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9.4 Oxygen Under Pressure

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During the last four decades, research in the area of oxygen toxicity has increased dramatically in response to requirements generated by increasing applications in therapy and in diving operations. The re-emergence and expansion of hyperbaric oxygen therapy (Davis & Hunt 1977, 1988, Thom 1989), coupled with more aggressive oxygen therapy of pulmonary insufficiency at atmospheric pressure (Beers & Fisher 1993, Sackner 1974), has caused large numbers of patients to be exposed to increased oxygen pressures. Concurrently, the expanded use of hyperoxia to decrease the duration and increase the effectiveness of decompression procedures in military and commercial diving operations (Lambertsen 1967) has exposed large numbers of normal men to increased oxygen pressures.

Along with greater application of hyperoxygenation, a better understanding has been achieved of the universal dependence of vital biological processes on the cellular antioxidant defense mechanisms that have evolved over the centuries of adaptation to the earth's atmospheric oxygen tensions (Gilbert 1964). It is now apparent that the same oxygen pressures required to sustain life would cause lethal oxygen poisoning in the absence of these mechanisms (Fridovich 1975, 1978, McCord & Fridovich 1978). Thus, antioxidant defenses that were once thought to be useful only under extreme and unusual circumstances now appear to be among the most basic of all biological processes.

The potential of oxygen free radicals for producing cellular damage was first appreciated by radiation biologists, and the possibility that the effects of oxygen toxicity may be initiated by a similar mechanism was first recognized by Gerschman and her colleagues (Gerschman 1964, Gerschman et al 1954). During the last two decades, investigations of oxygen radicals, their biological target sites, and opposing antioxidant defenses were greatly stimulated by indications that these species are also involved in ischemia-reperfusion injuries and other disease states (Halliwell 1987, McCord 1985, Repine et al 1987).

This chapter will describe the known effects of oxygen toxicity on specific organ functions in man over a range of useful oxygen pressures. It will also summarize what is known about the biochemical changes that precede overt toxic effects and the antioxidant mechanisms that oppose such changes. Safe and optimal exploitation of the useful properties of oxygen requires awareness of its toxic effects on susceptible organs and functions, and extension of inherent oxygen tolerance by any effective and practical means. A better understanding of the basic mechanisms of oxygen poisoning and opposing antioxidant defenses will aid in achieving these goals.

MANIFESTATIONS OF OXYGEN POISONING

The severity of oxygen poisoning increases progressively with elevation of the inspired P_{O_2} and with greater duration of exposure. At sufficient pressure and exposure duration, oxygen will cause initial functional impairment and ultimate chemical destruction of any living cell. Many of the diverse manifestations of oxygen poisoning are summarized in Fig. 9.4.1.

The pathologic effects of pulmonary oxygen toxicity include destruction of both capillary endothelium and alveolar epithelium, alveolar cell hyperplasia, edema, hemorrhage, arteriolar thickening and hyalinization, fibrin formation, atelectasis, and consolidation with severe impairment of gas exchange, hypoxemia and death (Bean 1945, Clark & Lambertsen 1971a). Manifestations of central nervous system (CNS) oxygen poisoning include effects ranging from localized muscle twitching to tonic–clonic generalized seizures and, with continued

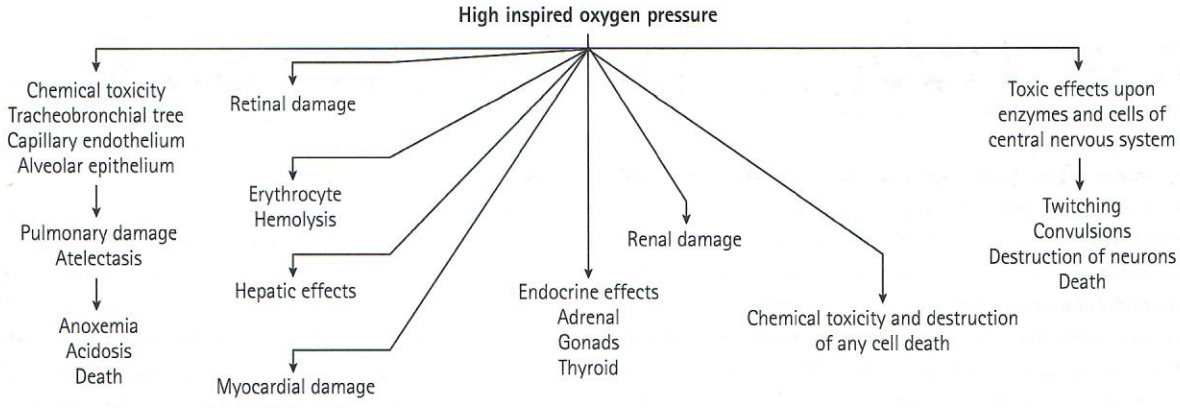

Fig. 9.4.1 Diversity and progression of toxic effects produced by exposure to increased oxygen pressures. (From Clark 1974, extending a concept used by Lambertsen 1965)

exposure past the onset of these signs, progressive neural destruction, permanent paralysis and death (Donald 1947, Lambertsen 1965). Effects elsewhere include retinal separation, destruction of visual cells and blindness (Beehler 1964, Nichols & Lambertsen 1969), erythrocyte hemolysis (Goldstein & Mengel 1969, Larkin et al 1972, Mengel et al 1964), renal damage (Hess & Menzel 1971, Resnick et al 1973) and myocardial damage (Caulfield et al 1972), as well as effects on the liver (Schaffner & Felig 1965, Schaffner et al 1966) and endocrine organs (Bean & Johnson 1954, Edstrom & Rockert 1962, Gersh 1945).

Detailed descriptions of specific organ pathology caused by prolonged exposure to toxic oxygen pressures are beyond the scope of this chapter. The sequence and characteristics of pathologic changes in the lung were described in a previous edition (Clark 1982). The nature and time courses of biochemical and morphologic changes that occur during recovery from pulmonary oxygen poisoning have been reviewed in detail by Thet (1986). An excellent, comprehensive review of the pathologic effects of oxygen toxicity in specific organs and tissues can be found in Balentine (1982).

When an intact animal or man is exposed to oxygen at toxic partial pressures, the sequence and severity of effects that occur in different organs and tissues are dependent upon interactions between the oxygen doses

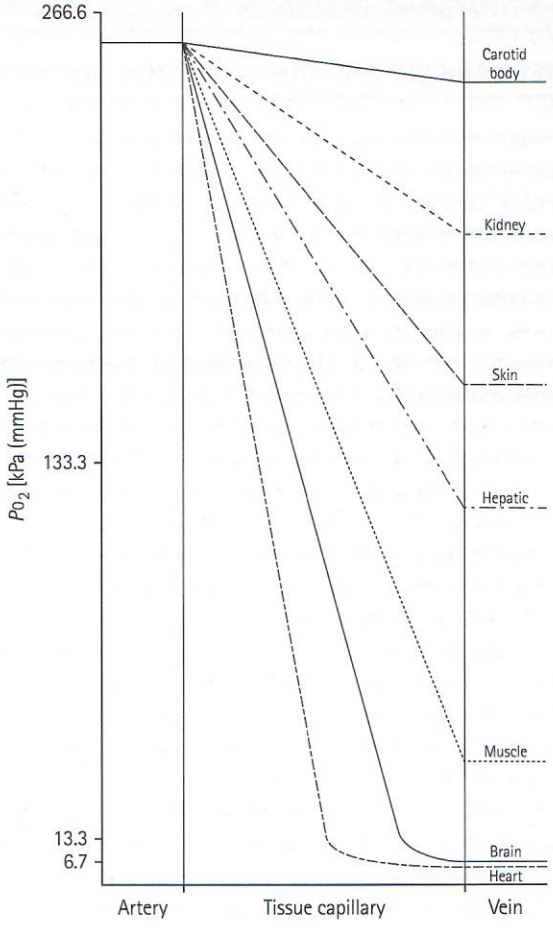

Fig. 9.4.2 Range of oxygen pressure in different organs and tissues during oxygen breathing at 352 kPa (3.5 ATA, 25 msw, 83 fsw). The curve for brain represents average measurements of arterial and internal jugular venous blood P_{O_2} in 16 conscious men (Lambertsen 1978). Venous values and capillary P_{O_2} changes for other organs and tissues are calculated from measured arterial values and tabulated values of tissue oxygen consumption and blood flow in man. Even within an organ or tissue, inequalities of metabolic rate and blood flow should cause local differences in P_{O_2}. Cells near the arterial end of a capillary are exposed to much higher P_{O_2} levels than other cells near the venous end. Pathologic states and drug effects on circulation or metabolism should be expected to alter the patterns shown here. (From Lambertsen 1965, 1978)

and relative susceptibilities of the exposed tissues. In turn, the oxygen dose of a specific tissue is determined by the balance that exists among factors such as the arterial P_{O_2}, blood flow, tissue metabolic rate and capillary density. Because these factors are not uniform throughout the body, specific organs and tissues are exposed to a range of oxygen tensions during oxygen breathing at any ambient pressure (Fig. 9.4.2) (Lambertsen 1978). Since pulmonary alveolar P_{O_2} exceeds even arterial P_{O_2}, the lung is exposed to higher oxygen tensions than any other organ. At oxygen pressures ranging from more than 50–202 kPa (0.5 to 2.0 ATA), exposure durations are limited by the development of pulmonary oxygen poisoning (Clark & Lambertsen 1971a,b). During exposure to oxygen pressures of 303 kPa (3.0 ATA) or higher, however, the most severe limitations are imposed by neurologic oxygen poisoning, as the relatively high susceptibility of brain tissue (Dickens 1946, Stadie et al 1945a,b) causes it to be adversely affected by oxygen tensions lower than those in most other organs (see Fig. 9.4.2).

MECHANISMS OF OXIDANT DAMAGE AND ANTIOXIDANT DEFENSES

FREE RADICALS AND OTHER REACTIVE SPECIES

Oxygen toxicity has been studied extensively for the past 40 years. Gerschman (1964) first proposed that oxygen toxicity is caused by the production of free radical intermediates in excessive concentrations during exposure to increased oxygen tensions. The partial reduction of oxygen by one electron to form superoxide ($O_2^{\bullet}$) and/or by two electrons to form hydrogen peroxide (H_2O_2) is the biochemical basis for the toxicity of oxygen (Freeman & Crapo 1982, Fridovich 1997). McCord and Fridovich (1978) recognized that the superoxide anion is a normal by-product of cellular metabolism and that its rate of generation in some reactions is enhanced by increased oxygen pressures.

The mitochondrial electron transport chain is a major subcellular source of superoxide, which is produced at both the ubiquinone and reduced nicotinamide adenine dinucleotide (NADH) sites at rates that increase in response to hyperoxia (Fisher 1988, Freeman & Crapo 1982, Jamieson 1989). Additional significant sources of superoxide during exposure to hyperoxia include the endoplasmic reticulum and microsomes (Fisher & Forman 1983). Superoxide may enter into reactions that produce other toxic species such as hydroperoxy and hydroxyl radicals and singlet oxygen (Fisher et al 1979). It is generally accepted that most of the oxidant damage to cellular components and membranes that occurs during exposure to hyperoxia is caused, not by direct actions of superoxide and hydrogen peroxide, but by the secondary generation of more reactive intermediates (Jamieson 1989).

Nitric oxide ($^{\bullet}NO$) is a free radical produced in vivo as part of normal physiology. Nitric oxide serves as an intercellular messenger that causes, among other things, smooth muscle relaxation and vasodilation. The free radical nature of $^{\bullet}NO$ is important for its interactions with heme iron-containing proteins, iron-sulfur clusters, and with other radical species. The biophysical properties of $^{\bullet}NO$ and oxygen are similar. Both are hydrophobic gases with similar solubility in biological fluids and ability to establish diffusion gradients (Zhang et al 1996). The reaction of $^{\bullet}NO$ with oxygen is relatively slow and hence oxygen is unlikely to compete successfully against any other potential $^{\bullet}NO$ target except in special environments. The reaction between oxygen and $^{\bullet}NO$ to produce toxic nitrogen species with higher oxidation potential may take place in hydrophobic environments such as the cell membrane where the concentration of both reactants is six- to 10-fold higher than in the cytosol or in the extracellular environment (Liu et al 1998b).

The reaction between $^{\bullet}NO$ and superoxide results in generation of peroxynitrite (ONOO-), which is a stronger oxidant than either $^{\bullet}NO$ or superoxide alone (Beckman & Koppenol 1996). The near-diffusion limited rate of this reaction [$k = 4\text{–}6.7 \times 10^9/M/s$] is more than three times faster than the reaction between superoxide and superoxide dismutase (Beckman et al 1990, Huie & Padjama 1993). Unlike superoxide and $^{\bullet}NO$, peroxynitrite is not a free radical. The physical properties of peroxynitrite allow it to readily traverse biological membranes (Beckman & Koppenol 1996, Denicola et al 1998, Marla et al 1997, Squadrito & Pryor 1998). Reaction between peroxynitrite and carbon dioxide leads to formation of a nitrocarbonate intermediate ($ONO(O)CO_2^-$) (Denicola et al 1996, Gow et al 1996, Lymar et al 1996, Uppu et al 1996). This moiety is a less potent one- or two-electron oxidant compared to peroxynitrite, but it is an efficient nitrating agent (Denicola et al 1996, Gow et al 1996). Because the tissue partial pressure of carbon dioxide increases slightly in diving and hyperoxic environments, a particularly high risk for reactions involving peroxynitrite may exist, given the fact that there is a concomitant hyperoxia. Elevations in tissue carbon dioxide tension are due to the diminished capacity of hemoglobin to carry carbon dioxide (Haldane effect), because oxyhemoglobin persists in the venous circulation.

The $ONO(O)CO_2^-$ species is capable of nitrosylating biomolecules such as thiols and uric acid (Koppenol 1998, Skinner et al 1998, Uppu et al 1996, White et al 1999). Nitrosated adducts of reduced thiols and uric acid, as well as nitrated adducts of glycerol, are biologically active •NO donors (Balazy et al 1998, Skinner et al 1998, van der Vliet et al 1998, White et al 1999). Hence, a pathway exists for augmented formation of endogenous vasodilators.

REGULATION OF NITRIC OXIDE SYNTHASE ACTIVITY AND THE RESPONSE TO HYPEROXIA

A growing body of evidence suggests that hyperoxia may alter steady-state •NO concentration in vivo. However, data are conflicting on whether •NO levels increase or decrease. The majority of studies indicate that hyperbaric oxygen increases •NO synthesis, but the biochemical basis is not fully understood (Fig. 9.4.3). Changes in intracellular Ca^{++} may play a role with regard to •NO synthesis in response to hyperoxia. CNS oxygen toxicity (seizures) can be diminished by nitric oxide synthase inhibitors, but it has not been clear whether this was due to a reduction of •NO concentration or simply due to lower O_2 delivery caused by the reduced cerebral blood flow from nitric oxide synthase inhibitors (Bitterman & Bitterman 1998, Chavko et al 1998, Oury et al 1992, Zhang et al 1993). Recently, rats exposed to O_2 at 505 kPa (5.0 ATA) were found to have increased intrasynaptosomal free Ca^{++} concentration which secondarily increased nitric oxide synthase activity (Wang et al 1998). Oxygen toxicity was inhibited by a nitric oxide synthase inhibitor, consistent with prior investigations, and also by a Ca^{++} channel blocker. These data suggest that hyperoxia triggers elevations of •NO synthesis via alterations of intracellular Ca^{++} levels, although other mechanisms are also possible. Ito et al (1996) speculated that hyperbaric oxygen may promote synthesis of •NO because of an apparent inhibition of arginase activity in brain and because of data indicating that hydrogen peroxide accelerates •NO synthesis. Several investigations have suggested that hydrogen peroxide may enhance the rate of the second step in the nitric oxide synthase reaction; conversion of *N*-hydroxy-L-arginine to citrulline and •NO, but this point is not well established (Clague et al 1997, Mittal 1993). If in vivo superoxide dismutase content were elevated by hyperbaric oxygen, enhanced •NO generation may be observed due to synthesis of hydrogen peroxide. However, it is generally viewed that superoxide dismutase achieves this effect by removal of superoxide, thereby increasing •NO half life (Hobbs et al 1994, Ignarro et al 1987). A more complex balance of •NO synthesis and destruction in relation to hyperoxia, with a net decrease in •NO activity, has been reported by Demchenko et al (2000).

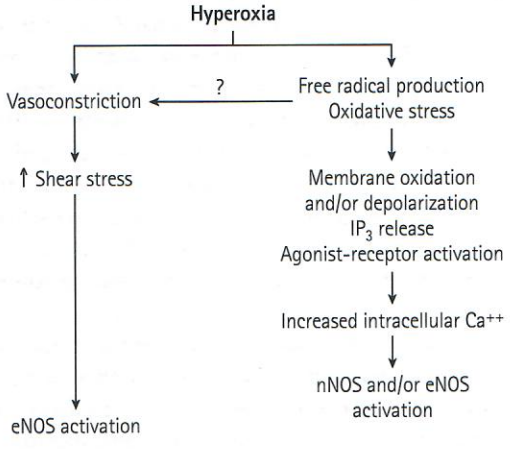

Fig. 9.4.3 Possible mechanisms for rapid activation of nitric oxide synthase by hyperoxia.

Three enzyme isoforms account for •NO production and they include neuronal •NO synthase (nNOS, Type I), inducible •NO synthase (iNOS, Type II) and endothelial •NO synthase (eNOS, Type III). Two isoforms, nNOS and eNOS, have traditionally been viewed as constitutive, although gene transcription and protein synthesis can be induced by environmental factors (Forstermann et al 1998). Type II NOS is constitutively expressed in some types of myocytes, glial cells, and airway epithelium, and synthesis is induced in many cell types by a variety of cytokines and lipopolysaccharides (Gath et al 1999, Guo et al 1995). All three isoforms require L-arginine, molecular O_2, and reduced nicotinamide adenine dinucleotide phosphate (NADPH) as cosubstrates and flavin adenine dinucleotide (FAD), flavine mononucleotide, tetrahydrobiopterin, heme and non-heme iron as cofactors (Perry & Marletta 1998).

Activities of nNOS and eNOS are controlled by variations of intracellular Ca^{++} concentration and subsequent calmodulin binding, whereas iNOS binds calmodulin tightly and is not regulated by Ca^{++}. All three isoforms bind to caveolins. Caveolin is the predominant protein component of specialized membrane structures termed caveolae, and there are three isoforms of caveolin (Gath et al 1999, Michel et al 1997). When agonists such as acetylcholine and bradykinin bind to membrane receptors, they elevate intracellular Ca^{++} which activates nNOS and eNOS (Ignarro et al 1987). Some data indicate that at least

for eNOS this process involves a Ca^{++} dependent dissociation of enzyme from caveolin, which allows for calmodulin binding and •NO synthesis. In time, as intracellular Ca^{++} falls to levels where calmodulin dissociates, the enzyme reassociates with caveolin and ceases catalytic activity (Feron et al 1998). Inhibition of iNOS by caveolin has not been demonstrated but tyrosine phosphorylation has been shown to inhibit iNOS, as well as nNOS activity (Bredt et al 1992, Dawson et al 1993). Factors other than caveolin which have been shown to regulate eNOS activity include fatty acid modification, phosphorylation, and binding by several proteins and cofactors (Russell et al 2000). Elevation of vascular shear stress, such as that caused by vasoconstriction in the face of constant flow rates, will cause eNOS dissociation from caveolin with enzyme activation that is independent of intracellular Ca^{++} (Ayajiki et al 1996, Dimmeler et al 1999, Fleming et al 1998, Rizzo et al 1998). Direct eNOS phosphorylation, or phosphorylation of other endothelial cell proteins, may also be a component of eNOS activation by shear stress and isometric contraction (Corson et al 1996, Dimmeler et al 1999, Fleming et al 1999). Activation by shear stress causes eNOS activity to persist for longer times and, possibly, at higher rates than does elevation of intracellular Ca^{++}. For example, in one comparison, shear stress-mediated activation caused a 13-fold increase in rate of •NO synthesis versus six-fold by elevating intracellular Ca^{++} (Corson et al 1996). There are overlaps between mechanisms of eNOS activation by receptor binding and by shear stress. Some agonists and shear stress will cause eNOS to bind the molecular chaperone, heat shock protein 90 (Hsp 90), which both renders eNOS activity independent of Ca^{++} and enhances activity (Garcia-Cardone et al 1998, Russell et al 2000). The role(s) for these activation pathways in hyperbaric hyperoxia requires study.

Studies with isolated nitric oxide synthase enzymes indicate their activities are influenced by redox state and specifically by O_2 tension. The Michelis constants (K_m) for O_2 were thought to be similar to other cytochrome P_{450}-like enzymes, ranging from 6 to 23 μM (Rengasamy & Johns 1996). However, during nitric oxide synthase catalysis, self-generated •NO reduces the ferric heme. Nitric oxide synthase activity is constrained by this process because when all substrates are replete, 70–90% of the enzyme is converted to the ferrous form which has only ~10% the activity as ferric nitric oxide synthase (Abu-Soud et al 1995, Hurshman & Marletta 1995). Oxygen tension may influence nitric oxide synthase activity by mediating ferrous-to-ferric conversion. Purified nNOS catalytic activity was reported to exhibit an apparent K_m for O_2 of ~ 400 μM and saturation at 800 μM (Abu-Soud et al 1996), values far greater than the K_m for binding O_2. More recently, Elayan et al (2000) reported the apparent K_m for O_2 for rat nNOS as 260 μM (achieved with 21 kPa O_2) (159 mmHg). Others have shown that type II iNOS has similar catalytic activity, with an apparent K_m for O_2 of 190 μM (Dweik et al 1998). Isolated eNOS activity has not been specifically studied with regard to O_2. Due to structural similarities among all three isotypes, Marletta et al (1998) have suggested that all respond similarly to substrate and cofactor availability. There are, however, many variables which may influence results of these types of experiments, such as enzyme isolation procedures and incubation conditions. For example, using high concentrations of iNOS under reducing conditions with dithiothreitol, Hurshman & Marletta (1995) reported little enhancement of activity with supernormal O_2 concentrations. Therefore, the physiologic relevance of ferrous-to-ferric NOS conversion and O_2 tension on activity of nitric oxide synthase is unclear.

Studies with intact cell systems have presented conflicting results with regard to the influence of O_2 on nitric oxide synthase activity. Aortic vascular endothelium was studied in static cultures (no shear stress due to flow) under conditions of maximum intracellular Ca^{++} availability [using the Ca^{++} adenosine triphosphatase (ATPase) inhibitor, thapsigargin] and the half-maximal rate of •NO synthesis occurred with 5 kPa (38 mmHg) O_2. Production was nearly at a maximum when cells were incubated with 19 kPa (145 mmHg) O_2 with nominal increase when incubated in medium saturated with 100% O_2 (93 kPa) (701 mmHg) (Whorton et al 1997). A different pattern with respect to O_2 was found with pulmonary vascular endothelial cells when Ca^{++} was not specifically manipulated. Again using static cultures, a significant elevation of •NO-mediated cyclic guanosine monophosphate (cGMP) synthesis was seen when O_2 partial pressure was increased from 5 to 20 kPa (40 to 150 mmHg), and still more was produced under normobaric hyperoxia (P_{O_2} 91 kPa; 680 mmHg) (Shaul & Wells 1994). However, these changes were not seen with mesenteric endothelium, leaving a possibility that O_2-associated enhancement of •NO synthesis is discretely a pulmonary endothelial phenomenon.

Studies with intact animals and with humans have provided a more consistent picture of O_2 influence on •NO production in lungs. Several investigators have proposed that elevations in •NO production by pulmonary endothelium may be the basis for O_2 induced dilation of pulmonary vasculature, affecting smooth

muscle tone via augmented cGMP synthesis (Cornfield et al 1996, Tiktinsky & Morin 1993). Humans exposed to 100% O_2 versus air were found to have ~ 38% more •NO in their exhaled air (Schmetterer et al 1997). However, studies have only focussed on O_2 in the range of severe hypoxia (ca < 3 kPa) (20 mmHg) to ambient pressure hyperoxia (100% O_2 at 101 kPa [1.0 ATA]). Moreover, ventilation with 21 to 50% O_2 caused little elevation of exhaled •NO (Dweik et al 1998). There have been no direct measurements of variation of nitric oxide synthase activity due to elevations of oxygenation in the systemic circulation. Indirect evidence based on measurements of flow showed that O_2 induced vasoconstriction was enhanced with nitric oxide synthase inhibitors (thus suggesting that •NO synthesis antagonized vasoconstriction) but other studies have failed to find an effect (Demchenko et al 2000, Harris et al 1996, Schmetterer et al 1997).

POSSIBLE ROLES OF FREE RADICALS IN VASCULAR EFFECTS AND TOXICITY OF HYPEROXIA

The mechanism for oxygen induced vasoconstriction is not known. It does not appear to be related to impaired production of •NO, although infusion of •NO-donor substances can ameliorate the diminished regional blood flow (Bitterman & Bitterman 1998, Demchenko et al 2000, Schmetterer et al 1997). Vasoconstriction occurs rapidly in response to hyperoxia and, as exposure to hyperbaric hyperoxia continues, vasconstriction resolves and flow is either restored or may even increase beyond normal. For example, in brain, hyperbaric hyperoxia initially causes vasoconstriction but later, shortly preceding the onset of oxygen induced convulsions, blood flow increases. This pattern of change has been noted in humans and several animal species (Bergo & Tyssebotn 1992, Jacobson et al 1963, Kurasako et al 2000, Lambertsen et al 1953b, Omae et al 1998, Plewes & Farhi 1983, Torbati et al 1978, 1979). New sources of •NO may influence the restoration of flow observed with prolonged exposures to hyperbaric oxygen (Demchenko et al 2000, Elayen et al 2000, Kurasako et al 2000). It appears that hyperbaric oxygen will increase the concentration of •NO-containing substances in both the pulmonary and systemic vascular beds. *S*-nitrosohemoglobin concentration was approximately doubled in arterial and venous blood of rats exposed to 303 kPa O_2 (3.0 ATA) versus 101 kPa O_2 (1.0 ATA) (Stamler et al 1997). Hence, it is possible that •NO-donating substances influence the biphasic pattern of changes in regional organ blood flow observed in response to hyperbaric oxygen.

PHARMACOLOGICAL ACTIONS OF HYPEROXIA

Platelet Derived Growth Factor

Hyperbaric oxygen was shown to augment synthesis of the beta receptor for platelet derived growth factor in wounds. The action was synergistic with platelet derived growth factor that was applied to experimental wounds in causing messenger RNA synthesis for the membrane-bound growth factor receptor (Bonomo et al 1998). The proximal agent causing up-regulation of transcription, for example, whether it may be one or more free radical(s) or molecular O_2, itself, is not yet known.

Reperfusion Injury

There is ample precedence that reactive species of O_2 and nitrogen are second messengers in vivo and that they serve to signal a variety of cellular responses. At least some apparent therapeutic effects of hyperbaric hyperoxia can be linked to this category of mechanisms. One of the reoccurring observations with animal studies of hyperbaric O_2 has been the benefit to treating various forms of reperfusion injury (Bitterman & Cohen 1989, Haapaniemi et al 1995, 1996, Horn et al 1999, Krakovsky et al 1998, Nylander et al 1987, Shandling et al 1997, Sirsjo et al 1993, Stavitsky et al 1998, Thom 1993, Thomas et al 1990, Ueno et al 1999, Wong et al 1996, Zamboni et al 1993). On first review, this is a paradox given the fact that hyperbaric oxygen is expected to accelerate production of reactive O_2 species, which commonly contribute to reperfusion injuries. However, neutrophils play a major role in the pathologic cascade after reperfusion and methods to impede adherence between neutrophils and reperfused endothelium are frequently beneficial (Virkhaus et al 1995). Inhibition of neutrophil β_2 integrins and their intercellular adhesion molecule (ICAM) endothelial counter-receptors can be achieved with hyperbaric oxygen (Buras et al 2000, Chen et al 1996, Tjarnstrom et al 1999, Thom 1993, Thom et al 1997, Zamboni et al 1993).

Reactive nitrogen species also play a role in reperfusion injuries. Physiologic increases in the concentration of •NO, typically within the nanomolar range, are protective against reperfusion and inflammatory responses (Murakami et al 1997). Nitric oxide can inhibit neutrophil-to-endothelial cell adhesion by inhibiting synthesis and expression of endothelial adhesion molecules such as ICAM, E-and P-selectins,

and also by inhibiting neutrophil cytoskeletal assembly and co-ordinated function of β_2 integrins (Banick et al 1997, Bhabra et al 1999, Johnson et al 1991, Kosonen et al 2000, Kubes et al 1991, Lindemann et al 2000, Liu et al 1998a, Radomski et al 1990, Sato et al 1999, Terada et al 1997).

INTERACTIONS WITH PLASMA MEMBRANES

The plasma membrane is an important site of interaction with free radicals, because many types of damage can be produced with a variety of functional consequences (Fig. 9.4.4), and additional toxic radicals, as well as other biologically active products, can be produced by the actions of membrane-bound enzymes (Freeman & Crapo 1982). Free radical damage to membranes can be expressed as lipid peroxidation, amino acid oxidation, protein strand scission and a variety of cross-linking reactions among lipids and proteins. Peroxidation of membrane unsaturated fatty acids, oxidation of structural proteins and inactivation of membrane-bound enzymes can increase membrane permeability and eliminate transmembrane ion gradients with the loss of secretory and other important membrane functions.

Lipid Peroxidation

Cell membranes are rich in polyunsaturated fatty acids which can react with free radicals and undergo hydrogen atom abstraction to generate lipid peroxides and lipid peroxy radicals that in turn undergo toxic reactions with many of the same cellular constituents acted upon by oxygen free radicals (Freeman & Crapo 1982, Fridovich & Freeman 1986). This process is potentiated by the presence of metals and can become autocatalytic after initiation to amplify the damage by oxidizing many molecules of polyunsaturated fatty acid per initiation event.

The hydrophobic nature of lipid radicals will cause most of these reactions to take place with membrane-associated molecules. The resulting peroxidation of membrane fatty acids may have adverse effects on membrane permeability and microviscosity. In addition, cross-linking and polymerization of membrane components caused by malondialdehyde, which is produced by peroxidation of fatty acids containing three or more double bonds, can alter critical membrane properties such as deformability, ion transport and enzyme activities (Fig. 9.4.4, Freeman & Crapo 1982).

Lipid peroxidation induced by exposure to hyperoxia has been demonstrated in rat cerebral cortical slices (Dirks & Faiman 1982, Kovachich & Mishra 1980) and correlated with partial inactivation of NaK-ATPase (Kovachich & Mishra 1981). An earlier onset of oxygen convulsions in mice than in rats was associated with greater levels of lipid peroxidation in the mouse cortical slices (Dirks & Faiman 1982). Normal and tocopherol deficient mice convulsed during a 1 h exposure to O_2 at 404 kPa (4.0 ATA), and both groups had increased brain levels of lipid peroxides (Jerrett et al 1973). The same exposure caused neither convulsions nor lipid peroxidation in the brains of tocopherol supplemented mice. However, brain lipid peroxide levels did not correlate with convulsions in rats exposed to O_2 at 303–606 kPa (3.0–6.0 ATA) (Becker & Galvin 1962). Potentiation of hyperoxia-induced lipid peroxidation by the presence of Fe^{2+} was observed in brain cortical

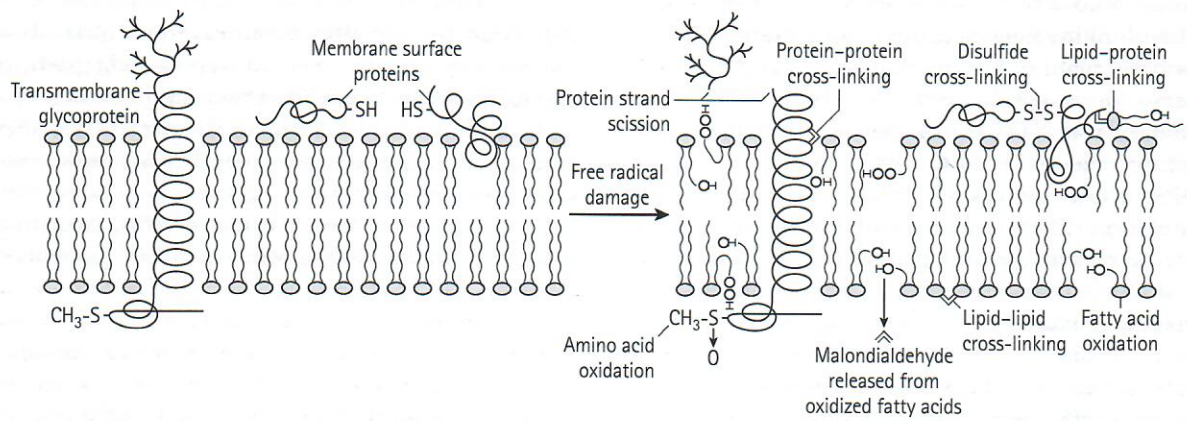

Fig. 9.4.4 Potential sites of free radical damage to plasma membranes. Free radicals can initiate lipid peroxidation, which produces short chain fatty acyl derivatives and the by-product malondialdehyde. Reactions with malondialdehyde can mediate a variety of cross-linking reactions. Free radicals can also catalyze amino acid oxidation, protein–protein cross-linking and protein strand scission. (From Freeman & Crapo 1982)

slices (Kovachich & Mishra 1981) and was also manifested as a linear relationship between degree of lipid peroxidation and endogenous iron content in homogenates from different areas of the brain (Zaleska & Floyd 1985). Additional support for an important role of iron in this potentiation is provided by the observation that the level of lipid peroxidation in rat brain homogenates exposed to hyperoxia was inhibited to a much greater degree by ceruloplasmin and deferrioxamine than by the addition of superoxide dismutase or catalase (Arai et al 1987).

Lipid peroxidation during exposure to hyperoxia has also been identified in the lungs and liver (Jamieson 1989). The rates of lipid peroxide formation in isolated perfused lungs from normal and tocopherol-deficient rats increased by about 50% and more than 500%, respectively, during exposure to O_2 at 404 kPa (4.0 ATA); Nishiki et al 1976). Exposure to 80–100% O_2 for 2–7 days also increased the rate of lipid peroxidation in lung tissues (Freeman et al 1982, Januskievicz & Faiman 1984, Webster et al 1987). The rate of lipid peroxidation is increased in isolated perfused liver exposed to O_2 at 404 kPa (4.0 ATA) (Nishiki et al 1976) and in liver tissue from rats exposed for 5 days to 80% O_2 at 101 kPa (1.0 ATA) (Webster et al 1987).

Metabolism of Arachidonic Acid

Actions of the membrane-associated enzymes cyclo-oxygenase and lipoxygenase on arachidonic acid, their primary substrate, initiate metabolic pathways that generate active radicals at several intermediate steps, and also produce a variety of biologically active products that include prostaglandins, thromboxanes and leukotrienes (Freeman & Crapo 1982). Although the degree to which radicals generated in this manner are involved in oxygen toxicity is not known, there are indications that products of arachidonic acid metabolism are involved at least indirectly.

Smith et al (1986) measured the concentrations of thromboxane and prostacyclin metabolites in bronchoalveolar lavage fluid obtained from mice exposed to O_2 at 101 kPa (1.0 ATA) for up to 4 days. While the concentration of thromboxane metabolite was not changed at any time, the prostacyclin metabolite concentration remained unchanged for 3 days and then increased steeply on day 4 of exposure. The administration of indomethacin, an inhibitor of cyclo-oxygenase, was associated with a reduced concentration of prostacyclin metabolite, increased lung damage and decreased survival time. The authors concluded that their data were consistent with inhibition of prostacyclin synthesis or with shunting metabolism of arachidonic acid through the lipoxygenase pathway. The increased concentration of prostacyclin metabolite on day 4 was attributed to concurrent endothelial cell destruction with the release of arachidonic acid in sufficient quantities to overcome the proposed decrement in prostacyclin synthesis.

In a subsequent study using the same animal model, Smith et al (1988) found a positive correlation between severity of lung damage and bronchoalveolar lavage fluid concentration of sulfidopeptide leukotrienes (predominantly leukotriene D_4). Neutrophils were not a major source of the leukotrienes, because similar increments in leukotriene concentration were also observed in neutropenic mice. Additional evidence in support of a role for lipoxygenase products in the development of pulmonary oxygen poisoning was provided by the observation that rats exposed to O_2 at 101 kPa (1.0 ATA) for up to 72 h had a progressive increase in lavage fluid concentration of leukotriene B_4 in association with increased numbers of neutrophils in lavage fluid and reduced activity of NADPH-cytochrome *c* reductase in lung microsomes (Taniguchi et al 1986). The administration of low and high doses of a lipoxygenase inhibitor provided concurrent dose-dependent reductions in leukotriene B_4 concentration and number of neutrophils in the lavage fluid. It also progressively reduced mortality and protected against the previously observed reduction in cytochrome *c* reductase activity. The authors concluded that the increased concentration of leukotriene B_4 acted as a chemoattractant for neutrophils which then contributed to the lung damage caused by exposure to hyperoxia.

Protein Damage by Free Radicals

The probability that a given protein will undergo free radical damage and the potential severity of that damage are determined by a variety of factors that include: the amino acid composition of the protein; the molecular location of susceptible amino acids and their influences on protein conformation and activity; the cellular location of the protein; and the nature of the attacking free radical (Ara et al 1998, Freeman & Crapo 1982). The functional significance of the resulting damage is also influenced by the availability and efficacy of mechanisms for reversal or repair of the damage. Amino acids that contain unsaturated bonds and sulfur atoms are readily reactive with free radicals. Thus, proteins containing tryptophane, tyrosine, phenylalanine, histidine, methionine, and cysteine are susceptible to amino acid modification by free radicals (Ara et al 1998, Freeman & Crapo 1982). The predominant

protein modification by peroxynitrite is with tyrosine residues to form 3-nitrotyrosine. Nitration of a protein is a selective process that is influenced by the location of the tyrosine and the electrostatic characteristics of its surroundings (Ara et al 1998). Cysteine can also be S-nitrosylated, although this is a more rapidly reversible alteration and, therefore, of less significance for impairing biological functions.

Protein degradation subsequent to damage from oxygen radicals was demonstrated by Davies and his colleagues (Davies 1987, Davies & Delsignore 1987, Davies & Goldberg 1987, Davies et al 1987a) in a system that employed ^{60}Co radiation in the presence of oxygen to generate hydroxyl and superoxide radicals. The results of these workers indicated a direct and quantitative relationship between protein damage by oxygen radicals and increased proteolytic susceptibility (Davies & Goldberg 1987). Subsequent investigation with rabbit erythrocytes has indicated that protein degradation induced by oxygen radicals precedes the appearance of detectable lipid peroxidation products and occurs independently of membrane damage associated with products of lipid peroxidation (Davies & Goldberg 1987). Wolff et al (1986) have suggested that membrane transport proteins may be unusually susceptible to oxygen radical attack.

Nitrated proteins are less readily proteolytically degraded and removed than are oxidized proteins (Davies & Goldberg 1987, Gow et al 1996). There appears to be a non-proteolytic pathway to repair nitrated proteins (Gow et al 1996, Kamisaki et al 1998). Although the enzyme(s) have not been specifically identified or characterized, decreasing levels of 3-nitrotyrosine have been described in an infant surviving from sepsis. Rodent tissues exhibit higher 'denitrase' activity after exposure to endotoxin or nitrative stress (Kamisaki et al 1998).

NEUTROPHILS AS A POTENTIAL SOURCE OF OXYGEN RADICALS

Activated neutrophils can release into the surrounding medium a variety of reactive species including superoxide, hydrogen peroxide, hydroxyl radical, hypochlorous acid, and peroxynitrite (Barry & Crapo 1985, Evans et al 1996, Jamieson 1989, Shasby et al 1982, Weiss & LoBuglio 1982). The intracellular location of most antioxidant defenses may make them less effective against oxygen radicals that are generated extracellularly (Barry & Crapo 1985, Jamieson 1989).

The hypothesis that neutrophils contribute to the development of pulmonary oxygen toxicity is supported primarily by observations made in animals during multiday exposures to O_2 at 101 kPa (1.0 ATA). In rats exposed for more than 2 days, neutrophils accumulate within the pulmonary vasculature, frequently adhering to capillary endothelial cells, and are also more numerous within the lung interstitium (Barry & Crapo 1985, Crapo 1986, Crapo et al 1980, Fox et al 1981). The accumulation of neutrophils in the lungs was associated with rapid exacerbation of lung damage (Crapo 1986) and, conversely, the pathologic effects of oxygen exposure were decreased by prior systemic depletion of neutrophils (Shasby et al 1982, Fox et al 1981).

Other studies have shown that, although neutrophil derived oxygen radicals can increase the severity of pulmonary oxygen poisoning, they are not a primary factor in its development (Crapo 1986, Jamieson 1989). Lethal exposures of rats to O_2 at 202 kPa (2.0 ATA) were not associated with increased numbers of neutrophils in bronchoalveolar lavage fluid (Glass et al 1986). In vitamin E deficient rats exposed to O_2 at 101 kPa (1.0 ATA), or in normal rats exposed after administration of disulfiram, survival times were significantly decreased in the absence of increased numbers of neutrophils in lavage fluid (Glass et al 1986). In contrast to the observation that neutropenia provided a degree of protection against pulmonary oxygen toxicity in rabbits (Shasby et al 1982), other investigators found that systemic neutropenia afforded no protection against hyperoxia induced lung microvascular injury in rats (Boyce et al 1989), rabbits (Laughlin et al 1986, Raj et al 1985) or lambs (Raj et al 1985).

ANTIOXIDANT DEFENSES

Biochemical defenses against oxygen derived free radical damage are essential for survival in an aerobic environment (Chance et al 1979, Forman & Fisher 1981, Freeman & Crapo 1982, Fridovich & Freeman 1986). Potential oxidant–antioxidant interactions in the lung are illustrated in Fig. 9.4.5. Fridovich & Freeman (1986) have characterized antioxidant defenses as a multilayered system that has evolved to counter the threat imposed by reactive intermediates initiated by the univalent reduction of molecular oxygen. The first line of defense in this system is avoidance of the univalent pathway by the action of enzymes, such as cytochrome oxidase, that can reduce molecular oxygen to water without producing reactive intermediates. Maintaining coupling of electron transfer in the mitochondrial respiratory chain, within cytosolic proteins that catalyze different oxidations such

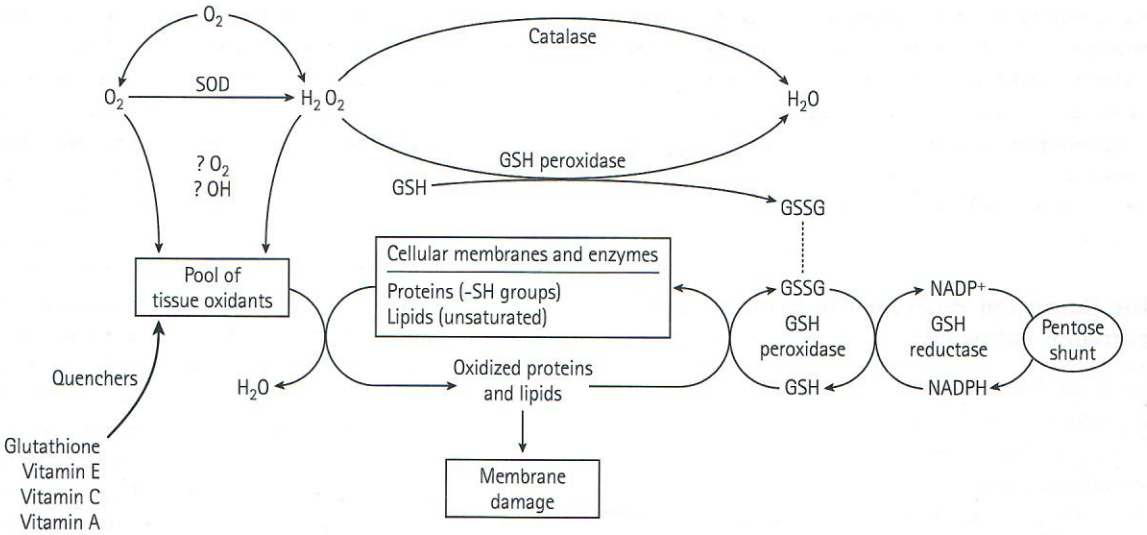

Fig. 9.4.5 Oxidant–antioxidant interactions in the lung. Possible metabolic events induced by the elevation of inspired P_{O_2} are represented. The sequence is initiated by enhanced generation of superoxide anion, H_2O_2, and possibly other active species such as singlet oxygen and hydroxyl radicals to form a pool of tissue oxidants. These oxidants may damage cell membranes and intracellular enzymes by oxidizing tissue proteins and lipids. The tissue oxidant pool is diminished and free radical chain reactions are stopped by interactions of quenchers with active species and oxidized tissue components. Superoxide anions can be removed specifically by superoxide dismutase (SOD). Damaged tissues may also be repaired by reduction of oxidized components by glutathione (GSH) to form oxidized glutathione (GSSG). Regeneration of GSH from GSSG may be accomplished by interaction with reduced nicotinamide adenine dinucleotide phosphate (NADPH), which is, in turn, restored by action of the pentose shunt pathway of glucose metabolism. The extent of lung damage may be determined by the net result of opposing radical-producing and radical-quenching actions with concurrent interactions between tissue-damaging and tissue-repairing processes. (After Fisher et al 1979)

as the cytochrome P_{450} and mono-oxygenases, as well as nitric oxide synthase, prevents electron transfer to oxygen. This reduces the pool of active radicals that must be opposed by other means.

Superoxide Dismutase

The second line of defense is provided by metalloenzymes, known as superoxide dismutases, which catalyze the dismutation of superoxide anion to form hydrogen peroxide (Freeman & Crapo 1982, Fridovich & Freeman 1986, see Fig. 9.4.5). The superoxide dismutase found in the cytoplasm of mammalian cells has divalent copper and zinc at its active sites, while manganese occupies the active sites of another superoxide dismutase found in the matrix of mitochondria.

The importance of superoxide dismutase as a component of the antioxidant defense system was first recognized and documented by McCord Fridovich (1978). Superoxide dismutases were found in all aerobic organisms, but not in anaerobes (McCord et al 1971). The augmented synthesis of superoxide dismutase was induced by increased exposure of facultative organisms to oxygen (Hassan & Fridovich 1977a), by increased intracellular production of superoxide radicals in response to agents such as paraquat (Hassan & Fridovich 1977b), and by metabolic changes causing greater dependence on aerobic metabolism (Hassan & Fridovich 1977c). Intracellular concentrations of superoxide dismutase were correlated with resistance to oxygen toxicity (Hassan & Fridovich 1977a,b,c), and mutants with deficient levels of superoxide dismutase were unusually susceptible to oxygen poisoning (McCord et al 1973).

Catalase and Glutathione Peroxidase

The third line of antioxidant defense is provided by enzymes, including catalase and glutathione peroxidase, that remove the hydrogen peroxide produced either indirectly by the dismutation of superoxide anion or directly by reoxidation of reduced flavoenzymes (Fridovich & Freeman 1986). Catalase is a heme protein that catalyzes the dismutation of hydrogen peroxide to water and oxygen. Hydrogen peroxide can also be reduced by glutathione peroxidase, an enzyme

which contains selenium in its active site. Glutathione peroxidase catalyzes the reduction of hydrogen peroxide to water by using as an electron donor the oxidation of glutathione to glutathione disulfide. The depletion of reduced glutathione is prevented by the action of glutathione reductase, which catalyzes the reduction of glutathione disulfide by oxidation of NADPH (Fig. 9.4.5).

Nitric Oxide and Peroxynitrite Removal and Scavenging Pathways

A bacterial •NO reductase has been described. This flavoprotein is capable of specifically removing •NO in bacteria (Gardner et al 1998, Hausladen et al 1998). A mammalian equivalent to the bacterial enzyme has not, as yet, been described. The selenium dependent glutathione peroxidase may serve as a peroxynitrite reductase, and selenoprotein P as a scavenger in mammalian cells (Arteel et al 1999, Burk & Hill 1999). As mentioned above, specific repair enzymes to cleave nitrated amino acids and yet preserve protein structure have been implicated in recent literature but not characterized or purified.

Termination of Active Radical Chain Reactions

A fourth line of defense is provided by biological antioxidants such as vitamin E which can react rapidly with chain propagating fatty acid radicals to form the stable alpha-tocopherol radical and terminate the chain reaction (Fridovich & Freeman 1986). Vitamin E is a series of tocopherol isomers that reduces lipid peroxy and other free radicals including superoxide, hydroxyl radical and singlet oxygen (Freeman & Crapo 1982). Vitamin E is positioned for maximum effectiveness against fatty acid radicals by hydrophobic properties which cause it to partition into biological membranes (Fridovich & Freeman 1986). Ascorbate (vitamin C) is also proposed to have antioxidant properties and may function to maintain tocopherols in the reduced active form (Freeman & Crapo 1982, Tappel 1969).

Reversal of Oxidant Damage

The reactivation of oxidized enzymes and the reduction of oxidized tissue components constitute the fifth and final line of antioxidant defense (Fridovich & Freeman 1986). These repair processes appear to be accomplished predominantly by interactions with reduced glutathione, and oxidized glutathione is produced as a by-product (Barron 1955; Fisher et al 1979). Evidence consistent with increased glutathione turnover in response to an elevation of tissue P_{O_2} has been obtained in vitro with the frog bladder preparation (Allen et al 1973) and the isolated lung preparation (Nishiki et al 1976).

The regeneration of reduced glutathione requires the presence of NADPH, which is in turn provided largely by reactions of the pentose shunt pathway of glucose metabolism (Fig. 9.4.5, Tierney et al 1977, Fisher et al 1979). The total lung content of the rate limiting enzyme of the pentose pathway, glucose-6-phosphate dehydrogenase, is increased in rats adapted to hyperoxia (Crapo et al 1978, Tierney et al 1973). Pentose cycle activity is also increased in isolated perfused rat lungs ventilated with O_2 at 505 kPa (5.0 ATA); Bassett & Fisher 1979). The involvement of the pentose cycle in the reversal of oxidant damage in the brain as well as in the lung is indicated by observations that pentose shunt activity is either little affected or increased concurrently with reduced activity of the tricarboxylic acid cycle in rat brain cortical slices (Kovachich 1978) and in isolated retinal tissue exposed to hyperbaric oxygen pressures (Brue et al 1978).

CORRELATION OF IN VITRO AND IN VIVO EFFECTS OF OXYGEN TOXICITY

EARLY STUDIES

Working concurrently on both sides of the Atlantic, Dickens (1946) in London and Stadie et al (1944, 1945a,b) in Philadelphia carried out extensive studies of oxygen poisoning in brain and other tissue preparations. They found that numerous enzymes and metabolic pathways could be inactivated by prolonged exposure to high oxygen pressures. Enzymes whose activity was dependent upon the presence of sulfydryl groups were particularly susceptible to oxygen poisoning (Haugaard 1946). Trace metals such as Cu^{2+} and Fe^{2+} were found to enhance the toxic effects of oxygen, whereas others such as Mn^{2+}, Co^{2+}, Mg^{2+}, and Ca^{2+} delayed enzyme inactivation by hyperoxia (Dickens 1946, Haugaard 1964).

Although these studies showed that oxygen uptake by in vitro preparations could be significantly decreased by oxygen exposure, they did not afford an explanation for the development of oxygen poisoning in intact animals. The respiration of brain slices and homogenates was significantly depressed only after exposure durations that were much longer than those required to cause convulsions in intact animals breathing oxygen at the same pressure. This discrepancy is even greater when it is recognized that the mean P_{O_2} of brain tissue in vivo is much lower than respired P_{O_2} (Fig. 9.4.2). Furthermore, the enzyme inactivation observed in vitro

was irreversible, while intact animals recovered rapidly when inspired P_{O_2} was reduced to normal after the initial hyperoxic seizure.

METABOLIC EFFECTS ON SUSCEPTIBILITY TO OXYGEN POISONING

Kovachich (1980) identified a variety of conditions that increase susceptibility to oxygen poisoning in rat brain slices and cause it to approach that found in intact animals (Fig. 9.4.6). These conditions include an increased potassium concentration in the incubation medium, addition of the depolarizing agent veratridine to the medium, uncoupling oxidative phosphorylation by addition of dinitrophenol, and omission of calcium ions from the medium. Although each condition acts by a different mechanism, all of them increase the respiratory rate of brain tissue and make it more susceptible to oxygen poisoning. Conversely, conditions such as the addition of Tris-buffer, an increased calcium concentration in the incubation medium, and cooling the medium from 37 to 32°C reduce respiratory rate and make brain tissue less sensitive to oxygen toxicity (Fig. 9.4.6).

Direct correlations of susceptibility to oxygen poisoning with metabolic rate have also been observed in intact animals. Poikilotherms are normally relatively resistant to oxygen toxicity, but become less resistant when they are warmed (Faulkner & Binger 1927). Certain insect species (lepidopterans) are more susceptible to oxygen poisoning during early larval development and pupation, when biosynthetic and metabolic activity is more intense than at other stages

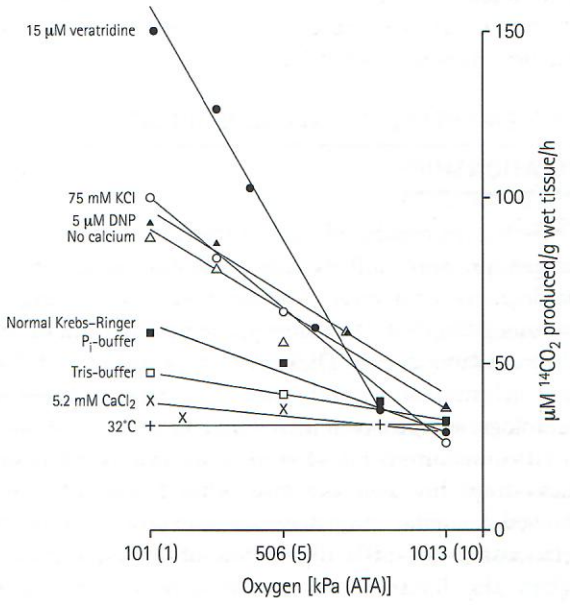

Fig. 9.4.6 Effects of increased glucose metabolism on susceptibility to oxygen poisoning in rat brain cortical slices. Each point represents the mean of five to seven experiments. All slices were incubated for 1 h at 37°C in Krebs–Ringer phosphate medium with the indicated changes, except for one group maintained at 32°C. The data show correlation of an increased metabolic rate at 101 kPa (1.0 ATA) of O_2 with enhanced susceptibility to oxygen poisoning at higher pressures. (From Kovachich 1980)

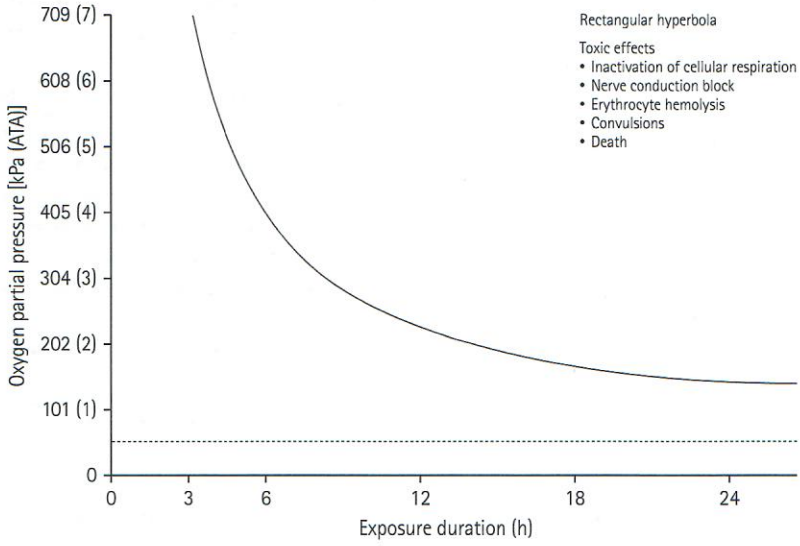

Fig. 9.4.7 Inspired oxygen pressure–exposure duration relationship for specific manifestations of oxygen poisoning. (From Clark 1974)

of development (Brown & Hines 1976). Alterations in metabolic rate produced by hypothermia (Grossman & Penrod 1949a), hibernation (Popovic et al 1964) and modifications of thyroid activity (Gersh & Wagner 1945, Grossman & Penrod 1949b) are associated with parallel changes in sensitivity to hyperoxic exposure.

OXYGEN PRESSURE–EXPOSURE DURATION RELATIONSHIPS

There is a hyperbolic relationship between the inspired oxygen pressure and exposure duration required to produce any of a variety of manifestations of oxygen poisoning (Fig. 9.4.7). Analyzing data obtained in many different laboratories, Dickens (1962) was one of the first investigators to recognize this relationship for neurologic symptoms in intact animals and man. Using in vitro measurements of respiratory rate in rat brain slices from his own and two other laboratories, he showed a similar oxygen pressure–exposure duration relationship for 50% depression of oxygen uptake. When the linear forms of these relationships are compared on log–log co-ordinates, however, their different slopes are obvious, as is the fact that neurologic symptoms occur much earlier than depression of oxygen uptake at the same oxygen pressure (Fig. 9.4.8).

The early in vitro measurements used by Dickens were obtained from brain slices incubated in normal Krebs–Ringer phosphate medium. It is now known that this medium maintains brain tissue in a resting state with a polarized plasma membrane and an oxygen uptake of about half that measured in the intact brain (Kovachich & Haugaard 1981). The metabolic rate of brain slices in vitro can be stimulated to approach that found in vivo by progressive increments of potassium concentration or by the addition of the depolarizing agent veratridine to the medium (Kovachich 1980). In such preparations, the linear expression of the pressure–duration relationship for hyperoxic depression of tissue respiration approximates the in vivo relationship more closely, with respect to both the location and the slope of the curve (Fig. 9.4.8). Although differences between inspired and brain oxygen tensions in vivo (Fig. 9.4.2) preclude direct comparisons of these curves, it appears that more accurate in vitro simulations of in vivo states can be obtained by the use of appropriate conditions.

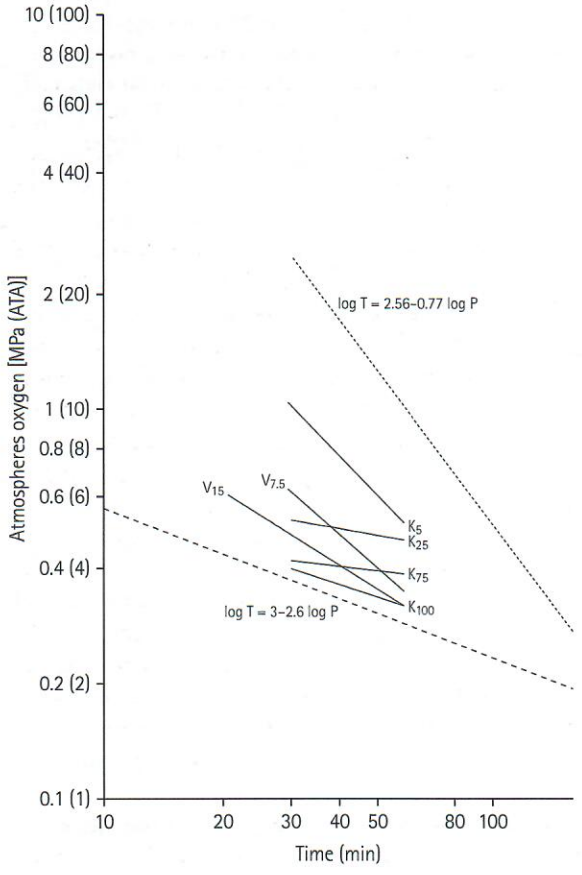

Fig. 9.4.8 Oxygen pressure–exposure duration relationships for in vivo and in vitro manifestations of oxygen poisoning. The dashed line on the left (log $T = 3 - 2.6 \log P$) represents the oxygen pressure–exposure duration relationship for neurologic symptoms in intact animals and man. The dashed line on the right (log $T = 2.56 - 0.77 \log P$) represents the corresponding relationship for 50% depression of oxygen uptake in rat brain slices incubated in normal Krebs-Ringer phosphate medium. The solid lines represent pressure–duration relationships for 25% decrements in $^{14}CO_2$ production in rat brain slices incubated in media with increased potassium concentration (K_{mM}) or with added veratridine ($V_{\mu M}$). As metabolic activity of the brain slices is progressively stimulated, the in vitro sensitivity to oxygen poisoning approaches that found in vivo. Dashed lines from data summarized by Dickens (1962). Solid lines from data of Kovachich (1980). (Adapted by Kovachich & Haugaard 1981 from a concept first used by Dickens 1962)

POTENTIAL LINKS BETWEEN INITIAL ACTIVE RADICAL REACTIONS AND SUBSEQUENT FUNCTIONAL DEFICITS CAUSED BY OXYGEN TOXICITY

Although the relevant active species and reactions have not yet been identified precisely, there is now general agreement that the effects of oxygen toxicity are initiated by the formation of oxygen derived free radicals and

their reactions with nearby cellular constituents (Freeman & Crapo 1982, Jamieson 1989). At the opposite ends of what will most likely prove to be multiple, concurrent pathogenetic pathways (Lambertsen 1978), many functional deficits associated with early, reversible degrees of oxygen poisoning have also been defined (see p. 372). However, relatively little is known about the many intermediate steps between the initial active radical reactions and subsequent functional derangements.

During the past four decades, several biochemical responses to hyperoxic exposure have been demonstrated in intact animals prior to the occurrence of overt toxic effects. Although such a finding per se does not indicate that the observed response is related to the cause of oxygen poisoning, it does establish the possibility of such a role. Until there is conclusive evidence for its exclusion, each of these early biochemical changes should be examined as a possible intermediate step between the formation of oxygen radicals and overt manifestations of oxygen poisoning.

ENZYME INACTIVATION BY OXIDATION OF ACTIVE SULFYDRYL GROUPS

In 1946, Haugaard showed that enzymes whose catalytic activity is dependent upon the presence of reduced sulfydryl groups are unusually sensitive to oxygen toxicity, and Tijoe & Haugaard (1972) later correlated the inactivation of such an enzyme by O_2 at 505 kPa (5.0 ATA) with the disappearance of active sulfydryl groups. The inactivation of sulfydryl groups in vivo was first demonstrated by Jamieson & van den Brenk in 1962. In the lungs of rats exposed to 505 kPa (5.0 ATA) O_2, dehydrogenase activity and sulfydryl content were reduced significantly after 15–30 min when no macroscopic and only slight microscopic changes were present (Jamieson & van den Brenk 1962, Jamieson et al 1963). After 45 min exposure, the lungs were damaged and disulfide content was increased. A reduced sulfydryl content was also associated with lung damage in mice exposed to hyperbaric oxygen (Waechter & Faiman 1982).

PROTEIN MODIFICATIONS FROM •NO AND PEROXYNITRITE

A number of specific proteins are modified by •NO. Factors which determine the selectivity of these reactions are under investigation. The proteins fall into four major categories:

1 iron containing heme and non-heme proteins;
2 proteins with iron-sulfur clusters;
3 proteins with labile cysteine residues; and
4 proteins with tyrosine residues that undergo one-electron oxidation.

Two amino acids, tyrosine and cysteine, have been shown to be major targets for peroxynitrite in proteins (Ischiropoulos 1998, Radi et al 1991). The reaction with tyrosine results in formation of 3-nitrityrosine, and the reaction with cysteine generates oxidized forms of cysteine and *S*-nitrocysteine (Balazy et al 1998, Ischiropoulos 1998, Radi et al 1991, van der Vliet et al 1998). Peroxynitrite will also selectively react with zinc-sulfur and iron-sulfur centers of proteins (Crow et al 1995, Keyer & Imlay 1997). Plasma proteins that have been found nitrated include ceruloplasmin, transferrin, α_1 antichymotrypsin, α_1 protease inhibitor and fibrinogen. Cellular proteins found to lose activity due to in vivo nitration include manganous superoxide dismutase, prostacycline synthase, tyrosine hydroxylase and calcium-ATPase (Klebl et al 1998, MacMillan-Crow et al 1996, Zou et al 1998).

PYRIDINE NUCLEOTIDE OXIDATION

Using a fluorometric technique to measure steady-state pyridine nucleotide oxidation–reduction levels in anesthetized rats breathing oxygen at high pressures, Chance et al (1965, 1966) found increased oxidation levels in the exposed liver, brain and kidney prior to the onset of convulsions. In rat liver, O_2 at 273 kPa (2.7 ATA) caused a detectable increase in the oxidation level compared with that in air. Changes in rat brain were smaller, but a distinct shift in the direction of oxidation was produced by 778 kPa (7.7 ATA) O_2. In both cases changes in the oxidation level appeared to be complete within 10–20 s after the start of hyperoxic exposure.

Employing a later refinement of the same method in conjunction with chronically implanted light guides, Mayevsky et al (1974) performed surface fluorometry of the brain cortex with simultaneous electroencephalographic (EEG) recording in awake rats exposed to O_2 at 606 kPa (6.0 ATA). The level of pyridine nucleotide oxidation was approximately the same whether the rats were compressed to 606 kPa (6.0 ATA) in O_2 alone, pretreated with succinate (which delayed the onset of convulsions) or compressed in O_2 with 1.5% CO_2 (which hastened seizures). However, the exposure duration required for pyridine nucleotide oxidation to reach its maximal value was lengthened after succinate administration and markedly shortened

by CO_2. In all three conditions, pyridine nucleotide oxidation preceded seizure activity.

INACTIVATION OF MEMBRANE TRANSPORT SYSTEMS

There are many indications that membrane-bound active transport systems are susceptible to oxygen inactivation. It is well established that glutamate uptake is dependent on a transport system that is linked to potassium transport (Kovachich & Haugaard 1981). In guinea pig brain cortical slices exposed to O_2 at 606 kPa (6.0 ATA) for 90 min, Kaplan & Stein (1957) found that glutamate uptake and potassium retention were both impaired. Similar observations were later made by Joanny et al (1970) in brain cortical slices exposed to oxygen pressures ranging from 101 to 1013 kPa (1–10 ATA). The active transport of sodium in the toad bladder preparation (Allen et al 1973, Miller et al 1976) and through isolated frog skin (Gottlieb & Cymerman 1970) is also impaired by exposure to hyperoxia. Allen et al (1973) concluded that the most likely mechanism of sodium transport inactivation by oxygen involved the intermediate formation of lipid peroxides.

CORRELATION OF CONVULSIONS AND CEREBRAL CORTICAL NaK-ATPASE INACTIVATION IN RATS

Kovachich et al (1981) showed that the activity of membrane-bound NaK-ATPase in brain cortical slices removed from rats after exposure to O_2 at 404 kPa (4.0 ATA) for intervals of 10–180 min is decreased significantly for at least 90 min before the onset of convulsions (Fig. 9.4.9). They also obtained preliminary evidence that modification of susceptibility to neurologic oxygen poisoning is associated with parallel changes in the rate of NaK-ATPase inactivation. Elevation of the inspired P_{CO_2} to 8 kPa (60 mmHg) in rats exposed to O_2 at 404 kPa (4.0 ATA) markedly hastens the onset of seizures and is accompanied by an earlier onset and increased magnitude of NaK-ATPase inactivation (Fig. 9.4.9). When rats are exposed to the same O_2–CO_2 atmosphere after 5 days of adaptation to an inspired P_{CO_2} of 8 kPa (60 mmHg), convulsions are delayed and the associated depression of NaK-ATPase activity is also ameliorated. These results are consistent with a gross increase in brain oxygen pressure caused by cerebral vasodilatation during exposure to hyperoxia with acute hypercapnia (Lambertsen et al 1955) and with partial reversal of this response following adaptation to chronic

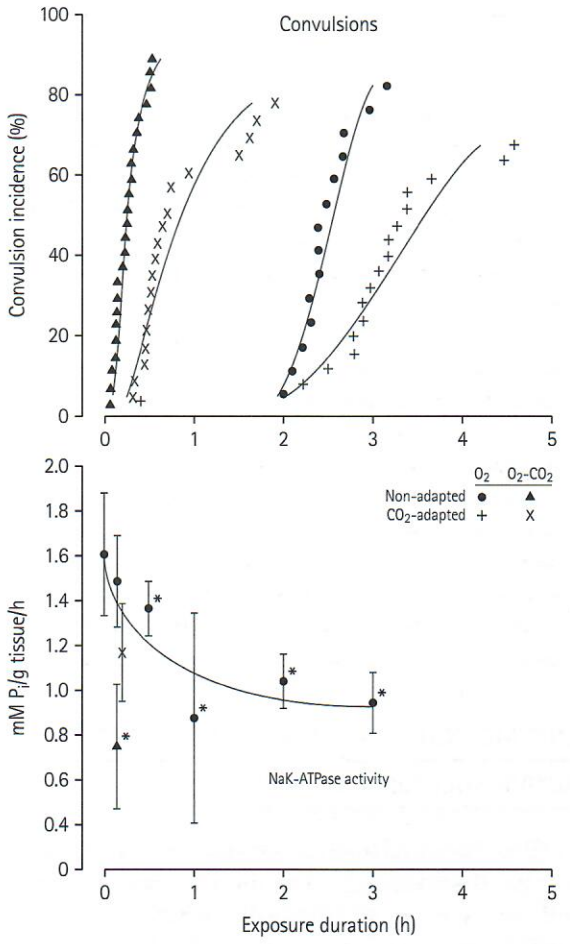

Fig. 9.4.9 Correlation of convulsion incidence and inactivation of cerebral cortical NaK-ATPase in rats exposed to O_2 and O_2–CO_2 at 404 kPa (4.0 ATA). Normal and CO_2 adapted rats were exposed at 404 kPa (4.0 ATA) to 100% O_2 and O_2–CO_2 ($P_{I_{CO2}}$ 8 kPa (60 mmHg)). Symbols for each of the four groups are shown on the graph. Each data point on the upper half represents the convulsion time of one rat. The curves are probability-log transformations of the data. Data points on the lower half are averages of NaK-ATPase activity in cortical slices removed from four to six rats after exposure to the conditions indicated by the symbol for durations shown on the graph. The brackets represent one standard deviation, and the asterisk indicates a statistically significant difference from control measurements in unexposed rats on the same day. The control point on the graph is an overall average of the individual controls. The curve was drawn by eye through the data for rats exposed to 100% O_2. The three points shown on the lower half for 10 min exposures to the indicated conditions have the same relative order as the corresponding convulsion incidence curves shown above. (Data shown in the bottom of the figure are from Kovachich et al 1981, and curves in the top half are from Clark 1981)

hypercapnia (Raichle & Stone 1971/72, Stone et al 1974).

Kovachich & Haugaard (1981) pointed out that membrane transport inactivation in brain cells could lead to extracellular accumulations of potassium and glutamate. Both effects could increase neuronal excitability and ultimately precipitate convulsions, because potassium is a depolarizing agent, and glutamate is thought to be an excitatory neurotransmitter.

HYPEROXIC DEPRESSION OF PULMONARY CAPILLARY ENDOTHELIAL CELL FUNCTION

The pulmonary capillary endothelial cell has been identified as an early and critical target cell in animals exposed to O_2 at 101 kPa (1.0 ATA); (Block 1988, Crapo 1986, Crapo et al 1980, Kistler et al 1967). Initial cytoplasmic changes are followed by fragmentation and cell destruction (Crapo 1986). Capillary endothelial cell damage has been cited as the cause of the increased microvascular permeability to protein and fluid that occurs during multiday normobaric oxygen exposures (Block 1988, Erdmann et al 1983, Hansen-Flaschen et al 1986, Matalon et al 1982, Newman et al 1983, Valimaki et al 1974, Wolfe et al 1974).

By means of plasma membrane transport processes or enzymic activity, pulmonary endothelial cells remove from the circulation and metabolize a variety of biologically active materials, including 5-hydroxytryptamine (5-HT), norepinephrine (NE) and angiotensin-converting enzyme (ACE) (Block 1988, Fishman & Pietra 1974, Ryan & Ryan 1977). Endothelial cell uptake of 5-HT by a carrier mediated, sodium dependent active transport process was significantly inhibited before the development of ultrastructural changes in rabbits (Dobuler et al 1982) and in cultured bovine endothelial cells (Block & Stalcup 1981, Block et al 1985) exposed to O_2 at 101 kPa (1.0 ATA). Endothelial cell uptake of 5-HT was also inhibited in isolated perfused lungs removed from rats exposed to O_2 at 101 kPa (1.0 ATA) for up to 48 h or at 404 kPa (4.0 ATA) for 1 h (Block & Fisher 1977a,b).

Uptake of both 5-HT and NE was depressed in isolated perfused lungs removed from rats exposed to O_2 at 101 kPa (1.0 ATA), (Block & Cannon 1978b). Both of these changes were significant within 12–24 h of exposure, and thus occurred long before the appearance of structural damage or symptoms of pulmonary oxygen poisoning. In contrast, clearance of imipramine by the isolated lung was not affected in rats that breathed O_2 at 101 kPa (1.0 ATA) for up to 48 h (Block & Cannon 1978a,b). These results are consistent with the active transport of 5-HT and NE by the pulmonary capillary endothelial cell, and with clearance of imipramine by passive binding (Fisher et al 1980).

The effect of hyperoxia on 5-HT clearance was found to be enhanced in lungs from vitamin E deficient rats (Block & Fisher 1977a,b). After only 12 h of oxygen breathing at 101 kPa (1.0 ATA), there was a 45% decrement in uptake of 5-HT. In rats exposed to O_2 at 404 kPa (4.0 ATA), 5-HT clearance was reduced by 30% after 45 min and by 45% after 60 min. Delayed reversal of the oxygen induced depression of 5-HT uptake in vitamin E deficient rats was indicated by a 30% decrement found 24 h after a 1 h exposure to 404 kPa (4.0 ATA) O_2.

Block (1978) has studied the effect of inspired oxygen pressure on recovery of 5-HT clearance in isolated perfused lungs removed from rats after varying periods of recovery from a 1 h exposure to O_2 at 404 kPa (4.0 ATA). At 1 h postexposure, 5-HT clearance was markedly depressed in the absence of structural lung damage. Clearance of 5-HT returned to the pre-exposure value within 4 h postexposure in rats that breathed air or 30% O_2, while 5-HT clearance was still significantly reduced at 24 h postexposure in rats that breathed 70% O_2 at 101 kPa (1.0 ATA) or air at 353 kPa (3.5 ATA).

The possible mechanisms that have been proposed to explain the observed hyperoxic depression of 5-HT clearance by pulmonary endothelial cells include: inhibition of the cellular energy sources needed for active transmembrane transport; inactivation of NaK-ATPase, which provides the required transmembrane sodium gradient; and alteration of the lipid environment in which the membrane carrier functions (Block et al 1986). Cultured bovine endothelial cells were used to evaluate the relative contributions of these possible mechanisms. Exposure to 95% O_2 and 5% CO_2 at 101 kPa (1.0 ATA) for 4–42 h had no effect on adenosine triphosphate (ATP) content or NaK-ATPase activity, but it did significantly decrease fluidity in two distinct lipid domains within the hydrophobic interior of the plasma membrane. The authors concluded that such a decrease in membrane fluidity could limit the movement, accessibility or function of the membrane carrier for 5-HT. Block (1988) later showed that resistance of endothelial cell plasma membranes to hyperoxic injury, as determined by the release of lactate dehydrogenase, could be increased by reducing the degree of unsaturation of constituent fatty acids.

In parallel with the hyperoxic depression of 5-HT clearance by pulmonary endothelial cells, plasma membrane activity of angiotensin-converting enzyme is also reduced by oxygen exposure at 101 kPa (1.0 ATA) in

intact rabbits (Dobuler et al 1982), isolated perfused rat lungs (Oparil et al 1987, Toivonen et al 1981) and cultured endothelial cells (Krulewitz & Fanburg 1984) before the appearance of ultrastructural changes. However, pulmonary endothelial ACE activity was not reduced in conscious adult sheep that had gas exchange abnormalities and pulmonary morphologic changes, including capillary endothelial damage, after exposure to O_2 at 101 kPa (1.0 ATA) for 3 days (Howell et al 1988).

EFFECTS ON METABOLISM OF GAMMA AMINOBUTYRIC ACID

Wood & Watson (1963) showed that brain gamma aminobutyric acid (GABA) concentration in rats exposed to O_2 at 606 kPa (6.0 ATA) is decreased significantly before the onset of convulsions and is further progressively decreased after mild and severe convulsions. These changes are rapidly reversible with nearly full recovery within 1 h after the end of the oxygen exposure. Furthermore, convulsions are delayed by intraperitoneal administration of GABA before hyperoxic exposure (Wood et al 1963). Subsequent, related studies showed that the rate of decrease in brain GABA concentration is correlated with susceptibility to oxygen convulsions in mice, hamsters, rabbits, rats and guinea pigs (Wood et al 1967), with exposures of mice to different oxygen pressures (Wood et al 1969) and with exposures of mice to different inspired P_{CO_2} levels at the same oxygen pressure (Wood et al 1969).

Although these results indicate prominent alterations in GABA metabolism during hyperoxic exposure, they do not establish a direct cause-and-effect relationship between a decrease in brain GABA concentration and induction of oxygen convulsions. Changes in brain GABA levels are not always accompanied by appropriate changes in convulsion times. Disulfiram delays the onset of seizures despite a concurrent decrement in brain GABA content (Alderman et al 1974, Faiman et al 1977). Although brain GABA levels are elevated by either amino-oxyacetic acid or hydrazinopropionic acid, these agents hasten the onset of convulsions and increase their frequency and severity (Alderman et al 1974). Hyperoxic effects on brain GABA metabolism may parallel the development of oxygen seizures without a direct causal relationship (Kovachich & Haugaard 1981) or may be related to concurrent alterations in ionic gradients across cell membranes (Radomski & Watson 1973).

It has also been proposed that oxygen effects on GABA metabolism may be part of a broader mechanism for convulsions that involves interacting effects on brain neurotransmitter functions (Colton & Colton 1985, Wood 1975). It is known that GABA is a major inhibitory neurotransmitter in the CNS, while glutamate is a major excitatory neurotransmitter (McGeer et al 1987). The primary source of GABA is decarboxylation of L-glutamic acid by glutamic acid decarboxylase, an enzyme known to be inhibited by hyperoxia (Tunnicliff & Wood 1974, Tunnicliff et al 1973). Glutamine synthetase, the major enzyme responsible for detoxification of glutamate to form glutamine, is also inactivated by oxygen radicals produced either by spontaneous oxidation of 6-hydroxydopamine or by exposure to hyperoxia (Schor 1988). Thus, brain concentrations of glutamate, the excitatory transmitter, are increased at the same time that concentrations of the inhibitory transmitter GABA are decreased. In this context, it is of interest that convulsions in mice exposed to O_2 at 505 kPa (5.0 ATA) were delayed by pre-exposure administration of an excitatory amino acid antagonist (Colton & Colton 1985).

EFFECTS OF OXYGEN POISONING IN MAN

HISTORICAL PERSPECTIVE

Although a comprehensive review of all human studies of oxygen poisoning is beyond the scope of this chapter, a brief historical account will provide perspective for the subsequent detailed descriptions of toxic effects on specific organ systems. In early investigations of human oxygen tolerance, Behnke et al (1935, 1936) measured circulatory, respiratory, and visual responses to oxygen breathing at ambient pressures of 101 to 404 kPa (1.0 to 4.0 ATA) and were the first to observe progressive contraction of visual fields during oxygen breathing at 303 kPa (3.0 ATA). Supporting the initial use of closed circuit oxygen rebreathing systems in military covert operations during World War II (Lambertsen 1947, Larson 1959), Donald (1947, 1992) in the Royal Navy and Yarbrough et al (1947) in the US Navy performed extensive studies of CNS oxygen tolerance in large numbers of subjects. These studies were primarily designed to determine the time courses for symptoms and signs of CNS oxygen poisoning at pressures up to 404 kPa (4.0 ATA) and, if possible, to identify a reliable preconvulsive manifestation of this operational hazard. During the decade after World War II, Lambertsen et al (1953a,b,c, 1955) at the University of Pennsylvania made detailed measurements of oxygen effects at

pressures up to 354 kPa (3.5 ATA) on blood gas transport, cerebral circulation and metabolism, respiration, and respiratory control. These measurements provided a physiologic basis for subsequent therapeutic applications of hyperoxia and initiated a continuing series of oxygen investigations that include both physiologic and toxic effects.

Early studies of human tolerance to pulmonary oxygen toxicity (Becker-Freyseng 1950, Caldwell et al 1966, Comroe et al 1945, Dolezal 1962, Ohlsson 1947) were conducted at oxygen pressures of 52 to 101 kPa (0.51 to 1.0 ATA) for durations ranging up to 110 h (Dolezal 1962). Extensive investigation of the potentially toxic effects of multiday exposures to oxygen pressures of 56 kPa (0.55 ATA) or less (Dubois et al 1963, Hall & Martin 1960, Helvey et al 1962, Michel et al 1960, Morgan et al 1961, Robertson et al 1964, Roth 1963) was stimulated by the selection of 100% O_2 at reduced ambient pressure as the initial cabin atmosphere for the US space program. In a study designed to complement previous investigation of human pulmonary oxygen tolerance, Clark & Lambertsen (1967, 1971b) measured the rate of development of pulmonary oxygen poisoning during O_2 breathing at 202 kPa (2.0 ATA). Results of this study along with available data from other laboratories (Caldwell et al 1966, Ohlsson 1947) were used to derive pulmonary oxygen tolerance curves (Clark & Lambertsen 1970, Lambertsen 1978) for use in therapy and diving. The available pool of human oxygen tolerance data was later expanded significantly by continuous oxygen exposures at 151, 202, 252, and 303 kPa (1.5, 2.0, 2.5, and 3.0 ATA) (Lambertsen et al 1987). In addition to providing the data required for improvement of the original pulmonary tolerance curves (Clark et al 1991b, 1999), these experiments were designed to obtain the information needed to derive equivalent curves for neurologic and other manifestations of oxygen poisoning. The human pulmonary oxygen tolerance database was also expanded by a series of 48 h exposures to air at 505 kPa (5.0 ATA) (106 kPa or 1.05 ATA O_2) (Eckenhoff et al 1987).

The early work of Donald (1947, 1992) and Yarbrough et al (1947) demonstrated that exercise and underwater immersion, separately or together, have adverse effects on CNS oxygen tolerance. After performing an additional series of experiments that incorporated these stresses, Lanphier & Dwyer (1954) established oxygen diving depth-time limits for the US Navy. These limits were later updated on the basis of an extensive series of more than 600 man-dives performed at the US Navy Experimental Diving Unit under simulated operational conditions that included a large number of single and multiple depth profiles (Butler 1986, Butler & Thalmann 1984, 1986). Subsequent experiments at the University of Pennsylvania were designed to complement the observations of symptoms and signs in working divers (Butler & Thalmann 1986) by measuring, under hyperoxic conditions, physiologic responses to exercise (Clark et al 1995a,b) and immersion combined with exercise (Gelfand et al 1994) or hypercapnia (Clark et al 1994).

LATENT PERIOD

During continued exposure to any toxic level of hyperoxia, overt manifestations of oxygen poisoning are preceded by a period in which there are no evident toxic effects (Lambertsen 1978). The duration of this 'latent' period is inversely proportional to the level of inspired P_{O_2} (Fig. 9.4.7). It is now known that there is no truly 'latent' period and that the biochemical effects of oxygen toxicity are initiated concurrently with the elevation of P_{O_2} (Clark & Lambertsen 1971a, Haugaard 1968, Lambertsen 1978). Nevertheless, this initial exposure interval represents an asymptomatic period of slowly developing toxicity from which recovery upon return to normoxia will be rapid and complete (Lambertsen 1978).

The nature, sequence and severity of toxic effects that follow the early asymptomatic period are determined by several interacting factors, including inspired oxygen pressure, duration of exposure, and individual susceptibility to oxygen poisoning. At oxygen pressures of 303 kPa (3.0 ATA) or higher, the tolerable duration of exposure is limited by the development of CNS oxygen poisoning; while at 202 kPa (2.0 ATA) or lower, the effects of pulmonary oxygen toxicity are usually dominant (Clark & Lambertsen 1971a,b, Lambertsen 1978).

SYMPTOMS AND SIGNS OF CNS OXYGEN POISONING

In an effort to identify preconvulsive manifestations of CNS oxygen poisoning, Donald (1947, 1992) exposed normal divers to oxygen pressures of 303 kPa (3.0 ATA) or higher until neurologic effects were experienced. These effects included the diverse symptoms and signs listed in Table 9.4.1. Unfortunately, the sequence of progression did not consistently include minor symptoms before the onset of convulsions. Even when a preconvulsive aura was experienced, it was sometimes followed so rapidly by seizures that it had little practical value as a warning.

Table 9.4.1 Effects of central nervous system oxygen poisoning in normal men. (Adapted from Donald 1947, 1992)

Facial pallor	Unpleasant olfactory sensations
Sweating	Unpleasant gustatory sensations
Bradycardia	Respiratory changes
Choking sensation	panting
Sleepiness	grunting
Depression	hiccoughs
Euphoria	inspiratory predominance
Apprehension	diaphragmatic spasms
Changes of behavior	Severe nausea
fidgeting	Spasmodic vomiting
disinterest	Vertigo
clumsiness	Fibrillation of lips
Visual symptoms	Lip twitching
loss of acuity	Twitching of cheek and nose
dazzle	Palpitations
lateral movement	Epigastric tensions
decrease of intensity	Syncope
constriction of visual field	Convulsions
Acoustic symptoms	
music	
bell ringing	
knocking	

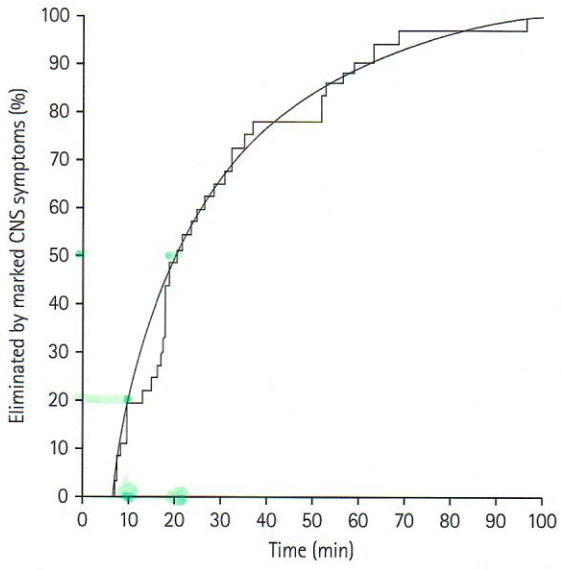

Fig. 9.4.10 Incidence of neurologic effects in 36 divers breathing O_2 at 373 kPa (3.7 ATA, 27 msw, 89 fsw) for intervals of 6–96 min. Each exposure was terminated when the diver experienced one of the neurologic effects listed in Table 9.4.1. (After Donald 1947)

Variations Between and Within Individuals

Using the first neurologic effect to occur as an index of CNS oxygen poisoning, Donald (1947, 1992) found an enormous variation in oxygen tolerance among different subjects exposed to the same conditions. This variability is expressed by the curve showing the incidence of neurologic effects in 36 divers exposed to O_2 at 373 kPa (3.7 ATA, 27 msw, 89 fsw) for durations of 6–96 min (Fig. 9.4.10). Attempts to correlate CNS oxygen tolerance with factors such as age, weight, physical fitness, smoking, alcohol ingestion, psychologic stability, or personality traits were all unsuccessful.

Even in the same diver, there was considerable variability in oxygen tolerance from day to day. The variation in exposure durations required to produce neurologic effects in a diver who breathed O_2 at 313 kPa (3.1 ATA, 21 msw, 69 fsw) once or twice a week over 90 days is shown in Fig. 9.4.11. Using the same data to construct a curve showing the percentage of exposures producing symptoms at specific durations (Fig. 9.4.12) indicates that variability in oxygen tolerance from day to day within the same subject was as great as that within a large group of subjects (Fig. 9.4.10). As with the variability between individuals, the cause of varying susceptibility to CNS oxygen poisoning within the same individual could not be found.

Hyperoxic Seizures

The seizure caused by oxygen toxicity is a generalized tonic-clonic convulsion (Lambertsen 1978). It may occur suddenly without warning or it may be preceded by an aura or a sequence of premonitory sensations. Onset of the convulsion consists of a rigid tonic phase, with abrupt loss of consciousness and powerful extension of the neck and all four extremities. An initial opening of the mouth permits insertion of a padded spacer between the teeth to prevent laceration of the tongue. The tonic phase is followed within about 30 s by a clonic phase which involves powerful repeated contractions of essentially all muscles for about 1 min before ending gradually. An apneic period which persists throughout both the tonic and clonic phases is followed by vigorous hyperventilation which is stimulated by retained carbon dioxide and a metabolic acidosis. If oxygen breathing has been discontinued, return of consciousness within a few minutes after the convulsion is usually followed by 5–30 min of gradual recovery of mental alertness. In contrast to the cerebral hypoxia that occurs during an epileptic seizure, brain hyperoxygenation is maintained during an oxygen convulsion by a high alveolar P_{O_2} in conjunction with marked hypercapnia and increased cerebral blood flow.

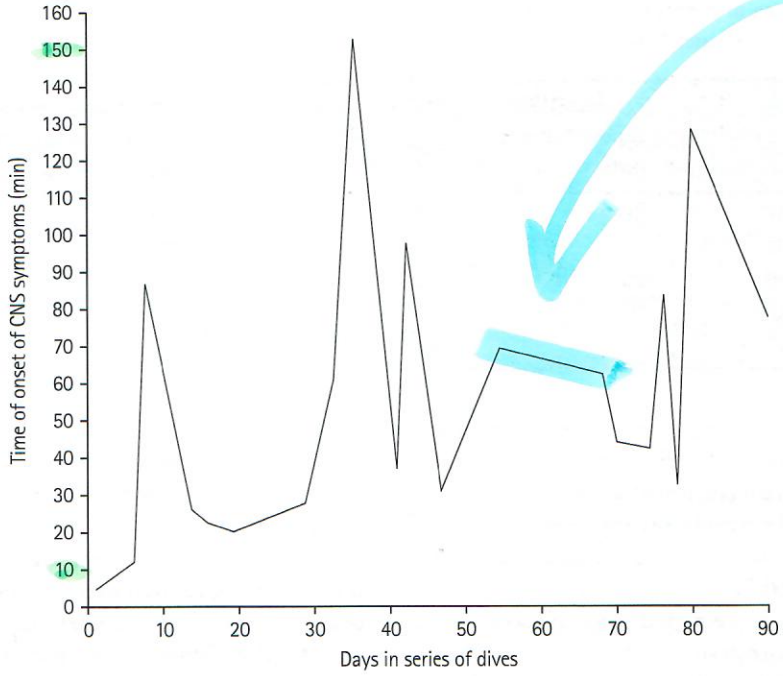

Fig. 9.4.11 Oxygen tolerance variability within the same individual on different days. On 20 different days over a total period of 90 days, the same diver breathed O_2 at 21 msw (70 fsw) until he experienced neurologic symptoms or signs. Exposure durations are indicated on the graph. (From Donald 1947)

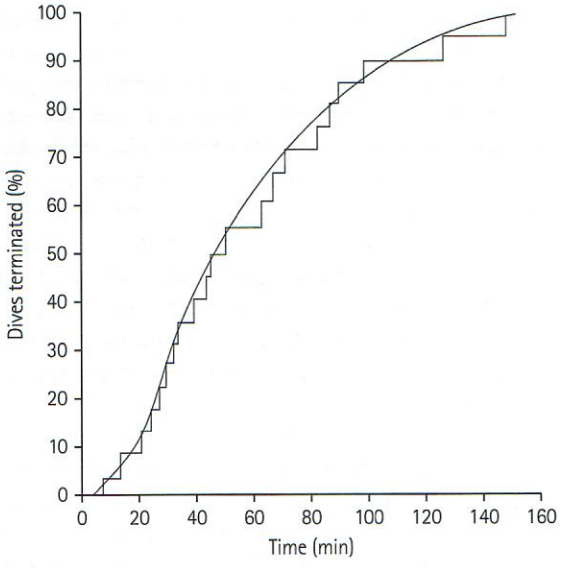

Fig. 9.4.12 Oxygen tolerance variability within the same individual. The data shown in Fig. 9.4.11 are replotted to indicate durations of individual exposures expressed as percentages of the total number of exposures. (From Donald 1947)

It has been shown repeatedly that, apart from the associated hazards of physical injury or drowning, a single oxygen convulsion does not produce harmful or residual effects (Donald 1947, Lambertsen 1978, Yarbrough et al 1947).

The Off-Oxygen Effect

Marked exacerbation of neurologic symptoms, including the occurrence of convulsions, has been observed within the first few minutes of air breathing after termination of hyperoxic exposure (Donald 1947, Lambertsen 1978, Young 1971). In a diver breathing oxygen at increased ambient pressures for therapy or decompression, this phenomenon creates a unique hazard in that an oxygen induced seizure with its concomitant period of breath-holding may begin during decompression. Fatal air embolism could occur in this situation unless decompression is stopped immediately and ambient pressure held constant until normal breathing is resumed.

Changes in Brain Electrical Activity

EEG provides an objective measure of brain electrical activity that has been studied as an index of CNS oxygen poisoning (Cohn & Gersh 1945, Gibbs et al 1935). Early workers found that, while the seizure itself was associated with generalized electrical discharges, gross EEG activity was not consistently altered prior to the actual event. More recently, continuous

Table 9.4.2 Comparison of previous and current single depth exposure limits for US Navy closed circuit oxygen scuba diving. (Adapted from Butler & Thalmann 1986)

Depth msw (fsw)	ATA	Previous USN exposure limits (min)	Current USN exposure limits (min)	Man-exposures within current limits	Toxicity episodes
6 (20)	1.61	110	240	153	12 Probable
9 (30)	1.91	45	80	40	0
10.5 (35)	2.06	25	25	87	1 Definite
12 (40)	2.21	10	15	40	1 Probable
15 (50)	2.52	–	10	58	1 Probable

EEG recording in 18 resting subjects exposed to O_2 at 303 kPa (3.0 ATA) for up to 3.5 h revealed no clinically evident changes in brain cortical electrical activity prior to the occurrence of a hypotensive syncopal episode in one subject and a typical oxygen convulsion in another (Lambertsen et al 1987).

Torbati et al (1981) recorded brain electrocortical activity in awake, unrestrained rats exposed to O_2 at 303, 404, or 505 kPa (3.0, 4.0, or 5.0 ATA). By filtering the record into individual frequency bands and plotting the time integral of rectified voltage against time for each band, they observed significant changes in the activity of individual bands up to 60 min before the appearance of the prominent electrical discharges that consistently preceded the onset of generalized seizures. However, Visser et al (1996) found no similar changes in the spectral analysis of an EEG recorded continuously in a diver who convulsed after breathing oxygen for 30 min at 283 kPa (2.8 ATA, 18 msw, 60 fsw). Neither on-line visual monitoring nor off-line quantitative analysis revealed any consistent differences between the EEG of the diver who convulsed and those of 23 other divers who had no symptoms or signs of CNS oxygen poisoning.

EFFECTS OF EXERCISE ON CNS OXYGEN TOLERANCE

The adverse effects of exercise on CNS oxygen tolerance are well known and are manifested primarily by the occurrence of convulsions at shorter exposure durations or at lower oxygen pressures than those experienced by men at rest (Butler 1986, Butler & Thalmann 1984, 1986, Donald 1947, 1992, Yarbrough et al 1947). Although this phenomenon was thoroughly documented in working divers over 50 years ago, the physiologic basis for the detrimental influence of exercise on CNS oxygen tolerance is still not known. The observation that immersion alone appeared to accelerate CNS oxygen poisoning even when the subject remained at rest (Donald 1947, 1992) raised the possibility that underwater work may decrease CNS oxygen tolerance more than an equivalent degree of exercise in a dry environment.

Recognizing that CNS oxygen toxicity is currently the primary limiting factor in US Navy closed circuit oxygen scuba diving operations, Butler & Thalmann (1984, 1986, Butler 1986) performed a large series of over 600 man-dives to provide an empirical basis for updating the then existing oxygen diving depth-time limits, which were based on work done by Lanphier & Dwyer (1954). Operational conditions were simulated as closely as possible while ensuring subject safety within the controlled experimental environment. Manifestations of CNS oxygen poisoning were classified as convulsion, definite or probable. The definite category included signs or symptoms such as confusion, aphasia, visual disturbances and muscle twitching, while the probable designation referred to symptoms such as nausea, dizziness, numbness, tingling, lightheadedness and poor concentration. The new depth-time limits for closed circuit oxygen scuba diving, which were incorporated into the *US Navy Diving Manual* (1991), are shown in Table 9.4.2 along with the previous limits for comparison and a summary of the experimental basis for the new limits.

In a subsequent series of experiments (Clark et al 1995b), oxygen-exercise effects on a variety of physiologic functions were studied in 10 subjects who performed intermittent exercise while breathing O_2 at 202 kPa (2.0 ATA) in a chamber. Two 30 min periods of moderately heavy exercise were alternated with rest and measurement periods of 30 min during a 120 min oxygen exposure. There were no indications of incipient convulsions or other manifestations of CNS oxygen poisoning. Continuous EEG monitoring at rest and during exercise showed no signs of increased neuronal excitability. These results appeared to be inconsistent with the results of one of the underwater oxygen-exercise profiles studied by Butler & Thalmann

(1986), in which 40 man-dives were carried out at [10.7 msw (34.8 fsw) 208 kPa (2.06 ATA)] with 30 min of exercise at a slightly lower workload. In contrast to the absence of CNS symptoms in the dry environment, four divers developed leg twitching at 25.0–29.5 min of exposure to give a 10% incidence of definite CNS symptoms. Possible reasons for the apparent inconsistency of results obtained under wet and dry conditions have been investigated (Clark et al 1994, Gelfand et al 1994, Lambertsen et al 2000). Measurements of ventilatory and cerebral circulatory responses to progressive hypercapnia (Clark et al 1994) and incremental exercise (Gelfand et al 1994) during partial and total immersion failed to identify a physiologic basis for a detrimental influence.

Despite the absence of CNS symptoms during intermittent exercise at 202kPa (2.0 ATA) in a dry chamber, arterial P_{CO_2} was consistently elevated during the periods of exercise (Clark et al 1995b). In a related series of experiments, performance of four consecutive levels of incremental exercise while breathing O_2 at 202 kPa (2.0 ATA) was associated with a progressive and nearly linear increase in arterial P_{CO_2} (Fig. 9.4.13) (Clark et al 1995b), in contrast to the progressive hypocapnia that usually occurs when incremental exercise is performed during air breathing at 101 kPa (1.0 ATA) (Clark et al 1980). The operational relevance of arterial P_{CO_2} elevation during oxygen breathing is that the associated increase in cerebral blood flow (Kety & Schmidt 1948; Reivich 1969) will deliver a higher oxygen dose to the brain (Lambertsen 1978, Lambertsen et al 1955). The expected relationship of cerebral blood flow to arterial P_{CO_2} during exercise was confirmed by direct measurement of a progressive increase in middle cerebral arterial blood flow velocity during incremental exercise while breathing O_2 at 202 kPa (2.0 ATA) (Clark et al 1995a, Lambertsen et al 2000). Such a mechanism could account for part, if not all, of the observed detrimental influence of exercise on CNS oxygen tolerance. Elevation of arterial P_{CO_2} during hyperoxic exercise was previously observed in men breathing O_2 at 202 kPa (2.0 ATA) (Salzano et al 1967) and in divers breathing a 55% N_2/45% O_2 mixture at 404 kPa (4.0 ATA) (Lanphier 1955; Lanphier & Camporesi 1982).

TOXIC EFFECTS OF OXYGEN ON THE EYE

Ocular manifestations of oxygen poisoning are affected by many variables in addition to inspired P_{O_2} and exposure duration (Nichols & Lambertsen 1969). These include the age of the exposed individual, the method of oxygen administration and the presence of latent or overt conditions that may alter susceptibility to oxygen poisoning. Major influences of each of these conditions are described below.

Retrolental Fibroplasia

This is a unique condition caused by exposure of the premature infant to hyperoxia. Initially, there is constriction of the developing retinal vessels,

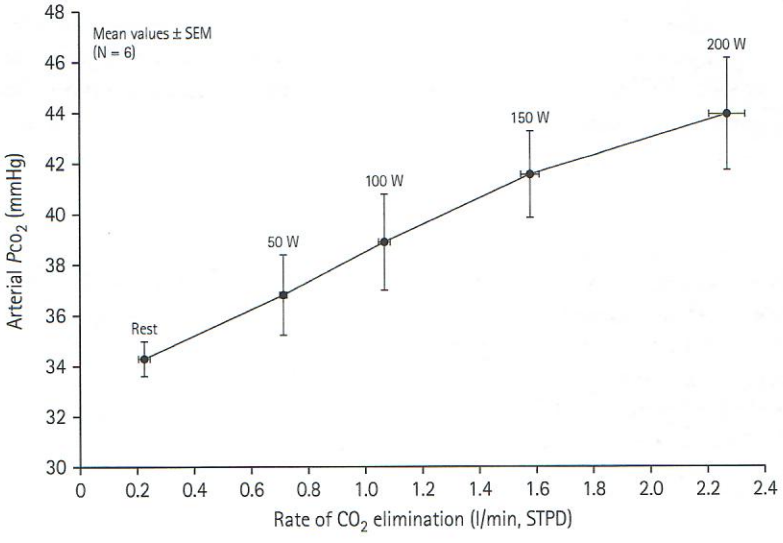

Fig. 9.4.13 Relationship of arterial P_{CO_2} to rate of CO_2 elimination (workload) during incremental exercise while breathing O_2 at 202 kPa (2.0 ATA). Exercise was performed on a bicycle ergometer at the indicated workloads (in watts) with the subject in a semirecumbent position. Average values for six subjects are shown. (From Clark et al 1995b)

followed by endothelial cell destruction and the arrest of retinal vascularization at an incomplete stage of development (Balentine 1982, Nichols & Lambertsen 1969). Later, after removal from hyperoxia, there is a disorganized and profuse vascular proliferation from the remaining endothelial cells. This produces a fibrous mass of vascular tissue which does not grow normally in proportion to the infant's growth, causing irreversible retinal detachment and permanent blindness. The condition can be prevented only by avoiding any elevation of arterial P_{O_2} beyond the normal range for air breathing at atmospheric pressure (James & Lanman 1976).

Effects on Peripheral Vision

Behnke et al (1936) observed a progressive loss of peripheral vision to the point of almost total blindness (tunnel vision) in a man who breathed O_2 at 303 kPa (3.0 ATA) for 3.5 h (Fig. 9.4.14). The change was reversible and recovery was nearly complete within 50 min after resumption of air breathing. Similar observations were later reported by Donald (1947) and by Rosenberg et al (1966). Although subsequent studies in animals demonstrated severe pathologic effects such as visual-cell death, retinal detachment and cytoid body formation (Balentine 1982, Nichols & Lambertsen 1969), little additional information about visual responses to hyperoxia was obtained in man for nearly 50 years after the initial observation of Behnke et al.

The early observation of Behnke et al (1936) was confirmed and considerably extended by a series of experiments, referred to collectively as Predictive Studies V (Clark et al 1987, Gelfand et al 1987, Lambertsen et al 1987, Pisarello et al 1987), in which organ-specific oxygen tolerance was evaluated in man during continuous oxygen exposures at 303, 252, 202 and 151 kPa (3.0, 2.5, 2.0 and 1.5 ATA). Peripheral visual fields were altered most consistently during the 303 kPa (3.0 ATA) exposures (Fig. 9.4.15). The visual field area remained near or above the pre-exposure control value for 2.5–3.0 h, and then decreased progressively until oxygen breathing was stopped at 3.5 h. The average decrement in visual field area at 3.5 h of exposure was 50%, with individual decrements of 74–91% in six subjects (Lambertsen et al 1987). Recovery was complete in all subjects within 45 min

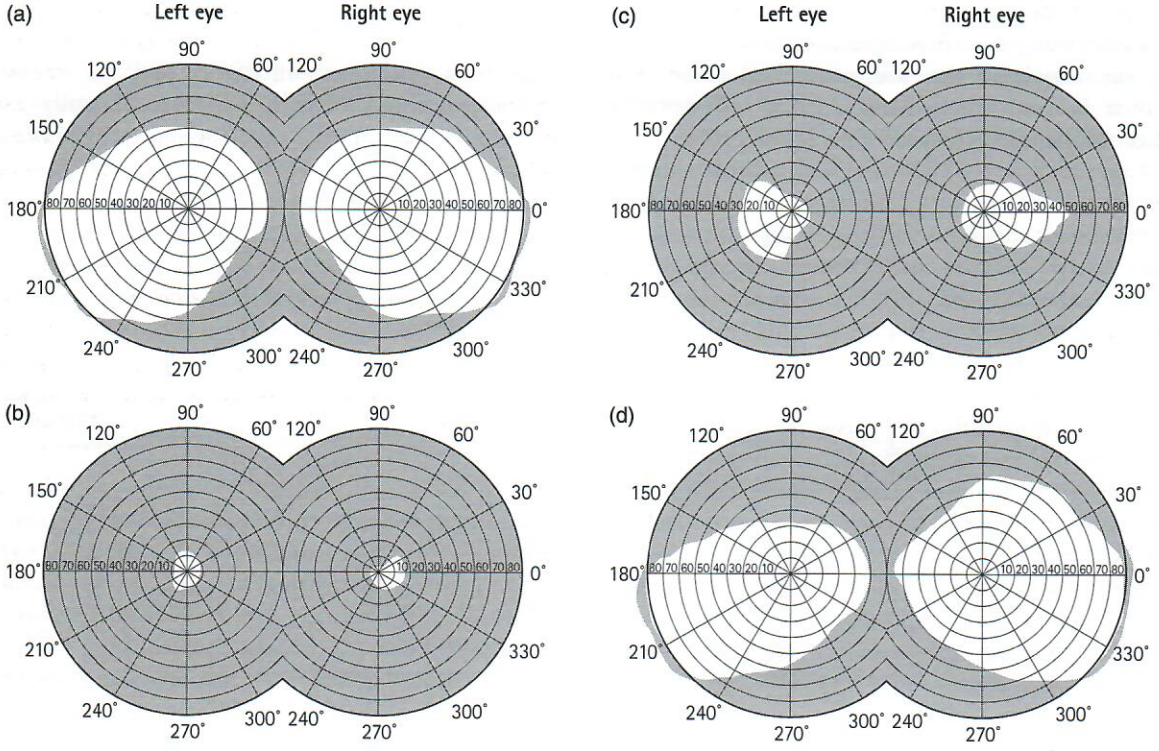

Fig. 9.4.14 Perimetric measurements of visual fields in the same subject before and after 3.5 h of oxygen breathing at 303 kPa (3 ATA). Normal pre-exposure visual fields shown in a. Visual fields shown in b, c and d, respectively, were obtained 5, 25 and 50 min postexposure. (From Behnke et al 1936)

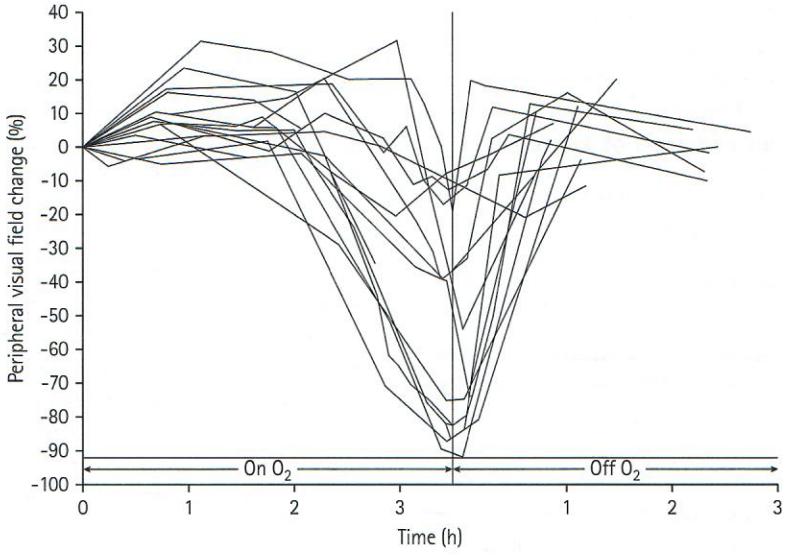

Fig. 9.4.15 Peripheral visual field changes in man during and after continuous oxygen exposure at 303 kPa (3.0 ATA) for 3.5 h. Progressive decrements in peripheral vision developed after a delay and reversed rapidly after termination of oxygen breathing. Associated decrements in electroretinogram b-wave amplitude were less consistent and generally of smaller magnitude. Visual acuity and visual evoked cortical responses were not changed. (From Lambertsen et al 1987)

after resumption of air breathing. Visual acuity and visual evoked cortical responses, both of which are determined primarily by central visual function, were not altered even in the subjects who had the largest decrements in visual field area.

Extreme susceptibility to visual loss during hyperoxic exposure was found in an individual who had recovered many years previously from retrobulbar neuritis in one eye (Nichols et al 1969). Progressive visual field contraction in the affected eye was first noted after about 4 h of oxygen breathing at 202 kPa (2.0 ATA) and was nearly complete within 6 h. Although most of the visual field returned within the first few hours of air breathing, complete recovery required more than 24 h. A similar degree of susceptibility to the visual effects of oxygen toxicity was observed in a diver who experienced progressive loss in peripheral vision, more extensive in the right eye than in the left, during a nearly 1 h period of decompression from 303 to 202 kPa (3.0 to 2.0 ATA) (Zal'tsman et al 1965). Despite continued oxygen breathing for an additional 2 h with gradual decompression to 101 kPa (1.0 ATA), normal visual fields were restored within 40 min after resumption of air breathing.

Effects on Retinal Electrical Activity

Noell (1955, 1962) and Bridges (1966a,b) have shown in rabbits the effects of prolonged exposure to hyperoxia on the electroretinogram (ERG), measured as the electrical response of the dark-adapted retina to a flash of light. Amplitude of the ERG was completely and reversibly suppressed by oxygen exposures ranging from nearly 2 days at 101 kPa (1.0 ATA) to less than 1 h at 707 kPa (7.0 ATA). Recovery of the ERG amplitude did not occur if the oxygen exposure was continued sufficiently beyond the time of complete extinction, presumably to the point of visual cell damage or death (Nichols & Lambertsen 1969, Noell 1962).

Reversible decrements in ERG amplitude have also been observed in normal men during prolonged exposures to oxygen pressures of 303, 252, 202, and 151 kPa (3.0, 2.5, 2.0, and 1.5 ATA); Clark et al 1988, Lambertsen et al 1987). Although it was anticipated that changes in ERG amplitude would correlate directly with concurrent changes in peripheral visual field area, this did not occur. Average decrements in ERG amplitude and visual field area were 13 and 39%, respectively, in 11 subjects who breathed O_2 at 303 kPa (3.0 ATA) for an average duration of 3.4 h (Clark et al 1988). Only the change in visual field area was statistically significant. In another group of 11 subjects, who breathed O_2 at 202 kPa (2.0 ATA) for an average 9.0 h, ERG amplitude and visual field area were significantly reduced by 38 and 9%, respectively. Postexposure recovery of ERG amplitude typically lagged significantly behind the return of visual field area to pre-exposure values. The observed lack of correlation for ERG and

visual field effects of oxygen toxicity may reflect different sites of action or biochemical characteristics of the cells involved (Clark et al 1988).

Hyperoxic Effects on the Lens of the Eye

Progressive myopia is an ocular manifestation of oxygen poisoning which has been observed in some patients receiving daily 90–120 min exposures to O_2 at 202–252 kPa (2.0–2.5 ATA) for a variety of chronic disease states (Anderson & Farmer 1978, Lyne 1978). Refractive changes occurred symmetrically in both eyes at a rate of about 0.5–1.0 diopter of myopia per month and appeared to progress throughout the duration of oxygen therapy. Changes as great as 5.5 diopters occurred in at least one individual (Lyne 1978). After the series of hyperbaric oxygen therapies was completed, recovery was often rapid for the first few weeks and then continued more slowly for periods ranging from several weeks to as long as a year (Fig. 9.4.16). Reversal was complete in most but not all patients (Anderson & Farmer 1978).

Butler et al (1999) published the case report of a 48 year old, closed circuit mixed-gas scuba diver who developed 1.5 diopters of myopia while accumulating 84.8 h of mixed-gas diving at a constant oxygen partial pressure of 131 kPa (1.3 ATA) over a period of 21 days. The myopia was associated with a decrease in intra-ocular pressure from a predive value of 1.9 kPa (14mmHg) to 0.8–1.1 kPa (6–8 mmHg) after the dive series. All changes reversed over a period of about 3 weeks. The myopic shift experienced by the same diver on a subsequent trip was ameliorated by reducing the constant P_{O_2} to 121 kPa (1.2 ATA) and inserting longer periods of normoxia between dives. Three divers on a third trip performed about 45 h of diving over 15 days at a constant P_{O_2} of 131 kPa (1.3 ATA). Using a Snellen eye chart and hand-held trial lenses, a myopic shift of approximately 0.5–1.5 diopters was observed by all three divers.

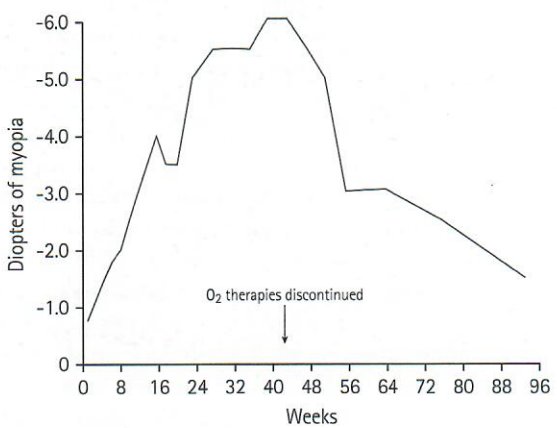

Fig. 9.4.16 Onset and reversal of progressive myopia during and after a 44 week series of oxygen therapies. Each therapy consisted of 1 h of oxygen breathing at 252 kPa (2.5 ATA) preceded and followed by 30 min periods of compression and decompression. One therapy was given each day. (From Lyne 1978)

The cause of the progressive myopia induced by hyperoxia has been attributed to a reversible change in lens shape or metabolism (Anderson & Farmer 1978, Anderson & Shelton 1987, Lyne 1978). Other possible causes of myopia which have been excluded include changes in corneal curvature or intraocular tension (Anderson & Farmer 1978) and change in the axial length of the eye (Anderson & Shelton 1987). Diabetic and elderly patients appear to have a higher incidence of myopia (Lyne 1978). Anderson & Shelton (1987) noted that hyperoxic myopia rarely occurred in non-diabetic patients under the age of 50. The incidence of progressive myopia is likely to be higher in patients who receive oxygen by hood or monoplace chamber than in those who receive it by face mask only, because the oxygen tensions of aqueous and lens tissue are much higher when both the corneal surface and arterial blood are exposed to an increased P_{O_2} (Anderson & Shelton 1987).

The results of animal experiments also indicate that ocular effects of oxygen toxicity may be more severe when the entire eye is exposed to the respired oxygen atmosphere than when hyperoxygenation occurs only via the arterial circulation. Following prolonged but non-lethal whole body exposures of guinea pigs to O_2 at 303 kPa (3.0 ATA), marked histopathologic changes were found in the corneal endothelium and lens epithelium as well as in the plexiform and inner nuclear layers of the retina (Nichols et al 1972). Cataract formation has also been demonstrated in mice 8–12 months after a 6 week period of exposure to O_2 at 303 kPa (3.0 ATA) for 2 h twice a week (Shocket et al 1972).

Accelerated progression of pre-existing nuclear cataracts and formation of new cataracts were observed in a group of 25 patients who received extremely prolonged series of hyperbaric oxygen therapies (Palmquist et al 1984). Daily therapies consisting of 1 h at 202–252 kPa (2.0–2.5 ATA) were given over periods of 2–19 months for totals of 150–850 exposures. All of the patients developed myopia, and all but one had a refractive change of at least 1.0 diopter. The overall mean value of maximal change in refraction was 3.0 diopters. Most patients who received at least 200 exposure hours had a partial reversal of myopia with

continued therapy. Of the 15 patients who had clear lens nuclei at the start of therapy, seven developed a nuclear cataract with reduced visual acuity during the therapy series, and eight of 10 patients who had pre-existing cataracts had an accelerated progression. The degree of myopic change was greatest in the patients who had clear lens nuclei at the start of therapy. Upon termination of therapy, the induced myopia was reversible in most but not all patients, and 11 patients had persistent myopia for at least 6 months. Only two of the patients who developed nuclear cataracts had a partial reversal of lens changes after termination of therapy. Palmquist et al concluded that the development of myopia appeared to be an early reversible manifestation of toxic effect on the lens, while nuclear cataract formation reflected a more severe and less reversible degree of damage.

Early cataract development in a 49 year old woman who received only 48 therapies over an 11 week period was recently reported by Gesell et al (2000). Each therapy consisted of O_2 breathing at 252 kPa (2.5 ATA) for 90 min with two 5 min air breaks. The patient was not diabetic or taking steroids. Cataract formation was associated with a myopic shift which continued to progress for over 4 months after the end of the therapy series, stabilized at 4–6 months post-therapy, and remained constant at 3.25 diopters for 8 and 11 months post-therapy. Her cataracts also stabilized and persisted for the same period.

CLINICAL MANIFESTATIONS OF PULMONARY OXYGEN TOXICITY

Pulmonary Symptoms

The symptoms of pulmonary oxygen poisoning appear to be caused by a tracheobronchitis that starts in the substernal or carinal area and spreads throughout the tracheobronchial tree (Clark & Lambertsen 1971a,b). In subjects breathing oxygen at pressures of 79–89, 101 and 202 kPa (0.78–0.88, 1.0 and 2.0 ATA), symptoms have been experienced as early as 6, 4 and 3 h, respectively. During oxygen breathing at 303 kPa (3.0 ATA), mild symptoms may be experienced within 1 h by sensitive individuals (Clark et al 1991b). Beginning as a mild tickling sensation that is accentuated by inspiration and occasionally induces a cough, the tracheal irritation becomes progressively more intense and widespread, in parallel with more frequent coughing. When extreme, the tracheal symptoms are characterized by a constant burning sensation which is exacerbated by inspiration and accompanied by uncontrollable coughing. The most severe symptoms are associated with dyspnea on exertion or even at rest.

Upon termination of hyperoxic exposure at 202 kPa (2.0 ATA), the intensity of symptoms usually diminishes rapidly within 2–4 h. Complete resolution of pulmonary symptoms occurs over about 1–3 days, although dyspnea on exertion may persist beyond this period. Concurrent upper respiratory tract infection within the next few weeks may cause an apparent recurrence of symptoms.

Physical Findings

Following exposure of normal men to O_2 at 202 kPa (2.0 ATA) until they developed severe symptoms and impairment of pulmonary function, physical examination of the chest was negative (Clark & Lambertsen 1971b). In only a few isolated instances, bubbling rales, fever and hyperemia of the nasal mucosa were found in volunteer subjects after multiday exposures to oxygen pressures of 79–96 kPa (0.78–0.95 ATA) (Clark & Lambertsen 1971a). However, abnormal physical findings including fever, alveolar atelectasis, thick tracheal secretions, coarse rales, rhonchi and bronchial breath sounds were observed in a group of five patients who received prolonged therapy for pulmonary insufficiency (Hyde & Rawson 1969). Although definite association of these abnormalities with pulmonary oxygen poisoning was prohibited by the coexistence of bacterial pneumonia in three of the patients, the original disease process had resolved in the other two at the time of observation.

Radiological Changes

Chest roentgenograms taken before and after the controlled production of pulmonary oxygen intoxication in normal men have not revealed significant differences (Clark & Lambertsen 1971a,b). However, much longer exposures of patients to O_2 at 101 kPa (1.0 ATA) have been associated with characteristic radiologic changes that developed during the course of oxygen therapy and cleared when inspired P_{O_2} was reduced below 86 kPa (0.85 ATA) (Hyde & Rawson 1969). These lesions first appeared as diffuse, bilateral pulmonary densities which then extended and coalesced to involve almost the entire lung with dense, bilateral opacification in the most severe cases.

EFFECTS OF OXYGEN TOXICITY ON PULMONARY FUNCTION

Measurements of pulmonary function in normal men exposed to oxygen pressures ranging up to 303 kPa (3.0 ATA) are summarized in Table 9.4.3. Significant

Table 9.4.3 Effects of hyperoxia on pulmonary function in normal men.

Ambient pressure (ATA)	Oxygen partial pressure (kPa)	Exposure duration (h)	Number of subjects	Indices of pulmonary oxygen toxicity	References
0.25	23	408	8	VC, FEV, Ca_{O2}, $Capa_{O2}$, chest radiograph	Morgan et al (1963b)
0.25	23	408	2	VC, FEV_1, %FEV, MBC, V_T	Morgan et al (1961)
0.26	24	120	2	VC	Roth (1963)
0.26	24	72	1	VC, FEV, ERV, IC, MBC, V_E, $\dot{V}_T$, chest radiograph	Hall & Martin (1960), Roth (1963)
0.26	25	336	6	VC, TLV, MBC, D_{LCO}, Pa_{O2}, chest radiograph	Helvey et al (1962)
0.31	26	72	2	VC, $\dot{V}_E$, P_{ACO2}, Ca_{CO2}, chest radiograph	Becker-Freysing (1950), Roth (1963)
1.0	30	45	4	^{99m}Tc-DTPA $T^1/_2$, BAL albumin ↑, BAL cells	Griffith et al (1986)
0.92	31	720	4	FEV, RV, MBC, D_{LCO}, Pa_{O2}	Robertson et al (1964)
0.34	32	336	3	VC, ERV, IC, FRC, RV, MBC, R_L, D_{LCO}, C_L, Raw, $\dot{V}_E$, Pa_{O2}, Pa_{CO2}, single-breath distribution, chest radiograph	Dubois et al (1963)
0.34	32	336	4	VC, P_{AO2}, Pa_{O2}, P_{ACO2}, Pa_{CO2}, chest radiograph	Morgan et al (1963a)
0.34	33	336	6	VC, TLV, MBC, D_{LCO}, Pa_{O2}, chest radiograph	Helvey et al (1962)
0.34	33	720	4	FEV, RV, MBC, D_{LCO}, P_{AO2}, Pa_{O2}	Robertson et al (1964)
1.0	40	45	4	^{99m}Tc-DTPA $T^1/_2$, BAL albumin ↑, BAL cells	Griffith et al (1986)
1.0	40	17	6	^{99m}Tc-DTPA $T^1/_2$, VC, TLV, FEV_1, D_{LCO}	Montgomery et al (1989)
1.0	45	168	2	V_E, Pa_{CO2}	Richards & Barach (1934)
0.50	49	336	6	VC, TLV, MBC, D_{LCO}, Pa_{O2}, chest radiograph	Helvey et al (1962)
0.50	49	24	6	VC, *f*	Comroe et al (1945)
1.0	50	45	6	^{99m}Tc-DTPA $T^1/_2$ ↓, BAL albumin ↑, BAL cells	Griffith et al (1986)
1.0	51	24	10	VC, *f*	Comroe et al (1945)
0.69	56	168	6	VC, *f*, chest radiograph	Michel et al (1960)
1.0	76	24	9	VC ↓, *f*	Comroe et al (1945)
1.0	79–89	53–57	6	VC ↓, *f*, chest radiograph	Ohlsson (1947)
1.0	91	65	2	VC ↓, P_{ACO2}	Becker-Freysing (1950)
1.0	96	42–110	12	VC ↓, $\dot{V}_E$ ↑, *f* ↑, pHa ↑, Pa_{O2} ↑, *Vt*, O_2 sat. $_a$, Pa_{CO2}, Ca_{CO2}	Dolezal (1962)
1.0	96	17	14	BAL albumin ↑, BAL cells	Davis et al (1983)

The symbols used in this table are defined in Table 9.4.4. Arrows indicate statistically significant changes and direction of change. (Modified from Clark & Lambertsen 1971a)

Table 9.4.3 (*Cont'd*)

Ambient pressure (ATA)	Oxygen partial pressure (kPa)	Exposure duration (h)	Number of subjects	Indices of pulmonary oxygen toxicity	References
1.0	99	24	34	VC ↓, f, O_2 sat.$_a$, Pa_{O2}, $\dot{V}_E$, chest radiograph	Comroe et al (1945)
1.0	99	30–74	4	VC ↓, TLV ↓, D_{LCO} ↓, D_M ↓, Q_L ↓, FEV, RV, V_C, P_{AO2}, Pa_{O2}, Pa_{CO2}, pHa, chest radiograph	Caldwell et al (1966)
1.0	101	17	6	^{99m}Tc-DTPA $T^1/_2$, VC, TLV, FEV_1, D_{LCO}	Montgomery et al (1989)
5.0	106	48	12	VC ↓, PEFR ↓, D_{LCO} ↓, PIFR, MEP, MIP, P_{AO2}, Pa_{O2}	Eckenhoff et al (1987)
1.5	151	17–19	9	Selected indices in Table 9.4.4	Clark et al (1987, 1999)
2.0	202	6–12	13	VC ↓, IC ↓, ERV ↑, FIV ↓, % FIV ↓, MMIF ↓, f ↑, Q_L ↓, D_{LCO} ↓, V_C ↓, pHa ↓, Ca_{O2} ↑, $Capa_{O2}$ ↑, FRC, RV, FEV, % FEV, MMEF, R_{aw}, R_L, V_t, Q_C, D_M, P_{AO2}, Pa_{O2}, Pa_{CO2}, Ca_{CO2}, chest radiograph	Clark & Lambertsen (1971b), Fisher et al (1968) Puy et al (1968)
2.0	202	11	1	FRC ↑, V_t ↓, f ↑, VC, Q_L	Burger & Mead (1969)
2.0	202	6–12	15	Selected indices in Table 9.4.4	Clark et al (1987, 1999)
2.5	252	5–6	8	Selected indices in Table 9.4.4	Clark et al (1987, 1999)
3.0	303	3–3.5	13	Selected indices in Table 9.4.4	Clark et al (1987, 1991b)

The symbols used in this table are defined in Table 9.4.4. Arrows indicate statistically significant changes and direction of change. (Modified from Clark & Lambertsen 1971a)

Table 9.4.4 Symbols used in Table 9.4.3 and their definitions.

BAL	Bronchoalveolar lavage	MIP	Maximal inspiratory pressure
Ca_{CO2}	Arterial carbon dioxide content	MMEF	Maximal flow rate during mid-expiration
Ca_{O2}	Arterial oxygen content	MMIF	Maximal flow rate during mid-inspiration
$Capa_{O2}$	Arterial oxygen capacity	O_2 $sat._a$	Arterial oxygen saturation
C_L	Pulmonary compliance	Pa_{CO2}	Arterial carbon dioxide tension
D_{LCO}	Carbon monoxide diffusing capacity of the lung	P_{ACO2}	Alveolar carbon dioxide tension
D_M	Diffusing capacity of the pulmonary alveolar membrane	Pa_{O2}	Arterial oxygen tension
		P_{AO2}	Alveolar oxygen tension
ERV	Expiratory reserve volume	PEFR	Peak expiratory flow rate
f	Respiratory rate	pHa	Arterial pH
FEV	Forced expired volume	PIFR	Peak inspiratory flow rate
FEV_1	1 s forced expired volume	Q_c	Pulmonary capillary blood flow
%FEV	Percentage of forced expired volume expired in 1 s	R_{aw}	Airway resistance
		R_L	Pulmonary resistance
FIV	Forced inspired volume	RV	Residual volume
FIV_1	1s forced inspired volume	^{99m}Tc-DTPA $T^1/_2$	Half-time for lung clearance of ^{99m}Tc-DTPA
%FIV	Percentage of forced inspired volume inspired in 1 s	TLV	Total lung volume
		V_c	Pulmonary capillary blood volume
FRC	Functional residual capacity	VC	Vital capacity
IC	Inspiratory capacity	$\dot{V}_E$	Expired minute volume
MBC	Maximum breathing capacity	V_t	Pulmonary parenchymal tissue volume
MEP	Maximal expiratory pressure	V_T	Tidal volume

effects and direction of change are indicated. Impairment of pulmonary function was not detected during multiday exposures to oxygen pressures of 50 kPa (0.5 ATA) or less. At pressures of 76 kPa (0.75 ATA) or higher, some aspects of pulmonary function were significantly affected, while others were not. Since studies carried out in normal men are necessarily limited to relatively early, reversible stages of oxygen poisoning, observed functional deficits indicate which parameters are most susceptible to oxidant damage. However, with continued exposure to a toxic inspired P_{O_2}, it is not likely that any index of pulmonary function will remain unaffected at some point in the progression towards severe hypoxemia and death.

The effects of continuous oxygen exposure at 151, 202, 252, and 303 kPa (1.5, 2.0, 2.5, and 3.0 ATA) on selected indices of pulmonary function are summarized in Table 9.4.5 (Clark et al 1991b, 1999). The measurements at 151, 202, and 252 kPa (1.5, 2.0, and 2.5 ATA) were obtained using the same methods in a single laboratory as part of Predictive Studies V (Lambertsen et al 1987), while those at 303 kPa (3.0 ATA) were obtained using similar methods in a nearby hospital laboratory as an early component of the same study (Clark et al 1991b). The results of these measurements are therefore uniquely suitable for comparison of toxic effects on pulmonary function over a range of useful oxygen pressures. Average exposure durations at 151, 202, 252, and 303 kPa (1.5, 2.0, 2.5, and 3.0 ATA) were 17.7, 8.8, 5.7, and 3.4 h, respectively. Overall, the data indicate that patterns of pulmonary function deficits vary with different combinations of oxygen pressure and exposure duration. They also show that lung mechanical function is impaired earlier and more prominently than gas exchange function by continuous oxygen exposure at each pressure (Clark 1988a; Clark et al 1991b, 1999).

Decrease in Vital Capacity

Forced vital capacity (FVC) was significantly decreased by 20–21% at 151 and 202 kPa (1.5 and 2.0 ATA), 12% at 252 kPa (2.5 ATA) and only 3% after partial recovery from exposure at 303 kPa (3.0 ATA). The decrease in vital capacity occurred entirely within the inspiratory component of this lung volume, since expiratory reserve volume was not changed or was increased significantly (Clark & Lambertsen 1971b). The impairment of inspiratory function in early pulmonary oxygen poisoning was also manifested by reductions of the 1 s forced inspired volume, the percentage of the total forced inspired volume that can be inspired in 1 s and the maximum mid-inspiratory flow rate (Clark & Lambertsen 1971b).

Effects on Expiratory Function

Maximal mid-expiratory flow rates (FEF_{25-75}) were reduced only at the three higher pressures with average decrements of about 19%, 31%, and 12% at 202, 252, and 303 kPa (2.0, 2.5, and 3.0 ATA), respectively. The density dependence of mid-expiratory flow rates (%Δ $Vmax_{50}$) was significantly reduced by about 18–20% after oxygen exposures at 151, 202, and 303 kPa (1.5, 2.0, and 3.0 ATA). An average decrement of 20%, which was measured after the 252 kPa (2.5 ATA) exposures, was not statistically significant in eight subjects. Representative HeO_2 and air flow-volume curves obtained in one subject before and after oxygen exposure at 303 kPa (3.0 ATA) for 3.5 h are shown in Fig. 9.4.17. The individual data reflect the observed significant, postexposure reductions in both the density dependence of maximal mid-expiratory flow rates and the peak expiratory flow rate on HeO_2 (Clark et al 1991b). The peak expiratory flow rate on air was not changed.

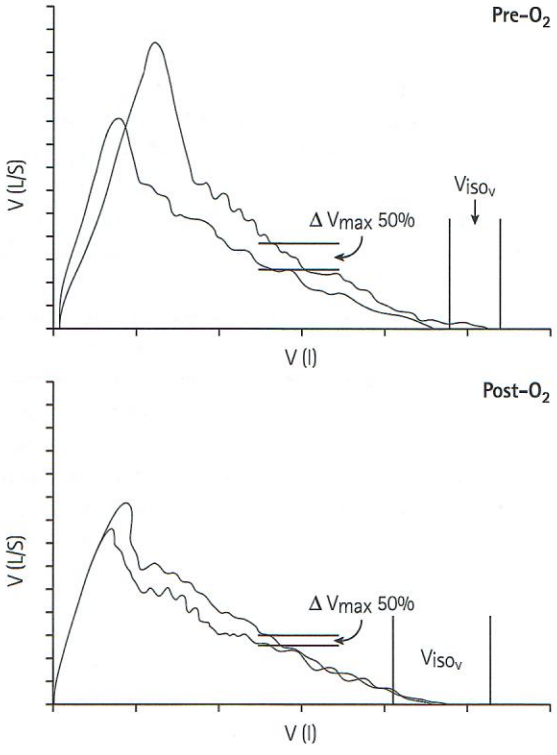

Fig. 9.4.17 Effects of oxygen breathing for 3.5 h at 303 kPa (3.0 ATA) on density dependence of maximum mid-expiratory flow rates and isoflow volume in one representative subject. Maximum expiratory flow curves for air and HeO_2 obtained before and after oxygen exposure are shown as artist's copies of original data. Flow curves for air (lower) and HeO_2 (higher) are superimposed for each measurement period. Postexposure reduction in density dependence of expiratory flow is represented by observed narrowing of the difference in mid-expiratory flow rates. (From Clark et al 1991b)

Variable Patterns and Degrees of Effects on Pulmonary Function

Although pulmonary mechanical function was altered significantly by all four oxygen exposures shown in Table 9.4.5, the patterns and magnitudes of effects varied markedly among different combinations of O_2 pressure and exposure duration. Relative changes in average expiratory and inspiratory lung volumes and flow rates are compared in Fig. 9.4.18 to illustrate some of these differences (Clark et al 1999). At the end of the 151 kPa (1.5 ATA) O_2 exposures, changes in lung expiratory function ranged from essentially no change in FEF_{25-75} (–1%) to a 25% decrement in peak expiratory flow rate (PEFR), while all four indices of lung inspiratory function were uniformly decreased by 22–23%. In contrast, at the end of the O_2 exposures at 202 kPa (2.0 ATA), decrements in three of the four measures of lung inspiratory function were relatively greater than the corresponding measures of expiratory function. A third pattern of effects is reflected by observations in two subjects who had unusually large changes in pulmonary function at 1.4 h after cessation of O_2 breathing at 252 kPa (2.5 ATA). Although average reductions in FVC and forced inspiratory vital capacity (FIVC) were nearly identical for these two individuals, decreases in 1s forced expired volume (FEV_1), PEFR and FEF_{25-75} greatly exceeded the corresponding inspiratory changes. It is likely that the different patterns of effects represent varying combinations of direct and indirect effects of oxygen toxicity.

Effects on Lung Elastic Properties

Average volume–pressure relationships measured at high lung volumes in 14 men before and after breathing O_2 at 202 kPa (2.0 ATA) for an average duration of 8.8 h are shown in Fig. 9.4.19 (Clark et al 1999). They are compared with a curve obtained from similar measurements in normal subjects at many lung volumes ranging from functional residual capacity to total lung capacity (Turner et al 1968). Although average values measured before O_2 exposure are on or near the curve, the postexposure value at a high lung volume is below the curve, reflecting a reduction in lung compliance. Concurrent values in vital capacity and total lung capacity were significantly reduced by 8–9%. Dynamic lung compliance was also significantly decreased by about 30% after 30–48 h of breathing at 99 kPa O_2 (0.98 ATA) (Caldwell et al 1966). Reduction in lung compliance from oxygen toxicity should be distinguished

Table 9.4.5 Effects of continuous oxygen exposure at 151, 202, 252 and 303 kPa (1.5, 2.0, 2.5 and 3.0 ATA) on selected indices of pulmonary function in man. Measured at the end of exposure or during early postexposure period. Values shown are percentage changes from early exposure or pre-exposure control values with the one indicated exception. (Clark et al 1991b, 1999)

	150 kPa (1.5 ATA) Mean ± SD (n)	200 kPa (2.0 ATA) Mean ± SD (n)	250 kPa (2.5 ATA) Mean ± SD (n)	300 kPa (3.0 ATA) Mean ± SD (n)
Duration (h)	17.7 ± 0.8	8.8 ± 1.7	5.7 ± 0.4	3.4 ± 0.3
Measurement Time	Endexposure	Endexposure	Postexposure	Postexposure
FVC	−20.4%* ± 11.6 (9)	−21.0%* ± 14.3 (14)	−11.9%* ± 15.6 (8)	−3.4% ± 5.2 (13)
FEV_1	−14.0%* ± 16.2 (9)	−22.2%* ± 22.0 (14)	−21.7%* ± 29.2 (8)	−6.1%* ± 5.0 (13)
FEF_{25-75}	−1.0% ± 27.0 (9)	−19.2%* ± 32.5 (14)	−30.8%* ± 34.3 (8)	−11.8%* ± 7.5 (13)
$\%\Delta Vmax_{50}$[a]	−19.9%* ± 22.9 (9)	−17.6%* ± 20.7 (8)	−20.4% ± 46.9 (8)	−18.4%* ± 14.5 (5)
$D_{L_{CO}}$	−10.8%* ± 8.5 (9)	−9.7%* ± 4.6 (15)	−7.7%* ± 4.4 (8)	−1.7% ± 9.3 (11)
(A–a) ΔP_{O_2} (mmHg)				
pre-	12.3 ± 5.4 (6)	20.6 ± 6.9 (15)	16.1 ± 2.0 (8)	NM
post-	24.7* ± 8.3 (6)	18.6 ± 6.8 (15)	18.9 ± 4.1 (8)	NM

Note: FVC, forced vital capacity; FEV_1, 1 s forced expired volume; FEF_{25-75}, maximal mid-expiratory flow rate; $\%\ \Delta V_{max\ 50}$, difference in maximal expiratory flow rates on helium–oxygen and air at 50% of forced expired volume expressed as percentage of the air flow rate; $D_{L_{CO}}$, lung carbon monoxide diffusing capacity; (A–a) ΔP_{O_2}, alveolar–arterial oxygen difference on air during exercise; NM, not measured
[a]Values in table expressed as actual change rather than percentage change from early exposure or pre-exposure control
*$P < 0.05$

from that caused by absorptional atelectasis during O_2 breathing at rest, especially at a low lung volume (Burger & Macklem 1968, Burger & Mead 1969). The former requires more than 5 h for complete recovery (Fisher et al 1968), whereas the latter is rapidly reversible by deep inspiration.

Airway Resistance Measurements

The value of airway resistance (R_{aw}), as measured in a body plethysmograph, is determined primarily by larger, more proximal airways which are more than 2 mm in diameter (Grippi et al 1997). There were no significant changes in large airway resistance after any of the oxygen exposures shown in Table 9.4.5 which is consistent with the conclusion that the observed flow limitations were caused by increments in resistance of the smaller, peripheral airways (Clark et al 1991b, 1999). A reduction in density dependence of flow is also consistent with increased small airway resistance (McFadden et al 1974). Despite the absence of detectable increments in large airway resistance in early pulmonary oxygen poisoning, it should be considered that continued exposure to a toxic level of hyperoxia will eventually produce prominent airway obstruction caused by edema formation or other mechanisms.

Effects on Pulmonary Gas Exchange

Pulmonary diffusing capacity for carbon monoxide ($D_{L_{CO}}$) was significantly reduced by about 8–11% after oxygen exposure at 151, 202 and 252 kPa (1.5, 2.0 and 2.5 ATA), but not after the relatively short exposures at 303 kPa (3.0 ATA) (Table 9.4.5). Despite the consistent reduction in $D_{L_{CO}}$, alveolar–arterial oxygen differences during air breathing were increased only after the relatively long exposures at 151 kPa (1.5 ATA), and then only when pulmonary capillary transit time was decreased by an exercise induced increase in cardiac output. Caldwell et al (1966) also observed arterial P_{O_2} reduction during air breathing after prolonged oxygen exposure at 99 kPa (0.98 ATA) when the limitations of transpulmonary diffusion were stressed by exercise. Although fatal hypoxemia is the ultimate outcome of pulmonary oxygen poisoning, significant widening of the alveolar–arterial oxygen difference during either oxygen or air breathing at rest is not an early effect of this condition in man (Caldwell et al 1966, Clark & Lambertsen 1971b, Clark et al 1999).

Measurements of pulmonary capillary blood volume (V_c) and diffusing capacity of the pulmonary alveolar membrane (D_m) after prolonged hyperoxic exposures at 202 and 99 kPa (2.0 and 0.98 ATA) are consistent with variation in the sequence of toxic effects on the alveolar–capillary diffusion barrier at different oxygen pressures and durations of exposure. Oxygen breathing at 202 kPa (2.0 ATA) for 6–11 h caused a 30% decrease in V_c with no significant change in D_m (Puy et al 1968), while 30–74 h of exposure at 99 kPa (0.98 ATA) decreased D_m by 30% without altering V_c (Caldwell et

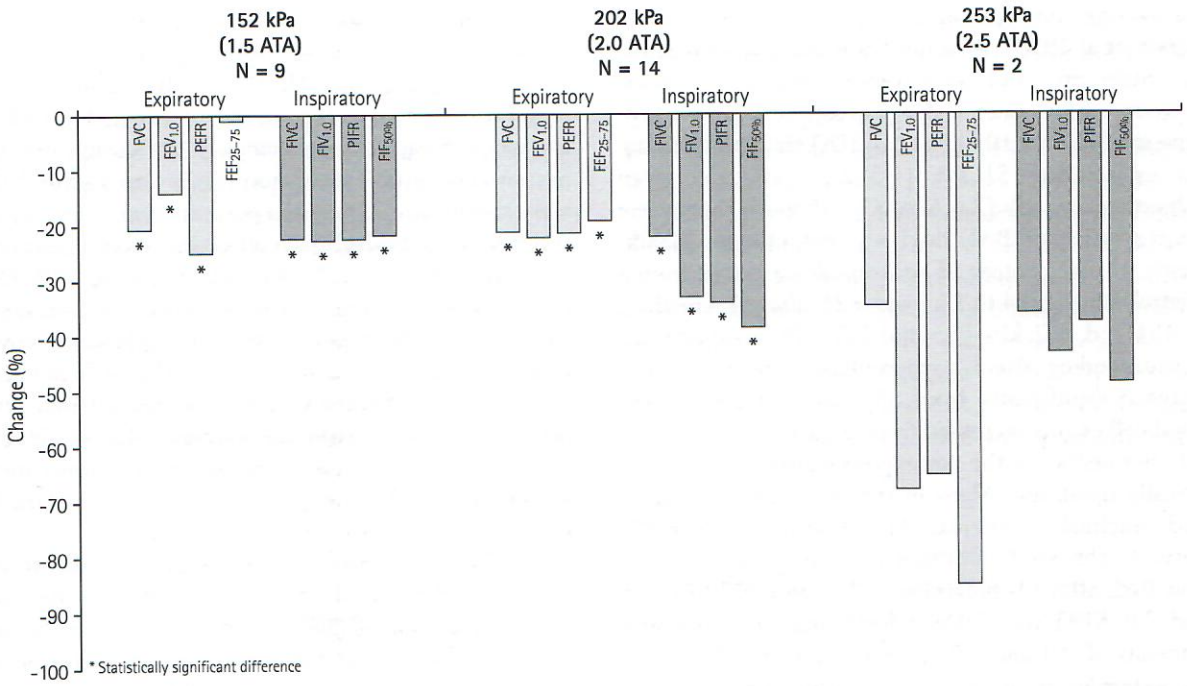

Fig. 9.4.18 Average changes in expiratory and inspiratory lung volumes and flow rates after O_2 exposures at 151, 202 and 252 kPa (1.5, 2.0 and 2.5 ATA). Numbers of subjects at each pressure are indicated. FVC, forced vital capacity; FEV_1, 1s forced expired volume; PEFR, peak expiratory flow rate; FEF_{25-75}, maximal mid-expiratory flow rate; FIVC, forced inspiratory vital capacity; FIV_1, 1s forced inspired volume; PIFR, peak inspiratory flow rate; $FIF_{50\%}$, maximal inspiratory flow rate at 50% of inspired volume. *$p < 0.05$. (From Clark et al 1999)

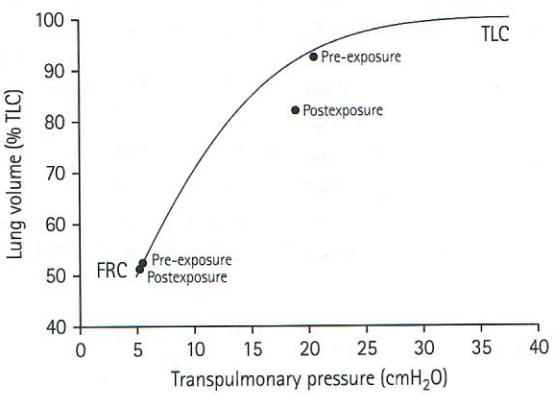

Fig. 9.4.19 Lung compliance before and after O_2 exposure at 202 kPa (2.0 ATA). Curve from data of Turner et al 1968 represents average volume–pressure relationships for 20 subjects between ages of 18 and 33 years. See text for discussion. (From Clark et al 1999)

al 1966). These results are consistent with vasoconstriction and capillary destruction as early events during exposure to very high, acutely toxic oxygen levels and with initial appearance of interstitial edema and increase in alveolar membrane thickness during longer exposures to a lower, less toxic inspired P_{O_2} (Clark & Lambertsen 1971a).

Evidence for Increased Alveolar–Capillary Permeability in Early Pulmonary Oxygen Poisoning

Davis et al (1983) studied the early effects of pulmonary oxygen poisoning on alveolar–capillary permeability by performing bronchoalveolar lavage (BAL) in 14 subjects before and after breathing 96–101 kPa (0.95–1.0 ATA) O_2 for 15.5–18.0 h (mean 16.7 h). Nine subjects experienced mild substernal discomfort during the last few hours of exposure, and six had mild erythema of the airways on bronchoscopic examination. Total recovered volumes of lavage fluid were similar before and after oxygen exposure, but albumin concentration increased significantly by 67% postexposure. The total protein and transferrin concentrations in the lavage fluid were also increased significantly postexposure by 90 and 111%, respectively. Neither the number nor type of inflammatory cells in the lower respiratory tract were changed by oxygen exposure.

Bronchoalveolar lavage has also been performed after breathing O_2 at 151 and 202 kPa (1.5 and 2.0 ATA)

for average durations of 17.6 and 9.7 h, respectively (Clark et al 1999). Because it was not always possible to obtain pre- and postexposure data in the same subjects, results were analyzed as different groups with nine subjects in a 101 kPa (1.0 ATA) air control group, six subjects at 151 kPa (1.5 ATA) O_2, and seven subjects at 202 kPa (2.0 ATA) O_2. Although the protein concentration of BAL fluid was not changed significantly, the percentage of neutrophils increased from a control value of 0.4 to 16.5 and 9.2% after O_2 breathing at 151 and 202 kPa (1.5 and 2.0 ATA), respectively. Corresponding alveolar macrophage proportions also changed significantly from 93.1 to 77.3 and 75.4%. Total cell counts increased from 9.2 to 11.1 and 20.3×10^4, but neither of the postexposure changes were statistically significant. Many of the postexposure subjects had tracheal erythema. An important difference between this study and that of Davis et al (1983) is that BAL after O_2 breathing at 151 and 202 kPa (1.5 and 2.0 ATA) was delayed for average postexposure intervals of 9.4 and 7.3 h, respectively, to allow time for extensive measurements of pulmonary and other physiologic functions, while the subjects studied by Davis et al (1983) had BAL immediately after O_2 exposure at 101 kPa (1.0 ATA).

Using clearance of inhaled technetium-labeled diethylene triamine penta-acetate (^{99m}Tc-DTPA), along with albumin concentration in BAL fluid, to assess lung alveolar–capillary permeability, Griffith et al (1986) exposed groups of four to six subjects to 21, 30, 40 or 50% O_2 for a mean time of 45 h. Several biochemical markers of cell injury in BAL fluid were also measured pre- and postexposure. The albumin concentration was not changed after inhalation of compressed air, but was significantly increased in a dose dependent pattern after exposure to 30, 40 or 50% O_2. The average half-time for lung clearance of ^{99m}Tc-DTPA was significantly reduced only after exposure to 50% O_2. There were no significant changes in total and differential cell counts in BAL fluid or in concentrations of lactate dehydrogenase, alkaline phosphatase, extracellular potassium, and several eicosanoids. The authors noted the apparent inconsistency of their unexpected observation that movement of relatively large albumin molecules (about 70 000 Daltons) into lung alveoli occurred after the 30 and 40% O_2 exposures in the absence of more rapid movement of ^{99m}Tc-DTPA (492 Daltons) in the opposite direction.

In an attempt to detect the earliest manifestations of pulmonary oxygen poisoning, Montgomery et al (1989) monitored pulmonary symptoms and several indices of pulmonary function in six normal subjects who breathed 21, 40 and 100% O_2 for 17 h periods administered in random order at intervals of at least 1 week. Postexposure assessments of pulmonary function included clearance of ^{99m}Tc-DTPA, vital capacity, total lung capacity, forced expiratory volume in 1 s (FEV_1) and single-breath carbon monoxide diffusing capacity. Serum concentrations of fibronectin and Factor VIII were also measured pre- and postexposure as indices of systemic toxicity. Although all of the subjects experienced typical substernal pain while breathing 100% O_2, none of the measured parameters were significantly altered after either the 40 or 100% O_2 exposures. However, an average arterial P_{O_2} of 61 kPa (459 mmHg) measured after breathing 100% O_2 for 20 min was substantially lower than the average value of 82 kPa (619 mmHg) measured previously in 10 normal men during O_2 breathing at 101 kPa (1.0 ATA); (Clark & Lambertsen 1971c).

Despite the apparent inconsistencies in the three studies cited above, the observed results indicate that increased alveolar–capillary permeability may be an early manifestation of pulmonary oxygen poisoning in man that precedes the occurrence of detectable changes in pulmonary mechanical function. Verification of this possibility will require a better understanding of the possible causes and functional significance of an increased albumin concentration in BAL fluid or an increased pulmonary clearance rate of inhaled ^{99m}Tc-DTPA. The results obtained to date indicate that the apparent early increment in alveolar–capillary permeability is readily reversible and not associated with measurable impairment of gas exchange function. However, progressive impairment of pulmonary epithelial and endothelial barrier functions may contribute to the later development and relatively slow reversal of a decreased pulmonary diffusing capacity for carbon monoxide (Clark et al 1999, Hyacinthe et al 1981).

Rate of Development of Pulmonary Oxygen Poisoning

At regular intervals during the continuous oxygen exposures of Predictive Studies V (Lambertsen et al 1987), each subject individually rated chest pain, cough, chest tightness and dyspnea as absent (0), mild (1+), moderate (2+) or severe (3+) (Clark et al 1991b, 1999). The average ratings of all four symptoms were combined for each subject group to derive overall 'pulmonary symptom' ratings and were plotted against exposure duration at 151, 202, 252, and 303 kPa (1.5, 2.0, 2.5, and 3.0 ATA; Fig. 9.4.20). Smooth curves drawn through the average symptom ratings show that rates of symptom development increased progressively at higher levels of inspired P_{O_2}. The curves

also show that pulmonary symptoms were moderately intense on the average by the end of the longer oxygen exposures at 151 and 202 kPa (1.5 and 2.0 ATA), but they were generally mild immediately prior to exposure termination at 252 and 303 kPa (2.5 and 3.0 ATA).

Concurrently with the periodic assessment of symptoms, the rates of development of pulmonary oxygen poisoning during continuous exposures at 151, 202 and 252 kPa (1.5, 2.0, and 2.5 ATA) were monitored by repeated performance of flow–volume maneuvers and spirometry (Clark et al 1999). Pulmonary function was evaluated objectively only before and after the 3.5 h exposures at 303 kPa (3.0 ATA); (Clark et al 1991b). Of the many lung volumes and flow rates that were measured, vital capacity was selected for objective comparison of toxic effects at 151, 202, and 252 kPa (1.5, 2.0, and 2.5 ATA), because it decreased progressively and significantly at all three oxygen pressures (Fig. 9.4.20) and because similar effects were observed in earlier studies at lower pressures (Table 9.4.3).

The curves in Fig. 9.4.20 were drawn by eye through average data for each pressure. Average rates of decrease in vital capacity were progressively greater at higher oxygen pressures. Comparison of pulmonary symptom and vital capacity curves for each pressure shows that the initial fall in vital capacity consistently preceded the onset of symptoms, and that prominent decrements in vital capacity were associated with mild symptoms.

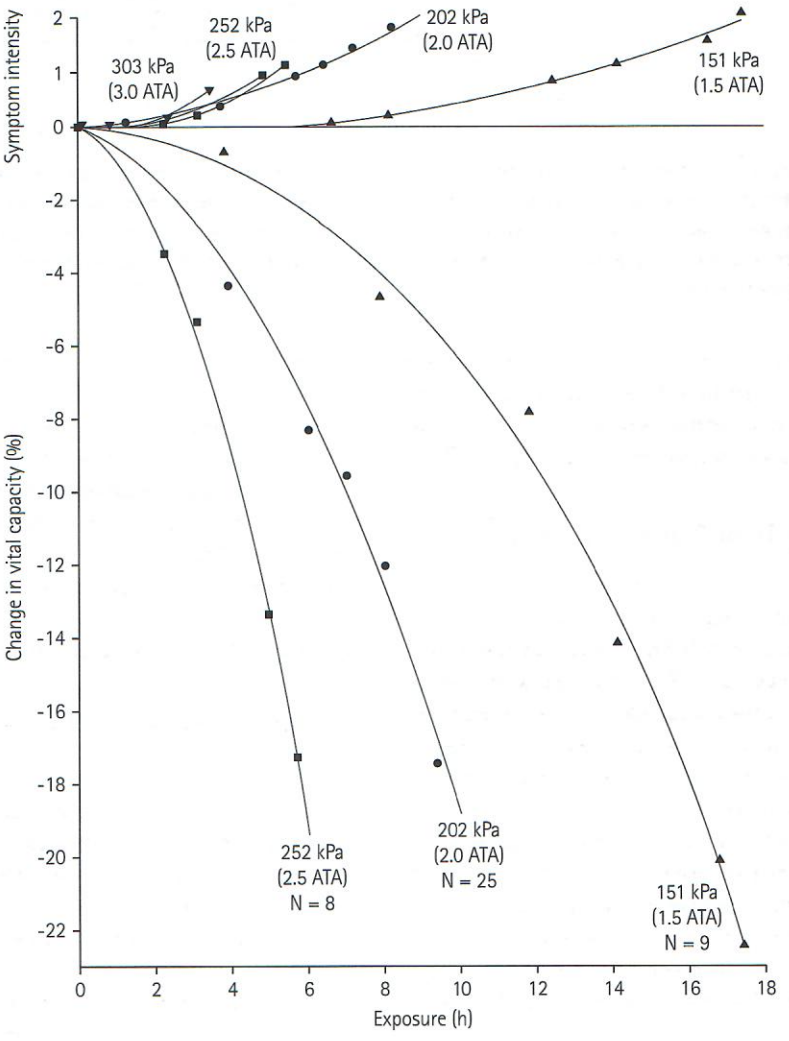

Fig. 9.4.20 Rates of development of pulmonary symptoms and vital capacity decrements in man during continuous exposure to oxygen pressures of 303, 252, 202, and 151 kPa (3.0, 2.5, 2.0, and 1.5 ATA). Average symptom intensities were determined as described in the text. Vital capacity was not measured during oxygen exposure at 303 kPa (3.0 ATA) to allow more time for monitoring CNS functions (Lambertsen et al 1987). Pulmonary function was evaluated 2–4 h after the 303 kPa (3.0 ATA) exposures (Clark et al 1991b). (From Clark et al 1999)

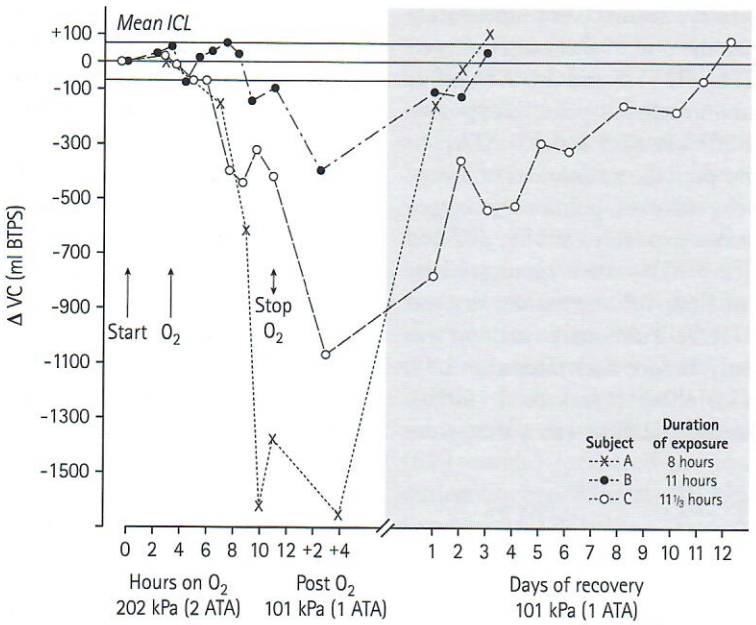

Fig. 9.4.21 Individual variation in rates of development of, and recovery from, pulmonary oxygen poisoning. The solid horizontal line indicates the control value of vital capacity for each subject, and the dashed lines are 95% confidence limits for the subject with the widest limits. The start of oxygen breathing is adjusted on the graph to allow all three exposures to end at the same time. Note that rate of recovery is not necessarily proportional to magnitude of decrement in vital capacity. (Adapted from Clark & Lambertsen 1971b)

As was found with CNS manifestations of oxygen poisoning (Fig. 9.4.10), the rate and magnitude of fall in vital capacity varied markedly among different individuals at the same oxygen pressure (Fig. 9.4.21).

Rate of Recovery from Pulmonary Oxygen Poisoning

Recovery from pulmonary oxygen poisoning is a complex process involving different rates of reversal of diverse toxic effects in different cells and tissues (Lambertsen 1978, 1988). Complete recovery includes reversal of the intracellular biochemical influences of oxygen toxicity along with recovery from tissue reactions to those influences. These components of reversal and recovery are expected to have different time courses, many of which cannot be measured directly. Recovery from functional deficit is likely to occur more rapidly than reversal or repair of structural damage.

The average rates of vital capacity recovery in eight subjects exposed to O_2 at 252 kPa (2.5 ATA), eight subjects exposed at 202 kPa (2.0 ATA) and nine subjects at 151 kPa (1.5 ATA) are shown in Fig. 9.4.22 (Clark 1988a, Clark et al 1999). The average vital capacity increased rapidly in all three subject groups during the first 5 h after exposure termination and returned to the pre-exposure control value within 15–30 h. It is of interest that the rates of vital capacity recovery appear to fall in the same order of magnitude as the preceding rates of decrease in vital capacity during oxygen exposure (Fig. 9.4.20).

The individual variability in the rates of vital capacity recovery for three subjects who breathed oxygen at 202 kPa (2.0 ATA) for 8–11.3 h is shown in Fig. 9.4.21. The subject who was exposed for 11.3 h recovered completely over a period of 11–12 days. A post-exposure recovery period of several weeks was observed in one subject who breathed oxygen for 74 h at 99 kPa (0.98 ATA); Caldwell et al 1966). The period of time required for complete recovery of vital capacity appears to be determined at least as much by the duration of the preceding exposure as by the magnitude of decrement. Oxygen exposures that are prolonged sufficiently to cause edema or other tissue reactions are likely to require longer recovery periods for complete resolution to occur (Clark 1988a).

As stated above, average DL_{CO} was significantly reduced after oxygen exposure at 151, 202, or 252 kPa (1.5, 2.0, or 2.5 ATA), but not after the shorter 303 kPa (3.0 ATA) exposures (Table 9.4.5). The data summarized in Fig. 9.4.23 show that small but statistically significant decrements in DL_{CO} persisted for at least 8–9 days after oxygen exposure at 202 or 252 kPa (2.0 or 2.5 ATA; Clark et al 1999). Average DL_{CO} was reduced

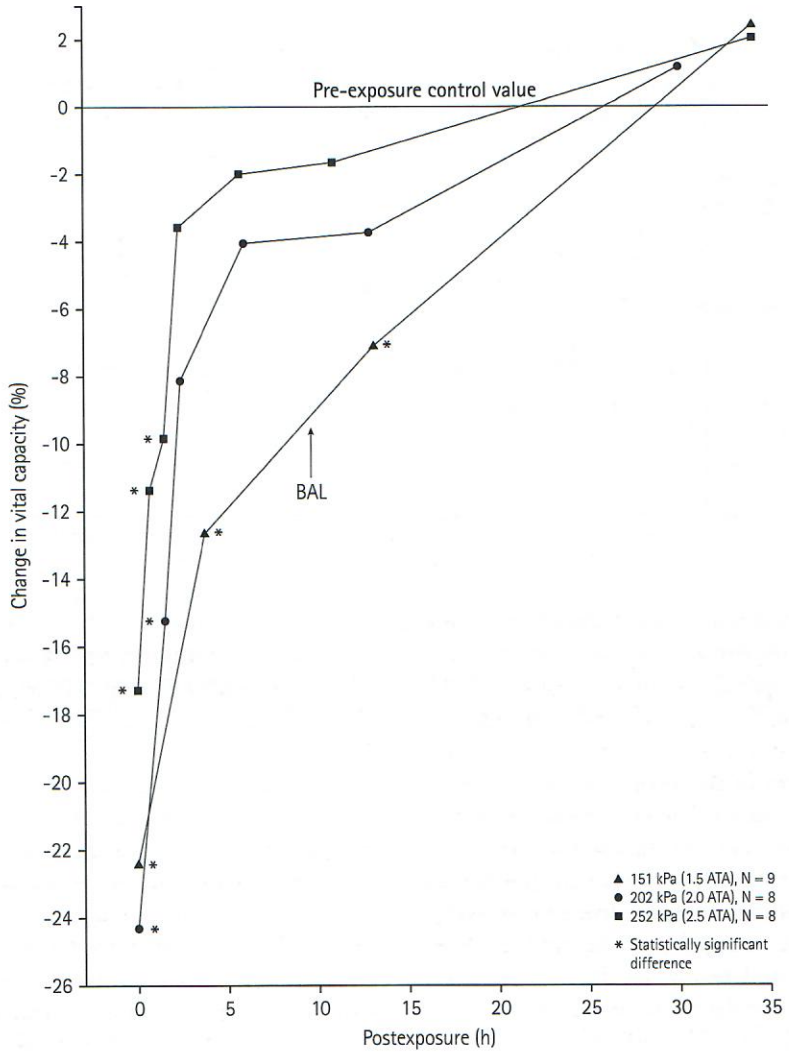

Fig. 9.4.22 Rates of vital capacity recovery after oxygen exposure at 252, 202, and 151 kPa (2.5, 2.0, and 1.5 ATA). Average exposure durations were 5.7 h at 252 kPa (2.5 ATA), 8.4 h at 202 kPa (2.0 ATA) and 17.7 h at 151 kPa (1.5 ATA). Lung volumes and flow rates were measured repeatedly during air breathing at 101 kPa (1.0 ATA) after termination of oxygen exposure. Pre-exposure control values were also measured at 101 kPa (1.0 ATA). Bronchoalveolar lavage (BAL) was performed in six of nine subjects at an average time of 9.4 h after the O_2 exposures at 151 kPa (1.5 ATA). Statistically significant decrements are indicated by an asterisk. (From Clark et al 1999)

significantly only at 13 h after oxygen exposure at 151 kPa (1.5 ATA). Extended follow-up measurements obtained from 2 weeks to 5 months after the oxygen exposures showed that average $D_{L_{CO}}$ values had returned fully or to within 2% of the pre-exposure control values. Slow recovery from oxidant effects on $D_{L_{CO}}$ is also indicated by the observation of Hyacinthe et al (1981) that more than 2 weeks were required for complete reversal of a 13% reduction of $D_{L_{CO}}$ in 10 divers who were intermittently exposed to O_2 at 40–81 kPa (0.4–0.8 ATA) during an 8–9 day period of decompression. Pulmonary diffusing capacity for carbon monoxide appears to be a sensitive index of complete recovery from pulmonary oxygen poisoning.

In addition to providing an indication of complete recovery, reductions in $D_{L_{CO}}$ have been found after multiday exposures to relatively low levels of hyperoxia that have no detectable effects on lung volumes and flow rates (Suzuki 1994, Suzuki et al 1991). In a 320 msw (1049 fsw) saturation dive, six divers were exposed to O_2 partial pressures of 42 and 50 kPa (0.42 and 0.5 ATA) for durations of 5.2 and 11.5 days, respectively (Suzuki et al 1991). Average $D_{L_{CO}}$ per liter of lung volume ($D_{L_{CO}}/VA$) was significantly reduced by

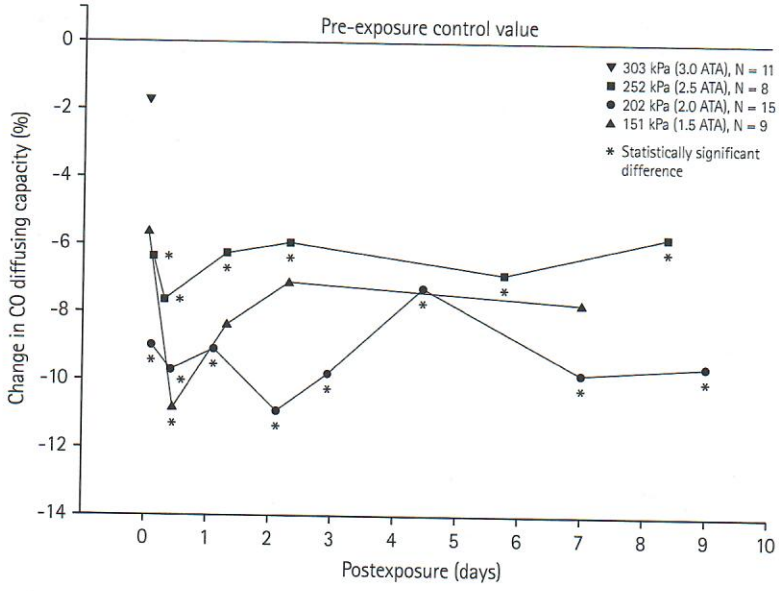

Fig. 9.4.23 Pulmonary diffusing capacity for carbon monoxide in man after oxygen exposure at 303, 252, 202, and 151 kPa (3.0, 2.5, 2.0, and 1.5 ATA). Average changes in carbon monoxide diffusing capacity relative to pre-exposure control values are shown, and statistically significant differences are indicated by an asterisk. Subsequent measurements of diffusing capacity in all subjects who were available for extended follow-up equaled or exceeded the pre-exposure control value. (From Clark et al 1999)

about 15% at the end of the dive and about 12% on postexposure days 4 and 13. The next measurement on postexposure day 48 was not significantly different from the pre-exposure control. In a subsequent 450 msw (1476 fsw) saturation exposure, six divers breathed O_2 partial pressures of 42 and 50 kPa (0.42 and 0.50 ATA) for durations of 10 and nearly 19 days, respectively (Suzuki 1994). Average $D_{L_{CO}}/VA$ reductions at postexposure days 0, 5, 10, 13, 35, and 55 were about 11, 17, 16, 13, 11, and 4%, respectively. The changes at 5, 10, and 13 days were statistically significant.

In an attempt to separate hyperoxia from filtration of venous gas microemboli in the pulmonary circulation as potential causes of $D_{L_{CO}}$ reductions found after decompression from deep saturation dives, Thorsen et al (1993) measured pulmonary function in eight subjects before and after a 28 day saturation dive to a pressure of 0.25 MPa (2.5 ATA). The O_2 partial pressure profile, designed to match that of three previous saturation dives to 3.7 MPa (37 ATA) (Thorsen et al 1990), consisted of 40 kPa (0.40 ATA) for the first 14 days with excursions to 75 kPa (0.74 ATA) for 2 h every second day, 50 kPa (0.50 ATA) for the next 12 days, and a gradual fall to 21 kPa (0.21 ATA) during the last 2 days in decompression. Average $D_{L_{CO}}$ was significantly reduced by 9.8% immediately after the dive, as compared to an 11.4% reduction in 18 subjects after the saturation dives to 3.7 MPa (37 ATA). Average forced midexpiratory flow rate (FEF_{25-75}) was also significantly reduced by 9.8% after the shallow hyperoxic dive, but not after the earlier deep dives. Pulmonary function measurements repeated 6–8 weeks after the shallow dive were not significantly different from predive control measurements (Thorsen et al 1993). However, long term follow-up measurements in the same eight subjects at 1 and 3 years postdive revealed that FEF_{25-75} was significantly reduced by average values of 8.7 and 9.3%, respectively (Thorsen & Kambestad, 1995). Concurrent $D_{L_{CO}}$ values were nearly equal to predive controls. The occurrence of what appear to be quantitatively small, irreversible changes in FEF_{25-75} after an earlier partial recovery is inconsistent with the relatively rapid and complete reversal of much larger changes in FEF_{25-75} found after continuous exposures to O_2 at 202 and 252 kPa (2.0 and 2.5 ATA) (Clark et al 1999). It is also difficult to link a specific deficit in pulmonary function with a presumed causal event that occurred 1 to 3 years previously without conclusively establishing the absence of other potential causes. More early and long term follow-up measurements of recovery from varying degrees of pulmonary oxygen poisoning are needed to reconcile the apparent discrepancies.

There are indications that cumulative effects of pulmonary oxygen toxicity may be produced by daily exposures which individually produce no overt changes in pulmonary function. Thorsen et al (1998) measured $D_{L_{CO}}$ and performed flow–volume loops at weekly

intervals in 20 patients who received hyperbaric oxygen therapy for a variety of non-pulmonary clinical indications. Each therapy consisted of three 30 min periods of O_2 breathing at 240 kPa (2.4 ATA) separated by two 5 min periods of air breathing. Measurements were performed before compression on days 1, 7, 14, and 21 of the therapy series. One day after the 13th therapy, FEV_1 and FEF_{25-75} were significantly reduced by 3.2 and 8.6%, respectively. Corresponding changes after 20 therapies were –4.4 and –10.3%, along with a 4.4% DL_{CO} reduction which reverted to a 3.4% decrease that was not statistically significant when corrected for a concurrent fall in hemoglobin concentration. Surprisingly, FEV_1 and FEF_{25-75} were still significantly reduced by 3.5 and 10.7%, respectively, 4 weeks after the end of the 21 therapy series. Although these changes were not clinically evident, additional measurements are needed to investigate the possible occurrence of cumulative effects from current therapy protocols.

Possible Interaction of Neurologic and Pulmonary Effects of Oxygen Toxicity

Two of eight subjects who breathed O_2 for 5–6 h at 252 kPa (2.5 ATA) had unusually large changes in pulmonary mechanical function with sharp drops in lung volumes and mid-expiratory flow rate during the last 2 h of exposure, continued decline in FEV_1 and FEF_{25-75} during the first postexposure hour, and nearly complete recovery over the next 3–4 h (Fig. 9.4.24; Clark et al 1999). A similar pattern of changes in vital capacity was also observed in one of the subjects previously studied during and after oxygen exposure at 202 kPa (2.0 ATA); Fig. 9.4.21).

The prominent magnitudes of the observed changes in lung volumes and flow rates, as well as their rapid rates of onset and reversal, are consistent with an exacerbation of localized manifestations of pulmonary O_2 poisoning by interaction with concurrent effects of CNS O_2 toxicity. The observed prominent decrements in pulmonary mechanical function could have been caused by vagally induced bronchoconstriction, which is also consistent with the sensation of chest tightness that was experienced by these subjects (Clark et al 1999). Results consistent with augmentation of vagal influences on cardiac function during oxygen breathing at 202, 252 or 303 kPa (2.0, 2.5, or 3.0 ATA) were also found in some individuals (Pisarello et al 1987).

HEMATOLOGICAL EFFECTS OF OXYGEN TOXICITY

Erythrocyte hemolysis has occurred in normal men during or after exposure to oxygen pressures ranging from 34 to 303 kPa (0.34 to 3.0 ATA). Red cell mass declined significantly and continuously in men who breathed 100% O_2 at an ambient pressure of 34 kPa (0.34 ATA) for 30 days (Larkin et al 1972). This effect appeared to be caused by a combination of erythrocyte hemolysis and suppression of erythropoiesis. The progressive decrement in red cell mass was arrested promptly upon return to normoxia, but prolonged recovery from the effects of hyperoxia was indicated by the observation that reticulocyte counts were still increased on day 14 postexposure.

At an ambient pressure of 101 kPa (1.0 ATA), oxygen breathing for only 4 h was associated with a small elevation of plasma hemoglobin concentration, which returned to normal within 24 h (Larkin et al 1970). A group of 44 US Navy diving school candidates who breathed O_2 for 30 min at 282 kPa (2.8 ATA) had small, but statistically significant, reductions in venous blood hematocrit and hemoglobin levels at 1 week postexposure (Bradley & Vorosmarti 1968). Their reticulocyte count was significantly increased at 2 days postexposure. However, similar changes were not found in smaller groups of experienced divers who breathed O_2 at 282 kPa (2.8 ATA) for 30–90 min.

Mengel et al (1964) also found variable hematological responses to hyperoxia in 23 subjects who breathed O_2 at 202–404 kPa (2.0–4.0 ATA) for intervals ranging from about 0.5 to 10 h. Only six of these subjects had accelerated erythrocyte autohemolysis after the hyperoxic exposure. The observation that suppression of erythropoiesis and erythrocyte hemolysis occurred consistently in normal men during multiweek exposures to low levels of hyperoxia (Larkin et al 1972), while more variable responses were found during much shorter exposures to higher oxygen pressures (Mengel et al 1964, Bradley & Vorosmarti 1968), can be explained by the involvement of cell maturation in the hemolytic process which appears to be an accelerated destruction of older erythrocytes (Lambertsen 1978). Structural changes in the cell membrane caused by lipid peroxidation, functional alterations in pathways of intracellular metabolism, and oxidative degradation of hemoglobin may contribute singly or in combination to hyperoxic erythrocyte hemolysis (Kaplan 1967).

Dise et al (1987) pointed out that the erythrocyte provides an ideal system with which to study the effects of hyperoxia on a pathway for membrane phospholipid fatty acid renewal, because it has a single membrane which lacks the capacity for de novo phospholipid and fatty acid synthesis, but has an intact pathway for deacylation and reacylation of endogenous membrane phospholipid in situ. The activity of this pathway could

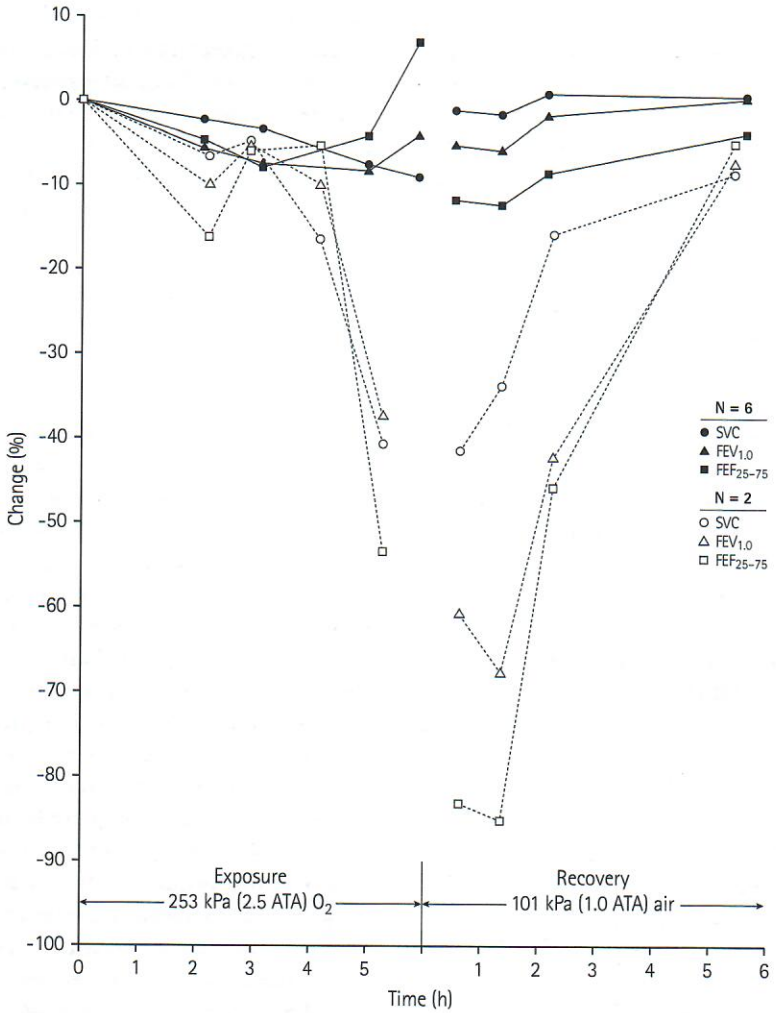

Fig. 9.4.24 Pulmonary function changes during and after O_2 breathing at 252 kPa (2.5 ATA) for 5–6 h. Average changes in slow vital capacity (SVC), one second forced expired volume (FEV_1) and maximal mid-expiratory flow rate (FEF_{25-75}) for two subjects who had unusually large deficits are compared with corresponding values in six other subjects who had much smaller changes. Control measurements at 252 kPa (2.5 ATA) were obtained during early oxygen exposure. Pre-exposure control measurements at 101 kPa (1.0 ATA) were used for the postexposure data. (From Clark et al 1999)

influence the ability of erythrocytes to tolerate hyperoxic exposure by replacement of oxidized fatty acids. Mengel & Kann (1966) found that peroxidation of erythrocyte membrane lipid occurred in vivo concurrently with erythrocyte hemolysis during exposure of vitamin E deficient mice to hyperbaric hyperoxia, but the role of lipid peroxidation in the hemolysis previously observed in human subjects (Mengel et al 1964) was not investigated.

In normal men exposed to O_2 at 303 kPa (3.0 ATA) for 3.5 h, Dise et al (1987) found that incorporation of [9, 10-^{3}H]oleic acid into membrane phospholipid by intact erythrocytes was significantly decreased by about 30% at 1 h postexposure. Partial recovery of activity at 24 h postexposure was established by in vitro assay using additional cells obtained from the same subjects. This reversible decrement in phospholipid fatty acid turnover preceded cell lysis or any detectable change in membrane phospholipid fatty acid composition, and it was accompanied by an increase in the cellular content of reduced glutathione. A similar inhibition of the pathway for acylation of membrane phospholipid in situ was previously found in intact erythrocytes obtained from sheep after prolonged exposure to O_2 at 101 kPa (1.0 ATA; Dise et al 1985).

Table 9.4.6 Probable sites of early functional impairment in oxygen poisoning. (Modified from Lambertsen 1978)

On basis of high tissue Po_2	On basis of exceptional susceptibility
Upper respiratory tract	Central nervous system
Endothelium of pulmonary veins, left heart and systemic arteries	Endocrine organs and tissues
Pulmonary alveoli and capillaries	Special sensory structures including eyes, ears and organs of balance
Arteriolar walls	Secretory glands
Renal glomerular capillaries	Renal tubular system
Carotid bodies	Liver
Choroid plexus	Hematopoietic tissues

TOXIC EFFECTS ON OTHER ORGANS AND TISSUES

Little is known about the effects and rates of development of oxygen poisoning in organs and tissues other than those described above. This paucity of information has not caused serious problems in the past, because practical applications of hyperoxia in diving operations and in therapy have been limited primarily by onset of pulmonary or CNS oxygen poisoning. However, as effective means for extending pulmonary and CNS oxygen tolerance are implemented, it will be necessary to determine the nature and time course of oxygen poisoning in other critical organs and tissues (Lambertsen 1978). Relatively early and severe functional deficits during prolonged oxygen breathing are predictable in organs that are exposed to nearly arterial levels of hyperoxia by virtue of a high blood flow, or in those that contain active transport systems or other functions which are exceptionally susceptible to oxygen toxicity. Several of these potential target sites, including but not limited to those discussed above, are listed in Table 9.4.6. Comprehensive evaluations of specific organ oxygen tolerance in man during continuous oxygen exposures at 151, 202, 252, and 303 kPa (1.5, 2.0, 2.5, and 3.0 ATA) were performed in Predictive Studies V to provide some of the information required for optimal operational and therapeutic applications of hyperoxia (Clark et al 1991b, 1999, Gelfand et al 1987, 1998, Lambertsen et al 1987, Pisarello et al 1987).

DEFINITION OF OXYGEN TOLERANCE IN MAN

In order to avoid the toxic effects of hyperoxia while fully exploiting each of its many potential applications, the rate of development of oxygen poisoning must be defined in man over a range of useful oxygen pressures. As indicated previously, the exposure durations at which a specific manifestation of oxygen poisoning occurs at different oxygen pressures can be described by a rectangular hyperbola (Fig. 9.4.7) (Dickens 1962). This empirical observation was used in conjunction with use of vital capacity change as an index of pulmonary oxygen poisoning to derive predictive curves which provided practical guidelines for applications of hyperoxia in diving and therapy (Clark & Lambertsen 1967, 1971a). Such predictive oxygen tolerance curves have been defined thus far only for the lung. The rationale and empirical basis for derivation of the original pulmonary oxygen tolerance curves from the limited data then available are summarized below.

The available database that is relevant to a definition of human tolerance to pulmonary oxygen toxicity has been greatly expanded since development of the original curves by additional information obtained at oxygen pressures of 303, 252, 202, 151, and 106 kPa (3.0, 2.5, 2.0, 1.5, and 1.05 ATA) (Clark et al 1991b, 1999, Eckenhoff et al 1987). Analysis and integration of the expanded database to provide an improved definition of respiratory–pulmonary oxygen tolerance is ongoing but incomplete (Lambertsen et al 1999). An adequate presentation of the database, tolerance redefinition, and relevant discussion, pending open literature publication, is beyond the scope of this chapter. The concepts that were used in the derivation of the original tolerance curves from a limited database (Bardin & Lambertsen 1970, Clark & Lambertsen 1967, 1970, Wright 1972) have been retained in the analysis and integration of the additional data.

QUANTITATIVE INDICES OF PULMONARY OXYGEN TOLERANCE

The definition of oxygen tolerance in man requires the use of sensitive indices to monitor the onset and rate of progression of a toxic process that will ultimately be lethal. Several measures of pulmonary function were

evaluated for this purpose in normal men who breathed O_2 at 202 kPa (2.0 ATA) until they developed obvious subjective and objective manifestations of pulmonary oxygen poisoning (Clark & Lambertsen 1971b). The pulmonary function indices that were significantly altered at a completely reversible degree of intoxication included vital capacity, inspiratory capacity, expiratory reserve volume, inspiratory flow rate, carbon monoxide diffusing capacity and lung compliance.

Decrease in vital capacity proved to be the best available index for monitoring rate of pulmonary effects degeneration in groups of men. During oxygen breathing at 202 kPa (2.0 ATA), it was reduced significantly at a time when symptoms were barely perceptible and it continued to fall throughout the exposure in association with increasing severity of symptoms (Clark & Lambertsen 1971b). It could be measured quickly and reproducibly in trained subjects. Furthermore, vital capacity measurements in men exposed to hyperoxia by many different investigators (Table 9.4.3) provided data relevant to pulmonary oxygen tolerance over a wide range of oxygen pressures. None of the other measures of lung function that were altered in early pulmonary oxygen poisoning fulfilled all of the above criteria as consistently as decrease in vital capacity.

Additional information (Clark et al 1987, 1991b, 1999) obtained since the original derivation of pulmonary oxygen tolerance curves (Clark & Lambertsen 1967, 1970) has shown that no single measure of pulmonary function is uniquely satisfactory for monitoring either the rate of development of pulmonary oxygen poisoning or the postexposure rate of recovery. For example, pulmonary mechanical function is impaired earlier and more severely than gas exchange function during continuous oxygen exposures over a range of useful pressures (Table 9.4.5), but pulmonary diffusing capacity for carbon monoxide appears thus far to be the most sensitive index of recovery from pulmonary oxygen poisoning (Fig. 9.4.23). Analysis and integration of this information is in final process (Lambertsen et al 1999).

THE UNIT PULMONARY TOXIC DOSE CONCEPT

Many practical applications of hyperoxia involve consecutive exposures to different oxygen pressures. Since oxygen poisoning occurs more rapidly at higher pressures, the same exposure duration does not produce equivalent degrees of intoxication at different levels of hyperoxia. The total dose of oxygen must therefore be described with respect to both the inspired P_{O_2} and the duration of exposure. This description has been facilitated by expressing exposures to different oxygen pressures in terms of an equivalent exposure to a standard reference level of hyperoxia. The 'unit pulmonary toxic dose' (UPTD) concept is designed to express any pulmonary toxic dose in terms of an equivalent exposure to O_2 at 101 kPa (1.0 ATA) (Bardin & Lambersten 1970, Wright 1972).

UPTD calculations are based on vital capacity measurements that describe the rate of development of pulmonary intoxication at oxygen pressures above 50 kPa (0.5 ATA). It is assumed that inspired P_{O_2} exposure duration relationships for specific percentage decrements in vital capacity are rectangular hyperbolae, as found for other manifestations of oxygen poisoning (Fig. 9.4.7) (Dickens 1962). It is further assumed that these hyperbolae describing pulmonary oxygen tolerance in man have vertical and horizontal asymptotes at zero time and 50 kPa (0.5 ATA), respectively.

An asymptote at zero time implies that onset and progression of pulmonary intoxication are immediate at an infinitely high oxygen pressure. The horizontal asymptote at 50 kPa (0.5 ATA) indicates that pulmonary function is not significantly impaired in men breathing oxygen at lower pressures. Its selection was based on the absence of detectable changes in vital capacity during exposures to the inspired P_{O_2}-duration conditions summarized in Fig. 9.4.25. Although the latter assumption cannot be made with certainty for indefinitely long exposures to low levels of hyperoxia, it is valid for at least 14 days at 49 kPa (0.49 ATA; Helvey et al 1962) and 30 days at 32 kPa (0.32 ATA) of O_2 (Robertson et al 1964).

A group of hyperbolic curves describing the time course of pulmonary oxygen poisoning in man at pressures above 50 kPa (0.5 ATA) is shown in Fig. 9.4.26. They were based on the then available measurements of rate of decrease in vital capacity during oxygen breathing at 202 kPa (2.0 ATA; Clark & Lambertsen 1971b), 99 kPa (0.98 ATA; Caldwell et al 1966) and 79–89 kPa (0.78–0.88 ATA; Ohlsson 1947). Linear forms of the same curves on log–log co-ordinates are shown in Fig. 9.4.27. Data points representing the specified vital capacity changes in 50% of the exposed subjects at each of the three pressures are plotted in such a way that asymptotes occur at zero time and 50 kPa (0.5 ATA) inspired P_{O_2}. The oxygen tolerance curves were defined by drawing parallel lines through each set of three data points. Each line represents all of the inspired P_{O_2}-exposure duration combinations that produce the designated change in vital capacity or, stated in other terms, that represent an equivalent number of UPTD units. The reference standard for

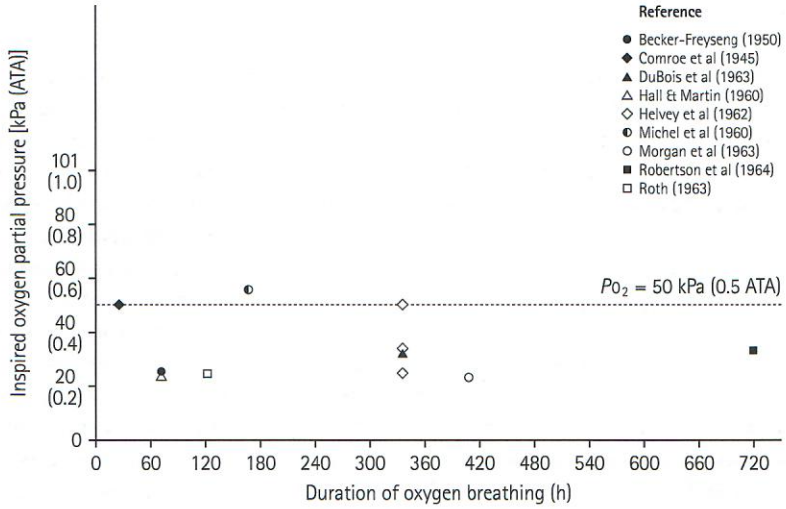

Fig. 9.4.25 Pulmonary oxygen tolerance studies in normal men which detected no objective evidence of pulmonary oxygen poisoning. Each symbol represents the average conditions of a separate study in humans. The numbers of subjects in each study are listed in Table 9.4.3. (From Clark & Lambertsen 1970)

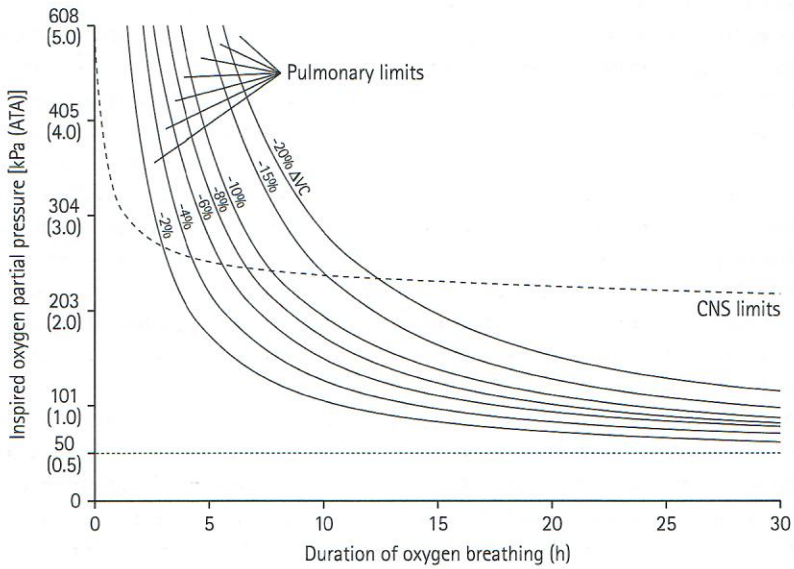

Fig. 9.4.26 Pulmonary and neurologic oxygen tolerance curves for continuous exposures of normal men. The pulmonary limits represent vital capacity changes in 50% of the exposed subjects. The curve defining CNS limits represents a 10% incidence of neurologic symptoms (Table 9.4.1). (Adapted from Lambertsen 1978)

UPTD calculations is duration of oxygen breathing at 101 kPa (1.0 ATA) expressed in minutes.

Applications and Limitations of Oxygen Dose Predictions

The ability to predict the degree of pulmonary effect associated with a planned hyperoxic exposure facilitates the safe and effective use of oxygen in diving, decompression and therapy. For example, a diver should not be permitted to accumulate large oxygen doses during routine decompressions, because repeated exposures to significant degrees of pulmonary intoxication may cause cumulative impairment of lung function. Furthermore, allowance should be made for additional hyperoxic exposure in the event that

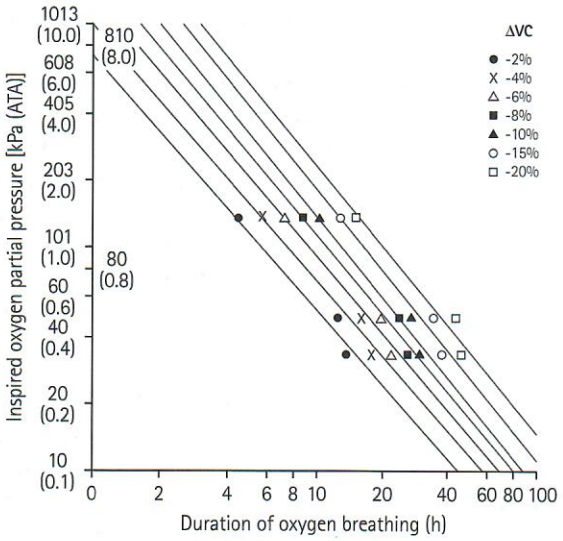

Fig. 9.4.27 Pulmonary oxygen tolerance curves on log–log co-ordinates. The hyperbolic curves shown in Fig. 9.4.26 are linear on logarithmic co-ordinates. Symbols represent the designated vital capacity decrements in 50% of the subjects studied by Clark & Lambertsen (1971b) at 202 kPa (2.0 ATA), Caldwell et al (1966) at 99 kPa (0.98 ATA) and Ohlsson (1947) at 79–89 kPa (0.78–0.88 ATA). See text for additional details. (From Clark & Lambertsen 1970)

hyperbaric oxygen therapy is required for decompression sickness near the end of the decompression period. An oxygen dose equivalent to a 2% decrement in vital capacity would be suitable for such an application. This would probably be associated with only mild symptoms and would be completely reversible after cessation of hyperoxic exposure.

On the other hand, an oxygen dose equivalent to a 10% decrement in vital capacity would be permissible during the treatment of severe decompression sickness, gas embolism or any other serious condition. This effect would also be expected to reverse completely after a recovery period of sufficient duration. Larger oxygen doses which may be associated with residual impairment of pulmonary function are justifiable in the therapy of truly life threatening conditions such as gas gangrene. Complete reversal of rapidly developing, complex vital capacity decrements as great as 40–45% of the control volume have been observed after prolonged oxygen breathing at 202 and 252 kPa (2.0 and 2.5 ATA; Clark & Lambertsen 1971b, Clark et al 1999). However, it would be unwise to assume that all individuals will recover fully from comparable degrees of toxic effect, especially when such effects are produced directly by prolonged oxygen exposures at 202 kPa (2.0 ATA) or lower.

Although the UPTD system has been used widely to provide guidelines for safe hyperoxic exposure in commercial and military diving operations (Edmonds et al 1992, Flynn et al 1981, Lemaire 1981), it still has the limitations that were recognized by its originators (Bardin & Lambertsen 1970, Wright 1972). Vital capacity decrements in any individual (Fig. 9.4.21) may be smaller or larger than the changes in 50% of the subjects on which UPTD predictions were based. A progressive decrease in vital capacity is usually associated with increasing severity of symptoms (Fig. 9.4.20), but some individuals have severe symptoms with small decrements in vital capacity, while others have prominent reductions with minimal symptoms (Clark & Lambertsen 1971b). The possible interactions of neurologic and pulmonary effects of oxygen toxicity in some individuals (Fig. 9.4.24) also deviate from average data.

Even more critical in operational applications of the UPTD concept is the persisting lack of adequate data describing rate of recovery from various degrees of pulmonary oxygen poisoning. An early awareness of this critical limitation and recognition of individual variability in the rate of recovery from pulmonary intoxication (Fig. 9.4.21) prompted a purposefully conservative approach based on the assumption that the pulmonary toxic dose is cumulative, with no recovery between successive hyperoxic exposures (Bardin & Lambertsen 1970, Wright 1972). Although this certainly leads to overestimation of toxic dose (Gardette & Lemaire 1977, Lemaire 1975), such an error is preferable to dangerous overexposure of an individual on the basis of a UPTD prediction that assumes an unrealistically rapid recovery rate.

Recovery rates for several indices of pulmonary function were measured after production of early, reversible degrees of pulmonary oxygen poisoning by continuous oxygen exposures at 151, 202, and 252 kPa (1.5, 2.0, and 2.5 ATA; Clark et al 1999). The results show that selected indices of pulmonary mechanical function returned to pre-exposure levels within 15–30 h (Fig. 9.4.22), while $D_{L_{CO}}$ as an index of gas exchange function required more than 1 week for complete reversal of clinically benign but statistically significant changes (Fig. 9.4.23). In addition to the need for more information about rates of recovery from acutely evident, reversible degrees of pulmonary oxygen poisoning, an equally important information gap is that concerning the chronic, long term effects of repeated exposures to subclinical or mild degrees of pulmonary intoxication.

There are indications that complete recovery does not occur between daily exposures to relatively mild

degrees of oxygen poisoning. Sterk & Schrier (1985) exposed six divers 5 days per week to a daily oxygen dose consisting of three 2 h exposures that, if combined and given continuously, would be expected to produce an unmeasurable decrease in vital capacity of about 2%. There were no changes in vital capacity or other spirometric measurements at the end of the first or second weeks. However, pulmonary symptoms were experienced intermittently during both weeks, marked fatigue occurred at the end of the each week, and paresthesias were experienced during the second week. When a second group of six subjects was exposed to a daily oxygen dose equivalent to nearly a 4% decrease in vital capacity, similar symptoms occurred earlier and were more severe. These exposures were stopped after only 1 week. Despite the increased severity of symptoms, spirometric measurements at the end of the week detected no objective changes in pulmonary function.

OTHER MATHEMATICAL DESCRIPTIONS OF OXYGEN TOLERANCE

Using an expanded data set which included vital capacity measurements (Eckenhoff et al 1987) that were not available when the UPTD concept was originally developed (Bardin & Lambertsen 1970), Harabin et al (1987) performed non-linear least-squares error analysis to assess statistically the variability of the available data and to calculate adjusted constants for the original predictive model by fitting the individual vital capacity data to the general hyperbolic equation:

$$\%\Delta VC = B_s(P_{O_2} - B_1)\ (t - B_2)^{B_3},$$

where P_{O_2} is inspired oxygen pressure in ATA, t is exposure time in min, B_1 is the P_{O_2} asymptote, B_2 is the time asymptote, B_3 is an exponent that was empirically set to be −1.2 by Bardin & Lambertsen (1970; with rearrangement the sign of B_3 is positive) and B_s is a derived individual slope parameter. The overall statistical best fit of the derived individual linear slope data was obtained when the following conditions were met:

1 an individual slope parameter B_s was estimated for each subject rather than an average slope;
2 the P_{O_2} asymptote $B_1 = 0.38$ rather than 0.5 ATA;
3 the time asymptote B_2 was essentially zero as before; and
4 exponent $B_3 = 1.0$ rather than 1.2.

The slope parameter exerted the largest influence on the model, with the exponent and P_{O_2} asymptote exerting statistically significant but much smaller influences.

The methods used by Harabin et al are essentially statistical tools for testing the overall consistency of available data. Their emphasis on fitting linear regressions to individual vital capacity data differed from the probit %ΔVC–log time regression on median data that was originally used by Clark & Lambertsen (1971b). To the extent that limitations of the original UPTD concept were imposed by the individual variability in available data, the non-linear least-squares analysis provided an objective, quantitative means for describing data and assessing individual variability in the assumption of linear O_2 dose–effect relationships.

An alternative approach (Arieli 1994) used a power relationship to describe mean percent decrease in vital capacity, along with other specific manifestations of oxygen toxicity, as a function of exposure duration and oxygen pressure. Arieli's use of a power function to describe the progression of oxygen poisoning was based on his assumption that it may relate to the reaction kinetics of the free radical intermediates that underly the basic mechanisms of oxygen toxicity. The following general expression for mean changes in vital capacity was derived:

$$\%\Delta VC = at^b\ P_{O_2}{}^c,$$

where t is exposure time in min, P_{O_2} is inspired oxygen pressure in ATA, and a, b and c are constants. Using the avaialble database (Clark & Lambertsen 1971b, Clark et al 1987, Eckenhoff et al 1987), the following values of the constants in the general equation were obtained:

$$\%\Delta VC = 0.0115t^{1.85}P_{O_2}{}^{4.56}.$$

By equating this expression with that derived by solving the equation for an oxygen pressure of 101 kPa (1.0 ATA), Arieli (1994) obtained the following equation for calculating an equivalent duration of oxygen breathing at 101 kPa (1.0 ATA) for any combination of exposure duration and inspired oxygen pressure, thus maintaining the 'UPTD' definition:

$$UPTD = tP_{O_2}{}^{2.46}.$$

This adaptation does not incorporate a P_{O_2} asymptote of 50 kPa (0.5 ATA), originally selected by Clark & Lambertsen (1970, 1971a), and still used as the inspired P_{O_2} below which toxic effects were not measurable (Lambertsen et al 1999). In addition, it is important to recognize that any mathematical expression used to describe the rate of development of oxygen poisoning is dependent upon and limited by the empirical data set upon which the analysis is based.

NEED FOR AN OBJECTIVE, PRECONVULSIVE INDEX OF CNS OXYGEN TOLERANCE

The onset times for the symptoms and signs of CNS oxygen poisoning (Table 9.4.1) have been determined in large numbers of men at oxygen pressures ranging up to 707 kPa (7.0 ATA); (Butler & Thalmann 1984, 1986, Donald 1947, 1992, Haldane 1941, Yarbrough et al 1947). However, many of the observed symptoms such as apprehension and nausea had no relationship to incipient convulsions, and signs such as muscle twitching, which did indicate that convulsions were imminent, did not always occur prior to the actual seizure. Butler & Thalmann (1986) classified the signs and symptoms of CNS oxygen toxicity that were experienced by their divers as 'probable' or 'definite' in an attempt to compensate for their varying reliability as harbingers of incipient convulsions. The actual occurrence of convulsions was placed in a third category, with serious implications in diving. Butler & Thalmann (1984, 1986) also found that some divers appeared to be unusually susceptible to oxygen convulsions, and took this into account in their recommended limits for closed circuit oxygen diving (Table 9.4.2). Although convulsions are an undesirable index of CNS oxygen poisoning, it is not always possible to avoid them even under laboratory or therapy conditions, and existing evidence reveals no indications of residual neurologic impairment (Lambertsen 1978).

EEG has been studied as an objective index of CNS oxygen poisoning (Cohn and Gersh 1945, Gibbs et al 1935), but EEG alterations prior to the onset of convulsions could not be demonstrated. This early observation was confirmed by failure to detect any preconvulsive changes in brain cortical electrical activity, as measured with 12 scalp electrodes using the International 10–20 System, in one subject who convulsed after breathing O_2 at 303 kPa (3.0 ATA) for 3 h (Lambertsen et al 1987). The same subject had normal hearing thresholds and no detectable impairment of cognitive and psychomotor performance at preconvulsive intervals of 4 and 30 min, respectively. Visser et al (1996) also recorded the EEG of a diver who convulsed while breathing O_2 at 283 kPa (2.8 ATA, 18 msw, 60 fsw). Neither on-line visual monitoring nor off-line quantitative analysis revealed any consistent differences between the EEG of this diver and those of 23 other divers who did not convulse.

In lieu of a better toxicity index, the occurrence of a 10% incidence of neurologic symptoms during exposure to oxygen pressures of 282–404 kPa (2.8–4.0 ATA); Donald 1947, Yarbrough et al 1947) was used to describe CNS oxygen tolerance in man (Fig. 9.4.26) (Lambertsen 1978). Asymptotes for this curve were considered to be zero time and an inspired oxygen pressure of 202 kPa (2.0 ATA). The latter asymptote was selected because no prominent neurologic effects were observed in normal men breathing O_2 at 202 kPa (2.0 ATA) for up to 12 h (Clark & Lambertsen 1971b). Although the absence of convulsions during similar oxygen exposures at 202 kPa (2.0 ATA) was later confirmed (Lambertsen et al 1987), reversible decrements in the retinal electrical activity in response to a light flash (ERG b-wave amplitude) have been observed consistently (Clark et al 1988, 1991a). At the present time, it appears that loss of peripheral vision (Fig. 9.4.15) and decrease in ERG b-wave amplitude are the best available quantitative indices of CNS oxygen toxicity for use in the development of improved CNS oxygen tolerance curves.

EXTENSION OF OXYGEN TOLERANCE

The onset and rate of progression of oxygen poisoning in vivo can be influenced by a variety of conditions, procedures and drugs (Clark & Lambertsen 1971a). Two major purposes for which these influences have been studied are the evaluation of their possible relevance to mechanisms of oxygen toxicity and the search for effective means of delaying the onset and decreasing the severity of intoxication in practical applications of hyperoxia. The effects of a large number of specific influences have been described in previous reviews (Clark 1982, Clark & Lambertsen 1971a, Deneke & Fanburg 1980, Frank & Massaro 1980, Haugaard 1968). Several potential means for the extension of oxygen tolerance (Lambertsen 1978) were discussed in a symposium (Clark 1988b) dedicated to that topic.

Any chemical agent that effectively delays the onset and progression of oxygen poisoning would be extremely useful in therapeutic and operational diving applications of hyperoxia. For maximal effectiveness, however, such an agent would have to be distributed throughout all body tissues to counteract the multiple, diverse effects of oxygen toxicity (Fig. 9.4.1), while at the same time remaining free of significant side-effects (Lambertsen 1978, 1988). For all of these reasons, the protective agents cited in previous reviews (Clark 1982, Clark & Lambertsen 1971a, Deneke & Fanburg 1980, Frank & Massaro 1980, Haugaard 1968) probably have only limited potential for practical applications.

An example of a chemical agent that was found initially to delay some of the effects of oxygen toxicity,

but later actually proved to enhance the progression of other effects, is provided by disulfiram, a drug used in alcohol aversion therapy. Early studies showed that disulfiram administration effectively delayed the onset of convulsions and lung damage in animals exposed to O_2 at 404 kPa (4.0 ATA; Faiman et al 1971, 1974). The drug appeared to be promising for use in man, because even large doses were nearly devoid of toxic effects (Ritchie 1975). However, continued investigations showed that survival time is significantly decreased in rats given disulfiram before exposure to O_2 at 101 kPa (1.0 ATA; Deneke et al 1979) or 202 kPa (2.0 ATA; Forman et al 1980). This detrimental action of disulfiram is apparently caused by its metabolism in vivo to diethyldithiocarbamate, an inhibitor of superoxide dismutase (Forman et al 1980).

OXYGEN TOLERANCE EXTENSION BY ALTERNATING HYPEROXIA AND NORMOXIA

In contrast to the present lack of truly effective pharmacologic measures for extension of oxygen tolerance, a currently useful procedure employs systematic alternation of hyperoxic exposure intervals with relatively brief normoxic intervals to increase markedly the total duration of tolerable exposure to a selected level of hyperoxia within a period of 24 h or longer (Clark 1974, Clark & Lambertsen 1971a, Hall 1967, Harabin et al 1988, Hendricks et al 1977, Lambertsen 1955, 1978, 1988, Widell et al 1974). Since this procedure involves the periodic, sequential elevation and reduction of oxygen tension rather than passage of a chemical agent across cellular membrane barriers, it is effective in all organs and tissues affected by oxygen poisoning. Although the rates of reversal of the effects of oxygen toxicity (Fig. 9.4.1) are not known, the observation that oxygen tolerance can be extended significantly by alternating intervals of normoxia for 5 min with hyperoxia for 20 min (Hall 1967, Hendricks et al 1977, Widell et al 1974) indicates that substantial recovery must occur more rapidly than the corresponding rates of development.

DEFINING OPTIMAL PRINCIPLES OF OXYGEN TOLERANCE EXTENSION BY INTERMITTENT EXPOSURE

The quantitative gains in oxygen tolerance achieved by intermittent insertion of normoxic intervals ranging from one-half to double the length of the hyperoxic interval in guinea pigs exposed to O_2 at 303 kPa (3.0 ATA) are shown in Fig. 9.4.28 (Hall 1967). The oxygen time indicated on the abscissa for intermittent exposure represents the sum of all the oxygen breathing intervals. Survival time is increased progressively as the normoxic interval is lengthened while holding the oxygen period constant.

Subsequent experiments were designed to define optimal principles of oxygen tolerance extension in the

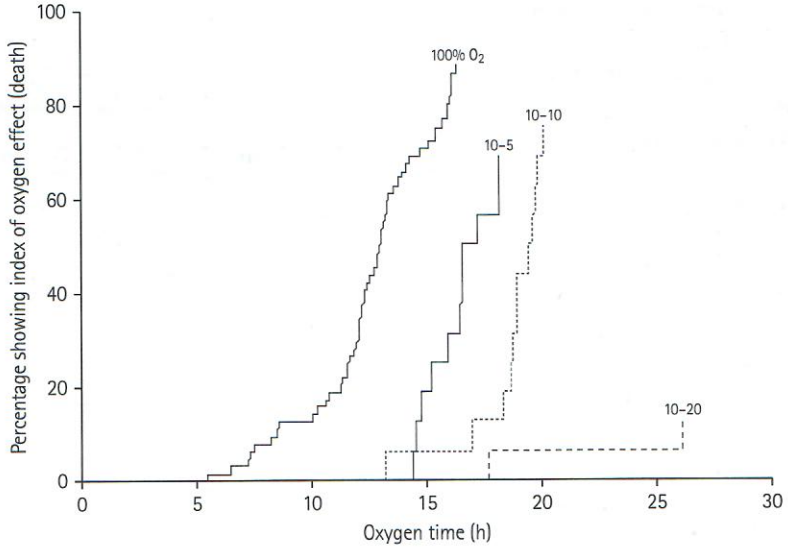

Fig. 9.4.28 Survival time in guinea pigs during continuous and intermittent oxygen breathing at 303 kPa (3.0 ATA). Oxygen time on the abscissa does not include intervals of normoxic exposure (7% O_2 in N_2 at 303 kPa, 3.0 ATA). (From Hall 1967)

rat by measuring survival time responses to systematically varied intermittent exposure patterns at oxygen pressures of 202 and 404 kPa (2.0 and 4.0 ATA; Clark & Lambertsen 1982, 1989). In general, oxygen: normoxic exposure patterns with the same ratio, i.e. 20:20 and 60:60 min or 20:10 and 60:30 min, provided similar extensions of survival time at either 202 or 404 kPa (2.0 or 4.0 ATA). This general rule did not apply when the oxygen period was so long that toxic effects were not readily reversible or when the normoxic interval was too short for adequate recovery from the preceding oxygen period. When a 60 min oxygen period was alternated with a 180 min recovery interval, 20 rats had no evident adverse effects from a cumulative oxygen exposure at 202 kPa (2.0 ATA) that was nearly equal to four times the average time to death for continuous exposure (Clark & Lambertsen 1982).

Possible Mechanisms of Oxygen Tolerance Extension by Intermittent Exposure

Frank et al (1989) found that exposure of rats to O_2 at 96–101 kPa (0.95–1.0 ATA) for 48 h, followed by a 24 h 'rest period' in air, made them able to tolerate 3–7 additional days of O_2 exposure with only slight pulmonary edema and no deaths. The induction of increased oxygen tolerance was associated with significant increments in lung activities of the antioxidant enzymes, superoxide dismutase, catalase, and glutathione peroxidase, during re-exposure to O_2 after the rest period. All rats survived re-exposure to O_2 even when the recovery period in air was reduced to 6 h. When the pre-exposure period was reduced from 48 to 36 h, only 83% of the rats allowed to recover in air for 24 h survived re-exposure for 3 days, and the survivors had macroscopic evidence of pulmonary damage. These results are relevant to possible mechanisms of oxygen tolerance extension by intermittent exposure, because they demonstrate that a sufficiently toxic oxygen exposure, followed by a suitable normoxic recovery period, induces the production of antioxidant enzymes upon subsequent exposure to hyperoxia.

Harabin et al (1990) measured brain and lung antioxidant enzyme responses in guinea pigs and rats that were exposed either continuously to O_2 at 282 kPa (2.8 ATA) or intermittently on a schedule that alternated 10 min oxygen periods with 2.5 min periods of air breathing (57 kPa O_2, 0.56 ATA). The convulsion and survival times were significantly longer during intermittent exposure than for continuous exposure in both species. The antioxidant enzyme activities in the brain and lung were compared at equivalent durations of continuous and intermittent oxygen exposure. Brain superoxide dismutase (SOD) activity was not significantly changed in either species. Catalase (CAT) activity was reduced in guinea pigs during both continuous and intermittent exposures. Glutathione peroxidase (GSHPx) activity was reduced in both species for both types of exposure. Lung SOD activity was increased during intermittent exposure in guinea pigs and during both exposures in rats. CAT activity was reduced in both species for both exposures, while GSHPx activity was reduced for both exposures in guinea pigs, but not in rats. In guinea pigs, antioxidant enzyme activities were generally either increased more or decreased less during intermittent than for continuous exposure. This relationship was less evident in rats. In neither species could the observed increments in convulsion and survival times during intermittent exposure be attributed primarily to changes in antioxidant enzyme activities. It is possible that a different schedule of intermittent exposure at 282 kPa (2.8 ATA) or some other pressure would provide a closer association between increased oxygen tolerance and antioxidant enzyme activities.

Extension of Oxygen Tolerance in Man by Intermittent Exposure

The application of alternating hyperoxic and normoxic exposure periods to extension of pulmonary oxygen tolerance in man is illustrated in Fig. 9.4.29 (Lambertsen 1978). A decrease in vital capacity was used as an index of pulmonary intoxication in men breathing O_2 at 202 kPa (2.0 ATA; Hendricks et al 1977). The results obtained for alteration of 20 min oxygen and 5 min normoxic periods are compared with similar measurements obtained during continuous exposure of a different group of subjects to O_2 at 202 kPa (2.0 ATA; Clark & Lambertsen 1971b). The comparison indicates that duration of oxygen breathing associated with a 4% decrement in vital capacity is more than doubled by the use of intermittent exposure.

For the purpose of testing in man the concept that equivalent gains in oxygen tolerance are provided by intermittent exposure patterns that have the same oxygen:normoxic ratio, vital capacity was measured repeatedly in six subjects who were exposed to O_2 at 202 kPa (2.0 ATA) on an intermittent exposure pattern that alternated 60 min oxygen periods with 15 min normoxic intervals (Clark et al 1990). The degree of oxygen tolerance extension provided by the 60:15 intermittent exposure pattern was then compared with that found for the 20:5 pattern previously used by Hendricks et al (1977). The rate of vital capacity reduction in four of the six subjects on the 60:15 pattern was nearly identical to the average rate of fall

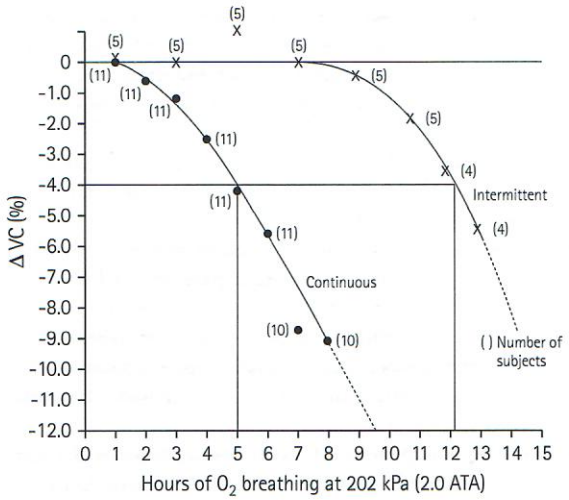

Fig. 9.4.29 Extension of pulmonary oxygen tolerance at 202 kPa (2.0 ATA) in man. The curve showing rate of decrease in vital capacity during continuous oxygen breathing was obtained from Clark & Lambertsen (1971b). The curve for intermittent oxygen exposure was adapted from Hendricks et al (1977), and the indicated duration of oxygen breathing represents a summation of all the intermittent, 20 min oxygen periods.

in five subjects on the 20:5 pattern, but vital capacity fell more rapidly in the remaining two subjects. These results are consistent with the interpretation that optimal use of intermittent exposure to extend pulmonary oxygen tolerance in man at 202 kPA (2.0 ATA) will require the use of oxygen exposure periods that are less than 60 min in duration (Clark et al 1990).

Prior to the completion of the intermittent exposures on a 60:15 pattern at 202 kPa (2.0 ATA), human oxygen tolerance extension by intermittent exposure was confirmed only for the lung. Using progressive reduction in the ERG b-wave amplitude as an index of toxic effects on the visual system, nearly equivalent degrees of oxygen tolerance extension were found for both the eye and the lung in the same subjects (Clark et al 1991a, Lambertsen et al 2000). The progressive decline in average b-wave amplitude preceded the average fall in vital capacity for both continuous and intermittent oxygen exposure at 202 kPa (2.0 ATA).

An early conceptual basis for composite oxygen tolerance extension by intermittent exposure was that the cumulative oxygen exposure required to produce overt manifestations of oxygen poisoning can be lengthened by periodic reversal of the subclinical toxic effects that develop during each successive oxygen exposure period (Lambertsen 1955). Although this concept has not been refuted, a comparative evaluation of two different patterns of intermittent oxygen exposure at 202 kPa (2.0 ATA) indicates that an additional mechanism of human oxygen tolerance extension must be involved (Clark et al 1992, Lambertsen et al 2000). Visual and pulmonary indices of oxygen poisoning were measured repeatedly in two groups of subjects who were exposed intermittently on either a 60:15 or a 30:30 pattern. The latter pattern was expected to provide greater extensions of oxygen tolerance, because it simultaneously halved the oxygen exposure period and doubled the normoxic recovery interval. The expected results were in fact observed at the end of the intermittent exposures, but the toxic effects associated with the 30:30 pattern appeared to exceed those for the 60:15 pattern at an earlier duration of exposure. This apparent early development and later reversal of overt toxic effects on the 30:30 pattern may reflect the existence of an additional, previously undetected mechanism for oxygen tolerance extension by intermittent exposure. Although the nature of this additional pathway is not yet known, it is likely that better understanding will provide opportunities for even greater gains in oxygen tolerance extension by intermittent exposure.

The extension of oxygen tolerance by intermittent exposure has been studied in man thus far only at an oxygen pressure of 202 kPa (2.0 ATA). Full exploitation of this practical and effective procedure in therapeutic and operational applications of hyperoxia will require similar studies of both pulmonary and CNS oxygen tolerance over a range of useful oxygen pressures. Some of the required baseline measurements during continuous oxygen exposures have been performed (Clark et al 1987, 1991b, 1999, Gelfand et al 1987, Lambertsen et al 1987, Pisarello et al 1987). It is anticipated that the greatest gains in oxygen tolerance and applied hyperoxygenation will be achieved by determination of the lowest effective level of hyperoxia for each specific application and by optimal use of programed intermittency to provide maximal exposure to that oxygen pressure (Lambertsen 1978, 1988). In the event that effective, non-toxic antioxidant drugs become available for administration to man, it is likely that their greatest usefulness will be in conjunction with intermittent exposure rather than in lieu of it.

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