NASA SP-47

SPACE-CABIN ATMOSPHERES

Part I—Oxygen Toxicity

NATIONAL AERONAUTICS AND SPACE ADMINISTRATION

NASA SP-47

SPACE-CABIN ATMOSPHERES
Part I—Oxygen Toxicity

A literature review by
Emanuel M. Roth, M.D.

Prepared under contract for NASA by
Lovelace Foundation for Medical Education
and Research, Albuquerque, New Mexico

Scientific and Technical Information Division 1964
NATIONAL AERONAUTICS AND SPACE ADMINISTRATION
Washington, D.C.

FOR SALE BY THE SUPERINTENDENT OF DOCUMENTS, U.S. GOVERNMENT PRINTING OFFICE
WASHINGTON, D.C. 20402 - PRICE 40 CENTS

Foreword

THIS REPORT, previously published as NASA Technical Note D–2008 in August 1963, is Part I of a study on *Space-Cabin Atmospheres*, conducted under sponsorship of the Directorate, Space Medicine, Office of Manned Space Flight, National Aeronautics and Space Administration. Part II, "Fire and Blast Hazards," is available as NASA SP–48. Future parts of this study will be: Part III, "Physiological Factors of Inert Gases," and Part IV, "One- versus Multiple-Gas Systems."

This document provides a readily available summary of the open literature in the field. It is intended primarily for biomedical scientists and design engineers.

The manuscript was reviewed and evaluated by leaders in the scientific community as well as by the NASA staff. As is generally true among scientists, there was varied opinion about the author's interpretation of the data compiled. There was nonetheless complete satisfaction with the level and scope of scholarly research that went into the preparation of the document. Thus, for scientist and engineer alike it is anticipated that this study will become a basic building block upon which research and development within the space community may proceed.

GEORGE M. KNAUF, M.D.
Deputy Director, Space Medicine
Office of Manned Space Flight

Introduction

> . . . But, perhaps we may also infer from these experiments, that though dephlogisticated air might be very useful as a medicine, it might not be so proper for us in the usual healthy state of the body; for, as a candle burns out much faster in dephlogisticated air than in common air, so we might, as may be said, live out too fast, and the animal powers be too soon exhausted in this pure kind of air. A moralist, at least, may say that air which nature has provided for us is as good as we deserve. . . .
>
> *Priestley, 1775* [135]

As soon as the first oxygen was made available for study, the toxic potency of this gas was recognized. The classical reviews by Stadie, Riggs, and Haugaard in 1944 [151] and by Bean in 1945 [8] cover in detail most of the work to this time. An unpublished brief review by Snapp and Adler [149] summarizes the more significant features of oxygen toxicity to the 1948 period. Most of the signs and symptoms and potential mechanisms of oxygen toxicity were well worked out by this time. Subsequent studies have been related primarily to the mechanism of oxygen toxicity and to studies of unusual environmental conditions employing high oxygen concentrations.

The present review is limited to the data on oxygen toxicity that are important in the analysis of space-cabin atmospheres. Of chief concern are the effects of oxygen at pressures below 1 atmosphere. Oxygen at higher pressures is discussed only to help elucidate mechanisms of toxicity in the space-cabin environments. Chapter 1 covers the molecular mechanism of oxygen poisoning. Subsequent chapters treat oxygen toxicity and mechanisms in animals and in man; the role of oxygen in atelectasis, blast effects, and the space radiation problem; drug therapy against oxygen toxicity; and consideration of oxygen toxicity in the selection of a space-cabin atmosphere.

Contents

CHAPTER 1

Molecular Mechanisms

PHYSICAL CHEMISTRY OF OXYGEN MOLECULE

A TRUE EVALUATION of the gross physiological responses to high oxygen tensions requires an understanding of the biochemical interactions of oxygen at a molecular level. The peculiar properties of the oxygen molecule are derived from its unusual electronic configuration. Pauling [129] was first to point out the paramagnetic nature of oxygen resulting from its two unpaired electrons. Pauli's exclusion principle requires that two electrons in the same orbital have opposite spins with neutralization of magnetic moments. Paramagnetism results from the magnetic moments presented by unpaired electrons of oxygen and free radicals.

Figure 1 demonstrates the electrons at the end of the axes of the P orbitals (X, Y, and Z). The two unpaired electrons are indicated by the arrows at P_Y and P_Z. The electrons of oxygen can form two 3-electron bonds (:O:::O:), but it is thought that oxygen gets its paramagnetic behavior from the presence of two unpaired electrons.

By virtue of its unusual electronic structure, oxygen has a high oxidizing potential which endows it with its properties as the ultimate oxidizing agent for the maintenance and, as we shall see, destruction of many living systems. The destructive oxidizing capacity of the oxygen molecule is kept in check by several peculiar aspects of its own structure and that of living systems with which it interacts.

Oxygen (O_2) is useful as a potential energy source because it is in reality a rather "sluggish" oxidizing agent which gives it an "energy storage" function. In 1940 Gorin [75] pointed out that the sluggishness of oxygen is probably due to the fact that it has to be activated to the free-radical state for its intracellular role. Michaelis [112] in 1949 postulated that the reduction of oxygen proceeds through several univalent steps which would imply free-radical intermediates. Using the electron magnetic resonance techniques of Sogo and Tolbert,[150] Commoner et al.[34] in 1957 demonstrated free radicals as probable intermediates in oxidation-reduction in chloroplast systems.

Szent-Györgyi [155] has recently reviewed the analogy between semiconductor systems and the conduction of electrons along proteins and oxidation-reduction enzymes of biological systems. Gerschman [62] has reviewed the possible reaction of oxygen with hydrogen to form the hyperoxal (hydroperoxo) free radical $HO_2\cdot$ or $H\cdot + OH\cdot$ with unpaired electrons (fig. 2). (The dot after a molecule represents a free radical capable of attacking many types of bonds.)

Gerschman [62] postulated that the activation energies predicted for the reduction of oxygen in univalent free-radical steps would tend to act as energy barriers preventing rampant oxidation of cellular components by free oxygen. (See fig. 3; ΔF_0 represents free-energy change.)

Once in active free-radical form, oxygen can react with many cellular components. The

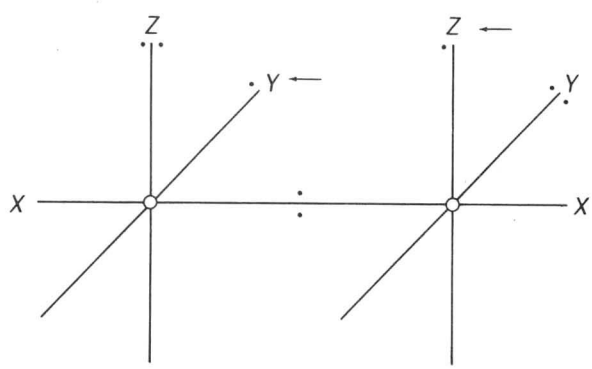

FIGURE 1.—*Electronic configuration of molecular oxygen* O_2. (AFTER GERSCHMAN.[62])

capacity of oxygen to partake in chain reactions with organic systems has been beautifully reviewed by Walling.[163] The initiation and prolongation of the chain process is reviewed subsequently. Free radicals, of course, may be generated by ionizing radiation as well as by metabolic processes. The interaction between oxygen effects and radiation has been known for years as "the oxygen effect" and is discussed in a later section. Effects of ozone toxicity appear to follow the same general free-radical mechanisms (Davis [41]).

ANTIOXIDANT DEFENSES

The defense of cellular systems against free radicals generated by oxidative processes is still poorly understood. It is very possible that somehow the aging processes of the entire body may well represent the progressive deterioration of antioxidant defense. The relation of destructive oxidative processes to the aging of red blood cells is discussed in a subsequent section. As will be seen, the generation of hydrogen and electrons by the degradation of carbohydrates and other energy sources contributes to antioxidant defense. The reduced form of triphosphopyridine nucleotide (TPNH) which finally results from these reactions reduces in turn the glutathione, cysteine, and other active reducing compounds within the cell. Other mechanisms also contribute to the antioxidant defense.

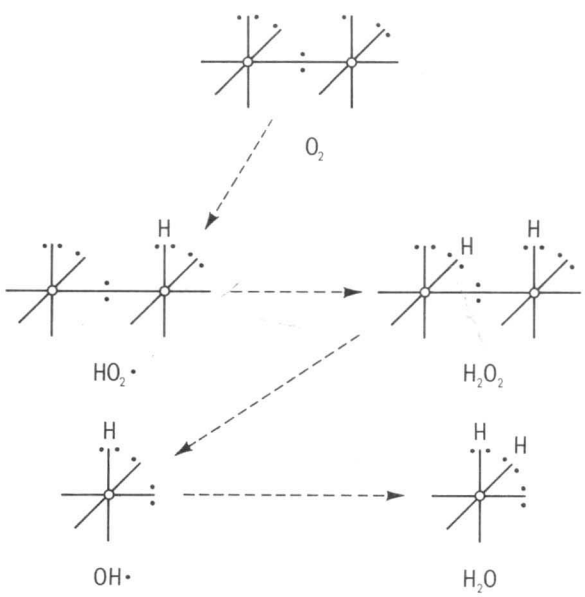

FIGURE 2.—*Hydrogen-oxygen species.* (AFTER GERSCHMAN.[62])

Chance [28] has recently pointed out the peculiar role of the terminal cytochromes as buffers for the oxidative system. Reductive changes in the terminal oxidases and proximal members of the respiratory chain occur at oxygen concentrations exceeding the critical level based upon cellular respiratory activity. The overabundance of terminal oxidases allows them to be oxidized by molecular oxygen and to leave, nevertheless, an adequate amount of the reduced form to carry on respiratory processes without measurable changes in the respiratory rate. By providing a storage of bound oxygen, this system probably buffers the cell in anoxic states as well.

Gerschman et al.[65] (1955) and Taylor [157] (1956) have demonstrated the role of vitamin E and the α-tocopherols as antioxidants in the cell. Indeed, some symptoms of vitamin E deficiency are probably those of toxicity to 0.2 atmosphere of oxygen, the normal sea-level condition. Animals deficient in vitamin E are very sensitive to high-oxygen environments.[110] The importance of this concept will become clearer in the discussion of recent experiments in space-cabin simulators.

Bacteria have been known for years to have antioxidant defenses. Porter [134] demonstrated that obligate anaerobes die in the presence of oxygen because they lack catalase. This is indeed the rationale for the new OHP (oxygen at high pressure) treatment of tetanus. Annear and Dorman [2] and Gordon et al.[74] demonstrated that hydrogen peroxide was indeed the lethal factor. High oxygen pressures can actually cause mutations,[55] possibly through the depolymerization of deoxyribonucleic acid (DNA)[70] via the peroxide or free-radical mechanism. This suggests that genetic stability depends on adequate antioxidant defense.

FREE-RADICAL CHAIN REACTIONS

It appears that oxygen toxicity and damage by ionizing radiation proceed by similar mechanisms. Both involve free-radical mechanisms. Excess levels of free radicals start

chain reactions typical of auto-oxidation processes. This concept is outlined below, where RH is a normal carbon-hydrogen bonded organic molecule, R· is a normal active biological free-radical intermediate, and RSH is a normal biologically active thiol group on an organic molecule.

I. Initiating steps

A. Ionizing radiation

1. Indirect (via water)

$$H_2O \xrightarrow{h\nu} H_2O^+ + E^-$$

$$H_2O^+ + H_2O \rightarrow H_3O^+ + OH\cdot$$

$$E^- + H_2O \rightarrow OH^- + H\cdot$$

2. Direct effect on biological molecules

$$RH \xrightarrow{h\nu} R\cdot + H\cdot$$

B. Biological reduction of O_2

$$O_2 \xrightarrow{H} HO_2\cdot \xrightarrow{H} H_2O_2 \rightarrow OH\cdot + H_2O \xrightarrow{H} 2H_2O$$

C. Oxidation of R· by O_2

$$R\cdot + O_2 \rightarrow RO_2\cdot$$

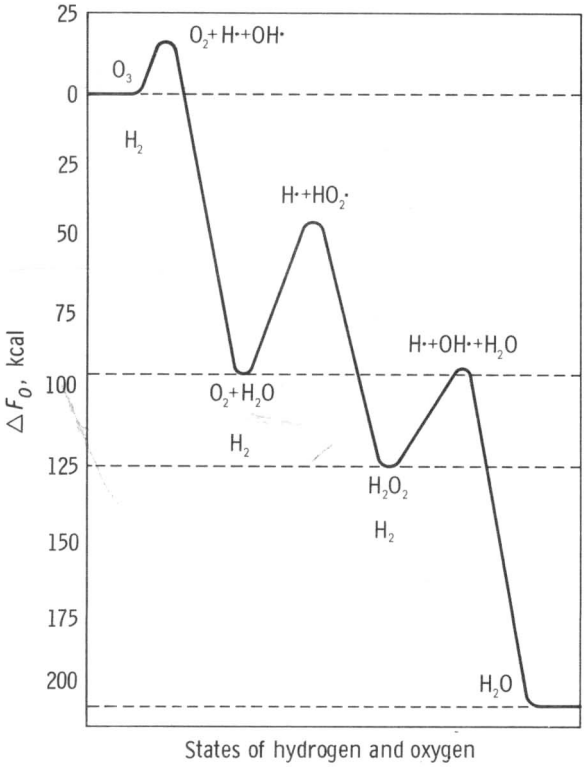

FIGURE 3.—*Reduction of oxygen by hydrogen. Dashed lines refer to stable or quasi-stable states.* (AFTER GERSCHMAN.[62])

II. Damaging steps (chain reactions)

A. $RH + HO_2\cdot \rightarrow R\cdot + H_2O_2 \rightarrow$ Step I*C* $\rightarrow$ Chain reaction

B. $R'O_2\cdot + RH \rightarrow R'O_2H + R\cdot \rightarrow$ Step I*C* $\rightarrow$ Chain reaction

C. $RSH + HO_2\cdot \rightarrow RS\cdot + H_2O_2$ { $\rightarrow$ Step I*C* $\rightarrow$ Chain reaction; $\rightarrow$ Step III*C* $\rightarrow$ Permanent damage or chain termination

III. Chain-terminating reactions (permanent damage or protection by stopping free-radical chain)

A. $R'O_2\cdot + R\cdot \rightarrow R'O_2R$

B. $R\cdot + R\cdot \rightarrow RR$

C. $RS\cdot + RS\cdot \rightarrow RSSR$

The propagation of free-radical chain reactions is characteristic of both types of insult. As Gerschman et al.[63] pointed out, effects of the reducing agents cysteamine, glutathione, and thiourea protect mice against radiation as well as against oxygen, even though at lower oxygen concentrations they may actually potentiate the oxygen effect. In the latter case, they probably are acting as pro-oxidants in presenting a cell with a thiol compound which is converted by O_2 to $RS\cdot + HO_2\cdot$ (step II*C* above). The sulfhydryl compounds cystamine and aminoethylisothiuronium (AET) act similarly.

Cobalt has been shown by Gilbert et al.[71] to destroy hydrogen peroxide, and by Gerschman et al.[63] to protect against 1 atmosphere of oxygen as well as against ionizing radiation.[126] Obligate anaerobes can actually grow in the presence of oxygen when cobalt is added to the medium.[42] The metal complexing agents ethylenediaminetetracetic acid (EDTA) and diethyldithiocarbamic acid (DEDTC) have been shown by many investigators[63, 85] to protect intact animals and enzymes against both irradiation and

high oxygen tensions. These agents possibly chelate out the heavy metals such as copper which catalyze peroxy free-radical reactions.

Thiols are probably not the only compounds involved in the generation of reactive free radicals. During the past few years there has been an increased interest in the effects of peroxidation products of lipids on biological systems.[106] Several investigators have demonstrated that lipid peroxides may be responsible for some of the effects of radiation.[91,101,118] In his study of OHP, Wollman [173] demonstrated a significant increase in cerebral lipid peroxides of OHP with no significant changes in —SH groups. Becker and Galvin [9] recently confirmed these findings, but noted that there is no correlation of the peroxides with convulsive activity of OHP cerebral toxicity. No lipid peroxide elevation was noted in oxygen partial pressures of 1 atmosphere or less. It is still possible, however, that focal increases in lipid peroxides may indeed play a role in the destruction of red blood cells and alveolar membranes in the lower toxic p_{O_2} range.

BIOLOGICAL VARIABILITY

Upon consideration of the mechanism of action, the biological variability in the effects of high oxygen concentrations, depending on concentration, species, protective agents, and so forth, becomes more rational. The survival equation of Williams and Beecher,[168] $T=aP^{-b}$, where T is time in hours, P is pressure (atm) of oxygen, and a and b are empirical constants, has been found by Gerschman et al.[63] to be valid for $a=102$ hours and $b=2.73$ from only 1 to 10 atmospheres of oxygen. A big change appeared at 0.7 to 1.0 atmosphere. If oxygen does produce active free-radical intermediates, a sudden increase in chain reaction rates would be expected at a very specific concentration range. This would be evidenced by a sudden increase in sensitivity to oxygen pressure. This critical-dose effect is also seen in X-irradiation of mice.[98] The concentration of antioxidants and chain-terminating thiol compounds [163] at critical cellular sites would, therefore, be expected to determine the specific gross pathological physiology. Especially in dealing with the lower concentrations of oxygen (<1 atm), one would expect gross irregularities in effect from small changes in oxygen concentration and cellular environmental factors.

The possibility of sensitizing agents (to be discussed later) further complicates the picture. Many of the "target organ" variabilities and moderating factors in oxygen toxicity are discussed below. Accepting the role of oxygen as an initiating agent in generating free radicals, what are the actual target molecules (RH and RSH) of these agents within the cell? They appear to be the enzyme systems and nucleic acids. The effects of oxygen on these systems will now be discussed.

CRITICAL TARGET MOLECULES

Early studies of the effect of high oxygen tensions on enzymatic activity implicated the oxidase-dehydrogenase enzyme group as the optimum intracellular target.[8,44,59,151] Both in tissue slices and in isolated enzyme systems, oxygen appears to be inhibitory. The generation of reducing agents by enzyme systems of the carbohydrate degrading systems or even exogenous reducing agents frequently relieved the oxygen inhibition. Often the coenzymes themselves were not the prime targets.[44] Metal ions such as those of manganese, cobalt, magnesium, and calcium, which preserved the general glycolytic pathway for generation of reducing agents, were effective in reducing damage.

The pyruvate oxidizing system appeared quite sensitive. The dehydrogenase part of the succinoxidase system was irreversibly damaged in the brain preparations, and the cytochrome system was only weakened after longer exposure. Lactic and malic dehydrogenases were weakly affected. Triosephosphate dehydrogenase (only in the absence of cozymase) was oxygen sensitive, as was choline oxidase, another —SH brain enzyme. The brain lactic and malic dehydrogenases, flavin adenine dinucleotide (FAD) systems, catalase, and hexokinase were not sensitive to oxygen. These experiments appear to invoke the reactions:

$$RSH+O_2 \rightarrow RS\cdot + HO_2\cdot$$

and

$$RSH+HO_2\cdot \rightarrow RS\cdot + H_2O_2$$

with possible irreversible changes to

$$RS\cdot + RS\cdot \rightarrow RSSR$$

where RSH represents thiol apoproteins or coenzymes.

The diverse enzymes which utilize the coenzyme A system for "2 carbon" transfers appear to be good targets for oxygen. It has been shown [68] that β-mercaptoethanolamine (β-mercaptoethylamine), a component of coenzyme A, will protect mice against oxygen poisoning and irradiation. The pyruvic oxidase system which is central in the metabolic cycle and uses coenzyme A is, indeed, very sensitive to oxygen,[44] especially in the presence of cupric ions.[85]

The lipid peroxides that have been discussed as possible intermediates in the free-radical chains have been recently shown to inactivate specific enzymes. These compounds have been studied primarily for their role in radiation damage,[91, 118] but their effects are, of course, also pertinent to the oxygen toxicity problem. Bernheim et al.[17] have demonstrated that the oxidation of mitochondrial fatty acids inactivates succinoxidase, cytochrome oxidase, and choline oxidase. Tappel and Zalkin [156] and Wills [170] confirmed the effects of these peroxide compounds on enzymes. These investigators have suggested that since the mitochondrial cytochromes and other hematin compounds are the most active peroxidation catalysts in animal tissues, the unsaturated fatty acids of the mitochondria are the most probable intermediates in oxygen inactivation of the respiratory chain.

A recent finding of Dixon et al.[45] indicates that the enzyme most sensitive to oxygen is cytochrome C reductase of the pig heart. These investigators found, in purified preparations, a half-life of 6 minutes at 38° C and 1 atmosphere, as compared to the most sensitive enzyme of Dickens,[44] succinic dehydrogenase, with a half-life of 3 hours under the same conditions.

Of interest is the mechanism of this oxidative inactivation. The apoprotein itself is oxidized; critical is the binding, not the oxidation of the prosthetic flavin group. Apparently, oxidation of the binding site prevents flavin attachment. Glutathione does not protect, nor do CN^-, Versene, or dipyridyl groups, even though this enzyme, contains iron. This inactivation mechanism is the clearest picture we have of a specific molecular effect. Destruction of this enzyme may be, indeed, the critical factor in oxygen action, the other enzymatic defects merely changing the redox potential of the intracellular environment to accelerate the oxidation of this rate limiting step.

It seems probable that the movement of electrons in neighboring molecules and in critical protein that composes cellular structural elements may also be interfered with by oxygen.[155] Any enzyme may, therefore, be potentially affected *in vivo*. As has already been mentioned, nucleic acid molecules may also be targets for the free-radical reactions.

The *in vivo* cellular environment, it must be remembered, does have antioxidants and reducing metabolites, which alter the oxygen effects from those detected *in vitro*. The array of the multienzyme systems on the matrix of mitochondria[79] may indeed protect terminal respiratory enzymes against oxygen damage. With the isolated pure compound cytochrome C, Theorell[158] demonstrated that the peptide helix so encloses the hemin plate as to completely shield this active site from oxygen, but not from electrons. Similar shielding from oxygen may be present in many of the enzyme systems of living cells.

The protective effect of hypophysectomy[25] and adrenalectomy, and the augmentation of oxygen poisoning by very high doses of adrenal cortical hormones, bespeak the role of these hormones in control of cellular energetic reactions.[66] The multiple sites of action of adrenal cortical hormones only confuse the picture in our attempt to understand the molecular basis of oxygen toxicity in the intact animal. Yet, the adrenal has been demonstrated to control the level of cerebral lipid peroxides in OHP.[9] There have been many other studies of vitamin deficiency and other metabolic stress in relation to oxygen toxicity, but none appear to have shed any light on specific molecular mechanisms. These are discussed in Chapter 7.

FOREIGN INTEREST

The Russians have been doing work on free-radical interrelations between oxygen toxicity and radiation exposure. They have been stressing free-radical reactions and have been looking for "oxygen content in tissue" as a measure of the effectiveness of the —SH drugs against radiation. A typical example is a recent paper on antiradiation effect of thiourea and monothiols,[76] reviewed in Chapter 6 of this report. The Russians appear to be continuing this oxygen-radiation tack.[77, 97, 98, 102, 125]

The Russians seem to have an interest in oxygen at high pressure (OHP), probably for submarine, scuba, and therapeutic purposes. Recent studies of brain metabolism in the 3- to 6-atmosphere range revealed a release of large quantities of ammonia which were lowered by administration of arginine. The researchers postulated that oxygen breaks down brain protein, releasing NH_2 groups, and the arginine scavenges it in the form of γ-amino butyric acid (GABA). It is of interest that Wood and Watson [174] of Toronto have recently demonstrated that GABA protects animals against the convulsions of OHP. The formation of glycogen in the brains of rabbits exposed to OHP is also being studied.[22] The work of Dickens [44] mentioned previously suggests that decreased glycolysis may be the ultimate causative factor in this glycogen increase.

It would thus appear that the molecular basis of oxygen toxicity may be related to the capacity of oxygen to (1) initiate free-radical reactions which interfere with enzymatic activity by direct reaction with apoproteins or coenzymes, and (2) modify the general redox potential within the cell and inhibit critical reactions. The signs, symptoms, and pathological physiology of oxygen toxicity, especially in the <1 atmosphere pressure range, appear sensitive to small changes in oxygen tension and to the metabolic state of the cells.

CHAPTER 2

Effects of High Oxygen Tension in Animals

IN GENERAL, it appears that oxygen toxicity falls into two target-organ classes: at <2 atmospheres, the respiratory tract is hit; at >2 atmospheres, the central nervous system is the key organ. In this review, the problems at <1 atmosphere will be emphasized.

Recent reviews of the <2 atmosphere range have been presented by Mullinax and Beischer [117] and DuBois.[49] They indicate that slight variations in test conditions from experiment to experiment are probably significant in the pathological physiology. This, of course, would be expected from the critical oxygen-tension factor postulated above for the <1 atmosphere condition. Great pains will, therefore, be taken to emphasize the critical details of the experiments.

TENSIONS OF 0.75 TO 1 ATMOSPHERE

Smith [148] studied the effects of 0.7 to 0.8 atmosphere (600 to 760 mm Hg) of oxygen on birds, mice, rats, and guinea pigs. He found that after 4 days the animals died with signs of "early stages of pneumonia" and hyperemia of the lungs and other organs. Elevation of oxygen pressure to higher levels hastened their death. At 0.4 atmosphere (306 mm Hg), no such pulmonary changes were found. Stadie et al.[151] and Bean [8] confirmed these results.

Clamann and Becker-Freyseng [10, 31] exposed 50 assorted animals to 0.80 to 0.87 atmosphere oxygen (601 to 607 mm Hg) for 7 days and found, besides severe pulmonary edema, mediastinal edema and pleural exudates. Cats and rabbits showed marginal emphysema. Lungs were hyperemic; alveoli were edematous, filled with red and white blood cells, and lined with a debris-filled membrane. This membrane adhered to vascular walls, extended into bronchioles, and appeared fibrinous in nature. Employing similar oxygen conditions, Pichotka [133] and Liebegott [104] described the same picture, as did Ohlsson.[122] Paine et al.[123] described similar findings in dogs in 0.75 to 1 atmosphere (570 to 760 mm Hg), but signs of right-sided heart failure were more evident.

Penrod [130, 131, 132] pointed out that rats and guinea pigs have endemic lung diseases which complicate pathological studies and suggested that cats be used. He found that by cannulating one bronchus and occluding the other during administration of 100 percent oxygen at several atmospheres for 3 hours, he could produce pathology similar to that described above in the open lung, but not in the occluded lung. He suggested that this result indicates a direct effect on the alveolar membrane and eliminates the hypothesis of a blood-borne toxin. Atelectasis is also found in the blocked lung. An oxygen pressure of 3 atmospheres for 4 hours tends to cause mucoid plugs in bronchioles and secondary atelectasis in cats. Repeated exposures to air during OHP reinflates the lung and decreases damage to the central nervous system by OHP. Positive-pressure breathing also alleviates the signs of lung damage. A recent study by Weir et al.[165] confirms all the above animal findings.

In a study [145] of lung pathology resulting from oxygen toxicity (1 atm), the electron microscope showed that the mitochondria became vacuolated. Treciokas, [161] however, suggests that these vacuolated structures are found in normal lungs and are probably not early signs of oxygen damage. The latest electron-microscope study of oxygen toxicity [27]

in mice exposed to 1 atmosphere of oxygen (95 to 100 percent) and 80 to 90 percent humidity revealed, after 3 to 6 days, an apparent patchy thickening of the alveolar wall due either to hypertrophy or fluid accumulation in the cells. The splitting of basement membrane and fluid vacuoles between endothelial cells and membrane were also seen. This damage is probably responsible for the passage of fluid from blood into the alveoli, though occasional fluid-filled alveoli were seen without these changes. Macrophages were occasionally seen to have the "mitochondrial vacuolization" of Schulz,[145] as were alveolar cells. These are usually present in normal lungs and may be fixation artifacts. The membranes in the alveoli contain an atypical fibrin similar to human "hyaline membrane" disease. No characteristic bacterial flora was seen.

Cells other than those in the lung have been shown to be damaged by oxygen at <1 atmosphere. Noell[121] has recently demonstrated that the electroretinograph (ERG) potentials are attenuated and disappear in rabbits exposed to high concentrations of oxygen at 1 atmosphere total pressure. Time of disappearance and rate of decline are dependent on actual oxygen pressure. The visual cells of the retina are sensitive to oxygen concentrations at 1 atmosphere or less at times when no other sign of systemic oxygen toxicity is evident. The following times were adequate for destruction of visual cells at 1 atmosphere total pressure: all animals exposed for 40 hours at 100 percent oxygen, 50 percent of animals in 4 days at 80 percent oxygen, 50 percent of animals in 7 days at 55 to 60 percent oxygen, and no animals in 12 days at 50 percent oxygen. The rabbit appears unusually sensitive, but young rabbits were more resistant than old. In mice, rats, and cats, death of the animal from other organ sensitivities occurred before cell death was visually evident.

The role of carbon dioxide in oxygen toxicity was studied by Lambertsen et al.[99] The early high carbon dioxide levels in tissues reported by others in the past were shown to be artifacts of the method of measurement. No true rise in carbon dioxide was found in dogs, rabbits, or cats until the onset of convulsions resulting from an environment of 3 to 4 atmospheres of oxygen. The hypothesis that the hemoglobin-carbon dioxide transport defect initiated by OHP is the primary cause of death was thereby discredited. Primary pulmonary damage and convulsive activity were thought to be the prime causes of carbon dioxide elevation. The potentiating effects of 2 to 3 percent carbon dioxide on the pulmonary damage from oxygen toxicity in the <1 atmosphere range has been discussed by Ohlsson.[122]

It can be seen that most of the pathology in animals exposed to the range of p_{O_2} from 0.75 to about 1 atmosphere involves the lung. The effect appears to be directly on the alveolar walls and leads to a cyanotic death. Other organs may well be involved at a chemical level, but there is little evidence of gross pathology. Rabbits appear to have retinas which are especially sensitive. It is possible that gross pathology would be seen in other organs if the animals would live long enough with their pulmonary insult. Carbon dioxide retention appears to be an aggravating factor rather than a prime force. The carboxyhemoglobin mechanism which was once in vogue appears to be only a complicating factor in the cerebral as well as the pulmonary aspects of oxygen toxicity. Atmospheric carbon dioxide in the 2 to 3 percent range does hasten death from pulmonary damage.

TENSIONS OF 0.20 TO 0.75 ATMOSPHERE

The studies described in the preceding section were at oxygen tensions from 0.75 to 1 atmosphere. Little work has been done at tensions in the 0.20 to 0.75 atmosphere range. A much overlooked study performed by Campbell[24] in 1927 sheds some light on the problems that currently face us. Campbell exposed cats, rats, mice, cavies, monkeys, and rabbits to oxygen tensions in the range of 0.6 to 3 times that in air, up to 59 days, with many environmental and physiological parameters under constant surveillance. Monkeys, cavies, rats, and mice tolerated an oxygen pressure of 420 mm Hg (60 percent oxygen) for these prolonged periods without symptoms or excessive weight loss, except for the cavies, which lost weight. Cats, however, when exposed to oxygen at only 300 mm Hg (40 percent) showed

symptoms of sleepiness and loss of appetite, and lost weight.

Pathological examinations of cat lungs showed "collapse and few catarrhal cells." This finding is of interest in that Penrod [131] later reported that cats under OHP have a tendency to produce mucoid plugs in small bronchioles and suffer atelectasis. Most animals demonstrated hemoglobin depression of 30 percent while cats showed slight rises in hemoglobin (fig. 4). The elevated hemoglobins and white blood cells in cats may indicate a tissue anoxia from atelectatic processes in the lungs. Oxygen tension in the abdominal cavities of cats was indeed elevated to a lesser degree than in the other animals (fig. 5).

The animals (cats not measured) all showed a normal or slightly depressed reticulocyte count. Only one reticulocyte count at an unknown point in the experiment is reported. A prussian blue study of the spleens of rats and mice showed greater pigment deposition, suggesting an excessive rate of breakdown of red blood cells. Thus, cells seemed to be hemolyzing with an inadequate reticulocyte response. The hemoglobin content per cell was slightly elevated in these animals. Carbon dioxide tensions in the abdominal cavity of all the animals were slightly elevated. The findings of possible hemolysis of red blood cells in the presence of elevated oxygen tensions in the tissues are of particular interest and will be discussed later.

In 1960 MacHattie and Rahn [108] studied the effect of nitrogen-free environments in the growth and reproduction of mice. The animals were maintained for 51 days in an atmosphere of oxygen at a total pressure of 197 mm Hg,

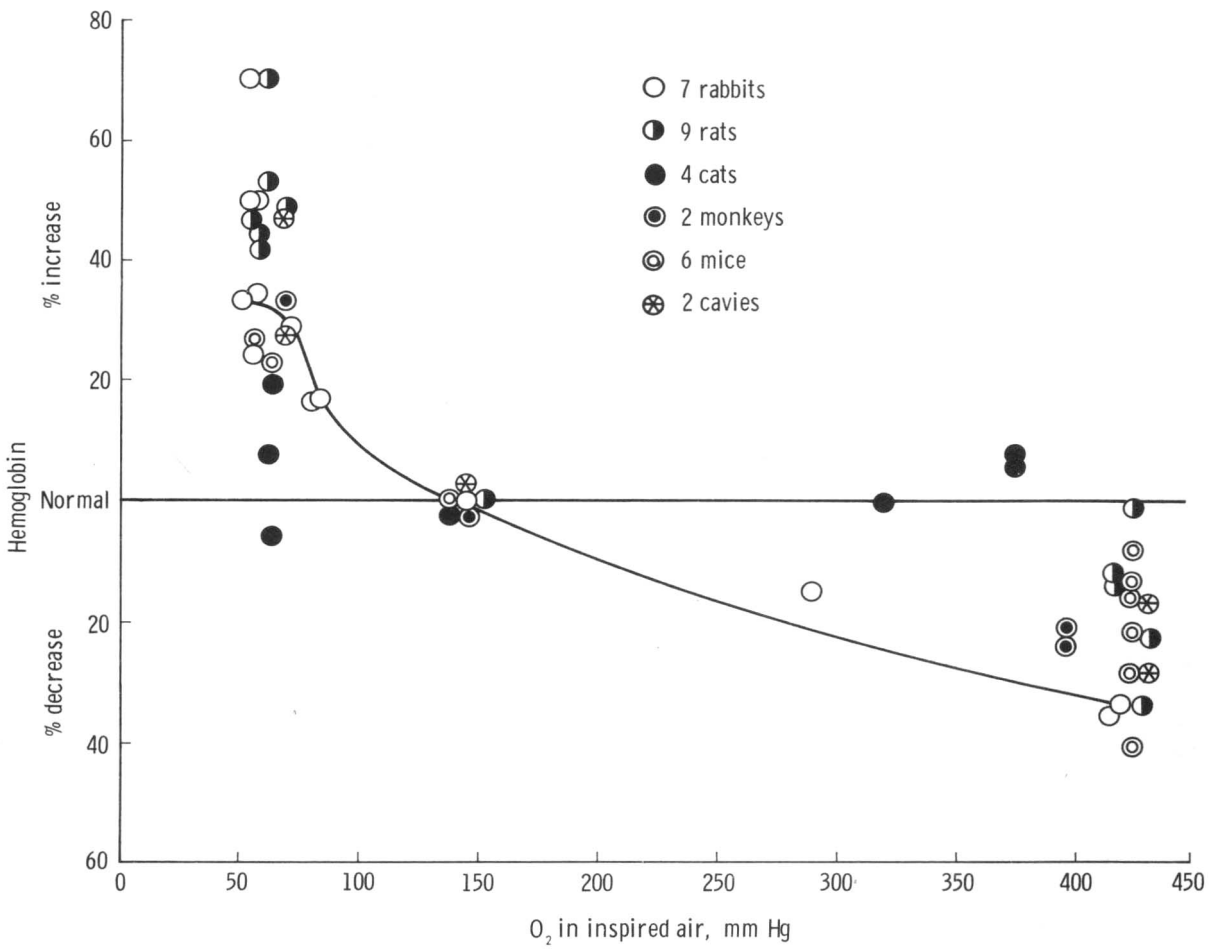

FIGURE 4.—*Relation between hemoglobin and oxygen pressure in the inspired air during prolonged exposures. The curve is drawn through points taken from one rabbit.* (AFTER CAMPBELL.[24])

742-152 O - 64 - 3

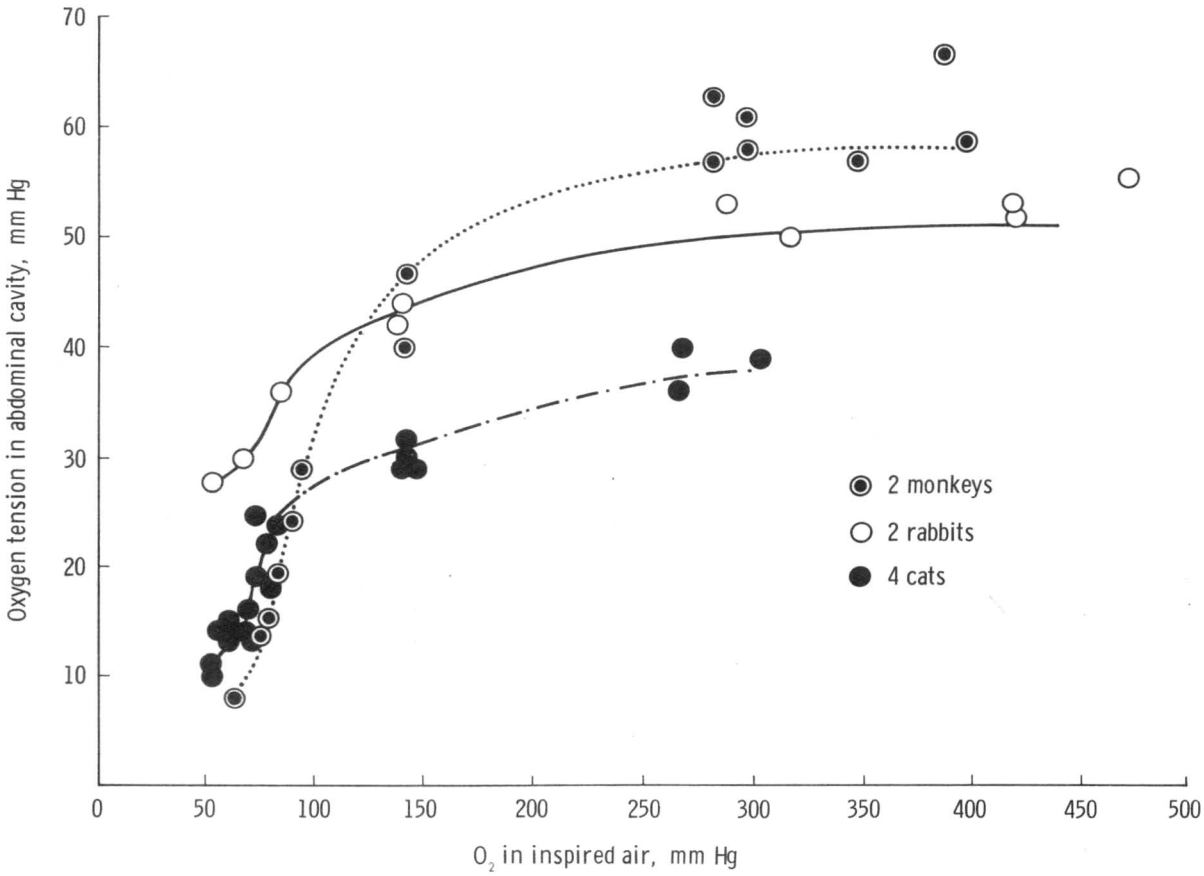

FIGURE 5.—*Relation between oxygen tensions in the abdominal cavity and oxygen pressure in the inspired air during prolonged exposures.* (AFTER CAMPBELL.[24])

providing a normal inspired oxygen tension. The carbon dioxide and nitrogen did not exceed 5 mm Hg for either gas. Under these conditions, animals appeared normal in most respects related to behavior, growth, and reproduction. In several cases, however, animals died of atelectasis within 48 hours of being placed in the chamber. Since it is more of a problem of nitrogen lack than oxygen excess, this atelectasis problem is discussed in Chapter 4.

Cook and Leon [36] have recently studied the threshold levels of p_{O_2} required for toxic effects in mice and male squirrel monkeys with temperature controlled at $25 \pm 5°$ C and relative humidity in the 87 to 91 percent range. Table 1 presents the results obtained with groups of 20 mice at each partial pressure. No purity analysis of the oxygen is reported. It would appear that the 570 to 646 mm Hg range is the threshold for mice. These results are similar to those in the older literature. Hemosiderosis was noted in the spleens of many of the animals, suggesting a hemolytic process; however, a mention that one of the controls showed the same defect tends to invalidate a hypothesis that the spleen pathology was of primarily hyperoxemic origin. Above 624 mm Hg, the classical lung pathology of OHP was found. The mice exposed to oxygen tensions of 358 and 548 mm Hg showed only occasional thickening of the alveolar membrane.

Several interesting new findings mark the autopsies of the mice exposed at 548 mm Hg. The first death occurred at 336 hours and the next one at 1,481 hours. The early death was discounted as due to "other factors." The other animals of this group demonstrated a progressive spastic type of paralysis starting at the 57th day. No damage to the anterior