Oxygen, pH, and mitochondrial oxidative phosphorylation

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Wilson DF, Harrison DK, Vinogradov SA. Oxygen, pH, and mitochondrial oxidative phosphorylation. J Appl Physiol 113: 1838–1845, 2012. The oxygen dependence of mitochondrial oxidative phosphorylation was measured in suspensions of isolated rat liver mitochondria using recently developed methods for measuring oxygen and cytochrome c reduction. Cytochrome-c oxidase (energy conservation site 3) activity of the mitochondrial respiratory chain was measured using an artificial electron donor (N,N,N’,N’-tetramethyl-p-phenylenediamine) and ascorbate to directly reduce the cytochrome c, bypassing sites 1 and 2. For mitochondrial suspensions with added ATP, metabolic conditions approximating those in intact cells and decreasing oxygen pressure both increased reduction of cytochrome c and decreased respiratory rate. The kinetic parameters [K_M and maximal rate (V_M)] for oxygen were determined from the respiratory rates calculated for 100% reduction of cytochrome c. At 22°C, the K_M for oxygen is near 3 Torr (5 μM), 12 Torr (22 μM), and 18 Torr (32 μM) at pH 6.9, 7.4, and 7.9, respectively, and V_M corresponds to a turnover number for cytochrome c at 100% reduction of near 80/s and is independent of pH. Uncoupling oxidative phosphorylation increased the respiratory rate at saturating oxygen pressures by twofold and decreased the K_M for oxygen <2 Torr at all tested pH values. Mitochondrial oxidative phosphorylation is an important oxygen sensor for regulation of metabolism, nutrient delivery to tissues, and cardiopulmonary function. The decrease in K_M for oxygen with acidification of the cellular environment impacts many tissue functions and may give transformed cells a significant survival advantage over normal cells at low-pH, oxygen-limited environment in growing tumors.

pressure in the cellular environment is essential to understanding cellular and tissue physiology.

The literature on the oxygen dependence of mitochondrial oxidative phosphorylation provides two different and completely incompatible sets of data and paradigms. One, based largely on the studies by Chance and coworkers (5, 9, 19, 20, 25), is that the critical oxygen concentration for bioenergetic function of mitochondria is ~0.05 Torr (0.08 μM). An important corollary of this lack of oxygen dependence ~0.05 Torr is that oxidative phosphorylation does not contribute to oxygen-dependent regulation of cellular metabolism or tissue function under physiological conditions. The second is based on the data showing that changes in cytochrome c reduction and energy metabolism respond to P_O2 changes at oxygen pressures as high as 30 Torr (15, 35–37, 40, 41). This value is near the mean P_O2 in the interstitial space of tissue and the mixed venous oxygen pressure. The corollary of the latter observation is that the mitochondrial oxidative phosphorylation, in addition to being the metabolic “power plant” of the cell, is highly responsive to changes in oxygen pressure under physiological conditions and, therefore, has a key role in regulation of metabolism and of the nutrient delivery system.

The reported experimental differences may have occurred, in large part, due to technical limitations of measurements of oxygen and reduction of the cytochromes. In many of the original experiments, “work arounds” were used to compensate for the technical limitations, which likely introduced sources of systematic errors that, in some cases, led to erroneous conclusions. In the present paper, we report measurements using most up to date technology for determination of oxygen pressures and cytochrome c reduction. Our results support the second paradigm: that the oxygen dependence of oxidative phosphorylation extends as high as to the mean oxygen pressures in tissue at a physiological pH of 7.4. Furthermore, we show that the oxygen dependence becomes lower at more acidic pH. The latter observation has far-reaching consequences, one of which is that the transformed cells in solid tumors may have significant metabolic advantage over the neighboring normal cells by being able to out-compete them for the limited supply of oxygen.

MATERIALS AND METHODS

Mitochondrial isolation. Mitochondria were isolated from the liver of 250-g male Sprague-Dawley rats. The rat was anesthetized (3% isoflurane), and, after the full anesthesia was achieved, the abdomen was opened. The liver was quickly removed, placed in 30 ml of cold isolation medium (0.22 M mannitol, 0.75 M sucrose, 0.5 mM EDTA, 5 mM MOPS buffer, pH 7.3), and minced with sharp scissors. The chilled tissue was then gently homogenized (40 ml medium/liver) with a motor-driven Teflon pestle in a glass vessel. Low-speed centrifugation (750 rpm, 8 min, Sorvall SS34 rotor) was used to remove the cell debris, and then the mitochondria were collected at a higher speed (7,500 rpm, 8 min). The mitochondria were washed...
twice by suspending the pellet in 70 ml, and then in 35 ml of the fresh medium, followed by centrifugation. Care was taken to leave behind all red regions (red blood cells) in the bottom of the mitochondrial pellets, so that the final material had no visual evidence of red cell contamination. The mitochondria were maintained on ice in a concentrated stock suspension (~2.5 ml) in isolation medium. Samples, obtained by 10- to 20-fold dilutions of the stock suspensions, provided the desired rates of oxygen consumption (between 0.3 and 0.9 Torr/s).

The investigators were trained in appropriate handling of rats. The experimental procedures were reviewed and approved by the University of Pennsylvania Animal Care and Use Committee before the study was carried out.

Measuring oxygen consumption and cytochrome c reduction. The mitochondria were suspended in an isotonic medium (0.22 M mannitol, 0.05 M sucrose) buffered with 15 mM 3-(N-morpholino)propanesulfonic acid (MOPS) and 15 mM tris(hydroxymethyl)amino-methane (Tris) at the indicated final pH values. Bovine serum albumin (Type 5, Sigma-Aldrich) was added to a final concentration of 1.5% by weight. \( O_2 \) was measured using the oxygen-dependent quenching of phosphorescence (23, 29, 31). A fiber-optic phosphorescence lifetime measurement instrument, operating in time domain (16, 30), and a phosphorescence probe Oxyphor G2 (7, 21, 31), bound to bovine albumin, were used to measure the oxygen pressure in the medium. Dunphy et al. (7) reported calibration of the oxygen dependence of albumin, were used to measure the oxygen pressure in the medium.

At the TMPD concentrations used in our experiments, the phosphorescence lifetime of G2 in the presence of 1.5% bovine serum albumin at zero oxygen (\( \tau_0 \)) and 22°C was 260 \( \mu s \), and the oxygen quenching constant was 200 Torr\(^{-1}\)s\(^{-1}\). We determined that \( \frac{N,N,N',N'-tetramethyl-p-phenylene diamine}{N,N,N',N'-tetramethyl-p-phenylene diamine} \) (TMPD) is able to act as an independent quencher of G2 phosphorescence. Consequently, \( \tau_0 \) was decreased in the presence of TMPD. At the TMPD concentrations used in our experiments, \( \tau_0 \) was ~160 \( \mu s \). In every experiment, \( \tau_0 \) was measured, and the measured value was used in subsequent conversion of the phosphorescence lifetime to oxygen pressure. Importantly, the oxygen-quenching constant (slope of Stern-Volmer calibration plot) is completely insensitive to independent quenchers, such as TMPD.

The measurement chamber was made of glass. After filling with mitochondrial suspension containing G2 (~2 \( \mu M \)) and the albumin, it was sealed with a ground glass stopper with an access port (1 mm in diameter, 1 cm long), filled with the medium. The unstirred medium in the access port excluded oxygen from the air from entering the reaction medium. The chamber had ~1-cm light path, held a total volume of 2.93 ml, and was equipped with a 4 mm \( \times \) 1.5 mm magnetic stirring bar. The chamber was placed inside a custom-constructed holder, equipped with a magnetic stirrer, and containing five fiber-optic ports. Two of the ports, positioned at the same level at the right angle to one another, served to interface 3-mm optical fibers to the phosphorimeter. The remaining three ports, whereby two ports (light sources) were positioned across from the third port (detector), were used to interface 3-mm fibers to an in-house constructed LED-based dual-wavelength spectrophotometer. This instrument was used to measure the state of reduction of cytochrome \( c \) by the change in absorbance at 550 nm, the peak of the \( \alpha \)-absorption band of cytochrome \( c \), and relative to the isosbestic point at 540 nm. The fraction of the cytochrome \( c \) that was reduced (\( f_{\text{red}} \)) was calculated, assuming cytochrome \( c \) became fully reduced when oxygen in the sample was depleted.

The light sources in the spectrophotometer were two LEDs (5 W) that could be switched on and off in ~1 \( \mu s \). The light from the LEDs was passed through band-pass filters (550 and 540 nm center wavelengths, 10-nm band pass) and coupled to the fibers, located on the same side of the sample chamber (see above), directly across from the fiber leading to the detector. The detector was an Avalanche Photodiode (Hamamatsu, 3.5-\( \mu s \) rise time). LEDs were modulated using a custom-built voltage-current converter, controlled by digital data acquisition board (NI USB-6251, National Instruments, 16 bit, 1 MHz). In operation, light of one LED was modulated by a square wave, consisting of six 200-\( \mu s \)-long light-on/50-\( \mu s \) light-off periods (total time 2.4 ms). The transmitted light was registered by the detector, whose output was digitized and analyzed on the fly. Modulation amplitude, attenuated due to light absorption, was averaged over six cycles. The second LED was activated immediately, and the measurement was performed in the same manner. After completion of the second measurement, a control TTL signal was issued to the phosphorometer, which activated the oxygen measurement operation. All three measurements (light intensities at 540 nm, 550 nm, and phosphorescence lifetime) were completed in ~200 ms, and the next sequence followed immediately. Because the operation of the two instruments was “time locked”, there was no time difference error when comparing the two sets of measurements. As a result, the oxygen and cytochrome \( c \) measurements are correlated in time with differences of <50 ms.

The phosphorimeter and dual wavelength spectrophotometer operations were programmed in C/C++ (Nokia). The data were analyzed using Origin 7 software (Originlab).

Measuring cytochrome-c oxidase activity. Cytochrome-c oxidase of the respiratory chain is responsible for oxygen consumption, reducing oxygen to water. The rest of the respiratory chain provides the necessary reducing equivalents through the reduction of cytochrome \( c \), but does not otherwise affect the electron transfer in the oxidase (phosphorylation site 3). Function of cytochrome-c oxidase can be effectively isolated from that of the rest of the respiratory chain by providing an alternative source of reducing equivalents for reducing cytochrome \( c \). An artificial reducing agent, TMPD, is available that can directly reduce cytochrome \( c \), while being rapidly re-reduced in the presence of millimolar levels of ascorbate. Under the measurement conditions, the oxidized form of TMPD, known as Wurster’s blue, is maintained at levels too low to affect our measurements. When mitochondria are supplemented with ascorbate and TMPD, the result is oxidation of ascorbate by molecular oxygen, with the reducing equivalents passing quantitatively through cytochrome \( c \) and cytochrome-c oxidase. This reaction is sensitive to site 3 inhibitors (cyanide, CO, etc.), but insensitive to inhibitors of sites 1 (such as rotenone) and 2 (such as antimycin A). The transfer of electrons from TMPD to oxygen is coupled to ATP synthesis, and ADP/O ratios between 0.5 and 0.9 have been reported for mitochondria treated with antimycin A to inhibit oxidation of endogenous substrates (14). Including antimycin A or rotenone in the assay medium had no effect on any of the measurements reported in this paper.

RESULTS

The oxygen dependence of respiration and reduction of cytochrome \( c \) measured in suspensions of rat liver mitochondria. Rat liver mitochondria were suspended in assay medium, and the rates of oxygen consumption and level of cytochrome \( c \) reduction were measured. Figure 1 shows data from a typical experiment. The data points are individual measurements, but, at 10 measurements per second, the individual points overlap to give an appearance of continuous lines. The oxygen pressures of interest were those <50 Torr (one-third of air saturation). The oxygen depletion curves are nearly linear when observed on this scale, and cytochrome \( c \) reduction begins while the oxygen pressure is still >10 Torr. Because mitochondrial preparations typically exhibit sufficient catalase activity and can rapidly decompose \( \text{H}_2\text{O}_2 \) into \( \text{O}_2 \) and water (catalase can be added to the medium to further accelerate the reaction), oxygen pressures could be cycled several times by injecting aliquots (typically ~5 \( \mu l \)) of \( \text{H}_2\text{O}_2 \) solution into the
stirred sample. For example, injection of 150 μM H2O2 resulted in the generation of 70–75 μM oxygen in 3–4 s. This permitted repetitive measurements of the oxygen depletion curve in each experiment. No evidence was observed for systematic differences between measurements during the initial oxygen depletion and those in later cycles.

The oxygen dependence of respiration and cytochrome c reduction at different pH values. Data from a representative measurement of cytochrome c reduction and oxygen consumption are shown in Fig. 2. The panels on the left are for mitochondria to which 1 mM ATP has been added, whereas those on the right are after the treatment with FCCP (p-trifluoromethylphenylhydrazone of carbonyl cyanide (+FCCP)), an uncoupler of oxidative phosphorylation. The figures include plots of the first derivatives of the oxygen data after smoothing the raw data by a 10-point fast Fourier transform (FFT) filter. These derivatives are the measures of the rates of oxygen depletion in the samples. The oscillations seen in the traces are largely due to instabilities in the rotation of the magnetic stirring bar. The signal-to-noise ratio in the oxygen decreases with increasing oxygen pressure (lower phosphorescence signal). The measurements shown are for the same mitochondrial preparation, making sure that the pH effects are not confounded with preparation-to-preparation differences in the mitochondria. The measurements shown are representative of more than 15 different mitochondrial preparations. All measurements were highly reproducible.

One notable observation is that, in coupled mitochondria in the presence of ATP, cytochrome c becomes progressively more reduced as pH becomes more alkaline. As seen in Fig. 2, the respiratory rate was the same at all three pH values at high oxygen pressures (>35 Torr), but cytochrome c was ~10% reduced at pH 6.9, and this increased to ~35% by pH 7.9. Addition of FCCP decreased the cytochrome c reduction at all pH’s to very low values (5–6%), eliminated noticeable reduction of cytochrome c at oxygen pressures >5 Torr, and eliminated decrease in oxygen consumption rate above ~3 Torr.

Relationship between oxygen dependence of the respiratory rate and \( f_{\text{red}} \). The oxygen dependence of the respiratory rate and the degree of cytochrome c reduction are both expressions of the oxygen dependence of oxidative phosphorylation. Increasing the \( f_{\text{red}} \) at a constant oxygen pressure increases the rate of respiration. Therefore, oxygen limitation results in a lower rate of oxygen consumption, but this decrease can be compensated for by an increase in the \( f_{\text{red}} \). The stoichiometric equation for site 3 of oxidative phosphorylation based on two-electron transfer model is:

\[
2c^{2+} + ADP + P_i + 1/2 O_2 + 2H^+ \rightarrow 2c^{3+} + ATP + H_2O
\]

The overall reaction is irreversible, and electron transfer is tightly coupled to ATP synthesis. In a mitochondrial suspension, the total cytochrome c content is constant, and the rate of respiration (\( v \)) is a function of the \( f_{\text{red}} \), \( P_{O2} \), and of the energy state \([ATP]/[ADP][Pi]\) (where brackets denote concentration). In the present studies, the energy state was set to a high value (+ATP) and presumed to be constant, facilitating study of the oxygen dependence of the reaction. The dependence on energy state will need to be addressed separately. The ideal approach to analysis of the present data would be by fit to a kinetic model that includes details of the cytochrome-c oxidase mechanism. This mechanism involves many reactions, however, and there is no generally accepted model. Instead, we use an empirical approach and fit the data using the familiar Michaelis-Menten kinetics.

The rate of the overall cytochrome-c oxidase reaction is a function of oxygen concentration and the amount (“concentration”) of reducing equivalents available to cytochrome-c oxidase. The reducing equivalents (electrons) are supplied to the cytochrome oxidase via cytochrome c, which is bound to the oxidase at a stoichiometric ratio of ~2 cytochrome c per cytochrome oxidase (cytochrome \( a_3s \)). Taking this into account, the reaction rate can be written as a function of oxygen concentration and \( f_{\text{red}} \). Previous measurements (37), verified by the present experiments, have shown that, when mitochondria are in the high energy state (+ATP) and under saturating concentrations of oxygen, the reaction rate is proportional to the square of \( f_{\text{red}} \) (see Fig. 3). Therefore, the overall reaction rate dependence can be expressed as:

\[
v(f_{\text{red}}, P_{O2}) = V_m \times \frac{P_{O2}}{K_m + P_{O2}} \times k \times f_{\text{red}}^2
\]

where \( f_{\text{red}} = [c^{2+}]/[c]_{\text{tot}} \), where \([c]_{\text{tot}}\) is the total concentration of cytochrome c, \( k \) is a constant, and \( K_m \) and \( V_m \) are the Michaelis-Menten parameters for oxygen (Michaelis constant and maximal rate, respectively).

The usual way to determine the \( K_m \) and \( V_m \) for a substrate in a formal two-substrate system would be to hold the concentration of one substrate constant and vary the second. In mitochondria, however, the level of reduction of cytochrome c can be measured, but not held constant, since this level changes...
during the depletion of oxygen. To obtain the respiratory rate extrapolated to fully reduced cytochrome c at each oxygen pressure, the measured rate \( v(f_{\text{red}} \cdot P_{O_2}) \) needs to be divided by the square of the fraction of cytochrome c reduced \( f_{\text{red}}^2 \) (Eq. 2). Therefore, with the reasonable assumption that reduction of cytochrome c does not alter oxygen binding affinity, the measured rate can be replaced by the rate extrapolated to 100% reduction of cytochrome c (Ro):
where $V_M$ and $K_M$ are the enzyme parameters for oxygen extrapolated to the complete reduction of cytochrome $c$. When $R_0$ is plotted against oxygen pressure, a hyperbola is formed, from which the values for $V_M$ and $K_M$ can be determined by least squares fitting.

Figure 4 shows typical graphs of $R_0$ vs. $P_{O_2}$, with one graph for each pH. The increase in the noise with increasing oxygen pressure is due to the intrinsically higher noise of the phorescence quenching method at higher oxygen pressures. (The signal becomes weaker and subsequently noisier as phorescence quenching method at higher oxygen pressures.)

Table 1 summarizes the kinetic parameters measured in media at the three different pH values. The values for the $K_M$ (±SD) for oxygen are $3.0 \pm 0.5, 12.3 \pm 3.7,$ and $17.8 \pm 5.4$ Torr at pH 6.9, 7.4, and 7.9, respectively. The values for pH 7.4 and 7.9 are significantly different from those for pH 6.9, as confirmed by the two-tailed $t$-test, with $P$ values of $\leq 0.0001$. Comparison of the fits for pH 7.4 and pH 7.9 gives the $P$ value of 0.058, not quite reaching the 95% confidence level.

The $K_M$ for oxygen in suspensions of mitochondria treated with FCCP was <2 Torr at all pH values and too low to be reliably measured in the present experiments.

**DISCUSSION**

Measurements of the cytochrome-$c$ oxidase reaction in intact mitochondria. The respiratory capacity of cytochrome-$c$ oxidase in intact mitochondria is high. When uncoupled from energy conservation, the turnover number for cytochrome $c$ is $\sim 160/s$ at 20% reduction (22°C), and this extrapolates to a turnover number of near 800/s at 100% reduction (35). In coupled mitochondria with added ATP (high-energy state), the turnover number is much lower, $\sim 3/s$ for 20% reduction and 80/s when cytochrome $c$ is fully reduced ($V_M$, Table 1). At saturating oxygen concentrations and the same levels of reduced cytochrome $c$, the difference in rate between coupled (plus added ATP) and uncoupled mitochondria can be attributed to the dependence on the energy state. The difference is at least 60-fold at the levels of cytochrome $c$ reduction (<20%) observed in intact cells and in vivo. This large dependence on the energy state, combined with the difficulty in experimentally controlling the energy state in suspensions of isolated mito-

![Fig. 3](http://jap.physiology.org/)

![Fig. 4](http://jap.physiology.org/)

**Table 1. The values of $K_M$ and $V_M$ for rat liver mitochondria (+ATP) suspended in media with pH values of 6.9, 7.4, and 7.9**

<table>
<thead>
<tr>
<th>Parameter (+ATP)</th>
<th>pH 6.9 ($n = 5$)</th>
<th>pH 7.4 ($n = 7$)</th>
<th>pH 7.9 ($n = 7$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$K_M$ for oxygen, Torr</td>
<td>$3.0 \pm 0.5$</td>
<td>$12.3 \pm 3.7$</td>
<td>$17.8 \pm 5.4$</td>
</tr>
<tr>
<td>$V_M$ (100% reduced cytochrome $c$), Torr/s</td>
<td>$29 \pm 25$</td>
<td>$31 \pm 12$</td>
<td>$39 \pm 11$</td>
</tr>
</tbody>
</table>

Values are means ± SD; $n$, no. of animals. $V_m$, maximal rate. The values for parameters were obtained by the least squares analysis of the dependence of $R_0$ (respiratory rate at 100% reduction of cytochrome $c$) vs. $P_{O_2}$ (Eq. 3) (Michaelis-Menten kinetics). These are the kinetic parameters for cytochrome-$c$ oxidase extrapolated to fully reduced cytochrome $c$. The values were tested for difference between different pH using a two-tailed-$t$-test. The $P$ values for the difference between the $K_M$ values corresponding to pH 7.4 and 7.9 and $K_M$ corresponding to pH 6.9 are $\leq 0.0001$. The $P$ value for the difference between $K_M$ values for pH 7.4 and 7.9 is 0.059.
chondria, limits quantification of this function. In the present paper, the energy state was set to a high value (+ATP) and assumed to be constant during the measurements.

**Relationship to the oxygen dependence of oxidative phosphorylation in intact cells.** Under physiological conditions (in cells), mitochondria consume oxygen only as needed to maintain the cellular energy state, [ATP]/[ADP][Pi], by replenishing the ATP that is being consumed by cellular processes. When the oxygen pressure in the cellular environment is decreased, the rate of ATP utilization (and of oxygen consumption) is initially not affected, because the decrease in PO2 can be compensated for by an increase in the fADP and/or a decrease in the energy state. Only when [ATP]/[ADP][Pi] becomes small enough that the rate of ATP consumption decreases does the rate of oxygen consumption decrease. Golub and Pittman (10) have recently reported that respiration in a thin planar muscle preparation has a Km for oxygen of 10.5 ± 0.8 Torr. This was measured by the rate of depletion of oxygen in the interstitial space following compression-induced expulsion of the red blood cells in the microcirculation. This Km is near that for rat liver mitochondria in the present studies. The higher values might be expected for resting muscle, where oxygen utilization rate is low, and the energy state is both high and strongly buffered by the creatine phosphate/creatine system. Earlier studies on suspensions of neuroblastoma C-1300 clone NB41A3 cells and of Tetrahy- 

mena pyriformis, a free-living protozoan (36), gave oxygen pressures for half-maximal respiratory rate of 1–2 Torr, but this is without correction for the increasing reduction of cytochrome c. Taking into account the changes in cytochrome c would give a KmA value that was substantially higher and consistent with the values for mitochondria in this paper.

An alternative method for estimating the activity of oxidative phosphorylation in intact cells is through measuring metabolic responses that are coupled to oxidative phosphorylation. One metabolic pathway that is tightly coupled to mitochondrial oxidative phosphorylation is the citric acid cycle (also called the tricarboxylic acid cycle). Koch and coworkers (22, 28) have reported measurements of the citric acid activity in cultured tumor cells at rigorously controlled oxygen levels and 37°C. The activity was quantified by the production of 14CO2 from 1–2 Torr measured for citric acid cycle activity, 1–2 Torr measured for respiration, because AMP is a potent allosteric activator of phos-

phorylation resulting in relatively large metabolic changes. Changes in the levels of AMP and ADP can markedly alter the activity of many important enzymes, both through their role as metabolites and by binding to, and regulating, enzyme activity. Increase in AMP would, for example, increase glycolytic activity, because AMP is a potent allosteric activator of phosphofructokinase I, the key control site of glycolysis.

The activity of AMP kinase (AMPK), a serine/threonine kinase capable of phosphorylating a number of regulatory protein kinases, is dependent on the levels of ATP, ADP, and AMP, but particularly AMP. Increase in AMPK activity results in increased rates of cellular catabolism, particularly catabolism associated with production of ATP, and in decreased rates of cellular anabolism. The list of metabolic pathways regulated by oxygen, pH, and Mitochondrial Oxidative Phosphorylation

**Oxidation pressures relevant to normal physiology.** Historically, oxygen levels in the arterial blood (>80 Torr) and mixed venous blood (typically 35–45 Torr) could be readily measured, but measurements of the “tissue” oxygen levels were much more difficult. Early researchers, such as Whalen and coworkers (34), reported oxygen pressures in tissues in vivo being very low, with large fractions of the tissue volume being at <5 Torr and many readings approaching zero. As technol-

ogy has improved, reports on measurements of very low oxygen in normal tissues have disappeared, and mean oxygen pressures increased to slightly below the mixed venous value. These changes were primarily due to improvements in the condition of the tissue, decrease in invasiveness of measure-

ments, better anesthetics, etc. Currently, there is reasonably good agreement among measurements using needle microelec-

trodes (3, 4), surface electrode arrays (12), phosphorescence quenching (10, 38), and electron paramagnetic resonance oxi-

drometry (18, 26). These measurements are consistent with a bell-shaped distribution of oxygen pressures in the tissue, with the maximum near below the mixed venous PO2 of 35–45 Torr, and very little or no tissue volume having oxygen pressures <10 Torr.

**Oxidative phosphorylation and regulation of metabolism by oxygen.** Dependence of mitochondrial oxidative phosphorylation on oxygen pressure at physiologically relevant pressures is critical to understanding regulation of metabolism and physiology. A change of only 2% in the energy provided by the respiratory chain for synthesizing ATP (decrease from 15.7 to 15.4 kcal/mol, for example) would decrease [ATP]/[ADP][Pi], by a factor of 1.65. In most cells, the free ADP concentrations in mitochondria are substantially lower than the concentrations of free ATP and Pi, so the ADP concentration would increase ~65%. Through the adenylate kinase reaction, the concentra-

tion of AMP would increase by 170%. Therefore, amplification of the signal is large, and small alterations in oxidative phosphorylation result in relatively large metabolic changes. Changes in the levels of AMP and ADP can markedly alter the activity of many important enzymes, both through their role as metabolites and by binding to, and regulating, enzyme activity. Increase in AMP would, for example, increase glycolytic activity, because AMP is a potent allosteric activator of phosphofructokinase I, the key control site of glycolysis.

The activity of AMP kinase (AMPK), a serine/threonine kinase capable of phosphorylating a number of regulatory protein kinases, is dependent on the levels of ATP, ADP, and AMP, but particularly AMP. Increase in AMPK activity results in increased rates of cellular catabolism, particularly catabolism associated with production of ATP, and in decreased rates of cellular anabolism. The list of metabolic pathways regulated at physiological oxygen levels found in the brain showed higher polarization, lower rates of ROS production, larger mitochondrial networks, greater cytoplasmic fractions of mitochondria, and larger mitochondrial perimeters than those cultured at higher oxygen levels. These authors used oxygen concentrations of oxygen pressures from 1 to 11% in the gas phase above the culture vessels, although the pericellular oxygen pressures were not measured.

Overall, the available data on cells in vitro and in vivo are consistent with the oxygen dependence of the mitochondrial oxidative phosphorylation observed in our experiments on mitochondrial suspensions.
by AMPK is large and still growing (1, 2, 6, 11, 13), but it is known to include glycolysis, fatty acid oxidation, and protein synthesis. Evans and coworkers (see, for example, Ref. 42) have implicated AMP-activated protein kinase in a variety of cellular signaling pathways, including Ca$^{2+}$ signaling and carotid body sensory activity.

Coupling of cellular metabolism to physiological oxygen pressures through oxidative phosphorylation helps explain a wide range of physiological responses to altered tissue oxygen delivery. This includes oxygen sensing by organelles, such as the carotid body (39), regulation of blood flow in the heart (17) and other tissues, and of many other oxygen-dependent functions. Clearly, oxidative phosphorylation is only one of several oxygen sensor systems in cells. Mitochondria are, however, widely distributed and react very rapidly to changes in oxygen pressure. The oxygen sensor role for oxidative phosphorylation is complementary to that of the other sensor systems, such as hypoxia inducible factors (24).

Physiological effects of the pH dependence of oxidative phosphorylation. The pH in the environment of cells in vivo is significantly altered under many physiological and pathophysiological conditions. Physiological conditions, such as the acidification of muscle tissue during heavy exercise, are transient, but, in many pathophysiological conditions, such as wound healing, chronic hypoxia of cardiopulmonary or vascular origin, and tumor development, the hypoxia can be of much longer duration. Many tumor cells exhibit increased glycolysis and lactate production, an observation that led Warburg to hypothesize that defective oxidative metabolism was the underlying cause of cancer (the Warburg hypothesis) (32). Metabolism of glucose to lactate provides only 2 ATP compared with the 34–38 ATP produced when the glucose is metabolized to CO$_2$ and water, so a shift to less efficient ATP production was thought to imply a defect in oxidative metabolism. The Warburg hypothesis has not been supported, as little evidence has been found for a defect in mitochondrial oxidative metabolism associated with tumor formation (33, 43). The phenomenon of increased lactate production in many tumors, however, has continued to generate hypotheses that link increased glycolysis to tumorigenesis (8, 43). Most evidence, however, indicates that the changes in glycolysis are the result of, not a cause, of tumorigenesis. The acidification observed in many solid tumors is likely to be a result of the suboptimal oxygen delivery, combined with altered gene expression in the tumor cells. Cells that can adapt to the more acidic environment would gain a substantial growth advantage through the decrease in $K_m$ for oxygen. These acid-tolerant cells, in addition to their increased glycolytic capacity and other adaptive features, could out compete their more differentiated and less adaptable neighbors for the limited amount of oxygen that is available.

Conclusions. First, mitochondrial oxidative phosphorylation is dependent on the oxygen pressure in the surrounding medium, and that dependence shows a $K_M$ of near 12 Torr (22 µM) at pH 7.4. For cells in tissues (normal mean pericellular oxygen pressures near 35 Torr), this would result in strong coupling between the pericellular oxygen pressure and mitochondrial energy metabolism under physiological conditions. The metabolic “power plant” of the cell, oxidative phosphorylation, also has a central role in the regulation of cellular and tissue responses to altered oxygen pressure and in control of blood flow. Second, oxidative phosphorylation is dependent on pH, with the oxygen pressures required for normal function decreasing about 10-fold per increase in one pH unit. This pH dependence has major consequences in physiological conditions with altered pericellular pH. Solid tumors, for example, often develop local environments that are both more acidic and have lower oxygen pressures. The cytoplasm of tumor cells may become more acidic than that of normal cells, allowing them to “out compete” the normal cells for the limited amount of oxygen that is available.

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DISCLOSURES

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AUTHOR CONTRIBUTIONS


REFERENCES


