Ozone: A review of recent experimental, clinical and epidemiological evidence, with notes on causation

Part 1

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Part 1 of this review is concerned with theoretical issues of ozone dosimetry, animal and cellular studies that illustrate the mechanism of action of ozone on living tissues, and with clinical studies. Animal studies have indicated that there are long term effects from low level long term ozone exposure. Clinical studies involve controlled ozone exposures on human subjects, both normals and asthmatics. Exercise concomitant with the ozone exposure increases the effect of the gas. It is concluded that the induction of an inflammatory response in the airway, both in the nose and in the lung, is the striking and earliest feature of ozone exposure. Current unexplained observations include: the dissociation between the inflammatory and function test response; the mechanisms of 'adaptation' and of airway hyperresponsiveness; and the phenomena that underlie the effect of ozone on maximal athletic performance.

Key Words: Air pollution, Asthma, Ozone, Photochemical oxidants, Trophic effects.

The bibliography on ozone is now so lengthy, and includes many hundreds of references to works published in the past six years, that any summary has to be highly selective. In this review, it is appropriate to emphasize some special aspects of ozone that have to be understood if an informed evaluation of 'causality' is to be made. The effects of any pollutant have to be considered in relation to possible acute effects on the one hand, and long term effects on the

L' ozone : Une revue des données épidémiologiques, cliniques et expérimentales récentes accompagnée d'un commentaire sur la causalité

RÉSUMÉ : La première partie de cette revue porte sur des questions théoriques de dosimétrie de l'ozone, des études cellulaires et animales qui illustrent le mode d'action de l'ozone sur les tissus vivants et, des études cliniques. Les études animales démontrent que de faibles niveaux d'exposition à long terme à l'ozone entraînent des effets à long terme. Les études cliniques comprennent des expositions contrôlées à l'ozone sur des sujets humains, sains et asthmatiques. L'exercice associé à une exposition à l'ozone accroît l'effet de ce gaz. On conclut donc que l'induction de la réaction inflammatoire dans les voies aériennes, dans le nez ou le poumon, est la caractéristique frappante et la plus précoce survenant après une exposition à l'ozone. Des observations courantes mais qui restent encore inexplicées comprennent: la dissociation entre la réponse inflammatoire et les résultats des épreuves fonctionnelles; les mécanismes «d'adaptation» et d'hyperactivité bronchique et le phénomène sous-jacent à l'effet de l'ozone sur la performance athlétique maximale.


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other. Thus, in the case of sulphur dioxide, the special sensitivity of some asthmatics to this gas at very low concentration might play some part in explaining the epidemiological evidence of increased hospital attendances in relation to it; however, the question of whether living in a region with generally higher ambient sulphur dioxide levels increases the risk of developing chronic obstructive lung disease is obviously different – and is hardly likely to be explained by the first observation.

In the case of ozone, animal exposures are valuable in relation to mechanisms of action, although recent human experimentation using bronchial lavage and even bronchial biopsies have made this information rather less relevant than formerly. Animal exposure data are of more contemporary importance in relation to the possible effects of longer term exposures because controlled human clinical studies of that phenomenon are impractical. Hence, in the following review, particularly close attention is paid to the effects in animals shown to follow exposures lasting over weeks or months. In clinical terms, the complex questions involved in the multifactorial disease of ‘asthma’ have to be reviewed if the strengths and weaknesses of the prima facie case in relation to ozone are to be appreciated. This is because, before a judgement of causality is attempted, not only must all collateral evidence have been reviewed, but the strengths of what may actually be tenuous linkages in the inferential argument must also be evaluated.

The question of ‘biological plausibility’ in coming to a judgement of causality is complex. In the case of ozone, it requires consideration of dosimetry, of differences between animal species, and of the pathology and pathophysiology of common conditions such as asthma.

These are the considerations that have determined the comparative emphasis in the following sections.

**ANIMAL AND CELLULAR STUDIES**

**Ozone dosimetry**

A considerable body of work has been published in relation to ozone dosimetry (the dose delivered to different regions of the airway) during the past few years; only the major conclusions are summarized in this section:

- Although somewhat different kinetic assumptions and geometric airway data have been used, all the models indicate that in all species the tissue dose of inhaled ozone (expressed as µg/cm²/min/µg of inhaled ozone) reaches a maximum in the terminal bronchiolar region. The net dose is highest at the first point of impact, namely the trachea.
- The maximally affected zone, in terms of tissue dose, in the human and in the rat is the 17th generation in the airway. For the human data, this calculation was based on a tidal volume of 800 mL and a breathing frequency of 15 breaths/min (1).
- The effect of exercise or increased ventilation is to increase the terminal bronchiolar regional dose slightly, and to increase significantly the pulmonary regional total dose and the dose in the proximal alveolar region.
- Differences among animal species have been considered in detail (2); discussion of these is beyond the scope of this review.
- Theoretical dosimetric calculations have been compared with measurements of ozone in the human respiratory tract under different conditions. Gerrity et al (3) measured ozone uptake in the extrathoracic airways of normal subjects and found that disappearance was as high as 40% on inspiration, and 92% on both inspiration and expiration. Both of these values fell as respiratory rate was increased. A surprising finding was that there was only a small difference between the uptake that occurred with nose breathing and that with mouth breathing. Hynes et al (4) found little difference between nose and mouth breathing in pulmonary function response to ozone.

These studies, taken as a whole, indicate that deposition modelling of inhaled ozone emphasizes the terminal bronchiolar and centriacinar regions as sites of maximal tissue deposition of the gas. Exercise, by increasing the lung volume and tidal volume and increasing the breathing frequency, would be expected to increase the tissue dose significantly. These factors would be operative in addition to the higher dose delivered under these circumstances.

**Acute exposures**

The principal effects observed after acute exposures of a variety of species to ozone concentrations less than 1.0 ppm are lung inflammation and changes in lung permeability (5-7) and increased airway responsiveness. There is increased mortality if the animal is subsequently challenged with a bacterial aerosol. The initial target of ozone appears to be lung fluid lining components and cell membranes (8). Reactive oxygen intermediates are involved in the cell damage, which is partly caused by auto-oxidation chain reactions. Increased permeability has been demonstrated after exposure of rats to 0.12 ppm or 0.4 ppm for two days (9) or in guinea pigs exposed to 1.0 ppm for 1 h (10). The increased airway responsiveness is generally considered to be a consequence of the induced inflammation, and O’Byrne and colleagues (11) found that neutrophil depletion inhibited the increased airway responsiveness after ozone exposure in dogs. However, a recent series of experiments on an isolated rat lung preparation (12) showed that ozone could increase airway responsiveness and damage airway epithelium in the absence of neutrophils before a microvascular leak was caused. The presence of neutrophils was shown to increase the magnitude of these effects of ozone.

The third effect, to increase mortality in mice if ozone exposure occurs before a bacterial aerosol is administered, is primarily due to its effect on macrophages. Recent experiments (13) using *Streptococcus zooepidemicus* in mice showed that 3 h of exposure to either 0.3 or 0.8 ppm ozone led to increased mortality, greater in one strain of mice than in another. The difference in sensitivity was shown to be due to a difference in the sensitivity of the alveolar macrophages in the two different strains of mice. It is the phagocytic...
efficiency of the macrophage that is impaired by the prior ozone exposure.

Evidence that ozone impairs the clearance mechanism of the lung was provided by the observation that ozone exposures of base levels of 0.06 ppm with daily excursions to 0.25 ppm in rats increased the retention of asbestos fibres (14). This was attributed to interference with macrophage function. Ozone exposure has also been shown to decrease the T lymphocyte and antiviral response (15).

Popper et al (16) recently pointed out that nonhuman primates appear to be more responsive to ozone at concentrations less than 1.0 ppm than are rats. This may be due to differences in nasal structure (and hence in delivered dose to the lung) or to differences in lung structure, since rats do not possess the many generations of respiratory bronchioles that exist in human and primate lungs.

This brief summary of the effects of acute animal exposures shown in animal experiments indicates that aggravation of inflammatory lesions, increased airway responsiveness and worsening of respiratory infections are effects that should be looked for in an exposed human population.

Other phenomena are described after acute ozone exposures to animals or to cells, usually to higher concentrations than those in ambient air. These include: changes in the nasal cavity and nasopharynx; assessments of the effects of the age of the rat on the outcome – in general younger rats seem more sensitive; no differences in effects between normal rats and rats with elastase-induced emphysema; studies of a wide range of biochemical effects, particularly on polysaturated fatty acids, antioxidants and proteins, and on the production of arachidonate metabolites; genotoxicity, mutagenicity and effects on carcinogenicity (although such effects can be shown, for instance, on DNA [17], there is no human correlate); and cytogenetic studies showing effects with in vivo exposures to as little as 0.4 ppm ozone.

Witschi (18) recently reviewed these observations, pointing out that the radiomimetic activity of ozone did make it a potential contributor to human lung cancer.

### Long term exposures

Human controlled exposures for longer than a few hours are not feasible; hence, the results of longer term animal exposures are very important. Since the primate lung appears to be more sensitive to ozone than the rat lung is, the evidence from primate exposures (*Macaca fuscata*us) will be considered first. Almost all of this work originated in the Primate Center in Davis, California. It was recently elegantly summarized and particularly well illustrated by this research group (19).

The exposures were generally to 0.25 ppm ozone for 8 h/day, seven days a week for 18 months. A very brief summary of their findings and conclusions follows:

- The initial inflammatory response is modified in subsequent exposures, but inflammatory cells in the peribronchial connective tissues persist during exposures that last up to one year.
- Epithelial and interstitial changes in distal airways are the most striking morphological changes in animals exposed for a year or more. There is a proliferation of nonciliated bronchiolar and type 2 alveolar epithelial cells. These changes start early and are evident after 50 h of exposure to 0.8 ppm of ozone.
- Necrosis of ciliated and type 1 pneumocytes occurs, and these cells are replaced by the cells noted above. The long term effect is therefore a 'remodelling' of the centriacinar airways by extension of bronchiolar cell types in airways that were formerly alveolar ducts.
- There is an increase of collagen localized in the peribronchial and centriacinar regions. This confirms earlier studies from the same laboratory in rats exposed to higher concentrations of ozone.
- The morphological changes induced by the ozone exposure were still present after a six-month postexposure period of living in filtered air. Quantitative morphology studies indicate that the lungs were more abnormal after the postexposure period than immediately after the ozone exposure.

Rat exposures of longer than two weeks have been studied in a number of centres. Using a 78-week exposure protocol of a base exposure of 0.06 ppm with spikes to 0.25 ppm. Chang et al (20) showed the following: that an acute response occurred in the centriacinar region, and that these changes partly resolved; that type 2 cells increased, and that the interstitium was increased; and that the basement membrane was thickened, but that centriacinar remodelling did not occur. Earlier observations from the same laboratory using a six-week exposure protocol of similar concentrations (21) noted that there was an increase in interstitium thickness and an increase in number of alveolar macrophages. Rat exposures to 0.95 ppm for 8 h/day for 90 days were found by Barr et al (22) to have resulted in airway remodelling, and the lesions resulting from chronic versus daily episodic exposures were similar (23).

Costa et al (24) demonstrated changes in lung function in rats after brief exposures, and more recently reported reductions in forced vital capacity (FVC) of rats after repetitive ozone exposures (25).

Soldiva et al (26) in Sao Paulo exposed 60 rats for six months to the ambient air and compared them with controls kept for the same length of time in a clean area. A monitoring station 200 m from the exposure location showed that ozone went up to 0.4 ppm, particulates up to 90 μg/m³, sulphur dioxide up to 0.025 ppm and carbon monoxide up to 4 ppm. The exposed rats had secretory cell hyperplasia in the airways, ultrastructural ciliary alterations and a more rigid nucleus – changes that caused mucociliary clearance impairment. Nasal resistance and inflammatory cells in bronchoalveolar lavage (BAL) were also increased in the exposed group.

### Ozone in combination with other chemicals

Since human exposures are rarely to a single pollutant, the question of interaction with other chemicals assumes some importance. In very detailed experiments on rats, Last (27) studied the effects of ozone and sulphuric acid aerosol alone and in combination. On all outcomes related to an inflamma-
Recent experiments have shown that after ozone exposure, changes in the nose detected by nasal lavage can be used as an indicator of changes in the lung. Graham and Koren (29) exposed 10 normal subjects to air or 0.4 ppm ozone for 2 h with exercise. Nasal lavage was performed before, immediately after and 18 h after exposure. BAL was also done at 18 h. Nasal polymorphonuclear neutrophils (PMN) increased 7.7-fold immediately after exposure and were still increased 6-fold at 18 h, which was identical to the increase in PMNs in BAL at this time. Albumin levels increased 3.9-fold in nasal lavage and 2.2-fold in BAL at 18 h. Comparison of the PMN data in nasal lavage and in BAL for each individual showed a significant quantitative correlation after the air exposure but not after ozone exposure.

Bascom et al (30) studied 12 asymptomatic subjects with a history of allergic rhinitis. They were exposed to 0.5 ppm of ozone in an exposure chamber without exercise or to clean air as a control. Nasal challenge was performed with four doses of ragweed or grass antigen. Symptoms were rated and nasal lavage was performed. Histamine and albumin concentrations were measured, and tobramycin/mixture esterase activity and cell counts performed. Ozone caused a significant increase in upper and lower respiratory symptoms; a mixed inflammatory cell influx with a 7-fold increase in nasal lavage neutrophils; a 20-fold increase in eosinophils; a 10-fold increase in mononuclear cells; and an apparent sloughing of epithelial cells. Albumin concentration also increased. However, all of this did not increase the response to allergen.

Frischer et al (31) recently reported some field studies on the nose. Forty-four children were studied from May to October 1991. Each had five to eight lavages. One hundred and forty-eight nasal lavages were performed on 14 days following high ozone exposure (more than 180 µg/m³ or 90 ppb) and 106 after low exposures (less than 40 µg/m³ or 70 ppb). A significant increase in PMN counts occurred following high ozone days (27.38X10³ versus 20.27X10³). Eosinophilic cationic protein also changed (3.49 µg/L on low days and 5.39 µg/L on high days); myeloperoxidase also changed (77.39 µg/L on low days and 138.6 on high ozone days). The authors concluded that “Ozone at ambient concentrations initiates a reversible inflammatory response of the upper airways in normal children”.

McBride et al (32) recently reported on a comparison between the nasal inflammatory responses of normal and asthmatic subjects. Exposures using a head dome were to 120 or 240 ppb of ozone or clean air for 90 mins with moderate exercise. They reported that the degree of inflammatory response, as judged by nasal lavage specimens, was considerably greater in the asthmatics, though pulmonary function test changes were not different between the two groups (and were minimal in degree).

**Effects on the normal lung**
Over the past 20 years, many human studies with controlled exposure to ozone have been reported. These are very briefly summarized here. It has been found that:

- FVC and forced expiratory volume in 1 s (FEV₁) of young normal subjects is lowered by a 6 h exposure with exercise to as little as 0.08 ppm of ozone (the current Canadian 1 h standard) (33).
- Considerable individual variation in response exists in normal subjects, but in a given individual, reproducibility is good. Hazucha (34) analyzed results from different laboratories involving a large number of normal subjects. He made the important observation that although one can talk of a “threshold” value for an individual, there is no such thing as a threshold value for a group. This is because, if a statistically significant shift in mean value of a group is considered to be the criterion, then in one individual the value will have fallen very significantly as a result of the exposure. This point is often misunderstood.
- The first effect of ozone is not to cause airway constriction, but to limit the maximal inspiration the individual can take (probably through a spinal reflex activated by irritant receptor stimulation) (35).
- The decrement in lung function continues the longer the exposure, at least over 6 h (33).
- Over a 4 h period, an exposure regimen starting at 0 ppm, increasing to 0.24 ppm at the maximum and declining to 0 ppm causes a greater change in FEV₁ than a constant exposure to 0.12 ppm for the whole period (36).
- When the FVC is lowered, airway inflammation is demonstrable. This was first shown by Selzter et al (37) and confirmed in additional studies by Koren et al (38). A recent report from Aris et al (39) involved BAL in 12 normal subjects 18 h after exposure to 0.20 ppm ozone with moderate exercise. There was evidence of induced inflammation as shown by an increase in polymorphs and in inflammatory mediators. In addition, morphometric studies based on bronchial biopsy material showed a 6-fold increase in neutrophils per cm² of tissue from the bronchial wall. Koren et al (40) recently summarized the time sequence of the inflammatory events in normal healthy subjects following a 2 h exposure to 0.4 ppm ozone for 2 h with moderate exercise. At the end of the exposure, PMNs, prostaglandin E₂ and interleukin-6 were all increased in the BAL fluid and were at higher levels than they were 18 h later. Fibronectin and urokinase-type plasminogen activator, both of which have been associated with fibrogenic processes, were at higher levels in BAL at
time that maximal work could be sustained. The mechanism of these effects is not precisely delineated: they may indicate that ventilation-perfusion mismatch in the lung has had the effect of lowering the arterial oxygen tension significantly. No direct measurements of arterial oxygen tension have been made however.

Effects on asthmatics

Early observations on asthmatics indicated that the percentage change in FEV₁ for a given ozone exposure was not much different from that in nonasthmatics. However, when the data are examined closely, it is clear that such a conclusion leads to an underestimation of the importance of the comparative effects. The recent observation that the nasal inflammatory response after ozone exposure is greater in asthmatic than in nonasthmatic subjects is noted above.

Kreit et al (49) studied nine asthmatic and nine normal subjects (Table 2). Subjects were classified as asthmatic if they had a history of reversible chest tightness and wheezing, a previous diagnosis of asthma made by a physician, and a concentration of methacholine required to double baseline specific airway resistance (PC100sRaw) of less than 1.5 mg/mL. All of these subjects were felt to have relatively mild asthma. Their ages ranged from 21 to 34 years; five were women and four were men. Baseline FEV₁ values varied from 2.58 to 5.32 L. The nine normals were aged 19 to 31 years, and comprised five women and four men; all had normal methacholine responsiveness. An exposure chamber was used. Exercise was done on a bicycle ergometer with minute ventilations of about 50 L/min. Ozone concentrations were 0.4 ppm. With initial values of lung function 30 to 40% lower in the asthmatics, the effects of a comparable absolute fall in ventilatory indices are necessarily more significant in terms of limiting exercise capability or in causing dyspnea. The methacholine responsiveness changed in the normals from a pre-exposure value of 32.3 mg/mL (required to double airway resistance) to a postozone value of 8.5 mg/mL. In the

<table>
<thead>
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<th>TABLE 1 Percentage change in inflammatory indicators following different exposures of ozone</th>
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<tr>
<td>Inflammatory indicator</td>
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<tr>
<td></td>
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<tr>
<td>Neutrophils</td>
</tr>
<tr>
<td>Fibronectin</td>
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<tr>
<td>Protein</td>
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<tr>
<td>PGE2</td>
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<td>LDH</td>
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<td>C3a</td>
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<td>PA</td>
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<td>Phagocytosis</td>
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<tr>
<td>Alpha-1-AT</td>
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<td>IL-6</td>
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Alpha-1-AT: Alpha-1 antitrypsin; C3a: Complement fragments; IL-6: Interleukin-6; LDH: Lactate dehydrogenase; ND: Not done; PA: Plasminogen activator; PGE2: Prostaglandin; Phagocytosis: (by macrophages). Data from reference 41.
nine asthmatics, the pre-exposure value was 0.52 mg/mL, and after ozone it fell to 0.19 mg/mL. Thus, although in each case there was a 4-fold change, in the asthmatics it was from an initially hyperresponsive level.

- A low ozone exposure of 0.12 ppm breathed for 1 h at rest was found by Molino et al (50) to lead to a reduction in dose of inhaled allergen for a given response in six of seven asthmatics. If confirmed in larger scale studies, this would have important long term implications. As noted above, Bascom et al (30) did not find that ozone exposures enhanced the effect of an allergen in the nose, although an inflammatory reaction had been induced.

**TABLE 2**

Changes induced by two-hour exposure to 0.4 ppm ozone with intermittent exercise

<table>
<thead>
<tr>
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<th>FVC (L)</th>
<th>FEV₁ (L)</th>
<th>FEV₁/FVC% (%)</th>
<th>FEF25-75 (L/s)</th>
<th>IC</th>
<th>sRaw</th>
</tr>
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<tbody>
<tr>
<td>Normals (n=9)</td>
<td>−0.48</td>
<td>−0.58</td>
<td>−3.7</td>
<td>−1.08</td>
<td>−0.49</td>
<td>+1.74</td>
</tr>
<tr>
<td>Mean initial values</td>
<td>5.11</td>
<td>4.39</td>
<td>86.8</td>
<td>4.92</td>
<td>3.22</td>
<td>5.93</td>
</tr>
<tr>
<td>Asthma (n=9)</td>
<td>−0.67</td>
<td>−0.81</td>
<td>−9.3</td>
<td>−0.92</td>
<td>−0.51</td>
<td>+8.92</td>
</tr>
<tr>
<td>Mean initial values</td>
<td>4.53</td>
<td>3.37</td>
<td>74.4</td>
<td>2.70</td>
<td>2.89</td>
<td>8.63</td>
</tr>
</tbody>
</table>

FEF25-75 = Maximal midexpiratory flow; FEV₁ = Forced expiratory volume in 1 s; FVC = Forced vital capacity; IC = Inspiratory capacity; sRaw = Specific airway resistance. Data from reference 49

Ozone in combination with other chemicals

Although initial studies had indicated that ozone and sulphur dioxide breathed together might enhance the effect of each, subsequent experiments failed to support this conclusion. Koenig et al (51) showed that pre-exposure to ozone enhanced the effect of sulphur dioxide in a group of 13 adolescent asthmatics. The sequences of exposure studied were: air followed by 100 ppb sulphur dioxide; 120 ppb ozone followed by 120 ppb ozone; and 120 ppb ozone followed by 100 ppb sulphur dioxide. Air-sulphur dioxide and ozone-ozone exposures did not cause significant changes in function, but ozone-sulphur dioxide exposure caused an 8% fall in FEV₁, a 19% increase in respiratory resistance, and a 15% decrease in V_max 50%.

**CONCLUSIONS**

There is a wealth of experimental data on the acute effects of ozone on the human subject. The first phenomenon observed is the induction of inflammation. Decrement in lung function are complex in origin, and may not follow closely the severity of the inflammation induced. There are many poorly understood phenomena, including the precise determinants of the individual response; the mechanism of ‘adaptation’; the phenomena that underlie the observed reduction in maximal oxygen uptake; and the consequences of ozone exposure in combination with other irritants. There is evidence that asthmatic subjects may have a greater inflammatory response than nonasthmatics. Although the degree of function test response is broadly similar between normals and asthmatics, the latter may be more severely affected because their initial pulmonary function may be lower, and their pre-exposure degree of airway responsiveness is greater.

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