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Overall and Regional Lung Function in Dogs Exposed Acutely to Ozone

MICHAEL S. MORGAN, PAUL MEYER, RICHARD HOLUB, AND ROBERT FRANK

Department of Environmental Health, University of Washington, Seattle, Washington 98195; Lawrence Livermore Laboratory, Livermore, California 94550; and Department of Environmental Health Sciences, Johns Hopkins School of Hygiene and Public Health, Baltimore, Maryland 21205

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Regional distribution of ventilation and overall function of the lungs were compared in anesthetized, paralyzed, mechanically ventilated dogs exposed to ozone. Three animals were exposed at each concentration (0, 0.13, 0.22, and 0.45 ppm ozone) for 3 hr. There were no significant changes in pulmonary flow resistance, dynamic compliance, or flow rates derived from maximum forced expiratory maneuvers. The distribution of inspired gas was determined using air containing ^{13}N and a positron camera to follow wash-in in each of 80 or more regions of the lung. The mean wash-in time ($\overline{T}$) and its standard deviation did not change in sham-treated dogs, but $\overline{T}$ increased by 40 to 100% in exposed animals. Dogs showing increases in $\overline{T}$ also had increased frequency dependence of compliance (correlation significant at $P < 0.05$). The changes in ^{13}N wash-in reflected less uniform distribution of ventilation, with the greatest disparity occurring between central and marginal regions. Altered regional mechanical function, in the absence of overall changes, suggested that the initial effect of ozone was on the small airways. The measurement may be a sensitive indicator of acute irritation in the lung periphery. © 1986 Academic Press, Inc.

INTRODUCTION

Because of the characteristic chemical formation and destruction rates of ozone, a common photochemical air pollutant, human exposure appears limited to periods of 1 to 3 hr per day (Severs *et al.*, 1977). Laboratory toxicity studies have, in general, been designed to account for this brief exposure. In experimental animals altered pulmonary function has been found following acute ozone exposures at 0.2 to 0.26 ppm by volume (Amdur *et al.*, 1978; Imove *et al.*, 1979; Watanabe *et al.*, 1973). The changes in airflow resistance, dynamic compliance, slow volume–pressure curves and diffusing capacity of the lungs in cats (Watanabe *et al.*, 1973) suggested that ozone affected airway caliber more than alveolar capillary transport or quasistatic lung elastic properties. At ozone concentrations of 0.5 ppm and above, structural changes in the peripheral airways, particularly at the bronchiolar level, have been observed in several species of animals after intermittent exposure for 7 days (Schwartz *et al.*, 1976). The lesions found in structural studies may have been associated with mechanical changes in those airways, but the functional measurements ordinarily used in inhalation toxicology are relatively insensitive to the properties of small airways (Macklem, 1971). Techniques which reflect regional, in contrast to overall, lung mechanical function are thought to be more sensitive to early dysfunction of small airways (Woolcock *et al.*, 1969) and thus may show ozone-related changes in the absence

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of effects on overall lung function. To test this hypothesis, we compared nearly simultaneous measurements of overall and regional lung mechanical function in dogs exposed to ozone concentrations ranging from 0.13 to 0.45 ppm. Regional function was assessed using an isotope-labeled gas and a three-dimensional lung imaging system with an on-line computer for information storage. In exposed dogs we found consistent changes in the regional distribution of ventilation but minimal effect on overall lung mechanics.

MATERIALS AND METHODS

Twelve kennel-raised foxhounds weighing between 16 and 20 kg were anesthetized with pentobarbital sodium, 25 mg/kg initially followed by 50 mg/hr. The dogs were then paralyzed with gallamine triethiodide (Flaxedil, Davis and Geck), 40 mg initially and then 20 mg/hr, and were ventilated via tracheostomy using a Harvard pump. The pump settings were constant throughout each experiment: the stroke rate was 30 per minute and the stroke volume was selected to maintain end-tidal CO_2 concentration at the level found during spontaneous breathing. A Malecot catheter (no. 12) was inserted through the fourth intercostal space for measurement of intrapleural pressure. A 50 cc pneumothorax was maintained throughout to prevent obstruction of the catheter. Pressure and flow were detected with variable reluctance transducers (Validyne Co., Northridge, Calif.) and a capillary flowmeter. Expired carbon dioxide, rectal temperature, and electrocardiographic signals were monitored to assess the stability of the preparation. After sacrifice by massive overdose of pentobarbital, the lungs were excised and examined for gross signs of edema.

Overall function. Pulmonary flow resistance (R_l) and dynamic compliance (C_{dyn}) were calculated from the pressure, flow, and integrated flow signals and reported as the mean of five consecutive breaths. Frequency dependence of compliance (ΔC_{dyn}) was assessed by briefly increasing the pump rate to 60 strokes per minute, and was defined as the difference between C_{dyn} at 30 and at 60 breaths/min. At regular intervals the lungs were inflated to 30 cm H_2O transpulmonary pressure and the tracheal cannula was connected to a 125 liter reservoir maintained at -70 cm H_2O gauge, for generation of maximum expiratory flow–volume curves. The reservoir pressure was held constant throughout the maneuver by a regulator and vacuum pump. Preliminary studies indicated that reservoir pressures of -40 cm H_2O or lower were sufficient to produce maximal flow in dogs of similar size at lung volumes less than 70% of vital capacity. Expired volume was derived by integration of the flow signal. Flow rates at 50 and 75% expired vital capacity ($\dot{V}_{max50}$ and $\dot{V}_{max75}$, respectively) were read and averaged for three maneuvers. Flow and volume were not corrected for gas decompression.

Regional ventilation. Every hour the animal was ventilated for 2 min with air containing approximately 1 mCi ^{13}N per liter of N_2. The radioisotope was produced from pure nitrogen by the photonuclear reaction $^{14}N(\gamma,n)^{13}N$ at the 100 MeV Livermore Electron Accelerator facility (Meyer *et al.*, 1975). It was rapidly transferred in a plastic bag to the experimental site and administered to the animal with the Harvard pump. The ^{13}N nucleus decays to stable ^{13}C by positron emis-

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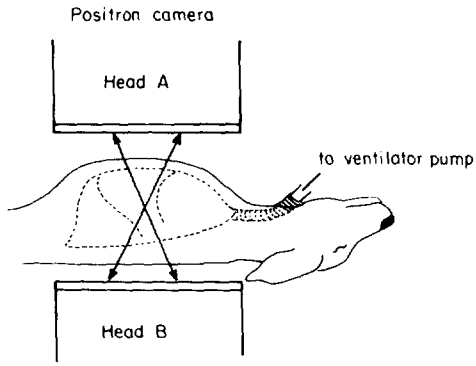

FIG. 1. The dog is immobilized in a plastic foam cradle with its chest centered in the camera field. The crossed arrows indicate two positron annihilation events, each producing a pair of opposing gamma photons which enter the detectors in coincidence and are counted. Events occurring at or near the plane located midway between camera heads are collected, while events outside this plane are interpreted as background.

sion with a half-life of 10 min. Back-to-back gamma rays are emitted upon positron electron annihilation and the gamma rays were detected by a dual NaI crystal positron camera. Since positron cameras have been described in detail in the literature (Anger, 1967), this discussion will be limited to the characteristics of the Livermore device (Behrin, 1972). Each of the detector heads (Fig. 1) consists of a 40-cm diameter NaI crystal, 1.25-cm thick, with an array of 37 photomultiplier tubes. An electronic circuit originated by H. O. Anger, Lawrence Berkeley Laboratory, provided x and y coordinates of the gamma photons detected in the crystal. Energy selection of photons, in this case 511 keV, is made by a differential discriminator. If both detectors register a signal within the coincidence window, the x and y signals of each camera head are digitized to 8 bit precision and stored in a PDP-15 computer (Digital Equipment Corp.). This defines a straight line between detectors on which the annihilation event occurred. Signals from a clock provided markers in real time. As used here, the Livermore camera has a spatial point source resolution (full width at half maximum) of 1.5 cm in the plane of focus. The focal plane of an event was established by back projection (Behrin, 1972) using the recorded data. This method defocused events which took place above or below the plane of interest, into a background field. Image-reconstruction algorithms now available were not used in this work.

The count rate of this camera is limited to about 5000 coincidences per second, primarily by the digitizing and computer storage rates. The maximum activity for the present experiments was kept below 2000 coincidences/sec. Solid angle corrections to the data were applied as a function of camera radius using standard methods. Nuclear decay corrections were applied.

The spatial resolution of the imaging system is limited in practice by the range of the ^{13}N positrons in the airways and parenchyma before annihilation occurs. The image does not have sharp boundaries but can reveal relative changes in regional ventilation dynamics provided the animal is not moved. The limit of the lungs' image was determined in two ways: first, with the dog between the camera

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heads a ^{22}Na point source was used to provide spatial markers before experiments began; later, data were discarded in locations where the count rate after the first wash-in was less than 10 to 15% of the highest count rate at any point in the chest field for that run. This method was verified using tubular Lucite phantoms containing ^{13}N. In all dogs the focal plane was located approximately midway in the dorsal–ventral axis, where activity was greatest.

Regional ^{13}N appearance rates were obtained from the stored positron camera data in the form of coincidence rate versus number of pump strokes (breaths) starting with the first appearance of radioactivity. The camera field was subdivided into smaller regions (see Fig. 2) whose number depended on spatial resolution, counting statistics, and size of the animal. Typically, the number of regions varied from 80 to 100 and the count rate after equilibration in each region ranged from 15 to 25 events/sec. Virtually all regions displayed an apparent plateau in activity, taken as equilibrium, which was reached in 1 min (30 breaths or less). The arrival time (T) was defined as the number of pump strokes required for each region to reach 95% of its plateau activity. Some regions whose maximum activity satisfied the criterion described earlier displayed no clear plateau (that is, their activity over the first 30 to 40 breaths rose to less than 90% of the maximum) and these data were discarded.

From each regional plot of count rate versus number of strokes, T was determined by visual curve fitting, taking as the plateau value the average over the last 10 to 20 breaths. The uncertainty was commonly not more than ±two strokes. Regional counts were normalized to the total lung area counts to eliminate activity variations between runs.

We chose to analyze the ^{13}N wash-in data by visual curve fitting, since computer techniques were no more reliable in rejecting technically faulty data. Measurements of arrival time were confirmed by an independent observer who had no

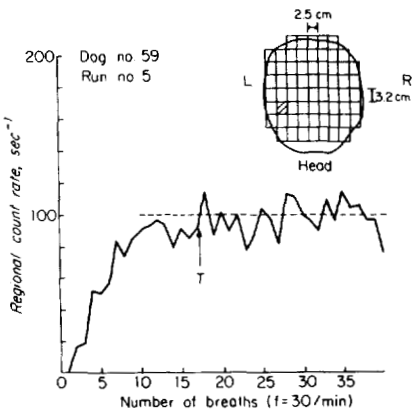

FIG. 2. Regional ^{13}N wash-in data for a run during the baseline period in one dog. The inset shows the rectangular grid superimposed on an outline of the lungs at the focal plane, estimated from dimensions measured at autopsy. The grid defines the regions for which wash-in data were analyzed. The arrival time T for the shaded region was determined as the point where the count rate first reached 95% of the visually estimated saturation level (dashed line). The arrival time is expressed as the number of breaths at 30 breaths/min.

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knowledge of the experimental conditions. Others have compared curve fitting with more sophisticated methods of quantifying regional wash-in curves obtained in this way and have found no improvement in results (Bunow *et al.*, 1979).

Exposure. Ozone was produced by ultraviolet irradiation of medical grade oxygen. The ozone was diluted with filtered, humidified air in a glass reservoir, where the ozone concentration was monitored using a chemiluminescent detector (Monitor Labs Model 8410, San Diego, Calif.). The mixture was withdrawn from the reservoir and pumped to the dog through Teflon tubing and stainless steel fittings to minimize ozone loss. The pump cylinder and piston were also Teflon coated. Ozone uptake between the reservoir and the tracheal cannula was less than 10%.

The 12 dogs were randomly assigned to one of four groups, each receiving either sham treatment or one ozone concentration: 0.13, 0.22, or 0.45 ppm. Each experiment consisted of a 2-hr baseline period in which the animal breathed filtered air followed by a 3-hr exposure; overall and regional functional measurements were made frequently throughout this time.

RESULTS

There were no consistent changes in R_1, C_{dyn}, $\dot{V}_{max50}$, or $\dot{V}_{max75}$ during the sham exposure or at any ozone concentration. Typical of these results for overall lung function are the measurements of C_{dyn} shown in Fig. 3. Gross examination of the lungs at autopsy revealed no signs of intraalveolar edema or other indications of acute inflammation or hemorrhage.

The mean ($\bar{T}$) and standard deviation (s) of the T distribution for each dog appear in Table 1. Each value is the combined result of two consecutive ^{13}N wash-in runs. During baseline, the scatter in $\bar{T}$ and s among the animals was comparable

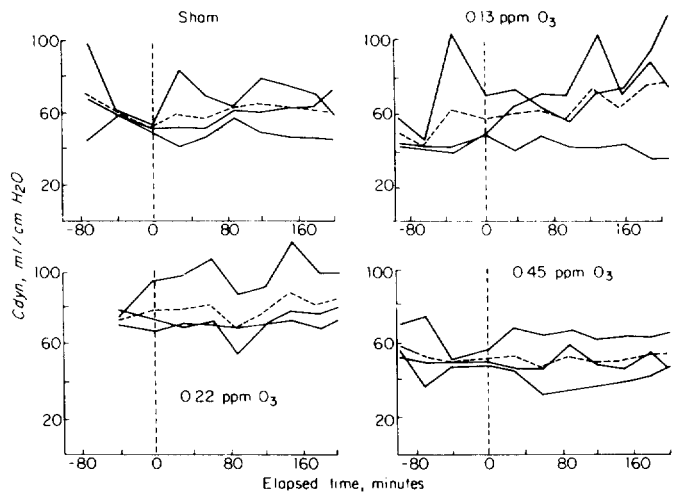

FIG. 3. Dynamic compliance (C_{dyn}) at 30 breaths/min during baseline and exposure. Exposure begins at the vertical dashed line. Solid lines represent individual animals and the dashed line shows the mean.

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TABLE 1
DISTRIBUTION OF ARRIVAL TIME (T), IN BREATHS

	Mean			Standard deviation		
O_3 ppm	Baseline	Exposure	Ratio	Baseline	Exposure	Ratio
0	14	13	0.9	8	9	1.1
	10	7	0.7	5	4	0.8
	11	14	1.3	9	10	1.1
		Mean	1.0		Mean	1.0
0.13	10	14	1.4	7	8	1.1
	9	12	1.3	5	8	1.6
	17	24	1.4	11	13	1.2
		Mean	1.4		Mean	1.3
0.22	25	27	1.1	13	11	0.8
	5	10	2.0	5	8	1.6
	10	12	1.2	6	8	1.3
		Mean	1.4		Mean	1.2
0.45	11	22	2.0	5	10	2.0
	15	15	1.0	11	10	0.9
	10	24	2.4	7	13	1.9
		Mean	1.8		Mean	1.6

to that found in normal human subjects (Butler and Mohler, 1979). Regions with small values of T were ventilated more rapidly than average, while those with higher values of T were ventilated more slowly than average. The breadth of the distribution is therefore a qualitative measure of uniformity, and may be characterized by the standard deviation of T.

The sham-treated animals had reproducible T distributions before and during exposure. While the response of individual dogs to exposure was variable, both $\overline{T}$ and s tended to increase with ozone concentration. The ratio of each parameter during exposure to its value during baseline illustrates this tendency, as shown in Table 1. The cumulative frequency distributions of T for all four experimental conditions are shown in Fig. 4. The changes in T were present within 1 hr of the onset of exposure to ozone.

Because relatively few measurements were made at each ozone concentration, the data for $\overline{T}$ and s were not sufficient for standard analysis of variance. As an alternative, we postulated that these two measures of regional function might be correlated with other parameters, such as frequency dependence of compliance or $\dot{V}_{max75}$, thought to reflect regional or small airways function. The results for ΔC_{dyn} and $\dot{V}_{max75}$ for each dog are given in Table 2, and reveal no consistent changes when considered alone that could be attributed to exposure. However, when changes in ΔC_{dyn} were compared to nearly simultaneous changes in $\overline{T}$ in each dog (Fig. 5), an association was apparent. A nonparametric test of correlation suitable for small samples of paired data, the Kendall rank correlation coefficient (Siegal, 1956), was significant ($P < 0.05$), having a value of 0.45. Further, dogs with the largest increase in $\overline{T}$ received 0.45 ppm ozone, although a clear dose–response relationship was not apparent. Other comparisons of $\overline{T}$ or s with ΔC_{dyn} or $\dot{V}_{max75}$ showed no significant association.

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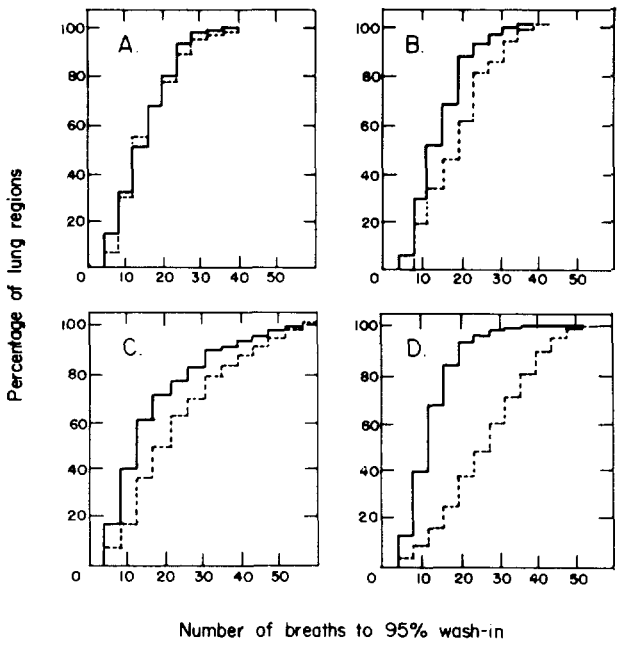

FIG. 4. Cumulative frequency distribution of arrival times in the four groups of dogs. Each distribution shows the percentage of regions in the camera field (see Fig. 2) having arrival times less than each range of T values. The abscissa is the arrival time T. The distributions represent the sum of the three animals in each group for the baseline (solid line) and exposure (dashed line) periods. Ozone concentrations: (A) 0 ppm; (B) 0.13 ppm; (C) 0.22 ppm; (D) 0.45 ppm.

DISCUSSION

We emphasize that larger groups of animals are needed to confirm the findings presented, and particularly to establish dose–response relationships.

Acute exposure to ozone in the range of 0.13 to 0.45 ppm produced no significant change in overall lung mechanics, even though ozone was delivered directly to the trachea, thereby avoiding the absorptive surfaces of the upper respiratory passages (Yokoyama and Frank, 1972). Thus dogs appear to be more resistant to the functional effects of ozone than cats exposed in a similar manner (Watanabe *et al.*, 1973). We selected dogs for our study because their lungs are large enough to fill nearly the entire field of the positron camera and are much larger than the limit of spatial resolution.

Changes in conventional functional tests have generally not been seen in healthy human subjects at concentrations below 0.5 ppm when exposure was at rest (Folinsbee *et al.*, 1978). When vigorous exercise has been superimposed, dose-related effects were noted at both 0.18 and 0.12 ppm (McDonnell *et al.*, 1983). At high levels of exercise with high flow rates and predominance of oral breathing (Niinimaa *et al.*, 1981), the scrubbing efficiency of the upper airways for ozone is functionally bypassed, a circumstance analogous to the situation in the present study.

It is possible to estimate the levels of ambient ozone which, when inhaled

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TABLE 2
FREQUENCY DEPENDENCE OF COMPLIANCE AND FORCED EXPIRATORY FLOW RATES

	ΔC_{dyn} (ml/cm H_2O)			$\dot{V}_{max75}$ (liters/sec)		
O_3 ppm	Baseline	Exposure	Change	Baseline	Exposure	Change
0	21	11	−10	4.0	4.0	0.0
	5	2.7	−2.3	3.7	2.7	−1
	2.7	−3	−5.7	4.7	4.8	0.1
		Mean	−6		Mean	−0.3
0.13	11	1	−10	2.4	3.4	1.0
	−7	13	+20	2.4	2.8	0.4
	2	2	0	3.0	2.5	−0.5
		Mean	+3		Mean	0.3
0.22	18	18	0	4.4	4.5	0.1
	10	20	10	3.9	3.3	−0.6
	16	21	5	5.0	4.9	−0.1
		Mean	5		Mean	−0.2
0.45	0	5	5	3.6	3.7	0.1
	10	19	9	3.0	2.6	−0.4
	16	15	−1	3.1	3.3	0.2
		Mean	4		Mean	0.0

through intact upper airways, would result in the tracheal concentrations used in this work. Table 3 shows the tracheal ozone concentrations and corresponding ambient levels for nose- and mouth-breathing dogs based on reported measurements of ozone uptake by those pathways (Yokoyama and Frank, 1972). For nose breathing at rest, the ambient concentrations range from 0.43 to 1.1 ppm, while for mouth breathing with exercise the ambient levels range from 0.14 to 0.51 ppm. Our experimental conditions are therefore a close approximation to ambient exposures during moderate exercise.

The parameter T was selected to quantify the regional ventilation since it involves no assumption of exponential wash-in kinetics and is determined when count rates, and hence statistical certainty, are highest. The arrival time must be inversely related to regional ventilation per unit regional lung volume, but this relationship is inversely proportional only for a single well-mixed compartment.

TABLE 3
AMBIENT OZONE CONCENTRATION EQUIVALENT TO TRACHEAL OZONE CONCENTRATION IN EACH DOG

Tracheal ozone (ppm)	Ambient ozone[a] ppm	
	Nose breathing at rest	Mouth breathing on exercise
0.13	0.43	0.14
0.22	0.65	0.25
0.45	1.1	0.51

[a] Estimated using fraction of ozone taken up by dog upper airways at different flow rates (Yokoyama and Frank, 1972).

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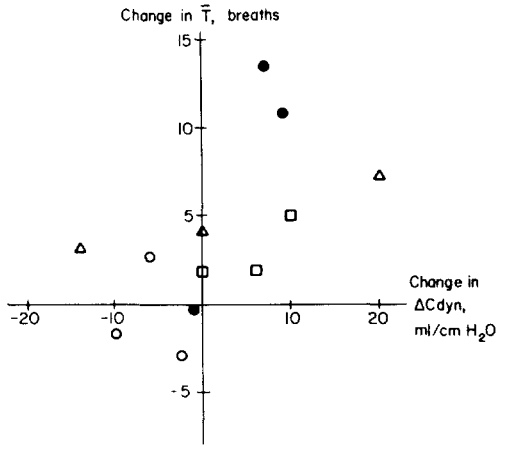

FIG. 5. Correlation between changes in mean arrival time $\bar{T}$ and changes in frequency dependence of compliance ΔC_{dyn} from baseline to exposure. Each point represents one dog. The correlation coefficient was 0.48, $P < 0.05$. Ozone concentrations: ○, 0 ppm; ▲, 0.13 ppm; □, 0.22 ppm; ●, 0.45 ppm.

We have no basis for taking our regions as single compartments. However, as dispersion about the mean of regional ventilation per unit regional volume reflects nonuniform distribution in the lung (Butler and Mohler, 1979), the dispersion of our T about its mean should have the same interpretation. In each of these experiments, overall ventilation per unit volume appeared to be constant over time since tidal volume was fixed by the pump and there was no evidence that functional residual capacity changed. Although thoracic gas volume was not measured directly, relative changes over time would have been reflected in altered plateau count rates over the entire chest for ^{13}N. Variations in the plateau were less than 15% and could be accounted for entirely by changes from run to run in the ratio of radioactive nitrogen to stable nitrogen in the gas inhaled by each dog. In addition, vital capacities from the forced expiratory maneuvers were stable. Based on these considerations and the absence of changes in C_{dyn}, we conclude that there were no significant variations in lung volume between ^{13}N wash-in runs. (C_{dyn} and ^{13}N wash-ins were measured at 30 breaths/min). Accordingly, the observed increases in both the mean and standard deviation of T associated with exposure to ozone are consistent with an increase in the dispersion of regional ventilation.

Thus, in contrast to the absence of changes in overall function, the regional distribution of inspired gas appeared to be affected by ozone, in a manner consistent with less uniform ventilation of the lung. Though less dramatic than changes in T, the associated rise in frequency dependence of compliance, that is, an exaggerated fall in C_{dyn} between measurements at 30 and 60 breaths/min, lends support to this interpretation (Ingraham and Schilder, 1967). The occurrence of marked abnormalities of ventilation distribution in the presence of normal flow resistance and static compliance has been described in patients with chronic bronchitis and asthma (McFadden and Lyons, 1968; Wollcock *et al.*, 1969) and in excised lungs with experimental obstruction (Brown *et al.*, 1969). These observa-

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tions were taken as evidence for constriction of peripheral airways, which have little influence on overall flow resistance (Macklem, 1971). Our data provide no direct evidence of peripheral airways effect, since when $\bar{T}$ increased during exposure, $\dot{V}_{max75}$ showed small and inconsistent changes. However, the absence of an association between $\bar{T}$ and R_1 may be seen as evidence that the caliber of the central airways was not affected at these concentrations.

An alternative possibility is that changes in regional elastic properties caused by ozone could have been responsible for the abnormalities in distribution of ventilation. Our results argue against this, since C_{dyn} at 30 breaths/min was unchanged and pulmonary edema, which can affect regional compliance (Cook *et al.*, 1959), was not visibly evident on autopsy. Work done in cats suggests that acute exposure to ozone in concentrations up to 1 ppm is not likely to alter the static volume/pressure behavior of the lungs (Watanabe *et al.*, 1973).

The positron images of the lungs taken during exposure to ozone were consistent with a shift in ventilation away from the perimeter of the lung. Increased T values were aggregated at the perimeter, while smaller T values were aggregated toward the center. The regions with elevated T values make up the "tail" on the right side of the frequency distributions shown in Fig. 4. This pattern occurred in every dog in which $\bar{T}$ and s were elevated during ozone exposure.

The marginal areas of the positron image field consist mainly of small airways and alveoli, while the central areas contain airways of all sizes and a relatively lower proportion of alveoli. If within each region of our image the same fraction of alveoli became slowly ventilated during ozone exposure, the central regions would display more rapid wash-in because of the greater influence of major airways. Conversely, the marginal regions would wash-in more slowly, since they have fewer airways in proportion to alveoli. This result would be observed whether peripheral airway caliber or local elastic properties had changed. Our data thus cannot be used to determine conclusively which of the two physiological mechanisms was at work, although airway changes seem more likely, based on structural studies (Boatman *et al.*, 1974).

The process by which ozone elicits these changes is not entirely clear. The response in dogs, especially in the periphery of the lungs, is mediated initially by the parasympathetic system (Gertner *et al.*, 1983a; Lee *et al.*, 1979); subsequently and at higher concentrations, histamine release is involved (Gertner *et al.*, 1983b). Prostaglandins of the cyclooxygenase pathway appear to play little role (Gertner *et al.*, 1983b).

The ability to detect changes in lung function in response to concentrations of ozone found in ambient air is a topic of considerable interest. The use of methods sensitive to nonuniformity in the lungs appears to detect changes in resting animals at ozone levels lower than those used in previous work which relied on tests of overall function. We found changes at levels close to the present National Ambient Air Quality Standard of 0.12 ppm averaged over 1 hr (U.S. Environmental Protection Agency, 1979). Such methods can also be applied to human subjects. Of the radioactive methods available, positron imaging with ^{13}N has several potential advantages. The short half-life permits measurements to be repeated hourly. The ability to detect low levels of radioactivity (roughly equal to

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natural background) reduces the accumulated dose for the procedure. Finally, ^{13}N is not taken up by the bloodstream in appreciable amounts in these brief exposures, so that no correction is needed for time-dependent background, as is the case when ^{133}Xe is used.

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