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Hypoxia: On the borderline between physiology and pathophysiology

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Hypoxia: On the borderline between physiology and pathophysiology. A foreword

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Molecular oxygen was not present when life began on Earth. However, within about 1 billion years, photosynthetic organisms appeared, and within a relatively short time, oxygen, a by-product of photosynthesis, became a dominant chemical entity in the atmosphere. Oxygen was then used to produce chemical energy in the form of adenosine triphosphate (ATP) in specific cellular compartments, the mitochondria. A *lack of oxygen* (hypoxia) is not very easy to define, because hypoxia may mean different things to different organs and organisms. Quite a number of physiological processes are actually geared to maintaining a *constant flow of oxygen*, for example to the heart, the skeletal muscles or the brain. In other words, biological systems have a number of protective devices that actually *prevent* the occurrence of harmful levels of hypoxia. These devices function as regulated and regulatory elements in the chain of transporters that carry oxygen from the environment to the mitochondria. The papers that comprise the present multi-author review are based on three symposia that were held in conjunction with the annual meeting of the Swiss Union of Biological Sciences that took place in Fribourg in 1989. The subjects that were dealt with embraced a broad spectrum of hypoxia-sensitive systems in man and other mammalian species. It was hoped that by putting these interdisciplinary contributions together, a scientific fermentation would occur which might not otherwise have arisen.

The transport of oxygen from the environment to the final consumer, the mitochondrion, involves the following forces: a) convectional transport of O_2 in the lung and in the blood; b) diffusional transport from the lung alveoli into the blood capillaries and out of the tissue capillaries to the mitochondria. It is obvious that a shortage of oxygen, i.e. a mismatch between oxygen supply and oxygen demand, can occur at all levels of this oxygen transport chain. One of the very first cellular structures that would sense such a diminished oxygen supply is, of course, the mitochondrion itself. Wilson discusses and reviews in his contribution the possible role of the *mitochondria* as *'oxygen sensors'* and concludes that in the *physiological range* of *oxygen pressures*, the oxygen-de-

pendence of mitochondrial oxidative phosphorylation is reflected in an altered ratio of the cytoplasmic concentrations of [ATP]/[ADP][P_i]. What remains unknown, however, is the exact way in which such an altered ratio of [ATP]/[ADP][P_i] is 'translated' into specific cellular events. In the heart and in the pancreatic B-cells – but up to now only in those tissues – a *voltage-independent* K^+*-channel* has been detected, which is *blocked* by ATP. A fall of cytosolic ATP should therefore lead to an extracellular accumulation of K^+, which is indeed observed. However, as Kléber points out in his article on the *electrical and mechanical function of the hypoxic heart,* intracellular acidification also has a direct and very pronounced effect on net K^+ efflux and extracellular K^+ accumulation. At the same time, an increase in cytosolic Ca^{2+} concentration leads to an uncoupling of electrical cell-to-cell connections with a consequent rise in electrical resistance between adjacent heart cells, which in turn causes an adaptive decrease in mechanical forces when the heart becomes hypoxic. Therefore, ATP is not the only factor that leads to an altered heart function under conditions of decrease in blood oxygen content (anemia) or reduced coronary blood flow (ischemia).

This contention is also supported by the studies of Dietrich, Mast and Elzinga, which were designed to find out how *force-related energy turnover* and *force generation* are related in conditions of normoxia and hypoxia. A quantitative measure of energy turnover can be obtained under *anaerobic conditions* from the changes of phosphocreatine and adenine nucleotides (ATP, ADP, AMP) as well as from lactate formation and, under *aerobic conditions,* from oxygen consumption, assuming a constant ratio between the production of ATP and the consumption of oxygen. From the entirety of the results obtained by Elzinga and his colleagues it has become clear that the *contraction-related energy demand* and the *economy of force production* are very much alike in normoxic and hypoxic hearts, and that the glycolytically produced ATP is also determined by the force-related energy turnover. Therefore, the mechanisms that couple the turnover of chemical and mechanical energy are not out of balance when oxygen becomes scarce.

So far, we have been concerned with adaptational changes of heart muscle cells in situations where hypoxia has already occurred, for example under conditions of severe ischemia, i.e. reduction of blood flow. Quite obviously, it is desirable to activate mechanisms that are geared to *preventing* the occurrence of a hypoxic condition, and this can be achieved by *decreasing* the *blood flow resistance* in an organ that is in danger of becoming hypoxic. One substance that is considered to be of relevance in this connection is adenosine, the role of which in the regulation of coronary blood flow is discussed in the paper by Schrader, Deussen and Smolenski. These investigators have provided several lines of evidence that tissue oxygenation is the major determinant for the production of adenosine by the heart; this is reflected in a close linkage between the release of adenosine and the P_{O_2} of the coronary venous blood. The generation of adenosine from adenosine monophosphate (AMP) is highly sensitive to the oxygen availability of the heart cells. Even a small mismatch between oxygen supply and oxygen demand leads to a release of adenosine, which in turn stimulates a coronary vasodilatation by acting from the *outside* on the smooth muscle cells of the coronary vessels. An additional safeguard against possible hypoxic damage is represented by the endothelial cells that release, as a function of the oxygen availability, vasoactive substances that act *inside* the blood vessels. This role of endothelial cells as 'oxygen sensors' is discussed in the contribution of Pohl. It is clear now that the endothelial cells themselves can perceive changes of the blood P_{O_2} and release, in response to this variation, vasodilatory substances, like prostacyclin or endothelium-derived relaxing factor (EDRF). Both substances lead to a relaxation of smooth muscle cells by activating a membrane-bound adenylate cyclase in the case of prostacyclin, or a cytosolic guanylate cyclase in the case of EDRF. Other factors that are released from the endothelial cells into the vessel lumen upon hypoxia, e.g. ATP, serotonin, bradykinin or substance P, elicit a release of EDRF or prostacyclin by specific receptor-dependent pathways. Exactly how these endothelium-derived vasoactive substances are released in an oxygen-dependent fashion is not known, but one essential step seems to be an increase in the cytosolic Ca^{2+} concentration that occurs under hypoxia in the P_{O_2}-range between 20 and 40 mm Hg. In summary, the blood flow to and within a given organ seems to be under the control of *chemical messengers* that act both from the side of the *parenchymal cells* and from the side of the *endothelium* to control rapidly and accurately the adequacy of blood flow under conditions of increased demand or reduced supply of oxygen.

A total of four papers were given on the subject of the *regulation of oxygen flow* to and *respiration of skeletal muscle.* Jones, Aw, and Sillau discuss in their contribution the resistances that determine the rate of oxygen transfer from blood to tissue. Their considerations are applicable to any physiological system in which the transfer of oxygen from location A to location B is determined by a series of resistances that molecular oxygen has to overcome in order to reach the mitochondria. One of the main conclusions that can be drawn from the experimental and theoretical analyses of the work of Jones and his colleagues is that one of the major resistances to oxygen transfer from the blood to the mitochondria is represented by the *grouping* or *clustering of the mitochondria* within a cell. This particular spatial arrangement leads to a *fourfold increase* of the pressure head of oxygen that is necessary to produce sufficient cytochrome oxidation in the mitochondrial clusters. Under conditions of chronic hypoxia this clustering appears to become less dense, which in turn *reduces* the P_{O_2}*-dependence* of cytochrome oxidation.

Could it be, then, that a highly trained marathon runner would have less densely packed mitochondria in his or her skeletal and cardiac muscle? A paper pertinent to this question was given by Hoppeler, Howald and Ceretelli. These researchers examined a number of structural features in biopsies of skeletal muscle in participants of an expedition to the Himalayas. The main findings of this investigation were that exposure to chronic hypoxia leads to a reduction in muscle fiber size that is in turn associated with a reduction of the *absolute quantity of contractile proteins* of skeletal muscle by about 10%. At the same time a *decrease in the absolute volume of mitochondria* close to 30% occurred during the mountaineering expedition. This reduction in total mitochondrial volume affected the subsarcolemmal mitochondria much more (−43%) than their interfibrillar siblings (−13%). A reduction of total mitochondrial volume could have led to a looser packing of the mitochondrial clusters, but it is not known whether this adaptation did in fact take place. It is of interest in this connection that the decrease in *maximal oxygen uptake capacity* (−5%) was much less than the fall in the absolute volume of mitochondria. Since the capillary network was spared from any catabolism, the average oxygen supply to the mitochondria was improved. This may be one of the reasons why the maximal oxygen uptake capacity was only mildly affected in this hypoxia study.

Along the same lines are the analyses of Ferretti, who discusses the factors that limit maximal exercise in acute and chronic hypoxia. Ferretti carefully subdivides the various resistances that determine the flow of oxygen from the lung to the mitochondria and concludes from his analyses that in hypoxia the contribution of the circulatory system to maximal oxygen transport decreases by about 30% in both acute and chronic hypoxia compared to normoxia. On the other hand, the elevated ratio of capillaries supplying muscle fibers leads to a fall of the diffusion resistance between blood capillaries and muscle mitochondria by about 60%. However, in view of the fact that the *absolute contribution* of the *'circulatory resistance'* exceeds that of the *'peripheral diffusion resistance'* by about 30% in normoxia and by about 100% in hypoxia, the decrease in peripheral oxygen flow resistance under hypoxic conditions is not sufficient to prevent *maximal oxygen uptake* from falling as inspiratory P_{O_2} decreases.

In considering oxygen flow to the working muscle under normoxia and hypoxia, it is of course important to know those factors that control respiration in skeletal muscle at rest. Chinet has addressed this question in his paper by comparing the dependency of oxygen consumption upon oxygen availability in the resting muscle. The major question that is of relevance here is that of the *regulation of oxygen consumption of the resting muscle;* a physiological process that allows the maintenance of a low energy cost at rest. The main process that leads to this type of adaptive behavior is a reduced flow of oxygen (and nutritive substrates) that may be brought about by *heterogeneities* in the *micro-circulation.* In other words, the availability of oxygen regulates muscle cell respiration in such a way that a *self-limiting system* is being generated that decreases oxygen consumption in the non-exercising muscle.

So far we have been concerned with the way in which a limited transfer of oxygen regulates basic cellular processes or the function of organs like heart and skeletal muscle. In addition, processes take place on a larger scale that protect the whole organism from the potentially deleterious effects of a shortage of oxygen. Two of these more general adjustments that occur upon *general* or *systemic* hypoxia are an increase in the production of red blood cells and a stimulation of alveolar ventilation. The regulation of red cell production (erythropoiesis) is affected by erythropoietin, a glycoprotein hormone that is synthesized in the kidney as a function of oxygen supply. Under conditions of hypoxia that may be brought about by a loss of red blood cells (anemia), or a fall of the P_{O_2} in the arterial blood (hypoxic hypoxia), the concentration of erythropoietin in the serum can increase by a factor of up to 1000. Scholz, Schurek, Eckardt and Bauer have established an experimental system using isolated perfused kidney preparations in order to define some of the *metabolic indicators* that may link hypoxia and increased erythropoietin production. Under hypoxic conditions the isolated kidney produced about the same amount of EPO as one sees under similar conditions in vivo, indicating that the oxygen sensor that governs erythropoietin production is located in the kidney itself. However, activation of the classical second messenger systems like adenylate cyclase, guanylate cyclase, protein kinase C-activation did not lead to a stimulation of the production of erythropoietin. Only an inhibition of the interaction between calcium and calmodulin was shown to markedly reduce the hypoxia-induced rise in the production of erythropoietin in this experimental model. From these results it would seem that a functioning calcium-calmodulin system is a necessary prerequisite for hypoxia-induced erythropoietin formation.

It is interesting to note here that a reduction in the *oxygen transport capacity* of the blood by exposure to carbon monoxide elicits a stronger erythropoietin response than a comparable degree of 'normal' anemia. The possible reasons for these different effects can be extracted from the article by Haab on the influence of carbon monoxide on oxygen transfer from the blood capillaries to skeletal muscle. What turns out to be of great importance for the transfer of oxygen from the blood to the mitochondria is the rate at which oxygen *dissociates* from hemoglobin during the capillary transit of red cells. In the presence of carbon monoxide the off-rate kinetics of oxygen are so much slower than they are normally, that much less oxygen can dissociate from hemoglobin than in conditions of 'normal' anemia. In the case of skeletal and cardiac muscle, the contribution of the *carrier function* of *myoglobin* to maximal oxygen consumption is also compromized by

carbon monoxide. In general terms, the analyses of Haab can be applied to any tissue because they highlight the importance of the kinetics of oxygen-release for normal oxygen delivery. In this way, carbon monoxide must not be merely regarded as a poison but also as a tool that can help the physiologist and the biochemist to unravel some of the mechanisms that are involved in oxygen transport.

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Contribution of diffusion to the oxygen dependence of energy metabolism in cells

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The oxygen dependence of mitochondrial oxidative phosphorylation extends to greater than 30 Torr, that is, well into the range of oxygen pressure in cells under normal physiological conditions [12–14]. This dependence can be most effectively discussed in terms of 'high' and 'low' ranges of oxygen concentrations.

The oxygen dependence of mitochondrial oxidative phosphorylation at oxygen pressures greater than about 10 Torr

In the 'high' oxygen pressure range (above about 10 Torr), most of the oxygen dependence is reflected in changes in the regulatory parameters for oxidative phosphorylation (cytoplasmic [ATP]/[ADP][Pi] and intramitochondrial [NAD^+]/[NADH]). In order to place the oxygen dependence of mitochondrial oxidative phosphorylation in this region of oxygen pressure in perspective, it should be remembered that the rate of ATP utilization by cells is essentially independent of oxygen pressure. Thus, in the 'high' oxygen pressure range, the response of mitochondria to a decrease in oxygen pressure to below that required to saturate the cytochrome *c* oxidase reaction is a transient decrease in the rate of respiration (rate of ATP synthesis). The decrease in respiration causes a temporary suppression of the rate of ATP synthesis, but the resultant excess of ATP utilization over ATP synthesis lowers the [ATP]/[ADP][Pi]. As the latter decreases, the respiratory rate is progressively stimulated (see for example Wilson [11]). If the oxygen pressure is held steady at the new value, the decrease in [ATP]/[ADP][Pi] continues until the rate of ATP synthesis again equals the rate of ATP utilization and a new steady state is attained. Normally, because the turnover of ATP is high and the pool small, these changes are rapid (maximally a few seconds). As noted above, the rate of ATP utilization is essentially independent of oxygen pressure, and therefore the steady state respiratory rate remains nearly constant as the oxygen pressure is lowered. Only when the oxygen concentration is too low to support the required rate of ATP synthesis even at minimal [ATP]/[ADP][Pi] does the respiratory rate decline. Thus, in the physiological range of oxygen pressure, the oxygen dependence of mitochondrial oxidative phosphorylation in cells is observed primarily in the cytoplasmic [ATP]/[ADP][Pi].

Mitochondrial respiration is also, however, dependent on the intramitochondrial [NAD^+]/[NADH] (see for example Wilson [11] and Erecinska [3]) and the latter is determined by the activity of the mitochondrial dehydrogenases. The activities of the dehydrogenases are highly regulated and, under some conditions, part of the effect of lowering the oxygen pressure is compensated for by decreasing [NAD^+]/[NADH] (reduction of the mitochondrial pyridine nucleotide pool). When the oxygen pressure is low enough that the combined changes in [ATP]/[ADP][Pi] and [NAD^+]/[NADH] can no longer stimulate respiration enough to keep the rate of ATP synthesis equal to its rate of utilization, the respiratory rate falls. This does not occur until after the conditions are reached which will, if sustained for significant lengths of time, result in severe cellular pathology.

The oxygen dependence of mitochondrial oxidative phosphorylation at low oxygen pressures

In the range of oxygen pressures where the changes in [ATP]/[ADP][Pi] and [NAD^+]/[NADH] can no longer compensate for the effects of decrease in oxygen pressure on mitochondrial respiration is, in general, below 10 Torr. In this region of oxygen pressures a decrease is accompanied by a decrease in the rate of oxygen consumption. The respiratory rate falls according to the equation for saturation kinetics:

$$v = V_{max} \times P_{O_2}/(P_{50} + P_{O_2}) \quad (1)$$

where v is the respiratory rate, V_{max} is the respiratory rate when the oxygen pressure (P_{O_2}) is saturating. Often this type of kinetic behavior is discussed in terms of an 'oxygen affinity'. In this case, however, the effective P_{50} is determined primarily by factors other than the affinity