Regulation of Tissue Oxygenation

Second Edition

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Regulation of Tissue Oxygenation

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Roland N. Pittman
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COLLOQUIUM SERIES ON INTEGRATED SYSTEMS PHYSIOLOGY: FROM MOLECULE TO FUNCTION TO DISEASE #65

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ABSTRACT

This presentation describes various aspects of the regulation of tissue oxygenation, including the roles of the circulatory system, respiratory system, and blood, the carrier of oxygen within these components of the cardiorespiratory system. The respiratory system takes oxygen from the atmosphere and transports it by diffusion from the air in the alveoli to the blood flowing through the pulmonary capillaries. The cardiovascular system then moves the oxygenated blood from the heart to the microcirculation of the various organs by convection, where oxygen is released from hemoglobin in the red blood cells and moves to the parenchymal cells of each tissue by diffusion. Oxygen that has diffused into cells is then utilized in the mitochondria to produce adenosine triphosphate (ATP), the energy currency of all cells. The mitochondria are able to produce ATP until the oxygen tension or P_{O_2} on the cell surface falls to a critical level of about 4–5 mm Hg. Thus, in order to meet the energetic needs of cells, it is important to maintain a continuous supply of oxygen to the mitochondria at or above the critical P_{O_2}. In order to accomplish this desired outcome, the cardiorespiratory system, including the blood, must be capable of regulation to ensure survival of all tissues under a wide range of circumstances. The purpose of this presentation is to provide basic information about the operation and regulation of the cardiovascular and respiratory systems, as well as the properties of the blood and parenchymal cells, so that a fundamental understanding of the regulation of tissue oxygenation is achieved.

KEY WORDS

cardiovascular system, respiratory system, blood, microcirculation, oxygen transport

Preface to the Second Edition

It has been five years since the first edition of this book was published, and it was thought that enough new work had been published in the field of oxygen transport and its regulation to justify preparing this new edition.

Two important developments related to the topic of this book have appeared since the first edition: a novel mechanism to delineate the role of oxygen in the local regulation of blood flow was introduced in 2013, and a study of the oxygen dependence of cellular respiration was reported in 2012.

The local regulation of blood flow and oxygen's role in it have a long history in physiology. This topic has been subject to intense study for over a century; however, within the past decade questions have arisen regarding whether current explanations need revision. The standard explanation of functional hyperemia is the metabolic vasodilator hypothesis in which multiple vasodilator molecules from the active tissue act on nearby arterioles to produce increased blood flow. In the mid-1990s the novel idea that red blood cells (RBCs), which carry oxygen, can also perform as mobile oxygen sensors was introduced: in one case it was proposed that ATP was released by the RBCs; in the other, NO (nitric oxide) was released. More recently a very different proposal has been made in which two small signaling radicals, NO (from the vascular endothelium) and O_2^- (from the active parenchymal cells), interact in an oxygen-linked way to modulate the interstitial concentration of NO, thereby controlling blood flow. It is worth noting that only for this most recent proposal have real time kinetic data of the vasodilator verified predicted responses. A new figure, with accompanying text, summarizes these different mechanisms.

The oxygen dependence of cellular respiration has long been thought to be an "on/off" process whereby mitochondrial oxygen consumption is independent of oxygen tension (PO_2) down to very low levels ($\approx$ 1 mmHg). This oft-repeated in vitro finding greatly simplifies the analysis and interpretation of studies in which tissues are subjected to low oxygen environments, mostly pathologic situations. Recently, the novel application of phosphorescence quenching to measure PO_2 and oxygen consumption in micro-environments has revealed that the PO_2-dependence of oxygen consumption *in situ* is quite different, so that oxygen consumption varies with PO_2 continuously over most of the physiologic range. This new and unexpected finding, corroborated by *in vitro* studies

from another laboratory, means that the classic concept of tissue hypoxia needs to be carefully reconsidered and possibly revised, with anticipated consequences for interpretation of physiologic and clinical data on tissue oxygenation, both in health and disease.

In addition to these new findings included in the present edition of the book, a small number of corrections which previously escaped the author's attention have been made right; about 20 new references have been added, and three new figures illustrating the new text have been added. It is hoped that readers will find the new material to be useful and provocative for their work and understanding of this key research area.

Contents

Acknowledgments

FIRST EDITION

It is a pleasure for the author to acknowledge the contributions of a number of people to the work presented in this book. Brian Duling introduced the author to the field of microcirculation, and in particular to the importance of oxygen transport and the role of oxygen in the regulation of blood flow. Aleksander (Sasha) Popel, and more recently Aleksander (Alex) Golub, have been long time colleagues, both of whom have contributed valuable ideas about oxygen transport through numerous discussions, published articles and research collaborations. Paul Grannis, my graduate dissertation advisor, served as an early role model for clarity of thought and organization, excellence in teaching, and a lifelong pursuit of science. The author owes much to the excellent work of the editorial and production staff of Morgan & Claypool Life Sciences, especially Dana Dreibelbis and Joe Cho.

SECOND EDITION

This new edition is dedicated to the memory of Brian Duling, whose energetic life in science ended much too soon. Sasha Popel and Alex Golub have continued to provide insight and clarity into many of the key topics included herein regarding the nature of oxygen in biological systems. The author is indebted to and offers grateful thanks to Kelsey Hideshima who contributed her artistic talents to produce the new figures in this edition, offered needed feedback on large sections of the text, and used her proofreading skills to ensure that typos were kept to a minimum. Any errors which might have escaped detection by others are the responsibility of the author. Again, the outstanding work of the editorial and production staff of Morgan & Claypool Life Sciences, especially Joe Cho and Jovan Carreon, are gratefully acknowledged.

CHAPTER 1

Introduction

In order to carry out the variety of activities required of cells which make up an organism, a continuous supply of energy in the form of adenosine triphosphate (ATP) is required. Cells prefer to make ATP by the process of oxidative phosphorylation which takes place inside the mitochondria and which has an absolute requirement for oxygen. Thus, the regulation of tissue oxygenation is a critical feature for survival of an organism, and various mechanisms have been put in place to ensure that all cells in the organism are afforded a supply of oxygen which is adequate to carry out cellular activities. The regulation of tissue oxygenation can be studied at several different levels of organization, ranging from the intact organism, to the collection of organs which make up the organism, to the cells which make up each organ, and finally to the molecules which are involved in the regulatory processes, from oxygen itself to various transport and signaling molecules that are at the smallest scale in the regulatory pathways. Understanding how oxygen transport works at the molecular scale and integrating the behavior at one level of organization to achieve the next level ultimately lead to an overall understanding of how tissue oxygenation is regulated. Although much can be learned at each level of organization, we will find that studies at the level of the microcirculation provide an interface between organ and cellular behavior, since it is at this level that one first has integration of vascular, blood, and cellular function.

Although the title of this book is *Regulation of Tissue Oxygenation*, it is not clear if "regulation" is the appropriate term to use when dealing with the lowest level of organization involving cells and signaling molecules, the microcirculation. Does the traditional approach of describing regulation in terms of one or more feedback loops linked to specific chemical mediators apply here or is this a procrustean approach with no clear endpoint? What variable(s) related to oxygen is/are regulated? Additional research at this level is needed to provide a more comprehensive understanding of how tissue oxygenation is determined and many of the key issues have been the subject of a recent review [92].

What is clear is that the maintenance of an adequate supply of oxygen requires the coordinated operation of the three major systems involved in oxygen transport: cardiovascular system, respiratory system, and blood. The supply of an adequate amount of oxygen to all cells of the body is one of the most important functions of the cardiorespiratory system. Because complete descriptions of the cardiovascular system, the respiratory system and blood can be found elsewhere [7, 13, 67], this presentation will focus on the most important aspects of these three systems that pertain to the topic of regulation of tissue oxygenation.

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CHAPTER 2

The Circulatory System and Oxygen Transport

The cardiovascular or circulatory system is designed to ensure the survival of all cells of the body at every moment and it does this by maintaining the immediate chemical environment of each cell in the body (i.e., the interstitial fluid) at a composition appropriate for that cell's normal function. The term "homeostasis" is used to denote the approximate constancy of the internal environment (Claude Bernard, 1878).

First consider the simple hypothetical case of a single spherical cell suspended in a large (>100 times the cell volume), well-stirred volume of aqueous medium in equilibrium with room air and containing other nutrients. Oxygen availability is often a limiting factor for cell survival, and oxygen is supplied to a cell by passive diffusion. As oxygen molecules diffuse into the cell, they are consumed, so that there is a progressive fall in oxygen concentration from the surface of the cell to the lowest concentration which occurs at the center of the cell. For a spherical cell with a typical diffusion coefficient for oxygen ($\approx 10^{-5}$ cm^2/s) and an oxygen consumption of resting skeletal muscle ($\approx 10^{-2}$ ml O_2 cm^{-3} min^{-1}), the critical size (radius) which is just adequately supplied with oxygen from the surrounding medium is about 1 mm. Thus, we find that diffusion puts an upper limit on the size of cells in regard to their need for oxygen.

Although diffusion is an efficient transport process over short distances (<100 μm) as seen by the average time required for a molecule to diffuse a distance x ($t \approx x^2/2D$), how can a much larger multicellular organism, such as the human body containing about 100×10^{12} cells, be adequately supplied with oxygen? For mammals, the bathing medium for cells is water and total body water is about 60% of body weight. For a 70-kg person, total body water is distributed among three compartments with the following approximate volumes: intracellular ≈23 l (33% of body weight); interstitial ≈16 l (22.5% of body weight); and circulating plasma ≈3 l (4.5% of body weight). Cells are bathed in interstitial fluid (ISF), but interstitial fluid volume is only a little more than half the intracellular fluid volume. Thus, ISF cannot be considered a large reservoir of fluid, and its composition is directly influenced by cellular metabolism.

An organism is faced with the following problem: How can the composition of ISF be maintained near its desired value? The solution of this problem is to introduce a circulatory system which

continuously refreshes the ISF by putting it in intimate contact with "fresh, reconditioned" fluid (i.e., arterial blood). The circulating blood must be brought close to the cells (<10 μm) since nutrient and metabolic waste exchange takes place by passive diffusion, a transport mechanism which is most efficient over short distances. Thus, the cardiovascular system uses bulk flow (convection) to reduce the effective distance between the pumping action of the heart and the various parts of an organism.

In order for this system to be practical and do its job efficiently, two important conditions must be satisfied: (1) there must be adequate blood flow through the smallest blood vessels, capillaries, which are in contact with the cells comprising a tissue; and (2) the chemical composition of the incoming blood must be controlled to be that which is desired in the ISF. The design and operation of the cardiovascular system fulfill these conditions. Two important functions of the cardiovascular system are to move material (the carrier is blood) and to move heat (tissue metabolism generates heat that must be brought from the body's core to the cutaneous vascular bed at its surface, where it is radiated away from the body).

2.1 DESIGN OF THE CARDIOVASCULAR SYSTEM

The systemic circulation and pulmonary circulation are connected in series through the four chambers of the heart, so that all the blood that is pumped from the left ventricle into the systemic organs eventually makes its way back to the right ventricle from where it is pumped into the lungs. The systemic organs (tissues) are connected in parallel, and the following statements are consequences of this parallel architecture: (1) the stroke volume ejected from the left ventricle is divided among the various organs, and a given volume of blood passes through only one organ before entering the venous outflow of the organ; (2) the arterial blood entering each organ has the same composition; (3) the blood pressure at the entrance to each organ is the same; and (4) the blood flow to each organ can be controlled independently (local regulation of blood flow).

The various organs and tissues can be classified as one of two broad types: (1) blood "reconditioners" and (2) "essential" tissues. The main purpose of the blood "reconditioners" is to maintain the composition of the ISF relatively constant under all conditions. In general, flows to these tissues exceed their metabolic needs. Examples of this type of tissue are the lung, which ensures proper exchange of oxygen and carbon dioxide; the kidney, which maintains electrolyte composition and fluid balance; the gut, which oversees nutrient absorption; and the skin, which is involved in temperature regulation. The "essential" tissues are those whose function is critical at all times. The blood flows to these tissues typically match their metabolic needs. Examples of this type of tissue are the heart,

which requires a continuous supply of energy to maintain its pumping activity, and the brain, which requires a continuous supply of nutrients and a need for the washout of metabolic products in order to maintain consciousness and carry out its critical functions. One can also add skeletal muscle during exercise to this list, since its energy requirements and needs for washout of metabolic products can be substantial.

2.2 HEMODYNAMICS

A requirement for the circulatory system to carry out its function of bringing blood close to cells so that the exchange of nutrients (e.g., oxygen) and wastes can take place by diffusion is that the blood be able to flow through the complicated networks of blood vessels in the various organs. In order to make a viscous fluid such as blood flow, whether through a single vessel, an organ or the entire systemic circulation, a pressure difference must be applied between the inflow and outflow of the network. The relationship between volumetric flow, Q, and the applied pressure difference, ΔP, is the fluid mechanical equivalent of Ohm's law for electrical circuits and is expressed as $Q = \Delta P/R$, where R is the resistance to the flow of blood. Although the myriad of series and parallel connections of blood vessels in a tissue is quite complicated, each element—a single vessel segment—is simple to deal with.

2.2.1 Flow of Blood through Single Vessels

Poiseuille's law for a viscous fluid quantifies the relationship among the volumetric flow of blood through a blood vessel, modeled as a circular cylindrical tube, the geometric properties of the tube and the flow properties of the blood. Poiseuille's law (1846) is usually expressed as:

$$Q = \pi a^4 \Delta P/8\eta l, \qquad (2.1)$$

where Q is volumetric flow, the factor $\pi/8$ arises from the circular cross-section, a is the radius of tube, l is the length of tube, η is the viscosity of the blood, and ΔP is the pressure difference between the ends of the tube, also called the driving pressure or perfusion pressure. It is noteworthy that the fourth power dependence of flow on radius means that blood flow is quite sensitive to changes in radius, which can vary in the circulatory system as vasomotor tone in vessels controlling flow (i.e., mainly arterioles) changes. It should also be noted that vessel length is generally constant for a given vessel and that viscosity is a property of blood related to the ease with which it can be made to flow.

From the relationship among Q, ΔP, and R, one finds that R depends on the geometry of the vessel and the viscosity of the blood as

$$R = 8\eta l/\pi a^4. \tag{2.2}$$

The average velocity of blood through a vessel can also be expressed in terms of the above factors. Conservation of flow leads to the conclusion that volumetric flow is equal to the product of average velocity, v, and the cross-sectional area of the vessel, πa^2:

$$Q = \pi a^2 v. \tag{2.3}$$

Thus, the average velocity can be expressed as

$$v = a^2 \Delta P/8\eta l. \tag{2.4}$$

For a Newtonian fluid flowing through a vessel or tube of circular cross-section, the radial dependence of velocity is described as "parabolic" due to the quadratic dependence of velocity on radial position, r:

$$v(r) = v_0 [1 - (r/a)^2], \tag{2.5}$$

where a is the internal radius of the tube, and v_0 is the maximum velocity that occurs on the axis ($r = 0$); the minimum velocity is zero at the wall ($r = a$; called the "no slip" condition).

2.3 STRUCTURE AND FUNCTION OF THE MICROCIRCULATION

The microcirculation deserves special attention since it is across the walls of these vessels that the exchange of oxygen, among other substances, takes place [101]. Furthermore, the arterioles, also known as the "resistance" vessels, are the primary site for control of blood flow. Thus, the blood vessels of the microcirculation play important roles in both the convective (arterioles) and diffusive (capillaries) transport of oxygen. These blood vessels are classified as arterioles, capillaries and venules and vary in diameter from about 100–200 μm for the largest arterioles and venules down to about 5 μm for capillaries. In terms of their structure, all these vessels possess an inner layer of

endothelial cells. In addition, the arterioles have a circumferential layer of vascular smooth muscle with which they can control blood flow and its distribution within organs. Venules typically have thinner layers of smooth muscle.

The primary function of the circulatory system is to exchange substances between blood and tissue, and these exchange processes take place in the microcirculation. The classes of vessels playing a role there are the arterioles (resistance vessels which regulate flow), capillaries (the primary exchange vessels) and venules (exchange and collecting vessels). The amount of flow through the capillaries appears to be regulated to maintain adequate tissue oxygenation. The regulation of blood flow appears to be accomplished by the coordination of several different mechanisms which affect the flow of blood through precapillary vessels.

2.4 TRANSCAPILLARY EXCHANGE OF SOLUTES

The transport mechanism of passive diffusion is a rapid and efficient mode of molecular exchange over the small distances (tens of micrometers) between the blood supply (capillaries) and tissue cells. Fick's first law of diffusion (1855) describes the net rate of transfer of a substance from a location of high concentration to one of lower concentration:

$$\Delta N/\Delta t = D\,A\,(\Delta c/\Delta x) = P\,A\Delta c, \qquad (2.6)$$

where $\Delta N/\Delta t$ is the amount of the substance exchanged per unit time, D is the diffusion coefficient for the substance through the capillary wall, A is the surface area available for diffusion (proportional to the number of blood-perfused capillaries), Δc is the concentration difference across the capillary wall or $\Delta c = c(\text{blood}) - c(\text{ISF})$, Δx is the thickness of the capillary wall (~1 μm), and P is the permeability of the capillary wall defined as $D/\Delta x$.

In regard to the permeability characteristics of the capillary wall, the wall is composed of a single layer of endothelial cells about 1 μm thick. For lipid-soluble substances (e.g., oxygen), the entire wall surface is available for diffusion. For water-soluble substances (e.g., glucose), there are small aqueous pathways equivalent to cylindrical pores 80–90 Å in diameter through which they may pass. Total pore area is about 1/1000 (i.e., 0.1%) of the surface area of a typical capillary. The permeability of the wall to a particular substance depends upon the relative size of the substance and the pore ("restricted" diffusion).

During times of increased activity in a tissue, there is a need for delivery of more nutrients to the active tissue, as well as a need to eliminate accumulated metabolic wastes that result from the

increased metabolism of the tissue. The amount of a substance which is exchanged between blood and tissue can be increased by having more of the anatomically present capillaries perfused with blood. This increases the surface area available for exchange and reduces the distance that exchanged molecules must diffuse, both of which increase the efficiency of diffusion. There is some controversy regarding whether it is the number of blood-perfused capillaries that is important or, in the case of oxygen exchange, whether it is the surface area of the capillary wall in contact with moving red blood cells. Under resting, baseline conditions, the equivalent of only a fraction (about 1/3 to 1/2) of the capillaries in a given tissue are being perfused at any given moment. During times of increased demand for nutrients and especially oxygen (e.g., heart and muscle tissue during exercise), more capillary pathways can be opened to flowing red blood cells. Whether a given capillary is open or closed depends on the contractile state of a region of smooth muscle (probably a terminal arteriole) located near the entrance to a capillary [65]. This view of capillary recruitment is the one proposed by Krogh [69], although in recent years this view has been replaced by the idea that most capillaries are perfused by red blood cells at any given time, and increased activity simply increases blood flow in capillaries of the active tissue [97].

2.5 REGULATION OF BLOOD FLOW

Since the convective supply of oxygen depends directly on blood flow, the regulation of tissue oxygenation depends critically on the regulation of blood flow. The cardiovascular system controls blood flow to individual organs (1) by maintaining the input pressure to each organ within narrow limits by the mechanisms designed to regulate arterial pressure and (2) by allowing each organ to adjust its vascular resistance (R) to blood flow to an appropriate value. The cardiac output (CO) is distributed among the various organs according to their respective resistances so that flow (Q) in an organ is given by:

$$Q = (\mathrm{TPR}/R)\,\mathrm{CO}, \tag{2.7}$$

where TPR is total peripheral resistance of the systemic circulation. There are three major mechanisms that control the function of the cardiovascular system: local, neural and humoral. They can work independently of each other, but there are also interactions among them. The local mechanisms are intrinsic to a tissue and will be described in more detail below. The neural mechanisms involve the central nervous system and rely primarily on the release of norepinephrine from the sympathetic nerve endings of the autonomic nervous system. Finally, the humoral mechanisms rely on circulating

vasoactive hormones, such as angiotensin II and epinephrine. It is important to recognize that the vasoregulation occurs in the resistance vessels. In the context of the regulation of tissue oxygenation, it is most appropriate to focus on the mechanisms that control blood flow at a local level.

2.5.1 Local Regulation of Blood Flow

The local mechanisms for regulating blood flow are intrinsic to the various tissues and can operate independently of neurohumoral influences [17, 110]. Local regulatory processes allow each tissue in the body some measure of autonomy to satisfy its current and particular requirements in regard to blood flow. Because the various organs and tissues of the body are connected in parallel, the cardiac output can be redistributed among the tissues should their relative need change by altering the resistance (R) to blood flow in the affected tissues.

The site of local regulation of blood flow is the microcirculation, which is composed of a network of blood vessels—arterioles, capillaries, and venules—whose functions are regulation of tissue perfusion and exchange of substances between blood and tissue. Although the topology of vascular networks is typically quite complex, as a first approximation, one can think of most networks as a collection of microcirculatory "units" connected in parallel, where each unit is composed of a feeding arteriole, several capillaries arising from the arteriole and a venule which collects the blood after molecular exchange has taken place between it and the interstitial fluid. Because of the parallel structure of the network, which is a collection of these microcirculatory units, it is possible to redistribute blood flow from one region to another within a tissue to accommodate any alterations in local metabolic needs.

Examples of local blood flow control processes are autoregulation, reactive hyperemia and active (or functional) hyperemia. The term "autoregulation" in this context refers to the tendency for organ blood flow to remain constant in the face of local changes in arterial or perfusion pressure. Autoregulation is observed in virtually every vascular bed. It is most pronounced in the brain and kidney and is prominent in the heart, skeletal muscle, intestine, and liver. Recall that flow (Q) equals perfusion pressure (ΔP = difference between inflow arterial pressure and outflow venous pressure, $P_a - P_v$) divided by vascular resistance (R) so that, as ΔP rises through the autoregulatory range ($P_a \approx$ 80–160 mm Hg in brain and kidney), R must increase to maintain constant flow. Reactive hyperemia refers to the elevated blood flow observed in an organ when flow is restored following a period of circulatory arrest (i.e., occlusion of the blood supply). Hyperemia is literally an excess of blood in a region. The magnitude of the hyperemia is related both to the duration of the occlusion period and to the pre-occlusion blood flow. Active (or functional) hyperemia refers to the increase in blood flow which accompanies an increase in the metabolic activity of an organ or tissue. It has been described in skeletal and cardiac muscle, brain, intestine, stomach, salivary glands, kidney, and

adipose tissue. The name of the hyperemia depends upon the specific function of the tissue (e.g., contraction hyperemia for muscle or secretory hyperemia for various glands). Each one of these examples of local regulatory processes can be linked to the regulation of tissue oxygenation.

2.5.2 Mechanisms of Local Regulation

Two major mechanisms have been proposed to account for the local regulatory phenomena described above: the myogenic mechanism and the metabolic mechanism. Although these mechanisms appear to act independently, the expression of each mechanism varies among tissues and some combination of each one is probably operative, depending on the particular intervention, i.e., altered perfusion pressure, flow, or tissue activity.

2.5.3 Myogenic Mechanism

The myogenic mechanism, in essence, states that vascular smooth muscle actively contracts in response to stretch, in an attempt to maintain circumferential wall tension, T, relatively constant in the resistance vessels. The relationship among wall tension (T), intravascular pressure (P), internal radius (a), and vessel wall thickness (w) is given by the law of Laplace (1805) for a cylindrical elastic tube: $T = Pa/w$. Thus, elastic blood vessels exposed to an increased intravascular pressure will become passively distended. The smooth muscle in the vessel wall responds by active contraction (leading to vasoconstriction) which tends to return wall tension near its baseline value and vascular caliber below its original value. The myogenic mechanism is sometimes referred to as pressure-related control of blood flow [16].

2.5.4 Metabolically Linked Mechanisms of Blood Flow Regulation

Tissue cells continuously utilize ATP as an energy source to maintain cellular function. The two most common ways in which ATP can be produced are by oxidative phosphorylation and glycolysis. Because oxidative phosphorylation is the primary pathway for most cells to generate ATP, cells have a continuous need for oxygen. In the presence of an adequate supply of oxygen (normoxia), the adenosine diphosphate (ADP) produced from the hydrolysis of ATP is rephosphorylated as part of the process of oxidative phosphorylation, and the contribution of glycolysis to ATP production is negligible.

When the activity of a tissue increases, blood flow through that tissue is observed to increase to a degree that is proportional to the increase in activity. Blood flow increases because the vascular smooth muscle of the arterioles relaxes in response to an increase in the concentration of one or

more vasodilator molecules. Several different hypotheses have been put forward to account for the observed vasodilation, but at the present time none of these possibilities has been able to account quantitatively for the increased perfusion, i.e., active or functional hyperemia. Figure 1 illustrates four of the most prominent ideas to explain the activity-induced increase in blood flow. Each of these hypotheses focuses on a different mechanism involving one or more different vasodilators.

2.5.4.1 Metabolic vasodilator hypothesis

The oldest of the hypotheses dates back to 1876 when it was proposed that increasing the activity of a tissue (e.g., skeletal muscle contraction) led to the elaboration of a vasodilator molecule, substance X, from the active parenchymal cells (see Fig. 1A). Over the years many attempts were made to identify "substance X" to no avail. By chemical analysis of arterial and venous blood associated with the active tissue, substances whose concentration was higher in venous blood were viewed as candidates for the vasodilator produced by the active parenchyma. In fact, a number of molecules were identified in this way, many of which had vasodilator properties. Examples are adenosine and lactic acid, associated with the development of hypoxia in the active cells. Other substances, such as potassium ion, carbon dioxide, and osmolarity, are associated with the increase in activity, but not directly with cellular hypoxia. Other potential chemical mediators of the vasodilation have also been proposed, but so far no one substance or even combination of substances has been able to account for the observed vasodilation. Because multiple potential chemical mediators of the vasodilation have been proposed, the concept of "redundancy" has been introduced [62, 73, 83] whereby some unknown combination of vasodilators interact in a way to provide the needed amount of blood flow and consequent oxygen supply to the active parenchymal cells. Although "redundancy" exists in the sense that there are undoubtedly multiple vascular smooth muscle and parenchymal cells involved in the functional hyperemic response, for there to be simultaneously present multiple chemical mediators of vasodilation, each with its distinct mechanism of action and dose-response relationship, the integration of these disparate inputs in just the correct way to match oxygen supply to oxygen demand would appear to be unreasonably complicated for such an important function. The increased tissue activity leads to an increase in ATP utilization and the resultant increase in oxidative and glycolytic metabolism lead to reduced PO_2 and increased production of lactic acid. If the supposed cellular hypoxia is the cause of an increased release of adenosine (through ATP degradation) and lactic acid, then restoration of blood flow (i.e., the functional hyperemia) should return the active cells to their baseline state of being adequately oxygenated, thereby removing the hypoxic stimulus that caused the production and subsequent elaboration of the vasodilators in the first place. In order for this hypothesis to be valid, all the parenchymal cells should constantly be on the brink

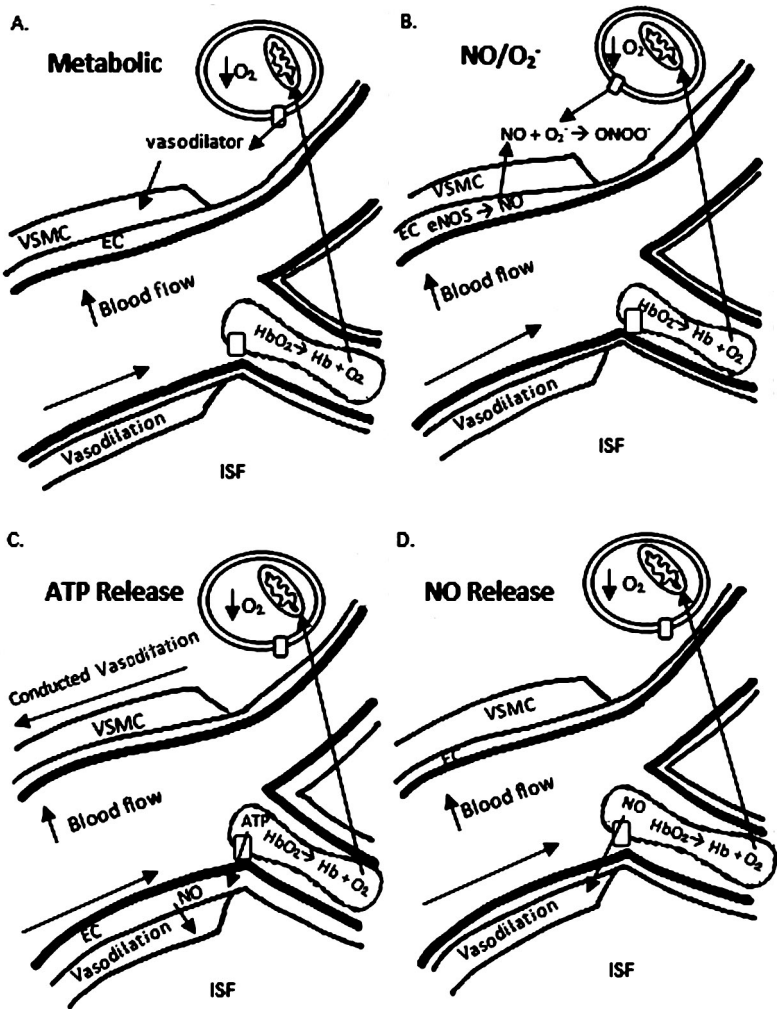

FIGURE 1: Schematic diagram of metabolically linked mechanisms for blood flow regulation in response to an increase in tissue activity. A common feature for all four mechanisms is that the increased activity causes more oxygen to be consumed, lowering intracellular and interstitial PO_2. This decreased PO_2 is then related ultimately to an increased concentration of the particular vasodilator involved in the vasomotor response, followed by an increase in blood flow. A. Metabolic mechanism; B. NO/O_2^- radical pair mechanism; C. ATP release from RBCs mechanism; D. NO release from RBCs mechanism.

of hypoxia, a state which does not appear to be borne out by experimental observation. In addition to the logical inconsistency regarding removal of the hypoxic stimulus, the fact that the metabolic vasodilator hypothesis cannot be falsified, as an unobserved vasodilator can always be proposed to explain any discrepancy in response, this hypothesis does not stand on a firm footing.

2.5.4.2 Erythrocyte as a mobile oxygen sensor hypothesis

In the mid-1990s the intriguing idea was proposed that the carrier of oxygen, the erythrocyte or red blood cell (RBC), was involved in the vasodilatory response to increased tissue activity. Two different hypotheses utilizing the release from the RBC of either ATP [28] or nitric oxide [117] were published at about the same time. A common feature of both proposals is that an increase in parenchymal cell activity leads to a reduction in PO_2 within the active cells, as well as in the adjacent interstitial fluid, and this reduction in PO_2 is propagated by diffusion, ultimately to the RBCs flowing through nearby capillaries which provide oxygen to the parenchymal cells. As oxygen is then released from the hemoglobin molecules inside the RBCs, a conformational change takes place whereby the hemoglobin goes from the oxygenated R (relaxed) state to the deoxygenated T (taut) state. From this point the two hypotheses differ in regard to the identity of the vasodilator and its mode of release. These ideas are illustrated in Fig. 1, panels C and D, respectively.

2.5.4.2.1 ATP release from RBCs The RBCs require a supply of ATP to run the Na-K ATPase (Na-K pump) which maintains the ionic gradients across the RBC membrane. Because RBCs do not contain mitochondria, they produce ATP by glycolysis and have an ATP concentration in the millimolar range. As the hemoglobin molecule transitions from the R to the T state, this conformational change is transmitted to the RBC membrane and leads to the release of ATP through a band 3 channel [60]. The ATP then moves through the plasma to the vascular endothelium, where it binds to P_2Y purinergic receptors, causing the production and release of NO, prostacyclin (PGI_2), and endothelium-derived hyperpolarizing factor (EDHF). These substances then produce a conducted electrical response which is conducted upstream to the arterioles, whose vasodilation is the cause of the observed functional hyperemic response (see Fig. 1C).

2.5.4.2.2 NO release from RBCs According to this version of the "RBC as a mobile O_2 sensor" hypothesis, as the RBCs pass through the lungs, NO produced by nitric oxide synthase in the endothelial cells (eNOS) is taken up into the RBC cytoplasm and is carried by the hemoglobin

as S-nitroso hemoglobin (SNOHb) at the cysteine-93 locus on the beta chains of hemoglobin. As in the ATP release hypothesis, when the hemoglobin becomes deoxygenated, there is a conformational change from the R to the T state and this causes NO to be released from the hemoglobin. NO then diffuses out of the RBC and to the arteriolar smooth muscle, where it produces vasodilation. The most serious issue with this hypothesis is that it is difficult to understand how the NO can escape from the RBC, given the very high affinity of the unliganded heme group for NO (see Fig. 1D). An interesting alternative to this hypothesis is that the source of the NO is NO_2^- ion which enters the RBC from the plasma and is acted on by deoxygenated hemoglobin which takes on the role of a nitrite reductase, enzymatically converting NO_2^- to NO [35]. How the free NO is able to exit the RBC cytoplasm without being scavenged by the hemoglobin remains an obstacle to understanding how this mechanism works in vivo.

2.5.4.3 Nitric oxide/superoxide radical pair interaction hypothesis

The endothelial cells continuously produce nitric oxide (NO) through the action of the constitutive enzyme eNOS on the substrates l-arginine and molecular oxygen. NO is a vasodilator and, for it to be "the" chemical mediator of functional hyperemia, a complementary molecule should be released from the parenchymal cells to neutralize some of the NO produced, thereby modulating the concentration of NO in the interstitium and, hence the degree of vasodilation. An obvious candidate for this complementary signaling molecule is superoxide (O_2^-), produced into the extracellular space by NAD(P)H oxidase in the plasma membrane of the parenchymal cells (e.g., sarcolemma of skeletal muscle cells) from its substrates oxygen and NADPH. Chemically, the radical pair NO and O_2^- react with each other rapidly and selectively (spin-prohibited interaction with other non-radicals), so that the NO concentration can be altered quickly by slight changes in the production of O_2^-. Under resting or inactive conditions, the parenchymal cell would be expected to be well oxygenated, so that the production of O_2^- would be high and thus neutralize much of the NO produced by the endothelium, resulting in a state of partial constriction and relatively low blood flow. When the activity of the parenchymal cells increase (e.g., increased skeletal or cardiac muscle contraction, increased neural activity in the brain, and digestion of a meal in the gastro-intestinal system) their oxygen consumption would increase, thereby lowering the local PO_2 and reducing O_2^- production; under these conditions NADH also moves from the cytosol to the mitochondria, reducing the other substrate for NAD(P)H oxidase. NO concentration in the surrounding interstitium would then increase, resulting in vasodilation and increased perfusion. When the parenchymal cell activity wanes and oxygen consumption decreases, PO_2 will increase and NADH will be shuttled from the

mitochondria to the cytosol, restoring the O_2^- production, $[NO]_{ISF}$ and blood flow to the "resting" level. A detailed description of this mechanism is presented by Golub and Pittman [39–41] and Fig. 1B provides a pictorial view of the processes involved.

2.5.5 Other Oxygen-Linked Issues of Flow Regulation

Several other observations related to the regulation of blood flow, and hence convective oxygen delivery, will be considered here as they have a direct impact on the regulation of tissue oxygenation. The question arises as to whether tissue P_{O_2} is closely regulated and whether oxygen plays a direct role in oxygen-linked flow regulation by acting directly on the vascular smooth muscle of resistance vessels. Duling and Berne [22] found that tissue P_{O_2} was regulated within a narrow range, even when the P_{O_2} of the superfusion solution flowing over the tissue under observation (e.g., hamster cheek pouch or cremaster muscle) was varied over a relatively wide range of tens of mm Hg. Thus, raising the P_{O_2} of the superfusion solution led to arteriolar constriction, but a relatively constant tissue P_{O_2}, suggesting that some components of the tissue and/or the arteriolar wall were sensitive to oxygen and communicated with the arterioles to limit blood flow and oxygen delivery to a desired level. Furthermore, subsequent experiments using a bicarbonate-buffered superfusion solution showed that in the presence of carbon dioxide, tissue P_{O_2} was still regulated but at a higher P_{O_2} than in the absence of carbon dioxide [19]. Duling [19] suggested that there were two possibilities that might explain this finding: (1) the oxygen supply might be regulated by a direct effect on vascular smooth muscle or (2) oxygen acts only through cellular metabolism and that the rate of oxygen delivery to cells, rather than the absolute P_{O_2}, would be regulated. Further *in vitro* [93] and *in vivo* [20] experiments raised serious doubts that vascular smooth muscle had the requisite sensitivity to oxygen, so that the blood and nearby tissue would need to become severely hypoxic (P_{O_2} of only a few mm Hg) before arteriolar smooth muscle would respond with relaxation. Cytochrome *c* oxidase, which has a high affinity for oxygen (low K_M or P_{50} of ~1 mm Hg) and is thus responsive to oxygen levels over a narrow range, was considered to be the oxygen "sensor" in the above studies. Duling [21] later considered that other oxidases and oxygenases, with higher K_M's or P_{50}'s and which serve other oxygen-linked processes, might take on the role of oxygen sensor.

Is tissue P_{O_2} really regulated in the sense of being a controlled variable in a feedback loop which involves a chemical mediator? There are several oxygen-linked systems which have been proposed for modulating blood flow [17]. Because the maintenance of tissue oxygenation is such an important feature for survival of the organism, it seems necessary that some mechanism must exist to ensure an adequate oxygen supply to all cells of the organism. Perhaps there is some overall

coordination of events which regulates blood flow to ensure delivery of oxygen and nutrients and removal of metabolic wastes, and leads to an oxygen level consistent with maintaining a balance between energy demand and production.

2.5.6 Conducted Vasomotor Responses

It has been found that the local vasomotor responses can spread from their point of origin to upstream and downstream sites by electrical conduction through gap junctions between endothelial and vascular smooth muscle cells [2, 110]. Since the metabolic responses most closely associated with the regulation of tissue oxygenation will be expressed and sensed initially in the terminal branches of the microvascular network (i.e., capillaries and terminal arterioles), their spread to upstream sites will typically lead to increased blood flow, and hence oxygen supply, through increased vasodilation of arterioles. Thus, the local signals confined to perhaps tens of micrometers can exert their influence over a much wider spatial domain of hundreds to thousands of micrometers, thereby recruiting many vessels in the network to participate in the hyperemia. The sensitivity of the vascular wall to various locally produced vasoactive substances (classic metabolic mechanism), shear stress (flow-induced release of NO from the endothelium), and stretch (myogenic mechanism) appears to vary along the vascular network and from organ to organ. The conducted vasomotor responses thus act to coordinate and integrate the regulation of tissue oxygenation.