Chapter 2
From the Atmosphere to the Mitochondrion: The Oxygen Cascade

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2.1 Introduction

Oxygen is essential for energy metabolism whereby the energy trapped in chemical bonds in food stuffs is transferred in a series of stepwise chemical reactions to be available for all cellular functions in the form of high-energy phosphate compounds (adenosine triphosphate and creatine phosphate). Whereas there are energy-generating processes that do not require oxygen, their contribution to the total energy homeostasis is minimal and is of limited applicability.

Food stuffs in the form of carbohydrates and fats are utilized by glycolysis, fatty acid oxidation and the citric acid (Krebs) cycle to generate initially the high energy phosphate compounds NADH and FADH in mitochondria. These are energy-rich because they contain a pair of electrons with high transfer potential. When these electrons are transferred to oxygen, energy is liberated. This energy is trapped with high efficiency in the form of ATP (adenosine triphosphate) by the process of oxidative phosphorylation.

ATP is used in an enormous variety of biological processes comprising both maintenance functions at the basal level (e.g. maintenance of cellular ion channels and gradients) and in energy consuming processes involving external work (such as muscular exercise). Because of the diversity of the intensity of the processes involving external work of the organism, a wide range of energy requirements need to be met. Hence, the oxygen transport system needs to deliver oxygen to organs and cells at rates that accommodate the widely varying metabolic requirements.

The requirements of the oxygen transport system from the atmosphere to all organs, tissues, cells and mitochondria include (Pennock and Attinger 1968):

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1. The processes need to be energy-efficient such that work done by cardiac and respiratory muscles is not wasteful and represents a relatively small proportion of the organism's total energy output.
2. The process needs to be sensitive to fluctuating demands of organs and cellular metabolic activity.
3. The process needs to be responsive to varying metabolic demands of different organs and be capable of matching distribution of blood flow regionally, to various organs and cells according to their function and metabolic demands.
4. The process needs to be efficient in allowing oxygen to penetrate from blood to metabolizing cells and to their mitochondria by diffusion (Pennock and Attinger 1968; Leach and Treacher 1992; Leach and Treacher 1998).

Various cells in various organs utilize oxygen at highly variable rates (Wagner, Venkataraman et al. 2011), and total body oxygen consumption varies over a ten-fold range from rest to maximal levels of exercise (Wagner 2011). The oxygen transport system needs to respond accordingly. When the system fails to supply oxygen to meet the prevailing demand, a state of *hypoxia* is said to exist. It can take a variety of forms which will be explored below.

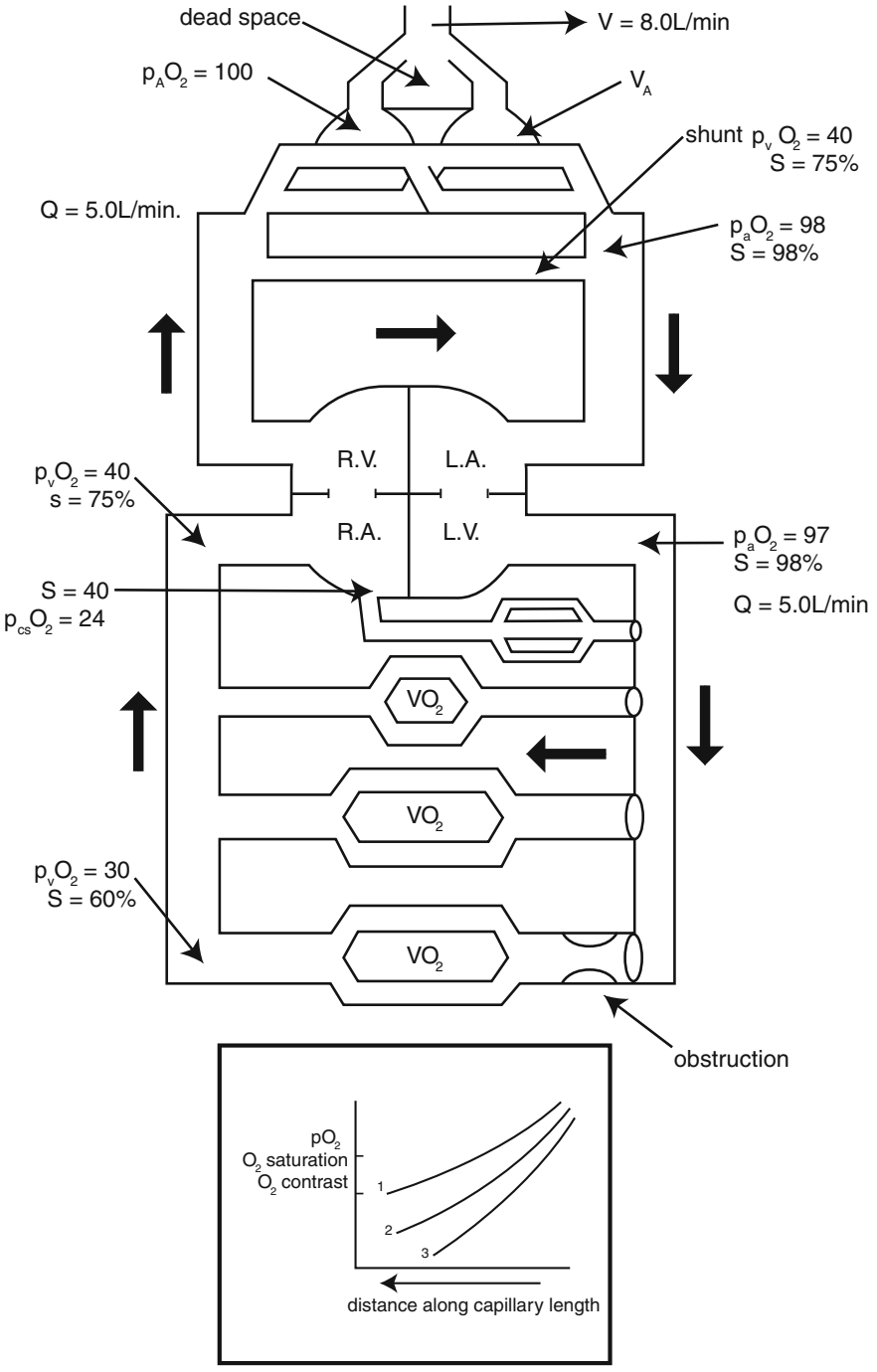
The whole system is schematically illustrated in Fig. 2.1.

2.2 The Components of the Oxygen Transport System

The oxygen transport system comprises the following consecutive processes (Leach and Treacher 1992; Treacher and Leach 1998; Schober and Schwarte 2012):

1. Mass transport by active convection of atmospheric air from the environment to the pulmonary alveolar spaces, powered by the contraction/relaxation cycling of the respiratory muscles whose action is regulated mainly by the medullary and pontine respiratory centers and peripheral chemoreceptors.
2. Passive diffusion across the alveolo-capillary membrane, through the plasma and across the erythrocyte membrane and binding to hemoglobin (HGb) "driven" by a partial-pressure gradient for oxygen ($p_AO_2 - pcO_2$).[1]
3. Mass transport by active convection of blood from the alveolar capillaries and the left heart through the vascular distribution system to all systemic capillaries, and return to the right heart, powered by the contraction/relaxation cycling of the myocardium, regulated by the autonomic nervous system, various hormones, vasoactive peptides, prostanoids and nitric oxide (NO) and other local vascular regulatory functions affecting the distribution of blood flow.

[1] The subscripts represent the following: A: alveolar; a: arterial; B: barometric; c: capillary; H2O: water vapor; v: venous.


dead space
V = 8.0L/min
p_AO_2 = 100
V_A
shunt p_vO_2 = 40
S = 75%
Q = 5.0L/min.
p_aO_2 = 98
S = 98%
R.V.
L.A.
p_vO_2 = 40
s = 75%
R.A.
L.V.
p_aO_2 = 97
S = 98%
S = 40
$p_{cs}O_2$ = 24
Q = 5.0L/min
VO_2
VO_2
p_vO_2 = 30
S = 60%
VO_2
obstruction
pO_2
O_2 saturation
O_2 contrast
1
2
3
distance along capillary length

◀ **Fig. 2.1** Conceptual schematic of the oxygen transport system comprising the respiratory and circulatory components. The figure includes some of the important, conventionally accepted values of oxygen partial pressures, blood hemoglobin oxygen saturations and gross flow rates of air and blood. In the respiratory system, alveolar ventilation, dead space and shunt components are shown. The schematic illustrates four representative systemic vascular beds, including that of the coronary circulation, showing its high extraction rates of oxygen extraction, and three other representative vascular beds in which oxygen is consumed and extracted from the blood. The bottom vascular bed illustrates one with major obstructions (e.g. atherosclerotic plaque) limiting its flow rates. The insert at the bottom illustrates conceptually the decrements of pO2, oxygen saturation and oxygen content along an idealized capillary under three sets of conditions: 1. normal perfusion; 2. limited perfusion relative to the tissue's oxygen consumption; and 3. inadequate perfusion resulting complete depletion of oxygen before reaching the downstream end of the capillary, resulting in hypoxia of some mass of cells. The abbreviations are: S: oxygen saturation, Q: cardiac output; V: ventilation; VO2: oxygen consumption; subscripts: a: arterial; A: alveolar; v; venous; cs: coronary sinus. RA: right atrium; RV: right ventricle; LA: left atrium; LV: left ventricle.

4. Passive diffusion from the capillary blood across the plasma membrane to the interstitial space, across the cell membrane, throughout the cytoplasm, facilitated by myoglobin (MGb) where present, and into the mitochondria driven by a partial pressure gradient for oxygen (mean capillary pO_2 – mean mitochondrial pO_2).

There is a critical link between the convective and diffusive phases in the respiratory processes and between the convective and diffusive phases in the circulatory processes. This link is the *oxy-hemoglobin equilibrium relationship,* represented by the oxygen dissociation curve of hemoglobin (ODC), illustrated in Fig 2.2.

The physiological importance of the oxygen dissociation curve

Oxygen is dissolved in water governed by a simple linear relationship between oxygen concentration and the partial pressure of oxygen to which the water is exposed (Henry's law). The solubility coefficient of oxygen in water at 37 °C is 0.03 ml/dL/mm Hg. Thus at normal sea-level atmospheric pO_2 is about 150 mm Hg, this would result in oxygen concentration in water at body temperature of about 4.4 mL/L (or in more conventional terms 0.44 mL/dL, or 0.44 volume %). This would not permit survival of a human organism. The evolution of hemoglobin with its ability to reversibly bind oxygen to its Fe^{++} iron moiety represents an enormous evolutionary adaptation to homeothermic existence and the ability to meet a wide range of rates of oxygen delivery to meet demands. A more comprehensive exploration of the biochemistry of hemoglobin is presented in a later chapter of this book by Mozzarelli.

Hemoglobin combines proteins (two alpha chains and two beta globin chains) and four porphyrin rings each containing an iron atom within a hydrophobic "pocket" and this arrangement greatly modifies the properties of the iron. When hemoglobin is exposed to very high pO_2 all the oxygen binding sites become oxygenated, i.e. binding oxygen molecules. When this occurs the hemoglobin becomes "*fully saturated"* and each *gram* of hemoglobin binds 1.34 ml of oxygen; this is said to be the *"oxygen capacity"* of hemoglobin. At the normal blood

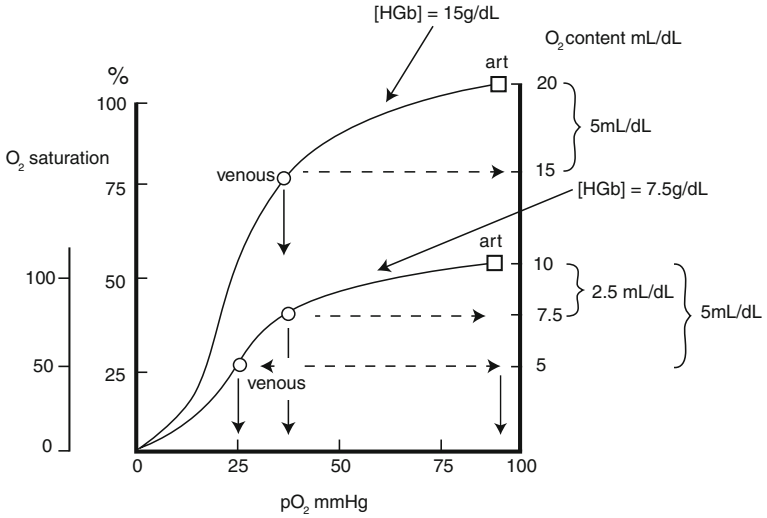


Fig. 2.2 A conceptual illustration of the oxygen dissociation curves of normal ([HGb] = 15 g/dL) and anemic ([HGb]=7.5 g/dL) blood. Notice that under "normal" conditions at a given flow rate 5 mL of oxygen can be extracted from 100mL of blood resulting in 25% desaturation at a venous pO2 of about 40 mmHg (the presumptive "normal"). Notice also that in the case of anemic blood the extraction of only 2.5 mL oxygen from 100 mL of blood will result in the similar venous blood parameters as in the "normal" case. In order to extract 5 mL of oxygen from 100 mL of perfusing blood requires a lower venous end tissue pO2 and saturation; i.e. greater desaturation. This would only be compensated for by increased blood flow. This is the fundamental basis of the problem in anemia potentially resulting in tissue hypoxia.

hemoglobin concentration of 150 g/L (or more conventionally expressed as 15 g/dL) blood fully saturated with oxygen has an oxygen concentration or *content* of approximately 20 mL/dL. When the blood hemoglobin concentration changes, its oxygen *content* also changes in proportion, but the oxygen capacity of each gram of HGb remains the same. Exposure of HGb to lower pO_2 environments results in the "detachment" of some of the oxygen bound, and this is governed by the *Oxygen Dissociation Curve (ODC).*

Oxygen becomes bound to the iron in a sequential manner such that the binding to the first iron atom promotes oxygen binding to the second, and so on, and that the binding to the fourth iron atom requires a greater increment of pO_2. The process of unbinding is the exact reverse of this process. These phenomena underlie the "sigmoid shape" of the oxy-hemoglobin dissociation curve (ODC). When oxygen saturation is plotted against the pO_2 the curve is initially rising steeply but flattens out as the pO_2 rises towards about 70 mm Hg or above, as shown in Fig. 2.1.

Two important advantages follow from this, namely that

1. When blood passes through the pulmonary capillaries it is exposed to the normally high pO_2 prevailing in the alveoli (normally about 100 mm Hg at sea level). This is sufficient to nearly fully saturate the hemoglobin. The fact that

the ODC is quite flat in the alveolar pO_2 ranges means that even at lower than normal alveolar pO_2's hemoglobin is only slightly desaturated. More importantly, the nature of the oxy-hemoglobin equilibrium relationship greatly facilitates the diffusion of oxygen across the alveolar-capillary wall, because as oxygen diffuses into the plasma, it immediately diffuses into the red cell where it becomes avidly bound to HGb. As a result, the plasma pO_2 remains low and there remains a large residual alveolar air-to-plasma pO_2 gradient while the intra-erythrocytic pO_2 remains low, despite the fact that a substantial amount of oxygen has been added to the HGb component. All of this favors the continuing diffusion of oxygen from alveolar air to capillary plasma and to the red cell HGb. Thus, far more oxygen can be loaded onto and transported by hemoglobin than in simple aqueous solution in blood.

2. When blood passes through systemic capillaries which are surrounded by actively metabolizing cells, this creates a low pO_2 environment. At these ranges of pO_2 the ODC is quite steep and any small decrease in pO_2 results in large decrement in oxygen saturation and release of oxygen first into the plasma and then out to the tissue cells. Here the amplification of diffusion described above is reversed. As oxygen leaves the plasma, pO_2 falls and that causes intra-erythrocytic pO_2 to fall, as well; the steep part of the ODC ensures that a relatively large volume of oxygen becomes detached from hemoglobin while still keeping the intra-erythrocytic pO_2 relatively high. The oxygen exiting the plasma phase is replaced by oxygen desaturation of the HGb within the red cell, thereby maintaining the plasma phase pO_2 still high required for continued outward diffusion of oxygen.

The "standard" oxyhemoglobin equilibrium relation (defined at T = 37 °C, pH = 7.4 and pCO_2 = 40 mm Hg) is defined as the oxygen saturation of hemoglobin at 50 %, known as the p50, or the oxygen saturation at the midpoint of the saturation scale, or the pO_2 required to *half-saturate* HGb. The normal value is approximately 27 mm Hg. The mathematical approximation of the ODC is the Hill equation (see Eq. 2.6 below). While the shape of the curve remains unchanged, a number of factors can modify the *position* of the curve by moving it horizontally either to the right or the left along the pO_2 axis. This comes about because of a quantitative change in the *affinity* of hemoglobin to oxygen. The important physiological modifiers of the ODC are temperature, pH, CO_2 concentration (pCO_2) and the intra-erythrocytic concentration of organic phosphates, particularly 2,3-diphospho-glycerate (2,3-DPG). Increases in each of temperature, pCO_2 and a decrease in pH result in a rightward shift, i.e. an increase in the pO_2 required to half-saturate HGb, or an increase in p50, also referred to as decreased affinity. A convenient mnemonic is that events occurring in exercising muscle result in a rightward shift, namely *h*ot, *h*acid, *h*ypercapnic. The rightward shift results in *increased unloading* at any given tissue pO_2 thereby rendering oxygen unloading more efficient. It also results in a smaller disadvantage in pulmonary oxygen loading, but this is usually obviated by improved ventilation and higher alveolar oxygen tension.

There is insufficient space for a full exploration of physiological importance of the intra-erythrocytic allosteric regulator, 2,3-DPG.

The human red blood cell is uniquely adapted to fulfill a single function, namely gas (oxygen, carbon dioxide, nitric oxide, etc.) transport in that it contains neither a nucleus nor mitochondria. It is "chock full" of tightly packed HGb molecules at a concentration of about 36 g/dL. Hence its energy metabolism depends on glycolysis for the generation of organic phosphates. 2,3-DPG is an intermediate product whose steady state concentration within the red cell is determined by the activity DPG mutase and DPG phosphatase. There is high affinity binding between beta chains of globin and 2,3-DPG. In the presence of a high concentration of 2,3-DPG, a conformational change occurs within the HGb molecule such that the p50 is increased. The range of the change in p50 that can be induced by changes in 2,3-DPG concentration within the red cell is substantial, 15–34 mm Hg (Nunn 1987). Functionally, a change in p50 from 27 to 34 mm Hg means that at a pO_2 of 40 mm Hg there is nearly 10 % greater desaturation of HGb giving off about 1.6 ml/dL more oxygen to the tissues (Murray 1976).

The effect of a reduction in intra-erythrocytic pH is partly mediated by a change in 2,3-DPG concentration, by inhibition of DPG mutase and regulation of DPG phosphatase, resulting in a rightward shift of increased p50.

An important distinction must be made between pO_2 and oxygen saturation on the one hand and oxygen *content* on the other. As noted above, each gram of HGb when fully saturated (high pO_2) is capable of binding 1.36 mL of oxygen. This implies that at the normal HGb concentration of about 15 g/dL blood contains almost 20 mL oxygen/dL. In its passage through an hypothetical vascular bed that takes up 5 mL of oxygen from each 100 mL of blood passing, the venous blood oxygen content will be 15 mL/dL, the venous oxygen saturation will be 75 % (i.e. [20–5]/20 × 100 %), and the venous pO_2 will be 40 mm Hg. However if the HGb concentration is reduced to e.g. 7.5 g/dL, the oxygen capacity when saturated will be about 10 mL/dL. In the same hypothetical example above, for example after blood loss and replacement by an infusion of a crystalloid the extraction of 5 ml oxygen/dL of blood passing venous blood oxygen saturation will be 50 %, pO_2 will be about 27 mm Hg and tissue oxygen supply will be compromised. Thus, the HGb concentration will define the oxygen capacity and content of the blood, *but not the oxygen saturation* which is defined exclusively by the ODC and the pO_2 to which the blood is exposed. This is illustrated schematically in Fig 2.2. In fact, in the example above, the physiological adaptation that ameliorates the potential hypoxia is that the ODC is shifted to right thereby contributing some "extra" oxygen unloaded at any given capillary blood pO_2.

What are the physiological consequences in pathological states?

Bank blood stored at 4 °C rapidly loses its 2,3 DPG resulting in a leftward shift and a p50 that may be as low as 15 mm Hg; under these circumstances oxygen unloading in the tissues is quite difficult and it takes at least 24 h for the transfused red cells to "rejuvenate" and to recover normal oxygen affinity. On the other hand, anemia (either chronic or acute) is accompanied by a rise in intraerythrocytic 2,3-DPG, resulting in a rightward shift with improved oxygen unloading in the tissues.

In the presence of severely reduced arterial pO_2 (as in high altitude or severe pulmonary disease) the effects are mixed; the rightward shift impairs loading in the lung, but promotes unloading in the tissues. In the presence of respiratory failure and CO_2 retention the acidosis and hypercapnia causes a rightward, shift, but when corrected to standard conditions the p50 and 2,3-DPG concentration revert to normal. A number of, but by no means all, inherited hemoglobinopathies manifest themselves by a conformational change in the globin chains and either a rise or fall in p50. Sickle cell anemia is a special case because in addition to a change in its oxygen affinity it combines dramatic changes in red cell membrane deformability and fragility, crystallization of the hemoglobin and a specific vasculopathy.

Three gases form special ligands to hemoglobin. Carbon monoxide is naturally produced in the body during porphyrin catabolism, but exposure to unphysiologically high partial pressures is characterized by displacement of the oxygen from heme iron, because of CO's excessively high affinity to heme (about 300 times that of oxygen). The binding of CO to the heme iron reduces the oxygen capacity and simultaneously causes a leftward shift of the residual oxygen binding. Cyanide similarly has a very high affinity binding to heme iron, and acts similarly to CO, but because of its even greater affinity to cytochromes in the respiratory electron transport chain, it inhibits oxygen consumption and energy production.

Lastly, the normal ligand, nitric oxide, has a very special role in vasoregulation in part through a cycling process between HGb iron and S-nitrosyl groups of globin (Lima, Forrester et al. 2010; Haldar and Stamler 2013). This is an extremely important process in cardiovascular signalling to regulate oxygen delivery.

The following types of hypoxia are recognized:

- Hypoxemia when the oxygen saturation of blood is subnormal;
- Tissue hypoxia when oxygen supply at the organ, tissue or cellular level is inadequate;
- Stagnant hypoxia when tissue hypoxia is caused by inadequate delivery by reduced blood flow (e.g. ischemia);
- Histotoxic or cytotoxic hypoxia when ATP production is impaired or stopped by an agent that interferes with the mitochondrial respiratory chain by competing with and binding to oxygen consuming sites (e.g. cyanide);
- Anemic hypoxia when oxygen delivery is deficient because of inadequate functional hemoglobin concentration of the blood, or reduction of the oxygen capacity by "occupation of" a significant proportion of binding sites by other ligands with higher affinity than that of oxygen.

The physiological consequences of hypoxia are far too numerous even to enumerate here. Suffice to say that they depend on its severity, the organism's tolerance and whether the hypoxia is sudden and short-lived, or is long standing such that adaptations and tolerance have developed to ameliorate some of its effects. Cellular tolerance of hypoxia may involve a variety of mechanisms, including "hibernation" to reduce metabolic activity, increased extraction of oxygen and adaptations of enzyme systems and gene expression to permit metabolic activity at lower levels of oxygen availability(Leach and Treacher 1992).

The following equations represent the components and their interactions of the system (Leach and Treacher 1992).

$$\textbf{Alveolar air } pO_2: \quad p_AO_2 = F_iO_2 \times (P_B - P_{H_2O}) - (p_ACO_2/RQ) \tag{2.1}$$

$$\textbf{Blood oxygen concentration}: \quad C_aO_2 = S_aO_2 \times [HGb] \times 1.36\} + p_aO_2 \times 0.0031 \tag{2.2}$$

$$\textbf{Oxygen delivery rate}: \quad DO_2 = QX\,C_aO_2 \tag{2.3}$$

$$\textbf{Oxygen extraction ratio}: \quad EO_2 = VO_2/DO_2 \tag{2.4}$$

These equations represent the following:

(Equation 2.1) *The alveolar air equation* represents the partial pressure of oxygen in alveolar air at the prevailing barometric pressure after accounting for the vapor pressure of water with which tracheal air becomes saturated at body temperature. It defines the oxygen partial pressure in the steady state accounting for oxygen extracted and CO_2 added by the respiratory gas exchange. This is the oxygen partial pressure with which blood in the pulmonary capillaries equilibrates during its rapid transit through the capillary. This oxygen partial pressure defines the oxygen saturation of hemoglobin according to the oxygen dissociation curve (ODC).

(Equation 2.2) Describes *blood oxygen concentration or content in the arterial blood* that arrives from the pulmonary circulation (not accounting for any loss through venous admixture). The oxy-hemoglobin saturation, as defined above, is the arithmetic product of the oxygen capacity of 1 gram of hemoglobin when fully saturated (1.36 mL/g) and the hemoglobin concentration, plus the additional amount of oxygen dissolved in physical solution in blood water at body temperature (0.0031 mLO_2/mL of water/mm Hg). It clearly shows the superiority of hemoglobin binding of oxygen over oxygen dissolved in the aqueous compartment of blood. The presence of 1g of hemoglobin when fully saturated multiplies oxygen transported by blood by over 40-fold. Fifteen g hemoglobin/100 mL of blood multiplies oxygen transported over 600-fold. The two equations combined illustrate the advantages of breathing oxygen enriched air mixtures when hemoglobin is not fully saturated when breathing atmospheric air, or at high altitude.

(Equation 2.3) Defines *the oxygen delivery rate* that describes the equal importance of the cardiac output and of the oxygen content of the blood in satisfying the oxygen need of the organism in a complementary manner. It does not, however, address the fine tuning of delivery required to satisfy varying oxygen demands of the various organs and how these may be satisfied by modulating the *distribution* of the cardiac output. When this equation is applied to an individual organ, the CO is replaced by the organ blood flow and this is the variable that is modulated over a wide range to meet the needs of the organ in question.

Implicit in the *Oxygen Extraction equation* (Eq. 2.4) is the fact that the proportion of oxygen that can be extracted from blood is finite and a significant concentration of oxygen must remain in blood returning to the venous system. The extraction ratio defines the residual oxyhemoglobin saturation, and according to the ODC, the oxygen partial pressure after the blood's transit through the systemic capillary beds. A finite pO_2 must exist at the end of the capillary bed to assure that a sufficient pO_2 gradient exists to maintain diffusion of oxygen from the capillary to cells at some distance.

At rest, the blood returning from the systemic circulation is normally about 70–75 % saturated and has a mixed venous pO_2 of approximately 40 mm Hg. Some organs extract a good deal more of the oxygen (e.g. the heart), while others extract less; some vary their extraction over a very wide range (e.g. skeletal muscle at rest and during exercise), whereas others (e.g. brain) maintain global oxygen consumption and extraction nearly constant.

The following sections will describe the four stages of the "oxygen transport system" and the 'oxygen cascade" from the atmosphere to the mitochondria.

It is very important to note that the critical link between the convective mass transport and diffusive processes in both the respiratory and circulatory phases of oxygen transport is the *oxy-hemoglobin dissociation (ODC)* relationship between the partial pressure of oxygen (pO_2) and the saturation of the hemoglobin and thereby the oxygen content of the blood. The Chapter by Mozarelli describes the mechanisms underlying and the relationship itself in great detail. For the current purposes it is important to note that the ODC greatly facilitates the loading of oxygen onto HGb in the pulmonary circulation exposed to high alveolar pO_2, and the unloading of oxygen in the capillaries in the systemic circulation by the continuous exposure of the blood to lower extravascular pO_2 by the continuously consuming cells in the capillaries' immediate environment.

2.3 The First Step in the Oxygen Cascade

2.3.1 Mass Transport from Environment to Alveolar Space

Atmospheric air containing 21 % oxygen at a total atmospheric pressure of 760 mm Hg at sea level has a pO_2 of approximately 159 mm Hg. Within the tracheo-bronchial tree the air is warmed to body temperature and saturated with water vapor which reduces the effective air pressure by 47 mm Hg; thus air entering the alveolus has a pO_2 of (713 mm Hg × 21/100) about 150 mm Hg. CO_2 is added to alveolar air at a partial pressure of 40 mm Hg, thereby diluting the alveolar air oxygen concentration and reducing the mean alveolar air p_AO_2 to approximately 100 mm Hg, as described above by the alveolar air equation (Eq. 2.1.).

2.3.1.1 Pulmonary Ventilation

Respiratory muscle activity of inspiration/expiration cycling maintains two–way airflow and averaged over several cycles, maintains a partial pressure of oxygen and carbon dioxide in the alveolar air of 100 and 40 mm Hg, respectively. In the face of marked changes in the metabolic consumption of oxygen and production of carbon dioxide the alveolar pO_2 and pCO_2 are maintained remarkably constant at these values by complex neural *regulation of the total and alveolar ventilation.*

Metabolic oxygen demand and carbon dioxide production vary from basal needs during restful sleep to maximal levels of exercise, over an approximately tenfold range (Murray 1976). A centrally located "oscillator" generates reciprocal stimulation/inhibition of inspiration/expiration cycling, located in the pons (Morschel and Dutschman 2009). This central rhythm is modulated in the medulla oblongata where the principal mechanisms regulating both respiration and the cardiovascular system are located. The principal respiratory regulation whereby arterial blood pCO_2 is maintained resides in an area on the medulla's ventral surface which is exquisitely sensitive to changes in pH, primarily resulting from unbuffered changes in pCO_2 in the cerebro-spinal fluid (CSF) bathing this region (Guyenet 2008; da Silve, Li et al. 2010; Nattie 2011). Minor changes in CO_2 in this region bring about marked changes in alveolar ventilation that result in maintenance of a near-constant alveolar and arterial blood pCO_2. The neural output from the medullary respiratory centers is conveyed by efferents from spinal motor nuclei to the diaphragm, intercostal and abdominal muscles. In addition to the medullary respiratory centers, neural input is conveyed by afferent fibers in the vagus (X) and glosso-pharyngeal (IX) nerves from the aortic and carotid body chemoreceptors to the nucleus of the solitary tract and then to the pontine and medullary integrative neurones. The aortic and carotid chemoreceptors are principally sensitive to significant changes in the pO_2 of arterial blood, but are also sensitive to changes in pCO_2. These chemoreceptors are primarily responsible for driving ventilation in response to reduction in the arterial blood pO_2, that occurs e.g. at high altitude or acute pulmonary injury. In addition, feedback information on lung deformation by stretch receptors is conveyed from parenchymal mechanoreceptors by the Vagus nerve, and from respiratory muscles from muscle spindles via afferents to the spinal cord.

In order to generate airflow into the lung, inspiratory muscle contraction overcomes three forces: 1. The elastic recoil of the lung and chest wall complex; 2. The frictional resistance to airflow in the airways and the frictional resistances between lung and thorax; and 3. Inertial airflow resistance which is primarily determined by the aggregate cross sectional area of the airways, according to Poisseuille's law (Murray 1976). The normal airways at resting breathing offer minimal resistance and the work of breathing represents a small proportion of the total body oxygen consumption. However, when an increase in ventilation and oxygen delivery are required by increased metabolic activity, the work of breathing is also increased. As ventilation increases by both augmented tidal volumes and respiratory rates, bulk airflow and flow velocity within the lung in

both directions is increased exponentially, the flow becomes turbulent, and the work of breathing becomes one of the limiting factors at maximal exercise because it consumes all the additional oxygen intake. Likewise, at near-maximal levels of ventilation expiratory muscle effort is required to maintain the high-velocity airflows at fast respiratory rates. The expiratory muscle contraction under these conditions may lead to dynamic compression of airways, especially those not fully supported by cartilage. The resulting compression increases airway resistance further, leading to deleterious positive feedback and air trapping. The resistance offered to airflow is varied according to the degree of contraction or relaxation of airway smooth muscle, sensitive to sympathetic innervation and agonists, various inflammatory mediators and NO-mediated dilators (Spina 2008). However under pathological conditions characterized by excessive airway smooth muscle contraction (e.g. asthma) airway resistance may increase many-fold increasing the work of breathing, even at low levels of ventilation, to an extent that it becomes a very large proportion of total body oxygen consumption (Ozier, Allard et al. 2011). Thus, pathological airway responses may impose major limitations on the ability of the oxygen transport system to respond to increased demands and thereby limiting exercise capacity (Ozier, Allard et al. 2011). The airways themselves are also capable of sensing oxygen and respond appropriately in a reflex manner (Peers and Kemp 2001; Waypa and Schumacker 2010).

The phenomenon of hypoxic pulmonary vasoconstriction is observed when alveolar air hypoxia occurs; this is observed globally at high altitude and locally when some lung units are hypoxic because of reduced local alveolar ventilation. This assures that blood flow is diverted to areas of the lung that are better ventilated. The mechanism is not fully known, but it involves oxygen sensing by vascular (probably endothelial) cells, signalling to trigger a functional response (Waypa and Schumacker 2010).

2.3.1.2 Pulmonary Blood Flow

The whole of the cardiac output (with the exception of the bronchial (arterial) flow) is driven by right ventricular contraction through the entire pulmonary circulation. At the inlet, pulmonary arterial pressure at rest is normally approximately 20/10 mm Hg and at the outlet, left atrial pressure is about 5−8 mm Hg and the mean pressure loss across the pulmonary circulation is about 12 mm Hg. The mean capillary pressures are estimated to be of the order 10−12 mm Hg, significantly lower than those in systemic capillaries. This assures that: 1. The alveolar spaces are kept dry by Starling forces. 2. The structural integrity of the alveolocapillary membrane is not disrupted. 3. The total resistance offered by the pulmonary circulation to blood flow is about one-fourteenth that of the high-pressure systemic circulation, permitting a low level of work by the right ventricle. As estimated from measurements of lung diffusing capacity, at any given time the entire active pulmonary capillary bed contains about 70 mL of blood (Ceridon,

Beck et al. 2010) distributed over an enormous surface area where blood and air are in intimate contact.

2.3.1.3 Ventilation: Perfusion Matching

Two components of the system, normally of minimal magnitude, do not participate in respiratory gas exchange: the *dead space* and the *shunt* components (see Fig. 2.1). The former comprises the anatomical dead space of the large airways and unventilated alveoli. The latter comprises true anatomical right-to-left shunts, and un-perfused alveoli, cumulatively referred to as *venous admixture*. In these components there is no exposure of blood to alveolar air. As long as these components are of negligible magnitude, respiratory gas exchange proceeds efficiently. Normal, fully efficient oxygenation of the blood requires that ventilation and blood flow be evenly matched throughout the lung and in nearly all lung units (West 1965). If perfusion is not matched to ventilation in individual lung units a mismatching occurs with deleterious effect upon gas exchange. When a lung unit is underventilated relative to its perfusion, the alveolar air and end-capillary pO_2 will be less than normal. When a lung unit is under-perfused relative to its ventilation, the alveolar air and end-capillary blood pO_2 will be above-normal. Thus collectively, under-ventilated lung units behave as shunts, and the underperfused lung units collectively behave like dead space. When there is significant mismatching of ventilation and perfusion of a substantial number of lung units, hypoxemia will result (West 1965).

It may appear counter-intuitive that over-ventilated lung units with above-normal pO_2 do not compensate for the effect of under-ventilated lung units whose pO_2 is below-normal. The explanation lies in the oxygen dissociation curve (ODC).

Above-normal pO_2 will not increase blood oxygen saturation and content because this already occupies the flat part of the ODC and full saturation is achieved at normal pO_2. Subnormal pO_2 on the other hand will result in reduced saturation and oxygen content. When blood is collected in the pulmonary veins from all lung units including those with mismatched ventilation and perfusion, the mixture will reflect the effect on the ODC of the subnormal blood oxygen content and saturation. The result is reduced oxygen saturation, content and pO_2 in arterial blood. While some local mechanisms are operative to regulate ventilation and perfusion of lung units such that ventilation and blood perfusion are more evenly matched (West 1965), for example by hypoxic pulmonary vasoconstriction locally, very significant mismatching of ventilation and perfusion (V_A/Q) by lung pathology results in hypoxemia and a corresponding effect on the oxygen transport system (West 1965), as indicated by Eq. 2.3.

2.4 The Second Step in the Oxygen Cascade

2.4.1 Diffusion from Alveolar Air to Blood in the Pulmonary Capillary

Four factors are important in efficient respiratory exchange at rest between alveolar air and capillary blood in the lung: 1. A large pO_2 gradient of approximately 100−40 mm Hg; 2. A large surface area available for gas exchange with a thin diffusion barrier; and 3. A favorable diffusion coefficient for oxygen. These three factors facilitate the near-complete equilibration of oxygen partial pressures during the rapid (approximately 0.7 s.) transit time of blood through the capillary bed. The fourth factor, the ODC facilitates the transfer of a large amount of oxygen by diffusion from alveolar air to solution in plasma and into the red cells by *rapid binding* to HGb. The initial rapid diffusion of oxygen is promoted by the high oxy-HGb affinity on the steep part of the ODC when the partial pressure gradient is greatest. As the blood arrives in the pulmonary capillary with a mixed venous pO_2 of about 40 mm Hg and about 70−75 % saturated, the binding of the fourth oxygen atom is fastest. As the gradient declines along the passage of blood through the capillary's length, equilibration occurs less rapidly, but is promoted by the flat top part of the ODC, even at varying alveolar oxygen partial pressures (Murray 1976; Nunn 1987). Equilibration of pCO_2 between alveolar air and capillary blood is even more rapid than that of oxygen, even at smaller partial pressure gradients (46−40 mm Hg), because of the higher diffusion coefficient.

When metabolic demands increase, as in exercise, fever, etc. both cardiac output and ventilation respond. This results in increased bulk flow and flow velocity of both air in airways and blood in the pulmonary circulation. The limitations thereby imposed on respiratory gas exchange (e.g. shortened capillary transit time) are ameliorated by a number of factors, including:

1. Recruitment of both alveolar surfaces and capillaries, and improved matching of ventilation and perfusion.
2. Widening of the alveolar-to-capillary partial pressure gradient by virtue of the increased oxygen extraction in the systemic circulation and the reduced venous blood pO_2 in the blood returning to the lung.
3. Rightward shift in the ODC of venous blood entering because of the lower blood pH and pCO_2.

This second step of diffusive gas exchange occurs passively, at the cost of moving air and blood by the work of the respiratory muscles and of the right ventricle satisfying the requirement of efficiency as noted above.

The processes in these first two stages of oxygen transport optimize the content component of Eq. 2.3 above. Important impairments would be represented by

1. Impaired respiratory function (neuro-muscular impairment, chest wall deformities, pleural masses, reduced lung compliance).

2. Reduced barometric pressure.
3. Pulmonary parenchymal or vascular disease.
4. Cardiac disease.
5. Central nervous system and spinal cord injuries or disease (e.g. motor neuron diseases).
6. Deficiency of functional hemoglobin (anemia, metHGbemia, carbon monoxide poisoning, hemoglobinopathies).

In summary: Pulmonary ventilation is regulated by altering the neural outputs to changing both the frequency and tidal volume. These changes result in alterations of the alveolar ventilation and thereby the volumes from which oxygen is extracted and carbon dioxide is added changing the steady state concentration of gases with which blood in the pulmonary capillaries equilibrates. Pathological conditions affecting the ability to maintain normal blood gas composition may have a major impact on the ability of the organism to provide adequate oxygen supply to meet all metabolic requirements of organs and tissues, resulting in hypoxia.

2.5 The Third Step in the Oxygen Cascade

2.5.1 Mass Transport from the Pulmonary to the Systemic Capillaries

2.5.1.1 The Cardiac Output

The cardiac output (CO) is the volume of blood pumped by each ventricle of the heart expressed as L/min. Its two components are the heart rate (HR) and the stroke volume (SV); the typical range being about 60 times/minute × 75 mL = 4,500 mL/min. From basal level at rest to maximal exercise the cardiac output can vary over an approximately five-fold range to about 22.5 L/min, by increasing HR over an approximately three-fold range and the SV over an approximately two-fold range. Thus, oxygen transport can be varied to meet a wide range of metabolic demands, (see Eq. 2.3.) and by modulating the distribution of flow among organs according to their activity. Thus, at rest blood flow to skeletal muscle is very low, but during vigorous exercise blood flow is augmented disproportionally to the exercising muscles and to the heart, and may be maintained or even temporarily curtailed to some organs, such as the abdominal organs. Alternatively, during digestion blood flow to the gastro-intestinal tract is increased to meet the secretory and absorptive needs. This permits the "economical" and efficient conservation of cardiac work required to augment the cardiac output satisfying the requirement described above.

2.5.1.2 The Regulation of the Heart Rate (HR)

The rate of spontaneous depolarization arising in the sino-atrial node in the right atrium determines the heart rate under normal circumstances. It is under the influence of the sympatho-adrenal system accelerating it and its vagal innervation slowing it. Normally, at rest it is under a predominant vagal influence and responds by increasing the HR both by inhibition of the vagal and augmenting the sympathetic influences. One of the clinically observable phenomena is the reciprocal HR response to an acute change in blood pressure mediated by the *baroreceptor reflex or baroreflex;* the receptors being located in the carotid sinus and the aortic arch. As the HR is accelerated under tachycardic conditions, ventricular function is altered *indirectly,* by shortening the time available for both filling and ejection. The ventricles adapt to such changes by altering their *contractility* (see below). The HR is not *directly* affected by the other mediators that are important in modulating ventricular function and, thereby the SV ejected.

2.5.1.3 The Regulation of the Stroke Volume (SV)

The determinants of the stroke volume produced can be described by terms borrowed from skeletal muscle mechanics: *preload, contractility and afterload*. Preload in this respect is represented by the end-diastolic volume of the ventricle. The afterload is represented by the load against which the contraction generates force; in this case the aortic and PA pressures, respectively for the left and right ventricles. As long as the rising intraventricular pressure is less than aortic and PA pressures, the contraction is isovolumic and myocyte shortening only stretches elastic connective tissue elements. Once intraventricular pressure reaches aortic and PA pressures, external shortening and ejection can begin. Thus the contraction does internal and external work. The total work is determined by all three elements: preload, afterload and the particular characteristic of the myocardium, its contractility (Hunter, Janicki et al. 1980).

There are two important mechanisms whereby the myocardium is capable of varying its tension generation and the magnitude of the stroke volume ejected: 1. That based on *Starling's Law* of initial length which is an inherent property of the heart itself and is determined principally by the preload; and 2. Changing *contractility* by external influences, namely the ability of changing the work performed from a given preload, against a given afterload (Sagawa, Suga et al. 1977; Baan, van der Velde et al. 1992).

Starling's law states that, over a certain range, the force generated by the ventricle and thereby the stroke volume ejected is related to the preload i.e. the volume contained in the relaxed ventricle at the end of filling, the *end-diastolic volume*. The underlying mechanism is related to the "sliding filament theory" of muscle contraction in that the extent of the overlap of the actin and myosin filaments before contraction begins determines the number of cross bridges that

can be formed and, consequently the magnitude of the force generated and work done.

In the intact organism changes in *contractility, or of inotropic state*, are of far greater significance than the Starling mechanisms. A change in *contractility* occurs under the influence of sympatho-adrenal stimulation, inotropic drugs, and is represented by an increase in the forces generated at any given preload and afterload. It is manifested by: 1. An increase in tension generated from the same preload, against a given afterload. 2. A greater *velocity* of shortening. 3. An increased stroke volume ejected from the same end-diastolic volume by contraction to a smaller end-systolic volume. The subcellular mechanism underlying increased inotropic state likely involves a rise in intra-cellular calcium concentration in systole, resulting in the greater engagement and faster cycling of more of the cross bridges participating. Of the two mechanisms, the Starling mechanism is involved when the increasing the inotropic state is not possible, namely in heart failure (Norman, ouriri et al. 2011; Little and Applegate 1993). Otherwise, the preferred mechanism to increase stroke volume is by changes in the *inotropic state* or *contractility*.

At normal heart rates the *duration of diastole* provides sufficient time for adequate filling to occur, but at elevated heart rates the period of diastolic filling is shortened and atrial contraction is required for adequate filling to occur, and sufficiently high diastolic compliance, i.e. ease of extension of the chamber, is of critical importance. During periods of sympatho-adrenal stimulation both heart rate and stroke volume are increased, the latter by the augmented contractility whereby the ventricle is capable of augmenting the stroke volume by both increased velocity of shortening and contraction to a smaller end-systolic volume in the shortened time available for a cardiac cycle. Thus, whereas tachycardia increases the number of times a stroke volume is ejected and, coincidentally limits the duration of each cardiac cycle, the simultaneous increase in contractility permits substantially increasing the stroke volume, thereby achieving a maximal cardiac output range of five-fold over basal.

The continuous flow of blood to the left heart is "boosted" at high pressure by left ventricular contraction and is propelled through the arterial distribution system to all the organs and to their capillaries. The distribution of blood flow to individual organs, and within them regionally, is under a complex regulatory system "designed" to optimize oxygen delivery to meet the organs' metabolic needs and respective non-metabolic functions. The regional distribution of the cardiac output (CO) is regulated by the respective organs' aggregate resistance comprising the degree of contraction/dilation of the smooth muscle of the arterioles. The principal regulators of arteriolar smooth muscle contraction/relaxation are the autonomic nervous system, various vasoactive peptide hormones (including endothelin and angiotensin), and the locally acting "autacoid" prostaglandins (including prostacyclin and thromboxane), as well as the local O_2 and CO_2 concentrations. The principal mechanism whereby relaxation of arteriolar smooth muscle is mediated involves the generation of nitric oxide (NO) which regulates the function of the intracellular enzyme soluble guanylate cyclase (sGC), the product of which, cyclic

guanosyl monophosphate (cGMP) in turn, inhibits cross-bridge cycling of vascular smooth muscle cells. The complexity of the regulation of blood flow in each organ is expressed at a variety of levels: the phenotypic diversity of vascular smooth muscle cells and their receptors specific to each organ, the type and density of receptors responsive to vasoactive peptides and to autonomic nervous system mediators, the hierarchy of all of these regulatory functions, and finally the respective organ's metabolic demand for oxygen and demand for blood flow specific to the organ's non-metabolic function (e.g. secretion, absorption, regulation of water and ionic composition and temperature).

In summary: The cardiac output is regulated through induced changes in the heart rate and the stroke volume. The heart rate is regulated by the sympathetic and parasympathetic systems; sympathetic influences have a positive chronotropic, whereas, through the vagal innervation parasympathetic influences have a negative chronotropic effect. The ability to alter contractility is a critically important property of the heart; in the face of effects that would limit the range of possible stroke volumes (reduced preload by encroachment on filling by a shortening of the diastolic period, reduction of the external shortening possible by increased afterload), it permits extending the range of stroke volume by increased tension generation at greater velocity and contraction to smaller end-systolic volumes.

As shown by Eq. 2.3. the cardiac output which can be varied over a five-fold range, is the central variable component in systemic oxygen transport. Normally, the oxygen content is relatively fixed and, unlike the cardiac output, is not variable on a moment-by-moment basis. Hence, the cardiac output is the principal variable whereby the deficiencies in the oxygen content of the blood can be compensated for to maintain or augment systemic oxygen transport.

2.6 The Peripheral Circulation

The regulation and function of the major components, the major arteries, the arterioles, the capillaries and veins are different and are discussed sequentially.

Blood flow in major distribution vessels, the arteries

The major distributing arteries have relatively thick walls and spirally arranged smooth muscle under sympathetic control. The function of the smooth muscle is largely to alter the compliance of these vessels and thereby to affect the profile of the pressure wave and the magnitude of the pulse pressure (i.e. the difference between systolic and diastolic blood pressures). The flow of blood is largely streamlined and these vessels offer relatively little resistance to blood flow. Hence, there is little pressure dissipated from the aorta to the smallest distribution arteries. Streamlined flow may become turbulent (with accompanying increase in flow resistance) at bifurcations and sites of partial obstructions protruding into the lumen. Branchings of the distributing arteries feed the various organs which are arranged in parallel, and flow to each organ is determined by the relative aggregate resistance the organs' arterioles present.

Blood flow and pressure in the arterioles

The arterioles arise from parent vessels by multiple branching to reach a size of approximately 100 μm in diameter. These vessels are invested by a relatively thick circular smooth muscle which is highly responsive to numerous vasoactive mediators acting through a variety of specific receptors. The degree of contraction of the smooth muscle determines the diameter of the arteriole, and thereby the resistance to flow it offers, according to Poisseuille's law (blood flow is directly proportional to the driving pressure and inversely proportional to the radius^4, the length and the viscosity of the fluid). In the present context, the single most important determinant of flow at any given driving pressure is the resistance due to changes in arteriolar radius. Thus, the level of the arterioles is the site of largest drop in perfusion pressure. The high-velocity flow in the arterioles is slowed down substantially in the arterioles.

The resistance in the arterioles serve three separate functions:

1. All the arterioles in the aggregate limit outflow from the major arteries, thereby maintaining high mean and diastolic arterial blood pressures.
2. Arterioles of each organ in the aggregate regulate the distribution of blood flow to individual organs.
3. Each arteriole in any given organ reduces the high arterial blood pressure to a level similar to the colloid oncotic pressure of the blood, thereby limiting the pressures to which the poorly supported capillary walls are exposed and facilitating the transcapillary fluid exchange based on Starling forces.

Arteriolar smooth muscle is under complex bi-directional control. It responds by *active contraction* (by increased cross bridge cycling) to a plethora of systemic autonomic nervous, as well as humoral systemic (endocrine: angiotensin, endothelin and local (paracrine: prostanoids) mediators, through a number of diverse receptor types distributed with great organ-to-organ variability. The opposite effect, dilation, is mediated principally by a single mechanism, namely the *inhibition* of cross bridge cycling. The common pathway of dilation involves the synthesis and release of NO which signals locally and downstream the action of soluble guanylate cyclase (sGC) to generate cyclic adenosine monophosphate (cAMP) which is the mediator effecting the inhibition of cross bridge cycling in the smooth muscle and consequent relaxation. In the critical organs with high metabolic activity (brain, heart, kidney, liver) the arterioles are also subject to additional controls regulating the arteriolar smooth muscle activity, sensitive to oxygen availability within the organ, the oxygen sensor likely being the mitochondria (Waypa and Schumacker 2010).

Blood flow in capillaries

Because of the high resistance in the arterioles, blood flow velocity is further reduced in the capillaries to facilitate gas exchange. The capillary walls consist of a single layer of endothelial cells supported by a basement membrane. The internal diameter is similar to, or even smaller than, the red blood cell (RBC) so that the cells need to deform in their transit through the capillary. The deformation serves

to mix the RBC contents thereby facilitating even distribution of oxygen and carbon dioxide within. The deformation required to permit RBC passage is also the cause of marked decline of the hydrostatic pressure along the length of the capillary. According to the Starling hypothesis, as a result of the hydrostatic pressure exceeding the colloid osmotic pressure difference across the capillary surface, there is net filtration along the first half of the capillary's length. As the hydrostatic pressure declines below that of the net colloid osmotic pressure across the capillary wall, reabsorption occurs along the distal half of the capillary, with a net zero balance in trans-capillary fluid exchange and a continuous circulation of extracellular fluid.

2.7 The Fourth Step in the Oxygen Cascade

2.7.1 Diffusion of Oxygen from Capillary Blood to Metabolizing Cells and Within the Cell to the Site of Consumption, the Mitochondria

The Fick equation of diffusion of a gas in a liquid medium describes the determinants of the flux as

$$\mathbf{V} = \mathbf{D} \times \mathbf{A} \times \{\Delta \mathbf{P} / \mathbf{d}\} \tag{2.5}$$

where A is the area available for diffusion; D is the diffusion constant for the gas; **ΔP** is the gas partial pressure difference; d is the diffusion distance, thus **ΔP/d** is the partial pressure gradient.

The simplest model of oxygen delivery is the Krogh model of two concentric cylinders; a cylinder of cells penetrated centrally by a capillary, approximately equidistant from all cells at the periphery of the tissue cylinder. This simplified model is useful to illustrate the maintenance of diffusive gas exchange along the same principles already described above for pulmonary capillary gas exchange. Oxygen is unloaded from hemoglobin and carbon dioxide is taken up (by three different forms of carriage) and a continuous diffusive flow of oxygen is maintained to all the cells within the capillary's territory. The functional (i.e. open) capillary density determines the diffusion distances from capillary blood to consuming cells and their mitochondria over a wide range of consumption rates, depending on their level of activity (Wagner, Venkataraman et al. 2011). As described above, the presence of HGb in the red cells facilitates diffusion by virtue of taking up oxygen from the plasma and binding, minimizing the increment of oxygen partial pressure per unit volume of oxygen received and extending the wide partial pressure gradient.

The blood entering the capillary with a high pO_2 begins to surrender its oxygen because it is surrounded by an immediate environment of lower pO_2, initially giving off oxygen dissolved in plasma, and followed by release of oxygen bound to

HGb. Because of the continuous consumption of oxygen by all the cells within the larger cylinder, the partial pressure of oxygen declines both longitudinally and radially from the "inflow" to the "outflow" end (see bottominset in Fig. 2.1). The principal force driving diffusion is the gradient in pO_2 from blood to the cells. The oxygen dissociation characteristics of HGb facilitate the rapid and efficient unloading of oxygen within the capillary. The cells at the extreme end of the cylinder and furthest from the capillary are at the greatest disadvantage, because their immediate environment is at the lowest pO_2.

The oxygen sink, the mitochondria, represent the extreme end of the diffusion path and the lowest local pO_2. Mitochondrial pO_2 in vivo is difficult to determine (Lanza and Sreekumaran Nair 2009, 2010), but it has been estimated in one study, using a novel methodology, to be in the range 30–40 mm Hg in vivo in the liver(Mik, Johannes et al. 2008), far higher than the critical pO_2 estimated earlier (Lanza and Sreekumaran Nair 2009). If this is indeed the case, then end-capillary pO_2 in the same range would be insufficient to drive diffusion from the distal parts of the capillary where the prevailing pO_2 may be as high as 30–40 mm Hg (Pitman 2011).

Of course, the Krogh cylinder model as the single source of oxygen for a collection of contiguous cells is a gross oversimplification of the real world of the complex networks of the microcirculation with continually opening and closing of individual capillaries and at any time there are excess capillaries supplying more than a single collection of cells. The arrangement facilitates altering the diffusion distances from capillary to cells and thereby optimize oxygen diffusion to the cells and mitochondria (Pitman 2011). The gradients existing in the microcirculation are far more complex than those illustrated by the Krogh cylinder model (Tsai, Johnson et al. 2003; Pitman 2011). Nevertheless, *directional changes* in the venous blood draining are useful directional indicators of the state of oxygenation of the organ in question. The complexity of the arrangement has been elegantly illustrated by Intaglietta and colleagues in tissues which can be conveniently transilluminated for intravital microscopic observation and quantitative analysis.

Using the core equation of the oxygen transport system as defined above by Eq. 2.3:

$$\mathbf{DO_2} = \mathbf{Q} \times \mathbf{CO_2}$$

Of the two critical determinants of oxygen delivery, oxygen content is principally dependent on hemoglobin concentration [HGb]. The prevailing pO_2 determines the oxyhemoglobin saturation (S_{O2}) according to the ODC (According to the Hill equation:

$$\mathbf{SO_2} = \{(\mathbf{pO_2})^{\mathbf{n}}\} / \{(\mathbf{pO_2})^{\mathbf{n}} + (\mathbf{p_{50}})^{\mathbf{n}}\} \tag{2.6}$$

where n = 2.6, is the Hill coefficient).

At this time Eqs. 2.3 and 2.4 need to be expanded by the application of the *Fick principle* which states that the rate of oxygen entering the capillary is equal to the sum of the rate oxygen exiting and that consumed:

$$\mathbf{Q} \times \mathbf{CaO_2} = \{\mathbf{Q} \times \mathbf{CvO_2}\} + \mathbf{V_{O_2}} \tag{2.7}$$

And the oxygen content in Eq. 2.7 is:

$$\mathbf{CO_2} = \{\mathbf{SO_2} \times [\mathbf{HGb}] \times \mathbf{1.36}\} + \{\mathbf{pO_2} \times \mathbf{0.0031}\} \tag{2.8}$$

And Eq. 2.4 is expanded as:

$$\mathbf{E} = \{(\mathbf{CaO_2} - \mathbf{CvO_2})/\mathbf{CaO_2}\} \tag{2.9}$$

Thus, E, the extraction ratio, is the fraction of oxygen *extracted* from the arterial blood content, will yield the oxyhemoglobin saturation in the "venous" blood emerging from the microcirculation, and the corresponding pO_2 at the end of the capillary is the pressure driving diffusion to the cells. The pO_2 of blood entering and leaving the capillary and the rate of oxygen extraction en route determine the *mean tissue* pO_2 in a complex manner, and directional changes are reflected in the end-capillary pO_2.

Another way of analyzing the oxygen transport system is by the relationship of oxygen delivery to oxygen consumption (Leach and Treacher 1992).[2] The basis of the analysis is plotting oxygen consumption against oxygen delivery. Normally over a certain range of decreasing oxygen delivery, consumption is relatively independent of the delivery. Within this range demand may be satisfied by increased extraction of oxygen, a widening of the arterio-venous oxygen content difference, to an extent that no significant hypoxia is incurred. However, when delivery is reduced further a point of inflexion is reached and oxygen consumption declines nearly linearly, as delivery continues to decline. The point of inflexion represents the conditions that are such that a significant "mass" of cells becomes so hypoxic that it is no longer capable of using oxygen and a shift to anaerobic metabolism occurs. In this analysis the hypoxic cells unable to consume oxygen are not identified, only that they are sufficient in magnitude that their effect becomes observable. At this point some of the indicators of "hypoxia", or anaerobiosis can be observed (e.g. lactate production, etc.) (Leach and Treacher 1998; Schober and Schwarte 2012).

The consequences of pathological failure of the system to deliver sufficient oxygen for the prevailing metabolic needs are reduction in the tissue pO_2 or hypoxia(Leach and Treacher 1998). This may be incurred under the following circumstances:

1. Of the two components of Oxygen Delivery rate, blood flow can be varied but the blood hemoglobin concentration is normally fixed at approximately 140–160 g/L. Hence the complementary response to a content deficiency is to augment blood flow,

[2] This is an excellent introduction to most aspects of oxygen transport.

a. Significantly decreased [HGb] results in reduced oxygen content and delivery and may result in *hypoxia* because of increased extraction and reduced end-capillary and mean tissue pO_2 unless the blood flow is increased proportionally.
b. The arterial blood oxygen content will be reduced in the presence of reduced alveolar pO_2, physiologically significant shunting, mismatching of ventilation and perfusion, and even in the presence of normal extraction, end-capillary and mean tissue pO_2 will be reduced, unless compensated for by augmented blood flow.
c. The arterial blood oxygen content will also be reduced in the presence of reduced alveolar pO_2, unless compensated for by augmented blood flow
d. Conversely, significantly increased [HGb] results in an increase in oxygen content and delivery, but at the cost a marked rise in blood viscosity requiring augmented cardiac work.
e. In some *obligate aerobic* organs (the heart) normal levels of oxygen extraction are nearly complete and further increments of extraction are limiting so that

    i. marked increase in work and metabolic oxygen demand must be satisfied by a proportional increment in blood flow;
    ii. restriction in blood flow *and* marked reduction in [HGb] in the face of a rise in work load and metabolic rate inevitably lead to tissue hypoxia;
    iii. a physiologically significant change in the oxyhemoglobin dissociation characteristics of the blood (increased affinity, reduction in p_{50}), in combination with reduced [HGb] may induce tissue hypoxia and limit the ability to respond to increased demand;
    iv. conversely, markedly increased [HGb] in the face of increased work load and metabolic need may lead to tissue hypoxia because the associated rise in blood viscosity may limit the flow increment.

2. The ability to respond to increased *systemic* demands by increasing the cardiac output may be limited by tissue hypoxia in the heart initiating a *vicious circle* whereby the organ's ability to respond by increasing the cardiac output over the normal dynamic range of five-fold is limited in the presence of functional impairment and abbreviated contractile reserve. The limitations may be due to:

    a. impaired contractility or *systolic dysfunction* may arise from a number of causes, including

        i. impaired energy metabolism (Rosca and Hoppel 2010), such as in coronary vascular disease, mitochondrial dysfunction, loss of effectively functioning myocyte mass (infarcts), myocardial disease, valvular heart disease, ischemia–reperfusion and stunning,
        ii. excessive afterload as in established hypertension,
        iii. myocardial hypertrophy (Machackova, Barta et al. 2006), by extending diffusion distances in myocardium,
        iv. heart failure (Little and Applegate 1993).

b. impaired filling or *diastolic dysfunction* (Bateman, Sharpe et al. 2003; Periasamy and Janssen 2008), as in

   i. myocardial hypertrophy(Machackova, Barta et al. 2006),
   ii. myocardial fibrosis,
   iii. heart failure.

c. idiopathic or disease-related complex cardiomyopathies, including

   i. diabetic cardiomyopathy (Boudina and Abel 2010),
   ii. sepsis-induced cardiomyopathy (Romero-Bernejo, Ruiz-Bailen et al. 2011; Fernandes and Cesar de Assuncao 2012)
   iii. heart failure,
   iv. stress-related cardiomyopathy (Richard 2011)
   iv. endothelial dysfunction (Endeman and Schriffrin 2004; Ding and Triggle 2005; Feletou and Vanhoutte 2006).

2.7.1.1 Cardiac Energetics and the Coronary Circulation

Because the heart is an obligate aerobic organ and because it plays a central role in responding to changing demands for oxygen by changing cardiac output over a five-fold range that require marked changes in its metabolic work, a brief consideration of the coronary circulation is of special interest.

The myocardium does work in generating tension and pressure during isovolumic systole and in shortening in ejection. These two factors and the level of contractility at which the work is performed determine the *myocardial oxygen consumption* (Crossman 2004).

2.7.1.2 The Special Characteristics of the Coronary Circulation

The myocardium is an obligate aerobic organ and requires continual delivery of oxygen to meet its metabolic activity that supports its work. At rest it receives about 5 % of the cardiac output to support all of its functions and consumes about 10 % of the body's energy output. It can tolerate hypoxia for only short periods because its ATP and CP (creatine phosphate) reserves are very limited. Hypoxia rapidly results in cessation of contractile activity.

Because the left ventricle generates high intraventricular pressure the intramural arteries that supply the subendocardial layers are subject to compression from the high intra-ventricular pressure, and flow within them may be completely interrupted at the peak of systole. Hence, perfusion of the subendocardial myocardium is largely restricted to ventricular diastole when the perfusing pressure is the aortic diastolic blood pressure, but intra-ventricular pressure is very low. As a result the subendocardial layers are especially vulnerable to hypoxic injury if flow is inadequate.

The coronary circulation is distinct in that it has the highest extraction of oxygen with especially low coronary venous pO_2 and oxygen saturation; at rest, oxygen saturation is of the order of 40 % and pO_2 is 25−30 mm Hg in coronary venous blood. This corresponds to the widest arterio-venous oxygen content difference in the body of about 11−12 mL/dL (Sheppard and VanHoutte 1979). This is reflected in the fact that myocardial oxygen consumption is a greater fraction of the total than blood flow. This high extraction limits the scope of increasing the heart's own oxygen delivery by substantially increasing further its oxygen extraction. Yet, the myocardium is capable of increasing its work output over a five-fold range. This can occur even in the presence of variable arterial blood pressure, the perfusion pressure for coronary flow. Moreover, the changes in heart rate that are evoked when the cardiac output needs to respond to increased demands will abbreviate the time available for myocardial perfusion during each cardiac cycle. The adaptive mechanism accounting for this capacity is the ability to increase markedly coronary blood flow, the other component of delivery, as well as the microcirculatory anatomy, the rich capillary network and the wide distribution of mitochondria and the presence of myoglobin.

Coronary blood flow is *auto-regulated* in that over a physiologically relevant range of arterial pressures coronary flow is largely independent of the pressure. But when the coronary arterioles are maximally dilated, flow is linearly related to pressure. The coronary circulation is also auto-regulated in the sense that coronary flow is adjusted over a large range by changing the arteriolar resistance to meet the prevailing demand for oxygen by the myocytes. Hence, the resistance at the level of the arterioles is highly variable. At rest basal coronary tone is quite large but it can be decreased several-fold by *active vasodilation*. The difference at any given perfusing pressure between the basal blood flow and that which can be achieved by *maximal active vasodilation is the coronary vasodilator reserve*. The magnitude of this normal reserve is the principal reason that the heart can respond to widely varying metabolic demands by increasing its own blood supply.

The physiological significance of the coronary vasodilator reserve lies in the pathophysiology of coronary vascular disease. There is practically no resistance to flow in the normal *majo*r distributing coronary arteries; the major site of resistance is downstream at the level of the arterioles. It is their maximal dilation that determines the coronary reserve. However, if there are atherosclerotic or other pathological obstructions in the larger arteries, adequate basal coronary flow to meet basal demands can only be achieved by a proportional *reduction* in arteriolar resistance. This abbreviates the range available for dilation in case of *enhanced demand*. The consequence is that the maximal achievable flow is limited and delivery cannot meet the demand associated with e.g. high level of exercise. Moreover, atheromatous plaques represent areas of endothelial dysfunction with further impairment of the vasodilator response.

A significant contributing factor that permits the normal heart to respond to large demands is its microcirculatory anatomy. The ratio of myocytes to capillaries is about unity, thereby assuring that diffusion distances are short, largely determined by the myocytes' diameter. In case of enhanced demand extra capillaries

can be recruited, but the important facilitating effect is the richness of mitochondria spread widely throughout the myocyte (Jones 1986), and the presence of myoglobin, facilitating oxygen diffusion within the myocytes themselves. When the heart hypertrophies by enlargement of the myocytes, diffusion distances become longer and this may affect oxygen supply to the myocytes adversely.

2.7.1.3 What Does a Mitochondrion Do?

The oxygen-consuming process in the mitochondrion is localized in the five sequential enzyme complexes embedded in the inner membrane, comprising the *mitochondrial respiratory chain* (Duchen 1999). Four of the five complexes provide reduced NADH transporting free electrons to the fifth complex, ATP synthase, where oxidative phosphorylation of ADP takes place. In the process oxygen is used to generate water and CO_2. Some of the oxygen is not completely oxidized and becomes the substrate for the generation of *reactive oxygen species* (ROS) at two of the membrane bound enzyme complexes (Gao, Laude et al. 2008). One such ROS is the superoxide anion ($O_2^{-\cdot}$). Normally there are a variety of powerful antioxidant defenses within the mitochondrion such that ROS production is effectively detoxified, although deficiencies of antioxidants are associated with ROS-mediated damage to the mitochondrial DNA (Gao, Laude et al. 2008; Rosca and Hoppel 2010).

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