2 Physical, Physiological, and Biochemical Aspects of Hyperbaric Oxygenation

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This chapter presents a basic scientific foundation detailing the important and interesting properties of oxygen, then surveys how these realities come into play under hyperbaric conditions. The sections involved are:

Introduction

Oxygen is the most prevalent and most important element on earth. A complete and in-depth discussion of the biochemical and physiological aspects of oxygen is available in Jain (1989b), but a brief description of how oxygen is transported and the basic physical laws governing its behavior will be useful for discussion in this book. The various terms frequently encountered in relation to oxygen include:

Partial pressure of a gas	p
Partial pressure of oxygen	pO_2
Partial pressure of oxygen in alveoli	pAO_2
Partial pressure of oxygen in arterial blood	paO_2
Partial pressure of oxygen in venous blood	pvO_2

Physical Basics

The atmosphere is a gas mixture containing by volume 20.94% oxygen, 78.08% nitrogen, 0.04% CO_2, and traces of other gases. For practical purposes air is considered to be a mixture of 21% oxygen and 79% nitrogen. The total pressure of this mixture at sea level is 760 millimeters of mercury (mmHg). Dalton's law states that in a gas mixture, each gas exerts its pressure according to its proportion of the total volume:

$$\text{partial pressure of a gas} = (\text{absolute pressure}) \times (\text{proportion of total volume of gas})$$

Thus, the partial pressure of oxygen (pO_2) in air is

$$(760) \times (21/100) = 160 \text{ mmHg}$$

Pressures exerted by gases dissolved in water or body fluids are certainly different from those produced in the gaseous phase. The concentration of a gas in a fluid is determined not only by the pressure, but also by the "solubility coefficient" of the gas. Henry's law formulates this as follows:

$$\text{concentration of a dissolved gas} = (\text{pressure}) \times (\text{solubility coefficient})$$

The solubility coefficient varies for different fluids and it is temperature-dependent, with solubility being inversely proportional to temperature. When concentration is expressed as volume of gas dissolved in each unit volume of water, and pressure is expressed in atmospheres, the solubility coefficients of the important respiratory gases at body temperature are as follows:
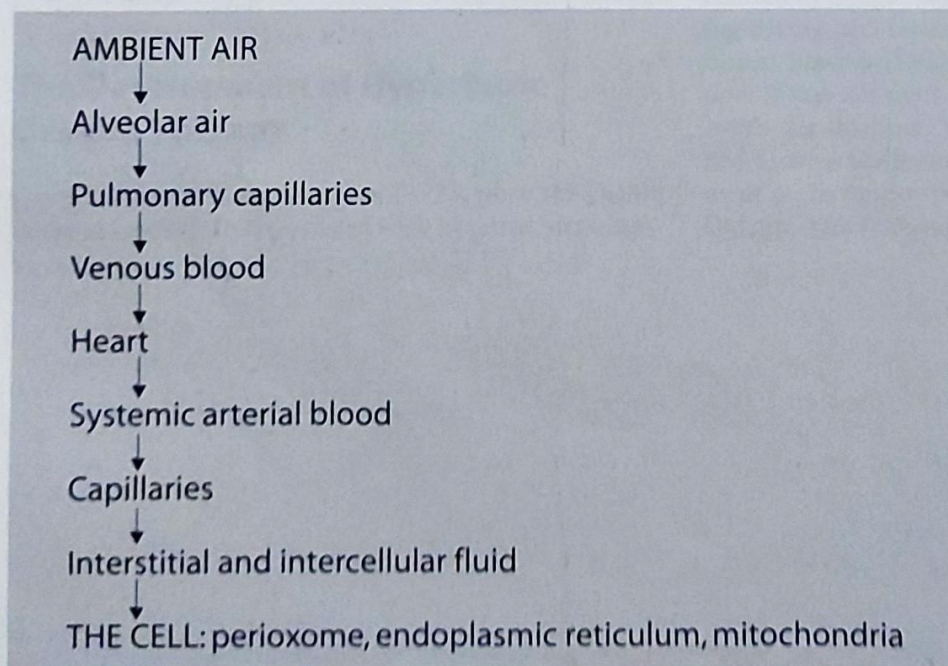
Oxygen:	0.024 ml O_2/ml blood atm pO_2
CO_2:	0.5 ml plasma/atm pCO_2
Nitrogen:	0.067 ml/ml plasma/atm pN_2

From this one can see that CO_2 is, remarkably, 20 times more soluble than oxygen.
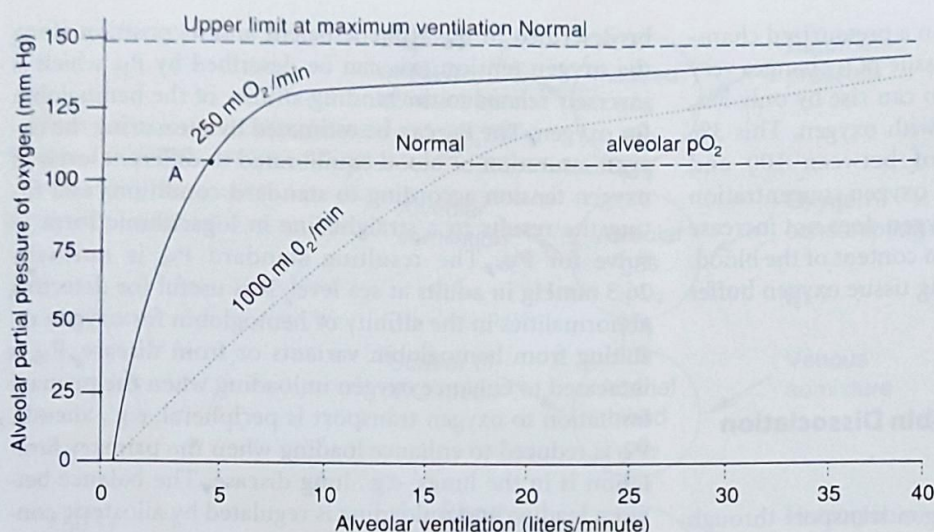
Physiology of Oxygenation

The Oxygen Pathway

The oxygen pathway is shown in Figure 2.1. It passes from the ambient air to the alveolar air and continues through the pulmonary, capillary, and venous blood to the systemic arterial and capillary blood. It then moves through the interstitial and intracellular fluids to the microscopic points,

Figure 2.1
The oxygen pathway.

AMBIENT AIR
↓
Alveolar air
↓
Pulmonary capillaries
↓
Venous blood
↓
Heart
↓
Systemic arterial blood
↓
Capillaries
↓
Interstitial and intercellular fluid
↓
THE CELL: perioxome, endoplasmic reticulum, mitochondria

of oxygen consumption in the perioxomes, endoplasmic reticulum, and mitochondria.

Ventilation Phase

Oxygen is continuously absorbed into the blood which moves through the lungs, and it thereby enters the systemic circulation. The effect of alveolar ventilation, and the rate of oxygen absorption from the alveoli on the pAO_2, are both shown in Figure 2.2.

At a ventilatory rate of 5 liters/min and oxygen consumption of 250 ml/min, the normal operating point is at A in Figure 2.2. The alveolar oxygen tension is maintained at 104 mmHg. During moderate exercise the rate of alveolar ventilation increases fourfold to maintain this tension, and about 1,000 ml of oxygen are absorbed per minute.

Carbon dioxide is being constantly formed in the body and discharged into the alveoli secretion is 40 mmHg. It is well known that the partial pressure of alveolar CO_2 (pCO_2) increases directly in proportion to the rate of CO_2 excretion, and decreases in inverse proportion to alveolar ventilation.

Transport Phase

The difference between pAO_2 (104 mmHg) and pvO_2 (40 mmHg), which amounts to 64 mmHg, causes oxygen to diffuse into the pulmonary blood. It is then transported, mostly in combination with hemoglobin, to the tissue capillaries, where it is released for use by the cells. There the oxygen reacts with various other nutrients to form CO_2, which enters the capillaries to be transported back to the lungs.

During strenuous exercise, the body oxygen requirement may be as much as 20 times normal, yet oxygenation of the blood does not suffer, because the diffusion capacity for oxygen increases fourfold during exercise. This rise results in part from the increased number of capillaries participating, as well as dilatation of both the capillaries and the alveoli. Another factor here is that the blood normally stays in the lung capillaries about three times as long as is necessary to cause full oxygenation. Therefore, even during the shortened time of exposure on exercise, the blood can still become nearly fully saturated with oxygen.

Normally 97% of the oxygen transported from the lungs to the tissues is carried in chemical combination with hemoglobin of red blood cells, and the remaining 3% in a dissolved state in plasma. It turns out that one gram of hemoglobin can combine with 1.34 ml oxygen from where it is removed continuously by ventilation. The normal concentration of hemoglobin is 15 g/100 ml blood. Thus, when hemoglobin is 100% saturated with oxygen, 100 ml blood can transport about 20 (i.e., 15×1.34) ml oxygen in combination with hemoglobin. Since the hemoglobin is usually only 97.5% saturated, the oxygen carried by 100 ml blood is actually 19.5 ml. However, in passing through tissue capillaries this amount is reduced by 14.5 ml (paO_2 40 mmHg and 75% oxygen saturation). Thus, under normal conditions, 5 (i.e. 19.5–14.5) ml of O_2 is transported to the tissues by 100 ml blood. On strenuous exercise, which causes the interstitial fluid pO_2 to fall as low as 15 mmHg, only 4.5 ml oxygen remains bound with hemoglobin in each 100 ml blood. Thus 15 (i.e. 19.5–4.5) ml oxygen is transferred by each 100 ml blood – three times the amount transferred under normal conditions. Since cardiac output can also increase up to six or seven times, for instance, in well-trained marathon runners, the end result is a remarkable 20-fold (i.e., 15×6.6 = approx. 100; 100/5 = 20) increase in oxygen transport to the tissues. This is about the top limit that can be achieved.

Hemoglobin has a role in maintaining a constant pO_2 in the tissues and sets an upper limit of 40 mmHg. It usually delivers oxygen to the tissues at a rate to maintain a

pO_2 of between 20 and 40 mmHg. In a pressurized chamber pO_2 may rise tenfold, but the tissue pO_2 changes very little. The saturation of hemoglobin can rise by only 3%, as 97% of it is already combined with oxygen. This 3% can be achieved at pO_2 levels of between 100 and 200 mmHg. Increasing the inspired oxygen concentration or the total pressure of inspired oxygen does not increase the hemoglobin-transported oxygen content of the blood. Thus, hemoglobin has an interesting tissue oxygen buffer function.

Shift of the Oxygen-Hemoglobin Dissociation Curve

Hemoglobin actively regulates oxygen transport through the oxygen-hemoglobin (oxyhemoglobin) dissociation curve which describes the relation between oxygen saturation or content of hemoglobin and oxygen tension at equilibrium. There is a progressive increase in the percentage of hemoglobin that is bound with oxygen as pO_2 increases. Bohr (1904) first showed that that the dissociation curve was sigmoid-shaped, leading Hill to postulate that there were multiple oxygen binding sites on the hemoglobin and to derive the following equation:

$$\left(\frac{\text{oxygen tension}}{\text{P50}}\right)^n = \frac{\text{oxygen saturation}}{100 - \text{oxygen saturation}}$$
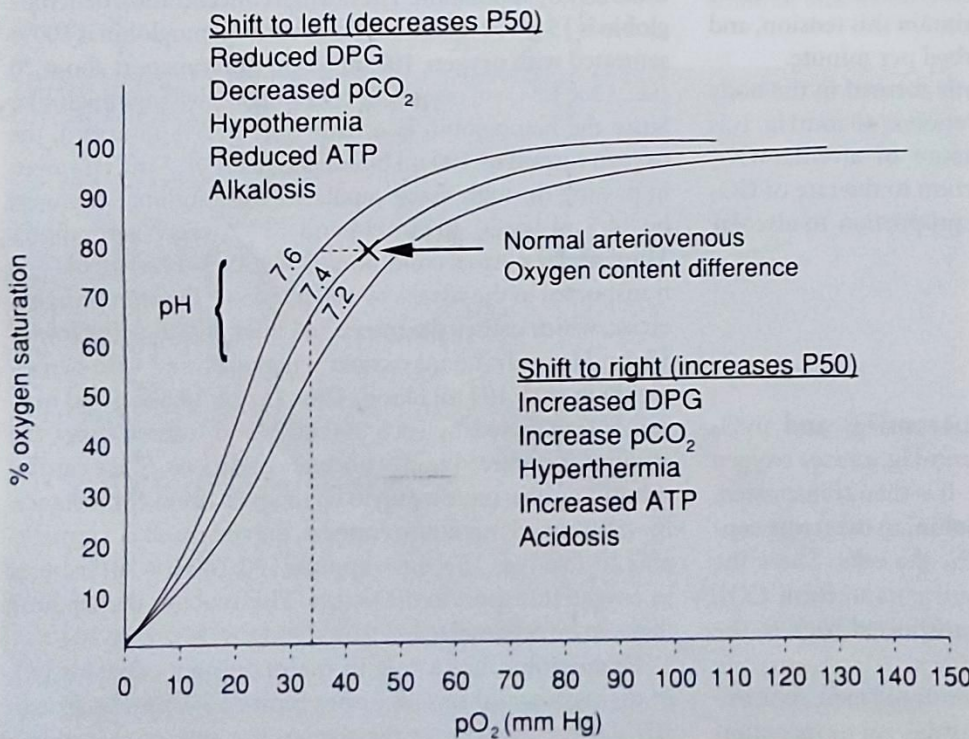
where P_{50} is the oxygen tension (in mmHg) when the binding sites are 50% saturated. Within the range of saturation between 15 and 95%, the sigmoid shape of the curve can be described in the Hill coefficient and its position along the oxygen tension axis can be described by P_{50} which is inversely related to the binding affinity of the hemoglobin for oxygen. The P_{50} can be estimated by measuring the oxygen saturation of blood equilibrated to different levels of oxygen tension according to standard conditions and fitting the results to a straight line in logarithmic form to solve for P_{50}. The resulting standard P_{50} is normally 26.3 mmHg in adults at sea level. It is useful for detecting abnormalities in the affinity of hemoglobin for oxygen resulting from hemoglobin variants or from disease. P_{50} is increased to enhance oxygen unloading when the primary limitation to oxygen transport is peripheral, e.g., anemia. P_{50} is reduced to enhance loading when the primary limitation is in the lungs, e.g., lung disease. The balance between loading and unloading is regulated by allosteric control of the P_{50} and chemoreceptor control of ventilation which is matched to diffusing capacities of the lungs and the tissues. Optimal P_{50} supports the highest rate of oxygen transport in health and disease.

A number of conditions can displace the oxyhemoglobin dissociation curve to the right or the left, as suggested in Figure 2.3.

Delivery of Oxygen to the Tissues

During transit from the ambient air to the cellular structures, the pO_2 of oxygen drops from 160 mmHg to a few mmHg in the mitochondria. This gradual drop is described as the "oxygen cascade" and is shown in Figure 2.4.

Shift to left (decreases P50)
Reduced DPG
Decreased pCO_2
Hypothermia
Reduced ATP
Alkalosis

Normal arteriovenous
Oxygen content difference

Shift to right (increases P50)
Increased DPG
Increase pCO_2
Hyperthermia
Increased ATP
Acidosis

Figure 2.3
Shift of the oxygen-hemoglobin dissociation curve. DPG, diphosphosglycerate
Source: Jain (1989b), *Oxygen in physiology and medicine*, Thomas, Springfield, by permission.
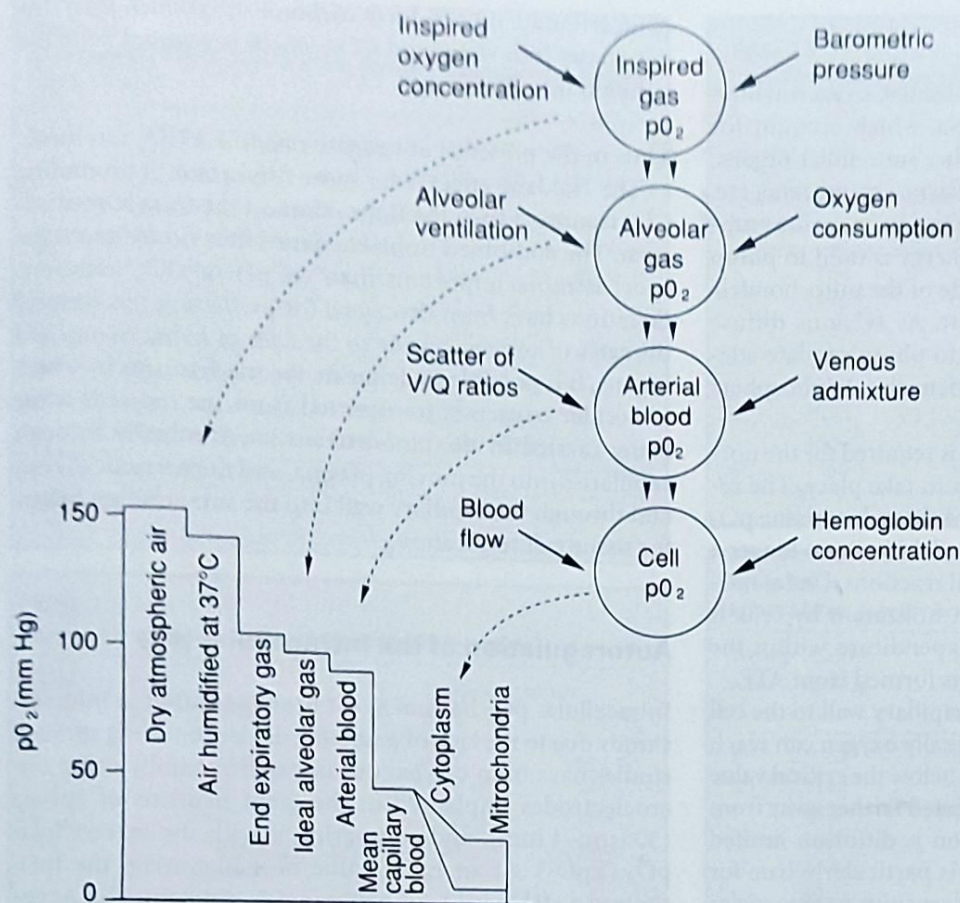
Figure 2.4 The oxygen cascade.

Oxygen Transfer at the Capillary Level

There is considerable resistance to oxygen transfer in the capillaries, and this is as significant as the resistance in the surrounding tissues.

Microvascular geometry and capillary blood flow are the most important factors responsible for regulating the oxygen supply to the tissues to meet the specific oxygen demands of organs such as the heart and brain. The tissues, of course, form the end point of the oxygen pathway. The task of the active transport system is to ensure an adequate end-capillary pO_2 so that passive diffusion of oxygen to the mitochondria is maintained.

Relation Between the Oxygen Transport and Utilization

The relationship between the transportation of oxygen and its utilization was first described long ago by Fick (1870). According to the Fick principle, oxygen consumption of the tissues (pO_2) is equal to the blood flow to the tissues (Q), multiplied by the amount of oxygen extracted by the tissue, which is the difference between the arterial and the mixed venous oxygen contents, $C(a-v)O_2$:

$$Oxygen\ Consumption\ (VO_2) = (Q) \times (C(a-v)O_2)$$

As the VO_2 of a given tissue increases, the normal response in the human body is to increase the local blood flow to the area, to maintain the local $(a-v)O_2$ content difference close to the normal range. A marked increase of $(a-v)O_2$, above 4–5 vol% is observed during physical exercise, as discussed further in Chapter 5. An increase of this magnitude in non-exercising individuals usually means an impaired circulation, inadequate to meet the increased demand of the tissues in some disease states, or it means that the oxygen content of the arterial blood is very low. The increased extraction of oxygen from the blood leads to a lower pO_2 compared to the normal level of 35–40 mmHg with O_2 saturation at 75%. Naturally the regional flow throughout the body is variable, and organs such as the heart and brain extract much more oxygen from the blood than do other organs. The brain makes up 2–3% of body weight but receives 15% of the cardiac output and 20% of the oxygen uptake of the entire body. Within the brain, cerebral blood flow and oxygen uptake vary according to the level of cerebral activity.

Oxygen Utilization in the Cell

The major site of utilization of molecular oxygen within the average cell is the mitochondria, which account for about 80%, while 20% is used by other subcellular organs, such as microsomes, nucleus, the plasma membrane, etc. Oxygen combines with electrons derived from various substrates to release free energy. This energy is used to pump H^+ ions from the inside to the outside of the mitochondria against an electrochemical gradient. As H^+ ions diffuse back, free energy is made available to phosphorylate adenosine diphosphate (ADP), and adenosine triphosphate (ATP) is generated.

Only a minute amount of oxygen is required for the normal intracellular chemical reactions to take place. The respiratory enzyme system is so geared that when tissue pO_2 is more than 1–3 mmHg, oxygen availability is no longer a limiting factor in the rate of chemical reactions. Under normal conditions, the rate of oxygen utilization by cells is controlled by the rate of energy expenditure within the cells, i.e., by the rate at which ADP is formed from ATP.

The diffusion distance from the capillary wall to the cell is rarely more than 50 µm, and normally oxygen can reach the cell quite readily. But, if pO_2 falls below the critical value of 1–3 mmHg, and if the cells are located farther away from the capillaries, the oxygen utilization is diffusion-limited and not determined by ADP. This is particularly true for cerebral white matter, which is very sensitive to hypoxia as well as hyperoxia.

Effect of Blood Flow

Since oxygen is transported to the tissues in the bloodstream, interruption of blood flow means that the amount of available oxygen to the cells also falls to zero. Under these conditions, the rate of tissue utilization of oxygen is blood-flow limited.

Effect of Oxygen-Hemoglobin Reaction on Transport of CO_2

This response, known as the Haldane effect, results from the fact that the combination of oxygen with hemoglobin causes it to become a stronger acid. This displaces CO_2 from the blood in two ways:

- When there is more acid, hemoglobin has less of a tendency to combine with CO_2 to form carbhemoglobin. Much of the CO_2 present in this form in the blood is thus displaced.
- The increased acidity of the hemoglobin causes it to release an excess of H^+ ions and these, in turn, bind with bicarbonate ions to form carbonic acid which then dissociates into water and CO_2, which is released from the blood into the alveoli.

Thus in the presence of oxygen, much less CO_2 can bind.

The Haldane effect is far more important in promoting CO_2 transport than the Bohr effect on the transport of oxygen. The combined Bohr-Haldane effect on oxygen transport is more important than on pH or CO_2 transport. Equations have been described for predicting the limits of the rates of oxygen supply to the cells of living tissues and organs. It is possible to delineate the mechanisms by which molecular oxygen is transported from the red cells while being carried in the bloodstream longitudinally through capillaries into the moving plasma, and thence radically out and through the capillary wall into the surrounding tissues for tissue cell respiration.

Autoregulation of the Intracellular pO_2

Intracellular pO_2 has not as yet been measured in humans, simply due to the lack of a suitable device for doing so. Such studies have been carried out in experimentally using microelectrodes implanted in the giant neurons of aplysia (500 µm–1 mm), and comparing it with the extracellular pO_2 (EpO_2). At an EpO_2 value of +20 mmHg, the IpO_2 showed a stable value of between 4.5 and 8 mmHg. At between 10 to 50 mmHg EpO_2, IpO_2 is kept fairly constant by "autoregulation." A simple, minimally invasive method for the analysis of intracellular oxygen in live mammalian cells is available. Loading of the cells with the phosphorescent oxygen-sensing probe, MitoXpress (Luxcel Biosciences Ltd, Cork, Ireland), is achieved by passive liposomal transfer or facilitated endocytosis, followed by monitoring in standard microwell plates on a time-resolved fluorescent reader (O'Riordan et al 2007). Phosphorescence lifetime measurements provide accurate, real-time, quantitative assessment of average oxygen levels in resting cells and their alterations in response to stimulation.

Hyperbaric Oxygenation

Theoretical Considerations

Hyperbaric oxygenation (HBO) involves the use of oxygen under pressure greater than that found on earth's surface at sea level. Units commonly used to denote barometric pressure include:

mmHg	millimeters of mercury
in Hg	inches of mercury
psi	pounds per square inch

Table 2.1
Comparison of Pressure Units in Range Encountered in Hyperbaric Therapy

ATA	Absolute mmHg (torr)	pressures (bar) Pa	fsw	msw	psi	atm
1	760	1.013	101.3	0	0	0	0
1.5	1140	1.519	151.9	16	5.16	7.35	0.5
2	1520	2.026	202.6	33	10.32	14.7	1
2.5	1900	2.532	253.2	50	15.48	21.05	1.5
3	2280	3.039	303.9	66	20.64	29.4	2
4	3040	4.052	405.2	99	30.97	44.0	3
5	3800	5.065	506.5	132	41.29	58.7	4
6	4560	6.078	607.8	165	51.61	73.3	5

Pa, kilo Pascal; Pascal (Newton per square meter) is the SI unit of choice.

Table 2.2
Range of Partial Pressures in Hyperbaric Therapy

Total pressure ATA	mmHg	Oxygen pressure ATA	mmHg
1	760	0.21	159.7
1.5	1140	0.31	239.4
2	1520	0.42	319.2
2.5	1900	0.53	394.0
3	2280	0.63	478.8
4	3040	0.84	638.4
5	3800	1.05	798.0
6	4560	1.26	957.6

Table 2.3
Ideal Alveolar Oxygen Pressures

Total pressure ATA	mmHg	pAO_2 breathing air mmHg	pAO_2 breathing 100% O_2 mmHg
1	760	102	673
1.5	1140	182	1053
2	1520	262	1433
2.5	1900	342	1813
3	2280	422	2193
4	3040	582	O_2 not adminis-
5	3800	742	tered at pressures
6	4560	902	> 3 ATA

Table 2.4
Effect of Pressure on Arterial O_2

Total pressure ATA	mmHg	Ideal dissolved oxygen content (vol%) Breathing air	Breathing 100% O_2
1	760	0.32	2.09
1.5	1140	0.61	3.26
2	1520	0.81	4.44
2.5	1900	1.06	5.62
3	2280	1.31	6.80
4	3040	1.80	O_2 not adminis-
5	3800	2.30	tered at pressures
6	4560	2.80	> 3 ATA

Values assume arterial pO_2 = alveolar pO_2 and that hemoglobin oxygen capacity of blood is 20 vol%.

kg/cm²	kilograms per square centimeter
bar	bar
fsw, msw	feet or meters of sea water
atm	atmospheres
ATA	atmospheres absolute

The only absolute pressures are those measured by a mercury barometer. In contrast, gauge pressures are a measure of difference between the pressure in a chamber and the surrounding atmospheric pressure. To convert pressure as measured by a gauge to absolute pressure (ATA) requires addition of the barometric pressure. A guide to these conversions is shown in Table 2.1. The range of partial pressures of oxygen under HBO is shown in Table 2.2, and the ideal alveolar oxygen pressures are shown in Table 2.3.

Boyle's well-known law states that if the temperature remains constant, the volume of a gas is inversely proportional to its pressure. Therefore, normal or abnormal gas-containing cavities in the body will have volume changes as HBO therapy is applied.

Density

As barometric pressure rises there is an increase in the density of the gas breathed. The effect of increased density on resting ventilation is negligible within the range of the 1.5–2.5 ATA usually used in HBO. However, with physical exertion in patients with decreased respiratory reserves or respiratory obstruction, increased density may cause gas flow problems.

Temperature

The temperature of a gas rises during compression and falls during decompression. According to Charles' law, if the volume remains constant, there is a direct relationship between absolute pressure and temperature.
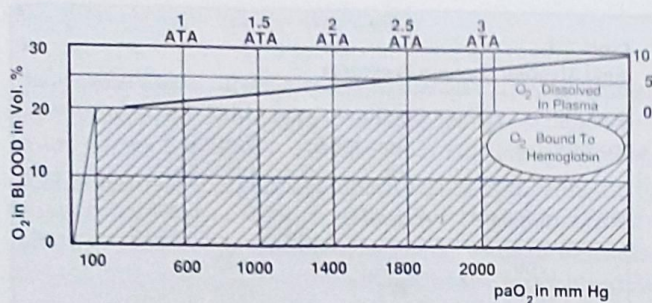
Figure 2.5
Oxygen uptake curve under HBO in humans.

Effect of Pressure on Oxygen Solubility in Blood

Only a limited amount of oxygen is dissolved in blood at normal atmospheric pressure. But, under hyperbaric conditions, as seen in Table 2.4, it is possible to dissolve sufficient oxygen, i.e., 6 vol% in plasma, to meet the usual requirements of the body. In this case oxyhemoglobin will pass unchanged from the arterial to the venous side because the oxygen physically dissolved in solution will be utilized more readily than that bound to hemoglobin. The typical arterial oxygen uptake under HBO is shown in Figure 2.5. Here the usual oxygen dissociation curve has been extended to include increases in oxygen content as a result of inspiring oxygen up to 3 ATA. The pO_2 simply rises linearly with rise of pressure.

Effect of HBO on Capillary Oxygen Pressure Drop

The oxygen extraction by average tissues of 5 vol% results in a remarkable pressure drop of 60 (100 down to 40) mmHg from the arterial end to the venous end of the capillary. At 2,000 mmHg the oxygen content is approximately 25 (20+5) vol%. The extraction of 5 vol% in this case causes a pressure drop of about 1,900 mmHg. Each of the differences in pO_2 represents the same number of oxygen molecules, in the first case carried by the hemoglobin and in the second case by the plasma. The metabolic requirement of the cells can ultimately be expressed as a certain number of molecules of oxygen per minute.

HBO and Retention of CO_2

When HBO results in venous blood being 100% saturated with oxygen, there is a rise in blood pCO_2 and a shift of pH to the acid side. This is due to loss of hemoglobin available to transport CO_2. This affects only the 20% of the venous content of CO_2 which is transported by hemoglobin. Excess CO_2 is transported by the H_2CO_3/HCO_3 mechanism, as well as by entering into physical solution in plasma. The elevation of cerebral venous pCO_2 is of the order of 5–6 mmHg when venous hemoglobin is 100% saturated with oxygen. CO_2 does not continue to rise in venous blood and the tissues as long as the blood flow remains constant, and presents no major problems.

Tissue Oxygen Tension under HBO

Various factors relating to tissue oxygen tension under HBO are:

- Arterial pO_2 is the maximum pO_2 to which any tissue will be exposed, and plays a major part in determining the pO_2 diffusion gradient driving oxygen into the tissues. Arterial pO_2 depends on the inspired pO_2.
- Arterial pO_2 content is the total amount of oxygen available. It depends on the inspired oxygen and the blood hemoglobin level.
- Tissue blood flow regulates the delivery of oxygen to the tissues.
- Tissue oxygen levels vary according to utilization of the available oxygen.

In a *typical* tissue, arteriovenous oxygen difference rises to 350 mmHg when 100% oxygen is breathed at 3 ATA. If the blood flow to the tissues is reduced by half, the corresponding values of capillary pO_2 will be 288 mmHg and 50 mmHg. But, of course, the oxygen requirement of different tissues varies. For example, the needs of cardiac muscle are ten times that of the skin.

Another factor is the vasoconstricting effect of HBO, which reduces the blood flow. Effective cellular oxygenation can be accomplished at very low rates of blood flow when arterial pO_2 is very high.

General Effects of HBO on the Healthy Human Body

The important general effects of hyperoxia on a healthy human body are listed in Table 2.5. The effects vary according to the pressures used, the duration of exposure, and the health of the subject. Unless otherwise stated, HBO refers to the use of 100% oxygen. The effects of HBO on each system, both in health and in disease, will be discussed in chapters dealing with disorders of those systems. As an introduction, some important effects are described here briefly.

Table 2.5
Effects of Hyperoxia

I. Oxygen transport and metabolism
   1. Inactivation of the hemoglobin role in oxygen and CO_2 transport
   2. The biological burning of normal oxygen metabolism

II. Respiratory system
   1. Suppression of carotid and aortic bodies with depression of ventilation
   2. Washout of N_2 with increased susceptibility to lung collapse

III. Cardiovascular system
   1. Bradycardia
   2. Decreased cardiac output and decreased cerebral blood flow

IV. Peripheral vessels
   1. Vasoconstriction of peripheral blood vessels: brain, kidney, eye
   2. Increased peripheral resistance

V. Metabolic and biochemical
   1. Increase of CO and H ions with decrease of PH in tissues
   2. Inhibition of cell respiration
   3. Inhibition of enzymatic activity: enzymes containing SH group
   4. Increase of free radical production

Cardiovascular System

The most important study on this subject is that by Bergö (1993) which was conducted on awake rats exposed to 100% oxygen at pressures ranging from 1–5 ATA. The cardiovascular observations were made as a background to the study of the effect of HBO on cerebral blood flow. Some of the conclusions drawn were as follows:

1. Increase of systolic blood pressure with fall of diastolic pressure. Although pulse pressure was increased, the mean arterial blood pressure remained constant.
2. Decrease of heart rate and cardiac output.
3. The number of cardiac arrhythmias increased with rising oxygen pressures and exposure duration.
4. Increase of peripheral vascular resistance

In human patients, HBO results in a decrease in cardiac output (CO), due to bradycardia, rather than a reduction in stroke volume. Blood pressure remains essentially unchanged. Blood flow to most organs falls in proportion to the fall of cardiac output except to the right and the left ventricles of the heart. There is no impairment of the function of any of these organs because the raised pO_2 more than compensates for the reduction of the blood flow. Vasoconstriction may be viewed as a regulatory mechanism to protect the healthy organs from exposure to excessive pO_2. Usually the vasoconstrictor response does not take place in the hypoxic tissues.

Dermal blood flow has been shown to decrease as a response to hyperoxia; it has been measured by laser Doppler flowmetry. It was also demonstrated that the reduction of blood flow did not occur in the vicinity of a chronic skin ulcer and that the vasoconstrictor response was restored after the ulcer had healed.

HBO is considered to modify fibrinolytic activity in the blood. To clarify the stage of fibrinolytic activation by HBO exposure, Yamami et al (1996) examined its alterations in human during and after the HBO exposure. Eight healthy female volunteers breathed oxygen at 284 kPa (2.8 ATA). Blood samples were collected before compression, shortly after compression to the pressure 284 kPa, shortly before the start of decompression, shortly after decompression, and then again 3 hours after decompression. The euglobulin fibrinolytic activity (EFA) and, the activities and antigens of both tissue-type plasminogen activator (t-PA) and plasminogen activator inhibitor-1 (PAI-1) were estimated. The PAI-1 activity and PAI-1 antigen showed significant decrease after compression to a pressure 284 kPa, before the start of decompression, and after decompression. The EFA level and t-PA activity rose significantly shortly after decompression, and 3 hours later returned on baseline. These findings suggest that fibrinolytic activity is elicited after HBO rather than during HBO.

Respiratory System

Hyperoxia suppresses the respiratory reactivity to CO_2. After an initial depression of respiration, there is hyperventilation. HBO reversibly depresses the hypoxic ventilatory drive, most probably by a direct effect on the carotid CO_2 chemoreceptors.

Usually there are no differences between forced vital capacities (FVC) and maximal expiratory flows before and after hyperbaric oxygen exposure while breathing dry or humidified oxygen. However, decreases in mean expiratory flow with steady FVC have been reported after 14 days of daily hyperbaric therapy (0.24 MPa) with although 80% of the patients were symptom free and remained so 1 year after the study (Mialon et al 2001). This toxicity is clinically insignificant in subjects free of inflammatory lung diseases. HBO therapy, though safe, is not entirely without effect on the lungs.

Nervous System

Vasoconstriction and reduced cerebral blood flow do not produce any clinically observable effects in a healthy adult when pressures of 1.5 to 2.5 ATA are used. Pressures higher than 3 ATA for prolonged periods can lead to oxygen convulsions as a result of oxygen toxicity. The effects of HBO are more pronounced in hypoxic/ischemic states of the brain. HBO reduces cerebral edema and improves the function of neurons rendered inactive by ischemia/hypoxia. The improvement of brain function is reflected by the improved electrical activity of the brain. The effect of HBO on cerebral blood flow is discussed in Chapter 17.

Microcirculation

HBO improves the elasticity of the red blood cells and reduces platelet aggregation. This, combined with the ability of the plasma to carry dissolved oxygen to areas where RBCs cannot reach, has a beneficial effect on the oxygenation of many hypoxic tissues in various circulatory disorders.

Biochemical Effects of HBO

Biochemical Marker of HBO

Urine methylguanidine (MG) which is known as a uremic toxin is synthesized from creatinine. Urine MG/urine creatinine/serum creatinine ratio is used as an index of MG synthesis rate which has been shown to increase during HBO therapy in human subjects and can be used as a marker of active oxygen products in vivo (Takemura *et al* 1990).

Effect of HBO on the Acid-Base Balance

Increased partial pressure of oxygen in the blood disturbs the reduction of oxyhemoglobin to hemoglobin. Of the alkali that neutralizes the transported CO_2, 70% originates from the hemoglobin. As a result of HBO and due to increased solubility of CO_2, there is retention of CO_2 leading to a slight rise of H^+ ions in the tissues.

HBO reduces excess lactate production in hypoxic states, as well as during exercise. This important subject is discussed extensively in Chapter 5.

Effect of HBO on Enzymes

Cyclo-Oxygenase Inactivation. This results in decreased production of prostacyclin by hyperoxic tissues. A study was made in human umbilical arteries by Yamaga *et al* (1984) who showed that brief exposure of arteries to hyperoxia resulted in a 30% decrease in activity of cyclo-oxy-genase, in contrast to a 49% increase in its activity throughout the hypoxic arterial segments.

Heme oxygenase (HO). This enzyme catalyzes the rate-limiting step in the oxidative degradation of heme to biliverdin. The isoform HO-1 is inducible by a variety of agents causing oxidative stress and has been suggested to play an important role in cellular protection against oxidant-mediated cell damage. A low-level overexpression of HO-1 induced by HBO exposure provides protection against oxidative DNA damage by further exposures to HBO (Rothfuss & Speit 2002).

Tyrosine Hydroxylase. Increased oxygen saturation of this enzyme leads to increased turnover of catecholamines..Hyperoxia inhibits phenylalanine and tyrosine hydroxylase.

Succinic Dehydrogenase (SDH) and Cytochrome Oxidase (CCO). These enzymes are activated by HBO. Their levels decline in the liver and kidneys of patients with intestinal obstruction. HBO after surgery led to the normalization of the levels of these enzymes.

Effect of HBO on Free Radical Production

The role of hyperbaric oxygen (HBO) therapy in free radical-mediated tissue injury is not clear. HBO has been shown to enhance the antioxidative defense mechanisms in some animal studies, but HBO has also been reported to increase the production of oxygen free radicals. Hyperoxia causes an increase in nitric oxide (NO) synthesis as part of a response to oxidative stress. Mechanisms for neuronal nitric oxide synthase (nNOS) activation include augmentation in the association with Hsp90 and intracellular entry of calcium (Thom *et al* 2002).

Effect of HBO on Cerebral Metabolism

The most important metabolic effects of HBO are on the brain. Most of the investigations of this topic have been prompted by the problem of oxygen toxicity. It is believed that the preconvulsive period of oxygen toxicity is characterized by alterations in several interrelated physiological functions of the brain, such as electrical activity, blood flow, tissue pO_2, and metabolic activity. The relation of these changes to the development of oxygen-induced convulsions has not yet been clarified. Nonetheless, several interesting observations have been made as a result of these studies which throw some light on the effect of HBO on cerebral metabolism in the absence of clinical signs of oxygen toxicity. Most of the cerebral metabolic studies are now done on human patients with various CNS disorders. Use of brain imaging in metabolic studies is described in Chapters 17, 19, and 44.

Glucose Metabolism

Studies on regional cerebral glucose metabolic rate (rCMRgl) in rats after exposure to pressures of 1, 2, and 3 ATA. show that the degree of central nervous system effects of HBO depend upon the pressure as well as the duration of exposure. Increased utilization of glucose in some neuronal structures precedes the onset of central nervous system manifestations of oxygen toxicity. Exposure of rats to 100% oxygen at 3 ATA causes an increase in rCMRgl, and this is related to the oxygen-induced preconvulsive pattern of the electrocorticogram.

In cats HBO (3 ATA for 60 min), has a definite effect on the glycerophosphate shuttle mechanism following acute blood loss. HBO stimulates the mitochondrial glycerol-3-phosphate dehydrogenase in the sensorimotor cortex and the medulla oblongata, providing glycerol-3-phosphate dehydrogenation. There is activation of the cytoplasm hydrogen delivery to the mitochondrial respiratory chain. In addition, there is prevention of a rise in glycerol-3-phosphate and NADH levels, as well as inhibition of glycerol-3-phosphate dehydrogenase, which limits lactate production.

Energy metabolism has also been found to be highly sensitive to raised pressures of oxygen, which can reduce the formation of ATP molecules considerably.

Ammonia Metabolism

Following injury to the brain, the activity of glutaminase increases sharply, providing a release of ammonia from glutamine and a rise in transcapillary transfer of ammonia into the brain tissue from the blood. At the same time there is activation of glutamate formation pathways under the effect of glutamine dehydrogenase, and decrease of glutamine formation due to inhibition of glutamine synthase. This also leads to a decrease in the amount of α-ketoglutarate. HBO at 3 ATA for 60 min prevents ammonia toxicity from increasing in the dehematized brain. The toxic effects of ammonia ions on the brain are eliminated via:

- stimulation of the activity of the mitochondrial GDG providing glutamate formation from α-ketoglutarate
- binding of ammonia with glutamate resulting in glutamine formation
- a decrease of glutaminase activity inhibiting the process of deaminization of glutamine – a potential source of ammonia
- transcapillary discharge of ammonia in the form of glutamine from the brain to the blood

Effect of HBO at Molecular Level

Effect on DNA

Dennog et al (1996) have investigated the DNA-damaging effect of HBO with the alkaline version of the single cell gel test (comet assay). Oxidative DNA base modifications were determined by converting oxidized DNA bases to strand breaks using bacterial formamidopyrimidine-DNA glycosylase (FPG), a DNA repair enzyme, which specifically nicks DNA at sites of 8-oxo-guanines and formamidopyrimidines. HBO treatment under therapeutic conditions clearly and reproducibly induced DNA damage in leukocytes of all test subjects investigated. Increased DNA damage was found immediately at the end of the treatment, while 24 h later, no effect was found. Using FPG protein the authors detected significant oxidative base damage after HBO treatment. DNA damage was detected only after the first treatment and not after further treatments under the same conditions, indicating an increase in antioxidant defenses. DNA damage did not occur when the HBO treatment was started with a reduced treatment time which was then increased stepwise. Speit et al (1998) have shown that HBO-induced DNA strand breaks and oxidative base modifications are rapidly repaired, leading to a reduction in induced DNA effects of > 50% during the first hour. A similar decrease was found in blood taken immediately after exposure and post-incubated for 2 h at 37°C in vitro and in blood taken and analyzed 2 h after exposure, suggesting similar repair activities in vitro and in vivo. When the same blood samples showing increased DNA damage after HBO in the comet assay were analyzed in the micronucleus test, no indications of induced chromosomal breakage in cultivated leukocytes could be obtained. The results suggest that the HBO-induced DNA effects observed with the comet assay are efficiently repaired and are not manifested as detectable chromosome damage.

Conclusions

The practical significance of many of the general effects of HBO is not clear. The study of the effects on cerebral metabolism was motivated by a search for the mechanism of oxygen-induced seizures. High pressures such as 6 ATA have been used which have no clinical relevance; the pressures for treatment of cerebral disorders usually do not exceed 2 ATA. The optimal pressure for treating patients with brain injury is 1.5 ATA. The cerebral glucose metabolism is balanced at this pressure. Raising the pressure even only to 2 ATA has unfavorable effects.

Generally HBO therapy is safe and well tolerated by humans at 1.5–2 ATA. The duration of exposure and the percentage of oxygen also have a bearing. No adverse effects are seen at 1.5 ATA for exposures up to 40–60 min.