Effect of Oxygen Concentration on Cellular Metabolism*

David F. Wilson, Ph. D.; and Maria Erećińska, M.D.

Experimental evidence is presented that mitochondrial oxidative phosphorylation is dependent on oxygen concentration in its physiologic range both in vivo and in vitro. Mitochondrial oxidative phosphorylation is considered to act as a tissue oxygen sensor important for controlling local vascular resistance.

Mitochondrial oxidative phosphorylation utilizes most of the oxygen consumed by cells, although in some tissues many other reactions combine to use a significant fraction of the total (perhaps 5 to 10 percent). Even more importantly, oxidative phosphorylation provides greater than 97 percent of the total ATP required for tissue survival and maintenance. To support this process, metabolic fuels such as fatty acids, amino acids and pyruvate are dehydrogenated, transferring the hydrogen atoms to NAD+ and FAD to give the reduced forms of the coenzymes (NADH and FADH2), while the carbon skeleton is released as CO2. Reoxidation of the reduced coenzymes is the first step of a multienzyme metabolic pathway, the final outcome of which is the reduction of molecular oxygen to water. The overall process is strongly exergonic (similar to combustion of hydrogen gas) and part (65 to 50 percent) of this available energy is used to synthesize ATP from ADP and inorganic phosphate (Pi), the remainder being released as heat. The overall process (oxidative phosphorylation) can be summarized:

\[
\text{NADH} + H^+ + 3 \text{ADP} + 3 \text{Pi} + 1/2 \text{O}_2 \\
\to \text{NAD}^+ + 3 \text{ATP} + \text{H}_2\text{O}
\]

Since cells continuously hydrolyze ATP to provide the energy necessary to do metabolic and mechanical work, they must have a constant supply of oxygen at tensions high enough to allow the mitochondria to synthesize ATP at the rate and energy level ([ATP]/[ADP][Pi]) required to maintain cellular homeostasis. The question then arises as to the oxygen concentration ([O2]) below which the functional capacity of mitochondrial oxidative phosphorylation is limited. This problem is of paramount importance, because if the mitochondria are saturated with oxygen at the concentrations found in tissue (ie, if their function is independent of [O2]), oxidative phosphorylation cannot act as a sensor of tissue oxygen concentration. On the other hand, if the mitochondria are not saturated at tissue oxygen concentrations, oxidative phosphorylation—and thereby tissue energy metabolism—is dependent on the oxygen tension, and the mitochondria act as an oxygen sensor.

Does Mitochondrial Oxidative Phosphorylation Act as a Sensor for Tissue Oxygen Concentration?

Many biochemical and physiologic parameters depend on tissue oxygen tension and, therefore, are potentially suitable for examining this question. One of the most dramatic is coronary flow. Over a wide range of cardiac work rates, coronary flow so precisely adjusts to oxygen utilization that the arterial-venous oxygen difference remains constant. This phenomenon is observed in isolated perfused hearts, which indicates that it is due to local control, not to external neural or humoral factors.

Changes in cardiac work involve the tissue energy metabolism because ATP consumption is directly correlated with work rate. Measurements of tissue metabolites show that the coronary flow increases as a nearly linear function of logarithm of the decrease in energy state ([ATP]/[ADP][Pi]) of the cardiac cells. It is possible to mimic the effect of global oxygen deficiency (ie, low oxygen tension) on oxidative phosphorylation by using specific inhibitors of the mitochondrial respiratory chain. When amytal (an inhibitor of the first energy coupling site) is added to the perfusion medium, there is an inhibition of respiration but the coronary flow increases. These changes are dependent on the concentration of amytal, are fully reversible and the maximal increases in coronary flow are nearly as large as during hypoxia. Measurements of tissue metabolites at various amytal concentrations show that coronary flow has the same relationship to the tissue energy state as is observed for changes in workload. In the case of amytal infusion, however, the increases in coronary flow are accompanied by decreases in oxygen consumption, leading to large increases in effluent oxygen concentration. This effect is also observed for other inhibitors of the mitochondrial respiratory chain such as cyanide and uncouplers of ATP synthesis, each of which is a potent vasodilator of coronary flow (Fig 1). This means that coronary flow is not regulated by [O2] per se but by the effect of changing oxygen concentration on the tissue energy state, a metabolic product of oxygen reduction by the respiratory chain. (The mechanism by which the change in tissue energy state affects the vascular resistance remains unknown.)

The enzyme of the respiratory chain which reacts with O2 is cytochrome c oxidase. The reduction of oxygen by cytochrome c oxidase is stoichiometrically coupled to ATP
synthesis, and this coupling results in the rate of cytochrome c oxidation (oxygen reduction) being dependent on the [ATP]/[ADP]/[Pi]. The mechanism of the coupled reaction is also such that the apparent affinity (Km) for oxygen is a function of both the state of reduction of cytochrome c and the [ATP]/[ADP]/[Pi]. In general, the apparent Km for oxygen increases as cytochrome c becomes more oxidized and as [ATP]/[ADP]/[Pi] becomes larger. Hence, the questions which must be addressed are: (1) Is cellular energy metabolism dependent on oxygen concentrations in the physiologic range? (2) What is the oxygen dependence of oxidative phosphorylation in isolated mitochondria and how does this relate to mitochondrial function in situ?

The Oxygen Dependence of Oxidative Phosphorylation in Suspensions of Cells

In suspensions of cells the oxygen diffusion barrier begins at the unstirred layer surrounding the plasma membrane, whereas in tissue it also includes the distance from the capillary to the plasma membrane. Thus, in cell suspensions, most of the barrier to oxygen diffusion has been removed, and all of the cells are exposed to the same oxygen concentration. The respiratory rate of cell suspensions is observed to have little dependence on oxygen concentration until below approximately 20 μM \( \text{O}_2 \) \( (\approx 12 \text{ mm Hg}) \). As the oxygen concentration decreases below this value, the respiratory rate decreases slightly, but the sharp decline usually associated with oxygen limitation occurs below 5 μM \( (\approx 3 \text{ mm Hg}) \), with an apparent Km of less than 1 μM \(^{17} \) \( (\approx 0.6 \text{ mm Hg}) \). Cytochrome c, however, becomes reduced as a continuous function of oxygen concentration below 150 μM \( (\approx 100 \text{ mm Hg}) \), with the reduction becoming more marked below 50 μM \( \text{O}_2 \) \( (\approx 30 \text{ mm Hg}) \). The cellular [ATP]/[ADP] ratio begins to decline below about 20 μM \( \text{O}_2 \). [Pi] cannot be measured for technical reasons. These results indicate that mitochondrial oxidative phosphorylation in cells is dependent on oxygen tension throughout the physiologic range and that the oxygen dependence is primarily expressed in the reduction of cytochrome c and decrease in [ATP]/[ADP]. Many different types of cells have been reported to show oxygen dependences similar to that described above. This includes neuroblastomas, kidney cells (BHK), ascites tumor cells, oligodendroglia, hepatocytes, and Tetrahymena pyriformis. Moreover, it has also been shown that the activities of metabolic pathways which utilize ATP are inhibited as the oxygen concentration is lowered.

The Oxygen Dependence of Oxidative Phosphorylation in Suspensions of Isolated Mitochondria

There have been many studies of the oxygen dependence of respiration by isolated mitochondria, but because of the choice of experimental conditions and/or technical shortcomings, it has been difficult in most cases to relate the measured relationship to the behavior of mitochondria in vivo. Since there are three coupling sites in the mitochondrial respiratory chain, evaluation of the \( \text{O}_2 \) dependence can be simplified by measuring only the oxygen dependence of the third site. This part of the respiratory chain (cytochrome c oxidase) is responsible for transfer of reducing equivalents from cytochrome c to molecular oxygen:

\[
2 \text{ cyt c}^+ + 2 \text{ H}^+ + \text{ADP} + \text{Pi} + 1/2 \text{O}_2 \\
\rightarrow 2 \text{ cyt c}^{2+} + \text{ATP} + \text{H}_2\text{O}
\]

An artificial electron donor (ascorbate plus N,N,N',N'-tetramethylparaphenylenediamine [TMPD]) can be used to reduce cytochrome c directly in a simple bimolecular reaction; the higher the concentration of TMPD, the more reduced cytochrome c is in the steady state. At a constant concentration of TMPD, the steady state of cytochrome c reduction is a balance between its rate of reduction by the electron donor (which is independent of oxygen tension) and its rate of oxidation by cytochrome c oxidase. When [ATP]/[ADP]/[Pi] is held constant, any oxygen dependence of the latter reaction then causes a change in the steady state of reduction of cytochrome c. In mitochondria oxidizing ascorbate and TMPD, the level of reduction of cytochrome c and the respiratory rate are found to have oxygen dependences which are strongly influenced by [ATP]/[ADP]/[Pi] (Fig 2). When [ATP]/[ADP]/[Pi] is less than 1 M \(^{-1} \), neither cytochrome c reduction nor the respiratory rate changes until the oxygen concentration is less than 1 to 2 μM, with apparent Km values of less than 0.5 μM. At high [ATP]/[ADP]/[Pi] values, however, cytochrome c is observed to undergo progressive reduction as the oxygen concentration declines below approximately 200 μM, and the respiratory rate begins to fall below approximately 50 μM \( \text{O}_2 \). Measurements at [ATP]/[ADP]/[Pi] and pH values approximating cellular conditions results in both cytochrome c reduction and the...
respiratory rate having oxygen dependences consistent with those observed in intact cells.

SUMMARY

Mitochondrial oxidative phosphorylation is dependent on oxygen concentration in the physiologic range, expressed in alterations of the redox state of cytochrome c and [ATP]/[ADP]. In cells at oxygen concentrations greater than about 50 μM (≈30 mm Hg), [ATP]/[ADP] is usually greater than 1 x 10^-2 M^-1 and cytochrome c is approximately 15 percent reduced. When the oxygen concentration is lowered, the capacity of the mitochondria to synthesize ATP becomes limited, and the rate of ATP synthesis (cellular respiration) temporarily falls below the rate of ATP utilization. This causes [ATP]/[ADP][Pi] to decrease and cytochrome c to become more reduced. Both of these changes act to increase respiration and continue until the rate of ATP production again equals its rate of utilization. At this point the respiratory rate is restored, cytochrome c is more reduced, and [ATP]/[ADP][Pi] is lower. The consequence of this chain of events is that, during gradual decline in Po2, the respiratory rate remains nearly constant until the [ATP]/[ADP][Pi] falls low enough that the rate of ATP utilization decreases. This results in measurements of the respiratory rate giving a very low apparent Km for oxygen. Tissue oxygen sensing occurs in part through the oxygen dependence of the cellular [ATP]/[ADP][Pi] and, therefore, occurs at oxygen tensions well above those resulting in suppression of the respiratory rate.

Our studies indicate that in cells utilizing primarily oxidative phosphorylation for ATP synthesis, the dependence of mitochondrial oxidative phosphorylation on the tissue Po2 is qualitatively the same. On the other hand, the nature of the coupling mechanisms which transmit information from the detector system (mitochondria) to the effector function, and consequently the final effect, may be different in different tissues. This could explain the well-known observation that in the heart, lowering the oxygen tension causes vasodilation, while in the lung, the same event causes vasoconstriction.

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DISCUSSION
The discussion began with the locus of the possible energy sensor which regulated the coronary circulation. It was pointed out that in the heart the sensor for the energy levels regulating coronary flow is probably located in the myocardium rather than in the coronary arteries. The experiments to prove that coronary circulation is controlled by the energy potential in the myocardial cell and not the vessel wall would require separate biochemical analyses of myocardial cells and the vascular wall, rather than, as was reported here, analyses of the heart tissue containing both vessels and myocardium. However, it seems unlikely that increasing the workload of the heart would be seen by the vessel wall in parallel to the cardiac contractile cell itself. The coronary flow is closely linked to the contractile tissue energy ratio [ATP]/[ADP][Pi] rather than myocardial oxygen uptake per se. The creatine phosphokinase system is the appropriate way to measure the phosphate energy potential in muscle tissue because the creatine phosphokinase reaction is near equilibrium and creatine and creatine phosphate are not bound extensively to cellular components (in contrast to ADP). It seems unlikely that the phosphocreatine is the intracellular sensor, because the mitochondria are primarily concerned with the adenine nucleotides, and the creatine/creatine phosphate system is present to facilitate transport of ADP through the cytoplasm. It is not clear how directly the coronary flow model can be related to the lung, because in the latter the location of the oxygen sensor is not apparent. For example, the pulmonary circulation appears to accept the entire cardiac output regardless of the energy requirement of the lung parenchyma. The use of carbon monoxide or cyanide as a metabolic inhibitor was not examined in the presented study. Carbon monoxide, which also binds to myoglobin and cytochrome P450, may act primarily through mechanisms other than inhibition of cytochrome c oxidase.

A question was raised about the susceptibility of the mitochondria to hypoxic damage. The discussion indicated that hypoxia resulted in lipase activation with the liberation of free fatty acids. Mitochondrial membranes accumulate these fatty acids with the result that mitochondrial oxidation becomes uncoupled from phosphorylation. However, the addition of bovine serum albumin restores coupling, so it is unlikely that the mitochondria were irreversibly damaged. Further, after an animal has been suddenly killed, the mitochondria remain viable for a relatively long period (hours at 0°C). Thus, it is unlikely that the mitochondria are the most labile portion of the cell in terms of irreversible damage to hypoxia.

Questions were raised about the possible influence of FCO2, pH, and oxygen radicals in the oxidation-phosphorylation signal. These factors have little effect on the phosphorylation state ratio (or more correctly, the free energy of hydrolysis of ATP). The experimental data show that coronary flow is correlated with the phosphorylation state ratio over a wide range of conditions, indicating that the latter is primarily responsible for regulation of flow. Both FCO2 and pH have direct effects on the vascular system and thus "fine tune" flow for a given phosphorylation state ratio. There is little evidence, at this time, that oxygen radicals contribute substantially to control of coronary flow except under pathologic conditions.

For the tissue phosphorylation state ratio to be an important in vivo regulator of the coronary circulation, there should be a clear change in the ratio over thephysiologic range of PO2 values. For example, the question was raised about the magnitude of cytochrome c reduction at low levels of O2 tension. However, it was pointed out that in some cell suspensions by the time oxygen had fallen from normal tissue values to 20 μM (PO2 = 12 mm Hg) the percentage of reduced cytochrome c had gone from the normoxic value of 15 percent to 25 percent. That is a large change (70 percent). In addition, the ratio of [ATP]/[ADP] had decreased. The change in the ratio is usually seen as an increase in [ADP] rather than a decrease in [ATP] because the concentration of the latter is 6 to 20 times higher than that of ADP. It has been proposed that adenosine, a known vasodilator, plays an important role in regulating coronary flow. Measurement of adenosine and its metabolites in the perfusion fluid indicates, however, that for flow changes induced by varied workloads, amylaid inhibition and uncoupler infusion there was no correlation between extracellular adenosine and flow. In hypoxia the concentrations of adenosine and its metabolites in the perfusate rise, and thus adenosine may contribute to reactive hyperemia.