The Metabolic Function of Oxygen and Biochemical Lesions of Hypoxia

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In this matter there must first be understood the substantial generation of the vital spirit which is composed of a very subtle blood nourished by the inspired air. The vital spirit had its origin in the left ventricle of the heart, and the lungs assist greatly in its generation. It is a rarefied spirit, elaborated by the force of heat, reddish-yellow (flavo) and of fiery potency, so that it is a kind of clear vapor from very pure blood, containing in itself the substance of water, air, and fire. It is generated in the lungs from a mixture of inspired air with elaborated, subtle blood which the right ventricle of the heart communicates to the left.—Michael Seretius, 1533

Among the fears which confront the practicing anesthetist, the dread of hypoxia is supreme. Dark blood, profound circulatory changes, pupillary dilatation, and subsequent failure to awaken are constant threats in the operating room. The causes and physiologic responses to hypoxia were reviewed in 1965.12

In this paper I propose to discuss the essential metabolic functions of oxygen, as well as the biochemical consequences of lack of oxygen.

In mammalian tissue dissolved or molecular oxygen may be utilized in three ways.90, 94

Oxygen transferases are enzymes which function to add both oxygen atoms to a substrate:

A + O₂ → AO₂

Mixed-function oxidases add one oxygen atom to the substrate, while the other atom yields water:

AH + 2 e⁻ + 2 O₂ → AOH + O⁻⁻ (H₂O)

The processes catalyzed by these two enzymes are not directly involved in the production of energy. Rather, they function in the biosynthesis of a number of biologically important molecules such as steroids, pigments, and fatty acids. This accounts for no more than 1 per cent of total mammalian oxygen consumption. Mixed-function oxidases are of increasing interest because of their function in microsomal drug metabolism, which can be inhibited in vitro and in vivo by carbon monoxide.126

The third system is that of electron-transfer oxidases, which may be depicted as catalyzing the following reaction:

4 e⁻ + O₂ → 2 O⁻⁻ (2H₂O)

These enzyme systems may be subdivided; the auto-oxidative flavoproteins react in the following manner:

Flavin H₂ + O₂ → flavin + H₂O₂

Hydrogen peroxide is removed by the action of catalase. It is not known what part of the cell subserves this reaction; however, it comprises only about 10 per cent of total oxygen uptake. The second form of electron-transfer oxidase is that which is responsible for approximately 90 per cent of mammalian oxygen consumption. This is the mitochondrial respiratory chain, which is the mechanism for converting substrate to energy. Since the spark of life may be said truly to reside within the mitochondrion, it is reasonable to discuss this organelle at this time.

The Mitochondrion

The mitochondrion (Gr. mitos, a thread; chondros, a grain) was first recognized as a distinct intracellular organelle less than a century ago. These structures are found in all aerobic cells of higher animals and plants, as well as in some algae, fungi, and protozoa, but do not reside in bacteria or blue-green algae.188
The mitochondrion may represent descendants of microorganisms which originally functioned as symbiotic parasites within the cytoplasm of larger cells. The intracellular locations of mitochondria, as well as their concentrations, vary widely from organ to organ. Rat liver cells each contain approximately 800 mitochondria 3 μ long and 0.5–1.0 μ wide. In the hepatocyte the mitochondria comprise approximately 20 per cent of the cell’s nitrogen content. The intracellular loci of the mitochondria are frequently near other structures which need their energy output; the skeletal muscle fibril is an example.

Mitochondrial function is intimately bound up with its structure, a diagrammatic representation of which appears in figure 1. The organelle contains all of the structural components necessary for the transduction of energy from substrate to adenosine triphosphate (ATP). It consists of two membrane systems which surround the inner lumen, known as the matrix. The space between the two membranes is 60–80 Å thick and does not communicate with the matrix. The outer membrane is smooth, while the inner one contains many infoldings, or cristae. The membranes are composed of 35–40 per cent lipid (more than 90 per cent of which is phospholipid) and 60–65 per cent protein. Most of the lipid serves in a structural capacity. About half the membrane protein is also structural in nature, while more than 25 per cent of the protein consists of the respiratory enzymes.

The inner membrane is covered with projecting tripartite subunits (fig. 2). Each of these “elementary particles” consists of a basepiece which forms the inner membrane, a spherical headpiece, and a stalk which connects the two. Most of the protein is located in the basepiece, while the locus of the system necessary for the synthesis of ATP from adenosine diphosphate (ADP) and inorganic phosphate (Pi) is the headpiece. The stalk functions to link electron transport and ATP synthesis. The structure of the mitochondrion and its subunits is exceedingly important. During oxidative phosphorylation (see below) changes in mitochondrial structure may be intimately involved, while the numbers of cristae are greater in tissues with increased metabolic rates.

Let us now turn our attention to the processes involved in the conversion of energy contained in the breakdown products of nutrition to the useful energy of ATP.

The Citric Acid Cycle

The input of energy to the mitochondrion is the citric-acid (Krebs, tricarboxylic-acid) cycle, which is depicted in figure 3. Acetyl CoA arising from the oxidation of pyruvate by mitochondrial pyruvate dehydrogenase enters the citric-acid cycle after combining with oxaloacetate. Nicotinamide adenine dinucleotide (NADH), produced during the oxidation of pyruvate to acetyl CoA, is reoxidized by the mitochondrial respiratory chain (see below). The central function of the citric-acid cycle is to yield electrons to the mitochondrial respiratory chain. This occurs at several dehydrogenase steps which yield NADH and re-
Reduced flavoproteins (fig. 3, steps 4, 6, 8, 10). These events may be depicted as follows:

\[
2 \text{NAD}^+ + 4 \text{H} \rightarrow 2 \text{NADH} + 2 \text{H}^+ \\
\text{Flavin} + 2 \text{H} \rightarrow \text{flavin H}_2
\]

NADH and reduced flavoprotein furnished by these dehydrogenase reactions are then oxidized by the mitochondrial respiratory chain, a process which makes available the energy conserved in ATP. Step 7 demonstrates the conversion of succinyl CoA to succinate and CoA, during which a phosphate bond initially appears as the terminal phosphate of guanosine-5-triphosphate. This, in turn, is transferred to ADP (adenosine diphosphate) by transphosphorylation, with the production of ATP. This means of forming ATP without the participation of the electron-transport chain is known as “substrate-level phosphorylation.” It is important to realize that the formation of carbon dioxide is not directly involved in the synthesis of ATP, since carbon dioxide results from decarboxylation, while ATP is formed by the oxidation of NADH or reduced flavoprotein. In considering the overall function of the citric-acid cycle, one might conceive of the carbon skeletons in the substrates simply as structures necessary for the delivery of hydrogen atoms to the mitochondrial respiratory chain.

Adenosine Triphosphate

In order to understand the purpose of oxidative metabolism, some attention must be paid to the means by which useful energy may be stored within the cell. Energy is utilized, transported, and stored in the form of high-energy phosphate bonds (∼P). Most of our concern will be with that ∼P which is the terminal phosphate of adenosine triphosphate. This compound might well be considered the “coin of the cellular realm.” The structure of ATP is shown in figure 4; the molecule consists of adenine, ribose, and three phosphates linked by pyrophosphate bonds. ADP and adenosine monophosphate (AMP) have similar structures except that they have two and one phosphates, respectively. Hydrolysis of ATP yields either ADP and phosphoric acid (H₂PO₄⁻) or AMP and pyrophosphoric acid (H₄P₂O₇⁻).

These latter two acids are called “P₆” or inorganic phosphate. ADP is usually not hydrolyzed to AMP and P₆; instead, myokinase (adenylic kinase) catalyzes the following reaction:

\[
2 \text{ADP} \rightarrow \text{AMP} + \text{ATP}
\]

Removal of the phosphate of AMP yields adenosine (adenine and ribose) and P₆.

Hydrolysis of the two phosphate bonds of ADP is accompanied by a free-energy change of −8 kcal/mole. It is because of the ability of ATP hydrolysis to furnish a considerable amount of free energy that these bonds are known as “high-energy” phosphate bonds. Other high-energy phosphate bonds and their free energy of hydrolysis are creatine phosphate (−10.6 kcal/mol) and phosphoenolpyruvate (−13.7 kcal/mol). Not all phosphate bonds have this property, and the hydrolysis of AMP, pyrophosphate, and glycerophosphate yields −3, −2, and −2.5 kcal/mol; for this reason, these bonds might be called “low-energy bonds.” The energy released by the hydrolysis of a high-energy phosphate bond allows reactions to proceed that would not otherwise be possible. These reactions, requiring the input of considerable energy to occur, share a common feature: the thermodynamic improbability of their occurring at all. It is by furnishing the energy to drive these reactions that ATP serves its role in maintaining life.

The values for free energy given above are those observed under standard physiochemical conditions, and are seen only when products and reactants are equimolar in concentration and at infinite dilution. Thus, under physiologic conditions, the magnitude of free-energy changes may be affected by the presence of
locations such as Mg$^{++}$ and Ca$^{++}$, as well as differences in relative concentrations of ATP, ADP, and P$_i$. Since ATP, ADP and P$_i$ are not uniformly distributed throughout the cell, a theoretical value for free energy may not be applicable to intracellular events.

Mitochondrial Respiration

The mitochondrial electron-transport (respiratory) chain is the means by which the energy contained within the substrates of the citric-acid cycle is transferred to the $\sim$P of ATP. The system consists of a series of compounds which can exist in either oxidized or reduced form (fig. 5). The components of the respiratory chain are found entirely within the mitochondrion; cytochromes and flavoproteins are bound to the cristae, while NAD$^+$ is located in solution within the matrix and intramembrane area. Physical separation of extramitochondrial NAD$^+$ results in lack of equilibration between these two fractions (see below). Excessive swelling or disruption of the mitochondrion causes immediate loss of mitochondrial NAD$^+$. Such treatment results in the rapid oxidation of exogenous NADH, a phenomenon not observed with intact mitochondria.

The respiratory chain transfers electrons from the substrate to cytochrome $a_3$ (cytochrome oxidase), the final member of the respiratory chain, which then transfers these electrons to oxygen. NADH dehydrogenase and succinic dehydrogenase transfer electrons from NADH and succinate to the remainder of the respiratory chain. Large amounts of coenzyme
Q (ubiquinone) are present in mitochondria; this substance can be reduced by either succinate or NADH, and is oxidized by preparations containing cytochrome oxidase activity. The cytochromes are proteins conjugated with iron-containing porphyrins. The ability of iron to exist in either the ferrous or the ferric state makes it possible for the cytochromes to shuttle electrons from coenzyme Q to molecular oxygen.

Substrates are electronegative compared with oxygen. As electrons move through the respiratory chain, they pass through a series of compounds with increasing oxidation-reduction potentials. Compared with the standard hydrogen electrode, NADH has a potential of $-0.32 \text{ v}$ and flavoprotein a potential of $-0.1 \text{ v}$; while cytochrome b is electroneutral. Cytochrome c has a potential of $+0.26 \text{ v}$, and cytochrome a a potential of $0.29 \text{ v}$. The potentials of cytochromes $c_1$ and $a_3$ are unknown; that of $a_3$ probably lies between $+0.29$ and $+0.81 \text{ v}$, which is the potential of oxygen. Energy may be liberated with each transfer of electrons, as in a battery. The transfer of two electrons from NADH to oxygen makes available a total free energy of 55 kcal, which can be trapped in the form of $\sim P$.

When the delivery of oxygen to the respiratory chain becomes impaired, each of the components becomes more reduced. Eventually, electron transfer slows and then ceases, at which point the energy contained within the substrates of the citric-acid cycle no longer can be made available. Later in this paper we shall see how these changes are measured and what magnitude of mitochondrial oxygen tension is necessary for normal energy production to continue.

**Oxidative Phosphorylation**

When mitochondria are incubated with substrate $O_2$, $P_i$, and ADP, the substrate is oxidized and ATP is produced. The process by which energy produced by respiratory-chain activity is stored in the form of ATP is known as "oxidative phosphorylation." Figure 5 shows the three sites at which energy is conserved. A redox potential difference of at least 0.22 v exists at these loci.

When $P_i$ and ADP are added to a suspension of mitochondria, a spurt of increased oxygen uptake occurs immediately. The amount of oxygen utilized rapidly can be measured and related to the ATP synthesized. Results of such investigations are expressed as the $P_i/O$ or ADP/O ratio. The former represents the amount of $P_i$ disappearing divided by the atoms of oxygen consumed. The latter is the ratio of ADP used to atoms of oxygen consumed. The ideal ratio is 3.0 for NADH-linked substrates and 2.0 when flavoprotein-linked substrates are consumed. The theoretical amount of free energy liberated when NADH is oxidized by oxygen is 55 kcal/mol. Since the theoretical energy conserved by the reaction

$$ADP + P_i \rightarrow ATP$$

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**Fig. 4.** Adenosine triphosphate. The vertical dashed lines indicate hydrolysis of the terminal and second phosphate groups. From Johnst. [9]
is 8 kcal/mol, oxidative phosphorylation has an efficiency of approximately 44 per cent.

**Magnitude of ~P Production by the Citric-Acid Cycle**

One can use the F/O ratio to calculate the theoretical yield of ATP resulting from the oxidation of acetyl CoA (see fig. 3). Reactions 4, 6, and 10 each result in the formation of three ATPs, and thus produce a total of nine ATPs. Reaction 7 produces one ATP and reaction 8, two molecules of ATP. Finally, mitochondrial oxidation of NADH produced in step 1 results in formation of three additional molecules of ATP. Thus, mitochondrial oxidation of acetyl CoA produces a total of 15 ~P bonds. When a molecule of glucose is metabolized (see below), two molecules of acetyl CoA result; this makes possible the formation of 30 ~P bonds per molecule of glucose. An additional 6 ~P bonds are synthesized when glycolysis occurs in the presence of oxygen (see below). In the presence of adequate oxygen supply, 36 ~P bonds are synthesized for each mole of glucose consumed. The complete combustion of glucose to CO₂ and water yields approximately 700 kcal/mol; an assumption of 8 kcal/mol/~P bond yields an efficiency of 41 per cent. It should be remembered that considerations of the relative concentrations of the compounds involved (see above) indicate that these calculations may be erroneous, and calculations of efficiency are probably meaningless at the present time.

**Respiratory Control**

In normal mitochondria, oxygen utilization by the respiratory chain is intimately linked or coupled to phosphorylation. Uncoupling agents (e.g., 2,4-dinitrophenol and bis-dydroxycoumarin) are capable of dissociating respiratory-chain function from phosphorylation. They therefore prevent the formation of ATP, while having little effect on electron transport or oxygen uptake. They may also function to stimulate the activity of ATPase, which converts ATP to ADP.

The obligatory coupling of oxidation to phosphorylation has permitted characterization of distinct metabolic states٢٨,٢٩ (table 1). Freshly-obtained mitochondria (state 1) have a slow respiratory rate, limited mainly by the supply of phosphate acceptor (ADP). NAD⁺, flavoproteins, and cytochromes b and c are partially reduced. A brief period of stimulation of oxygen uptake occurs following the addition of ADP; however, when endogenous substrates are exhausted, respiration decreases. These “starved” mitochondria (state 2) show complete oxidation of the redox pairs of the respiratory chain, since the absence of substrate prevents movements of electrons into these compounds. Addition of substrate immediately allows electrons to flow through the respiratory chain and, in the presence of phosphate acceptor, rapid oxygen utilization occurs (state 3). Oxygen uptake is limited only by the maximal rate at which the electron chain can transfer electrons. In the presence of excess oxygen and substrate, rapid oxygen uptake will persist until all available ADP has been phosphorylated. At this point (state 4), respiration will be slow, NADH will become almost completely reduced, while flavoprotein and cytochromes b and c will show partial reduction. State 4 is known as the “state of respiratory control”; the ratio, state 3 oxygen uptake: state 4 oxygen uptake, is called the “respiratory control index,” and is a measure of
the integrity of the mitochondrial suspension. Loss of respiratory control is manifested by an increased rate of mitochondrial oxygen utilization in the absence of ADP (state 4). The phenomenon has been observed in mitochondrial suspensions treated with commonly-used anesthetic agents. Decreased respiratory control, a very sensitive index of mitochondrial integrity, need not be accompanied by uncoupling of oxidation from phosphorylation. Although respiration may be abnormally high in the absence of phosphate acceptor, the addition of ADP can still be followed by a normal amount of phosphorylation. Mitochondria in state 4 can be returned to state 3 by the addition of ADP. These cycles may be repeated until all available oxygen has been utilized, at which point state 5 (anaerobic) occurs. Respiration is absent and all respiratory-chain components are fully reduced.

MECHANISM OF OXIDATIVE PHOSPHORYLATION

Oxidative phosphorylation has been the subject of a number of excellent reviews. Three basic approaches have attempted to elucidate the mechanism of oxidative phosphorylation. The chemical-intermediate model proposed that the transfer of electrons through the respiratory chain is coupled to the synthesis of a number of high-energy intermediates; these, in turn, are coupled to the synthesis of ATP. In this approach, depicted below, A and B represent two adjacent members of the respiratory chain (e.g., NAD+ and flavoprotein), between which phosphorylation occurs and energy is conserved. X and I are hypothetical intermediates. X might be either free histidine or a histidine moiety on a mitochondrial protein.

\[ \text{A} + \text{I} \rightarrow \text{A} + \text{I} \]
\[ \text{A} + \text{I} + 2 \text{H} \rightarrow \text{AH}_2 + \text{I} \]
\[ \text{AH}_2 + \text{I} + \text{B} + \text{X} \rightarrow \text{A} + \text{BH}_2 + \text{I} + \text{X} \]
\[ \text{I} + \text{X} + \text{P} \rightarrow \text{X} + \text{P} + \text{I} \]
\[ \text{X} + \text{P} + \text{ADP} \rightarrow \text{ATP} + \text{X} \]

In the absence of ADP, the last reaction cannot take place; binding of the inhibitory component I to one of the components of the respiratory chain prevents rapid electron transfer. Addition of ADP allows this inhibition to be released, following which oxygen uptake rapidly increases. There are two main difficulties with this concept. In spite of intensive investigation, none of the intermediates has been isolated. In addition, this approach fails to consider the importance of structural changes occurring in the mitochondrion during its energy cycle.

Peter Mitchell recognized some of these difficulties and proposed an alternative chemiosmotic model. This approach postulates that the chemical reactions involved with ATP synthesis are spatially and chemically separate from electron transfer. He proposed that the mitochondrial membrane, an insulating osmotic barrier of very low proton conductance, is the instrument by which oxidation and phosphorylation are coupled. The model postulated that the primary event is not the formation of a high-energy bond; rather, electron transport results in the movement of protons through the cristae or coupling membrane. The coupling membrane is impermeable to ions, which results in the establishment of a concentration gradient of protons and an electrical gradient. This chemical and membrane potential is used to synthesize ATP by reversal of an ATPase system located within the membrane. In the absence of ADP (state 4), the electrical potential continues to build up until its magnitude prevents further electron transport, thus establishing respiratory control. Classic uncouplers cause the coupling membrane to become a conductor of protons, and uncoupling results from the short-circuiting of the proton current.

The third model hypothesizes that mitochondrial respiration produces a change in the conformation of the tripartite repeating unit (fig. 6). Free energy produced during electron transfer is preserved in the form of conformational energy which can drive ATP synthesis. Green has called the three structural states "nonenergized," "energized," and "energized-twisted." In the nonenergized conformation, the headpiece-stalk collapses

Fig. 6. Conformations of the tripartite repeating unit are portrayed in the column on the right. High-resolution electron micrographs on which the reconstructions are based are shown on the left. From Green.
OXYGEN AND BIOCHEMICAL LESIONS OF HYPOXIA

Nonenergized (aggregated)

Energized

Energized twisted
onto the basepiece, while two apposed cristae compress the headpiece–stalk until no space exists between them. Electron transfer results in the extension of the headpiece–stalk away from its basepiece while it remains attached to the apposed cristae. Addition of P₄ during mitochondrial respiration results in the formation of the energized-twisted conformation; the headpiece–stalk remains extended and the headpiece separates from its basepiece. The latter changes result in a major rearrangement of the membrane into a tubular form, and the basepieces appear curved. The nonenergized conformation is thermodynamically stable, while the energized and energized-twisted structures are metastable. Because of this, they spontaneously revert to the nonenergized structure during anaerobiosis or following the addition of a respiratory inhibitor. These metabolic conformations continually relax and are regenerated. Therefore, if one blocks the energy necessary for generation of these metastable configurations, the number of energized repeating units rapidly decreases. Addition of ADP initiates oxidative phosphorylation and reduces the fraction of mitochondria with an energized conformation. This rapid rate of relaxation produces an increased turnover rate and is responsible for the ability of ADP to stimulate respiration. Addition of uncouplers reduces stability of these metastable conformations, which makes it impossible for them to serve as adequate sources of free energy for ATP synthesis.

Relation of Oxygen Availability and Mitochondrial Function

We are now in a position to consider the intramitochondrial events which transpire in the face of decreasing oxygen availability. In the presence of excess oxygen, the velocity of oxygen consumption is independent of oxygen concentration. With diminution of oxygen concentration, cytochrome oxidase becomes increasingly reduced. This is followed by reduction of all the respiratory-chain components. As mitochondrial respiration or electron flow decreases, ATP synthesis is diminished. At this point, energy conservation previously controlled by the concentration of ADP becomes limited by diffusion of oxygen to the mitochondrial respiratory chain. Consideration of these factors prompts two questions: 1) At what point is reduction of the mitochondrial respiratory-chain components observed? 2) At what level of oxygen availability will mitochondrial respiration diminish?

Critical Oxygen Tension†

Although it is extremely difficult to measure mitochondrial oxygen tension directly in vivo, Chance’s group has made use of the fact that the spectral characteristics of the mitochondrial respiratory-chain components are altered by their redox state. These changes permit the spectrophotometric or fluorometric measurement of the redox state of the respiratory chain in the intact animal or in vitro. In contrast to the in-vivo situation, in-vitro measurements of oxygen tension are relatively easily accomplished with a vibrating platinum electrode. Chance studied mitochondrial particles prepared from heart muscle during the oxidation of succinate to fumarate. Oxygen concentration was measured continuously with a vibrating platinum electrode; oxygen consumption was calculated as the change in oxygen concentration with respect to time. Absorbency at 445 mₜₜ was used to measure the reduction of cytochrome oxidase, while fumarate could be measured by means of its ultraviolet absorption at 250 mₜₜ. Since fumarate production should be the mirror image of oxygen consumption, the latter recording was used to check the accuracy of the platinum electrode. As mitochondrial respiration proceeded, the rate of oxygen utilization and the level of cytochrome oxidase reduction remained constant until oxygen tension was approximately 2 torr. At this critical oxygen tension, respiration began to decrease; a respiratory rate of half-normal was reached at a mitochondrial oxygen tension of 0.5 torr. Cytochrome oxidase was half reduced when mitochondrial oxygen tension was 1 torr. Isolated brain mitochondria behaved similarly. When oxygen tension was 0.5 torr, NAD⁺ showed a 10 per cent level of reduction; when oxygen tension was approximately 0.2 torr, oxygen tension at which respiratory rate begins to decrease.

† Critical oxygen tension is the O₂ tension at which respiratory rate begins to decrease.
NAD⁺ was 50 per cent reduced and oxygen uptake began to decrease.⁶²

Critical oxygen tension is naturally affected by the respiratory rate.⁷⁷,⁸³ However, since more respiratory enzymes are available in state 3 than in state 4, this change is minimized. For example, when kidney mitochondria undergo a state 4 → state 3 transition and oxygen uptake is increased fivefold, the critical oxygen tension rises from 0.6 to only 0.9 torr.⁷⁷

The critical oxygen tension may be lower than the oxygen tension at which respiratory-chain component reduction is first observed. An overabundance of cytochrome oxidase in the mammalian respiratory chain permits it to change the ratio of oxidized/reduced compound while, at the same time, sufficient concentration of the oxidized form remains to enable normal respiration to continue. This phenomenon is known as the "cushioning effect." Theoretical calculations or the oxygen affinity of the respiratory chain as a whole yield a critical oxygen tension of 1.07 torr.⁸³

Can the redox state of respiratory-chain components be measured in vivo? Chance and his co-workers⁶¹,⁶² have supplied an ingenious answer. When irradiated with an ultraviolet beam of 335–340 mμ NADH fluoresces with an emission peak of 463 for mitochondrion-bound NADH and 480 mμ for free NADH. This occurs in vivo and in vitro and permits continuous observation in vivo of the redox state of the NAD⁺/NADH system. Coincident spectrophotometric measurements of hemoglobin saturation allow the investigator to relate oxygen saturation (or tension) to respiratory-chain function. Furthermore, the relation between a change in oxygen tension and an alteration in mitochondrial respiratory-chain redox potential in an in-vitro system may be extrapolated to the in-vivo situation and thus allow estimation of mitochondrial oxygen tension in vivo. The latter means of estimating changed tissue PO₂ is far more useful at low oxygen concentrations, at which the methods for measuring hemoglobin saturation are inadequate. When a rat breathes 4 per cent oxygen, NADH reduction is approximately 50 per cent, capillary PO₂ is 12 torr, and intracellular oxygen tension is 0.1–0.2 torr.⁶¹,⁸³

That mitochondria function normally when their oxygen tension is less than 5 torr must not be taken as license to allow arterial hypoxemia. Indeed, the oxygen tension of blood within the capillaries serves as a driving force, permitting the diffusion of this final electron acceptor from erythrocytes through plasma into tissue and finally into the mitochondrion. One can relate arterial and venous PO₂ to the minimum oxygen tension at which mitochondrial cytochrome oxidase functions normally. Stainsby and colleagues⁸²,⁸³ exposed dogs to decreasing concentrations of inspired oxygen while gastrocnemius-plantaris muscle oxygen consumption was measured. At rest, muscle oxygen uptake began to decrease when arterial oxygen tension was 60 torr and venous oxygen, 25 torr. During exercise, capillary density increased and critical oxygen tensions were 40 and 10 torr in the arterial and venous systems, respectively. These data indicate the existence of a gradient from venous blood to mitochondrion of at least 5 to 20 torr, depending on the state of muscular activity.
Whalen has noted that the muscle normally operates so close to its critical oxygen tension that blood flow may limit oxygen consumption. This permits consideration of muscle oxygen tension as a regulator of heat production. The magnitude of this gradient within the central nervous system may also be demonstrated. Thus, although the redox state and oxygen consumption of brain mitochondria in man remain normal when oxygen tension is 3 or 4 torr, unconsciousness supervenes when venous oxygen tension is less than 18 torr.

**Anaerobic Metabolism**

The mitochondrion does not represent the sole means by which cellular energy may be derived. ATP-yielding reactions occur in the cytoplasm, and will now be discussed. The Embden-Meyerhof, or glycolytic, pathway is shown in figure 7. This metabolic pathway converts glucose to pyruvate and serves two purposes: it supplies substrates for the citric acid cycle and furnishes a source of ATP when oxygen supplies are inadequate. When oxygen is available, it moves slowly and feeds pyruvate into the citric-acid cycle after it has been converted to acetyl CoA. Following glucose through this scheme, we observe the initial loss of one molecule of ATP/mol glucose (steps 1, 5). The six-carbon fragment of glucose is split to two three-carbon fragments in step 6, and two molecules at ATP/mole of glucose are produced at step 8. Another two molecules of ATP/mole of glucose are produced at step 11. The last two steps represent substrate-level (glycolytic) phosphorylation.

As mentioned above, the intact mitochondrial membrane is impermeable to NADH. Thus, direct equilibrium between intramitochondrial NADH and the cytoplasmic NADH produced by glycolytic step 7 does not exist. In other words, the NADH produced within the cytoplasm by the oxidation of glyceraldehyde-3-phosphate to 1,3-diphosphoglyceric acid does not have direct access to the mitochondrial respiratory chain for reoxidation. However, two "shuttle systems" function to permit regeneration of cytoplasmic or glycolytic NAD$. $\alpha$-Glycerophosphate dehydrogenase is located within the cytoplasm; $\alpha$-glycero-

Phosphate oxidase is located within the mitochondrion. The former enzyme permits the reduction of dihydroxyacetone phosphate to $\alpha$-glycerophosphate while NADH is reoxidized to NAD$^+$ (reaction 13). $\alpha$-Glycerophosphate readily penetrates the mitochondrion, where it is reoxidized to dihydroxyacetone. The mitochondrial membrane is permeable to this substance, which diffuses back into the cytoplasm. An additional "shuttle system" is made possible by the equilibrium between malate and oxaloacetate. When cytoplasmic NADH increases, the ratio of cytoplasmic malate/oxaloacetate is raised. The mitochondrial membrane is permeable to both these substrates, and therefore mitochondrial malate and oxaloacetate will reflect changes in this cytoplasmic ratio. Increased oxidation of mitochondrial malate will permit restoration to normal of the cytoplasmic malate/oxaloacetate ratio and the cytoplasmic NADH/NAD$^+$ ratio.

The mitochondrial oxidation of each molecule of $\alpha$-glycerophosphate (a flavin-linked substrate) generates an additional two molecules of ATP. Thus, glycolysis occurring in the presence of adequate oxygen supplies (aerobic glycolysis) generates a total of six molecules of ATP/molecule of glucose consumed. When aerobic glycolysis and the citric-acid cycle are combined, a total of 36 molecules of ATP results from the degradation of one molecule of glucose (see above).

When oxygen availability decreases, the mitochondrial respiratory chain fails and, of course, this shuttle system becomes inoperative. For this reason, the NADH produced in reaction 7 is no longer able to transfer its reducing equivalents of the mitochondrial respiratory chain. NADH is then reoxidized within the cytoplasm by lactate dehydrogenase, which reduces pyruvate to lactate acid (step 12). Since the citric-acid cycle is inoperative, the only energy storage possible occurs at steps 8 and 11; a total of two $\sim P$ bonds/mole of glucose result. Thus, among the biochemical symptoms of hypoxia are increased lactate production and decreased energy synthesis.

**Glycolytic Control**

It is intuitively obvious that if energy production is not to be seriously decreased when
Fig. 7. The Embden-Meyerhof glycolytic scheme and the α-glycerophosphate shuttle. Black circles represent carbon atoms; O, H and OH groups are shown only where they participate in the reactions of the compound. Iodoacetate and fluoride inhibit at locations shown by dashed lines. From Jöbsis.33

Oxygen availability is limited, the rate of glycolysis must be capable of increasing. The ability of decreased respiration to stimulate glycolysis (Pasteur effect) has been known for years. What is the metabolic signal involved in this phenomenon? Inspection of figure 7 discloses that steps 8 and 11 depend on the presence of ADP. These steps, involved with glycolytic phosphorylation, have a lower affinity for ADP than does the mitochondrial respiratory chain. This decreased affinity prevents the rapid operation of glycolysis when normally-functioning mitochondrial respiration and oxidative phosphorylation keep ADP lev-
els low. As oxidative metabolism fails, the concentration of ADP increases, and glycolytic phosphorylation occurs. Increased ADP concentration further stimulates glycolysis by acting on rate-limiting steps or control points. Lowry and co-workers produced cerebral ischemia by decapitation. The cerebral content of all substrates, cofactors, and enzymes involved in the Embden-Meyerhof pathway were assayed before and as long as 20 minutes after ischemia. Metabolic rates were calculated by measuring changes in ATP, phosphocreatine, glucose, glycogen, and lactate. Decapitation resulted in a four- to sevenfold increase in glycolytic rate, the onset of which was characterized by decreases in glucose, glucose-6-phosphate and fructose-6-phosphate, and by increases of all substrates from fructose diphosphate to lactate. These data suggest that increased glycolytic rate results from facilitation of the phosphorylation of fructose-6-phosphate (phosphofructokinase, step 5). Phosphofructokinase is normally inhibited by ATP. When ATP formation lags behind the rate of ATP utilization, ADP and P_1 increase. The activity of myokinase results in a greater increase in AMP. The combination of increased P_1, ADP, and AMP increases phosphofructokinase activity; the products of phosphofructokinase activity, namely fructose diphosphate and ADP, both serve as deinhibitors of enzymatic activity and further increase its activity. Since glucose and ATP are not at equilibrium with ADP and glucose-6-phosphate at the onset of stimulation, and since the rate of glycolysis can be markedly increased, hexokinase may function as another control point.

Sacktor and colleagues administered Indoklon (bis 2,2,2-trifluoroethyl ether) to mice in order to produce convulsions. Glycolytic flux increased by at least a factor of 3. During the transition to a new steady state, the concentrations of glucose and hexose monophosphates decreased, while fructose-1,6-diphosphate increased, again indicating activation of phosphofructokinase. This was accompanied by increased concentrations of ADP, AMP, and P_1.

Interaction of Glycolysis and Respiration

We are now in a position to take an overall view of metabolic control. In the presence of oxygen, glycolysis feeds substrate into the citric-acid cycle, which furnishes reducing equivalents to the mitochondrial respiratory chain. Mitochondrial respiration occurs at a rate determined by metabolic needs (i.e., ADP concentration), and oxidative phosphorylation proceeds smoothly. Increased metabolic needs signaled by elevated concentrations of ADP produce state 4→state 3 transition, respiratory activity is increased, oxidative phosphorylation proceeds more rapidly, and an increased rate of ATP synthesis is established. When oxygen availability decreases, respiratory-chain activity is diminished and oxidative phosphorylation can no longer keep pace with ATP utilization. The concentrations of ADP, AMP, and P_1 increase within the cytoplasm, while that of ATP is diminished. This releases phosphofructokinase from its inhibition by ATP and glycolysis is stimulated. The increased glycolytic flux partially compensates for the decreased energy yield of this process. NADH produced by glycolysis is no longer able to transfer its reducing equivalents to the mitochondrion via the shuttle systems and therefore must be reoxidized by the reduction of pyruvate to lactate. As a result of increased glycolytic flux and inability of mitochondrial reoxidation of NADH, lactate concentration within the cell rapidly increases.

Effects of Hypoxia on the Central Nervous System

The anesthesiologist is intimately concerned with the metabolic state of the central nervous system. The information already furnished in this paper will allow us to understand changes which have been observed in the presence of cerebral hypoxia. Work performed more than two decades ago demonstrated that when dogs inspired less than 11 per cent oxygen, cerebral lactic acid concentration began to increase. At this point, slowing of electroencephalographic activity was observed. When inspired oxygen was less than 7 per cent, a decreased level of phosphocreatine developed, indicating that anaerobic metabolism was not sufficient
Fig. 8. Changes in blood gases, base excess, and lactate during three minutes of respiratory arrest in rats and following reestablishment of ventilation are shown in the left column. Changes in concentrations of phosphocreatine and ATP in rat brain are shown on the right. From Kaasik et al.

Fig. 9. Cytoplasmic NADH/NAD⁺ ratios in rat brain during and after a three-minute period of respiratory arrest. From Kaasik et al.
to meet energy demands. Lolley demonstrated that when 9–11-day-old rats inhaled 100 per cent nitrogen, the cerebral concentrations of ATP and phosphocreatine decreased, while that of AMP increased. Kasik et al. produced hypoxia in anesthetized rats by means of respiratory arrest (figs. 8, 9). Their data demonstrated a rapid increase in arterial lactate with an accompanying decrease in pH. The concentration of ADP in brain increased by 75 per cent, while that of AMP was augmented more than tenfold. The concentrations of ATP and phosphocreatine decreased and the cerebral NADH/NAD+ ratio increased. Upon reoxygenation, the concentrations of ATP, ADP, AMP, and the ratio NADH/NAD+ were restored to normal within two minutes. Phosphocreatine returned to the control value in five minutes, and the lactate levels were normalized within ten minutes. With hemorrhagic hypotension (mean arterial blood pressure 25–35 torr), phosphocreatine and ATP concentrations again decreased, while the levels of lactate and AMP and the NADH/NAD+ ratio increased. Again, restoration of normal cerebral perfusion restored ATP, ADP, AMP, and the NADH/NAD+ ratio to normal within two minutes. Phosphocreatine was somewhat slower, and the return of lactate and pyruvate was delayed even more. Similar findings in the presence of hypovolemic hypotension were reported by Siesjö. When mean arterial blood pressure was less than 40 torr, the expected changes in ATP, ADP, AMP, phosphocreatine, and the NADH/NAD+ ratio in cerebral tissue were observed. Less extreme levels of hypotension produced only slight changes in lactate concentration. The latter findings might indicate the presence of mild focal hypoxia, during which aerobic energy production could supply a normal amount of ATP. They could also demonstrate the cushioning effect (see above), in which reduction of respiratory chain components is not accompanied by decreased mitochondrial respiration or the need for increased glycolysis.

When increased cerebrospinal fluid pressure lowered cerebral perfusion pressure to 30–35 torr, increased lactate, pyruvate, ADP, AMP, and NADH/NAD+ were observed, while the concentrations of ATP and phosphocreatine in brain tissue decreased. When a less severe increase in cerebrospinal fluid pressure was produced, changes in lactate and pyruvate only were observed.

When terminal oxidase activity in cat brain was diminished by administration of cyanide, lactic acid increased and phosphocreatine concentration decreased.

Metabolic changes indicative of cerebral oxygen lack have been observed during convulsions. Electrical seizures in spontaneously-breathing mice increased the cerebral metabolic rate by a factor of 3. Phosphocreatine and ATP concentrations decreased, while lactate increased. The same observations were made when seizures were produced by inhalation of Indoklon.

When mice were paralyzed and ventilated during an electrically-induced seizure, the alterations in phosphocreatine, ATP, and lactate were minimized, indicating that maintenance of cerebral oxygenation by adequate pulmonary ventilation enabled the organ to meet increased metabolic demands. Similar findings in canine brain constituents during seizures in the presence of adequate ventilation have been described by Beresford. It is not surprising, therefore, that when man undergoes electroshock therapy, adequate ventilation prevents a significant decrease in cerebral venous oxygen tension, while increased cerebral lactate production is not observed.

**Nondestructive Studies**

When man is studied, obvious considerations make it necessary to deduce intracellular events from examination of blood entering and leaving an organ. Thirty years ago, Gibbs' group demonstrated that when arterial blood and cerebral venous blood were analyzed for concentrations of glucose, oxygen, carbon dioxide, and lactate, the utilization of glucose could be accounted for in terms of carbon dioxide and lactate production. When isocapnic hypoxia was produced by allowing man to breathe 6.9–7.5 per cent oxygen (P\textsubscript{O\textsub{T}} 35 torr, \textsubscript{P}\textsubscript{\textsubscript{O}_{2}} 27 torr), cerebral glucose consumption was increased, and the proportion of this substrate converted to lactate increased. Changes in cerebral oxygen consumption were not observed, findings similar to those of other work.
ers. However, the value for oxygen uptake represents that of the entire brain, and subtle changes in regional oxygen utilization cannot always be detected. Since the amount of energy produced by anaerobic glucose degradation is less than that realized in the presence of oxygen, a marked increase in glucose consumption and lactate production was more easily detected.

It is likely that hypoxia-induced cerebral lactic acidosis is responsible for the increased cerebral blood flow observed during hypoxia. Thus, when isocapnic hypoxia is produced in the dog, cerebral vasodilatation is a threshold phenomenon and occurs when PaO2 is 50 torr, a value corresponding to the point at which cerebral cortical acidosis may be detected. Adenosine (see below) produced during hypoxia may be another mediator of decreased cerebral vascular tone, since unpublished observations suggest an increase in cerebral venous adenosine concentration during severe hypoxia in the dog.

Relation between Cerebral Function and Metabolism

The effects of hypoxia on cerebral function encompass a wide spectrum of deficits, from the most subtle personality changes to convulsions, syncope, and organ death. When the cerebral circulation of man was acutely interrupted, blurring and greying of vision and narrowing of visual fields, sometimes progressing to complete blindness, occurred within five seconds. Within ten seconds, consciousness was lost, an event followed by tonic and clonic convulsions. Although ischemia was maintained for as long as 100 seconds, subjects awakened within 15-20 seconds after release of the cuff. They were confused, excited, dazed, and euphoric for the next 20 seconds; often they denied unconsciousness but were amnesic for the period involved. Within two minutes they were able to walk unassisted out of the room. More subtle changes, including loss of peripheral vision, dimming of vision, mental confusion, disorientation, impaired performance and judgment, restlessness, insomnia, yawning, sighing, dizziness, lethargy, irritability, nausea, vomiting, and impaired memory and mental performance, have been described by numerous authors.

Changes in the electroencephalogram accompanying hypoxia have been frequently observed. Meyer et al. demonstrated that when hypoxia is produced by inhalation of a low-oxygen gas, electroencephalographic slowing appeared when cerebral venous oxygen tension was less than 19 torr; anoxia was probably the cause of postictal depression. Changes in cerebral function can progress to central-nervous-system death.

It has been suggested that severe neurologic reactions to anoxia may be delayed for days or weeks after the original exposure, and during the intervening time between hypoxic coma and recovery, intellectual and neurologic recovery may appear complete.

An early attempt to relate cerebral function to metabolic events was that of Lennox et al. who showed that unconsciousness occurs when the cerebral venous blood of man has an oxygen tension of less than 18 torr. Chance and co-workers demonstrated that when a low inspired concentration of oxygen was administered to the rat, pulmonary ventilation stopped when cerebral NADH reduction was 90 per cent complete. When the animal was ventilated with oxygen, NADH reduction abruptly decreased; spontaneous respiration commenced when NADH was 50 per cent reduced. Electrical activity of the brain was observed to cease when NADH reduction was 80-90 per cent complete. Convulsions occurred when cerebral ATP concentration was reduced to approximately 50 per cent of normal. Since the brain's ability to synthesize ATP was restored following reoxygenation, it was concluded that damaging effects of hypoxia were due mainly to reduction of ATP level.

Function of Adenosine

The polarity of ATP, ADP, and AMP prevents their diffusion from the intracellular space to vascular tissue. However, adenosine, which is produced by removal of the final phosphate from AMP, is freely diffusible. It has been proposed that this substance functions as a mediator of organ circulation. When adenosine is infused into the arterial supply of skeletal muscle, vasodilatation occurs; ischemia of
skeletal muscle produces increased release of this compound. Early work by Berne's group demonstrated that decreased coronary vascular resistance produced by hypoxia is accompanied by increased concentrations of inosine and hypoxanthine in coronary sinus blood. Since ischemia produced increased levels of adenosine, as well as inosine and hypoxanthine, in cardiac muscle, it was postulated that failure to observe adenosine in venous drainage was a result of the action of erythrocytic adenosine deaminase. When 8-azoguanine (an inhibitor of adenosine deaminase) was used, adenosine appeared in the perfusates of isolated cat heart and guinea pig heart subjected to severe hypoxia. Continuous release of small amounts of adenosine from normal myocardium has been observed; increased release accompanies reactive hyperepemia. These considerations impelled Berne's group to consider adenosine as the mediator of coronary blood flow, and the means by which increased metabolic demands are immediately transformed into a signal for increased perfusion.

Utility of Lactate Determination

The preceding discussion has largely concerned changes occurring within the cell or, indeed, even within the mitochondrion itself. It is obvious that the clinician desiring to gain information concerning the state of oxygenation of his patients cannot make use of these findings. For this reason, assessment of hypoxia by means of blood lactate measurement has been extremely popular. That the lactate produced within the cytoplasm can rapidly gain access to the intravascular compartment has been amply demonstrated. Arterial lactate concentration increased during hemorrhagic shock. In one study, hemorrhagic and endotoxic shock were uniformly lethal when blood lactate concentration exceeded 10 mEq/L. Changes in blood lactate concentration occur during exercise when the "threshold of anaerobic metabolism" is reached, a phenomenon which may be demonstrated by continual measurement of respiratory gas exchange. When the rectal temperature of the dog was increased to 41°C, arterial lactate concentrations rose sharply. Similar findings were not observed in cerebral venous blood, a phenomenon likely to be the result of lack of equilibration of lactate between cerebral tissue and blood. During myocardial hypoxia produced by coronary insufficiency, lactate concentration increased in coronary venous sinus blood. When myocardial metabolism was stressed by electrically increasing cardiac rate, lactate release into coronary venous blood was not changed in normal individuals. However, in eight of 12 patients with coronary-artery disease such a maneuver increased coronary venous lactate levels. Similar findings have been made in a patient with angina pectoris whose coronary arteriograms were normal.

Excess Lactate

In the late 1950's, Huckabee introduced the concept of "excess lactate." This approach to assessment of adequacy of oxygenation has stirred a great deal of controversy and warrants some discussion. During hypoxia, lactate is produced by the reduction of pyruvate by cytoplasmic NADH:

\[ \text{Pyruvate} + \text{NADH} + \text{H}^+ \rightleftharpoons \text{lactate} + \text{NAD}^+ \]

Rearrangement of this equation yields:

\[ \frac{\text{Lactate}}{\text{Pyruvate}} = K \frac{\text{NADH} \times \text{H}^+}{\text{NAD}^+} \]

Huckabee proposed that when hypoxia occurs the increase in mitochondrial NADH/NAD+ is reflected by an increased ratio of lactate/pyruvate. Although lactate and pyruvate could both change, hypoxia was signaled by an increased ratio of lactate/pyruvate. Excess lactate was that amount of lactate not predicted by the initial concentrations of lactate and pyruvate (and therefore the initial concentrations of NADH and NAD+). The formula for excess lactate was thus:

\[ X_L = (L_L - L_I) = \frac{(P_F - P_I)}{(L_I/P_I)} \]

when \( X_L \) = excess lactate, \( L_L \) and \( P_I \) = initial lactate and pyruvate concentrations, and \( L_F \) and \( P_F \) = final lactate and pyruvate concentrations. Considerable data of Huckabee support the contention that excess lactate reflects the state of cellular oxygenation. When man inhaled 10 per cent oxygen, excess lactate was
increased in arterial blood. This may have represented an interaction between respiratory alkalosis and hypoxia, since isocapnic hypoxia produced by the inhalation of 6.9–7.5 per cent oxygen produced no significant changes in arterial excess lactate in man. The oxygen debt produced by inhalation of low-oxygen gases or by exercise correlated better with excess lactate than with lactate alone. When myocardial hypoxia was produced in the dog by inhalation of less than 10 per cent oxygen, excess lactate appeared in coronary sinus blood. The infusion of lactate, pyruvate, or glucose, as well as hyperventilation, produced increases in lactate but not in excess lactate. Since these stimuli should not have been associated with hypoxia, excess lactate was deemed a more valid index of oxygen lack than lactate. Contrary to these findings, hyperventilation has been associated with the appearance of excess lactate in other studies.

Other investigators have made similar observations. Inhalation hypoxia and anemia in both animal and man may be associated with augmented levels of arterial excess lactate. Broder and Weil studied excess lactate as a metabolic index of the severity of shock in man. A value of more than 4 mEq/l was likely to be associated with a fatal outcome. However, data presented several years later by the same group indicated that measurements of lactate alone served as a better prognostic index of survival.

Discriminant function analysis was performed using data obtained from man in circulatory shock. The possibility of failure of prediction was 12 per cent based on lactate, whereas it increased to 21 per cent with lactate/pyruvate and 19 per cent with excess lactate. In hypotensive man, lactate and excess lactate increased when arterial oxygen tension was reduced below 50 torr owing to pulmonary insufficiency, a finding not made with pulmonary insufficiency alone.

What are the objections to the use of excess lactate? These involve two concerns: 1) there are theoretical objections to the concept and derivation of excess lactate; 2) both lactate and excess lactate may increase as the result of nonhypoxic stimuli.

The first objection is to the assumption made by Huckabee that blood lactate/pyruvate, cytoplasmic lactate/pyruvate, cytoplasmic NADH/ NAD+, and mitochondrial NADH/NAD+ are all in equilibrium. Data of Lowry and Passonneau suggest that during hypoxia it is "doubtful that pyruvate and lactate are maintained near equilibrium during peak glycolysis." Alterations in pH in vivo produce changes in the lactate/pyruvate ratio not predicted by equilibrium conditions. The equilibrium constant of the lactate dehydrogenase reaction is approximately 10,000 at a pH of 7.4, while that value predicted from actual measurements in mammalian tissues is only 10. Remember also that the mitochondrial level of NADH is governed by factors other than oxygen availability (see table 1). Furthermore, oxygen lack exerts its main effect on the respiratory chain, and therefore intramitochondrial NAD+ is the only fraction directly affected. Communication with the cytoplasmic component of NAD+ relies on the operation of shuttle systems already discussed. Thus, the ratio of cytoplasmic NADH/NAD+ depends upon relative rates of hydrogen's entry into and exit from the cytoplasmic pool of NAD+.

Ethanol in normal subjects causes the appearance of "excess lactate" because the rate of entrance of hydrogen by way of alcohol dehydrogenase into the cytoplasmic pool of NAD+ exceeds the rate at which it can be transferred to the mitochondrion for oxidation to water. When the NAD+/NADH system is fluorometrically assayed in vivo, increased oxidation within the gastrocnemius and gracilis muscles of the dog is observed during muscular activity, while lactate is being released.

This lactate formation does not represent hypoxic stimulation of anaerobic glycolysis, but is probably the result of an imbalance between the rates of pyruvate production by aerobic glycolysis and pyruvate utilization in the citric acid cycle. On the other hand, under some circumstances active muscle may actually take up lactate.

Fluorometric measurements of tissue NADH concentrations before, during, and after anoxia have been correlated with analysis of tissue lactate and pyruvate and indicate that changes in the lactate/pyruvate ratio lag considerably behind changes in the NADH/NAD+ ratio. These data indicate an inadequate speed of operation of the shuttle.
system. In some tissues, excess lactate may be produced because the rate of glycolysis is unchecke by respiration; this represents a weak Pasteur effect, and is not a manifestation of hypoxia. Equilibrium of erythrocytic and plasma lactate and pyruvate and diffusion of lactate from some brain tissues to venous blood may be limited.124 Finally, Harris53 has observed that the calculation of excess lactate is based on a mathematical error, for while Huckabee stated that lactate is a function of the product of two quantities, he expressed it as a function of the addition of these substances.

Many investigators have expressed serious doubts regarding the equivalence of blood lactate and the oxygen debt. An exogenous load of lactate or pyruvate presented to the dog was immediately followed by increased oxygen uptake, part of which was the result of metabolic alkalosis. It was impossible to account for the administered lactate or pyruvate exclusively in terms of the increased oxygen consumption. The increased oxygen uptake was reduced by more than 50 per cent in the presence of beta-adrenergic blockade.190 The investigators felt that the oxygen cost of exogenous loads represented a resultant of several calorigenic processes; beta-adrenergic stimulation, alkalinization, and a “metabolic” mechanism. Noting the absence of a relationship between lactate removal and oxygen consumption following exercise or hypoxia, Alpert15 stated that “excess O2 consumption of exercise and the removal of lactate are incidentally and not causally related.” Cain21 and Thomas121 have demonstrated that excess oxygen uptake during hypoxia is not related to the peak levels of excess lactate. Harris, in an excellent review of this subject,42 has stated that we must face “the possibility that the concentration in blood does not determine the magnitude” of the oxygen debt.42

These theoretical objections do not detract from the utility of this concept, since in most circumstances severe hypoxia will be accompanied by an increased lactate/pyruvate ratio and excess lactate. However, one must consider that there are factors other than hypoxia capable of increasing excess lactate, as well as lactate. In such cases, measurement of both lactate and excess lactate will lead to erroneous conclusions; some of these factors are discussed below.

**Changes in Lactate Not Associated with Hypoxia**

Administration of cyclopropane to healthy volunteers elicited no evidence of systemic hypoxia; however, increases in arterial lactate (but not excess lactate) were observed.4 Both lactate and excess lactate increased in the arterial blood of volunteers receiving diethyl ether, a phenomenon that could be largely blocked by propranolol. Since this drug would not tend to modify any hemodynamic causes of regional hypoxia, it is likely that these changes represent a reaction to stimuli other than hypoxia.

In the past few years, a number of cases of lactic acidosis without obvious hypoxic etiology have been reported. These have been associated with use of the antidiabetic agent, phenformin,55,117 as well as with starvation.15 Lactic acidosis, which is often fatal, may appear spontaneously90,200 and may even have a genetic component.59

**Effects of Carbon Dioxide and pH on Lactate Metabolism**

One of the most difficult areas of interpretation concerns the effect of altered pH on lactate concentration. Although calculations based on the lactic dehydrogenase reaction indicate that alkalosis should decrease the lactate/pyruvate ratio,194 numerous investigators have found increased concentrations of lactate and increased lactate/pyruvate ratios to be associated with alkalosis.5,49,49,165,206,207 Eichenholz et al.44 showed that hyperventilation increased the arterial lactate concentration in dogs, a change which actually resulted in an abnormally low pH. Mechanical hyperventilation with carbon dioxide added to the inspired gas mixture produced no changes in lactate concentrations. Later work showed that respiratory alkalosis (PaCO2 15 torr) in the dog resulted in increased arterial lactate concentration within ten minutes, a change which restored arterial pH towards normal but did not produce acidosis.65 The latter investigators concluded that Eichenholz may have
combined hyperventilation with hypotension. Changes in lactate and the lactate/pyruvate ratio have been observed in man when $P_{A_{CO_2}}$ is 10–20 torr. The converse has been demonstrated; respiratory acidosis in the dog ($P_{A_{CO_2}}$ 98–119 torr, pH 6.9–7.1) reduced arterial lactate concentration. Furthermore, when carbonic anhydrase was antagonized by acetazolamide during mechanical hyperventilation, arterial pH, $P_{CO_2}$, and carbon dioxide content returned towards normal and lactic acidosis was abolished.

The site of lactate production during alkalosis remains in doubt. One group believes that it is derived from increased erythrocytic glycolysis. Well-oxygenated liver slices showed increased lactate/pyruvate ratios in the presence of metabolic alkalosis, while electron transfer by liver mitochondria remained normal. Again, this indicates the erroneous conclusions that can follow the oversimplified use of the lactate/pyruvate ratio as an index of the redox state of the respiratory chain. Increased lactate production by rat liver and kidney slices, as well as adipose tissue, during metabolic alkalosis has been demonstrated. Respiratory alkalosis in cat brain slices and minces had similar effects. Administration of carbon dioxide to a brain suspension inhibited aerobic glycolysis. Thus, it appears that many organs are capable of increasing lactate production in the face of either respiratory or metabolic alkalosis.

The mechanisms are reasonably well elucidated. Examination of the isolated heart showed that respiratory and metabolic alkalosis increased lactate production. Glucose-6-phosphate and fructose-6-phosphate levels were diminished, while fructose-1,6-diphosphate and dihydroxyacetone phosphate were increased. No changes in ATP, ADP, AMP, or P$_i$ were observed, while creatine phosphate increased slightly. The authors concluded that alkalosis increased glycolysis by stimulating the activity of phosphofructokinase, an enzyme extremely sensitive to small changes of pH in the physiologic range. In addition, carbon dioxide may be directly incorporated into tissues; changes in availability of carbon dioxide can alter the activity of the citric-acid cycle, since carbon dioxide may be directly converted into substrates of the citric-acid cycle of bacteria and mammals.

It is not surprising that the combination of alkalosis and hypoxia produces alterations of lactate metabolism more profound than those occurring with hypoxia alone. Takano demonstrated that when isocapnic hypoxia was produced in free-breathing dogs, arterial lactate did not increase until $P_{A_{CO_2}}$ was less than 35 torr. However, hypocapnic hypoxia was associated with increased lactate at an arterial oxygen tension of 50 torr. Cain observed an increased response of arterial lactate to hypoxia when $P_{A_{CO_2}}$ was lowered, and concluded that this represented the additive effects of hypoxia and alkalosis on glycolysis. When elevated temperature increased metabolic needs beyond the point of circulatory adequacy, elevation of arterial lactate and excess lactate was greater in the presence of hypocaopia.

We may conclude that systemic hypoxia usually will be accompanied by increased arterial lactate and excess lactate. Whether this is seen in the venous blood draining a hypoxic organ will depend largely on the ability of lactate to diffuse from tissue to blood. The converse, however, is not necessarily true, and increased lactate or excess lactate alone is not always diagnostic of hypoxia.

These considerations have been extremely important in recent considerations of the effects of hyperventilation upon cerebral oxygenation. This might be impaired by hypocaopia-induced vasoconstriction, as well as by operation of the Bohr effect. Functional changes such as hallucinations and impaired flicker fusion have been demonstrated to result from hyperventilation. Alterations in the electroencephalogram of hyperventilating man have been described; these were manifested when jugular venous oxygen tension was less than 21 torr. The expected interaction between hypoxia and hypocaopia in eliciting electroencephalographic abnormalities has also been observed. Examination of metabolic changes in the human brain by sampling of arterial and jugular venous blood has disclosed the similarities between hypocapnia and hypoxia. However, the latter studies may not have demonstrated the full magnitude of changes, because of incomplete equilibration of lactate between ve-
ous blood and brain tissue. This objection might be overcome by direct studies of brain tissue or cerebrospinal fluid. Leunen and Demester observed that increased cerebral tissue lactate accompanied hyperventilation in the rat. Plum and Posner showed that hyperventilation caused increased lactate and excess lactate to accumulate in the cerebrospinal fluid of dogs. The degree of lactate acidosis was proportional to the decreased carbon dioxide tension, and was augmented by hypoxia. The changes were seen when jugular venous oxygen tension approached 17–19 torr, a level which has produced unconsciousness in man, and which would theoretically predict significant impairment of cerebral oxygenation.

It might be argued that these changes in lactate concentration in brain tissue and cerebrospinal fluid serve as a mechanism to compensate for respiratory alkalosis. However, other changes indicate that this cannot be the sole explanation. When $P_{\text{aCO}_2}$ was 10 torr, canine brain lactate concentration was trebled and ATP was decreased. The change in ATP concentration was more than doubled when anemia accompanied hyperventilation. When brain tissue specimens of the cat and rat were examined following hyperventilation to $P_{\text{aCO}_2} < 20$ torr, the concentrations of lactate and excess lactate were elevated. Fluorometric measurements demonstrated increased reduction of the NAD+/NADH system. Although increased reduction of the mitochondrial respiratory chain was indicated, the levels of phosphocreatine, ATP, and ADP remained constant, while only a small increase of AMP occurred. Thus, cerebral hypoxia was manifested by increased reduction of the respiratory chain, but was not so significant as to compromise energy storage. Plum et al. demonstrated that 60 per cent of the increase in cerebrospinal fluid lactate accompanying hyperventilation was abolished when hyperventilation was combined with hyperbaric oxygenation. This indicated that more than half the augmentation of cerebral lactate resulted from hypoxia, while the remainder stemmed from the action of alkalis on glycolysis. As might be predicted from these data, administration of hyperbaric oxygen to hyperventilating man eliminated the electroencephalog-
metabolic requirements of the infant brains of some species.

It would also be reasonable to suppose that interference with glycolysis might alter these findings. Samson and Dahl have demonstrated that the survival of anoxic newborn rats is sharply curtailed following injection of the glycolytic inhibitor, iodoacetic acid. On the other hand, pretreatment with glucose will prolong survival of anoxic rats.38

The increased glycolytic function provided by a glucose load ameliorates changes in myocardial function produced by coronary ischemia in the intact dog.15, 20 In the isolated perfused heart, beating ceased within 15 minutes after anoxia, and no recovery was observed with reoxygenation. When glucose was administered during anoxia a slow cardiac rate was established and recovery was seen following reoxygenation. When fumarate or oxaloacetate functioned as electron acceptor, cardiac rate increased sixfold.24 Finally, in the intact rat the myocardial production of ATP and lactate was augmented and cardiac function improved in the presence of anoxia in those rats that had hearts with glycogen stores.174

HYPOTERMIA

For the clinician, the most important means of modifying the response to hypoxia is hypothermia. The ability of hypothermia to lower metabolic needs of the central nervous system has been documented many times.16, 35, 122, 132, 161, 165, 192 It has been estimated that a decrease in body temperature of 10°C is accompanied by a two- to threefold diminution in cerebral oxygen demands. During circulatory arrest, the rate of decline of cerebral ATP was diminished by hypothermia.144 Changes in the cerebral concentrations of ATP, ADP, and AMP in rat brain were unaltered by nitrous oxide, halothane, diethyl ether, cyclopropane, fentanyl, thiopental, phenobarbital, or amobarbital. Lactate and the NADH/NAD+ ratio were unaffected by administration of the inhalation agents; they were both slightly decreased following barbiturate anesthesia.144 These measurements do not disclose turnover rates, and Brunner has performed dynamic studies of the rate of cerebral ~P utilization during the 15-second period following decapitation. He demonstrated decreased rates of energy utilization in the presence of diethyl ether, halothane, or hypothermia. Michenfelder,122, 132 on the other hand, studied cerebral energy utilization for a longer period following decapitation. Changes in cerebral oxygen uptake pro-
duced by thiopental, nitrous oxide, or halothane were not accompanied by diminished rates of ATP utilization, while in hypothermic (30°C) dogs, the rate of cerebral ATP utilization was 40 per cent of control. These data suggest that drug-induced diminution of cerebral oxygen uptake does not necessarily confer protection against hypoxia. Decreased oxygen utilization during anesthesia may represent only the metabolic consequences of diminished cerebral function, while the oxygen requirements for maintenance of brain-cell viability remain unaltered. Examination of ATP depletion in the first few seconds following decapitation served as an overall index of total cerebral energy requirements (i.e., function and viability), while analysis of ATP changes in the first minutes following decapitation (after cerebral function had ceased) provided an index of requirements for cell viability. Unlike anesthetics, which diminish the oxygen consumption associated with cerebral function only, hypothermia decreased both this rate and the oxygen consumption necessary for cell viability. It appears that protection against hypoxia is definitely provided by hypothermia, while that offered by anesthetics is problematical, at best.

OTHER FACTORS

Certain animals are able to survive apnea during diving.24,125 This results from the ability to alter the distribution of cardiac output so that only those organs with the highest requirements for oxygen are perfused. In other species, a profoundly lowered metabolic need enables survival during hypoxia. Thus, hypoxia-induced changes in cerebral ATP, phosphocreatine, glycolgen, and glucose concentrations within the central nervous systems of fish and frogs are 20 times slower than in mammals; changes in the turtle brain are 50 times slower.126 For much the same reason, the turtle is able to withstand a dose of cyanide 50 times greater than would be toxic in the mammal.12 These changes are modified by circulatory factors, and the turtle is able to withstand anoxia produced by inhalation of 100 per cent nitrogen for 18 hours, while anoxia following excision of the heart leads to respiratory arrest in a little more than an hour.11 Differences between anoxic and ischemic anoxia are also observed in the dog.128 In the anaerobic turtle, alterations in pH are minimized, since a large volume of alkaline coelomoc fluid with a bicarbonate concentration three times greater than that of plasma is able to buffer fixed acids.128

Mammals capable of hibernation have tolerance to hypoxia. This has been observed in both the hibernating and the normothermic nonhibernating ground squirrel, and may represent an ability of the animal’s heart to function at low oxygen tensions.21

Adaptation to hypoxia is possible. Rats exposed to simulated high altitude produced by decompression had a longer period of spontaneous respiration when subsequently exposed to 100 per cent nitrogen.22 Hearts of cattle born and raised at an altitude of 4,250 meters were found to have increased numbers of mitochondria, as well as increased activity of enzyme systems concerned with oxidation, while mitochondrial size did not change.127 These changes increased the likelihood that an oxygen molecule might find an oxidative site. In contrast, continuous exposure of adult rats to a simulated altitude of 18,000 feet for two to four months had no effect on the lactic dehydrogenase activity of the isozyme patterns of brain, heart, liver, diaphragm and gastrocnemius muscle.124 When 21-day-old rats were exposed to 100 per cent nitrogen, 50 per cent survived an exposure lasting 61 seconds. When, however, the rats were exposed to 100 per cent nitrogen for 30 seconds, allowed to recover in air for ten minutes, and then retested with 100 per cent nitrogen, the 50 per cent survival time was increased to 93 seconds. This finding was not observed if pre-exposure preceded the test exposure by more than an hour. Pretreatment with iodoacetate reduced survival times in both groups to 8 seconds. Pretreatment with sodium pyruvate also prolonged survival. Although the levels of cerebral ATP in the two groups were equal at the beginning of the test period for hypoxia, the rate of decline of ATP was less and the rate of glycolysis was greater in the pre-exposed group.42 It was hypothesized that a brief exposure to hypoxia facilitated glycolysis during
the test period, making possible more efficient energy production and thus conserving ATP.

References

10. Austin WG, Greenberg JJ, Plecinini JC: Myocardial function and contractile force affected by glucose loading of the heart during anoxia. Surgery 57:839–845, 1965


69. Glass GH, Synder FF, Webster E: The rate of decline in resistance to anoxia of rabbits, dogs and guinea pigs from the onset of viability to adult life. Am J Physiol 140:605-615, 1944


74. Granholm L, Siesjö BK: The effects of hypercapnia and hypocapnia upon the cerebrospinal fluid lactate and pyruvate concentrations and upon the lactate, pyruvate, ATP, ADP, phosphocreatine and creatine concentrations of cat brain tissue. Acta Physiol Scand 75:257-266, 1969


120. Lemnox WG, Gibbs FA, Gibs EL: Relationship of unconsciousness to cerebral blood flow and to anoxemia. Arch Neurol Psychiatry 34:1001-1013, 1933
124. Lowry OH, Passonneau JV, Hasselberger FX, et al: Effect of ischemia on known substrates and cofactors of the glycolytic path-
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133. Michenfelder JD, Theye RA: The effects of anesthetics and hypothermia on canine cerebral ATP and lactate during anoxia produced by decapitation. Anesthesiology 33:430-439, 1970


156. Preswick G, Reivich M, Hill FD: The EEG effects of combined hyperventilation and
188. Takano N: Role of hypocapnia in the blood lactate accumulation during acute hypoxia. Resp Physiol 4:32-41, 1968
190. Thews G: Implications to physiology and
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CSF LEAK This report documents the occurrence of cerebrospinal fluid leakage from the subarachnoid space through the site of lumbar puncture. In the three reported cases, scintiscans performed following injection of radioactive iodinated serum albumin (131I) into the subarachnoid space showed migration of the solution cephalad and caudal out of the subarachnoid space into the peridural space. (Lieberman, L. M., Tourtellotte, W. W., and Newkirk, T. A.: Prolonged Post-lumbar Puncture Cerebrospinal Fluid Leakage from Lumbar Subarachnoid Space Demonstrated by Radioisotope Myelography, Neurology 12: 925-929, 1971.) ATRACTER'S NOTE: Only one of the three patients described appears to have had symptoms, presumably from the demonstrated spinal-fluid leak, consisting of severe occipital headache and nausea when assuming the upright position. Unfortunately, the authors failed to report the caliber of the needles used or to indicate the possible correlation between the degree of leak and the size of the needle.