Quantifying the role of oxygen pressure in tissue function

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It has been suggested that oxygen had reached significant levels in the earth’s oceans as early as 2.5 billion years ago and has rapidly increased to near current levels about 50 million years later (6). The appearance of oxygen dramatically changed the chemical composition of the oceans and atmosphere, setting the stage for the development of life-forms using oxygen chemistry for many of their basic energy and synthetic requirements. Oxygen from the environment could be used to obtain large amounts of chemical energy from relatively small amounts of material. This permitted life-forms to store enough energy “on board” to support motility and extended periods between feeding, especially critical to higher life-forms. Moreover, oxygen chemistry is relatively benign, and organisms can live for tens and even hundreds of years, continuously using very large amounts of oxygen, without accumulation of life-threatening levels of undesirable oxygen metabolites.

In order for motile animals to attain sizes of more than a few thousand cells, it is necessary to extract oxygen from the environment and distribute it to every cell of the body. In higher animals this is an engineering marvel, a closed circulatory system that extracts oxygen from the environment and pumps oxygenated fluid through an intricate vascular network that reaches all of the cells of the body. It is not surprising that the fields of biochemistry and physiology originated from early studies of the chemistry of oxygen, which was discovered independently by Joseph Priestley in 1774 (12) and Carl Wilhelm Scheele in 1772, and of blood flow, where the first accurate descriptions are attributed to Ibn al-Nafis in 1242 and William Harvey in 1628. In humans, oxygen delivery to the individual cells of tissue is critically important from conception to death. If at any point in our lives there is a serious malfunction in the oxygen delivery system, pathology results, or if other events, such as trauma, occur that interferes with oxygen delivery, this exacerbates the pathology. The nature of the pathology associated with malfunction of the oxygen delivery system is dependent on the tissue affected and the severity of oxygen deficit. A very incomplete list of medical problems in which oxygen delivery plays a substantial role includes miscarriages, birth defects, asthma, traumatic injury, peripheral vascular disease (diabetic and other), individual organ failure, loss of vision (age-related macular degeneration), and last, but not least, death itself.

Given the long history of research on oxygen and its extraordinary medical importance, it is surprising how much remains to be understood about the functional requirements that drove the evolutionary design of the oxygen delivery system and about the metabolic basis of the tissue pathology. It is generally agreed that most cells require oxygen to produce metabolic energy as well as to produce many other metabolic and regulatory metabolites. There are, however, inconsistent data and widely differing hypotheses concerning how much (what pressure or concentration) is required to support cellular metabolism and how the oxygen levels in cells within tissues are regulated. There is a wide disparity in the published data and in the hypotheses used to interpret the cell and tissue responses to changes in oxygen pressure.

As the scientific community rapidly expanded during the 20th century, the number of scientists and general understanding of metabolism increased exponentially. Initially, both biochemists and physiologists gave high priority to understanding the role of oxygen in cellular metabolism and oxygen delivery to tissue. Biochemists focused on the role of oxygen in the metabolism, identifying and characterizing oxygen metabolites, the enzymes using oxygen and oxygen consumption. Physiologists focused on the tissue responses to changes in oxygen levels, such as vascular resistance and blood flow, as well as on oxygen-sensing activity as exemplified by the carotid and arterial bodies. Divergence of opinion appeared as the physiologists reported dependence of physiological function on oxygen pressure throughout the physiological range, up to and including the oxygen pressures in arterial blood. This contrasted to reports by biochemists that there was no significant alteration in oxygen consumption until the oxygen pressures fell too low to be reliably measured. Beginning in the 1950s, biochemist study of oxygen was aided by the introduction of oxygen electrodes, an advance that greatly facilitated oxygen measurements. Innovative improvements in oxygen electrode technology included improving the measurement of stability and accuracy by isolating electrodes from the biological media with inert, oxygen permeable membranes by Clark (3) and miniaturizing the electrodes to where they could be inserted into individual cells by Whalen and coworkers (18, 19). When applied to the tissue of anesthetized animals, these miniaturized electrodes reported that the intracellular and pericellular oxygen pressures were similar and typically between zero and about 5 mmHg, much lower than the mixed venous values of 30–40 mmHg. In parallel developments, the rate of oxygen consumption by isolated cells were reported by Longmuir (9) to remain unchanged as the oxygen pressure lowered from air saturation (146 mmHg) to about 2 mmHg. In suspensions of isolated mitochondria, this independence of oxygen pressure was reported to extend down to 10^{-7} mmHg (2, 10, 11). Based on this and similar data, by the mid-1970s, the following were concluded. First, cells in tissue and isolated cells have all the oxygen they need for normal function if the oxygen pressure is above about 2 mmHg, and in tissue (in cells) the oxygen pressures are typically well below the mixed venous values. Regulation of the oxygen delivery system is designed only to avoid regions of anoxia within the tissue. Second, mitochondria are not sensitive to oxygen pressure changes above 1 mmHg and, therefore, cannot serve as oxygen sensors for the regulation of physiological functions such as breathing, blood flow, or biological adaptations to hypoxia.
These biochemical conclusions were reconciled with the physiological data by assuming that in tissue the oxygen measured in the vascular system was very different from that in the cells. Tissue heterogeneity, oxygen diffusion gradients, and/or unequal flow through individual vessels in the capillary network were assumed to introduce large differences between vascular and cellular oxygen pressures.

These conclusions were incorporated into the textbooks and became widely accepted, but data inconsistent with the conclusions began appearing, driven by steady developments in oxygen electrode technology and other complementary oxygen measurement methods. The following are some examples. First, cells in tissue have all the oxygen they need for normal function if the oxygen pressure is above 2 mmHg, and in tissue the oxygen pressures are much lower than the mixed venous values. In 2002, Baumgärtl and coworkers (1) reported that when care is taken to relieve tissue compression due to electrode insertion, the resulting oxygen histograms for kidney and brain showed maxima near the mixed venous value, and, in fact, there were very few oxygen measurements <10 mmHg. This is consistent with measurements made (1) in the vasculature of a resting skeletal muscle using oxygen-dependent quenching of phosphorescence and selective placing the phosphorescent probes in either the blood plasma or the interstitial space (22) and 2) in brain tissue when measuring oxygen using either oxygen-dependent quenching of the phosphorescence quenching (5) or using oxygen-sensitive electron paramagnetic resonance probes (14, 15) and 3) with data obtained in a variety of tissues using an oxygen-sensitive binding of the nitroimidazole, EF5 (7, 8). Thus the earlier reported that low-tissue oxygen values appear to have arisen from a combination of the anesthetic used, traumatic injury to the tissue during preparation, and other technical artifacts arising from tissue preparation and measurement. Second, mitochondria are not sensitive to oxygen pressure changes above 1 mmHg and, therefore, cannot serve as oxygen sensors for physiological regulation of breathing, blood flow, etc.; as expressed by Chance (2), “the individual mitochondria are either in the normoxic state 3 or the anoxic state 5.” It is technically very difficult to determine the oxygen dependence of mitochondrial and cellular oxygen consumption. The oxygen concentrations involved are low enough that they are difficult to measure, and the rates of oxygen consumption are high. As a result, the oxygen concentrations change very quickly unless there is a mechanism for continuously replenishing that which is being used, and oxygen electrodes have substantial (in s) response times. The experimental designs were limited to steady-state conditions, in which oxygen was slowly added to the medium to minimize the rate of change in oxygen concentration. Degn and Wohlrab (4) first reported that the rate of oxygen consumption by suspensions of isolated mitochondria was much more dependent on oxygen concentration when the mitochondria were metabolically coupled than when they were uncoupled. This observation was confirmed for isolated mitochondria (21) and extended to suspensions of isolated cells (13) using oxygen-dependent quenching of phosphorescence (16, 17). This was a newly developed oxygen measurement method for which the response time of <100 ms made possible accurate measurement of the rate of oxygen depletion from the medium. Measurements of the cellular energy state (ATP and ADP) and of the level of reduction of cytochrome c within the mitochondrial respiratory chain, a sensitive reporter of the coupling of electron transport to ATP synthesis (20, 21), indicated that the oxygen sensitivity of oxidative phosphorylation extended to near-mixed venous oxygen pressures.

This brief overview indicates some of the basis for the current controversy regarding the role of local (cellular) oxygen pressure in tissue biochemistry and physiology. Consensus appears to have been reached that the levels of oxygen in the tissue are much higher than originally reported by Whalen and coworkers. Under normal conditions the mean oxygen pressures in the vasculature and interstitial spaces of most tissues are 30–40 mmHg with very small fractions of the total volume with oxygen pressures <10 mmHg. The oxygen dependence of cellular oxidative phosphorylation, however, remains highly controversial. Quantitative knowledge of that dependence is critical to the understanding of not only cellular biochemistry but also a wide range of physiological functions that help to regulate both metabolism and the oxygen delivery system. Is mitochondrial oxidative phosphorylation dependent on the oxygen pressures in normal tissues? If so, then oxidative phosphorylation is an important player in the regulation of all aspects of cellular and tissue function, including the responses, physiological and pathological, to changes in oxygen levels in the cells. If not, then oxidative phosphorylation is affected only when the oxygen levels become too low to sustain life and is, therefore, not significantly involved in the physiological responses of cells to changes in oxygen supply, and the latter must be attributed to other reactions of oxygen.

It can be said that science is primarily based on measurements, and progress is correlated with the accuracy and reliability of those measurements. A rapidly increasing number of methods for oxygen measurement, each with different strengths and weaknesses, are becoming available and the technical limitations of each evaluated. As these methods mature, we can expect rapid progress in quantifying oxygen in tissues and cells and, as a result, important advances in understanding oxygen biochemistry and physiology and its role in human pathologies.

In their article, Golub and coworkers (4a) provide important data related to the design and function of the oxygen delivery system. The authors measure oxygen in the small arterioles of the microcirculation and in the pericellular (interstitial) space surrounding the vessels by introducing an oxygen-sensitive phosphorescent probe into each compartment and using a specially designed microscopic system to measure the phosphorescence lifetimes. Understanding oxygen delivery to tissue requires this information, since it is necessary for defining the functional role of arterioles in the microvascular tree that delivers the oxygen to the individual cells of tissue. Phosphorescence quenching is well suited to this application because it is minimally invasive, and the oxygen measurements can be made through a microscope. The design of a microscopic system capable of high spatial resolution and measuring phosphorescence decay at that resolution is, however, technically demanding. The importance of this article is not only in the measurements made but also in the attention to experimental detail in design of the instrumentation and execution of the measurements. Thorough evaluations of each possible source of error in the method are included. These, along with comprehensive controls, give confidence that the results are state of the art and provide a firm foundation on which to build.
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REFERENCES