Archives of Environmental Health: An International Journal
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/vzeh20

Respiratory Uptake of Ozone in Dogs

Eiji Yokoyama MD [b] & Robert Frank MD [a]

[a] Department of Environmental Health , School of Public Health and Community Medicine, University of Washington , 98195, Seattle , USA

[b] Institute of Public Health , Tokyo , Japan

Published online: 02 May 2013.

To cite this article: Eiji Yokoyama MD & Robert Frank MD (1972) Respiratory Uptake of Ozone in Dogs, Archives of Environmental Health: An International Journal, 25:2, 132-138, DOI: 10.1080/00039896.1972.10666149

To link to this article: http://dx.doi.org/10.1080/00039896.1972.10666149

Respiratory Uptake of Ozone in Dogs

Eiji Yokoyama, MD, and Robert Frank, MD, Seattle

We measured the uptake of ozone by the surgically isolated upper airways in 11 anesthetized, paralyzed, mechanically ventilated dogs. The gas was administered either by nose or mouth, each exposure lasting 20 to 30 minutes. The relative uptake of O_3 was found to be inversely related to concentration and flow rate, and was higher nasally than orally. Changes in nasal flow resistance, induced by aerosols of histamine and phenylephrine (Neo-Synephrine) hydrochloride, had little effect on the process. The removal of O_3 from inspired air by the lower airways and parenchyma was measured in five additional dogs that were mechanically ventilated through a tracheal cannula. The concentration ranged from 20 to 85 pphm, the tidal volume was constant, and the pump frequently was either 20 or 30 cycles per minute. Under these circumstances, the rate of uptake varied between 80% and 87%.

THE level of the respiratory passages reached by a toxic gas may be expected to influence the nature of the response. Previously, we reported that radioactive sulfur dioxide ($^{35}SO_2$) was almost completely taken up by the surgically isolated upper airways in dogs if the gas was administered by nose at a continuous flow of 3.5 liters/min.[1,2] The uptake of $^{35}SO_2$ differed for the nasal and oral passages and was sensitive to the rate of flow, particularly if the gas was administered by mouth. One of us,[3] using a similar preparation, has also shown that the nasal uptake of SO_2 exceeds that of nitrogen dioxide.

In the present study we extended the observations to ozone, comparing its uptake by the nasal and oral passages of dogs at different concentrations and rates of flow. We utilized concentrations of the gas encountered in urban and industrial settings. We also measured the uptake of O_2 by the lungs starting at the level of the trachea. Thus far, the reports available on the uptake of O_3 in animals[4,5] and man[6] have not distinguished between the transfer rates for the upper and lower portions of the respiratory system.

Methods

We studied mongrel dogs of both sexes weighing 13 to 18 kg (28.7 to 39.7 lb). The animals were anesthetized with intravenously administered sodium pentobarbital (initial dose, 30 mg/kg; maintenance dose, 30 to 70 mg every hour), paralyzed with intravenously administered succinylcholine chloride (initial dose, 3 mg/kg; maintenance dose, 10 to 14 mg every hour), and were mechanically ventilated through a tracheal cannula by means of a respiratory pump. Ambient, humidified air and 50% oxygen were administered alternately at 30- to 40-minute intervals.

The methods of isolating and exposing the upper airways are shown schematically in Fig 1. The technique for isolating the upper airways was similar to that used for studying the

Submitted for publication Dec 20, 1971; accepted Feb 11, 1972.

From the Department of Environmental Health, School of Public Health and Community Medicine, University of Washington, Seattle. Dr. Yokoyama is now at the Institute of Public Health, Tokyo.

Read in part before the American Industrial Hygiene Conference, Toronto, May 28, 1971.

Reprint requests to Department of Environmental Health, School of Public Health and Community Medicine, University of Washington, Seattle 98195 (Dr. Frank).

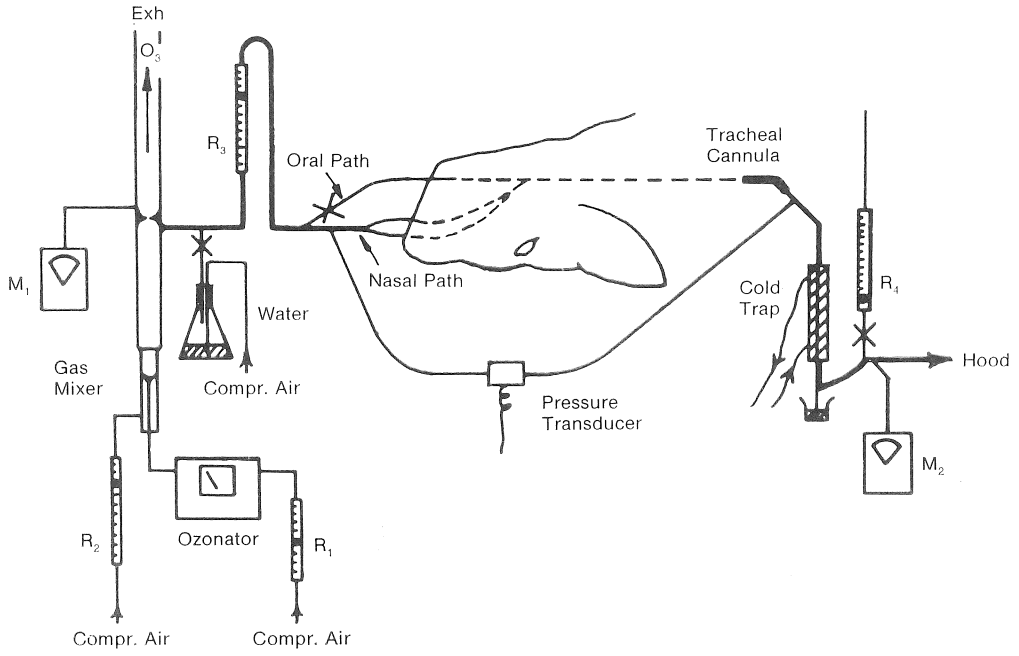

Fig 1.—Schematic diagram of the method of exposing the isolated upper airways of dogs to O_3. Mast O_3 analyzer, M; rotameter, R.

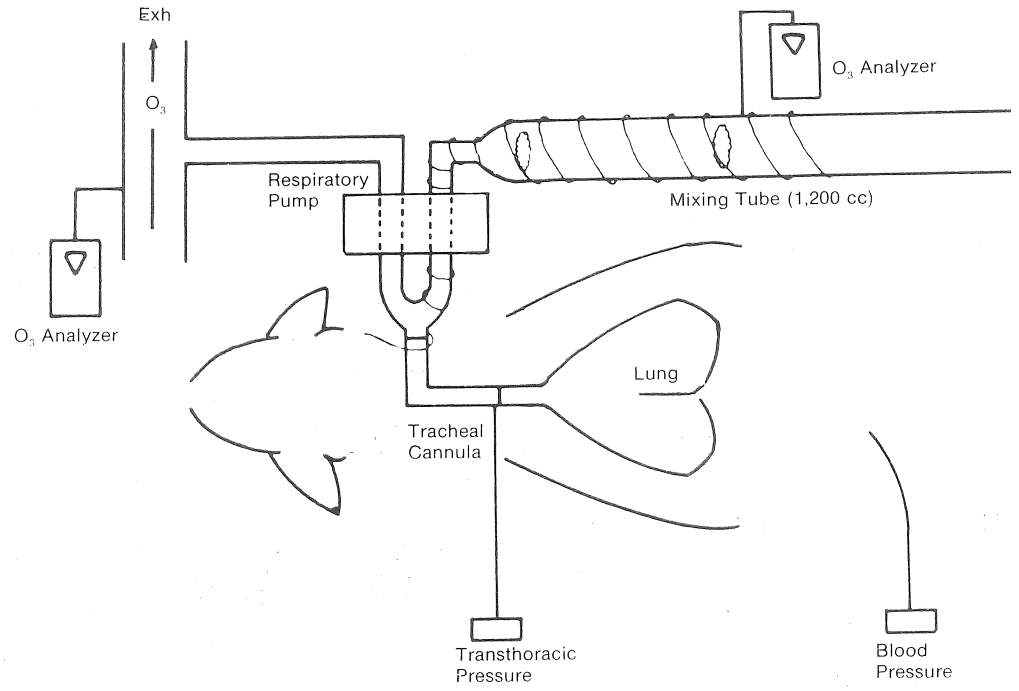

Fig 2.—Schematic diagram of the method of exposing the lower airways to O_3 and of determining expired O_3 concentrations. The two baffles in the mixing tube are indicated by the ovals with scalloped edges.

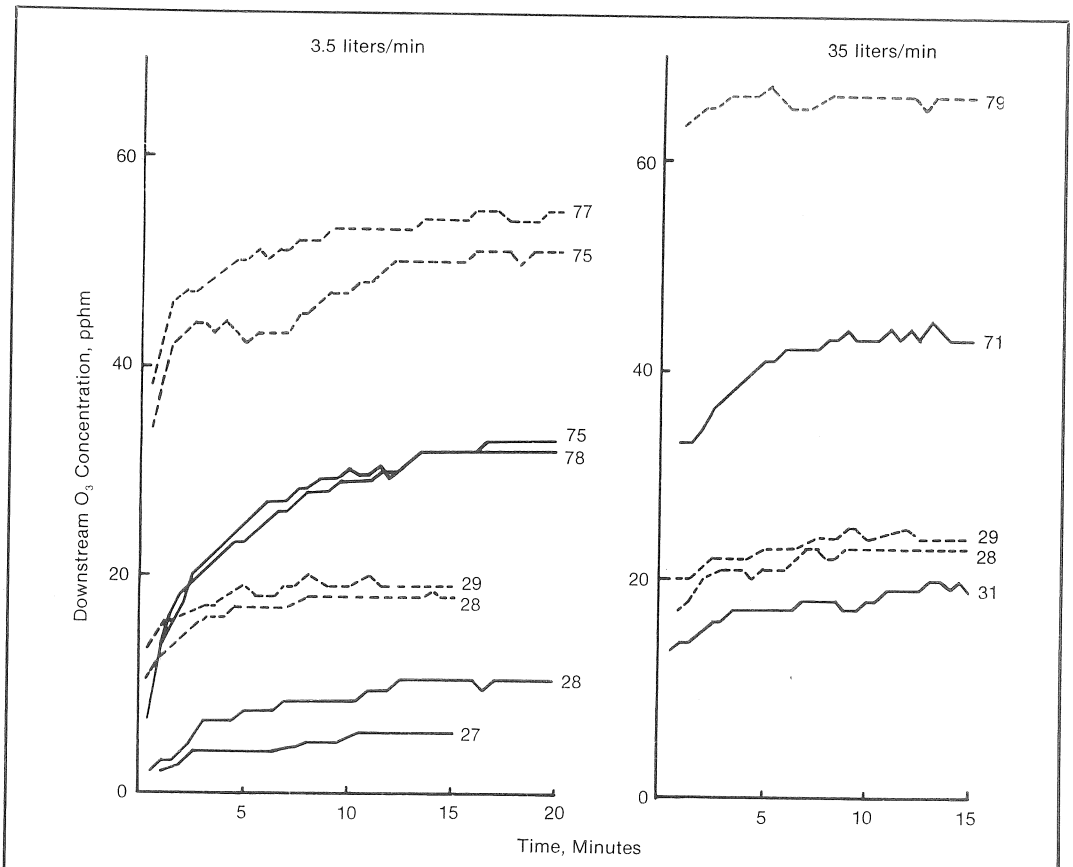

Fig 3.—Data from a single experiment: Time-course of changes in O_3 concentration beyond the isolated upper airway segment. Flow was continuous, entering the segment either by nose (**solid lines**) or mouth (**dotted lines**). Numbers at the end of each curve represent the upstream concentrations.

uptake of $^{35}SO_2$,[1,2] except for the elimination of the face mask. Instead, we fastened a glass tube (0.7 cm inside diameter [ID]) into each nostril by means of a quick-setting, powerful adhesive (Aron Alpha) that is effective on moist surfaces. The oral glass cannula (0.7 cm ID) was inserted to a point just inside the front teeth after the tip of the tongue was extended and sewn to the inferior margin of the jaw. The lips were sealed tightly with the adhesive. The nasal and oral cannulas were connected to the source of O_3; each pathway could be clamped shut independently. The muzzle, except for the nostrils, was splinted with an elastic bandage to prevent ballooning of the cheeks during oral flow. The tracheal end of the isolated airways was cannulated several centimeters below the larynx. A cold-trap (4 to 6 C) was placed downstream to the cannula to condense the water vapor released by the upper airways; the trap was placed proximal to the downstream Mast O_3 analyzer (M_2, Fig 1). At the outset, we tested each preparation for leaks by comparing flow rates through rotameters placed upstream and downstream to the isolated segment (R_3 and R_4, Fig 1). The maximal leak noted at peak flows was less than 3%: (upstream flow − downstream flow/upstream flow) × 100.

We used an ultraviolet O_3 generator. The O_3 was diluted to the desired concentration with compressed, filtered, metered air and administered through wide-bore glass tubing at room temperature. The upstream and downstream concentrations of O_3 were monitored continuously with separate Mast analyzers (M_1 and M_2, Fig 1). The analyzers were calibrated with the neutral potassium iodide method,[7] and were checked against the same source of gas before and after each experiment. The analyzers required 1.5 to 2.0 minutes to reach a constant reading starting from zero concentration. The O_3 or the filtered air could be directed through either the nose or mouth: the oral path is

shown clamped in Fig 1. The excess O_3 was vented (at point *Exh*, Fig 1). Each exposure lasted 20 to 30 minutes. Before each exposure, the appropriate pathway was flushed 20 to 30 minutes with humidified, ambient air at room temperature. Two ranges of flow were used: 35.0 to 45.0 liters/min (high) and 3.5 to 6.5 liters/min (low). The upstream concentration and the flow rate were monitored throughout during each exposure. A total of 118 exposures was made on 11 dogs.

The pressure gradient between the nasal and tracheal cannulas (transnasal pressure) and the arterial blood pressure were measured continuously with differential transducers and recorded with a thermal oscillograph.

For the exposure of the lower airways (five dogs), the lungs were ventilated alternately with humidified, ambient air or O_3 by means of separate respiratory pumps. The piston and cylinder of the pump used to administer O_3 were coated with Teflon. During the exposure to O_3, the expired gas and the gas emanating from the external dead space (equipment) were led into a glass mixing tube[1] (4.8 cm ID, 72 cm in length) containing two baffles, one at the proximal end and the other in the midsection (Fig 2). The concentration of O_3 was monitored with a Mast analyzer at a point beyond the second baffle. The expiratory valve of the respiratory pump and the mixing tube were maintained at about 37 C by means of heating tape to prevent the condensation of water vapor. The respiratory pump, at frequencies of 20 to 30 per minute and tidal volumes of 100 to 200 ml, adsorbed 2% to 20% of the incoming O_3. Under these circumstances, about ten minutes was required to reach a constant, preselected concentration beyond the pump. Once that concentration had been achieved, the O_3-respiratory pump was connected to the tracheal cannula in substitution for the ambient-air pump, and the exposure was begun. Each exposure lasted 15 to 20 minutes; the uptake measured during the last four to five minutes of the period is reported. The airway pressure was measured continuously with a differential transducer as an index of respiratory mechanical behavior, and was recorded with the thermal oscillograph. The tidal volume was selected from the ventilation graph of Kleinman and Radford,[8] based on a respiratory frequency of 20 breaths per minute; a correction was made for the volume of the external dead space. The gas was administered at two separate frequencies, 20 cycles per minute and 30 cycles per minute, but at a constant tidal volume. The purpose was to compare the uptake of O_3 at two average rates of flow and residence times in situ, at comparable lung volumes. To estimate the concentration of O_3 in the gas coming just from the lungs, we assumed there was complete mixing of the pulmonary and external dead space aliquots within the mixing chamber. We used the following computation:

$$(1)\quad (V_T \times C_{exp}) = (VD_{ext} \times C_{insp}) + (V_T - VD_{ext})\, C_L$$

$$(2)\quad C_L = \frac{(V_T \times C_{exp}) - (VD_{ext} \times C_{insp})}{V_T - VD_{ext}}$$

where V_T is the tidal volume measured in milliliters; VD_{ext}, external dead space volume, ml; C_{exp}, concentration of O_3, ppm, in mixed expired gas; C_{insp}, concentration of O_3, parts per hundred million (pphm), in inspired gas and in the gas contributed by the external dead space; C_L, concentration of O_3, pphm, in gas coming from the lung.

Two ranges of O_3 concentration were administered: 70 to 85 pphm (high) and 20 to 40 pphm (low).

Results

Upper Airways.—Following the onset of exposure, the downstream concentration of O_3 rose sharply during the first several minutes and leveled off after 15 to 20 minutes (Fig 3). The leveling off occurred sooner when the gas was administered orally or the flow rate was high (35 to 45 liters/min). We could not assess the early rapid changes in downstream concentration of O_3 owing to the slow response of the Mast analyzer. Consequently, we have expressed the uptake of O_3 during only the last four to five minutes of exposure when the analytic readings were steady. The average rates of uptake of O_3 by the nose and mouth are shown as functions of the concentration and flow rate in Fig 4 and Table 1. Nasal uptake exceeded oral uptake at both flows ($P < .01$). The uptake of O_3 both nasally and orally was inversely related to flow ($P < .01$), as well as to concentration ($P < .01$), except when the gas was directed through the mouth at the higher flow.

There were no changes in blood pressure or heart rate attributable to O_3.

Nasal Decongestion and Congestion.—Following administration of phenylephrine (Neo-Synephrine) hydrochloride, transnasal pressure fell to 15% to 43% (Fig 5). By comparison, the changes in uptake were random and did not exceed 10%. The drug

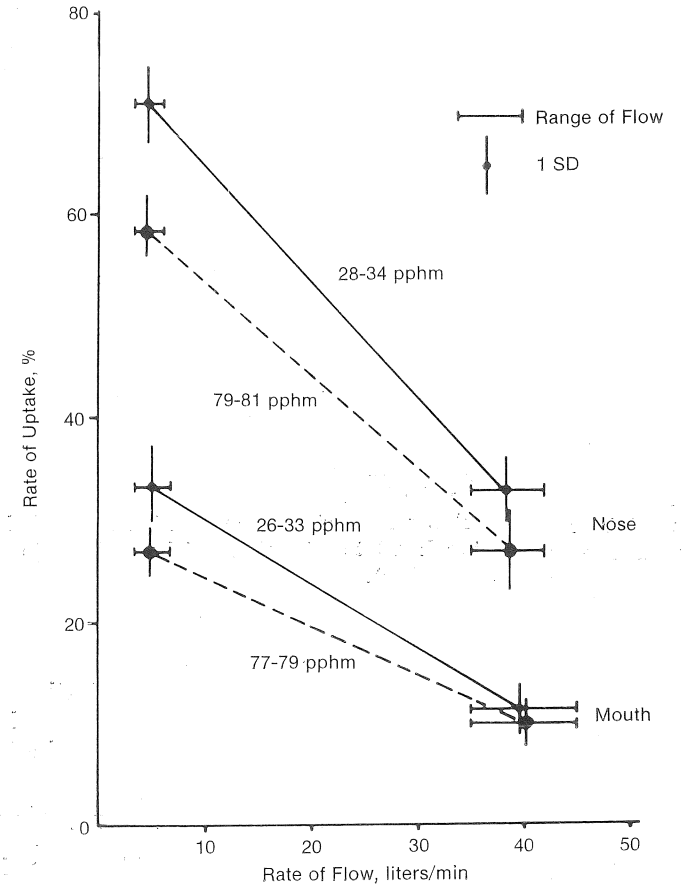

Fig 4.—Data collected on 11 dogs after 15 to 20 minutes of exposure showing the influence of concentration, flow rate, and pathway on the uptake of O_3.

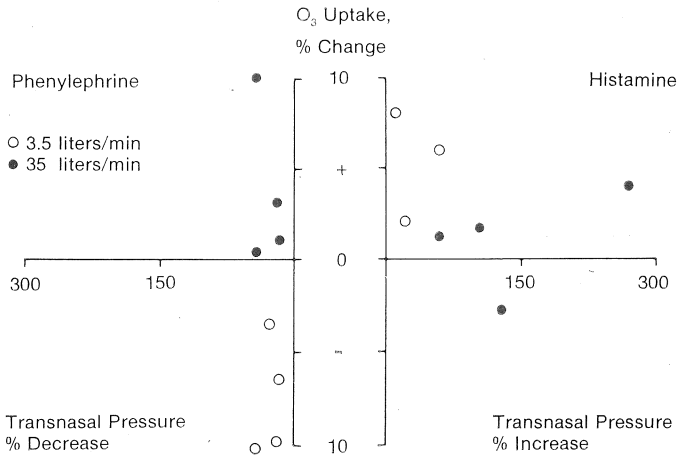

Fig 5.—Percentage change in the rate of nasal uptake of O_3 under conditions of increased or decreased transnasal pressure (at constant flow), the latter produced by aerosols of histamine or phenylephrine.

caused an increase in blood pressure in most of the animals.

The increase in transnasal pressure induced by histamine ranged in six experiments from 12% to 270%. In five instances, there was an associated increase in O_3 uptake which did not exceed take which did not exceed 8%. There were no significant changes in blood pressure or pulse rate at the time the O_3 was administered.

Lower Airways and Parenchyma.—The average rates of uptake of O_3 for all animals during the last four to five minutes of exposure are shown in Table 2. At both pump frequencies the relative uptake, as in the experiments on the upper airways, tended to be slightly higher at the lower concentration; however, this tendency was not statistically significant. For each concentration of O_3, the average uptake fell as the respiratory frequency (and flow rate) increased; the change was probably significant for the higher concentration of gas ($P < .05$).

The blood pressure and heart rate did not change significantly during the exposures.

In three animals, the peak-to-peak changes in airway pressure were increased by the histamine aerosol without any alteration in the uptake of O_3 (six exposures); at the time the uptake of gas was measured, the airway pressure changes were elevated about 20%-40%.

Comment

The nose was apparently more efficient than the

Table 1.—Influence of Flow Rate and Concentration of O_3 on Percentage Uptake of Gas by Isolated Upper Airways (11 Dogs)

	O_3, 26-34 pphm			O_3, 78-80 pphm				
	1: 3.5-6.5 liters/min	2: 35-45 liters/min	1 — 2	3: 3.5-6.5 liters/min	4: 35-45 liters/min	3 — 4, *P*	1 — 3, *P*	2 — 4, *P*
Nose	71.7 ± 1.7* (22)†	36.9 ± 2.7 (16)	<.01	59.2 ± 1.3 (17)	26.7 ± 2.1 (15)	<.01	<.01	<.01
Mouth	33.5 ± 1.9 (12)	11.6 ± 1.8 (9)	<.01	26.8 ± 1.4 (15)	9.8 ± 1.4 (12)	<.01	<.01	NS
Nose minus mouth, *P*	<.01	<.01		<.01	<.01			

* All values are mean ± SE.
† Numbers in parentheses are numbers of experiments.

Table 2.—Influence of Ventilatory Rate and Concentration of O_3 on Percentage Uptake of Gas by Lower Airways (Five Dogs)

O_3 Concentration, pphm	Uptake, % 2.5-4.2 liters/min* (20 Breaths per Minute†)	3.8-6.3 liters/min* (30 Breaths per Minute†)	*P*
20-40	87.4 ± 1.3‡ (21)§	83.0 ± 2.8 (9)	NS
70-85	84.2 ± 1.3 (11)	79.8 ± 1.3 (7)	<.05

* Ventilatory rate.
† Respiratory frequency.
‡ All values are mean ± SE.
§ Numbers in parentheses are numbers of experiments.

mouth in removing O_3 from the airstream, as has been shown for $^{35}SO_2$. The O_3 scrubbing efficiency of each pathway was greater at the lower flow and lower concentration. When changes in nasal vascularity were induced with aerosols of phenylephrine and histamine, the rate of uptake of O_3 was influenced only slightly. Our results underscore the importance of the type of breathing, independently of the ambient concentration of the O_3, in determining how much gas reaches the lower airways. In exercise (mouth open), the exposure of the lungs is likely to be much greater than can be accounted for by the increased ventilatory volume. A similar finding has been reported for $^{35}SO_2$.[2] This increase in exposure of the lower airways and parenchyma, reflecting both greater ventilatory rate and greater penetration by the gas, may account for the exaggerated effects of O_3 on pulmonary function during exercise[9] and for the impaired performance of athletes noted during periods of photochemical smog.[10]

Of the two gases, O_3 and SO_2, the former penetrates the upper respiratory system more readily. At low concentrations, O_3 also induces more peripheral functional[11] and structural changes than does SO_2.[12,13] The differences in rates and sites of uptake shown by the two gases probably reflect at least in part their different solubilities in water: at 25 C and 1 atmosphere of pressure, 0.0014 gm of O_3[14(p257)] compared with 0.4 gm of SO_2[14(p258)] dissolve in 100 ml of water. Another factor that may influence the rate of transfer of O_3 from air to the mucosal lining is its capacity for undergoing chemical transformation in the liquid phase. Whereas the fate of O_3 following transfer to the mucosal surface is uncertain, ^{35}S is found within the blood minutes after the onset of exposure of the upper airways to $^{35}SO_2$.[15] Part of the ^{35}S is in physical solution as a gas, which is thereupon excreted into the lungs, possibly from the pulmonary capillaries.[1]

Jordan and Carlson[4] observed that rabbits and dogs exposed to 10 ppm of O_3 by tracheal cannula developed pulmonary edema more readily than if breathing the gas directly by nose; they estimated that the nose removed about 75% of the gas. Hallett[6] reported that human subjects breathing 1 to 3 ppm of O_3 exhaled from 25% to 75% of the inspired concentration. Vaughan et al[5] administered about 0.45 ppm (45 pphm) or less of O_3 to the isolated upper airways

(nasal path) of beagles at a continuous flow of 3 liters/min and found that the uptake of the gas was virtually complete; however, the rate of uptake fell to about 78% when the initial concentration was 2 to 6 ppm. Our finding of a lower rate of uptake at comparable concentrations (26 to 34 pphm) and flows (3.5 to 6.5 liters/min) may reflect a difference in experimental procedure. Vaughan et al used a plastic (Mylar) bag to collect the downstream gas for analysis, whereas we sampled the airstream directly through Teflon tubing. We repeated their procedure of using a plastic bag and found that some O_3 was lost, presumably from adsorption on the walls, even if the bag was initially "conditioned" by exposure to the gas.

If we combine the results on the uptake of O_3 by the upper and lower airways (and parenchyma), and assume there are no significant differences in the uptake of the gas between continuous and cyclic flow as long as the average rate of flow is the same for the two circumstances, we then estimate that at least 90% of the O_3 in inspired air is removed during quiet breathing by the respiratory system. A significant fraction of the O_3 that is exhaled is probably from the dead space compartment; that is, it never reaches the alveolar level. The precise amount and, perhaps more importantly, the site of removal will be influenced by three factors, which have been examined in this study: whether breathing is by nose or mouth, the rate of flow, and the concentration of the inspired gas. A theoretical model for predicting the magnitude of transfer of O_3 at different levels of the lungs is being prepared.[16]

This study was supported by Public Health Service research grant EC00442.

The glass mixing tube containing two baffles, as depicted in Fig 2, was designed by Robert J. Charlson, PhD, Department of Civil Engineering and Geophysics, University of Washington, Seattle.

References

1. Frank NR, Yoder RE, Yokoyama E, et al: The diffusion of $^{35}SO_2$ from tissue fluids into the lungs following exposure to dogs to $^{35}SO_2$. *Health Phys* **13**:31-38, 1957.

2. Frank NR, Yoder RE, Brain JD, et al: SO_2 (^{35}S labeled) absorption by the nose and mouth under conditions of varying concentration and flow. *Arch Environ Health* **18**:315-322, 1969.

3. Yokoyama E: Uptake of SO_2 and NO_2 by the isolated upper airways. *Bull Inst Public Health* **17**:302-306, 1968.

4. Jordan EO, Carlson AJ: Ozone: Its bactericidal, physiologic and deodorizing action. *JAMA* **61**:1007-1012, 1913.

5. Vaughan TR Jr, Jennelle LF, Lewis TR: Long-term exposure to low levels of air pollutants. *Arch Environ Health* **19**:45-50, 1969.

6. Hallett WY: Effect of ozone and cigarette smoke on lung function. *Arch Environ Health* **10**:295-302, 1965.

7. Byers DH, Saltzman BE: Determination of ozone in air by neutral and alkaline iodide procedure. *Amer Industr Hyg Assoc J* **19**:251-257, 1958.

8. Kleinman L, Radford EP Jr: Ventilation standards for small mammals. *J Appl Physiol* **19**:360-362, 1964.

9. Bates DV, Bell GM, Burnham CD, et al: Short-term effects of ozone on the lung. *J Appl Physiol* **32**:176-181, 1972.

10. Wayne WS, Wehrle PF, Carroll RE: Oxidant air pollution and athletic performance. *JAMA* **199**:901-904, 1967.

11. Young WA, Shaw DB, Bates DV: Effect of low concentrations of ozone on pulmonary function in man. *J Appl Physiol* **19**:765-768, 1964.

12. Scheel LD, Dobrogorski OJ, Mountain JT, et al: Physiologic, biochemical, immunologic and pathologic changes following ozone exposure. *J Appl Physiol* **14**:67-80, 1959.

13. Bils RF: Ultrastructural alterations of alveolar tissue of mice. III. Ozone. *Arch Environ Health* **20**:468-480, 1970.

14. Washburn EW (ed): *International Critical Tables for Numerical Data, Physics, Chemistry and Technology*. New York, McGraw-Hill Book Co Inc, 1928, vol 3.

15. Yokoyama E, Yoder RE, Frank NR: Distribution of ^{35}S in the blood and its excretion in urine of dogs exposed to $^{35}SO_2$. *Arch Environ Health* **22**:389-395, 1971.

16. McJilton C, Thielke J, Frank R: Ozone uptake model for the respiratory system, abstracted. *Amer Industr Hyg Assoc J*, to be published.