Ozone Interactions with Lung Tissue
Biochemical Approaches

The likelihood that ozone ($O_3$) represents a significant health hazard to man is potentiated by the ubiquity of its environmental occurrences. Significant quantities of $O_3$ appear in airplane cabins at altitudes greater than 30,000 feet [1], and $O_3$ forms in proximity to high voltage electrical equipment such as that utilized for air and water purification systems [2]. Of most importance is the fact that over 90 per cent of measured oxidant in photochemical smog is ozone [3]. Although the effects on human lungs of oxidizing pollutants in concentrations found in urban smog are still poorly understood [4], there is considerable experimental pathophysiologic evidence pertaining to $O_3$ toxicity in animal lungs. Elucidations of mechanisms of $O_3$-induced lung injury should continue to improve our knowledge of the cellular biology of the lung and ultimately better our care of human subjects with pulmonary diseases. Furthermore, a better understanding of the phenomena of adaptive tolerance to $O_3$ injury and prevention of $O_3$ injury by antioxidant substances (such as vitamin E) may have significant public health implications.

The biochemical effects of $O_3$ on the lung have been investigated in various ways. These have included attempts to identify: (1) molecular mechanisms of $O_3$ interaction with pulmonary tissue; (2) metabolic perturbations caused by acute exposures in the range of 1 to 10 ppm $O_3$ for several hours; (3) metabolic perturbations caused by chronic exposures in the range of 0.1 to 0.8 ppm $O_3$ for days to months; (4) an explanation for the decreased susceptibility of the lung to $O_3$ in previously exposed animals and (5) factors that increase susceptibility of the lung to $O_3$-induced injury. Since the appearance of several reviews on this general topic [2,4–10] new information has become available concerning the effects of levels...
of O₃ approaching urban environments on various metabolic processes in the lung. This is a summary of recent research on the biochemical effects of O₃ on the lung.

**Molecular Mechanisms of O₃-Induced Damage.**

The molecular lesions produced by O₃ must occur at subcellular levels of biologic organization. Most of the accumulated information on the biochemical basis of O₃-induced lung damage has been related to its oxidant properties. O₃ is one of the most powerful oxidizing substances known. Only fluorine has a more electronegative oxidation potential. In biosystems, functional groups readily oxidized by O₃ include -SH, -NH₂, phenolic -OH and -CHO. Ozone may react with polyunsaturated (olefinic) lipids, thus inducing lipid peroxidation. One degradation product of peroxidized lipids, malonaldehyde, has been shown to increase in the lungs of animals exposed in vivo to as little as 0.8 ppm O₃ for a week [11]. The peroxidative process leads to the formation of free radical intermediates which may, in addition to further lipid peroxidation, cause deteriorative diene conjugations, ozonide, carbonyl and aldehyde formations, and SH group oxidations [7–9,12]. Thus, O₃-induced cell damage mechanisms have been likened to the fairly well recognized effects of free radical formations seen in radiation-induced reactions [13]. These reactions result in a number of deteriorative molecular processes including intra- and intermolecular cross-linking of proteins and nucleic acids [14].

The most commonly proposed mechanism is O₃ reaction with phospholipid micelles of cell membranes and/or intracellular lipid constituents causing changes in membrane permeabilities, leakage of cell contents and loss of intracellular compartmentalization [15–18]. Oxidant-induced red blood cell hemolysis in vitamin E-deficient red cells has been attributed to lipid peroxidation [19,20]. Alternatively, O₃ may react with extracellular (viz. surfactant) and/or membrane and intracellular lipids to produce lytic products that may be more cytotoxic than O₃ itself.

Although there is a paucity of direct evidence, oxidations and/or oxidation products most probably represent the molecular mechanisms of O₃ cytotoxicity. Reactive intermediates of molecular oxygen (viz. superoxide and hydroxyl radicals and singlet oxygen) may be involved. The oxidation theory is buttressed by studies designed to demonstrate the ability of water soluble and/or lipid soluble antioxidants to reduce O₃ toxicity. These studies have shown protective effects of supplemented antioxidant substances such as -SH compounds (viz. glutathione), tocopherols, aromatic amines (viz. p-aminobenzoic acid), and ascorbic acid on both animal survival [6,21–25] and on lung biochemical marker enzyme activities (which reflect the magnitude of oxidant-induced lung damage) [25].

Unresolved are the problems of determining the most important cellular constituents that O₃ oxidizes and defining the contributory role and nature of potential amplification systems such as cytotoxic lipid peroxides and lysosomal enzymes. In addition, the role of a number of neurohumoral factors and inflammatory mediators of inflammation, coagulation and immune phenomena derived from plasma proteins and from cells, which may initiate or amplify inflammatory cascades to perpetuate cell injury, need to be further clarified.

It must be concluded that molecular mechanisms of O₃-induced lung injury under in vivo exposure conditions are incompletely understood. At the present time the molecular mechanisms of O₃-induced lung damage cannot be distinguished from other types of lung injury, including those resulting from other forms of noxious environmental exposure.

**Acute High-Dose Effects.**

High-dose cytotoxic exposures (viz. 1–4 ppm X hours) have generally demonstrated deteriorative changes in metabolic parameters in the lung. As depicted in Figure 1, these biochemical changes can be caused either by direct O₃-induced cytotoxicity or via amplification systems consisting of inflammatory mediators derived from plasma proteins and from cells. Biochemical changes have included decreased activities of almost all enzymes assayed, including key -SH enzymes such as glucose-6-phosphate dehydrogenase [20] and succinate dehydrogenase [27], membrane-bound enzymes such as the plasma membrane 5'-nucleotidase [5] and lung microsomal membrane P-450 [28]. Respiratory control and phosphorylation activities of mitochondria are also severely compromised by acute exposure to O₃ [16], as is the production of surfactant, measured by decreased lecithin synthesis [29], and the activity of secretory lysozyme [30]. These severe depressions in enzymatic activities almost certainly represent the biochemical correlates of acute O₃-induced lung cytotoxicity.

If acute exposure to O₃ is not lethal and animals are allowed to recover, some parameters of lung metabolism become greatly augmented. This phenomenon appears coincident with initiation of reparative and proliferative phases of lung injury [31,32]. Discordances to this general theme in the literature are probably related to differences in the duration and magnitude of exposures to O₃ and to studies being performed at different stages of lung injury-repair processes.

**Chronic Low-Dose Effects.**

Exposure of animals to O₃ levels of 0.1 to 0.8 ppm (which are close to those occurring in urban smog) have caused increases in
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Figure 1. Acute effects of high-dose O₃ exposure on the lung. O₃ is thought to cause lung cell injury via deteriorative primary oxidative reactions and/or via activation of amplification systems such as the products of lipid peroxidation (lipid peroxides), mediators of coagulation and/or inflammation and lysosomal enzyme systems. "□" represents intrinsic defense systems protecting the cells against either primary (e.g. phenolic antioxidants) or secondary (e.g. glutathione peroxidase, α-1 antitrypsin) injury. In general, the stage of acute cell injury is accompanied by depressions in cellular biochemical activities.

liver metabolism within 24 to 48 hours. As depicted simplistically in Figure 2, reflections of this enhanced metabolic activity include increases in glucose-6-phosphate dehydrogenase activity [31], needed for nucleotide synthesis and the generation of reducing equivalents required for reductive biosynthesis; increases in mitochondrial enzyme activities [33], needed for energy requirements to subserv reductive signaling and proliferative processes; and increased DNA, RNA and protein synthesis [5,34-36]. These low-dose (0.1 to 0.8 ppm) mediated increases in lung metabolic activities appear to reflect cellular reparative and proliferative responses to O₃-induced cellular injury processes [32].

The site of augmented lung cellular enzyme activities is not well defined. Low-dose (0.1 to 0.8 ppm) O₃ exposure initially induces increased cell turnovers, causes selective increases in certain cell types such as Clara cells and type II cells, and elicits the accumulation of inflammatory cells [37-40]. It seems certain that even low levels of O₃ exposure can elicit damage in ciliated cells of the terminal and respiratory bronchioles, and type I cells of the proximal acinar regions [37-40]. This, in turn, induces a proliferation of Clara cells and type II cells and elicits inflammatory cell infiltration from the blood, and monocyte/macrophage proliferation from the blood and resident lung pools. Both processes would be expected to increase over-all lung tissue metabolism. Biochemical augmentations could thus reflect O₃-induced lung injury, i.e., a correlation exists between concentration of O₃ exposure and increased activities of a number of parameters of lung metabolic activities [11,18,31].

Diffuse acute or chronic lung injury is often accompanied by a tissue reaction occurring in the connective tissue of the supportive structures of the lung. The ultimate end-result is either resolution of the early edematous inflammatory exudate or development of fibrogenic responses. Collagen, a major component of lung connective tissue, is of fundamental importance in maintaining the structure of conducting airways, blood vessels, and the alveolar gas exchanging portions of the lung. After injury, it serves as a scaffold for the reconstitution of alveolar architecture. Total lung collagen reflects a balance between controlled production and degradation processes. Observations in rats indicate that ozone concentrations of 0.5 to 0.8 ppm increase lung prolyl hydroxylase activity, a key enzyme concerned with collagen synthesis, within 24 hours after the onset of exposure [41,42]. The implication of this finding is that collagen synthesis is significantly higher in O₃-injured lungs, at least during early phases of exposure to low concentrations of O₃.

As previously mentioned, O₃ exposure causes the
accumulation of inflammatory cells in the lung, as do exposures to other respiratory irritants such as cigarette smoke. Macrophage accumulation predominates in low-dose exposures. Biochemically, this is reflected by an increase in activities of lysosomal enzymes in the lungs of O₃-exposed animals [15,18]. Release of inflammatory cell lysosomal phospholipases and proteases could potentiate O₃-induced cell injury. Currently the role of accumulated inflammatory cells in mediating or amplifying O₃-induced damage remains undefined. It may be that the increased collagen synthesis noted early in O₃-injured lungs is related to inflammatory cell accumulations and localized release of fibroblast stimulating factors [43,44].

During the early injury and repair phases, lung collagen synthesis is stimulated more than is noncollagen protein synthesis [41,42]. By the third day of exposure the increased prolyl hydroxylase activity is accompanied by measurable increases in lung hydroxyproline content, an indicator of total lung collagen content. These findings complement morphologic observations that prolonged exposures of experimental animals to O₃ result in mild fibrosis of lung parenchyma [45,46]. Further studies of molecular mechanisms of connective tissue repair after exposures to O₃ (alone or simultaneously with other environmental noxious agents) would help to predict the chronic effects of O₃ exposure on human lungs.

The possibilities that chronic low-dose O₃ exposure or intermittent high-dose O₃ exposures may cause accelerated aging has been discussed in the literature [5]. In addition, as O₃ seems capable of accelerating cell division in some cell types [40], it seems possible that O₃ could act as a promoter of carcinogenesis [47]. Thus far there have been no specific biochemical mechanisms proposed for these phenomena.

Decreased Susceptibility to Lung O₃ Damage. Information concerning modifiers of O₃-induced lung injury is of considerable importance insofar as overall considerations of oxidant health hazards are concerned.

Studies have shown that pre-exposure of animals to O₃ at concentrations as low as 0.3 ppm or brief sublethal exposure to higher concentrations result in decreased susceptibility to exposure to subsequent lethal edemogenic concentrations of O₃ [48]. This phenomenon is called the tolerance effect [2,6,27,32]. The lowest pre-exposure doses of O₃ demonstrated to induce tolerance are approximately the same threshold levels that induce augmentation of biochemical parameters in the lung, suggesting that cellular biochemical changes are responsible for induction of the tolerance state. Tolerance to the acute, lethal edemogenic effect of O₃ is not of significance with respect to the harmful effects of O₃ in...
photochemical smog because the test doses of $O_3$ causing lethal edemogenic effects are considerably higher than the 0.8 ppm or less found in photochemical smog. In addition, the state of tolerance to lethal edemogenesis does not confer protection against either airway physiologic manifestations of $O_3$-induced bronchospasm [49], presumably mediated by airway nerve reflexes, or against the inflammatory changes in the bronchioles and proximal acini caused by low levels of $O_3$. In fact, emphysema has developed in experimental animals who have been repeatedly exposed to levels of $O_3$ that would produce tolerance to acute edemogenic concentrations of $O_3$ [6].

Recent biochemical and pathologic observations have revealed a more important aspect of decreased susceptibility to $O_3$ exposures. This is a phenomenon we refer to as adaptation. As previously mentioned (Figure 2), low-level $O_3$ exposures (0.1 to 0.8 ppm) cause augmentations of a number of parameters of lung metabolism. These augmentations reach a maximum 2 to 4 days after exposure has begun [31,32]. If the concentration of $O_3$ is low enough (0.1 to 0.5 ppm) the biochemical changes diminish during continued exposure [32,40]. A most important aspect of this adaptive phenomenon is that its effectiveness is concentration-dependent. In rats, adaptation is complete at the 0.1 to 0.5 ppm level; the biochemical and morphologic abnormalities readily apparent up to seven days are no longer detected after a month (our unpublished observations). The process of adaptation obviously has great significance relative to the long-term effects of photochemical smog on human populations. Although continuous low-level $O_3$ exposures (0.1 to 0.5 ppm) would thus appear to cause reversible biochemical and morphologic abnormalities in the rodent, there is thus far only a suggestion that helpful adaptations to chronic low-level $O_3$ exposures occur in man [50]. Its general applicability is currently being tested in nonhuman primates.

Finally, it has already been noted that administration of a wide variety of antioxidant substances provide a relative protection against $O_3$ exposure in experimental animals. As illustrated in Figure 2, low-level $O_3$ exposures also elicit increases in a number of potential biochemical mechanisms related to tissue oxidant susceptibility. The enhanced pentose shunt [25,31], glutathione peroxidase [11] and superoxide dismutase [51] activities would all be expected to ameliorate the effects of $O_3$ on lung tissue, provided that $O_3$-induced free radical formation, lipid peroxidation and -SH oxidation were significant factors in initiating and amplifying the lung damage. The relevance of this biochemical finding for man is yet to be determined.

**Increased Susceptibility to Lung $O_3$ Damage.** A number of factors have been experimentally shown to augment $O_3$ toxicity. Exercise [52] and tracheostomy [53] cause potentiation probably as a result of increasing the concentration of $O_3$ being delivered to the lung parenchyma at any given ambient level. Deficiencies of vitamin E probably potentiate $O_3$-induced damage by reducing available lung lipid antioxidant defense systems [22,23,25]. Viral and bacterial infections [45], thyroid hormone [54], aerosolized hydrogen peroxide [55], nitrogen dioxide [56] and agents known to modify microsomal mixed-function oxidases [57] have all been reported to augment experimentally induced $O_3$ toxicity.

Caution must be exercised in interpreting the overall affects of environmental pollutants, such as $O_3$, on parameters of lung biochemical activities. Both $O_3$ toxicity and changes in biochemical activities subsequent to $O_3$ exposures show considerable variability related to differences in species, strain, age, sex, nutritional status (e.g., dietary vitamin E and fatty acids constituents), method of exposure, humidity, temperature and respiratory mechanics. Considering the marked anatomic differences in the respiratory tract between species or even between people, it is clearly difficult to predict the fraction of the ambient $O_3$ that reaches distal lung parenchyma. An obvious demonstration of the diminishing inspired concentrations of $O_3$ that reach the distal lung parenchyma comes from recent scanning electron microscopy observations of lungs of monkeys exposed to 0.2 to 0.8 ppm $O_3$ for a week [58]; exposures to 0.2 ppm caused lesions limited to the proximal generation of respiratory bronchioles whereas exposures to 0.8 ppm caused damage to distal orders of bronchioles and centriacinar regions, i.e., the junction of conducting airways and parenchymal gas exchange tissue. The possible relationship of these observations to the development of centrilobular emphysema in man exposed to photochemical smog may be important to determine.

**Further Considerations.** Clarification of the critical effects of $O_3$ on lung tissue requires better understanding of the cellular biology of the lung. The ability of low-level $O_3$ exposure to stimulate several different metabolic pathways results from chemical interactions of $O_3$ with lung tissue. Biochemical "indicators" of inflammation, reparative and/or fibrogenic responses appear to quantitatively reflect the magnitude of $O_3$-induced changes. The role these processes play in the pathogenesis of such human diseases as chronic bronchitis, emphysema, interstitial fibrosis and pulmonary carcinoma must be determined by further research.

Measurement of sequential changes in lung biochemical parameters appears to represent an important index for characterizing tissue responses to injury and repair processes in the lung. Determinations
of these parameters would appear to represent a useful technic for assessing the toxic effects of inhaled environmental agents and the efficacy of therapeutic modalities such as antioxidants and anti-inflammatory compounds. An important challenge facing the lung biochemist will be to unravel the nonspecific biochemical changes in the lung caused by injury due to tissue inflammation and reparative processes from the specific inciting molecular and cellular mechanisms of the injury itself.

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REFERENCES


36. Werthamer S, Penha PD, Amaral L: Pulmonary lesions in-


