New perspectives on hyperoxic pulmonary toxicity—
a review

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A. INTRODUCTION

Pulmonary oxygen toxicity continues to be a subject of interest because of its relevance to clinical therapy and to underwater operations. Of greatest concern are the effects of oxygen exposures up to, but not exceeding 1 atm. Exposure to greater pressures (hyperbaric oxygen) is associated with the precipitation of convulsive seizures and represents a very special circumstance. For these reasons the subject of this review is restricted to oxygen concentrations not greater than 1 atm. In this article oxygen partial pressures in the range of 0.95–1.0 atm (often termed normobaric oxygen) is referred to as hypoxia. When oxygen tensions below this range are discussed, the actual percentage is given.

The first part of this paper discusses the acute effects of elevated oxygen partial pressure. It is evident from numerous reviews of this topic (1–5) that the biochemical events that underlie acute oxygen toxicity are incompletely understood, and that advancement of knowledge in this area has been slow. This paper summarizes the most recent data concerning the mechanisms that are traditionally considered in reviews of oxygen toxicity: generation of free radicals and cellular metabolic derangements. On the other hand, it is recognized that a number of pertinent studies, such as those dealing with the interaction between oxygen and the blood coagulation system, or relating to pulmonary damage associated with conditions other than oxygen exposure, have not received wide attention. That work has also been included here because it identifies other biochemical systems that may be affected by oxygen and affords evidence that diverse factors, acting through a common mechanism, may all lead to a similar form of pulmonary injury.

The second part of this paper deals with the subject of chronic oxygen toxicity. The picture of this condition is dominated by a form of pulmonary fibrosis that strongly resembles the lung pathology associated with a variety of other disorders (6–8). In this article the similarities between the effects of oxygen and other fibrotic processes are considered in some detail. Furthermore, several mechanisms that may be responsible for development of pulmonary fibrosis are examined. These include cellular responses, an autoimmune reaction, and alterations of collagen metabolism.

Thus the aim of this article is to develop a unified description of the biological response to elevated oxygen partial pressure—a description that incorporates not only often-described classic features but also a number of related observations that have been neglected in previous reviews. In addition it refers to a large body of information dealing with the chronic, fibrotic effect of oxygen, which is not usually discussed in reviews of acute pulmonary oxygen toxicity. It is hoped that this integration of information, coupled with a modest amount of speculation on the author’s part, will generate new perspectives regarding pulmonary oxygen toxicity and will result, as Pope (9) says, in a review

Where order in variety we see,
And where, tho’ all things differ, all agree.
B. ACUTE PULMONARY TOXICITY OF HYPEROXIA

1. Characteristics

Acute pulmonary oxygen toxicity is the condition that results from exposure of man or animals to oxygen concentrations of 90%–100% for a period of several days. Little has been added to the description of this condition since it was reviewed by Clark and Lambertsen (4), although its characteristics have been confirmed (10–13) and summarized a number of times (1, 3). The following summary is based on these earlier papers.

The initial stage of acute pulmonary oxygen toxicity has been termed the exudative phase and is characterized by a perivascular and interstitial inflammatory response that includes damage to capillary endothelium with exudation of edema fluid, polymorphonuclear leukocytes, and eventually macrophages. The edema is associated with widening of the interalveolar septa and a thickened air-blood tissue barrier. Alveolar hemorrhage develops and there is fibrin deposition in alveoli (hyaline membranes). Loss of alveolar lining cells (type I lining cells) also can occur during this phase.

As the disease progresses it reaches a stage referred to as the proliferative phase, characterized by resolution of the inflammatory exudate and increased cellularity of the interstitium due to proliferation of macrophages and fibroblasts, as well as by proliferation of the cuboidal type II alveolar lining cells that serve to reepithelialize the denuded alveolar basement membrane. By the time a typical laboratory animal such as the rat is near death, a substantial number of pulmonary capillaries have been destroyed, and embolization of some arterioles has occurred. Depending on the species, however, the destruction of type I alveolar lining cells and the proliferation of the type II cells may not be extensive (14, 15).

The cause of death in acute pulmonary oxygen toxicity is somewhat controversial. There is little argument that, terminally, transfer of gas between blood and alveoli is impaired as a direct result of the damage to capillaries, alveoli, and the thickened interstitium (4). Furthermore, at the time of death animals are markedly hypercarbic and acidotic (16, 17). The controversy arises in regard to the question of arterial blood oxygenation. The classic view holds that animals exposed to hyperoxia die because of, or accompanied by, hypoxemia (16, 18). Such animals exhibit gasping and cyanosis, and they have low arterial PO2 values. On the other hand there are now at least two studies showing that arterial oxygen tension in sheep (19) or rabbits (20) may be well above normal at the time of death. These results suggest that even the events occurring during the agonal stage of acute oxygen toxicity are not well defined.

2. Species variation

In general, the response of most species to hyperoxia is similar (4), but there are differences among species that have to be considered when interpreting the results of experiments. The rat is the animal most often studied, but mice, hamsters, and guinea pigs also find their way into the oxygen exposure chamber. Guinea pigs, dogs, and rats showed approximately the same sensitivity to hyperoxia with a median time to death (LT50) in the range of 65–80 h (21, 22), although immature rats (60–90 g) survived 4-to-6-wk exposures. On the other hand, mice and monkeys are considerably less susceptible. Mice have an LT50 of about 126 h (22) and most monkeys survive more than 100 h; many individuals surviving more than 300 h (4, 22). There is also marked resistance to the lethal effects of hyperoxia in several nonmammalian species. Frogs did not succumb after 62 d (23); turtles survived between 10 and 23 d (24); and chicks (15-to-50-days old) were still alive after 28 days (25) of exposure to 1 atm O2. Except in
special circumstances, however, these animals are not used to study pulmonary oxygen toxicity.

There also appear to be species-related differences in the pattern of damage that occurs in response to hyperoxia. In the mouse, rat, and guinea pig (7, 26), endothelial cells are the first structures to show damage. The development of endothelial damage in monkeys and man (7, 27, 28) closely resembles that in rodents. However, destruction of type I alveolar lining cells and proliferation of type II cells in rats (14, 29) is far less prominent and occurs later than in the monkey or man (7, 15, 27, 28). Furthermore, as is discussed in more detail later, the ability of certain substances to induce protection against (30) or sensitization to (31) the acute effects of hyperoxia is also species dependent.

In addition to interspecies differences, data in rats indicate that there are also intraspecies variations; one kind of variation is that occurring between different laboratories or investigators. Thus one laboratory consistently finds that 70% of Sprague-Dawley rats exposed to 95% $O_2$ die within 72 h (10, 11, 30, 32, 33), while others report a substantially lower sensitivity: 30%–40% dead in 72 h (34, 35), or 50% dead in 79 h (16, 31, 36, 37). These differences may probably be explained on the basis of animal strain or minor differences in exposure conditions (e.g., temperature and humidity).

Another sort of intraspecies variation is that occurring within a single laboratory or a single experiment. Thus in the first case mentioned above, whereas 70% of animals died in 3 days, it is also true that 30% of those animals survived the exposure and in fact might even be regarded as a relatively resistant subpopulation. This view is supported by the results showing that under exactly the same exposure conditions 13% of the rats survived for 7 days (38). In another laboratory and under similar conditions 85% of the rats died within 4 days, but the remaining 15% survived for more than 6 days (39). This phenomenon may be an important consideration in analyzing the effects of substances that modify survival (10, 21, 39), because the treatments may act in a number of alternative ways.

3. Mechanisms of injury

a. Free radicals

There exists a very large body of evidence indicating that the acute pulmonary changes brought about by oxygen or by certain substances such as paraquat and bleomycin are caused by the action of oxygen free radical species. Most of this evidence has been discussed thoroughly in earlier reviews (1–3).

In most biochemical reactions, dioxygen (normal $O_2$) ultimately accepts four electrons to form water or the stable hydroxyl anion. However, there are many intermediate, enzyme-catalyzed reactions in which it accepts either a single electron to form the superoxide anion ($O_2^-$), or two electrons to form hydrogen peroxide (40). Superoxide and peroxide can subsequently interact to form the hydroxyl radical ($\cdot$OH). These various metastable species of oxygen are referred to as oxygen radicals or activated oxygen, and are highly oxidative species.

This process of oxygen radical formation is part of the normal biochemical machinery of many, if not all, cells. The free radical is the active form of oxygen produced by, and used in, reactions such as hepatic microsomal hydroxylations catalyzed by mixed-function oxidases (41), the electron transfer reactions that constitute the mitochondrial respiratory enzyme system (41), the hydroxylations of lysine and proline that occur during collagen synthesis (42), and the synthesis of prostaglandins from arachidonic acid (43). Active phagocytosis as well as
cytotoxicity of cells such as macrophages and leukocytes is also associated with generation of superoxide anion (44–46).

Despite the fact that activated oxygen species are unavoidable byproducts of essential biochemical reactions, their influence must be limited to prevent undesirable oxidations. In accord with this requirement, cells come equipped with a battery of enzymes that function to scavenge oxygen radicals. Two of these are the superoxide dismutases, which reduce the superoxide anion to peroxide, and catalase, which reduces hydrogen peroxide to water (47).

Glutathione (GSH) is an important sulfhydryl compound in cells that reduces (and is oxidized by) oxygen radicals in a reaction involving GSH-peroxidase. GSH, in turn, is regenerated through the action of GSH reductase, which requires reduced nicotinamide adenine dinucleotide phosphate (NADPH) as the electron donor (reductant). Next in this cascade, NADPH is regenerated through operation of the hexose monophosphate shunt, in which the oxidation of glucose-6-phosphate (G-6-P) is catalyzed by the enzyme G-6-P dehydrogenase (G-6-PDH) (48).

Superoxide dismutase, catalase, GSH, and the cascade responsible for maintaining adequate supplies of reduced GSH are collectively referred to as the tissue antioxidant system.

In the absence of such an antioxidant system, the free radicals could cause undesirable oxidative reactions, including peroxidation of cell membrane lipids, and protein sulfhydryl oxidation and cross-linking (49). The most popular explanation for the effect of hyperoxia is that elevated oxygen tension increases the rate of free radical formation to a level that exceeds the ability of the antioxidant system to reduce them, so that the radicals accumulate and produce cell damage.

A substantial body of strong, though circumstantial, evidence for this theory of hyperoxic injury is based largely on observed parallels between the activity of the lung’s antioxidant system and the animal’s susceptibility to oxygen. For example, maneuvers that increase resistance of rats to oxygen—preexposure to 85% O₂ (50, 51), or treatment with endotoxin (21, 30, 33), propylthiouracil (52), or low-dose (250 mg/kg) diethyldithiocarbamate (39)—also increase the content of several antioxidant enzymes in lung. Agents that increase susceptibility all reduce the antioxidant potential of animals’ lungs, either by lowering the activity of antioxidant enzymes [disulfiram (53), high dose (>300 mg/kg) diethyldithiocarbamate (53), and dexamethasone (52)], or by decreasing GSH levels [thyroxine (52)]. Relatively resistant subgroups of animals surviving lethal oxygen exposures also have elevated antioxidant levels (39).

Additional evidence that oxygen radicals are the agents responsible for pulmonary oxygen toxicity comes from experiments with the herbicide paraquat. This compound is metabolized to a free radical form, which then reacts with oxygen to generate superoxide radical and regenerate the original paraquat molecule (54). Moreover, paraquat produces acute (and chronic) pulmonary damage that has many features in common with the pathology produced by hyperoxia (13). Other relevant observations are that treatment of rats with regimens that induce antioxidant enzymes and raise an animal’s resistance to hyperoxia also protect it against paraquat toxicity (55, 56); that nonlethal doses of paraquat sensitize animals to hyperoxia (13); and that a low level of paraquat in drinking water given to rats increases pulmonary antioxidant activity (55). Bleomycin, an antineoplastic mixture of cytotoxic glycopeptide antibiotics of fungal origin, also causes pulmonary injury in humans (57, 58). When instilled into the trachea of rats or hamsters it produces acute inflammatory changes and chronic effects resembling those of paraquat (59, 60). This agent, like paraquat, is associated with generation of active oxygen species in vitro (61).

Ozone (O₃) is another substance associated with the generation of free radicals (62) and is one that, like oxygen, causes an acute pulmonary inflammatory reaction and thickening of the alveolar wall (63, 64). At least in vitro, ozone has been shown to react with and alter protein
and lipid in a manner similar to that of oxygen or paraquat (65). These observations provide
additional circumstantial support for a role of oxygen radicals in pulmonary injury.

Very recently this circumstantial evidence for the role of oxygen free radicals has been
bolstered by direct demonstration of oxygen radical production in tissue in vitro, measured as
the level of cyanide-resistant respiration (66). Using this method it was shown that oxygen
radical production doubled when lung slices were incubated in 85% O₂, and that the electron
transfer reactions in mitochondria contribute substantially to this response (66).

b. Role of inflammatory cells

Although the earliest detectable event in the development of pulmonary oxygen toxicity is
endothelial damage (14), it is rapidly followed by the appearance of large numbers of alveolar
and interstitial macrophages and polymorphonuclear leukocytes (3, 8). Because it is known
that these cells produce superoxide radical as part of their normal metabolic activity (43, 46),
it has been suggested that this additional source of oxidant species either causes or amplifies
the injury during exposure to hyperoxia. The results of a morphometric study in rats fail to
support the idea that inflammatory cells initiate lung damage during exposure to hyperoxia
(17); in this study, endothelial cell damage was detected before the volume of PMNs or
macrophages had increased. On the other hand, in an in vitro system hyperoxia stimulated
alveolar macrophages to synthesize and release a chemotactic factor that attracts polymor-
phonuclear leukocytes (67). Furthermore it has been observed that endotoxin treatment, which
increased the resistance of rats to hyperoxia, also reduced the number of macrophages in the
lungs of exposed animals (33). As noted under Free radicals, however, the protective effect
of endotoxin can be attributed to the induction of pulmonary antioxidant enzymes, and it
seems just as likely that the number of macrophages might have been smaller because the
injury was less severe. Thus the suggestion that inflammatory cells play an important role in
the development of acute hyperoxic pulmonary toxicity is plausible, but the available experi-
mental evidence is weak.

c. Specific cellular derangements

A number of studies, many of them reviewed by Mustafa and Tierney (1), have been directed
toward determining the cellular or subcellular sites of hyperoxic damage, but most of these
efforts have been disappointing. There is weak evidence for early damage to mitochondria (1,
68), but this is not a consistent finding (69). The inhibitory effect of hyperoxia on isolated
phagocytic cells is not in good agreement with the effect of O₂ on these cells in vivo (70). The
results of all of these studies remain compatible with a mechanism of hyperoxic damage that
involves attack by oxygen radicals, and they offer no support for a different biochemical basis
for the toxicity of oxygen.

d. Role of prostaglandins

The prostaglandin family of substances has a role in a wide range of physiological and
pathological processes, and it is not surprising that investigators have sought to determine
whether these compounds contribute to the development of acute pulmonary oxygen toxicity.
One of the principal reasons for suspecting that oxygen might act by altering prostaglandin
metabolism is, as mentioned earlier, because superoxide anion is involved in the oxidation of
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arachidonic acid to prostaglandins (43). However, there is very little support for the suggestion that prostaglandins have such a role.

In two studies, administration of indomethacin, an inhibitor of prostaglandin synthesis, failed to change the susceptibility of rats to hyperoxia (11, 52). When rats were fed a diet high in fat (21%, experimental; 5%, normal lab chow) their susceptibility to hyperoxia was markedly increased without a decrease in pulmonary antioxidant levels (71). To explain this effect it was suggested that the high polyunsaturated fatty acid content of the diet could have promoted increased synthesis of prostaglandins, and that these were responsible for the increased mortality. However, when rats were fed high fat diets that had either high or low contents of high polyunsaturated fatty acid, both groups of animals exhibited the same (elevated) mortality, while only those fed the high polyunsaturated fatty acid diet had elevated concentrations of prostaglandins in lung (71). Therefore there seems to be no support for the view that prostaglandins have a critical role in the development of acute hyperoxic toxicity.

e. Oxygen and coagulopathy

There are data to suggest that the components of the blood intrinsic coagulation system may be a site of attack by hyperoxia, but little mention of this work has been made in major reviews of oxygen toxicity. This conclusion is based on at least two lines of circumstantial evidence. First, when rats were exposed to hyperoxia for 27 h, 85% of the animals survived but showed signs of consumptive coagulopathy: clotting time was prolonged; the concentrations of coagulation factors XII, VII, and VIII were reduced; and fibrin monomers were demonstrated in 29% of the survivors (31, 37). Rabbits exposed to hyperoxia for 66 h also show signs of a mild coagulopathy characterized by increased amounts of fibrin degradation products but without prolongation of the clotting time (18). In another study, exposure of guinea pigs to 100% O₂ for 48 h induced a mild state of hypercoagulability and consumption of precursors of fibrinolysin (72).

Second, certain species of animals (e.g., frog, turtle, duck, and immature chicken) that cannot initiate the blood intrinsic coagulation cascade because they lack factor XII (Hageman factor) are resistant to hyperoxia (31, 37). Furthermore, rats that have chronic respiratory disease have very low levels of factor XII, have prolonged clotting times, and are markedly resistant to hyperoxia (37). Thus it has been suggested that hyperoxia-induced activation of the intrinsic coagulation system may be a prerequisite for production of acute pulmonary toxicity. These observations are of importance in attempting to explain how endotoxin increases the resistance of animals to hyperoxia, discussed later in subsection 3g.

Treatment of rats with lead (Pb²⁺) before exposure to hyperoxia has an interesting and unexpected effect that appears to be related to oxygen-induced effects on the intrinsic coagulation system. Ionic lead, known to enhance the coagulopathy induced by endotoxemia (73), also greatly enhanced the hypercoagulability caused by hyperoxia (37). In addition, it reduced the median survival time of the animals from 72 h to 29 h (16, 36, 37). However, the lead-treated animals succumbed to an entirely different form of death than did controls: after lead, death seemed unrelated to pulmonary embarrassment because the animals did not exhibit the changes in pulmonary histology, acidosis, hypoxemia, or hypercarbemia that usually characterize acute death in hyperoxia. Apparently the animals died as a result of the severe coagulopathy rather than impaired pulmonary function. Therefore these studies describe an altogether different kind of acute hyperoxic toxicity and call attention to the need for specifying the nature of the manifested toxicity when attempting to investigate agents that alter the susceptibility to hyperoxia.
f. Other mechanisms

Not all investigators agree that the toxic effects of hyperoxia result from increased levels of activated oxygen. Fee (74) has argued that such is not the case, pointing out 1) that the reaction of superoxide dismutase with superoxide ion leads to the formation of peroxide; and 2) that the level of superoxide dismutase does not always correlate with the oxygen stress to which an organism is exposed, because the enzyme is found in some strictly anaerobic species, whereas it is absent from certain aerobic cells. Fee favors a model in which pulmonary damage is a result of direct oxidation of enzymes by dioxygen, a view that has been expressed by others (75). Although these arguments have merit, they do not address the relationship between susceptibility to oxygen and the activity of glutathione and its regenerating system.

Another alternative to the oxygen radical theory is based on the effects of a high fat diet, mentioned earlier. Regardless of its potential to affect prostaglandin synthesis, the high fat diet reduced the ability of rats to survive in a hyperoxic atmosphere but did not produce a corresponding change in lung antioxidant levels (71). This lack of association between antioxidants and survival hardly represents a serious challenge to the oxygen radical theory, but it does suggest that other mechanisms may contribute to the acute effects of hyperoxia.

In summary, while oxygen radicals cannot be implicated in every case of acute diffuse pulmonary injury, and although superoxide dismutase is not always found in association with resistance to oxygen toxicity, no alternative mechanism has been convincingly shown to mediate the damaging effects of hyperoxia. Furthermore, as discussed above, the link between hyperoxic injury and free radicals is very strong. While oxygen radicals seem to be the most likely agents of hyperoxic toxicity, the site of their action is still very uncertain; cell membranes, mitochondria, and the blood coagulation system are possible targets.

g. Mechanisms of tolerance and the action of endotoxin

Tolerance, or more accurately, reduced susceptibility to hyperoxia has been achieved in experimental animals (almost always in rats) principally by two methods: preexposure to concentrations of oxygen that are substantially less than 100% (suboxic) for a period of several days (21, 50, 51, 76, 77), and administration of endotoxin (10, 11, 21, 30, 33, 38). It is notable that neither of these maneuvers affects the antioxidant levels or the susceptibility of mice (21, 30).

After rats are exposed to 85% O₂ for 7 days their lungs show pathologic changes indicative of early toxicity (76) and contain elevated levels of antioxidant enzymes (21, 50, 51), and the animals are less susceptible to 100% O₂. As discussed earlier, all available evidence suggests that protection is afforded by the enhanced antioxidant activity. This induction of antioxidant enzymes presumably represents a reaction to increased concentrations of oxygen radicals. Furthermore, it appears that increased resistance to oxidative damage, produced by exposure to low levels of the toxic agent, is independent of systemic influences, because resistance to ozone-induced injury can be elicited unilaterally by ventilation of one lung with air while the other is ventilated with a low concentration of ozone (78).

Although the protection against hyperoxia afforded by endotoxin is also associated with increased antioxidant activity, it differs in several important respects from that produced by preexposure to suboxic oxygen concentrations. Resistance to hyperoxia after endotoxin is not associated with any detectable histologic damage to the lung (11). Also, endotoxin has a beneficial effect even when administered up to 36 h after starting exposure to hyperoxia (20). Another unique feature of the resistance conferred by endotoxin is that the rise in pulmonary
antioxidants does not occur until after oxygen exposure has begun; endotoxin by itself does not induce increased amounts of antioxidant enzymes, but instead it appears to enhance the lung’s ability to increase these enzymes in response to the hyperoxic stress.

The relationship between pulmonary toxicity and activation of the intrinsic coagulation system discussed above may represent a basis for the action of endotoxin. Endotoxin itself causes a consumptive coagulopathy (73), and a dose of endotoxin that protects rats from hyperoxia (about 1/50 the LD₅₀) may, without any serious effects of its own, deplete clotting factors. Thus, it may produce a state similar to that existing in rats with chronic respiratory disease or in animals that are naturally deficient in factor XII—a state of reduced coagulability that is associated with extraordinary resistance to hyperoxia (31, 37).

h. Adult respiratory distress syndrome

One of the topics that has received scant attention in past reviews of hyperoxic effects is the relationship between acute pulmonary oxygen toxicity and the clinical condition known as adult respiratory distress syndrome (ARDS). Consideration of this relationship is important because it can influence the way we regard the etiology of pulmonary oxygen toxicity.

Adult respiratory distress syndrome is a clinical syndrome characterized by a pattern of pulmonary damage and impairment that, in both acute and chronic phases, cannot be distinguished from classic pulmonary oxygen toxicity. The remarkable aspect of this condition is that it can be caused by a constellation of disorders, some of which are apparent from the alternative names that have been given to this disease: “shock lung,” “traumatic wet lung,” “diffuse alveolar damage,” “respiratory insufficiency syndrome,” “congestive atelectasis,” and “progressive pulmonary consolidation” (7). It arises from hemorrhagic shock, trauma, sepsis, complicated abdominal surgery, cardiopulmonary bypass, viral pneumonia, diabetes, drug coma, and Goodpasture’s syndrome (7, 8). Recent research suggests that ARDS arises as a result of endothelial damage that may be triggered by a sequence of events involving activation of complement, generation of molecules that are chemotactic for neutrophils, aggregation and adherence of neutrophils to the pulmonary microvascular endothelium, and release of toxic products (elastase, lysosomal cathepsins, oxygen radicals) from the neutrophils (79).

While most authorities agree that in its acute form the underlying disease is the stimulus that evokes the pulmonary lesions, there is a good deal of ambiguity about the progression of ARDS to the chronic stage. This is because the usual treatment for ARDS, when the patient’s arterial Pₒ₂ falls below 45 mmHg, is ventilation with oxygen-rich gas, typically 55%-77% O₂ (8). Clinical studies afford strong evidence that the acute phase of ARDS is largely self-limiting in patients surviving longer than a few days, and that the chronic form of the condition is actually a direct result of hyperoxic therapy (7, 8).

An important point revealed by the similarity between ARDS and acute pulmonary oxygen toxicity is that surgery, hypovolemic shock, drug overdose, near-drowning, breathing of elevated oxygen concentrations, bacteremia—a diverse collection of precedents—all result in a remarkably similar, if not identical, clinical entity. It is unlikely that oxygen radicals initiate the sequence of injurious events leading to pulmonary pathology in each case. It is more likely that each circumstance is associated with a specific biological action: cytotoxicity, release of humoral mediators, activation of complement or the coagulation cascade, or stimulation of the immune system. These in turn act on a final common target, resulting in a stereotyped response. Understanding these pathways and learning the nature of the common step should be a goal of future research on acute pulmonary damage of all etiologies.
C. CHRONIC PULMONARY OXYGEN TOXICITY

1. Definition and description

Chronic pulmonary oxygen toxicity is difficult to define. It can be thought of as the progressive pulmonary damage that is seen during exposure to elevated oxygen concentrations (not necessarily 100%) after treatment times that are longer than those associated with death of individuals exposed to pure oxygen. Thus the chronic phase is seen in species such as the monkey, which usually survive the acute phase of oxygen toxicity (80); in individuals that belong to a relatively resistant subpopulation of a susceptible species (22, 38, 81, 82); in individuals belonging to a resistant age group of an otherwise susceptible species—e.g., very young rats (83–85); or in susceptible animals exposed to only moderately elevated oxygen concentrations, which produce only mild acute lesions and are not lethal (76, 83, 84, 86). In humans, information about chronic exposures is available from patients, with or without underlying pulmonary impairment, treated with a wide range of oxygen concentrations (7, 8, 28, 87). This variety in the way chronic oxygen exposures have been performed does nothing to simplify understanding the changes that occur in this disorder.

A prominent feature of chronic oxygen toxicity is fibrosis and, because this term is used extensively, it may be desirable to define it. The first meaning of fibrosis is simply the histological impression of excessive fibrous connective tissue. In this case fibrosis is merely a histopathologic diagnosis. What is recognized microscopically as fibrous connective tissue is principally the protein collagen, quantitatively the most important component of this tissue. However, a diagnosis of fibrosis does not necessarily mean that abnormal amounts of collagen can be demonstrated by chemical analysis of the tissue. The second definition of fibrosis is the set of pathologic processes (i.e., fibroblastic proliferation, increased rates of collagen synthesis) that produce the abnormal increase in the amount of fibrous connective tissue. It is important to note that neither the appearance of, nor the process of, fibrosis is necessarily connected to a particular physical or physiological dysfunction (88, 89).

The character of chronic pulmonary oxygen toxicity appears to depend, at least, on the species (or subpopulation) in which it is studied, and it may even depend on conditions used in the exposure. In spite of the fact that mice are reasonably susceptible to the acute toxic effects of oxygen (21, 81), they are especially resistant to chronic pulmonary oxygen toxicity. Animals that survived the acute toxic phase had minimal proliferative changes after 14 days of hyperoxia (90).

In rats, whether young animals or surviving adults treated with 1 atm O₂, or adults exposed to 0.8–0.9 atm O₂, the characteristic chronic lesions are destruction of alveolar walls with resulting emphysema (22, 81, 83, 86, 91) and a variable amount of fibrosis of the remaining parenchyma, particularly around larger blood vessels (86, 92). Some investigators hold the view that this dilatation of the air spaces is not the result of alveolar wall destruction, but that it represents only overdistension of alveoli and therefore is not true emphysema (82, 91). However, at least one group has considered that the oxygen-induced changes in the rat were similar enough to authentic emphysema to be a suitable model of pulmonary obstructive disease (84). These workers produced similar changes in dogs by exposing them to 0.6 O₂ atm for 5 wk.

Emphysematous changes have not been reported in every case of chronic oxygen exposure of rats (29). In one study, adult rats breathing 0.8 atm O₂ for 28 days had only edema and thickening of the alveolar walls, whereas young rats treated with 1 atm O₂ for 34 days showed emphysematous changes associated with ruptured alveolar septa of normal thickness (83).
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In humans and monkeys the picture of chronic pulmonary oxygen toxicity is quite different. The characteristic feature of chronic pulmonary oxygen toxicity is pulmonary fibrosis, which can progress to a degree of severity that results in death due to inadequate exchange of gas between the lungs and blood (7, 8, 28, 87, 93, 94). During the acute phase in monkeys, as in more susceptible species, resolution of the exudative phase is apparent as the proliferative phase develops (15). After 7 days, type II alveolar lining cells constituted 95% of the alveolar epithelium, whereas they represent only 15% of this tissue in normal animals. In addition, numerous fibroblasts were evident in the interstitium. After 12 days, type II pneumocytes had entirely replaced the type I cells, interstitial edema was decreased, and the volume of interstitial collagen fibers was increased (15). Similar changes are known to occur in patients receiving prolonged oxygen therapy (7, 8, 93). In such patients, hyperplasia of type II pneumocytes was prominent after 6 days, and replacement of type I cells by the former was virtually complete after 10 days.

Information about the progression of oxygen toxicity beyond this stage comes principally from clinical studies. However, the lung damage resulting from lengthier exposure to oxygen cannot be attributed with certainty solely to hyperoxia, because such exposures often occur as treatment for pulmonary dysfunction that initially arises from shock, trauma, or infection, i.e., ARDS (7, 8). If we accept the premise that late ARDS is actually the same as chronic pulmonary oxygen toxicity (see below), then it appears that the continued exposure of humans to elevated oxygen partial pressures does lead to rapidly progressive severe pulmonary fibrosis.

After 14 days of ventilation with oxygen concentrations of 55%–77%, the lungs of patients have a surprisingly chronic appearance. Histologic examination shows fibrosis with only slight septal thickening but with extensive involvement of alveolar ducts. In patients who died after prolonged respiratory distress the duct lumens were nearly or completely filled with fibrotic tissue, often giving the impression of microabscesses when infection was also present. The histologic impression of fibrosis in ARDS has been confirmed by measurements that demonstrate an increased lung content of the protein collagen, the principal component of fibrous connective tissue (95). Clinically, patients have tachypnea, decreased pulmonary compliance, and increasing hypoxemia. A study of patients who received more than 14 days of oxygen therapy for ARDS indicated that the cause of death could be classified as alveolar duct fibrosis in 80% of the cases (8). Death usually occurred within 32 days of beginning oxygen therapy (7, 8, 95).

2. Causes of pulmonary fibrosis

a. General considerations

In addition to its association with oxygen therapy, clinical pulmonary fibrosis is seen in connection with a number of diseases and as a side effect of certain drugs, and insight into the cause of this condition may be achieved by noting the similarities and differences between the actions of oxygen and other causes of the fibrotic process. After the discussion below concerning the specific features of ARDS, idiopathic pulmonary fibrosis, and the fibrotic conditions produced by several miscellaneous agents, there is little attempt to distinguish among the three designations, “pulmonary oxygen toxicity,” “ARDS,” and “pulmonary fibrosis.” The reason for allowing such generalization is not to obscure differences, but to emphasize similarities. The least amount of overlap among these diseases exists in their clinical features. More similarity is seen in their morphology, and even more in the possible biochemical mechanisms
responsible for fibrosis. Ultimately it may become possible to regard them as variations of a single entity.

b. Adult respiratory distress syndrome

As noted before, the later stages of ARDS provides the only model for chronic oxygen toxicity because of the paucity of animal studies involving such long-term exposures. Its principal features have also been described. Although the exact etiology of fibrosis in ARDS may seem indeterminate because the initial pulmonary insufficiency can stem from a variety of disorders, there is strong circumstantial evidence that oxygen is the principal causative agent. Thus, Pratt et al. (8) found that the proliferative lesions—septal fibrosis, alveolar cuboidal epithelium, and an increased number of macrophages—were all more strongly associated with duration of respiratory treatment than with the duration of the total illness. However, it is important to remember that the initial pulmonary injury in ARDS is the result of a condition that has no connection with hyperoxia, yet it produces acute pathology that strongly resembles hyperoxic toxicity. Therefore it is likely that the pathology of chronic ARDS represents an additive interaction between the initial pulmonary damage and oxygen therapy.

c. Idiopathic pulmonary fibrosis

The following description of idiopathic pulmonary fibrosis (IPF) is based on the excellent review of this subject by Crystal et al. (6). Idiopathic pulmonary fibrosis differs from most of the other types of fibrosis discussed in this section because it develops gradually (96) and does not involve an exudative acute phase, although a proliferative change (cuboidalization of the alveolar epithelium) does occur. It is characterized by a low-grade inflammatory response in the same areas that eventually show fibrosis, the interalveolar septa and the peribronchial regions. The volume-pressure relationship of the lung reveals decreased compliance (less volume at a given transpulmonary pressure), the opposite of the change seen with emphysema. During the midcourse of IPF patients experience distress only during exercise, and this discomfort is associated with an increased gradient between the alveolar and arterial oxygen tensions. These findings, supported by other evidence (97), indicate that during the intermediate stage IPF causes a ventilation-perfusion imbalance. The disease progresses over a period of months or years, and death is associated with pulmonary impairment resulting from fibrotic destruction of the lung parenchyma.

d. Miscellaneous agents

Both paraquat and bleomycin were identified earlier as drugs that cause acute changes that resemble the effects of hyperoxia. In addition, their chronic effects in man and experimental animals also resemble those of oxygen, and both have been used to produce laboratory animal models of human pulmonary fibrosis.

In the case of paraquat, an LD₅₀ dose of this drug causes a fulminating acute response with substantial destruction of pulmonary architecture and death of half the animals in the first few days. During the chronic phase of its action in rats, paraquat causes alveolar wall destruction, and perivascular and peribronchial fibrosis are evident within a week (98). This pattern of injury is reminiscent of that due to chronic oxygen treatment in this species and does not seem especially surprising, because, like oxygen, paraquat is thought to act by generating oxygen radicals (54).
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Bleomycin has been used in experimental studies more extensively than paraquat, and its effects have been followed for longer periods of time. Although bleomycin causes pulmonary fibrosis when given systemically (99), it is usually instilled intratracheally because this technique produces a more reproducible response (100).

Bleomycin appears to have similar effects in both hamsters and rats and produces a mild acute response, which resembles that elicited by hyperoxia (59). Interstitial fibrosis is seen after a week (98), is quite marked by four weeks (59, 100), and is judged severe after about 60 days (59). Thereafter it does not progress but remains prominent for 180 days or longer (100, 101). Thus, bleomycin-induced fibrosis appears to be self-limiting rather than rapidly progressive as in the case of ARDS. Another feature of bleomycin toxicity is an increased number of eosinophils in the circulation and lungs (59), and it has been suggested that this effect reflects the involvement of an immune response. Although there are some differences between the fibrosis induced by bleomycin and that in human oxygen toxicity, the bleomycin-treated rat or hamster still is considered to represent a suitable model for studying human pulmonary fibrosis (102).

Ozone (O₃) is another agent that has been used to produce an animal model of human pulmonary fibrosis. A serious drawback to the use of this gas is the complexity of the apparatus required for reproducible exposure of animals to low concentrations of ozone for a long period of time (103). Rats exposed to 0.8 ppm O₃ develop an acute pulmonary exudative reaction with less edema and tissue destruction than is caused by paraquat (98). Fibrosis is seen after 7 days (104) and is found in the proximal portion of the alveolar duct, proximal alveoli, and in the distal parts of terminal bronchioles (98, 103). This pattern of fibrosis is slightly different than that produced by oxygen in humans, which involves the entire length of the alveolar ducts. In addition, rats exposed to ozone, as to dioxygen itself, develop dilatation of pulmonary air spaces, which is not a feature of human oxygen toxicity (1). However, the rapid development of extensive fibrosis is more like the human disease than is the bleomycin model. Because it is thought that ozone acts by generating oxygen radicals (62), the toxicity of this agent can be considered one more example of fibrosis caused by a strong oxidizing species.

3. Fibrosis and collagen metabolism

a. Collagen content of fibrotic tissue

The biochemical correlate of fibrosis (evaluated histologically) is an increase in lung content of the protein collagen, as measured by chemical methods. In fact, a detectable rise in collagen content should be considered a requisite finding for concluding that a disorder involves a fibrotic process. Another biochemical change that should be expected in connection with fibrosis is an increased rate of collagen synthesis by the tissue, although a decreased rate of collagen degradation also could lead to accumulation of fibrous connective tissue.

In the case of oxygen-treated rats, tissue collagen is increased after 14 days (91, 105, 106). Ozone-induced pulmonary fibrosis in animals is also associated with increased collagen content of lung (104), as well as with an increased rate of collagen synthesis by lung minces in vitro (98, 103). Bleomycin, like ozone, increases both collagen content (59, 100, 107) and the in vitro rate of collagen synthesis (98, 108, 109). Paraquat did not increase lung collagen content after 7 days (98), probably because the tissue was sampled too soon after administration of the drug. However, the rate of collagen synthesis was increased in lung tissue from paraquat-treated rats after only 3 days (110), and normal lung slices treated with paraquat in vitro also synthesized
more collagen than did control slices (111). In virtually every study in which it has been investigated, the fibrotic agents have been found to stimulate the synthesis of collagen to a greater extent than the synthesis of noncollagenous protein (60, 88, 98, 102, 103, 108–112).

In the case of human chronic pulmonary oxygen toxicity, Zapol et al. (95) found marked collagen accumulation in patients who died of ARDS and had received more than 2 weeks of oxygen therapy. In the case of IPF, however, where the histologic diagnosis indicates severe fibrosis, the chemically measured collagen content has not been found to be abnormal (6, 113). Furthermore, the rate of collagen synthesis by lung biopsy tissue in vitro was not different from that seen with lung tissue from patients without fibrosis (113). Thus the expected correspondence between tissue collagen content and degree of fibrosis does not seem to hold for IPF. To explain this discrepancy it was suggested that the morphologic appearance of fibrosis involves no net increase in the amount of lung collagen but is due only to ‘‘rearrangement’’ of existing collagen. Because the ratio of type I to type III collagen was found to be increased, it was proposed that the less-prominent type III collagen fibers were degraded, whereas type I fibers, which form prominent bundles, were synthesized (113).

Whereas this question has not yet been entirely resolved, a convincing explanation has been offered (114) that is based on a similar difficulty in demonstrating increased tissue collagen in lungs from ARDS patients (95). Apparently as a result of inflammation, the weight of noncollagenous components increases in parallel with the increase in collagen. Under these conditions collagen per unit lung weight would not be greater than normal, whereas collagen per whole lung would be. In addition, ARDS and IPF both exhibit a patchy distribution, and results that are representative of the whole lung should be based on fairly large tissue samples (95). Therefore, in studies of IPF, an increase in lung collagen measured chemically may have been obscured because the analyses were performed with biopsy specimens or the small number of autopsy samples, and because the results were expressed on the basis of lung weight.

At this point it seems appropriate to suggest caution in thinking about the significance of collagen deposition in fibrosis. Collagen is only one component of fibrous connective tissue, albeit the one most likely to be seen histologically. However, little attention has been paid to the contributions of such other components as elastin, proteoglycans, and fibronectin, all of which play important roles in determining the characteristics of connective tissue (115–117). For a complete understanding of fibrotic disease we shall have to consider the entire tissue, not merely one of its proteins.

b. Collagen remodeling

Besides quantity, the type and age of collagen may also be important in determining the effects of fibrous connective tissue accumulation. There is evidence that in fibrosis the increase in collagen is the net result of a dynamic process involving the synthesis of new collagen and simultaneous degradation of older collagen. This process, referred to as ‘‘remodeling,’’ results in fibrous tissue that has altered characteristics. In fact, increased degradation of collagen appears to be an early effect of elevated oxygen. In rats exposed to hyperoxia, increased amounts of hydroxyproline, presumably representing degraded collagen, were found in endobronchial washings after 24 h (34, 35) and in the urine after 60 h (106). In addition, the content of collagen in the lungs of these rats actually decreased during the first 60 h but subsequently increased as fibrosis developed (106). The increased number of pulmonary macrophages present during certain stages of the fibrotic process may partly explain the enhanced degradation of collagen, because they are a source of collagenase, the enzyme that specifically catalyzes
that process (118). Fibroblasts themselves, however, appear to possess all the necessary machinery for both synthesis and catabolism of collagen (112).

While relative rates of destruction and synthesis clearly affect the age and amount of collagen in fibrous connective tissue, they also affect its chemical nature, because new collagen synthesized during fibrosis in animal models (98) or in humans (6, 113, 119) has a higher ratio of type I to type III collagen than does the older, normal connective tissue. Therefore, the newly synthesized collagen would be expected to have different mechanical properties (6). It has also been suggested that, whether related to age or type, newly synthesized collagen is disorganized or more loosely structured, possibly due to changes in the cross-linking of the collagen molecules, and in this way it confers different properties on the fibrous connective tissue (91, 105, 113).

c. Relationships between fibrosis and pulmonary function

The significance of pulmonary fibrosis lies not in the accumulation of collagen itself, but in the effect it has on pulmonary function and in its relationship to the mortality of chronic disease. It is generally felt that collagen accumulation can affect function in two ways. First, an increased quantity of fibrous connective tissue, sufficient to distort pulmonary architecture, must have undesirable effects on pulmonary function. Collagenous tissue is noncompliant and should at least change mechanical properties of the lung. Also, alveolar walls thickened with collagen fibers clearly cannot be expected to permit normal exchange of gases between alveoli and blood. Second, even if the quantity of collagen is not increased, remodeling could alter the properties of the connective tissue, thereby affecting function.

In spite of these rationales for expecting a conspicuous correspondence between fibrosis and altered function, the correlation between them is poor (120). In the rat model of chronic oxygen fibrosis, β-aminopropionitrile, an inhibitor of collagen cross-linking, reduced accumulation of collagen in lung but increased the enlargement of alveoli caused by oxygen exposure under these conditions (105).

After administration of bleomycin to rats or hamsters, pulmonary compliance was decreased after fibrosis was well established, but it returned to normal, although the fibrosis did not diminish (88, 100). In addition, penicillamine, another inhibitor of cross-linking, reduced fibrosis in bleomycin-treated hamsters but had no effect on the course of compliance changes (121).

There is also poor correspondence between fibrosis and functional change in patients with idiopathic pulmonary fibrosis. During the midcourse of this disease, fibrosis does not correlate well with the observed reductions in vital capacity, diffusing capacity, or resting arterial Po2. On the other hand there is good correlation between fibrosis and either the degree of respiratory difficulty experienced during exercise or the increase in the alveolar-arterial oxygen difference measured under those conditions (6).

Because of these considerations it appears that measurement of mechanical properties of the lung do not provide good indexes of fibrosis, and that physiological measurements must be chosen carefully if they are to be useful indicators of chronic disease that has not yet reached the terminal stage.

d. Biochemical mechanisms in pulmonary fibrosis

Nonspecific injury. One of the simplest ways of looking at the fibrotic effect of injurious agents such as oxygen or ozone is that they stimulate collagen deposition as a result of direct cellular injury caused by oxidative reactions. However, simple local injury does not explain
the acute pulmonary damage caused by trauma and sepsis, or by substances such as paraquat and bleomycin, which can produce their effects after systemic administration. It also cannot explain why fibrosis occurs idiopathically or progresses rapidly at a time when the inflammatory response has subsided. It is clear that more complex explanations must be sought.

**Direct effects of oxygen on collagen synthesis.** One of the most straightforward biochemical mechanisms that may contribute to fibrosis caused by oxygen, ozone, and perhaps by paraquat, is the direct action of oxygen or oxygen radicals on the synthesis and processing of collagen. This possibility is suggested by the fact that molecular oxygen, via free radical intermediates, participates in several reactions that are involved in the production of new extracellular collagen (42, 122). The hydroxylation of proline and lysine are two such reactions that occur intracellularly (123–125). Hydroxylation of peptidyl prolyl residues is essential for converting collagen to a form that can be secreted into the extracellular space (125), and the hydroxylation of lysine influences the kinds and degree of cross-linking of collagen fibers (115). These facts suggest that elevated oxygen tensions could enhance synthesis and extracellular maturation (cross-linking) of collagen.

Some experimental evidence supports this concept. Slices of rat lung incubated in 0.95 atm O₂ synthesized collagen at a higher rate than did control slices in air (126). Sponge granuloma tissue (representing subcutaneous connective tissue) from rats, incubated in 0.95 atm O₂, also produced collagen at a higher rate than air controls (127, 128). When paraquat was added to slices of incubating rat lung, it stimulated collagen synthesis more than it did the synthesis of noncollagenous protein (111). Furthermore, when superoxide dismutase was added to the incubation medium, paraquat no longer stimulated protein synthesis. The in vitro studies with hyperoxia must be interpreted with caution however, because large gradients of gas tension, as well as solutes, exist in incubating tissue slices (129). This means that a substantial portion of a tissue slice incubated in air may be hypoxic. In one study with rat lung, the finding that the newly synthesized collagen had a low ratio of type I collagens to type III also complicates interpretation of these results (126).

There are also arguments against the direct oxygen effect. One is that the proline hydroxylation reaction apparently does not limit the rate of collagen synthesis (130, 131). Another is that hyperoxia does not increase the degree of prolyl hydroxylation or the rate of maturation of collagen produced by connective tissue in vitro (132). Furthermore, because oxygen tensions have to be reduced to very low levels before proline hydroxylation is impaired sufficiently to reduce collagen secretion (133), it is doubtful that an oxygen tension above 0.2 atm would markedly influence this synthetic step. These considerations suggest that increased collagen synthesis mediated by a direct action of oxygen is probably of minor importance in the genesis of fibrosis.

**Paradoxical hypoxia.** An interesting mechanism of fibrosis has been suggested that is based on the observation that exposure of young rats to 0.84 atm O₂ for up to 84 days increased collagen content, lactate concentration, and the activities of the enzymes lactate dehydrogenase and prolyl hydroxylase in lung (134). According to the “paradoxical hypoxia” hypothesis, hypoxia (via oxygen radicals) produces nonspecific local injury leading to inflammation. The high metabolic activity of inflammatory cells then produces (paradoxically) a localized region of hypoxia, resulting in glycolysis and formation of lactate. Because lactate is known to activate prolyl hydroxylase (135), it has been suggested that increased prolyl hydroxylase activity leads to enhanced synthesis of collagen. Mustafa and Tierney (1) have reviewed evidence indicating
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that lactate accumulation could also occur because hyperoxia damages mitochondria, reduces ATP production, and thus stimulates glycolysis.

This concept, however, contains a number of weaknesses. The most serious one, mentioned in the previous section, is that there is good evidence that prolyl hydroxylase activity does not limit the rate of collagen synthesis (130, 131). Furthermore, according to this view, progressive fibrosis would require a persistent intense inflammatory response or continuing mitochondrial damage, and these conditions do not appear to exist after the exudative phase of acute oxygen toxicity has begun to resolve.

Hyaline membranes. It has been recognized for some time that hyaline membranes are a characteristic early pathological finding in ARDS (7, 28). Pratt et al. (8) noticed that the alveolar duct fibrosis that occurred in such patients had a distribution that was the same as that of the hyaline membranes that preceded them. In addition, when slices of lungs from mice that survived 0.9 atm O₂ for 6 days were cultured, collagen fibers were formed directly under the hyaline membranes (32). These observations have led to the suggestion that the fibrin in the hyaline membranes acts as a stimulus for fibroblastic proliferation and collagen secretion (136).

Immunologic mechanisms. There are data suggesting that pulmonary fibrosis represents an autoimmune disorder, but so far the evidence for such a mechanism is not strong. In idiopathic pulmonary fibrosis, lymphocytes from patients produce a migration inhibition factor when exposed to type I human lung collagen, and they are also able to lyse collagen-coated erythrocytes (137). These results suggest that in patients a cell-mediated immune response to autologous type I collagen may occur. The production of migration inhibition factor or similar lymphokines may be responsible for stimulating collagen synthesis (138–140) because they promote the accumulation of macrophages, which secrete a fibrogenic factor (141).

In the studies mentioned above, inactivating the T lymphocytes (137) prevented production of migration inhibition factor by, and the cytotoxicity of, the lymphocytes from IPF patients. Furthermore, rats given bleomycin also developed a cellular sensitivity to type I collagen (142), and antilymphocyte globulin administered to similarly treated animals reduced collagen accumulation (143). T cells are also thought to play a role in the fibrosis associated with pulmonary sarcoidosis (144). In animals with bleomycin-induced fibrosis, the number of circulating and extravascular pulmonary eosinophils was elevated at the same time that the rate of collagen synthesis was highest (59, 145). Because eosinophilia is thought to be associated with T cell activation (146), this observation affords further evidence for the involvement of an immunologic process.

Results of other studies may be regarded as compatible with the operation of an immunologic mechanism in fibrosis. Antiserum to polymorphonuclear leukocytes enhanced fibrosis and the rate of collagen synthesis in rats given bleomycin (60, 147), suggesting that these cells normally act to mitigate the fibrotic response. However, the observation that bleomycin causes a typical fibrotic reaction in the nude athymic mouse, an animal that lacks T cells and exhibits a poor immune response, is damaging to this hypothesis (148).

e. Antifibrotic agents

Substances with clinical antifibrotic activity are being sought, but no material yet investigated has the ideal combination of low toxicity and ability to specifically inhibit synthesis of collagen. Endotoxin, which increases the resistance of rats to acute oxygen toxicity, also has been reported to reduce fibrosis in this species. When rats that survived 7 days of hyperoxia were
examined 31 days later, animals that had been treated with endotoxin had a lower lung collagen content than did control animals (10, 38).

Indomethacin had no effect on fibrosis in rats 14 days after administration of bleomycin but significantly reduced the amount of collagen found at 60 days (59). The reduction in collagen content that occurred between the 14th and 60th days appeared to be the result of a reduction in collagen synthesis, while collagen degradation continued at a high rate. This study suggests that prostaglandins play a role in the fibrotic process.

An apparently naturally occurring antifibrotic factor is the high-molecular-weight substance, or substances, that reduces fibroblast proliferation and preferentially inhibits collagen synthesis. It has been extracted from lung (149) and detected in lung tissue cultures (150), and its activity is reduced in the lungs of bleomycin-treated animals (149, 150).

When β-aminopropionitrile, penicillamine, or p-aminobenzoate (PABA) were given to rats 2 days after treatment with bleomycin, the drugs reduced the amount of salt-insoluble (highly cross-linked) collagen formed in response to the fibrotic stimulus (102). However, in this study PABA exhibited high toxicity—the mortality of animals treated with PABA plus bleomycin was twice that seen in animals that received bleomycin alone.

Glucocorticoids have been used as antifibrotic drugs, and they decrease the collagen accumulation caused by bleomycin in animals (107, 109). It is not entirely clear, however, whether they preferentially inhibit the synthesis of collagen (151). In lung minces from ozone-exposed rats, methylprednisolone inhibited the increased rate of collagen synthesis, but it inhibited noncollagenous protein synthesis to the same extent (103). In one study, glucocorticoids affected synthesis of all protein equally in mouse granuloma tissue (152). However, a greater weight of evidence, from studies with either granuloma tissue or fibroblast cultures, indicates that these steroids do inhibit collagen synthesis preferentially (151, 153, 154).

D. CONCLUDING REMARKS

The two phases of oxygen toxicity in man seem to be concerned with quite distinct processes: in acute toxicity pulmonary architecture is not severely changed, but gas exchange is impaired by widening of the air-blood barrier, alveolar flooding, and atelectasis. In the chronic disease, pulmonary structure is markedly altered by rapid fibrosis. Yet there also seems to be a link between the two, because many agents other than oxygen elicit changes that resemble both stages of the disease. On the other hand, the fact that pulmonary fibrosis develops in response to so many different conditions suggests that the route to fibrosis via acute oxygen toxicity is only one of many leading to a final common pathway. An autoimmune reaction is a possible candidate for such a common mechanism. Finally, the study of abnormal fibrotic processes should include not only collagen but other connective tissue components as well.

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