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IS OXYGEN AN ESSENTIAL NUTRIENT?

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THE CHALLENGE

Oxygen is essential for the survival of almost all eukaryotes. One can argue that oxygen is responsible for the development of cellular compartmentation and that it serves as the engine of morphogenesis in higher animals. For the

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development of cellular organelles, membranes are needed to segregate oxygen-susceptible reactants in a milieu of oxygen, albeit at low and varying partial pressures. The organs of gas exchange, e.g. lungs and gills, the heart and circulatory system, and the peripheral capillary bed, have developed primarily to provide oxygen to cells. The concomitant development of unique hemoproteins, such as hemoglobin and myoglobin, augments the oxygen transport capacity of the circulatory fluid and provides for the transport and transient storage of oxygen. A large variety of oxygen-utilizing enzymes play central roles in the diverse functions of different cells. They range from oxygen-reacting enzymes that are required for generation of energy by the mitochondrial respiratory chain to other enzymes that aid in the synthesis of humoral regulators and mediators, such as the prostaglandins and physiologically active steroid hormones, to name but a few examples.

However, can one consider oxygen as a nutrient? Webster's dictionary (103) defines a *nutrient* as *furnishing nourishment; promoting growth*. By this definition one might consider oxygen as a nutrient. It not only promotes growth but also often serves as a regulatory molecule for metabolism. On the other hand, in a recently published textbook on nutrition (70) was the following statement: "One may partition the nutrients into six categories: proteins, carbohydrates, lipids, vitamins, major minerals, and trace elements. This excludes a consideration of water and oxygen as nutrients, since no special efforts are required to produce and provide them." This difference of interpretation stimulated us to answer the challenge by Robert E. Olson (the editor of this series) to write this review.

Oxygen is ubiquitous on the Earth's surface and it is the subject of immense chemical, physiological, and clinical literature. A recent computer search identified 130,718 bibliographic references in the last 25 years that responded to the search term "oxygen." The rich history of research involving oxygen stems from the original observations of Lavoisier (68), Priestley (89), and Scheele (94). In the intervening two centuries, oxygen has attracted the attention of leading scientists and has often played a fundamental role in their contributions to chemistry and biology (35). Therefore, any attempt to summarize the current status of research on oxygen is necessarily biased and superficial. In this brief article we have selected those aspects of oxygen chemistry and physiology that we know best and that we believe provide new insights on the role of oxygen in eukaryotes—in particular, its role as a nutrient.

THE SOURCES OF OXYGEN

The energy for life derives from the large free energy difference between hydrogen and oxygen. The energy of radiation from the sun is used in the

photosynthetic processes of plants to separate water into oxygen and hydrogen. The hydrogen is stored combined with carbon in what are classically called nutrients (i.e. fat, carbohydrate, and protein) while the oxygen is released to the atmosphere as a diatomic molecule. Practically all living organisms that occupy the surface of planet Earth today, including the plants themselves, require molecular oxygen for the metabolism of the macro-nutrients to obtain the energy needed for growth, reproduction, movement, and survival. Thus, to designate one of these two reactants, hydrogen, as a more important nutrient than the other reactant, oxygen, is not logical.

The original atmosphere of the Earth presumably contained the lighter elements in approximately the same proportions as found in the universe, namely hydrogen at 86.687%, helium 13.18%, oxygen 0.09%, carbon 0.03%, and nitrogen 0.01% (69). Because hydrogen was predominant, it combined with all the other elements, except He, to produce an atmosphere consisting primarily of H_2, He, H_2O, CH_4, and NH_3. The lighter elements, H_2 and He, escaped from the Earth's gravitational field first; loss of the former is equivalent to oxidation of the Earth. Radiation reaching the Earth, over a period of time, dissociated hydrogen from combination with the other elements; some bound as well as free hydrogen also was lost, leaving an enrichment of the heavier, more abundant elements, i.e. oxygen, carbon, and nitrogen. There was no free O_2 in the atmosphere. The subsequent outgassing of the outer layers of the Earth then contributed to the atmosphere the small amount of oxygen required for initiating life processes in which oxygen combined with carbon to form CO_2 (51). CO_2, with N_2, formed the next stage of our atmosphere. Understanding the evolution of the atmosphere of Earth and the complex chemistry associated with progressive changes remains an active area of speculation (69). The linkage to the development of life, as we understand it, in particular the key role played by oxygen, remains a challenge for scientific investigation. Life presumably developed in the original reducing atmosphere using oxidation-reduction reactions dependent on such elements as sulfur and iron; but at some point photosynthesis developed and reactions were established for the dissociation of water, thereby markedly increasing the availability of oxygen for chemical and biological reactions (60). The oxygen in the atmosphere is believed to represent but 0.1% of the oxygen content of the Earth. The greatest store of oxygen remains combined with minerals in the solid crust and in water.

Oxygen in the atmosphere is continually being replenished by photosynthesis but consumed by oxidation of organic compounds, including respiration by living organisms including the small plants that produce it, in the sea as well as on land, at a rate of about 0.04% per year. Since the concentration of O_2 in the atmosphere is constant over time (based on measurements taken during a limited time scale of less than 100 years), and presumably uniform

over the Earth's surface, production and consumption are considered equal. Therefore if photosynthesis stopped and the consumption of oxygen remained constant, presumably the concentration of atmospheric oxygen would fall at this same rate, requiring 2500 years to disappear. What forces have led to the current "steady state" level of 21% oxygen in the atmosphere? What were the kinetics of transition from the original reducing atmosphere of primordial Earth to the present time and can one anticipate further increases in oxygen concentration in the atmosphere? Immense amounts of oxygen are available in mineral compounds. It is the energy available from the oxygen and hydrogen that is needed for life, rather than the molecules themselves.

THE UNIQUENESS OF OXYGEN

In 1964 P. George (34) summarized the advances in our understanding of the physical chemistry of oxygen by writing a tribute for the fiftieth anniversary of the publication of *The Fitness of the Environment* by L. J. Henderson (50). George's classic presentation itemizes the remarkable advances in our understanding of the physical-chemical properties of oxygen that justify its role as the ideal agent to serve as the terminal oxidant for life processes. George concludes that the physical properties of oxygen, combined with its thermodynamic and kinetic properties, place oxygen in a unique position so "that no other element could effectively replace it." Central to this theme is the large free energy release available in discrete increments that occurs during the reduction of oxygen to water. The four one-electron equivalent stepwise reduction of molecular oxygen to water results in a great variety of chemical species as intermediates (48, 65).

In biological systems the one-electron reduction of oxygen results in (*a*) the formation of the free radical superoxide anion, $O_2^{\cdot-}$. For example, during the reaction of some flavoproteins or hemoproteins with oxygen or during the redox cycling of quinones, significant amounts of superoxide are formed. The microbicidal role of superoxide formed during the "oxygen burst" of phagocytosis is an essential component of the response to inflammation. The protonated form of the superoxide anion, the perhydoxy radical, is the dominant form at neutral pH and is more invidious because of its increased lipid solubility. (*b*) The two-electron reduction state of molecular oxygen is hydrogen peroxide. This compound is generally formed by a dismutation of the perhydroxy radical in a reaction catalyzed by the enzyme superoxide dismutase (32, 74). Hydrogen peroxide can dissociate (in the presence of a suitable metal) to form an electrophilic oxene intermediate together with water (7, 47). Of course, this highly reactive form of oxygen can also arise from the heterolytic cleavage of molecular oxygen. (*c*) Most reactive of the reduction intermediates of molecular oxygen is the strongly oxidizing hydroxyl

radical, $HO^{\bullet}$, i.e. the three-electron reduced state of molecular oxygen (16). Classic Fenton reaction chemistry or the metal (iron) catalyzed Haber-Weiss reaction leads to this species of oxygen. Lastly, (*d*) the four-electron reduced form of molecular oxygen is water.

Although oxygen radicals are recognized to be short-lived, their destructive potential is frequently stabilized in the form of organic peroxides (ROOH) that can lead to peroxy radicals ($ROO^{\bullet}$) by hydrogen abstraction or alkoxy radicals ($RO^{\bullet}$) following interaction of the peroxide radical with an electron-rich molecule, such as a polyunsaturated fatty acid.

For many years, the study of these intermediates has been the predominant activity of those concerned with oxygen metabolism. Intense interest in this area has resulted in a rapid proliferation of Symposia and the establishment of new scientific societies (The Oxygen Society, The Society for Free Radical Research, to name but two) and associated journals dedicated to understanding the role of oxygen intermediates in chemistry and biology. The reactivity and associated toxicity of the highly reactive oxygen species formed during the reduction of oxygen may be major contributors to the pathogenesis of many chronic degenerative diseases (21).

NUTRITIONAL REQUIREMENTS AND THE BALANCE OF OXYGEN UPTAKE

The uniqueness of molecular oxygen is due to (*a*) its omnipresence as a gas; (*b*) its limited solubility in water, and (*c*) its specialized kinetic properties, particularly in association with metal ions such as iron and copper. Although molecular oxygen is used by higher animals in the synthesis of a great variety of important chemical compounds that serve as constituents and regulators of cellular metabolism, the amount of oxygen used for these reactions is minor compared to that consumed for energy production. A certain amount of oxygen is present in carbohydrate and protein (less in fat) and of course in water, but it is present in a reduced state with a limited potential for energy release. For example, the daily water intake of a normal adult human contains about three times as many molecules of oxygen as are consumed in respiratory metabolism. Oxygen-containing functional groups are present in most organic chemicals where they provide the necessary sites for recognition (by enzymes, immune cells, and receptors), as well as sites for conjugation (to increase the hydrophilicity of molecules) or for esterification (for storage or targeting signals).

Oxygen As a Gaseous Nutrient

A specified amount of O_2 is required for all energy needs by mammals. Any transient anaerobic energy used is eventually replaced from aerobic sources.

As a nutrient, O_2 has no substitute—unlike fat, carbohydrate, and protein, which are metabolically interchangeable. Furthermore, oxygen has a relatively limited concentration, in terms of moles per unit volume, in the ambient atmosphere and a relatively low solubility in water. Whereas an individual can ingest more solid and liquid nutrients than needed immediately and store these in the body as fat or glycogen, sufficient to maintain nutrition for days, the total possible body stores of molecular oxygen in humans can support metabolism for only several minutes. This property places stringent requirements on O_2 transport from the environment to cells. It also means that O_2 delivery must be adjusted within seconds for any changes in metabolic rate in order to maintain cell pO_2 and avoid cell death.

Within a cell, O_2 is transported by physical diffusion at a rate that is proportional to, and in the direction of, the concentration gradient of the oxygen multiplied by the diffusion coefficient of the O_2 in the cytoplasm (33, 88). If the innermost region of a cell is to be adequately supplied with O_2, its distance from the surface is restricted by the following equation (29, 31):

$$[O_2]_{surface} - [O_2]_r = \frac{metabolic\ rate}{6d} (r_{surface} - r). \quad 1.$$

For this calculation the cell is considered as a sphere, $[O_2]_{surface}$ is the oxygen concentration at the surface of the cell, and $[O_2]r$ is the concentration inside the cell at radius r; both are in moles per cubic centimeter. Metabolic rate is in moles/(cc $\times$ sec) and is considered uniform throughout the cell; ($r_{surface}$) is the radius of the spherical cell and r is a radial distance from the center, inside the cell, both in centimeters; and d is the diffusion coefficient of O_2 inside the cell in cm^2/sec and is also considered homogenous. This equation dictates that the radius of such a cell can be no larger than the value obtained when pO_2 at the center of the cell is so low that the rate of O_2 reduction by the mitochondria falls to zero. The minimal pO_2 at which a mitochondrion will continue to reduce O_2 is thought to be low, possibly less than 1 mm Hg, but this value is in dispute (14, 17, 53, 82, 92, 106–110). One can approximate this condition by setting $[O_2]$ at the center equal to 0 mm Hg; this will give the maximal radius for the cell. Choosing average values for a normal human kidney cell exposed to air, the pO_2 at the cell surface = 150 mm Hg, i.e. $[O_2]_{surface}$ of the cell = 1.2×10^{-4} mol/cc; the metabolic rate = 5.8×10^{-8} mol/cc, and the diffusion of oxygen $d = 1.03 \times 10^{-5}$ $cm^2 \cdot sec$ (29, 30). Substituting these values in Equation 1 and solving for radius r, one obtains a value of 60 μm.

However, two other considerations must be applied when evaluating the slope of the oxygen gradient within the cell. Inspection of Equation 1 shows that decreasing the metabolic rate of the cell increases its maximal possible

size proportionally (52). First, as O_2 diffuses into a metabolizing cell, it is consumed at each point and reduces the pO_2, which in turn reduces the diffusion gradient for further flux into the cell. Thus the higher the metabolic rate, the steeper the pO_2 gradient and the larger the pO_2 difference between the surface of the cell and the center. One might consider exceptions to this rule: For example, plant cells, whose rate of metabolism is slow, or fat cells, in which metabolism takes place only in the thin layer of cytoplasm surrounding the lipid droplet, may become much larger. Some cells have developed in their cytoplasm O_2-binding pigments such as myoglobin, which are believed to facilitate O_2 flux and thus increase the effective diffusion constant and reduce the pO_2 gradients in the cell. Second, the concentration of the oxygen-reacting enzymes in a cell may not be trivial. For example, in a liver cell the average concentration of cytochrome c oxidase is about 15 nmol per gram wet weight of tissue (15 μM) and of cytochrome P450 is about 50 nmol per gram wet weight of tissue (50 μM). Thus as the oxygen diffuses from the surface to the center of a cell, the rate of oxygen utilization rapidly becomes dependent on the concentration of the oxygen-reacting enzyme, i.e. the first-order reaction velocity constant changes to a second-order constant.

The Delivery of Oxygen

As life forms evolved into multicellular organisms with convective systems that in effect surround each cell with an oxygen-containing environment, they were able to grow in size. Claude Bernard immortalized this concept in his "millieu interieur" (6). Pasteur (86) noted that lack of O_2 produced changes in the structure of yeast, and he concluded that O_2 was the key to differentiation. Warburg (102) later extended this principle, believing that the lack of O_2 caused cells to de-differentiate. Certainly the need to maintain an optimum pO_2 at the cell surface is the prime determinant of the mechanical systems that maintain the milieu interieur in multicellular animals. For example, some insects conduct ambient air to the cells with small tubes (tracheoles) (76); some marine animals pump sea water to each cell (55). Higher vertebrates have developed a closed double-capillary exchange system that equilibrates a circulant (e.g. blood, hemolymph) with the surrounding air or liquid and moves this equilibrated transport fluid to capillaries around each cell where O_2 diffuses from the circulant to each cell (97). The most highly developed O_2 exchange systems include: (*a*) an external exchanger (lungs, gills, skin, etc.) that equilibrates ambient oxygen with a (*b*) circulant that is convected mechanically (heart and circulatory system) to (*c*) an internal exchanger, the tissue capillary beds, which equilibrate O_2 with the cell surfaces. The circulant in higher animals contains pigmented proteins that react reversibly with O_2, taking up and releasing a large amount of O_2 for a relative small change in O_2 partial pressure. In mammals this pigment,

hemoglobin, is contained within cells (erythrocytes) and raises the blood O_2 concentration to some 100 times the concentration of dissolved O_2. CO_2, which is produced in the cells, is carried in the opposite direction by the blood, mainly as HCO_3^-. This gives the blood a CO_2-carrying capacity even greater than that for O_2. The uptake of O_2 and the release of CO_2 in the lungs, and the reverse in the peripheral capillaries, mutually facilitate each other in an integrated physicochemical process. The O_2-carrying capacity of the blood can be regulated by altering the concentration of erythrocytes.

The mechanisms of O_2 uptake from the ambient are closely controlled to maintain constant blood pO_2 (30), but in a manner ensuring that unnecessary ventilatory effort is not exerted. The rate of pumping air or water past the external capillary exchange bed depends on the metabolic demand (30, 35). Although the ventilatory rate in humans decreases in sleep, a minimal level has to be maintained for survival. Water-breathing animals are further compromised by the low solubility of O_2 in water. Sea water equilibrated with the air contains about one thirtieth as much O_2 as the atmosphere and thus requires that 30 times as great a volume of sea water flow past the gill capillaries as the volume of air pumped in and out of the lung of an equivalent air-breathing animal. Active fish, such as the tuna, must keep swimming to force water past their gill capillaries fast enough to maintain their metabolism (22).

Meeting the Demand for Oxygen

The rate of metabolism is set by the organisms's need for energy for maintenance, movement, and growth; the transport system is subsidiary and is required to provide whatever O_2 is demanded by the cells. Sensors for O_2, the chemoreceptors of the carotid and aortic bodies (20, 56, 79), monitor the arterial blood and regulate minute ventilation of the lungs (or gills) so as to oxygenate the blood regardless of the cardiac output and pulmonary blood flow (28). The control of respiration acts to keep arterial blood pO_2 and pCO_2 constant, rather than regulating the amount of oxygen taken up. The activity of tissue cells is controlled independently by the volitional or vegetative nervous systems, which alter oxygen consumption and carbon dioxide production while the transport system adjusts to these needs. The mechanism by which the peripheral chemoreceptors sense blood pO_2 is not clear at this time (83). An extremely sensitive center in the medulla oblongata monitors arterial pCO_2 and can cause a doubling of ventilation for a rise in pCO_2 of only 1 mm Hg. Regulation of O_2 delivery is hierarchical at successive levels of arborization throughout the circulatory systems in order to maintain pO_2 in the individual cells within the required range.

The transport of O_2 from the peripheral capillaries to cell mitochondria is poorly understood; many experimental approaches have been used (40–43,

54, 66, 71, 87, 98), and a worldwide organization, the International Society for Oxygen Transport in Tissue (ISOTT) is dedicated to its study. The pO_2 in blood entering the cellular capillary bed is normally 100 mm Hg while the pO_2 at the mitochondria is certainly less than 15 mm Hg (19). This drop of pO_2 of some 85 mm Hg within the cells is the largest decline in the entire path from ambient air to the mitochondrial cytochrome c oxidase. The capillary walls, which are one cell thick, are so thin that their resistance to gaseous diffusion is negligible, or at least technically immeasurable (although some investigators disagree) (33, 54). Increased metabolic demand or decreased local O_2 from other causes produces increased capillary blood flow, but the most effective change is the opening up of closed or resting capillaries (95). Skin blood flow, which can be much greater than nutritional needs in order to promote heat loss, can vary more than 100-fold. Peripheral skeletal muscles have extremely low resting capillary blood flow, but with exercise and the associated metabolic demands the blood flow increases enormously (41, 53). Athletic training increases muscle capacity for work by increasing the number of mitochondria and, of necessity, the capillary bed to supply them.

Can one establish a similarity between the demand for oxygen and other nutrients? Other nutrients are taken into the body and transported from the environment to the cells by a system similar to that used by O_2, except that the blood-ambient exchanger is in the intestinal wall. Transport of other nutrients across the epithelium is by active and passive transport and endocytosis, and not simply by diffusion as in the alveolar capillary. However owing primarily to their much higher concentration in ingestates, the movement of nutrients from the absorption site to the cells is hardly ever a critical problem. A cell may die from lack of oxygen long before it can starve because of a failure of nutrient transport. For example, the normal arterial concentration of glucose is 5.5 mM and the normal arterial pO_2 is 100 mm Hg, equivalent to 0.12 mM O_2. In addition, 6 molecules of O_2 are required to oxidize 1 molecule of glucose so that from the viewpoint of supplying metabolic needs, the concentration of glucose is 275 times greater than that of O_2 in the extracellular fluid at the surface of the cell. Thus, oxygen turnover is the highest among essential nutrients.

OXYGEN-REACTING ENZYMES

The relative amounts of oxygen consumed by the tissues of an adult human male are directly related to the content and capacity of the mitochondrial respiratory chain (100). Skeletal muscle, which makes up about 42% of body weight, uses about 30% of oxygen consumed at rest, and over 86% of oxygen consumed during heavy work. The abdominal organs use 25% and the brain

uses 20% of the oxygen consumed at rest. Their relative contributions proportionately decrease during heavy work. Thus one can quantify the respiratory activity of organs and cells under different conditions of stress and physical challenge. Of interest is the relationship, first described by Drabkin (23), that correlates the percent distribution of cytochrome c in organs of the body with the relative amounts of oxygen utilized by various organs during heavy work, i.e. 82.6% of the cytochrome c of man is located in skeletal muscle. A similar relationship exists for heart, brain, and abdominal organs.

Howard Mason (personal communication) has cataloged over 350 different classes of enzymes in biology that react with oxygen. Of these about 150 different types may be present in mammals, although many are shared with lower forms of life. This calculation does *not* include the large number of isoforms (isoenzymes) that subdivide a specific class of oxygen-reacting enzyme. These enzymes include oxidases, oxygenases, hydroxylases, and peroxidases. They consist of hemoproteins, flavoproteins, copper proteins, and proteins containing metals such as molybdenum, manganese, cobalt, vanadium, etc. Clearly, Nature has devised many different approaches to capture the potential energy present in oxygen.

Cytochrome Oxidase

Quantitatively, mitochondria are the major consumers of oxygen in most organisms. In mammals more than 90% of the oxygen utilized is thought to be consumed via cytochrome oxidase of the mitochondrion. Understanding the enzymology and physiological role of the respiratory chain electron transport carriers of mitochondria, in particular the reaction with oxygen of cytochrome c oxidase, has served as a driving force in biochemical research for the last 70 years, since the pioneering observations of Warburg (101) and Keilin (62).

How does cytochrome oxidase reduce molecular oxygen to water? Understanding the mechanism(s) by which a single electron transfer pathway provides the needed four electrons for this reaction has challenged researchers and has commanded the most sophisticated techniques of biophysics and biochemistry (15). Knowledge of the metal content of mammalian cytochrome oxidase has been well established for over three decades. Four prosthetic groups are present: two heme-containing domains, a and a_3, and two copper-containing domains, Cu_A and Cu_B. But how do these oxidation-reduction centers relate to one another and to the overall reaction of oxygen reduction? In spite of ingenious and demanding efforts, a detailed description of the intermediary events associated with the oxidation of reduced cytochrome c, concomitant with the reduction of oxygen to water, is not yet available. Controversy surrounds findings proposing the existence of a peroxy-intermediate (105), the formation of a reactive hydroxyl radical ($OH^{\bullet}$) intermediate (77), or linkage to a proton-motive "O-cycle" (78, 80).

Introduction of the techniques of molecular biology has revealed the structural complexity of mammalian cytochrome c oxidase (12). Two decades ago, one would never have guessed that cytochrome oxidase is a mega-protein composed of 13 polypeptides (58, 75) of which some subunits are encoded by the mitochondrial genome while other subunits are coded by the nuclear genome (10). Clearly, coordination of synthesis of these different peptides and the intricacies of import and assembly of the subunits are central to understanding the function of cytochrome oxidase. Identification of the protein subunits associated with the heme and copper metal centers has been facilitated by comparison of orthologous proteins in prokaryotes (e.g. *Paracoccus denitrificans,* which contains only three subunits) with the cytochrome c oxidases of eukaryotes (9). But this comparison has identified a function for only four of the thirteen subunits. What are the roles of the remaining subunits and how do they contribute to the mechanism of electron transport and the associated reactions of energy conservation (111)?

An unexpected finding is that isoforms of at least three subunits of mammalian cytochrome oxidase exist and are expressed in a tissue-specific manner (57, 63). Recent studies have defined a heart form (H), with one set of subunits dominant in heart and skeletal muscle, and a liver form (L) with a different set of subunits present in liver, kidney, and brain. And these differences appear to influence the catalytic activities of the respective cytochrome c oxidases.

A thorough consideration of oxygen as an essential nutrient must include a discussion of its metabolism and the properties of the enzymes that interact with it. Clearly, we have much more to learn about cytochrome oxidase, the central enzyme of oxygen metabolism.

Cytochrome P450s

Another class of hemoproteins present in many cells is the cytochrome P450 superfamily. These hemoproteins catalyze an oxygenase reaction for the incorporation of molecular oxygen into a wide spectrum of organic chemicals (26, 39, 73). At present, there is detailed knowledge of 209 members of the P450 gene superfamily (81). The P450 superfamily is composed of 35 different gene families of which 12 families exist in all mammals examined to date. (A P450 is assigned to a specific gene family when its protein sequence has greater than 40% similarity to the protein sequence of a related P450.) These 12 families can be further dissected into 23 subfamilies, of which 17 have been mapped to human chromosomes. (A P450 is assigned to a specific gene subfamily when its protein sequence has greater than 55% similarity to the protein sequence of a related P450.) This inventory of the P450s represents only those P450s that have been cloned and sequenced. One can predict that three- to four-times as many P450s remain to be characterized.

The P450s are oxygenases, specifically monooxygenases. They function by

activating molecular oxygen to form an electrophilic species of oxygen [proposed to be a caged hydroxyl radical (104)], concomitant with the oxidation of reduced pyridine nucleotide (NADPH). The P450s contain cysteine as a ligand for the iron of the heme. The thiolate coordination bond that is formed is an identifying signature for this type of hemoprotein, and it is responsible for the absorbance band at about 450 nm that is formed when carbon monoxide reacts with the reduced hemoprotein (85).

Most interesting is the diversity of oxygen-dependent reactions catalyzed by different P450s. These reactions touch nearly every aspect of biology and medicine. An exhaustive listing of reactions catalyzed would reveal the central role that specific P450s play in cell development, differentiation, and death. Briefly, P450s catalyze critical biosynthetic reactions in plants leading to the formation of natural pesticides and insecticides (the phytoalexins) or the synthesis of plant hormones responsible for flavors, coloring pigments, flowering, or fruit ripening (64). In insects the P450s confer resistance to insecticides and they are responsible for the omega hydroxylation of fatty acids in the synthesis of waxes (1, 91). But it is in mammalian tissues that the P450s have been best studied and where we understand in greatest detail their role in the metabolism of endogenous as well as exogenous chemicals (39, 44). Initial studies revealed the role of adrenal P450s in the regio- and stereo-specific hydroxylation of steroids (25). Today one recognizes that the cascade of oxygen-dependent reactions associated with the metabolism of cholesterol to androgens and estrogens or to mineralo- or glucocorticoids involves at least five different P450s present in steroidogenic tissues (27). Of primary importance are the large number of different P450s with broad substrate specificity that participate in the metabolism of xenobiotics. The P450s play a critical role in the metabolic activation of chemicals for the initiation of chemical carcinogenesis or cellular toxicity (38). Of equal importance is the role of specific P450s in the metabolism of polyunsaturated fatty acids, such as arachidonic acid, to unique epoxides or the omega oxidation of prostaglandins, leukotrienes, and medium chain-length fatty acids (13). P450s catalyze the hydroxylation of vitamin D to its physiologically active form (8) as well as the oxidation of retinoids (90). The P450s may play a role in the odorant response of nasal tissue (93) and the mood-modifying role of neurosteroids in brain (2).

Genetic polymorphism is now recognized as a significant contributor to alterations in the function of specific P450s (37). The presence of varient alleles may explain the genetic susceptibility of individuals for lung cancer (61) or sensitivity to specific drugs (72). Clearly, those enzymes that react with oxygen are not immune from genetic variability in a manner that would alter their contribution to metabolism.

One of the most compelling arguments favoring identification of oxygen

as an essential nutrient is the listing of oxygen-dependent reactions catalyzed by different P450s and the recognition of the role played in cellular homeostasis by many of the metabolites that are formed.

Other Oxygen-Reacting Enzymes

Regrettably, neither time nor space permits us to describe the fascinating enzymology and biology associated with the many other enzymes present in mammalian tissues that react with oxygen. Textbooks and journals are dedicated to many of these physiologically important enzymes. Knowledge of their presence and the key role that they play in metabolism reinforces the premise that oxygen is necessary for life.

NUTRITIONAL DISEASES

Malnutrition or the Limitation of Oxygen Supplied to the Tissues

Nutritional diseases are generally caused by a reduction of an essential nutrient at the cellular level resulting from a failure of some step in its uptake from the environment, distribution to the site of need, or function in the cell. Well-known examples are the avitaminoses—diseases caused by lack of a vitamin in the diet, or failure to absorb or use it. Is there an equivalent nutritional disease associated with a lack or limitation of oxygen? Most cells in higher eukaryotes are delicately balanced between anaerobiosis and aerobiosis. Interference with the supply of blood to an organ results in hypoxia and the rapid onset of ischemia leading to a shifting of metabolism from a highly efficient energy-producing mode to the deleterious outpouring of acidic metabolites. A concomitant shift in ion distribution accompanies this collapse of the "energy charge" in the cell. One must conclude that the concept of malnutrition can be applied to oxygen depletion and that the consequences can be as insidious and devastating as the lack of a vitamin, essential fatty acid, or amino acid.

Reduction in the intake of food, starvation, can be tolerated by humans for weeks; limitation of water uptake can be tolerated for days; but a limitation of oxygen can result in irreversible changes after a few minutes. If the uptake of one foodstuff (glucose) is restricted, the body can call upon its reserves of other foodstuffs, including stored fat and protein. If water intake is reduced, the body can draw on fluid stored in the extracellular space. No such alternative sources exist as a backup for oxygen depletion. Some tissues, particularly muscle, can turn to glycolysis for a transient alternate source of energy, of ATP, while other critical organs, such as the brain, cannot.

The condition of limited oxygen uptake can be caused by a dysfunction at

any step in the transport of oxygen from inspired air to the cell. Some examples of a limited oxygen uptake where physiological adaptation has occurred are (30):

1. Reduced oxygen tension in the inspired air: This occurs at high altitude or breathing a polluted atmosphere.
2. Decreased alveolar ventilation of the lungs: Examples are the reduced minute ventilation in poliomyelitis, hemothorax, or pleural effusion.
3. Reduced O_2 content of the blood leaving the lungs: This can result from incomplete equilibration of O_2 between alveolar gas and blood leaving the individual capillaries such as in chronic pulmonary disease (52) or emphysema.
4. Decreased oxygen delivery to the capillaries: Anemia, obstruction of flow at the arterial level as in atherosclerosis or failure to pump the blood due to cardiac failure can limit delivery of oxygen to cells. This may result in angina pectoris and myocardial infarction; in the brain, it results in stroke; and in peripheral muscles, it results in intermittent claudication.
5. Handicapped transport from capillary blood to mitochondria: This process is primarily diffusion controlled, so the flux of O_2 is basically proportional to dpO_2/dx, but its local metabolism slows down the flux. Myoglobin, which binds O_2 reversibly and may act as a carrier molecule in a facilitated diffusion schema, can be poisoned.

O_2 lack can be acute or chronic. An acute fall in arterial pO_2 produces an increased rate of lung ventilation and increased cardiac output and arterial blood pressure in seconds. A similar decrease in tissue pO_2 causes dilation of peripheral arterioles in order to supply more O_2 to the cells. If the regulatory response is not rapid enough, or the stress is too great, there will be cell damage and ultimately death.

Chronic nonfatal reduction in the oxygen supply to a cell produces slower changes in the oxygen delivery system—an adaption. The blood hemoglobin concentration is increased by increasing the number of red cells. This takes days to weeks and is mediated through pO_2-sensing cells primarily in the kidney medulla that detect the lowered pO_2 and produce a hormone, erythropoietin, which stimulates bone marrow to produce more red blood cells. The affinity of hemoglobin for O_2 can be altered; the ventilation of the lungs can increase as well as the strength of the heart. Chronically lowered pO_2 in muscles produces an increased capillary bed. In athletic training, the metabolic reserve of the muscles rises because the concentration of mitochondria per cell increases and this demands more capillaries.

In recent years emphasis has shifted from seeking an understanding of the consequences of limiting the supply of oxygen to defining the deleterious

effects of reestablishment of oxygen to a tissue that has been subjected to anoxia. Oxygen radicals are generated during the reintroduction of oxygen to a tissue, such as the reperfusion of the ischemic myocardium (59). As a result, a peroxidation of cellular lipids may modify the properties of cellular membranes or an oxidation of key cellular proteins may produce conformational change or denaturation with a concomitant loss of enzymatic activity. The result of these irreversible injuries to the cell is necrosis. The source of oxygen radicals may be the infiltration of the tissue by phagocytes (4), the reestablishment of a functional mitochondrial electron transport system (84), or purine catabolism by xanthine oxidase (5). Clearly, a complex series of intracellular events occurs when oxygen is reintroduced to a cell, and more than one causative agent contributes to the resultant death of a cell. Oxygen radicals may play a similar role in the posttraumatic neuronal necrosis following brain or spinal cord injury (99).

A variant of the situation in which the supply of oxygen to a cell is limited occurs when a modification of the mitochondrial respiratory chain impedes the utilization of oxygen (45). The consequences of inhibition of cytochrome oxidase by carbon monoxide or cyanide are well known. In recent years there has been an increased recognition of mitochondrial dysfunction caused by genetic changes that result in a failure of the mitochondrial respiratory chain to express competent proteins (24). This mitochondrial pathophysiology and the associated clinical manifestations, called mitochondrial diseases, now number in the hundreds, and many are associated with changes of cytochrome c oxidase (11, 24, 36, 49). Deficiencies of both nuclear-encoded as well as mitochondrial-encoded subunits of cytochrome oxidase have been identified as causative agents of these pathological conditions. Of interest is the possible linkage of these deficiencies in cytochrome c oxidase to the role of cardiolipin and coenzyme Q in the function of the respiratory chain.

Overnutrition or Oversupply of Oxygen to Cells

An excess of some nutrients can be harmful and can produce a nutritional disease. Obesity might be called such a condition. An increase in tissue pO_2 can damage cells and is a disease of modern technology resulting from an increase of inspired pO_2. After the discovery of oxygen by Priestley in 1777 and the recognition that it supported life, physicians began to use it as therapy—just as many foodstuffs have been used—but the therapeutic effects were minimal so enthusiasm declined quickly, particularly since some toxic effects were noted. The O_2 transport system regulates the delivery of O_2 to peripheral cells to maintain pO_2 within a narrow range, from about 1 mm Hg to about 15 mm Hg (18).

If a healthy adult breathes essentially pure O_2 at sea level, his lungs become irritated (67); a rat exposed in the same way will die in a matter of hours;

newborn humans will develop retrolental fibrodysplasia. Chronic exposure of rats to high oxygen can diminish or destroy the ability of the carotid bodies to monitor low arterial blood pO_2 (79). These are but a few examples of the deleterious effects of inspiring higher than normal O_2 concentrations. Which of these undesirable effects is simply a result of high $[O_2]$ and which are the result of the action of oxygen radicals produced by the higher pO_2 is the subject of intense research (16). In either case, O_2 is both an absolute requirement for life and at the same time a toxin, giving rise to the oxygen paradox.

THE OXYGEN PARADOX

Ames & Gold (3) estimate that "the DNA hits per cell per day from endogenous oxidants are normally $\sim 10^5$ in the rat and $\sim 10^4$ in humans." Oxygen radicals are thought to account for the major share of agents causing this damage. Thus, there is a paradox. Oxygen is essential to life, yet we must balance this positive effect with the recognition that oxygen may also limit life processes. The literature on the potential negative effects of oxygen radicals is vast (21). Oxygen radicals reportedly contribute to the processes of aging, the promotion of cancer, the establishment of atherosclerosis, the initiation of inflammation and the consequent rheumatoid diseases, and on and on. The term "oxygen (or oxidative) stress" (96) has been coined to encompass the physiological and pathological situations that result from increased cellular loads of oxygen radicals. No cellular constituent is immune to oxidative modification initiated by oxygen radicals. Lipids, proteins, nucleic acids, and carbohydrates all fall victim to damage following exposure to radical or oxidant exposure (21, 46).

CONCLUDING REMARKS

This review is a blend of two different scientific approaches to the question of whether or not molecular oxygen fulfills the definition of an essential nutrient. The physiologist approaches the question from the perspective of cellular and subcellular physiology with emphasis on the availability, transport, and distribution of oxygen. The biochemist focuses on the molecular aspects of oxygen and its role in enzymology and metabolism. We remain divided in our conclusion: The physiologist, from his perspective of organismic biology, favors including oxygen as an essential nutrient. Its delivery to cells and mitochondria is the most critical of all nutrients; the stores of it in the body are enough for only a few seconds of metabolism. On the other hand, the biochemist notes that oxygen is involved in a plethora of reactions that extend well beyond those of a classical nutrient. Thus, oxygen is too important to life to be categorized simply as a nutrient.

As a nutrient oxygen is unique. The circulatory-respiratory transport system monitors and regulates arterial pO_2 in order to supply the body's needs continuously and rapidly. O_2 can be viewed as the architect of phylogenetic development. The critical balance of an "oxygen limited" cellular metabolism places oxygen at the fulcrum point for dictating the energy charge required for homeostasis. One is struck by the diversity of reactions in which oxygen participates during the synthesis of hormonal mediators and structural elements and in xenobiotic metabolism. Lastly, one must consider the dualism of oxygen. Oxygen is essential for life, although it carries the risk of destroying the very life for which it serves as the source of useful energy.

The challenge has been met, but the question remains unanswered.

We apologize to our many friends and colleagues for the failure to cite their many contributions to oxygen metabolism and physiology, but it would be an impossible task to highlight even a small portion of the elegant science that has contributed to our present understanding of oxygen chemistry and physiology, and thus we have, of necessity, been highly selective and somewhat arbitrary in our focus.

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