on the effects of human exposure to pollutants, singly and in combination. A unique

environmental control facility combined with an interdisciplinary research team provide the capability to conduct this research.

The basic design of pollutant exposure studies should seek to maximize information relevant to public health. The tests for effects of smog must be reliable and sensitive, the experimental air environment must be rigorously controlled and; equally important, the manner in which subjects are exposed to the experimental environment must realistically simulate actual air pollution exposures if results are to be relevant. These constraints impose experimental design complications and necessitate focusing attention on several distinct problems: environmental control, pollutant generation, environmental monitoring, physiological testing, and evaluation of symptoms and clinical observations. The following sections describe approaches to each of these problems.

FACILITIES

Environmental Chamber

Studies are performed in the Rancho Los Amigos Clinical Environmental Stress Testing Laboratory. This facility consists of a

stainless steel-sheathed controlled environment chamber, approximately 28 square meters in area, accessible through a five square meter double-door lock compartment which contains lavatory facilities and through which air is exhausted. The main chamber contains physiological test equipment and can hold four or five subjects at the same time. Data recording and monitoring equipment are located outside the chamber in an adjacent laboratory area. (Figure 1) Air flows in an approximately laminar manner through the main chamber from ceiling to floor at a rate that provides a complete change of air every five minutes, and is then exhausted without recirculation. The air is highly purified and can be adjusted to simulate a wide range of meteorological conditions (Table 1). The air purification unit (Mine Safety Appliances, Inc., Evans City, Pennsylvania) contains high-efficiency particulate filters, a catalytic oxidation unit for conversion of carbon monoxide and hydrocarbons to carbon dioxide, and chemical filters containing activated charcoal (Barnebey-Cheney, Inc., Columbus, Ohio) and aluminum oxide pellets impregnated with potassium permanganate (Purafil, Inc., Chamblee, Georgia). The air conditioning unit consists of refrigerant

coils for cooling and dehumidification, followed by intermittently operating heaters and steam injectors controlled automatically to maintain desired levels of temperature and humidity.

Pollutant Generation

Each pollutant gas in introduced through its own stainless steel inlet line into the purified air in the chamber inlet duct. Complete mixing occurs before the air reaches the main chamber producing uniform concentrations throughout the chamber (within five percent of the mean value). Carbon monoxide, nitric oxide, nitrogen dioxide and ozone have been studied. CO may be introduced directly from a cylinder of pure gas through a flowmeter system with a solenoid-actuated shutoff valve, which operates automatically in case of power failure. NO is similarly introduced from a cylinder containing 10 percent of the gas in nitrogen. The diluted mixture minimizes air oxidation of NO entering the chamber duct. NO_2 is introduced by bubbling nitrogen gas through a cylinder of liquid N_2O_4 and metering the resulting N_2-NO_2 mixture through a specially designed flow control apparatus. Ozone is generated using an ozonator (Welsbach T-408) which ionizes oxygen in purified air flowing

between two charged plates. No contaminating nitrogen oxides are produced using this technique. All pollutants can be generated in concentration ranges realistically simulating ambient conditions and concentrations can be controlled to within 10 percent of the expected value.

Environmental Monitoring

The chamber air environment is monitored continuously utilizing instruments and techniques equivalent to those used in ambient air monitoring networks. Instruments are calibrated as recommended by the California Department of Public Health and the California Air Resources Board[4] and cross comparisons are made with analytical laboratories of the latter agency. Two monitoring instruments, each operating on a different principle, are used for each gaseous pollutant under study. Ozone and nitrogen oxides are monitored using chemiluminescent analyzers (Models 612 and 642, REM Scientific, Santa Monica, California), which provide fast response and freedom from interference by other pollutants. Total oxidants (i.e., ozone) and

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[4] Recommended Methods, Air and Industrial Hygiene Laboratory, California Department of Public Health, Berkeley, 1968.

nitrogen oxides are also monitored by the neutral potassium iodide solution and Saltzman reagent methods, respectively, using continuous-flow colorimetric analyzers (Model K-76, Beckman Instruments, Fullerton, California). Carbon monoxide is monitored by a nondispersive infrared analyzer (Mine Safety Appliances) and by an oxidative electrochemical analyzer (Model 2100, Energetics Science, Inc., Elmsford, New York). A light-scattering, single particle counter (Royco Instruments, Model 225, Menlo Park, California) monitors particulates in five subranges between 0.5 and 10 microns in diameter.

METHODS

Physiological Testing

An initial target of any air pollutant challenge is the respiratory tract, which is thus the center of attention in tests of effects of exposure. Other areas of interest include hematology, blood enzyme biochemistry and psychomotor performance. Insult by pollutants can be manifested at various sites in the respiratory system. Broncho-constriction in the large airways, maldistribution of ventilation due to hypersecretion in small airways, constriction of alveolar units,

and diffusion impairment due to edema are possible effects. A variety of pulmonary tests is required to examine the various possibilities. The tests employed in this study are described below.

Flow-volume curves are recorded using a low-resistance spirometer (Electro-Med 780). Partial and maximum forced expiratory maneuvers are performed. Partial forced expirations are initiated at 65 percent of vital capacity. These tests may be affected more by mild bronchoconstriction than are full-vital capacity forced expirations.[9] The parameters measured are forced vital capacity (FVC), one-second forced expiratory volume (FEV_1), peak expiratory flow rate ($\dot{V}max$), and flow rates at 50 percent and 25 percent FVC ($\dot{V}_{50}$, $\dot{V}_{25}$) for partial and maximum flow-volume curves. These measurements give an easily obtained, relatively reproducible evaluation of overall pulmonary mechanical performance, but provide little information on the mechanisms responsible for any observed changes.

Airway resistance (R_{aw}) and thoracic gas volume (TGV) are determined in a whole-body plethysmograph using the method of DuBois et al.[10,11] The measurement of R_{aw} is more sensitive to bronchoconstriction than

maximum-flow measurements, but it is also more difficult to perform and less stable. These problems similarly affect the measurement of TGV which, however, may be useful for detecting gas trapped as a consequence of airways dysfunction (in combination with a gas-dilution lung-volume determination).

Total respiratory resistance (R_t) is determined by the forced oscillation technique.[12] The method of Goldman[13] is used to eliminate the need to achieve or simulate resonance. To eliminate the phase shift introduced by the Fleisch pneumotachograph at higher frequencies, a new phase-compensation technique[14] is used to ensure correct relationships of the flow and pressure signals. Resistance is measured at pressure perturbation frequencies of 3, 6, 9 and 12 hertz. This measurement is affected by changes in upper-airway configuration, which may complicate detecting changes in pulmonary airways per se. The method is believed to be capable of detecting asynchronous mechanical behavior (unequal regional ventilatory time constants) as predicted by Otis[15] which otherwise can be documented only by the considerably more difficult measurement of dynamic lung compliance.

Closing volume (CV) is determined by the single-breath nitrogen washout method[16] using a linear nitrogen analyzer (Med-Science 505). This test is believed to be sensitive to changes in small airways in dependent lung regions. It determines the lung volume at which closure of a significant number of small airways presumably occurs and also provides an estimate of residual volume (RV) and total lung capacity (TLC) through the expired nitrogen concentration[17] and an estimate of the uniformity of ventilation distribution through the slope of the alveolar plateau.[18]

Static and Dynamic Lung Compliance (C_{st}, C_{dyn}) are measured from recordings of transpulmonary pressure and respiratory flow and volume. Transpulmonary pressure is measured by the esophageal balloon method of Milic-Emili, et al.[19] Flow at the mouth is measured by a pneumotachograph (Fleisch) and volume by a spirometer (Electro-Med 780). Adequate dynamic response of the system has been verified at frequencies up to 100 breaths/minute. Dynamic compliance in the tidal range is measured in a series of at least 10 breaths each at normal frequency and at 20, 40, 60, 80, and 100 breaths/minute with tidal volume

monitored and kept constant at 0.75 liter. Static compliance is measured by closing a mouth shutter intermittently during an inspiration from functional residual capacity (FRC) to TLC, followed by an expiration to RV. Static compliance determinations are made in triplicate and each is preceded by an inspiration to TLC to give a consistent volume history.

Compliance measurements are indispensable for documentation of changes in the mechanical characteristics of the lung, particularly the development of unequal time constants. Unfortunately, the measurements are somewhat unstable and require considerable effort on the part of subjects and investigators. In this study these tests are performed only on a subgroup of subjects selected for motivation and performance.

Pulmonary Diffusing Capacity ($D_{L_{Co}}$) is determined by the single-breath carbon monoxide method.[20] A test gas containing 0.15 percent CO and 10 percent helium in air is used. In the calculation of $D_{L_{Co}}$ correction was made for back pressure of CO due to significant levels of blood carbon monoxide hemoglobin. Helium is analyzed using a

thermal conductivity meter (W. E. Collins, Inc., Braintree, Massachusetts) and CO using an electrochemical analyzer (Energetics Science, Inc., Model 2700). Reproducibility of this test is poor under conditions of this study (heat and intermittent exercise), but the test offers the potential to detect changes in the blood-air interface (such as alveolar edema) which might otherwise go undetected.

Oxygen consumption is measured at rest and during exercise on a constant-load bicycle ergometer (Model 844, Quinton Instruments, Seattle, Washington) at a level yielding 65 percent of predicted maximum oxygen consumption.[21] Expired air is collected in meteorological balloons and emptied into a spirometer (Collins 120-liter) to determine total expired volume. Gas samples are analyzed for oxygen using a paramagnetic analyzer (Beckman E-2) and for carbon dioxide using a gas chromatograph (Beckman GC-M). Oxygen consumptions are calculated after the method of Consolazio, et al.[22] A telemetry system (Spacelabs, Inc., Chatsworth, California) records an exercise electrocardiogram during this test.

Carboxyhemoglobin concentration (COHb) is estimated using the method of Gaensler and co-workers.[23] The subject holds a breath for 20 seconds to allow equilibration of CO between alveolar air and blood, then expires a sample of alveolar air into a container. CO is measured with an electrochemical analyzer (Model 2100, Energetics Science). The air CO concentration may be directly related to carboxyhemoglobin concentration. The test is performed prior to exposure in the chamber to verify that the subject has not received an inordinate ambient pollutant exposure and performed again at the conclusion of the chamber exposure period.

Symptomatology. Each subject is interviewed by the project physician immediately after exposure concerning symptoms, through the use of a standard questionnaire. Subjects also keep a standard record of symptoms during and after exposure. Caution is exercised in interpreting symptoms since these are not blind studies (see Experimental Protocol).

Clinical. An attending physician is present in the chamber area during the study and is able to view its progress by means of closed-

circuit television. In addition, heart rate and EKG are monitored via telemetry. Within the immediate chamber area are located a DC defibrillator, endotracheal tubes, bag resuscitator, oxygen, and drugs which would be necessary for treatment of an acute cardiovascular or respiratory emergency.

Development of significant chest pain during exercise, significant cardiac irritability, intractible wheezing, or questionable EKG changes in any subject, constitutes an indication for termination of the study for that subject and a thorough physical examination, with whatever subsequent treatment is deemed appropriate by the attending physician. Such an examination and treatment may be carried out either in the examining room immediately adjacent to the chamber or on the pulmonary unit of the hospital located 200 feet from the chamber.

In the event that untoward symptoms or intercurrent illness occurs, the ultimate decision as to whether or not a subject is to continue on in a study is made by the attending physician.

EXPERIMENTAL PROTOCOL

The exposure protocol has been designed to simulate as realistically as possible the ambient exposure of a person working outdoors on a smoggy summer day. A two-hour exposure period is realistic in that high ambient pollutant concentrations usually persist about that length of time. Intermittent light exercise (sufficient to approximately double minute volume) during exposure gives a realistic level of ventilation (to which pollutant dose is proportional) during work. Elevated temperature is an additional stress factor frequently present during air pollution episodes, and consequently introduced into the experimental situation. The design provides for successive days' exposures, as deleterious effects of exposure may be cumulative. These requirements are incorporated into the protocol in a cost-effective manner that tests several subjects on a given day and requires staggered exposure and testing periods, precluding blind studies or control measurements on the same day. Thus, two or three days of sham control runs (exposures to purified air) precede the pollutant exposure so reliable baseline values of the measured parameters can be obtained.

A test series may be reasonably designed to support or reject the null hypothesis that no effects of pollutant exposure at realistic levels will be detected in volunteer subjects. Results supporting the null hypothesis are useful to regulatory agencies in setting air pollution standards. This approach provides a simple method to test for combined effects of two or more pollutants: a single pollutant is tested initially, a second pollutant is added to the first in the next test cycle, etc. Exposure times may also be increased to simulate two days of pollutant exposure in one day's testing. If no effects are found, even under the "worst" exposure conditions, valuable information relevant to standard-setting may be obtained. If effects are found at some point, additional studies will be required to determine if they are attributable to a single pollutant, to a combined effect, or to cumulative effects of repeated exposures. If minimal effects are found in group of "normal" subjects, the experimental plan provides for testing a group of well-specified "hyper-reactive" subjects as the next step. These subjects are characterized by a pre-study history of

cough, chest discomfort or wheezing, associated with allergy or exposure to air pollution.

Table 2 describes the detailed experimental protocol incorporating the features described. A shorter protocol, eliminating certain tests (marked with an asterisk) is also used. This protocol retains tests considered relatively simple to perform and likely to detect effects of exposure. The shorter test series requires a briefer training period for subjects and thus results in subject groups more representative of larger populations. Some tests from the comprehensive protocol may be added to the short protocol when subjects have sufficient performance ability.

Statistical Analysis

Experimental data are subjected to repeated one-way variance analyses. Post hoc comparisons using the Newman-Kuels test[24] are made when significant F values are found. For each parameter comparisons are made among controls, first day of exposure, and second day of exposure; for each subject as well as each subject group. A few significant differences due to random variation may be found because

of the number of statistical comparisons being made; therefore, all observed statistically significant changes must be examined critically for physiological significance.

Subjects

Ethical and legal considerations require that the utmost care be exercised in human experimentation. Risk is inherent in this work as well as other research,[25] but can be minimized by taking all reasonable precautions consistent with satisfactory performance of the study.

The investigators serve as the first subjects for each exposure study. Pollutant exposure levels selected never exceed the highest documented ambient levels. The exposure environment is constantly monitored by the technician operating the pollutant generating equipment. A physician is continuously in attendance to observe subjects and their electrocardiograms are monitored via telemetry outside the chamber. A view port and a closed-circuit television system are available for directly observing the subjects. The attending physician and the chamber engineer check for hazardous conditions and, if they are encountered, take corrective measures or stop the study.

Prospective subjects are screened by a physician who is not associated with the study. A standardized psychological evaluation (Minnesota Multiphasic Personality Inventory)[26] is administered. After a full explanation of the procedure, including known risks and discomfort, informed consent is obtained. The consent form fully describes the procedures and risks, and specifies that the subject may withdraw from the study at any time. All subjects receive a small gratuity for their services.

RESULTS AND DISCUSSION

In comparison to previous experimental studies[4,5,6,7,8] more rigorous control of experimental variables was possible in the present study by using a unique environmental control chamber and current monitoring technology. During the experimental period, the environmental control system allowed good control of the temperature and humidity. The system removed interfering pollutant gases and particles from the incoming air (see Table 1). Added pollutant gases were typically controlled within ± 10 percent over the experimental period. Previous work has usually not had the capability to control the environment within these limits.

In comparison to previous work, more comprehensive biological information was sought using the present experimental design. Additional biological measures included detailed tests of lung mechanics as well as biochemical and behavioral parameters. During and after exposure clinical features were specified according to a standard format by both the subject and project physician. Details of the following subject variables are reported: age, sex, number of non-smokers, number and type of smokers, health status, socio-economic level, any known unusual ambient pollution exposure history during the experiment--this included estimation of blood carbon monoxide hemoglobin before each run to help screen for unknown excessive exposure.

A combination of air pollutants of known or suspected health significance was selected. Concentrations were chosen on the basis of recorded ambient levels for the California South Coast Air Basin (metropolitan Los Angeles and adjacent areas). Duration of pollution exposure and associated environmental temperature and humidity were also surveyed and considered in the design. The resulting protocol

as described in this paper represents an attempt to mimic "worst case" conditions of summer exposure in the Basin. Four-hour exposure periods were chosen to compress two successive daily episodes of two-hours each. Thus, four hours exposure on two successive exposure days were used to mimic actual pollution episodes of up to four days' duration. Although the goal was to use a combination of pollutant gases, considerations of medical safety directed that effects of the potentially most-toxic gas be established before adding others. Because, in the concentrations chosen, O_3 was considered potentially the most toxic, it was tested the first week; NO_2 was added the second week and CO the third.

We have conducted a series of experimental studies using this core protocol. Detailed findings on our first eight subjects exposed to ozone at realistic levels, alone and in combination with other pollutants, are described in a second article in this issue.[27] The definite symptoms and significant functional decrement found in some of these subjects indicated that the exposure time should be shortened. Therefore the core protocol was modified to provide a two-hour instead of a four-hour exposure period. Results of studies on additional subjects using

the shortened exposure time will be presented in Part III.[5] Several general observations which are documented in Part II are of interest and will be described here:

1. We have found that at least some lightly exercising individuals develop discomfort and measurable effects when exposed to realistic concentrations of ozone.

2. It is apparent from studies made to date that some individuals are not noticeably affected by ozone doses two or three times greater than those at which other individuals experience symptoms and measurable respiratory dysfunction. Thus, if only group comparisons are made, risks to more sensitive individuals may go undetected -- a matter of concern in experimental and in epidemiologic studies.

3. One striking result in exposure studies conducted to date is that, generally, pulmonary tests that are simplest to perform are most reliable in demonstrating changes. "Reliability" in this sense means that changes observed after exposure are significant compared to the normal test-to-test variability under control conditions. These tests include FVC, FEV_1, $\dot{V}_{50}$, total respiratory resistance by the oscillatory

[5] Hackney, et al.: Experimental Studies on Human Health Effects of Air Pollutants: III -- Two-Hour Exposure to Ozone Alone and in Combination with Other Pollutant Gases. (Manuscript in preparation)

method and the slope of the alveolar plateau of the closing-volume tracing. This finding implies that more complex tests are not essential to studies concerned primarily with documenting exposure effects. Although these tests are simple to perform, unfortunately interpretation of underlying physiological mechanisms is complicated; therefore, when the goal of testing is analysis of such mechanisms, more complex test procedures such as C_{st} and C_{dyn} are required.

Experimental studies on human health effects of air pollutants are by nature controversial. Results will be closely examined by many, including proponents of both lower and higher pollution standards, as well as by representatives of various non-medical disciplines. Hopefully, detailed documentation can help to dispel doubts and promote progress. For example, the accuracy of reported pollutant exposures is frequently questioned. In this regard we report not only means, but also variance of pollutant gas concentrations, reference methods for calibration of pollutant monitoring devices and details of calibration of these devices. In a further effort to provide detailed documentation, we have filed tables of key data with the sponsoring agencies in the

form of an "Operational Summary." Finally, to foster standardization and promote communication, we have visited and maintained contact with all the laboratories known to us in North America, doing this kind of human experimental work.

In summary, the purpose of this article will be restated: To document an experimental approach to a comprehensive study of air pollution human health effects, and the scientific and practical rationale for the approach.

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* Deceased

BIBLIOGRAPHY

1. Chambers, L.A.: Classification and extent of air pollution. Air Pollution, Vol. 1, Academic Press, New York, 1968, p. 1.

2. Edmonton Air Pollution Survey, December 1969 - November 1970, Environmental Health Service Division, Alberta Department of Health, Edmonton, Canada, 1971.

3. Atkins, D.H.F., Cox, R.A., and Eggleton, A.E.J.: Photochemical ozone and sulphuric acid aerosol formation in the atmosphere over Southern England, Nature, 1972, *235*, 372.

4. Air Quality Criteria for Photochemical Oxidants. NAPCA #AP-63, pp. 9-13, section 4A, col. 1, par. 1.

5. Bates, D.V., Bell, G., Burnham, C., Hazucha, M., Mantha, J., Pengelly, L.D., and Silverman, F.: Problems in studies of human exposure to air pollutants. Can.Med.Assoc.J., 1970, *103*, 833.

6. Bates, D.V.: Air pollutants and the human lung. Amer. Rev. Resp. Dis.,1972, *105*, 1.

7. Bates, D.V., Bell, G.M., Burnham, C.D., Hazucha, M., Mantha, J., Pengelly, L.D., and Silverman, F.: Short-term effects of ozone on the lung. J.Appl.Physiol, 1972, *32*, 176.

8. Hazucha, M., Silverman, F., Parent, C., Field, S., and Bates, D.V.: Pulmonary function in man after short-term exposure to ozone. Arch. Environ. Health, 1973, *27*, 183.

9. Bouhuys, A., Hunt, V.R., Kim, B.M., and Zapletal, A.: Maximum expiratory flow rates in induced bronchoconstriction in man. J. Clin. Invest, 1969, *48*, 1159.

10. Du Bois, A.B., Botelho, S.Y., Bedell, G.N., Marshall, R., and Comroe, J.H.: Rapid plethysmographic method for measuring thoracic gas volume. J. Clin.Invest, 1956, *35*, 322.

11. Du Bois, A.B., Botelho, S.Y., and Comroe, J.H.: New method for measuring airway resistance in man using a body plethysmograph. J. Clin. Invest., 1956, *35*, 327.

12. Du Bois, A.B., Brody, A.W., Lewis, D.H., and Burgess, F.: Oscillation mechanics of lungs and chest in man. J. Appl. Physiol., 1956, *8*, 587.

13. Goldman, M.D., Knudson, R.J., Mead, J., Peterson, N., Schwaby, J.S., and Wohl, M.E.: Simplified measurement of respiratory resistance by forced oscillation, J. Appl. Physiol., 1970, *28*, 113.

14. Yamashiro, S.M., Karuza, S.K., and Hackney, J.D.: Phase compensation of Fleisch pneumotachographs, J. Appl. Physiol., 1974, *36*, 493-495.

15. Otis, A.B., McKerrow, C.B., Bartell, P.A., Mead, J., McIlvoy, N., Selverstone, N.J., Radford, E.P.: Mechanical factors in distribution of pulmonary ventilation. J. Appl. Physiol., 1958, *8*, 427.

16. Anthonisen, N.R., Danson, J., Robertson, P.S., Ross, W.R.D.: Airway closure as a function of age. Resp. Physiol, 1969-70, *8*,58.

17. Buist, A.S., and Ross, B.B.: Predicted values for closing volumes using a modified single-breath nitrogen test, Am. Rev. Resp. Dis. 1973, *107*, 744.

18. Buist, A.S., and Ross, B.B.: Quantitative analysis of the alveolar plateau in the diagnosis of early airway obstruction. Amer. Rev. Resp. Dis., 1973, *108*, 1078.

19. Milic-Emili, J., Mead, J., Turner, J.M., and Glauser, E.M.: Improved technique for estimating pleural pressure from esophogeal balloons. J. Appl. Physiol., 1964, *19*, 207.

20. Ogilvie, C.M., Foster, R.E., Blakemore, W.S., and Morton, J.W.: A standardized breathholding technique for clinical measurement of the diffusing capacity of the lung for carbon monoxide. J. Clin. Invest., 1957, *36*, 1.

21. Astrand, P-O., and Rhyming, I.: A nomogram for calculation of aerobic capacity (physical fitness) from pulse rate during submaximal work. J. Appl. Physiol., 1954, *7*, 218.

22. Consolazio, C.F., Johnson, R.F., and Pecora, L.J.: Physiological measurements of metabolic functions in man. McGraw-Hill, New York, 1963.

23. Jones, R.H., Ellicott, M.F., Cadigan, J.B., and Gaensler, E.A.: The relationship between alveolar and blood carbon monoxide concentrations during breathholding. J. Lab. Clin. Med., 1958, *51 (4)*, 553.

24. Winer, B.J.: Statistical principles in experimental design, McGraw-Hill, New York, 1962.

TABLE 1

CHAMBER ENVIRONMENTAL CONTROL FACTORS

	DESIGN SPECIFICATION		ACTUAL PERFORMANCE	
PARAMETER	AMBIENT[f]	CHAMBER	AMBIENT	CHAMBER[h]
Temperature, ^{o}F	25-110	Within ± 1, range 14-110	25-110	Within ± 1, range 15-130
Relative Humidity	10%-100%	Within ± 4%, range 10%-100%	10%-100%	Within ± 5%, range 20%-100%
Total Number[a] Particles per $ft.^3$	$\geq 10^6$	$< 10^5$	$\geq 10^6$	200-400[b] $2 \times 10^3 - 10^4$ [c] $2 \times 10^4 - 10^5$ [d]
CO, parts per million (ppm)	30	2	20[g]	1
NO, ppm	2	.01	1[g]	0.02
NO_2, ppm	1	.01	0.7[g]	< 0.01
O_3, ppm	0.7	.01	0.4[g]	< 0.01
SO_2, ppm	1	.05	1[e]	0.01[e]
Hydrocarbons, ppm	30	5	100[e]	5[e]

(a) Particles with diameter ≥ 0.5 μm
(b) No one in chamber
(c) One to four subjects at rest
(d) One to four subjects exercising
(e) When deliberately challenged at or above the ambient level
(f) Extreme ambient conditions experienced in Los Angeles area
(g) Maximum concentration typically found outside of the environmental control facility
(h) Over at least a six-hour period

25. Kennedy, H.W.: Legal aspects of human exposure to atmospheric pollutants. Arch. Environ. Health, 1963, *6*, 99.

26. Minnesota Multiphasic Personality Inventory. The Psychological Corporation, New York, N.Y. 10017.

27. Hackney, J.D., Linn, W., Mohler, J., Pedersen, E., Breisacher, P., and Russo, A.: II. Four-hour exposure to ozone alone and in combination with other pollutant gases. (published in this issue Amer. Rev. Resp. Dis.)

TABLE 2

EXPERIMENTAL PROTOCOL

OVERALL EXPOSURE SCHEDULE

Week 1 - O_3; Week 2 - O_3 + NO_2; Week 3 - O_3 + NO_2 + CO

WEEKLY SCHEDULE

Monday, Tuesday (Wednesday)*	Control (clean air) - 4 subjects
Thursday, Friday	Pollutant exposure - 4 subjects

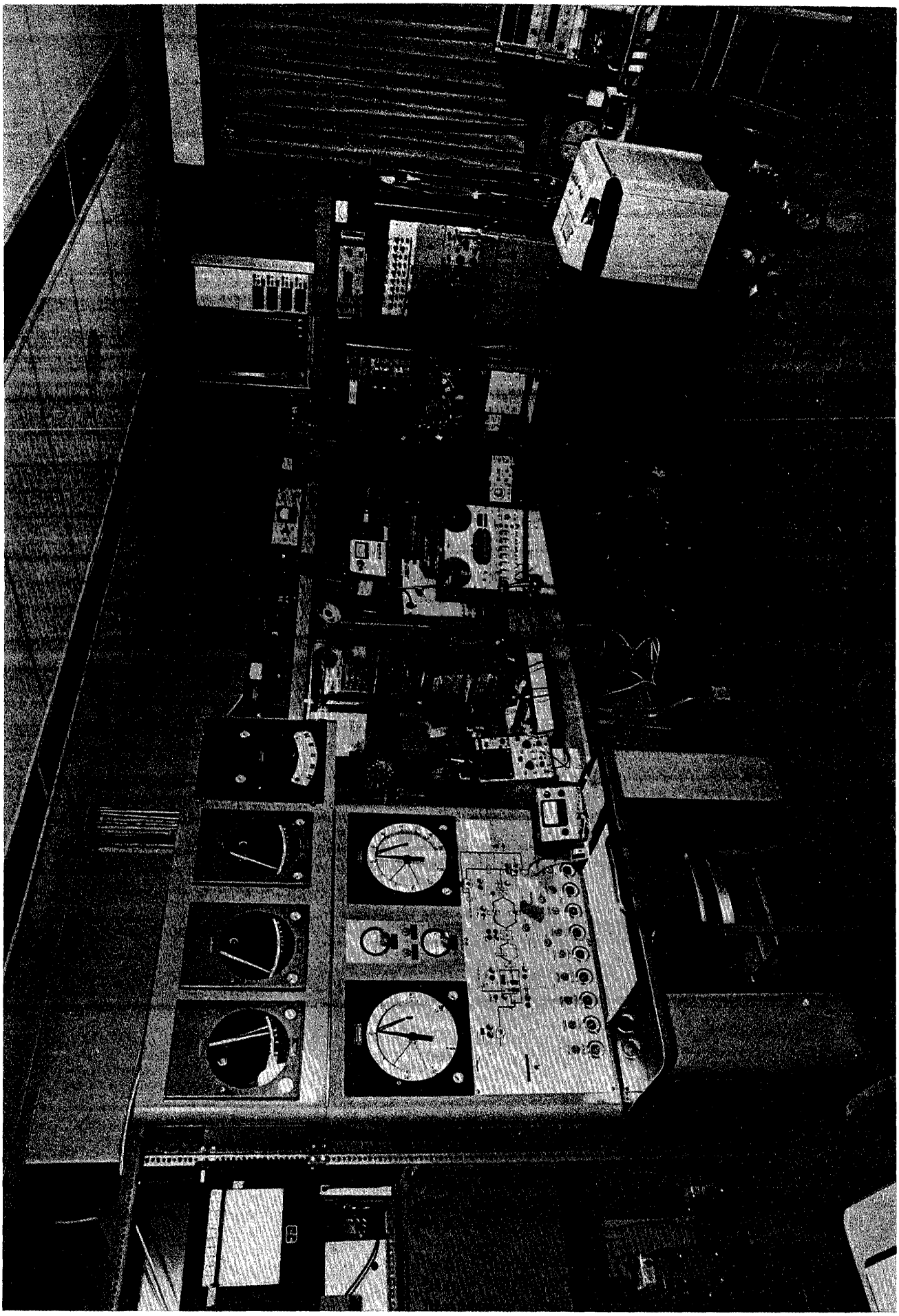
DAILY SCHEDULE

Subject No.	Begin Exposure	Begin Test Cycle
1	0700 Hr.	0845 Hr.
2	0800	0945
3	0900	1045
4	1000	1145

INDIVIDUAL SUBJECT SCHEDULE

TIME (Hr., min.)	Procedure
-0:01	COHb
0:00	Enter chamber, exercise first 15 min. of each half hour
1:45	Last rest period; psychomotor performance test*
2:00	Respiratory resistance (forced oscillation)
2:05	Flow volume maneuvers
2:10	Closing volume
2:15	Body plethysmography*
2:22	Lung compliance*
2:40	Exercise testing*
2:58	COHb
3:00	DL_{co}*
3:15	Exit chamber, venous blood sample
3:20	Physician interview and examination

* Deleted in abbreviated test protocol



CHAPTER II

EXPERIMENTAL STUDIES ON HUMAN HEALTH EFFECTS OF AIR POLLUTANTS: II -- FOUR-HOUR EXPOSURE TO OZONE ALONE AND IN COMBINATION WITH OTHER POLLUTANT GASES

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EXPERIMENTAL STUDIES ON HUMAN HEALTH EFFECTS OF AIR POLLUTANTS:

II. FOUR-HOUR EXPOSURE TO OZONE ALONE AND IN COMBINATION WITH OTHER POLLUTANT GASES

SUMMARY

Eight adult male volunteers were exposed to ozone singly and in combination with nitrogen dioxide and carbon monoxide under conditions simulating ambient air pollution exposures. Four "normal" men showed few or no effects in repeated exposures. Four male volunteers with a history of "hyper-reactive" airways, but with normal baseline pulmonary function spirometric studies, developed definite symptoms and pulmonary function decrement after ozone exposure.

EXPERIMENTAL STUDIES ON HUMAN HEALTH EFFECTS OF AIR POLLUTANTS:

II. FOUR-HOUR EXPOSURE TO OZONE ALONE AND IN COMBINATION WITH OTHER POLLUTANT GASES

INTRODUCTION

Ozone (O_3) is a major component of photochemical smog. High smog levels occur frequently in the Los Angeles region and are reported in many other urban areas. Other pollutant gases include nitrogen dioxide (NO_2) and organic peroxides.[1] Ozone is one of the most powerful oxidizing agents in smog and the fact that it is well-known for toxicity in industrial exposures[2] makes it a pollutant of great concern in air quality protection. Bates and his co-workers have reported[3,4] that normal adults performing intermittent light exercise developed marked pulmonary function decrement in two hours' exposure to ozone at concentration levels of 0.75 parts per million (ppm), and measurable decrement in similar exposures at 0.37 ppm. For comparison, the highest one-hour average oxidant concentration reported in the Los Angeles area between 1963 and 1973[5] was 0.71 ppm. On the other hand, subjective experience in the Los Angeles area suggests that the majority of

citizens are not markedly affected by ambient oxidant concentrations near 0.5 ppm. Furthermore, other experimental exposure studies[6,7] have not found marked effects or changes in spirometric measures at concentrations of 0.5 ppm and below. Possible explanations for these apparent discrepancies include difference in exposure time, subject activity and subject sensitivity, as well as the possible presence of interfering substances in the environment. The present study was undertaken to repeat the previous work under more highly controlled conditions and to expand the scope of investigation to include blood biochemistry and psychomotor performance testing, as well as pulmonary studies. The experimental plan called for testing "normal" subjects first, and then testing suspected "hyper-reactive" subjects if only minimal effects occurred in normals.

METHODS

The detailed experimental protocol and rationale are given in the preceding article.[8] Four "normal" male volunteer subjects (with no pre-study history of cough, chest discomfort or wheezing associated with allergy or exposure to air pollution), were recruited

from the project investigators or technical staff (designated Group 1) and were tested five days per week for three successive weeks. The first three days of each week were devoted to control runs (exposure to purified air); pollutant exposures took place on the fourth and fifth days. Conditions were designed to simulate a composite of extreme ambient conditions experienced during a Los Angeles summer. Exposures were: first week, 0.50 ppm O_3; second week 0.50 ppm O_3 plus 0.30 ppm NO_2; third week, 0.50 ppm O_3 plus 0.30 ppm NO_2 plus 30 ppm carbon monoxide (CO). The variability of the mean concentration of each pollutant over different exposure days was ±5 percent of the nominal value. During all exposure periods, maximum variability of pollutant concentrations was ±0.06 ppm for O_3 and NO_2 and ±3 ppm for CO. Temperature was 31°C and relative humidity 35 percent. Each exposure lasted four hours before testing was started and continued during the following hour, or for the period required to complete testing. Although similar ambient conditions occur in the Los Angeles region, the exposure time was approximately twice as long as commonly experienced during severe ambient pollution episodes. This exposure was

designed to support or reject the null hypothesis that no effects would be found under the "worst" applied stress. Parameters evaluated were forced vital capacity (FVC), one-second forced expiratory volume (FEV_1), partial and maximum forced expiratory flow-volume curves, total respiratory resistance (R_t) by the forced oscillation method, closing volume (CV), alveolar plateau slope from single-breath nitrogen test (delta nitrogen or ΔN_2), plethysmographic thoracic gas volume (TGV) and airway resistance (R_{aw}), 3-breath rebreathing residual volume (RV),[9] blood carboxyhemoglobin (COHb) concentration estimate, resting and exercise oxygen consumption ($\dot{V}O_2$), static and dynamic lung compliance (C_{st}, C_{dyn}), and pulmonary diffusing capacity ($D_{L_{CO}}$). Data were analyzed by repeated-measures one-way analysis of variance, comparing the individual's and the group's performance on control days, first exposure days, and second (successive) exposure days. All lung volumes are expressed as BTPS. Smokers were not asked to alter their smoking habits, but no smoking was permitted in the main chamber or during testing.

Because only minimal effects occurred in the "normal" group, four additional male subjects (designated Group 2) were recruited from the project investigators or technical staff and were tested as described above, except pollutant exposures were modified. These subjects had normal FVC, FEV_1, and CV, but had pre-study histories of cough, chest discomfort or wheezing, associated with allergy or exposure to air pollution. Marked effects developed among some of these subjects during the first exposure to 0.50 ppm O_3. Accordingly, the second 0.50 ppm exposure period (week 1, day 5) was shortened to two hours for three of the four subjects. In light of the results in week one, the protocol was further modified to look at dose-response relations rather than possible effects of the additional pollutants NO_2 and CO.

RESULTS

Biochemical and behavioral findings are reported elsewhere.[4,5]

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[4] Buckley, R.D., Clark, K., Posin, C., and Hackney, J.D.: Some effects of ozone inhalation on human erythrocyte metabolism. Accepted for publication. *Arch Env Health*

[5] Pedersen, E. E., Breisacher, P., Patterson, J., and Hackney, J.D.: Psychophysiological and psychomotor assessment of environmental stress effects due to pollution. In preparation.

Few significant pulmonary function changes or respiratory symptoms were detected in Group 1. The physiological significance of the changes in pulmonary function data is in doubt, since the changes occurred in the less stable parameters, (R_t, C_{dyn}, $D_{L_{Co}}$), were small in magnitude and did not occur consistently throughout all exposures. Statistical analyses for this group are given in Table 1. In Group 2, numerous changes were observed. Significant changes in group data are displayed in Figures 1 - 4 and in Table 2. Because of the marked differences between "normal" and "reactive" subjects, individual responses are discussed below. Height, weight, age, and other characteristics are given in Table 3.

Subject #01, Group 1

Symptoms: mild, transient pharyngitis during some exposures. No significant pulmonary function changes.

Subject #03, Group 1

Symptoms: slight chest tightness during exposure on some occasions, post-exposure fatigue and drowsiness. No significant pulmonary changes.

Subject #04, Group 1

Symptoms: slight substernal soreness during some exposures, slight fatigue and drowsiness during exposure. No significant pulmonary function changes.

Subject #06, Group 1

Symptoms: slight substernal soreness during some exposures, nasal discharge and productive cough during others, post-exposure headache and fatigue, chest pain during exercise testing with exposure to $O_3 + NO_2 + CO$. The chest pain was accompanied by minimal electrocardiogram S-T segment depression, causing exercise testing to be terminated on this occasion. This subject also showed a small but significant increase in total respiratory resistance when all exposure values were compared with all control values.

Subject #07, Group 2

This subject had a history of mild bronchospasm subjectively associated with exposure to certain plant species and/or air pollution. On exposure to 0.5 ppm O_3, he developed substernal pain, coughing, sputum, wheezing and malaise. The symptoms developed after about one

hour of exposure and persisted throughout the day. Observed pulmonary changes included reduction in FVC, FEV_1, $\dot{V}_{50}$, and $\dot{V}_{25}$; reduction in TLC; increase in RV; increase in airway resistance and total pulmonary resistance, decrease in static lung compliance, and increased delta nitrogen (indicating less uniform ventilation distribution). Exposure time for this subject was decreased to two hours on the second day of 0.5 ppm O_3 exposure. Symptoms and function changes observed were similar to those of the first day, but less severe. However, sputum with dark blood streaking was observed on one occasion. No significant adverse effects of exposure to 0.25 ppm O_3 were detected, but changes similar to those described above occurred with exposure to 0.37 ppm O_3. With the latter exposure, RV and static compliance did not change significantly and other changes were smaller than those observed in the 0.5 ppm exposure.

Subject #08, Group 2

This subject had a history of mild wheezing and dyspnea associated with outdoor exercise in polluted air. His symptomatology under O_3 exposure was essentially similar to that described for subject #07;

however, symptoms were relatively mild on the first day of exposure to 0.5 ppm and became markedly worse on the second day, even though exposure time was shortened to two hours. Severity of physiological changes generally paralleled that of the symptoms, suggesting a cumulative effect of two successive days' exposure in this subject. Physiological changes observed were similar to those in subject #07 except that in subject #08 RV did not change.

Subject #09, Group 2

This subject had a history of mild asthma. Upon exposure to 0.5 ppm O_3 he developed cough, wheezing, substernal pain, headache, muscular aches, and malaise, which persisted several hours after exposure. Observed physiological changes included loss in FVC and maximum flow rates, loss in TLC without significant change in RV, increase in airway and total pulmonary resistances, and marginal increase in delta nitrogen. Similar symptoms and smaller physiological changes were observed on exposure to 0.37 ppm O_3. The subject also developed a viral syndrome immediately following this exposure which lasted three days. Serum titers*

* Acute and convalescent viral titers were measured by compliment fixation using a micro titre method and read as tube dilutions. All titers were performed by the Communicable Diseases Laboratory of the Los Angeles County Health Department.

during the acute illness were positive for influenza A and B at 1:8 dilution and for adenovirus at 1:15. After 12 days influenza A was unchanged, influenza B became negative at less than 1:8 and adenovirus had decreased to 1:8. Only mild symptoms and no physiological changes were found on exposure to 0.25 ppm O_3.

Subject #10, Group 2

This subject had mild clinical asthma, subjectively exacerbated by air pollution, for which he inhaled epinephrine aerosols occasionally. His baseline pulmonary function test including FVC, FEV_1, $\dot{V}_{50}$, $\dot{V}_{25}$, delta nitrogen, CV, CC, RV, and TLC were normal, however. On exposure to 0.5 ppm O_3 he developed marked wheezing, cough and substernal pain. His forced vital capacity, expiratory flow rates and total lung capacity were slightly reduced. His airway resistance, which was abnormally high before exposure, was elevated further. Total respiratory resistance became frequency dependent, and the frequency dependence persisted throughout the remainder of the study. Dynamic compliance also became frequency dependent after the first exposure (Figure 5). Further evidence of maldistribution of ventilation caused by the first 0.5 ppm exposure

was a marked increase in delta nitrogen. The second 0.5 ppm exposure was shortened to two hours for this subject, and observed symptoms and function decrement were milder. Slight function decrement was observed with 0.37 ppm exposure but not with 0.25 ppm exposure.

Although individual responses of the asthmatic subjects to the exposures were variable, several consistent patterns emerged; these are discussed below.

Forced vital capacity, forced expired volume at one-second, and maximum expiratory flow rates. These parameters were consistently reduced in sensitive subjects. Contributing factors appeared to be increased airway resistance, reduced inspiratory capacity due to substernal pain, and tendency to cough during forced expirations. As these measurements were highly reproducible under control conditions, observed changes with exposure attained higher levels of statistical significance than with most other tests.

Lung volumes. Total lung capacity was consistently reduced in sensitive subjects. Residual volume, as determined by three-breath

nitrogen dilution,[9] single-breath nitrogen dilution,[10] or plethysmography,[11] increased significantly in one subject and was unchanged in the others. These changes appear to be due to pain produced by attempting to reach extremes of lung volume.

Single-breath nitrogen test. This test determines phase-4 volume (closing volume), presumably affected by changes in dependent peripheral airways; and phase-3 or alveolar platueau slope (delta nitrogen), presumably affected by significant changes in ventilation distribution anywhere in the lung. No significant changes in closing volume or closing capacity (CV plus RV) were found in any subject in this study, whereas delta nitrogen values increased with exposure in all sensitive subjects - slightly in some and markedly in others.

Total pulmonary resistance. Further investigation is required before results of this test can be evaluated in depth, since normal limits and test-to-test variability have not been fully worked out. Significant changes definitely occurred with ozone exposure, however. Resistance determined by forced oscillation increased at all oscillation

frequencies in three of four sensitive subjects and in one "normal" (Group 1) subject in whom no other significant physiological changes were found. The increases in oscillatory resistance in the sensitive subjects generally correlated with increases in airway resistance measured plethysmographically. In the fourth sensitive subject, total resistance, which was already elevated in comparison to other subjects, did not increase overall but became markedly frequency-dependent, as did dynamic lung compliance, strongly suggesting significant small-airways dysfunction resulting in nonuniform regional ventilatory time constants under exposure conditions.

Lung compliance. One sensitive subject showed a reduction in static compliance with exposure, and a corresponding drop in dynamic compliance at all frequencies without significant frequency-dependence. Another subject developed frequency dependence as previously indicated. No other statistically significant changes in compliance were found.

Exercise testing. No consistent changes in resting or exercise oxygen consumption were found with exposure. One "normal" subject

developed possible angina and minimal EKG changes during exercise when exposed to CO plus oxidants, as previously described.

Pulmonary diffusing capacity. Reproducibility of this test was poor in this study as compared to normal conditions (resting subjects, normal room temperature). In both Groups 1 and 2, slight decreases in diffusing capacity occurred on the second day of exposure as compared to the first day of exposure, when results were essentially unchanged from control values.

Symptomatology. Symptoms were absent in Group 1 during control runs, but some were reported during exposures. A low frequency of occurrence of symptoms in Group 2 during control runs increased dramatically with ozone exposure at 0.5 and 0.37 ppm. Symptom scores for each subject on each day were assigned by the project physician based on an interview using a standard questionnaire. Symptoms of cough, wheezing, sputum production, substernal pain, dyspnea, fatigue, headache, laryngitis, and nasal discharge were scored. Each was given from

0 to 4 points, based on severity, during each of the three time periods -- during exposure, remainder of the exposure day, and the following morning. Less specific symptoms such as malaise and muscular aches were not scored. In Group 2, symptom scores were strongly correlated with observed physiological changes except in the 0.37 ppm O_3 exposure, in which symptoms were more severe than would be expected, and out of proportion on the basis of physiological findings. The 0.37 ppm exposure was during the third week and the severity of these symptoms was greater than for the 0.5 ppm exposure of the first week.

DISCUSSION

A relatively broad range of sensitivity to ozone has been demonstrated, with observed exposure effects correlating well with subjects' own opinions concerning their sensitivity. Although the number of subjects tested is small, the results support the hypothesis that individuals with pre-existing pulmonary hyper-reactivity are more severely affected by exposure and thus more at risk in polluted

environments. All four subjects with any history of pulmonary hyper-reactivity were significantly affected by 0.37 ppm O_3, while all four subjects without such a history were affected minimally or not at all by 0.50 ppm O_3. In the latter subjects, addition of a second oxidant pollutant, NO_2 at 0.3 ppm, did not produce additional detectable effects. This does not, however, preclude the possibility that additive or synergistic effects of exposure to O_3 and NO_2 in combination may occur at higher NO_2 concentrations, at higher relative humidity, or in more sensitive subjects. Addition of CO to the pollutant mixture also failed to produce detectable effects other than increases in blood carboxyhemoglobin levels.

A comparison of the present results with the findings of Bates, et al.,[3,4] is in order. As illustrated in Table 4, the symptoms experienced and most of the pulmonary function changes observed were similar in the two studies. Discrepancies appear primarily in areas where methodology differed between the two studies, thus they may not be actual differences in physiological response to exposure.

One might infer that Bates' Canadian subjects, although they had no history of respiratory disease, were more sensitive on the average, since they experienced function decrement similar to the present (Los Angeles) Group 2 subjects. The present Group 1 subjects, also with no history of respiratory disease, experienced virtually no function decrement. Several possible explanations for this apparent discrepancy may be suggested. Random variation in sensitivity among individuals might be a factor, given the small numbers of subjects tested. Nominally similar ozone concentrations might actually have been significantly different in the two studies since monitoring instruments based on different analytical principles were used. Additional unknown pollutants modifying the effect of ozone might have been present in one study but not the other. This possibility seems more likely in the case of the Canadian study, in which air purification and monitoring apparatus was less extensive than in the present study. Finally, the relative lack of response in the present subjects could have been due to tolerance developed through

long residence in an oxidant-polluted area. This possibility is supported by the observation of tolerance development in animals.[12]

Further study of health effects of air pollutants is required. More detailed information on dose-response characteristics of ozone exposure over a wide range of concentration is needed for larger numbers of normals, hyper-reactors, and individuals with chronic respiratory disease. Possible synergistic effects of ozone with other pollutants have been reported[13] and require investigation. Information is also lacking on effects of oxides of nitrogen and numerous other pollutants. More rigorous epidemiologic investigation of air pollution episodes is also called for since experimental evidence now exists that many individuals are likely to be significantly affected by pollutant doses received during such episodes.

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BIBLIOGRAPHY

1. Chambers, L. A.: Classification and extent of air pollution, Air Pollution, *Vol. 1*, Academic Press, New York, 1968, p. 1.

2. Stokinger, H. E.: Evaluation of the hazards of ozone and oxides of nitrogen. Arch. Industrial Health, 1957, *15*, 181.

3. Bates, D. V., Bell, G., Burnham, C., Hazucha, M., Mantha, J., Pengelly, L. D., and Silverman, F.: Short-term effects of ozone on the lung, J. Appl. Physiol., 1972, *32*, 176.

4. Hazucha, M., Silverman, F., Parent, C., Field, S., and Bates, D. V.: Pulmonary function in man after short-term exposure to ozone, Arch. Environ. Health, 1973, *27*, 183.

5. Ten year summary of California air quality data, State of California, Air Resources Board, Sacramento, 1974.

6. Langerwerff, J. M.: Prolonged ozone inhalation and its effect on visual parameters, Aerospace Med., 1963, *34*, 479-486.

7. Goldsmith, J.R. and Nadel, J. A.: Experimental exposure of human subjects to ozone, J. Air Pollut. Contr. Assoc., 1969, *19*, 329-330.

8. Hackney, J. D., Linn, W. S., Buckley, R. D., Pedersen, E. E., Karuza, S., Law, D. C., Fischer, D. A.: Experimental studies on health effects of air pollutants - design considerations. (This issue)

9. Rahn, H., Fenn, W. O., and Otis, A. B.: Daily variations of vital capacity, residual air, and expiratory reserve, including a study of the residual air method, J. Appl. Physiol., 1949, *1*, 725.

10. Buist, A. S., and Ross, B. B.: Predicted values for closing volumes using a modified single-breath nitrogen test, Am. Rev. Resp. Dis., 1973, *107*, 744.

11. Du Bois, A. B., Botehlo, S. Y., and Comroe, J. H.: New method for measuring airway resistance in man using a body plethysmograph, J. Clin. Invest., 1956, *35*, 322.

12. Stokinger, H. E., Wagner, W. D., and Wright, P. G.: Studies on ozone toxicity. I. Potentiating effects of exercise and tolerance development, Arch Ind. Health, 1956, *14*, 158-160.

13. Hazucha, M., Parent, C., and Bates, D. V.: Combination effect of ozone and sulfur dioxide on pulmonary function in man, Fed. Proc., 1974, *33*, 350.

TABLE 1

RESULTS OF ANALYSIS OF VARIANCE FOR PHYSIOLOGICAL PARAMETERS, GROUP 1
Exposures: O=0.5 ppm O_3; N=0.3 ppm NO_2; C=30 ppm CO

F=statistic for analysis of variance
dF=degrees of freedom for analysis of variance
P=probability of control-exposure difference being due to chance

PARAMETER	EXPOSURE	F	dF	P
FVC	O	2.04	2,6	NS
	ON	4.17	2,6	NS
	ONC	<1.0	2,6	NS
$\dot{V}_{50}$	O	1.25	2,6	NS
	ON	<1.0	2,6	NS
	ONC	<1.0	2,6	NS
$\dot{V}_{25}$	O	<1.0	2,6	NS
	ON	<1.0	2,6	NS
	ONC	n/a	n/a	n/a
RV	O	1.18	2,6	NS
(single br)	ON	<1.0	2,6	NS
	ONC	7.73	2,6	<.05(a)
TLC	O	6.79	2,6	<.05(b)
(single br)	ON	<1.0	2,6	NS
	ONC	<1.0	2,6	NS
DL_{CO}	O	11.1	2,22	<.01(c)
	ON	<1.0	2,22	NS
	ONC	15.9	2,22	<.01(b)
Rt	O	<1.0	2,6	NS
(3 Hz)	ON	<1.0	2,6	NS
	ONC	7.95	2,6	<.05(b, d)

PARAMETER	EXPOSURE	F	dF	P
CV	O	<1.0	2,22	NS
	ON	3.12	2,22	NS
	ONC	<1.0	2,22	NS
Cdyn	O	<1.0	2,78	NS
(norm. freq)	ON	<1.0	2,72	NS
	ONC	1.79	2,72	NS
Cdyn	O	5.62	2,78	<.01(c
(60/min)	ON	<1.0	2,72	NS
	ONC	2.42	2,72	NS
Cdyn	O	1.45	2,78	NS
(100/min)	ON	<1.0	2,78	NS
	ONC	<1.0	2,78	NS
Cstat	O	<1.0	2,22	NS
	ON	1.06	2,22	NS
	ONC	<1.0	2,22	NS
TGV	O	5.67	2,16	<.05(b d
(RV)	ON	4.07	2,16	<.05(d d
	ONC	<1.0	2,16	NS
TGV	O	5.47	2,16	<.05(b d
(TLC)	ON	5.11	2,16	<.05(b d
	ONC	2.06	2,16	NS

(a) Decreased first day of exposure

(b) Increased second day of exposure

(c) Decreased second day of exposure

(d) Increased first day of exposure

TABLE 2

MEAN VALUES AND RESULTS OF VARIANCE ANALYSIS FOR PHYSIOLOGICAL PARAMETERS

GROUP 2

F=statistic for analysis of variance
dF=degrees of freedom for analysis of variance
P=probability of control-exposure difference being due to chance

PARAMETER	O_3 Exposure	Control	Exposure 1	Exposure 2	F	dF	P
FVC	.50	5.03	4.55*	4.69*	5.17	2,22	<.05
	.25	5.08	4.94	5.13	<1.0	2,22	NS
	.37	5.07	5.04	none	<1.0	1,11	NS
FEV_1	.50	3.96	3.54**	3.68	(a)		
	.25	4.07	4.03	4.11	<1.0	2,22	NS
	.37	4.07	3.95	none	2.15	1,10	NS
$\dot{V}_{50}$	.50	4.32	3.42*	3.27*	4.06	2,14	<.05
	.25	4.09	4.24	4.33	2.01	2,14	NS
	.37	4.15	4.10	none	<1.0	1,8	NS
$\dot{V}_{25}$	.50	1.99	1.50	1.20*	6.06	2,6	<.05
	.25	1.72	1.87	2.42	2.26	2,10	NS
	.37	1.80	1.78	none	<1.0	1,7	NS
$\dot{V}_{50}$	.50	4.55	3.14*	3.05*	5.34	2,16	<.05
(partial)	.25	4.66	4.56	4.60	<1.0	2,18	NS
	.37	4.42	3.52**	none	8.49	1,9	<.01
$\dot{V}_{25}$	.50	1.83	1.15**	1.19**	7.67	2,22	<.01
(partial)	.25	2.00	1.71	1.72	1.39	2,20	NS
	.37	1.62	1.39**	none	2.22	1,11	<.01
CC	.50	2.35	2.27	2.47	3.08	2,20	NS
	.25	2.33	2.36	2.20	1.05	2,22	NS
	.37	2.21	2.28	none	2.07	1,11	NS

(a) Insufficient data, some subjects unable to complete specified number of tests satisfactorily

*=significant change from control, p <.05 **=significant change from control, p <.01

TABLE 2

(continued)

PARAMETER	O_3 Exposure	Control	Exposure 1	Exposure 2	F	dF	P
ΔN_2	.50	0.73	1.60*	1.03	4.21	2,20	<.05
	.25	0.67	0.71	0.63	2.07	2,22	NS
	.37	0.69	0.84	none	2.13	1,11	NS
TLC	.50	6.84	6.42	6.18*	3.96	2,20	<.05
(single br)	.25	6.91	6.85	6.77	<1.0	2,22	NS
	.37	6.85	6.83	none	<1.0	1,11	NS
RV	.50	1.69	1.88	1.76	1.23	2,20	NS
(single br)	.25	1.66	1.64	1.58	1.51	2,22	NS
	.37	1.60	1.74*	none	8.17	1,11	<.05
R_{aw}	.50	1.26	1.70	1.65	2.50	2,22	NS
(FRC)	.25	1.09	1.24	1.11	<1.0	2,22	NS
	.37	1.35	1.49	none	2.75	1.11	NS
Cdyn	.50	.196	.187	none	<1.0	1,32	NS
(norm. freq)	.25	.241	.227	.266	<1.0	2,68	NS
	.37	.224	.222	none	<1.0	1,36	NS
Cdyn	.50	.179	.153	none	1.68	1,30	NS
(100/min)	.25	.184	.189	.172	<1.0	2,60	NS
	.37	.173	.127	none	3.07	1,39	NS
DL_{co}	.50	41.8	42.4	38.8	2.18	2,20	NS
	.25	41.4	40.4	49.8**	57.8	2,16	<.01
	.37	40.1	45.4**	none	19.4	1,11	<.01

TABLE 3

SUBJECT CHARACTERISTICS

	I.D. NO.	AGE, YR.	HT., IN.	WT., LB.	SMOKING HISTORY PACK-YEARS[1]	PRE-STUDY HISTORY OF SMOG SENSITIVITY[2]	HISTORY OF ASTHMA[3]	HISTORY OF ALLERGY[4] PERSONAL	FAMILY
GROUP 1	01	49	70	185	17	0	0	0	0
	03	36	70	185	34	0	0	0	0
	04	44	74	195	4 †	0	0	+	0
	06	42	69.5	185	50*‡	0	0	+	0
	MEAN	43	71	188					
	(S.D.)	(5.4)	(2.1)	(5)					
GROUP 2	07	36	68.5	155	0	+	0	+	+
	08	29	68	157	30	+	+	+	+
	09	41	74	177	20*	+	+	+	+
	10	30	68	172	0	+	0	0	0
	MEAN	34	69.5	165					
	(S.D.)	(5.6)	(2.9)	(11)					

* - Still smoking: all others have been non-smokers for at least four years prior to study.

†=now smokes pipe
‡=also smokes and inhales cigars

1 - 1 pack year = 1 pack of cigarettes per day for 1 year.
2 - Defined by symptoms of cough, wheezing, or chest discomfort when outside on high pollution days. (L.A. area
3 - Defined by spontaneous attacks of bronchospasm requiring bronchoidilator therapy separated by asymptomatic intervals.
4 - Defined by symptoms of wheezing, gastroenteritis, or dermatitis when in contact with specific antigens or a history of chronic rhinitis, post nasal drip, or hayfever.

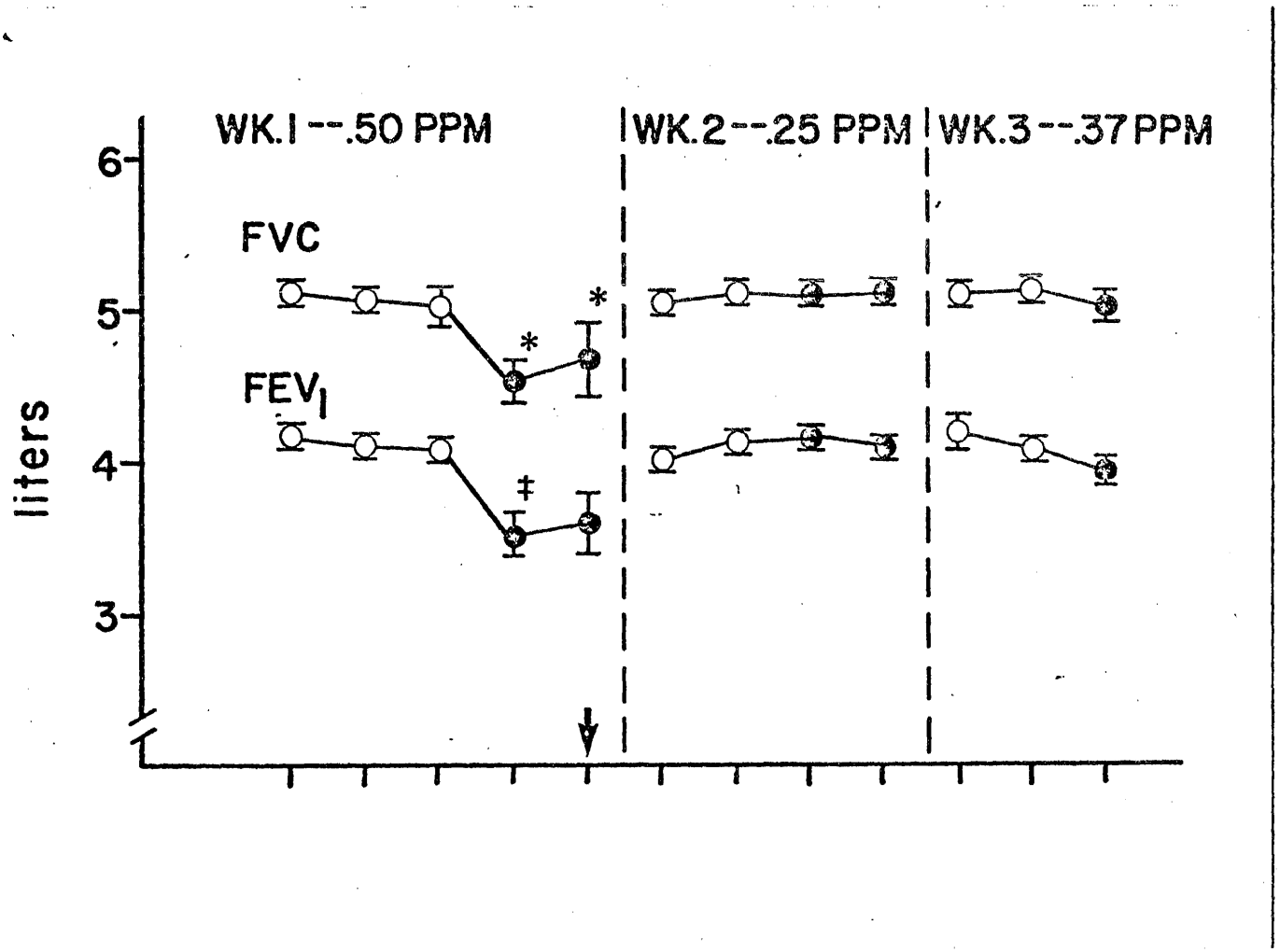

FIGURE 1

Daily group means (± one standard error) for FVC and FEV_1, Group 2. Open circle - control; black circle = ozone exposure. * = significant change from control, $p < .05$; ‡ = significant change from control, $p < .01$.

† Exposure time decreased from 4 to 2 hours in 3 of the 4 subjects.

TABLE 4

Parameter	Bates et al	Present study
FVC	-	-
FEV_1	-	-
$\dot{V}_{50}$	-	-
CV or CC	+	0
TLC	0	-
RV	+	0
Cough	+	+
Substernal pain	+	+
Wheezing	+	+
Laryngitis	+	+

Comparison of typical effects of O_3 exposure found in the present study and in previous work of Bates et al [3,4].

+ = increased (or symptoms observed)

- = decreased

0 = unchanged in most or all cases.

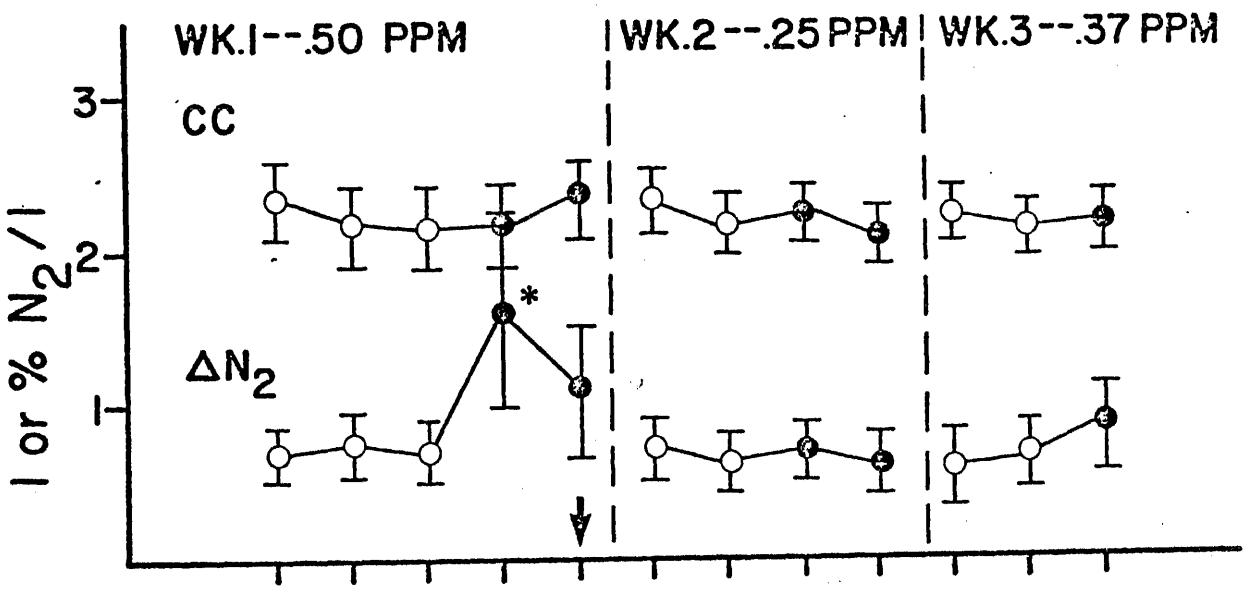

FIGURE 2

Daily group means (± one standard error) for closing capacity and delta nitrogen, Group 2. Open circle = control; black circle = ozone exposure. * = significant change from control, $p < .05$.

↓ Exposure time decreased from 4 to 2 hours in 3 of the 4 subjects.

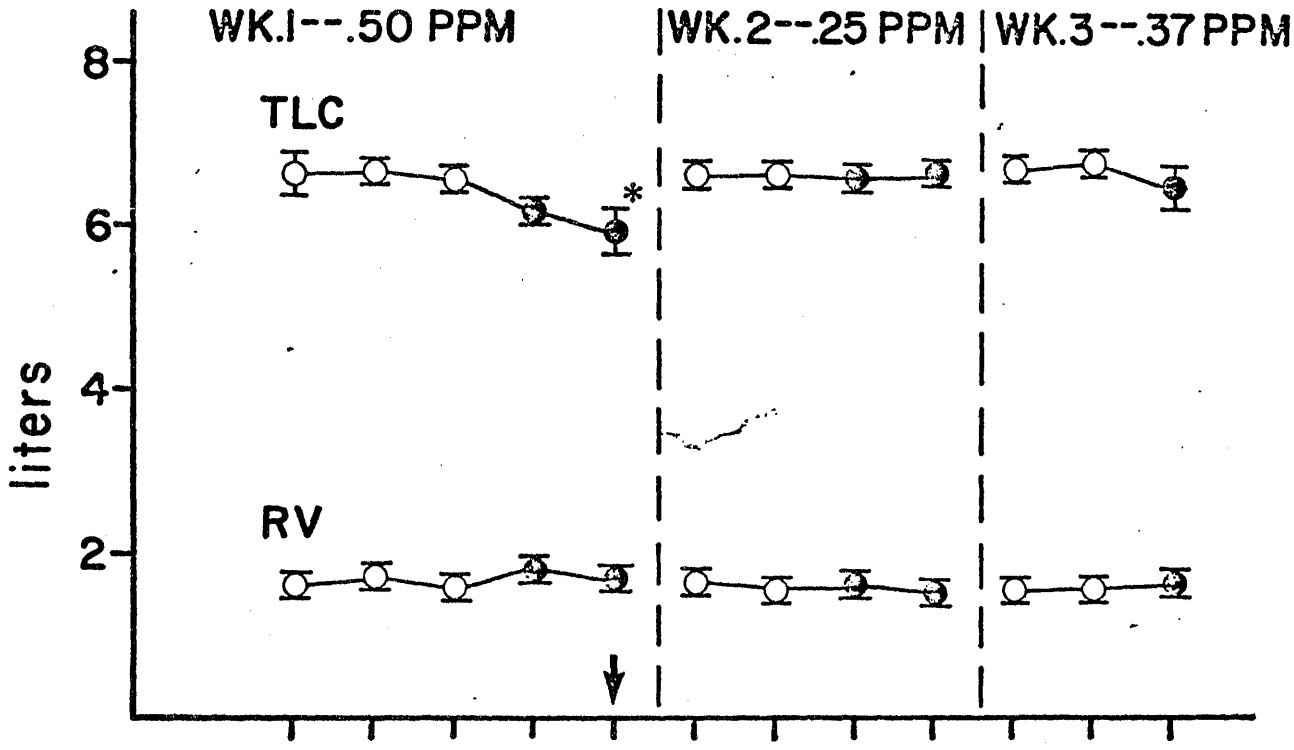

FIGURE 3

Daily group means (± one standard error) for lung volumes calculated from single-breath nitrogen tracings. Open circle = control; black circle = ozone exposure. * = significant change from control, $p < .05$.

+ Exposure time decreased from 4 to 2 hours in 3 of the 4 subjects.

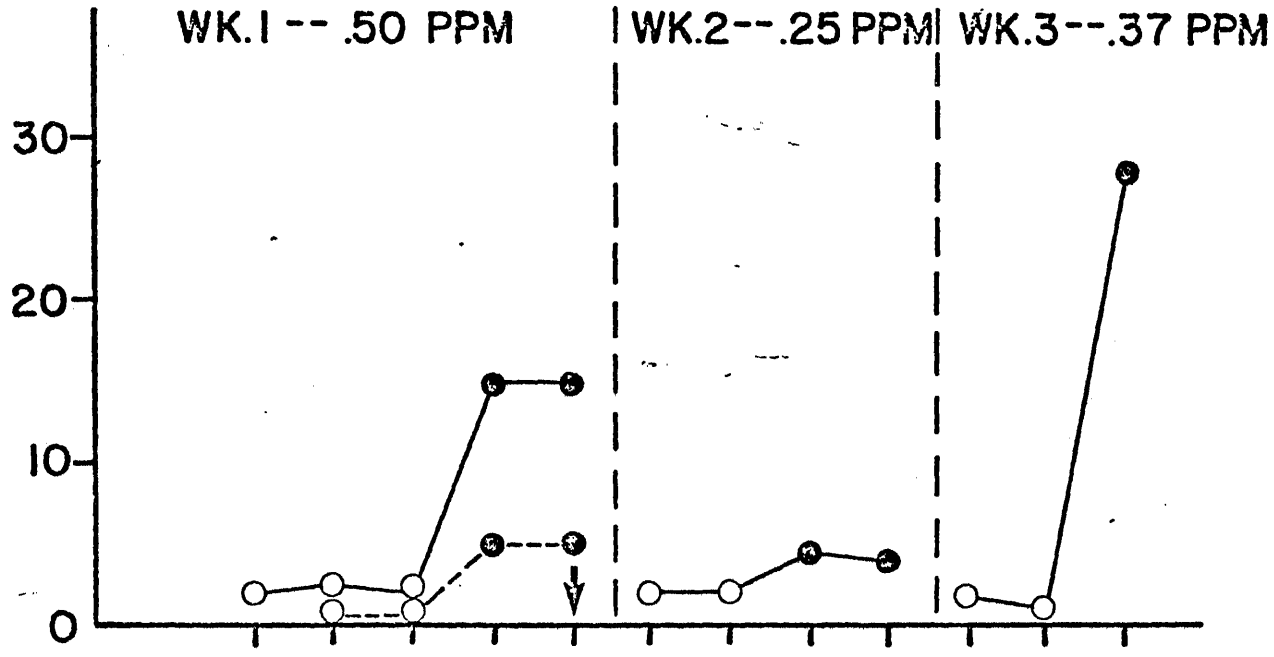

FIGURE 4

Daily mean symptom scores related to ozone exposure for Group 1 (dotted line, week 1 only) and Group 2 (solid lines). Open circle = control; black circle = ozone exposure.
↓ Exposure time decreased from 4 to 2 hours in 3 of 4 subjects of Group 2.

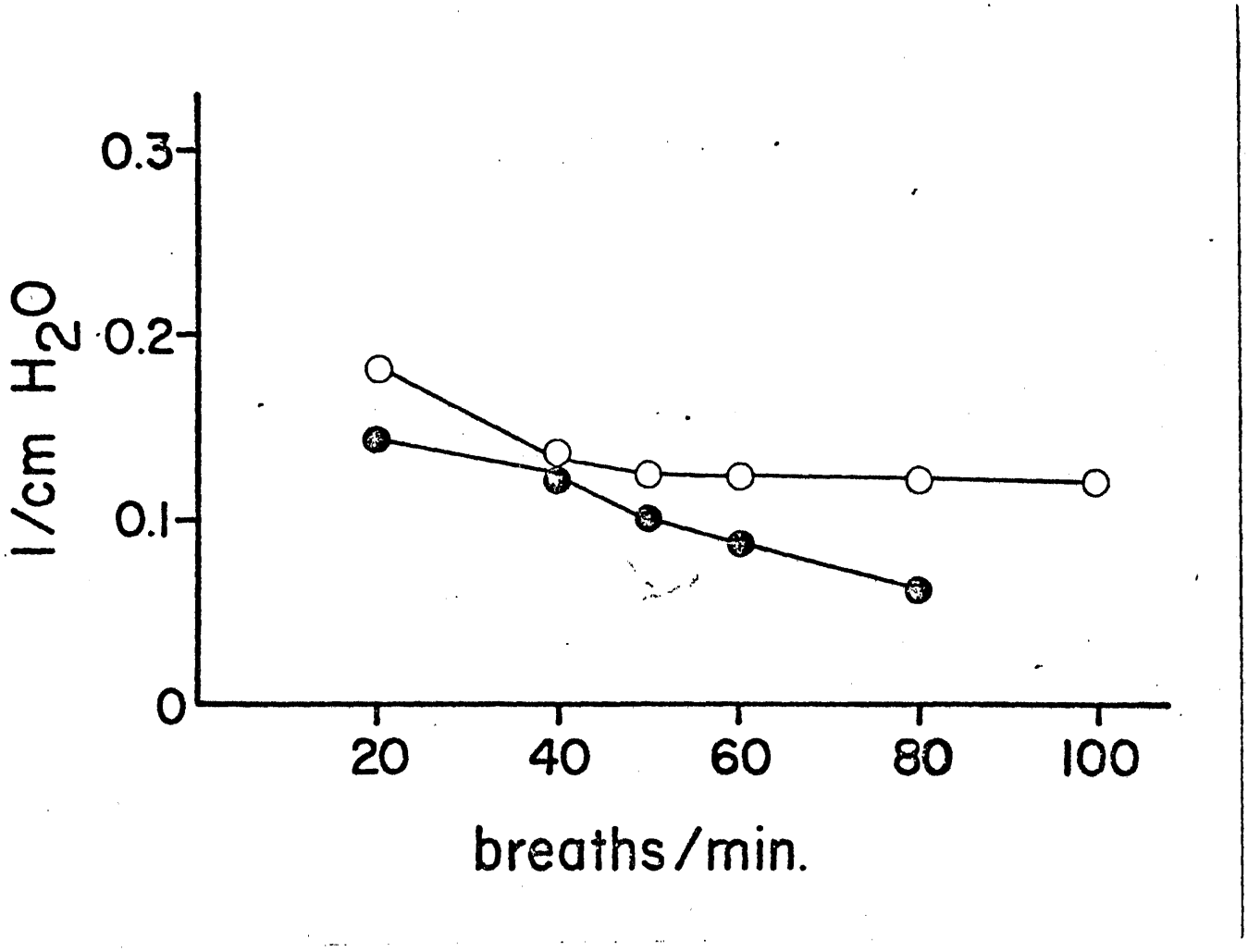

FIGURE 5

Dynamic compliance vs. breathing frequency, Subject 10. Open circle = mean of control runs for week 1; black circle = first day of 0.50 ppm ozone exposure. Subject could not perform maneuver at 100 breaths/min. under exposure.

CHAPTER III

EXPERIMENTAL STUDIES ON HUMAN HEALTH EFFECTS OF AIR POLLUTANTS: III -- TWO-HOUR EXPOSURE TO OZONE ALONE AND IN COMBINATION WITH OTHER POLLUTANT GASES

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ABSTRACT

Adult male volunteers were exposed to ozone (O_3) at 0.25, 0.37, or 0.50 parts per million (ppm), and to ozone in combination with nitrogen dioxide (NO_2) and carbon monoxide (CO), with secondary stresses of heat, intermittent light exercise and repeated exposure. Few significant physiological changes, and only mild symptoms, were found with 0.25 ppm O_3, with 0.25 ppm O_3 plus 0.30 ppm NO_2 or when 30 ppm CO was added to the latter mixture. With 0.37 ppm O_3, more symptoms were present and some subjects developed significant pulmonary function decrement. With 0.50 ppm O_3, most subjects had symptoms and about half showed significant pulmonary function decrement. In reactive subjects exposed on two successive days, changes were usually significantly greater the second day, indicating that effects of successive exposures were cumulative.

Introduction

Ozone (O_3) is one of the most powerful oxidizing agents among common photochemical oxidant air pollutants and is known to be highly toxic in industrial exposures; thus it may present a significant health hazard in relation to air pollution. Bates and coworkers found that volunteers having no history of respiratory disease and living in an area with little oxidant pollution developed significant respiratory symptoms and function decrement when exposed for two hours to ozone at concentrations as low as 0.37 parts per million (ppm), with intermittent light exercise.[1,2] In previous work in this laboratory,[3,4] similar studies were conducted on volunteers living in the Los Angeles area, where ambient ozone concentrations of 0.5 ppm or higher are possible. In these studies, four "normal" subjects (without a pre-study history of cough, chest discomfort or wheezing associated with allergy or air pollution exposure) failed to react significantly to O_3 exposure at 0.50 ppm, even when exposed for four-hour periods on two successive days, with nitrogen dioxide (NO_2) (0.3 ppm) and carbon monoxide (CO) (30 ppm) added, at elevated temperature (31°C), and with intermittent light exercise.

Four "reactive" subjects (with a pre-study history of cough, chest discomfort, or wheezing associated with allergy or air pollution exposure, but with normal baseline pulmonary function studies) after a similar exposure exposure to 0.50 ppm O_3 alone for four hours, developed significant pulmonary function decrement and symptoms severe enough to restrict normal activity. The same subjects did not react appreciably to 0.25 ppm O_3 but did react somewhat to 0.37 ppm O_3. The degree of pulmonary function decrement found in some reactive subjects led us to decrease the exposure time from four hours to two hours. The present studies use a two-hour exposure time and were undertaken to better define the range of sensitivity and dose-response characteristics in "normal" and "reactive" populations. Such information is required as a basis for setting realistic air-quality standards for the protection of public health.

Methods

The controlled-environment exposure facility and detailed experimental protocol used for these studies have been described previously.[3] Thirteen volunteer subjects, recruited from the project professional

and technical staff, participated in one or more exposure groups (Table 1). Group 3, consisting of seven "normal" subjects (without a pre-study history of cough, chest discomfort or wheezing associated with allergy or air pollution exposure), was exposed to 0.50 ppm O_3 in a one-week study. (Groups 1 and 2 are described in a previous report[4]). Groups 4 and 5 included both "normal" and "reactive" subjects. In a three-week study, Group 4 was exposed to 0.25 ppm O_3 the first week, to 0.25 ppm O_3 plus 0.30 ppm NO_2 the second week, and to 0.25 ppm O_3 plus 0.30 ppm NO_2 plus 30 ppm CO the third week. Group 5 was exposed to 0.37 ppm O_3 in a one-week study. During each week of studies, the first two or three days were devoted to sham exposures (exposures to purified air) to establish baseline values of the measured parameters, and the final two days were devoted to pollutant exposures (the given concentration of pollutant added to purified air).

Each subject was exposed in the controlled-environment chamber for two hours, then performed the battery of physiological tests while still under exposure. During the two-hour period, the first 15 minutes of every 30 was spent exercising at a level sufficient to approximately

TABLE 1

SUBJECT CHARACTERISTICS

I.D. NO.	EXPOSURE GROUP(s)	AGE, YR.	HT., IN.	WT., LB.	SMOKING HISTORY PACK-YEARS[1]	PRE-STUDY HISTORY OF SMOG SENSITIVITY[2]	HISTORY OF ASTHMA[3]	HISTORY OF ALLERGY[4] PERSONAL	FAMILY
03	3	36	70	185	34	0	0	0	0
07	4	36	68.5	155	0	+	0	+	+
08	5	29	68	157	30*	+	+	+	+
09	4,5	41	74	177	20†	+	+	+	+
10	4,5	30	68	172	0	+	0	0	0
11	3,4	30	72	155	0	0Π	0	0	0
12	3	28	72	166	0	0	0	0	+
13	3	28	74.5	155	0	0	0	+	+
14	3	34	69.5	150	0	0	0	0	0
15	3,4	22	65	128	1*	0Π	0	+	+
16	3,4	30	72	175	7.5*	0Π	0	0	0
17	4,5	36	66	142	0	0	0	0	0
18	5	27	70	150	0	0	0	0	0

Π-Following the chamber study a close re-evaluation of their history suggested that these subjects should be classified as smog sensitive.

* - Still smoking: all others have been non-smokers for at least four years prior to study.

†=now smokes pipe

1 - 1 pack year = 1 pack of cigarettes per day for 1 year.

2 - Defined by symptoms of cough, wheezing, or chest discomfort when outside on high pollution days. (L.A. area)

3 - Defined by spontaneous attacks of bronchospasm requiring bronchoidilator therapy separated by asymptomatic intervals.

4 - Defined by symptoms of wheezing, gastroenteritis, or dermatitis when in contact with specific antigens or a history of chronic rhinitis, post nasal drip, or hayfever.

double the volume of ventilation per minute, as compared to the resting level. Exercise consisted of walking briskly or riding a bicycle ergometer having a work load of 150 to 200 kilogram-meters per minute. Each subject also recorded symptoms and subjective impressions on a standard form. After completing the physiological tests, the subject left the exposure chamber and was immediately examined by the project physician. A venous blood sample was taken for biochemical analysis and the subject was interviewed regarding symptoms according to a standard questionnaire. Symptoms experienced during the remainder of the day were also recorded on a standard form.

Physiological parameters evaluated were forced vital capacity (FVC); one-second forced expiratory volume (FEV_1); partial and maximum forced expiratory flow-volume curves; total respiratory resistance by forced oscillation at 3, 6, 9, and 12 Hz (Rt); closing volume (CV); lung volume estimates, and slope of the alveolar plateau (ΔN_2 per liter) from the single-breath nitrogen test; carboxyhemoglobin (COHb) concentration estimate by breath analysis; and single-breath carbon monoxide diffusing

capacity ($D_{l_{CO}}$). Supplementary physiological tests included plethysmographic thoracic gas volume (TGV) and airway resistance (Raw) (Groups 4 and 5), static (Cst) and dynamic (Cdyn) lung compliance (Group 5), and residual volume (RV) by rebreathing (Group 3). Psychomotor performance was evaluated in Group 4 through measurement of reaction time, heart rate variability, and tracking performance, in a combined central and peripheral tracking task.[4] Data for each exposure group were analyzed using a repeated-measures, one-way analysis of variance. The analysis was designed to detect any statistically significant differences in group performance among three conditions -- sham exposure (control), initial ozone exposure, and second (successive) ozone exposure. Where significant F values were found, the Neuman-Kuels[5] test was used to determine which pair-wise differences contributed to the effect.

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[4] Pedersen, E. E., Patterson, J., and Hackney, J. D., results in preparation.

Results

Group 3. This "normal" group showed decreases, after O_3 exposure, in forced expiratory parameters including FVC, FEV_1, flow rate at 50 percent FVC on maximum and partial forced expiratory flow-volume curves ($\dot{V}_{50}$ and $\dot{V}_{50}$ P, respectively), and flow rates on flow-volume curves at 25 percent FVC ($\dot{V}_{25}$ and $\dot{V}_{25}$ P). Closing volume and closing capacity as measured by the single-breath nitrogen test did not change, but the alveolar nitrogen plateau slope (ΔN_2) increased, indicating less uniform distribution of ventilation with exposure. Residual volume measurement by rebreathing nitrogen dilution[6] gave larger values than were obtained from the single-breath nitrogen RV estimate, but neither measurement indicated significant changes in RV with exposure. Total lung capacity (TLC) decreased due to the decrease in vital capacity. Total pulmonary resistance increased at all oscillation frequencies with exposure.

The group changes in physiological measurements did not achieve statistical significance until the second exposure day in many cases,

and in most cases the decrements in function were markedly worse on the second day. The group changes were primarily due to subjects 11, 13, and 16. The remaining four subjects (including one who had been previously exposed and found nonreactive) showed no changes or very slight changes, and experienced few or no symptoms. Predominant symptoms in the reactive subjects were cough, substernal discomfort, and malaise. Biochemical analysis showed evidence of oxidation changes in erythrocyte and plasma enzymes and increased erythrocyte fragility.[7]

Physiological results for Group 3 are summarized in Table 2 and Figure I.

Group 4. This was a mixed group including one subject unreactive by history and previous testing (No. 15), one subject unreactive by history but not previously tested (No. 17), two subjects unreactive by history but reactive by previous testing (Nos. 11 and 16), and three subjects reactive by history and previous testing (Nos. 07, 09, and 10). Ozone exposure concentration was 0.25 ppm, or half that used previously. No consistent physiological changes attributable to exposure effects and

TABLE 2

MEAN VALUES AND RESULTS OF VARIANCE ANALYSIS FOR PHYSIOLOGICAL PARAMETERS, GROUP 3 (0.50 ppm O_3 EXPOSURE)

dF = degrees of freedom; NS = change not significant at .05 level; * = significant change from control, p <.05; ** = significant change from control, p <.01

PARAMETER	CONTROL	EXPOSURE 1	EXPOSURE 2	F	dF	P
FVC	5.15	5.03	4.75**	6.46	2,38	<.01
FEV_1	4.46	4.25	3.86**	7.58	2,38	<.01
$\dot{V}_{max}$	12.0	11.7	10.9 *	3.52	2,38	<.05
$\dot{V}_{50}$	5.95	5.73	4.84**	7.33	2,34	<.01
$\dot{V}_{25}$	2.69	2.39*	2.20**	6.86	2,34	<.01
$\dot{V}_{50}P$	6.42	6.06	5.12**	13.5	2,34	<.01
$\dot{V}_{25}P$	2.68	2.54	2.21**	6.68	2,38	<.01
CC	1.85	1.81	1.86	<1.0	2,38	N.S.
ΔN_2	0.68	0.75	1.04*	4.64	2,38	<.05
TLC (single-br.)	6.64	6.44	5.97**	5.29	2,38	<.01
RV (single-br.)	1.41	1.32	1.38	2.42	2,38	N.S.
RV(rebreathing)	1.66	1.66	1.70	<1.0	2,40	N.S.
Rt (6 Hz.)	3.39	3.52	4.77**	5.39	2,98	<.01
$D_{l_{co}}$	39.2	38.8	not available			

TABLE 3

MEAN VALUES AND RESULTS OF VARIANCE
ANALYSIS FOR PHYSIOLOGICAL PARAMETERS, GROUP 4

Exposure Conditions: O = 0.25 ppm O_3; N = 0.3 ppm NO_2; C = 30 ppm CO

PARAMETER	EXP. CONDITION	CONTROL	EXPOSURE 1	EXPOSURE 2	F	dF	P
FVC	O	4.82	4.80	4.79	<1.0	2,40	N.S.
	ON	4.88	4.88	5.01**	6.85	2,32	<.01
	ONC	4.66	4.64	4.68	<1.0	2,32	N.S.
FEV_1	O	3.94	3.95	3.96	<1.0	2,38	N.S.
	ON	3.94	3.96	4.03*	4.09	2,30	<.05
	ONC	3.77	3.74	3.79	1.67	2,32	N.S.
$\dot{V}_{50}$	O	4.66	4.69	4.84	1.50	2,12	N.S.
	ON	4.63	4.47	4.48	<1.0	2,10	N.S.
	ONC	4.58	4.58	4.63	<1.0	2,10	N.S.
$\dot{V}_{25}$	O	2.01	2.07	2.10	<1.0	2,12	N.S.
	ON	1.91	1.85	1.87	<1.0	2,10	N.S.
	ONC	1.86	1.77	1.92	<1.0	2,10	N.S.
$\dot{V}_{50}P$	O	5.12	4.91	5.10	1.45	2,12	N.S.
	ON	4.67	4.62	4.92	2.55	2,10	N.S.
	ONC	4.91	4.93	4.83	<1.0	2,10	N.S.
$\dot{V}_{25}P$	O	2.11	2.07	2.10	<1.0	2,12	N.S.
	ON	1.91	1.85	1.93	<1.0	2,10	N.S.
	ONC	1.87	1.78	1.92	<1.0	2,10	N.S.
CC	O	1.88	1.85	1.77**	10.7	2,40	<.01
	ON	1.98	1.93	1.93	<1.0	2,34	N.S.
	ONC	1.63	1.64	1.65	<1.0	2,34	N.S.

dF = degrees of freedom; NS = change not significant at .05 level; * = significant change from control, p <.05; ** = significant change from control, p <.01

TABLE 3
(continued)

PARAMETER	EXP. CONDITION	CONTROL	EXPOSURE 1	EXPOSURE 2	F	dF	P
ΔN_2	O	0.70	0.68	0.66	<1.0	2,40	N.S.
	ON	0.75	0.74	0.72	<1.0	2,34	N.S.
	ONC	0.72	0.75	0.72	<1.0	2,32	N.S.
RV (single-br.)	O	1.30	1.37	1.32	2.13	2,40	N.S.
	ON	1.40	1.38	1.36	1.74	2,34	N.S.
	ONC	1.15	1.11	1.11	2.01	2,34	N.S.
TLC (single-br.)	O	6.27	6.25	6.33	1.34	2,40	N.S.
	ON	6.50	6.53	6.66**	7.91	2,34	<.01
	ONC	6.16	6.14	6.12	<1.0	2,34	N.S.
Rt (6 Hz.)	O	3.25	3.83**	3.07	7.11	2,94	<.01
	ON	3.33	3.50	3.28	<1.0	2,82	N.S.
	ONC	3.35	3.65	3.54	<1.0	2,80	N.S.
$D_{1_{co}}$	O	40.4	42.0	34.2*	4.61	2,40	<.05
	ON	45.0	36.6**	39.5*	6.27	2,34	<.01
	ONC	36.2	38.4	38.0	<1.0	2,32	N.S.

did not change significantly with exposure, but Rt was increased on both exposure days, relative to control values. Symptoms reported by Subject No. 08 were similar to those in the reactive subjects of Group 3. The remaining subjects most frequently reported upper-airway irritation and coughing. Oxidative blood biochemical changes were detected in this group, but were not as severe as in groups exposed to 0.5 ppm O_3.

Physiological results for Group 5 are summarized in Table 4 and Figure V.

TABLE 4

MEAN VALUES AND RESULTS OF VARIANCE ANALYSIS FOR PHYSIOLOGICAL PARAMETERS, GROUP 5 (0.37 ppm O_3 EXPOSURE)

dF = degrees of freedom; NS = change not significant at .05 level; * = significant change from control, $p < .05$; ** = significant change from control, $p < .01$

PARAMETER	CONTROL	EXPOSURE 1	EXPOSURE 2	F	dF	P
FVC	5.37	5.29	5.35	<1.0	2,8	N.S.
FEV_1	4.29	4.19	4.24	1.11	2,8	N.S.
$\dot{V}_{50}$	4.94	4.74	4.68	<1.0	2,8	N.S.
$\dot{V}_{25}$	2.15	1.9	1.9	(not available)		
$\dot{V}_{50}P$	5.49	4.66	4.50	3.03	2,8	N.S.
$\dot{V}_{25}P$	2.18	1.86	1.86	10.3	2,8	N.S.
CC	1.97	2.04	2.02	<1.0	2,8	N.S.
ΔN_2	0.80	0.79	0.81	<1.0	2,28	N.S.
TLC (single-br.)	6.81	6.66	6.75	<1.0	2,26	N.S.
Raw (FRC)	1.40	1.55	1.48	<1.0	2,28	N.S.
Rt (6 Hz.)	3.24	4.01**	3.88**	7.25	2,26	<.01
Cdyn (20/min)	0.282	0.288	0.290	<1.0	2,80	N.S.
Cdyn (100/min)	0.136	0.130	0.129	<1.0	2,98	N.S.

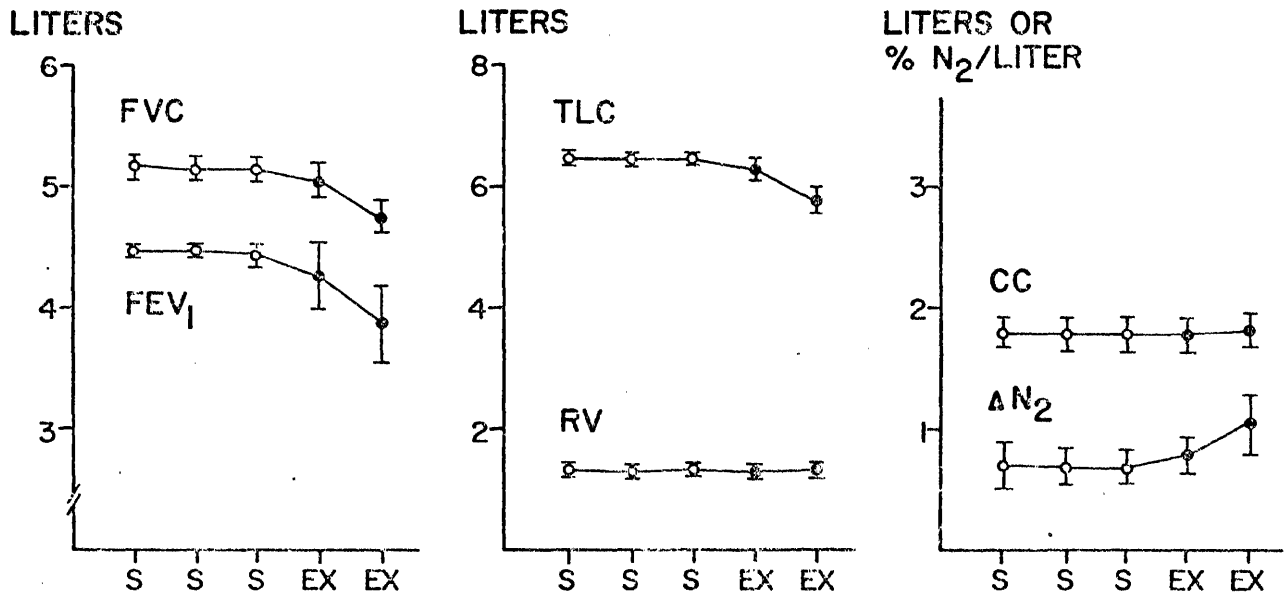

FIGURE I

Daily variation of pulmonary parameters in Group 3, mean ± one standard error. S = sham exposure, EX = 0.5 ppm O_3 exposure. TLC, RV, CC, and delta nitrogen calculated from single-breath nitrogen tracings.

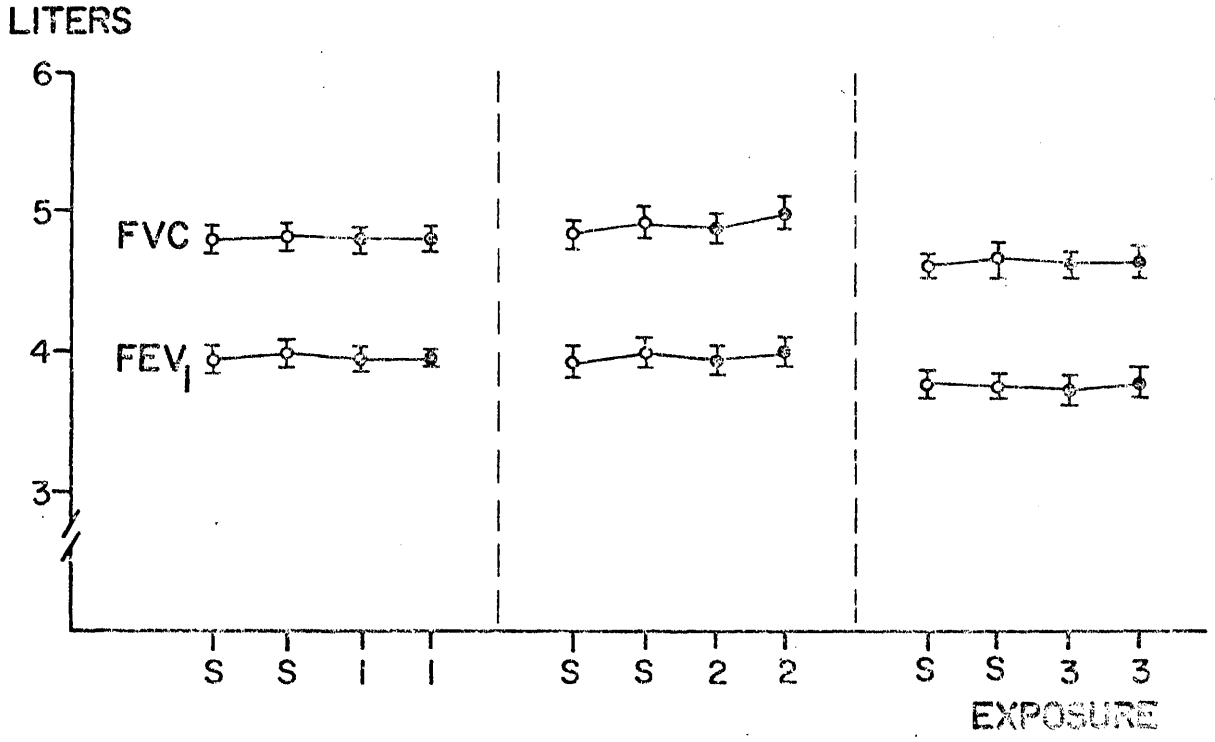

FIGURE II

Daily variation of forced expiratory parameters in Group 4, mean ± one standard error. Exposure conditions: S = sham; 1 = 0.25 ppm O_3; 2 = 0.25 ppm O_3 + 0.30 ppm NO_2; 3 = 0.25 ppm O_3 + 0.30 ppm NO_2 + 30 ppm CO. Successive weeks of study are separated by broken lines. Subject 09 absent week 3; subject 11 absent week 2.

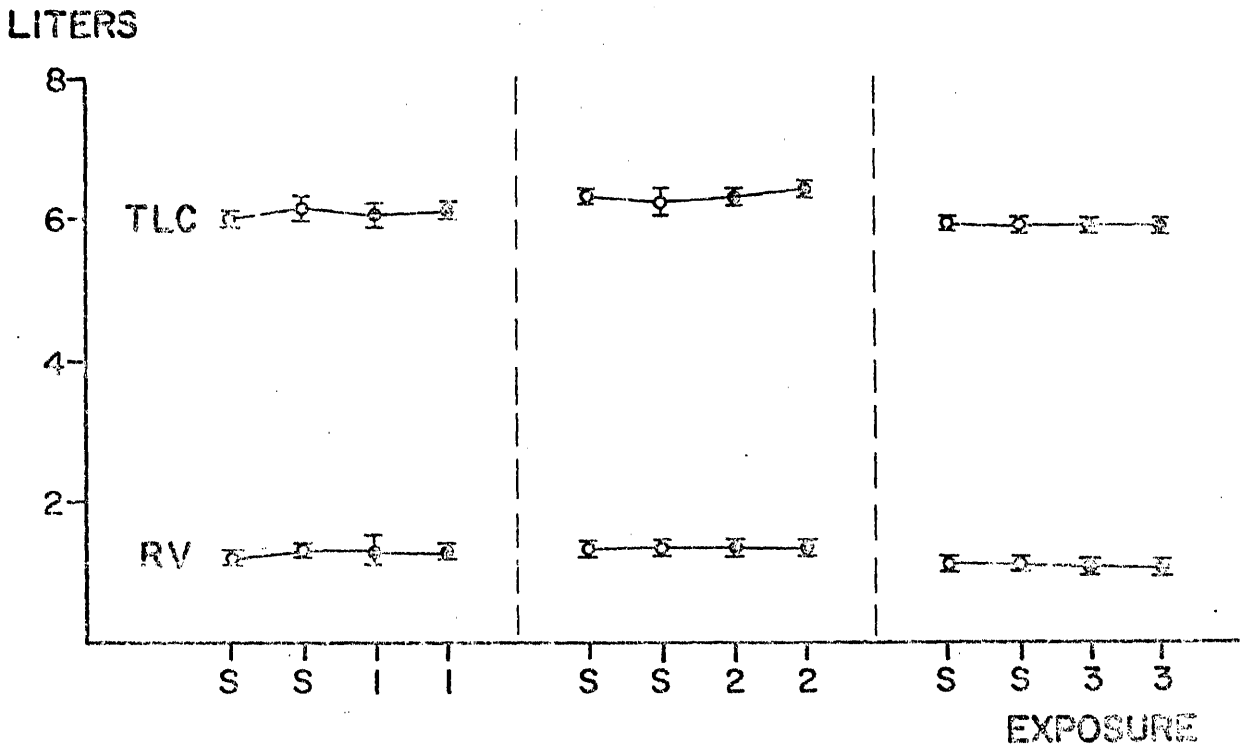

FIGURE III

Daily variation of lung volumes, calculated from single-breath nitrogen tracings, in Group 4, mean ± one standard error (See Figure II for explanation).

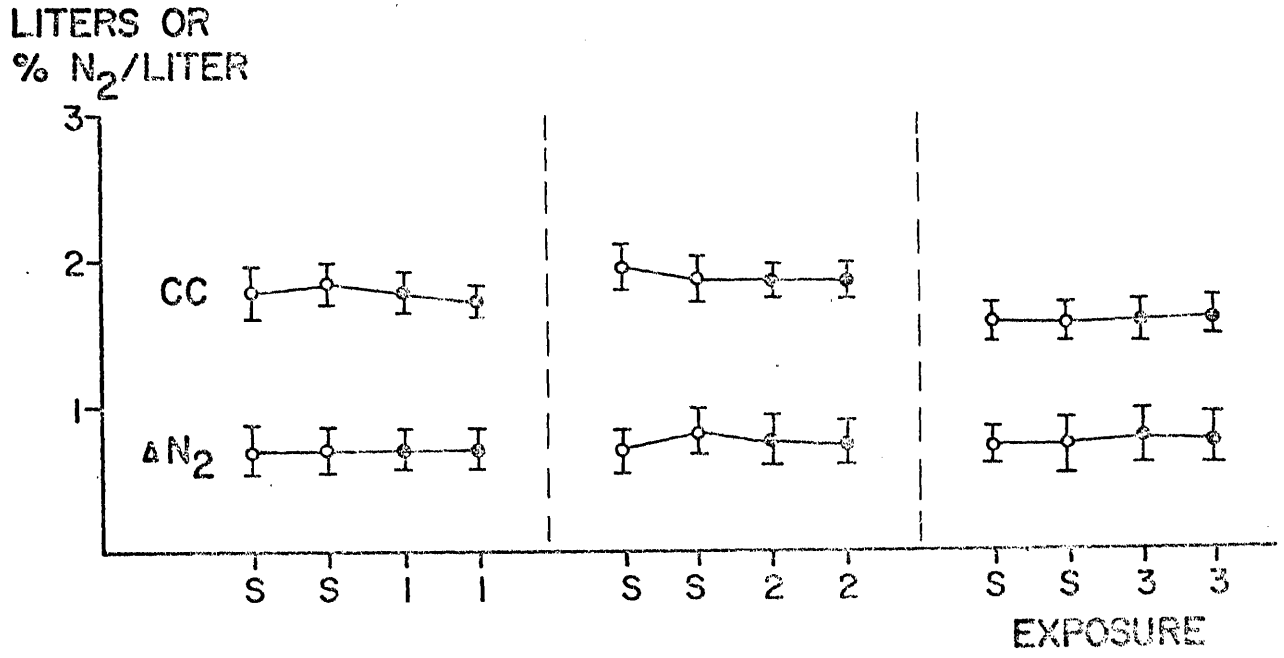

FIGURE IV

Daily variation of closing capacity and delta nitrogen in Group 4, mean ± one standard error (See Figure II for explanation).

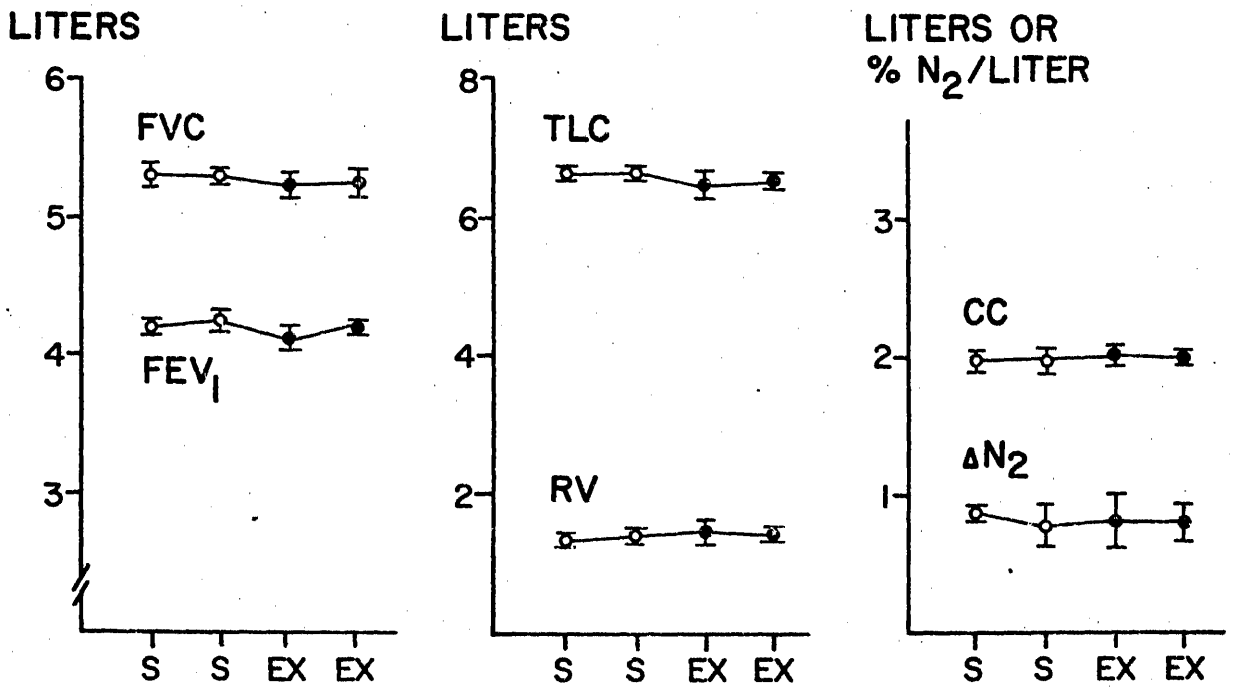

FIGURE V

Daily variation of pulmonary parameters in Group 5, mean ± one standard error. S = sham exposure; EX = 0.37 ppm O_3 exposure. TLC, RV, CC, and delta nitrogen calculated from single-breath nitrogen tracings.

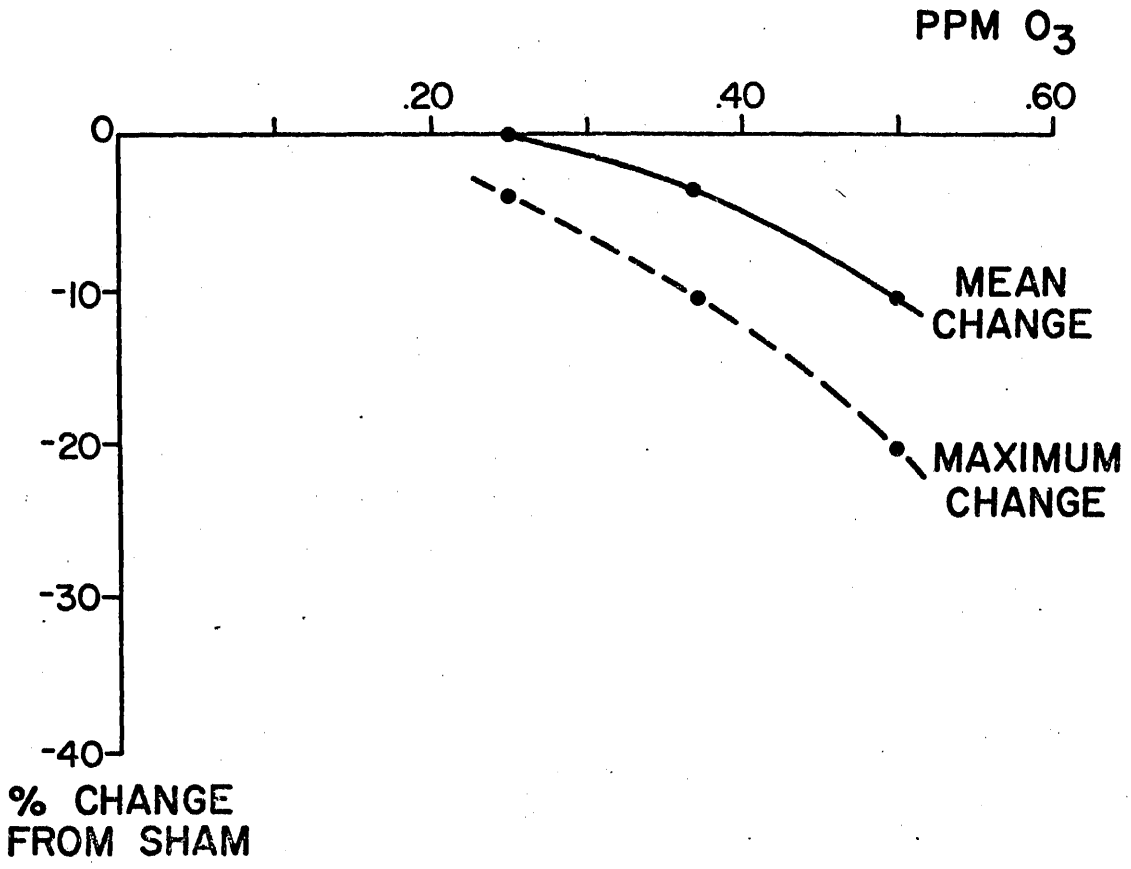

FIGURE VI

Dose-response behavior of FEV_1 in subjects exposed to O_3. Mean and maximum changes from control values observed in all subjects tested at given concentrations.

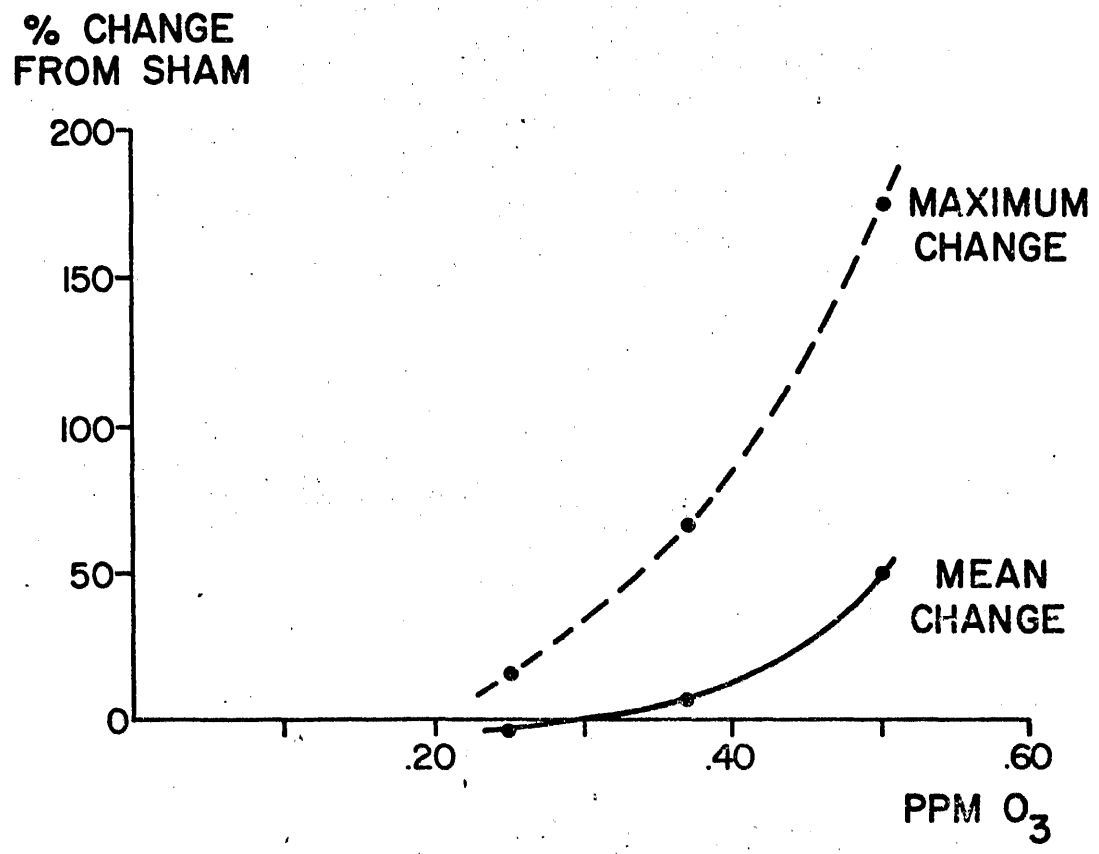

FIGURE VII

Dose-response behavior of delta nitrogen in subjects exposed to O_3. Mean and maximum changes from control values observed in all subjects tested at given concentrations.

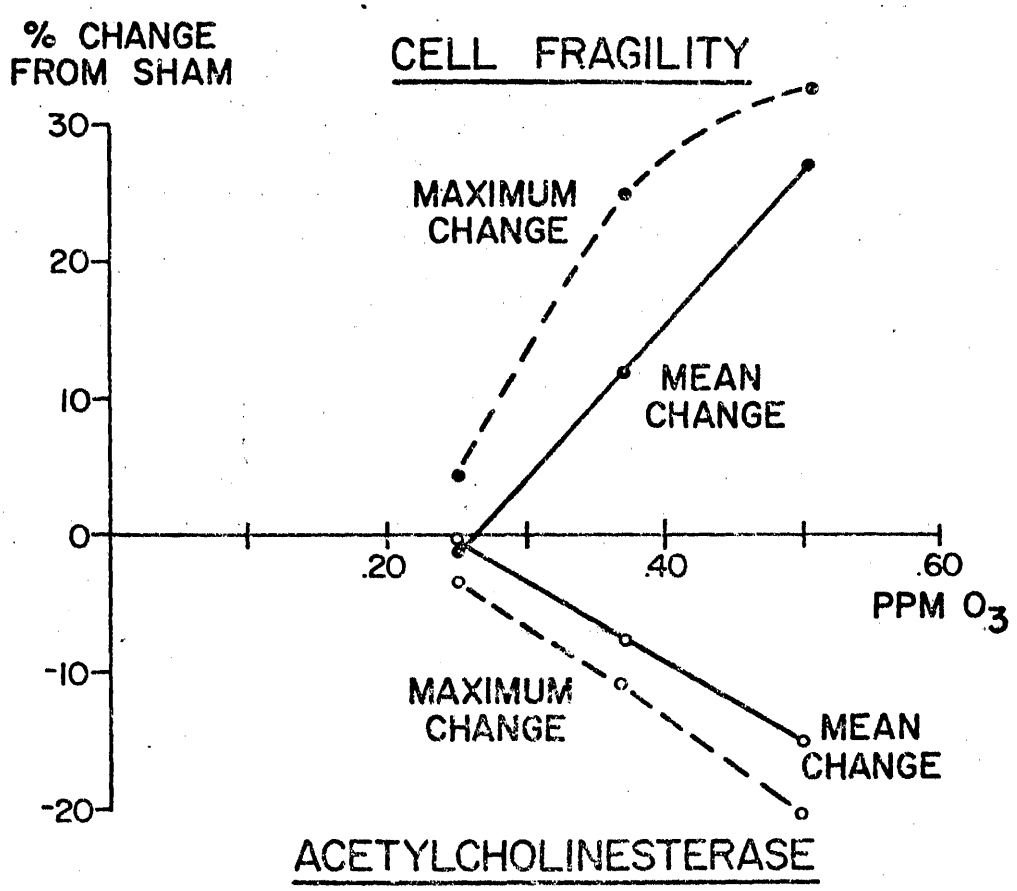

FIGURE VIII

Dose-response behavior of selected erythrocyte parameters in subjects exposed to O_3. Mean and maximum changes from control values observed in all subjects tested at given concentrations. Black circles = osmotic fragility of cells; open circles = cell membrane acetylcholinesterase level.

DISCUSSION

The foregoing results together with those reported previously from this laboratory[4] allow formulation of dose-response curves for effects of ozone exposure (Figures VI - VIII). Responses are expressed in terms of changes in the more stable physiological and biochemical parameters. Equating these observed responses with "significant deleterious effects on health" is not completely straightforward, since the underlying mechanisms are not fully understood. However, from a practical standpoint the observed physiological changes may reasonably be considered to represent significant health effects, since (a) the changes are qualitatively similar to those observed in certain pulmonary disease states, and (b) in studies to date, significant physiological changes have always been accompanied by significant clinical illness (respiratory symptoms severe enough to inhibit normal activity). While biochemical changes have been observed in asymptomatic as well as symptomatic subjects, the dose-response curves for those

biochemical parameters expected to show an immediate response to oxidant exposure are remarkably similar to the curves for the physiological parameters.

The given dose-response curves are to be considered first approximations only since they are based on small samples, neglect differences in exposure time, and do not distinguish between initial and cumulative exposures. Two methods have been used for deriving curves from individual dose-response data. In the first, a best-fit straight line is derived from all individual data points using the method of least squares. In the second, the mean observed response is plotted for each concentration studied and a smooth curve drawn through the resulting three points (Figures VI - VIII). A "maximum response" curve is obtained similarly by plotting for each concentration the response of the most reactive individual studied at that concentration. For the physiological parameters, the maximum individual response is taken as the difference between the mean of all measurements made during the exposure in which the subject showed the most severe reaction, and the mean of all control

measurements for the same week. For the biochemical parameters, only one measurement can be made per exposure, so the maximum response is taken as the largest individual percentage difference between an exposure value and the immediately preceeding control value. The mean dose-response curves generated by either of the above methods suggest a "zero-effect threshold" concentration of 0.25 to 0.30 ppm. This level is exceeded for one hour or more at least 20 days per year in parts of the Los Angeles area[8] and is not uncommon in other metropolitan areas, such as Toronto.[9] Since the exposure conditions which were studied simulate light, outdoor, physical work, a significant and widespread public-health risk related to ozone or other photochemical oxidant pollution is implied. Furthermore, many individuals are considerably more sensitive than the average, and thus may be at risk at significantly lower levels. The degree of risk to populations not studied, such as children, the elderly, or chronic pulmonary disease patients remains to be determined.

BIBLIOGRAPHY

1. Bates, D. V., G. Bell, C. Burnham, M. Hazucha, J. Mantha, L. D. Pengelly, and F. Silverman: Short-term effects of ozone on the lung. *J Appl Physiol*, 32: 176, 1972.

2. Hazucha, M., F. Silverman, C. Parent, S. Field, and D. V. Bates: Pulmonary function in man after short-term exposure to ozone. *Arch Environ Health*, 27: 183, 1973.

3. Hackney, J. D., W. S. Linn, R. D. Buckley, E. E. Pedersen, S. Karuza, D. C. Law, and D. A. Fischer. Experimental studies on health effects of air pollutants: I. Design Considerations. (submitted to *Am Rev Resp Dis*) 1974.

4. Hackney, J. D., W. S. Linn, J. G. Mohler, E. E. Pedersen, P. Breisacher, and A. Russo: Experimental studies on human health effects of air pollutants: II. Four-hour exposure to ozone alone and in combination with other pollutant gases. (submitted to *Am Rev Resp Dis*) 1974.

5. Winer, B. J.: Statistical principles in experimental design. McGraw-Hill, 1962.

6. Rahn, H., W. O. Fenn, and A. B. Otis: Daily variations of vital capacity, residual air, and expiratory reserve, including a study of the residual air method. *J Appl Physiol.*, 1: 725, 1949.

7. Buckley, R. D., J. D. Hackney, K. Clark, and C. Posin: Some effects of ozone inhalation on human erythrocyte metabolism. *Fed Proc.*, 33(3): 334, March 1974.

8. California Air Resources Board: Ten-year summary of California air quality data. Sacramento, 1974.

9. Ontario Air Pollution Control Department. 1971 - Quoted Proceedings "Canadian Conference on Air Pollution" Gravenhurst, Ontario, June 1974.