UC Irvine

UC Irvine Previously Published Works

Title

Enhancement of ozone-induced lung injury by exercise.

Permalink

<https://escholarship.org/uc/item/47z5n96v>

Journal

Journal of toxicology and environmental health, 16(6)

ISSN

0098-4108

Authors

Mautz, WJ
McClure, TR
Reischl, P
et al.

Publication Date

1985

DOI

10.1080/15287398509530792

Copyright Information

This work is made available under the terms of a Creative Commons Attribution License, available at

<https://creativecommons.org/licenses/by/4.0/>

Peer reviewed

ENHANCEMENT OF OZONE-INDUCED LUNG INJURY BY EXERCISE

William J. Mautz, Thomas R. McClure, Peter Reischl,
Robert F. Phalen, T. Timothy Crocker

Air Pollution Health Effects Laboratory, Department of
Community and Environmental Medicine, University of
California, Irvine, California

Rats were exposed for up to 3.75 h to 0.20–0.80 ppm O₃ under conditions of rest and treadmill exercise up to 30 ml/min, 20% grade, to assess the importance of exposure duration, O₃ concentration, and exercise on lung tissue injury. Focal lung parenchymal lesions increased in abundance and severity in response to the three variables; however, exercise was the most important. Lesion response to exercise was greater than that predicted by a simple proportion to estimated effective dose of O₃. The results emphasize the importance of including exercise in assessment of possible adverse health effects of exposure to airborne pollutants.

INTRODUCTION

The dose rate of an inhaled pollutant is elevated by exercise due to increased respiratory ventilation; however, toxic effects may be further enhanced during exercise ventilation because of deeper penetration and redistribution of inhaled compounds in lung tissues (Silverman et al., 1976; DeLucia and Adams, 1977). Ozone is a prominent component of photochemical air pollution, and its toxic properties are well documented. Humans experimentally exposed to ozone at concentrations observed in urban air pollution exhibit decreases in maximum work performance and decrements in pulmonary function, including development of rapid-shallow breathing patterns and declines in timed forced expiratory volumes (Bates et al., 1972; Hazucha et al., 1973; Follinsbee et al., 1975, 1977, 1978; Silverman et al., 1976; DeLucia and Adams, 1977; Adams et al., 1981; Adams and Schelegel, 1983; McDonnell et al., 1983). Pulmonary-function decrements observed following exposures during exercise are greater than for exposures to the same concentrations at rest. For example, ozone concentrations as low as 0.37 ppm can produce effects during exercise that are not observed for

This investigation was supported by the California Air Resources Board (AO-129-32, A2-129-33) and the Electric Power Research Institute (RP 1962-1).

We thank C. Beaucage, S. Bucher, C. Bufalino, D. Daniels, P. Diller, J. Kenoyer, T. Kurosaki, R. Lejniaks, and T. Nguyen for technical assistance, J. Davis and J. Whittenberger for reviewing the manuscript, and B. Ulery for typing the manuscript.

Requests for reprints should be sent to William J. Mautz, Department of Community and Environmental Medicine, University of California, Irvine, California 92717.

that concentration at rest (Bates et al., 1972; Hazucha et al., 1973; Silverman et al., 1976). Particularly sensitive subjects have been reported to respond to ozone concentrations as low as 0.15 ppm inhaled during heavy exercise (DeLucia and Adams, 1977). It is apparent that ozone influences exercise performance, induces an alteration in the normal pattern of ventilation, and can reduce maximum work performance.

Lung tissue damage from acute ozone inhalation has been documented in detail by histopathological studies of rodents and dogs exposed at rest for periods ranging from a few hours to several days (Stephens et al., 1973, 1974a,b,c, 1978; Schwartz et al., 1976; Shami et al., 1982; Last et al., 1983; Crapo et al., 1984). Alveolar ducts and alveoli are primary sites of injury. Exposure to ozone concentrations less than 1 ppm is associated with focal lesions in which alveoli contain shed type 1 epithelial cells as well as macrophages and inflammatory cells (lymphocytes and polymorphonuclear leukocytes) that enter the site of injury to remove killed cells. Alveolar duct walls and alveolar septae may also become thickened by edema fluid containing plasma proteins that pass from capillaries into the interstitial space within duct walls and septae. These responses typically begin within hours of initial exposure, and for single exposures the subsequent repair of tissue damage proceeds over a period of several days (Stephens et al., 1973, 1974a,b,c, 1978; Boatman and Frank, 1974; Shami et al., 1982). Rodents exposed to ozone while exercising demonstrate increased toxic responses, including reduction in lethal ozone concentration, increased susceptibility to bacterial infection, and increased lung tissue levels of reduced glutathione, a biochemical index of oxidant exposure (Stokinger et al., 1956; Gardner et al., 1974; Fukase et al., 1978).

Exercise is a common part of everyday life, and it is important to consider the increased response resulting from exposure during exercise to urban air pollution. In humans and animals, exercise has been shown to modify pulmonary responses to ozone exposure, but there have not been studies of the direct effects of exercise exposure respiratory tissue damage. We used a simple quantitative histopathologic technique for comparing ozone damage in rats under different experimental conditions of exercise. The purpose of this study was to quantify the influence of different conditions of exercise exposure on lung-tissue injury induced by ozone. Single exposures of rats were performed to compare responses to varying concentrations of ozone during rest and exercise of varying duration and intensity.

MATERIALS AND METHODS

Treadmill Exposure System

The exposure system, described in detail elsewhere (Mautz et al., 1985), was constructed from a modified 10-runway Quinton 42-15 ro-

dent treadmill with control of both speed and grade of running. The treadmill was enclosed to contain the exposure atmosphere. Inner surfaces of the 10 runways were lined with stainless-steel sheet to minimize reaction with ozone, and stainless-steel baffles and screens (1.3 cm mesh) at inlet ports were added to distribute the atmosphere stream uniformly in the runways and prevent rats from contacting inlet ports. Ducts at the rear of the treadmill exhausted the atmosphere to an outdoor vent. Average oxygen consumption ($\dot{V}_{O_2}$) of exercising rats was determined by measurement of atmosphere flow through the treadmill using a laminar flow meter (Fleisch number 4, Dynascience, Blue Bell, Pa.), and analysis of metabolic gas fractions upstream and downstream of the treadmill using a mass spectrometer (Perkin-Elmer model 1100, Pomona, Calif.), and application of standard open-flow respirometry equations (Mautz et al., 1985). The $\dot{V}_{O_2}$ of exercising rats was measured 5 times over each 5-min interval during the exposures.

Exposure atmospheres were produced by mixing ozone with clean air and delivering the mixture to each runway of the treadmill. Air was initially compressed and purified by passage through a bed of aluminosilicate spheres impregnated with $KMnO_4$ (Purafil, Atlanta, Ga.) and a Del-Monix gas scrubbing filter (Deltech, Newcastle, Del.), then decompressed, passed through a HEPA (high-efficiency particulate absolute) filter, and humidified by controlled injection of water vapor to 40% relative humidity. Ozone was produced by passing medical-grade oxygen through an electrostatic discharge ozone generator (Sander Ozonizer, Type III, Osterberg, West Germany) and diluting it into the humidified air stream. The atmosphere was passed to a 1-m³ stainless-steel chamber where initial exposures of rats at rest were performed. For exercise exposure experiments, the test atmosphere was conducted from the chamber through paired Teflon-lined flexible ducts to a stainless-steel manifold, which supplied the treadmill runways. Samples of the exposure atmosphere were repeatedly drawn from each runway upstream of the stainless-steel screens, and ozone concentration was measured throughout the exposures by ultraviolet spectrophotometry in a Dasibi model 1003-AH ozone analyzer (Glendale, Calif.). Ozone analyzers were calibrated before and after exposure with a Dasibi calibrator, and periodically instruments were calibrated using an ultraviolet photometric standard at the Dasibi factory and at the California Air Resources Board El Monte laboratory.

Histopathology

Rats deeply anesthetized with sodium pentobarbital (250 mg/kg) were killed by exsanguination through the abdominal aorta. The trachea and lungs were removed and fixed by airway perfusion with 10% neutral-buffered formalin at 30 cm fluid pressure for 72 h (McClure et al., 1982). Lung injury resulting from ozone exposures was quantified in

a procedure similar to cascade level 1 stratified sampling analysis of parenchymal lesions (Last et al., 1983). Apical, middle, and caudal lobes of the right lung were separated and cut along the main lobar bronchus. A block containing a half lobe was prepared from the caudal lobe by cutting in a plane parallel to the center line of the lobar bronchus. Paraffin-imbedded tissues were sectioned at 6 μm and stained with hematoxylin and eosin. Total parenchymal cross-section area of a lung section from each rat was measured using a dissecting microscope with a 100-square ocular grid calibrated with a stage micrometer. Large bronchi and vessels were excluded, and total parenchymal area was recorded for calculating percent of the area involved in lesions. The section was then examined systematically with a compound microscope using an ocular grid whose subdivisions covered a region of 0.1×0.1 mm and included sections of about 3–5 alveoli. If any grid space contained part or all of a lesion, the whole space was recorded as a lesion. The sum of lesion areas divided by the total parenchymal area provided an estimate of percent parenchyma occupied by lesions. Lung lesions had a focal distribution and varied in severity. The simplest lesions were foci of three to five alveoli in which one to five cells were found in alveolar spaces. Other lesions had similar cellular deposits associated with thickening of alveolar septa by increased numbers of nuclei and eosinophilic material denoting inflammation and interstitial edema. Lesion types were defined based on these morphological differences as follows:

Type I. Free cells of any type in alveolar spaces with no apparent change in septal walls. Control animals exhibited these features to a small degree (1–3% cross-section area); however, the finding was categorized as a lesion because the incidence increased following exposure to ozone.

Type II. Alveolar duct walls and alveolar septa thickened due to infiltrating cells. Free cells may or may not be present.

Histopathologic analyses were blind, as the reader did not know exposure histories. Rats received from the supplier occasionally showed evidence of pulmonary infections consisting of peribronchial lymphocytic follicles or perivascular infiltrates of inflammatory cells. Lung sections were graded by infection criteria, and animals exhibiting perivascular cuffing associated with interstitial inflammatory disease were excluded from the analysis. Approximately 10% of the animals in control and ozone-exposed groups had to be rejected, and the rejection rate was not correlated with ozone exposure. In rare cases, tissue preparations were rejected because of the presence of a lung tumor or accidental puncture during dissection.

Following exposure, rats were sacrificed when the injury and repair

response was maximally visible. To determine the best postexposure time for assessment, an initial sample of 20 rats was exposed for 4 h at rest to 0.8 ppm O_3 , and groups of 4 animals were sacrificed at 0, 1, 2, 4, and 8 d postexposure. Parenchymal lesion areas reached a peak 2 d after ozone exposure, and recovery was essentially complete by 8 d. A 2-day postexposure time period before sacrifice was then adopted for all other analyses. A second test established that sections sampled from different lung lobes of the same animals did not exhibit significant differences in measured lesion areas. Single sections from upper, middle, and caudal lobes of animals exposed for 4 h to 0.8 ppm ($n = 4$) or 1.2 ppm O_3 ($n = 4$) were compared. The effect of O_3 concentration was highly significant for both type I ($F = 14.6$, $p < 0.005$) and type II ($F = 10.3$, $p < 0.005$) lesion areas, but there was no significant effect of lung lobe, nor was there significant interaction (two-way ANOVAS, analyses of variance).

Animals, Training, and Exposure Protocol

Experimental animals were barrier-reared Sprague-Dawley rats (Hilltop Lab Animals, Inc., Scottsdale, Pa.). The animals were shipped in filter barrier containers, and housed at the laboratory in isolation units supplied with Purafil-scrubbed and HEPA-filtered air. Rats were held in wire-bottomed cages over beds of rock salt, which dried the feces and urine and suppressed ammonia production. Rats were received at age 6 wk and held at the laboratory for 1 wk before exposure. Exercise exposures were preceded by 3 d of training runs on the treadmill. Training was designed to acquaint the animals with the treadmill apparatus and establish that they could complete the experimental exposure protocols. A few individuals that were unable to complete the training program could be identified on the first day and were excluded from the experiment. Training for intermittent exercise exposures consisted of a progressive exercise program beginning on d 1 with 5 min of running at 10.7 m/min alternating with 5 min of rest for 1 h. On d 2 rats alternated 15 min of running with 15 min of rest for 1.5 h, and on d 3 they alternated 30 min running with 15 min rest for 2.75 h. Training protocol for continuous exercise exposures was: d 1, continuous running for 3.0 h at 8 m/min, 20% grade; d 2, 30 min running at 15 m/min, 20% grade alternating with 5 min rest for 2.9 h; and d 3, continuous running for 3.0 h at 15 m/min, 20% grade. Trained animals were randomly assigned to experimental groups.

Four exercise exposure experiments under different exercise conditions were performed:

1. Effects of ozone exposure during rest and intermittent exercise at two exercise durations. Exposure groups were:
 - a. Clean air control, exercise schedule 2: alternate 30 min running

- at 10.7 m/min, 0% grade with 15 min rest for 3.75 h (total time at rest 75 min, total time running 150 min).
- b. O₃ exposure, continuous rest in the treadmill, 3.75 h.
 - c. O₃ exposure, exercise schedule 1: alternate 15 min running at 10.7 m/min, 0% grade with 30 min rest for 3.75 h (total time at rest 150 min, total time running 75 min).
 - d. O₃ exposure, exercise schedule 2: alternate 30 min running at 10.7 m/min, 0% grade with 15 min rest for 3.75 h (total time at rest 75 min, total time running 150 min).

Ozone concentrations were 0.20 ± 0.02 ppm and 0.38 ± 0.02 ppm. Oxygen consumption ($\dot{V}_{O_2}$) of rats was measured in the 0.20 ppm exposure.

2. Effect of ozone concentration during continuous exercise at 10.7 m/min level grade, for 3.75 h. exposure groups were (a) clean air control, (b) 0.20 ± 0.02 ppm O₃, (c) 0.35 ± 0.02 ppm O₃, (d) 0.50 ± 0.02 ppm O₃, and (e) 0.80 ± 0.05 ppm O₃.

3. Effect of exposure duration during continuous exercise at 15 m/min, 20% grade. Ozone concentration was 0.35 ± 0.02 ppm. Exposure groups were (a) clean air control, 3.0 h, (b) O₃ exposure for 0.5 h (c) O₃ exposure for 1.0 h, (d) O₃ exposure for 2.0 h, and (e) O₃ exposure for 3.0 h.

4. Effect of exercise intensity during exposure to 0.35 ± 0.02 ppm O₃. Rat groups exercised at different work rates up to 30 m/min, 20% grade, $\dot{V}_{O_2}$ was measured, and exposure duration adjusted to equalize estimated effective doses of ozone by fixing the quantity (O₃ concentration) · (duration) · (average $\dot{V}_{O_2}$). Because the duration of the exposure at highest intensity exercise was likely to be limited by the stamina of the rats, the highest work rate group was tested first. This group was given brief (0.13 h) rest periods at 0.5 h intervals, and the exposure was terminated when the rats became exhausted. In subsequent exposures of other groups at lower exercise intensity, sample $\dot{V}_{O_2}$ measurements during the exposures were used to predict durations that would give the same effective dose as the highest intensity exercise group. Effective dose estimates were subsequently calculated based on all recorded concentration, $\dot{V}_{O_2}$, and duration data. Exposure groups were:

- a. Clean air control, $\dot{V}_{O_2}$ measurements during rest and running at exercise levels listed below.
- b. O₃ exposure, continuous rest, 3.42 h.
- c. O₃ exposure, running 8 m/min, 0% grade for 2.75 h.
- d. O₃ exposure, running 15 m/min, 20% grade for 2.33 h.
- e. O₃ exposure, running 30 m/min, 20% grade for 1.5 h with 2 intervening rest periods totaling 0.25 h.

Parenchymal lesion areas resulting from the exposures were ana-

lyzed with analysis of variance or stepwise linear regression (Dixon and Brown, 1979).

RESULTS

Parenchymal lesion areas induced by ozone in rest and intermittent exercise exposures are shown in Table 1. Running at 10.7 m/min approximately doubled $\dot{V}_{O_2}$ over resting rates (Mautz et al. 1985), and under the two intermittent exercise schedules tested, average $\dot{V}_{O_2}$ measured during the 0.2-ppm O_3 exposures was increased by a factor of 1.37 in exercise schedule 1 and 1.60 in exercise schedule 2. Exercise was strongly associated with elevated lesion areas in all exposures (Table 2), and in many cases the duration of exercise (schedule 2 versus schedule 1) also had a significant effect on lesion areas. At 0.20 ppm ozone, exposure at rest did not induce type II lesions or significant change in type I lesion areas; at this concentration type II lesions were found only in rats running on exercise schedule 2.

The effect of ozone concentration during continuous exercise exposure at 10.7 m/min for 3.75 h is shown in Table 3. Stepwise regression analysis (Dixon and Brown, 1979) using concentration raised to the powers 1, 2, and 3 as independent variables resulted in linear relationships of lesion areas to concentration: type I = $1.19 + 5.16$ (concentration), $r = 0.77$, $F = 33.8$, $p < 0.001$; type II = $-0.93 + 8.91$ (concentration), $r = 0.82$, $F = 58.3$, $p < 0.001$. Injury from intermittent exercise

TABLE 1. Lung Parenchymal Lesions Induced by 3.75-h Ozone Exposures of Resting and Intermittently Exercising Rats^a

		Average $\dot{V}_{O_2}$ (ml/kg · min)	Type I lesion (area %)			Type II lesion (area %)		
			Mean	SD	<i>n</i>	Mean	SD	<i>n</i>
0.20 ppm O ₃								
Clean air control,								
exercise schedule 2	37.1	1.6	0.6	5	0.0			5
Continuous rest	24.6	2.0	1.2	4	0.0			4
Exercise schedule 1	33.7	3.4	1.6	5	0.0			5
Exercise schedule 2	39.4	4.8	0.9	6	1.2	1.2		6
0.38 ppm O ₃								
Clean air control,								
exercise schedule 2		1.1	0.5	8	0.0			8
Continuous rest		2.2	0.6	9	0.3	0.5		9
Exercise schedule 1		4.3	1.4	9	1.5	0.8		9
Exercise schedule 2		5.4	1.6	8	3.9	2.4		8

^a Total exposure duration was 3.75 h and exposure groups differ in time duration of exercise (10.7 m/min, 0% grade) versus rest (see text).

TABLE 2. Significance Tests (One-Way ANOVA) for Ozone and Exercise Effects on Parenchymal Lesion Areas

Experiment, a priori test	Type I lesion		Type II lesion	
	<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>
0.20 ppm O ₃				
O ₃ exposure versus control	9.1	0.001	5.1	0.025
Rest O ₃ exposure versus control		NS ^a		NS ^a
Exercise versus rest	10.8	0.005		NS
Exercise schedule 2 versus schedule 1	4.6	0.05	9.9	0.01
0.38 ppm O ₃				
O ₃ exposure versus control	37.7	0.001	52.4	0.001
Rest O ₃ exposure versus control	4.3	0.05		NS
Exercise versus rest	30.0	0.001	20.1	0.001
Exercise schedule 2 versus schedule 1		NS	41.2	0.001

^a NS, not significant.

exposure to 0.2 and 0.35 ppm O₃ (Table 1) was more severe than from continuous exercise exposure to 0.35 ppm O₃ (Table 3).

In continuous-exercise (15 m/min, 20% grade) exposures to 0.35 ppm O₃ for increasing durations, type II lung lesions were first detected in exposures of 1 h (Table 4). Both lesion types showed large increases between 1 and 2 h exposure duration; however, stepwise regression analysis using duration raised to the powers 1, 2, and 3 yielded relations with linear terms [type I = $1.59 + 0.67(\text{duration})$, $r = 0.71$, $F = 44.3$, $p < 0.001$; type II = $-0.43 + 1.25(\text{duration})$, $r = 0.81$, $F = 82.6$, $p < 0.001$]. Average $\dot{V}_{O_2}$ during running at 15 m/min, 20% grade, was elevated over resting rates by a factor of about 2.3

Effects of exercise intensity at constant effective dose on lung lesion areas are shown in Table 5. Following the highest intensity exercise exposure, each subsequent exposure was terminated based on O₃ concentration and estimated metabolic gas exchange to achieve equivalent index of effective dose, (O₃ concentration) · ($\dot{V}_{O_2}$) · (duration).

TABLE 3. Effect of Ozone Concentration on Lung Parenchymal Lesions in Continuously Exercising Rats^a

Rat group exposure	Type I lesion (area %)			Type II lesion (area %)		
	Mean	SD	<i>n</i>	Mean	SD	<i>n</i>
Clean Air Control	1.9	0.1	9	0.0	0.0	9
0.20 ppm O ₃	1.8	0.2	10	0.2	0.1	10
0.35 ppm O ₃	2.6	0.3	10	1.1	0.4	10
0.50 ppm O ₃	4.2	0.4	9	4.5	0.4	9
0.80 ppm O ₃	5.3	0.6	10	6.2	0.9	10

^a Rats ran at 10.7 m/min and level grade for 3.75 h.

TABLE 4. Effects of Exercise Exposure Duration on Lung Parenchymal Lesions Induced by 0.35 ppm Ozone^a

Rat exposure group	Duration (h)	Average $\dot{V}_{O_2}$ (ml/kg · min)	Type I lesion (area %)			Type II lesion (area %)		
			Mean	SD	<i>n</i>	Mean	SD	<i>n</i>
Clean air control	3.0	51.6	1.8	0.1	10	0.0	0.0	10
Ozone	0.5	62.2	2.0	0.1	10	0.0	0.0	10
Ozone	1.0	55.5	1.7	0.1	9	0.2	0.1	9
Ozone	2.0	58.4	3.4	0.4	8	2.6	0.7	8
Ozone	3.0	59.7	3.6	0.2	9	3.2	0.3	9

^a Rats ran continuously at 15 m/min and 20% grade.

The exercising O₃ exposures gave similar values of the index; however, the resting exposure was slightly underestimated. Despite the similarity of effective doses of O₃, increasing levels of exercise during exposure had a large effect on O₃ lesion areas; by comparison to the lowest intensity exercise exposure, the highest intensity exercise increased type I lesion areas by a factor of 2.7 and type II areas by a factor of 10. Lesion areas of O₃ exposure groups regressed stepwise against the independent variables average $\dot{V}_{O_2}$ raised to the powers 1, 2, and 3 yielded second-order relationships: type I = $0.87 + 0.0009(\dot{V}_{O_2})^2$, $r = 0.78$, $F =$

TABLE 5. Effect of Exercise Intensity on Lung Parenchymal Lesions Induced by 0.35 ppm Ozone^a

Rat group	Duration (h)	Average $\dot{V}_{O_2}$ (ml/kg · min)	$(\dot{V}_{O_2}) \cdot (O_3) \cdot$ (duration)	Type I lesion (area %)			Type II lesion (area %)		
				Mean	SD	<i>n</i>	Mean	SD	<i>n</i>
Clean air control (rest; run 15 and 30 m/min, 20% grade)	1.58	60.4	0.0	2.1	0.5	9	0.0	0.0	9
Ozone (rest)	3.42	30.5	36.5	2.3	0.9	9	0.2	0.2	9
Ozone (run 8 m/min, 0% grade)	2.75	44.5	42.8	2.4	0.6	10	0.5	0.4	10
Ozone (run 15 m/min, 20% grade)	2.33	54.8	44.7	3.2	1.1	8	2.4	2.2	8
Ozone (rest and run 30 m/min, 20% grade)	1.75	75.4	46.2	6.4	2.1	10	5.0	2.5	10

^a Rats ran at different speeds and grades and exposure duration was adjusted to give the same effective dose to each group. Similar effective doses were estimated by the proportional quantity (ppm O₃) · (exposure duration) · (oxygen consumption rate).

55.8, $p < 0.001$; type II = $-1.13 + 0.0011(\dot{V}_{O_2})^2$, $r = 0.77$, $F = 51.0$, $p < 0.001$.

DISCUSSION

Our results demonstrate that exercise greatly enhanced ozone damage to the lung. Exercise is expected to be an important modifier of the toxic effects of inhaled pollutant compounds, and a number of investigations have shown that exercise exposure to pollutant compounds exacerbates induced changes in pulmonary function of humans (Silverman et al., 1976; DeLucia and Adams, 1977; Adams et al., 1981). With the development of exercising animal models, inhalation toxicologic studies may address questions that cannot be approached with human subjects, such as exercise effects on histopathologic and biochemical changes. Relative differences in the effects of exposure conditions can be determined in laboratory animals and used to assess the importance of factors such as concentration, duration, exercise, or presence of copollutant compounds. The purpose of performing exercise exposures of animals is to increase inhaled dose rate and to change dose distribution in a manner analogous to conditions of human exercise exposure. In the absence of specific information on dose-distribution changes and relative tissue sensitivity between species, the most reasonable approach to similarity of exercise conditions is equivalent factor increases in ventilation and metabolic gas exchange above resting rates. Most of the treadmill workloads in this study were mild exercise with walking gaits that could be sustained up to 3–4 h. Metabolic rates were elevated above rest by factors of 1.5–2.5 (Tables 1, 3, 4, 5). The most intense exercise level, 30 m/min, 20% grade (Table 5), could not be sustained for more than 1 h, so rats were exercised with 2 intervening rest periods. The $\dot{V}_{O_2}$ during running periods of this exposure was 82.4 ml/kg · min or about 3 times average $\dot{V}_{O_2}$ of rats at rest (Tables 1 and 5; also see Mautz et al., 1985). In terms of increment in metabolic gas exchange, the moderate exercise levels in this study were analogous to human walking or light manual labor (Consolazio et al., 1963).

Short-term single and intermittent ozone exposures of rats at rest have documented lung histopathological changes at concentrations ranging as low as 0.1 ppm (Stephens et al., 1974 a,b; Schwartz et al., 1976; Plopper et al., 1979). In our exposures of rats at rest to 0.20 and 0.35 ppm O_3 , parenchymal lesions were observed at 0.35 ppm (Table 1). Exercise exposure increased lesion areas and induced lesions at 0.20 ppm, a concentration that resulted in no detectable effects at rest. These concentrations are similar to O_3 levels at the lower limits for detection of human pulmonary-function changes. In resting exposures of human volunteers, significant pulmonary-function decrements were not observed below 0.5 ppm; however, exercising exposures have

demonstrated effects at concentrations down to 0.12 ppm (Hazucha et al., 1973; Silverman et al., 1976; DeLucia and Adams, 1977; Folinsbee et al., 1978; McDonnell et al., 1983).

The increases in rat parenchymal lesion areas in our exercising exposures were large, and it is important to consider whether the changes have a simple relationship to ozone dose increase due to exercise ventilation, or whether other factors were important in modifying the response. To examine this question, we considered estimates of inhaled ozone dose in the exercise protocols. It was not possible to measure minute ventilation of the exercising rats directly; however, in mild exercise not involving anaerobic metabolism, ventilation is proportional to metabolic gas exchange (Wasserman et al., 1981). Increases in ventilation relative to resting rate can be estimated by relative increases in $\dot{V}_{O_2}$ compared to rest. Effective dose is the product of concentration, minute ventilation, and exposure duration. A unit proportional to effective dose can be defined as the product of ozone concentration, exposure duration, and the ratio of exposure $\dot{V}_{O_2}$ to resting $\dot{V}_{O_2}$. Lesion area data from all exposures (Tables 1, 3, 4, and 5) are plotted as a function of this index in Fig. 1. In exposures where $\dot{V}_{O_2}$ was not directly measured, values from equivalent exercise conditions were used. If concentration, ventilation, and exposure duration had linear and equally important effects on response, then data would fall on a straight line connecting clean-air control data. In such an idealized model, any factor increase in one of the effective dose variables results in an equivalent factor increase in response magnitude above clean air control. It is unlikely that percent parenchymal lesion areas will bear truly linear relationships to dose variables, but deviations from the idealized model can illustrate important features of individual variables. Among the various experiments illustrated in Fig. 1, two pairs were performed under closely matching conditions, and similar results were obtained. These exposures were (1) rest exposures (open and solid circles) at effective dose indices 1.2 and 1.4: 0.35 ppm O_3 for 3.42 h versus 0.38 ppm O_3 for 3.75 h; and (2) exercise exposures (open square and adjacent symbol T) at effective dose indices 1.5 and 1.7: 0.35 ppm O_3 during continuous exercise at 15 m/min, 20% grade for 2.0 versus 2.3 h.

Other exposures show different responses to exposure variables. As exposure duration increased during continuous exercise exposure to 0.35 ppm O_3 (T symbols), lesions were first detected at 1 h and a strong response was evident at 2 h. There is a suggestion that the response to increasing duration was sigmoid; however, little improvement over a linear fit was found using higher order regressions. Extended (48-h) exposures of rats at rest have shown lung tissue changes similar to 8-h exposures (Stephens et al., 1974), but over time periods appropriate for maintaining continuous exercise in our study, exposure duration had a large effect on response. The response to changing O_3

FIGURE 1. Lung parenchymal zone lesion areas as a function of ozone effective dose based on relative increment in metabolic rate (see text). Data are group means from rat exposure experiments. Dotted lines are intermittent exercise exposures (Table 1): circles, continuous rest; solid triangles, exercise schedule 1; solid squares, exercise schedule 2. Open symbols are effect of exercise intensity at constant effective dose (Table 5). T symbols are effect of time duration of exposure (Table 4). C symbols are effect of O_3 concentration (Table 3). Arrows on the ordinate indicate values for clean-air control exposures.

concentration during continuous exercise exposure (C symbols) was similarly linear.

Parenchymal lesion areas in the concentration dose-response experiment were reduced compared to similar effective doses in the duration dose-response test, but the latter was performed at higher intensity exercise. Exercise exerted the strongest effect on lesion response. Exercise exposures at increasing workloads but shorter duration (Table 5) resulted in dramatic increases in lesion areas despite equivalent effective doses (Fig. 1, open symbols). Intermittent exercise exposures (dotted lines, Fig. 1) had an unexpectedly large influence on tissue injury. At each of the two concentrations tested (0.2 and 0.38 ppm O_3), rat groups were exposed for the same total time duration, and the difference between groups was the duration of exercise versus rest during exposure. Increments in effective dose index for the different exercise schedules were small, but the effect on lesion areas was large. In contrast, the concentration dose-response experiment (C symbols, Fig. 1) using the same exercise work rate and total time duration but continuous exercise throughout exposure resulted in a much reduced response at similar O_3 concentrations. Studies of human pulmonary function impairment in relation to effective O_3 dose have identified concentration as the primary important variable (Folinsbee et al.,

1978; Adams et al., 1981). Our results suggest that exercise ventilation may be more important than concentration in inducing tissue damage in rodents and that intermittent increases in ventilation associated with bouts of rest and exercise can be a significant modifier of the response. The effective dose concept provided a reasonable model for response to duration and concentration when exercise intensity was held constant, but it was not readily applicable when exercise level was a variable.

The principal conclusions of this study are that (1) exercise exposure to ozone concentrations characteristic of urban smog episodes greatly increased lung tissue damage in laboratory rats, and (2) the increases were greater than expected from simple proportion to inhaled dose. Although the mechanisms for a synergism between ozone exposure and exercise are not clear, ozone inhaled in relatively slow and shallow breathing at rest may be preferentially absorbed in the upper airways (Yokoyama and Frank, 1972). During exercise, deep and rapid inspirations and possibly oral breathing may deliver higher concentrations of ozone to the deep lung. The impact of changes in dose distribution within the respiratory system can thus be far greater than expected from a simple increase in respiratory ventilation. Our data imply that exercise is an important factor in assessing the possible adverse health effects of oxidant air pollutants. This concept is recognized in human pulmonary function testing, and we have extended it to include quantitative assessment of lung tissue damage in the rat model.

REFERENCES

- Adams, W. C., and Schelegel, E. S. 1983. Ozone and high ventilation effects on pulmonary function and endurance performance. *J. Appl. Physiol. Respir. Environ. Exercise Physiol.* 55:805–812.
- Adams, W. C., Savin, W. M., and Christo, A. E. 1981. Detection of ozone toxicity during continuous exercise via the effective dose concept. *J. Appl. Physiol. Respir. Environ. Exercise Physiol.* 51:415–422.
- Bates, D. V., Bell, G., Burnham, C., Hazucha, M., Mantha, J., Pengelly, L., and Silverman, F. 1972. Short term effects of ozone on the lung. *J. Appl. Physiol. Respir. Environ. Exercise Physiol.* 32:176–181.
- Boatman, E. S., and Frank, R. 1974. Morphologic and ultrastructural changes in the lungs of animals during acute exposure to ozone. *Chest* 65:95–115.
- Crapo, J. D., Barry, B. E., Chang, L., and Mercer, R. R. 1984. Alterations of lung structure caused by inhalation of oxidants. *J. Toxicol. Environ. Health* 13:301–322.
- Consolazio, C. F., Johnson, R. E., and Precora, L. J. 1963. *Physiological Measurements of Metabolic Functions in Man*. New York: McGraw-Hill.
- DeLucia, A. J., and Adams, W. C. 1977. Effect of O₃ inhalation during exercise on pulmonary function and blood biochemistry. *J. Appl. Physiol. Respir. Environ. Exercise Physiol.* 43:75–81.
- Dixon, W. J., and Brown, M. B., 1979. *BMDP Biomedical Computer Programs*. Berkeley: University of California Press.
- Folinsbee, L. J., Silverman, F., and Shephard, R. J. 1975. Exercise response following ozone exposure. *J. Appl. Physiol. Respir. Environ. Exercise Physiol.* 39:996–1001.
- Folinsbee, L. J., Horvath, S. M., Raven, P. B., Bedi, J. F., Morton, A. R., Drinkwater, B. L., Bol-

- duan, N. W., and Gliner, J. A. 1977. Influence of exercise and heat stress on pulmonary function during ozone exposure. *J. Appl. Physiol. Respir. Environ. Exercise Physiol.* 43:409-413.
- Folinsbee, L. J., Drinkwater, B. L., Bedi, J. F., and Horvath, S. M. 1978. The influence of exercise on the pulmonary function changes due to low concentration of ozone. In *Environmental Stress*, eds. Folinsbee, L. J., Wagner, J. A., Borgia, J. F., Drinkwater, B. L., Gliner, J. A., and Bedi, J. F. pp. 125-145 New York: Academic.
- Fukase, O., Isomura, K., and Watanabe, H. 1978. Effects of exercise on mice exposed to ozone. *Arch. Environ. Health* 33:198-200.
- Gardner, D. E., Illing, J. W., Miller, F. U., and Coffin, D. L. 1974. Enhancement of effect of exposure to O₃ and NO₂ by exercise. *Toxicol. Appl. Pharmacol.* 29:129-130 (abstr.).
- Hazucha, M., Silverman, F., Parent, C., Field, S., and Bates, D. V. 1973. Pulmonary function in man after short-term exposure to ozone. *Arch. Environ. Health* 27:183-188.
- Last, J. A., Gerriets, J. E., and Hyde, D. M. 1983. Synergistic effects on rat lungs of mixtures of oxidant air pollutants (ozone and nitrogen dioxide) and respirable aerosols. *Am. Rev. Respir. Dis.* 128:539-544.
- Mautz, W. J., Phalen, R. F., McClure, T. R., and Bufalino, C. 1985. A rodent treadmill for inhalation toxicological studies and respirometry. *J. Appl. Physiol. Respir. Environ. Exercise Physiol.* 58:673-679.
- McClure, T. R., Diller, P. J., and Phalen, R. F. 1982. An airway perfusion apparatus for whole lung fixation. *Lab. Anim. Sci.* 32:195-196.
- McDonnell, W. F., Horstman, D. H., Hazucha, M. J., Seal, E., Haak, D., Salaam, S. A., and House, D. E. 1983. Pulmonary effects of ozone exposure during exercise: Dose-response characteristics. *J. Appl. Physiol. Respir. Environ. Exercise Physiol.* 54:1345-1352.
- Plopper, L. D., Dungworth, P. L., Tyler, W. S., and Chow, C. K. 1979. Pulmonary alterations in rats exposed to 0.2 and 0.1 ppm ozone: A correlated morphological and biochemical study. *Arch. Environ. Health* 34:390-395.
- Schwartz, L. W., Dungworth, D. L., Mustafa, M. G., Tarkington, B. K., and Tyler, W. S. 1976. Pulmonary responses of rats to ambient levels of ozone. Effects of 7-day intermittent or continuous exposure. *Lab. Invest.* 34:565-578.
- Shami, S. G., Thibodeau, L. A., Kennedy, A. R., and Little, J. B. 1982. Recovery from ozone-induced injury in the lungs of the Syrian golden hamster. *Exp. Mol. Pathol.* 36:57-71.
- Silverman, F., Folinsbee, L. J., Barnard, J., and Shepard, R. J. 1976. pulmonary function changes in ozone interaction of concentration and ventilation. *J. Appl. Physiol. Respir. Environ. Exercise Physiol.* 41:859-864.
- Stephens, R. J., Freeman, G., Stara, J. F., Coffin, D. L. 1973. Cytological changes in dog lungs induced by chronic exposure to ozone. *Am. J. Pathol.* 73:711-726.
- Stephens, R. J., Evans, M. J., Sloan, M. F., and Freeman, G. 1974a. A comprehensive ultrastructural study of pulmonary injury and repair in the rat resulting from exposures to less than 1 ppm ozone. *Chest* 65:115-135.
- Stephens, R. J., Sloan, M. F., Evans, M. J., and Freeman, G. 1974b. Alveolar type 1 cell response to exposure to 0.5 ppm O₃ for short periods. *Exp. Mol. Pathol.* 20:11-23.
- Stephens, R. J., Sloan, M. F., Evans, M. J., and Freeman, G. 1974c. Early responses of the lung to low levels of ozone. *Am. J. Pathol.* 74:31-57.
- Stephens, R. J., Sloan, M. F., Groth, D. G., Negi, D. S., and Lunan, K. D., 1978. Cytologic response of postnatal rat lungs to O₃ or NO₂ exposure. *Am. J. Pathol.* 93:183-200.
- Stokinger, H. E., Wagner, W., and Wright, P. 1956. Studies of ozone toxicity. 1. Potentiating effects of exercise and tolerance development. *AMA Arch. Ind. Health* 14:158-162.
- Wasserman, K., Whipp, B. J., and Davis, J. A., 1981. Respiratory physiology of exercise: Metabolism, gas exchange, and ventilatory control. In *International Review of Physiology, Respiratory Physiology III*, Vol. 23, ed. J. G. Widdicombe, pp. 149-211. Baltimore: University Park Press.
- Yokoyama, F., and Frank, R., 1972. Respiratory uptake of ozone in dogs. *Arch. Environ. Health* 25:132-138.