CRITICAL P_{O_2}(s) IN OXYCONFORMING AND OXYREGULATING ANIMALS: GAS EXCHANGE, METABOLIC RATE AND THE MODE OF ENERGY PRODUCTION

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I. Introduction

The response of animals to declining oxygen tensions has for long been a matter of interest. The bulk of investigations focussed on the changing mode and rates of oxygen consumption. Based on these data it has been recognized early that animals may show different patterns of oxygen consumption in response to changes in ambient P_{O_2}. Some keep their oxygen consumption more or less constant in a wide range of P_{O_2} and are called oxyregulators. Others reduce their oxygen uptake with decreasing oxygen tensions and have, consequently, been termed oxyconformers (58).

This clear distinction has been questioned by Mangum and van Winkle (42), who suggested that a high variety of intermediate responses may be found among invertebrates and constructed a mathematical model to fit these measurements. Herreid (33) proposed to include the responses of both oxyregulators and oxyconformers into a general model of metabolic transition phases. He assumed that animals exhibit one variable critical P_{O_2} above which oxygen uptake remains constant but below which the resting rate of oxygen consumption starts to decline and becomes oxygen dependent.

However, oxygen consumption represents only the aerobic mode of energy production. Changes in oxygen uptake will remain poorly understood unless the contribution of anaerobic mechanisms to total energy output is also considered. During progressive hypoxia anaerobic metabolism may supplement aerobic energy production and, thereby, may indicate an insufficient oxygen supply. A number of studies have been published following Herreid's review in 1980 which provide more detailed information on the mechanisms of energy production in correlation with the changing rate of oxygen consumption and other physiological processes. These analyses have been performed on the cellular and systemic level, some of them include calorimetric measurements. Based on these developments, the concepts of oxyconformity, oxyregulation, and the critical P_{O_2} can now be readdressed and can be extended towards a general concept of metabolic regulation in response to changes in the ambient oxygen tension. The aim of this review is, therefore, to characterize metabolic transition phases in declining oxygen tension, and to identify the relationship between oxygen availability, oxygen transport, and the mode (aerobic versus anaerobic) and rate of energy metabolism. It will focus on those studies where these interrelationships have been considered. The discussion is restricted to quiescent animals and their "standard metabolic rate" in an attempt to exclude the perturbing effect of muscular activity on the pattern of oxygen

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consumption changes in hypoxia. The search for unifying principles should then allow to compare the metabolic pattern of oxyconforming and oxyregulating animals by using established terminology. This study is not intended to review the literature on ventilatory and circulatory mechanisms exploited by the animals to cover their oxygen requirements. Excellent reviews of these aspects have been published (33,22).

II. Anaerobiosis: Cytosolic versus Mitochondrial Mechanisms

In order to assess the total energy expenditure of an animal over the whole range of ambient oxygen tensions, the contribution of anaerobic metabolism to ATP provision must be considered. Depending upon the energy requirements during anaerobiosis different mechanisms may be used. Knowing these mechanisms the onset and degree of anaerobiosis can be monitored based on the exact determination of the various anaerobic metabolic end products (19).

Many marine invertebrates, *e.g.* various species among annelids, molluscs, crustaceans and sipunculids, reduce their energy needs during periods of severe hypoxia. In the transitional early phase of anaerobiosis (Figure 1) the energy turnover is still elevated as compared to late anaerobiosis. ATP production occurs by anaerobic glycolysis with the concomitant accumulation of alanine, D- or L-lactate or opines (alanopine, strombine, octopine, tauropine) (20), and the degradation of the phosphagen, *e.g.* phospho-L-arginine in molluscs, crustaceans and some sipunculids. Anaerobic mitochondrial metabolism also contributes and uses malate as a substrate which is replenished by the cytoplasmic transamination of aspartate. The amino group is transferred to pyruvate, thus giving rise to alanine. In the mitochondrial matrix malate undergoes disproportionation to oxaloacetate and fumarate. Further metabolism of oxaloacetate via citrate formation delivers NADH for fumarate reduction, *i.e.* both oxidation and reduction of malate lead to an increase in the succinate pool. This increase initiates the decarboxylation of succinate via the methymalonyl-CoA pathway finally yielding propionate which is the definitive end product and which is released into the surrounding medium.

During chronic, severe hypoxia mitochondrial ATP production predominates over that in the cytosol. Glycogen is now the major substrate, but the metabolite flux deviates from glycolysis at the phosphoenolpyruvate branchpoint giving rise to the formation of malate which again serves as a substrate for the anaerobically working mitochondria (86,63).

This complexity of anaerobic metabolism is not found in other invertebrate groups (*e.g.* arthropoda) (84). Among vertebrates the Embden Meyerhof Parnas pathway prevails even during long periods of environmental hypoxia (86). Although volatile fatty acid formation is not found in these groups, anaerobic mitochondria may, nonetheless, support or contribute to ATP production, giving rise to ethanol formation in some cases and/or leading to succinate accumulation by use of fumarate reductase activity. As a corollary, lactate (opines) and succinate may, in most cases, be used as cytosolic and mitochondrial markers of an anaerobic metabolism.

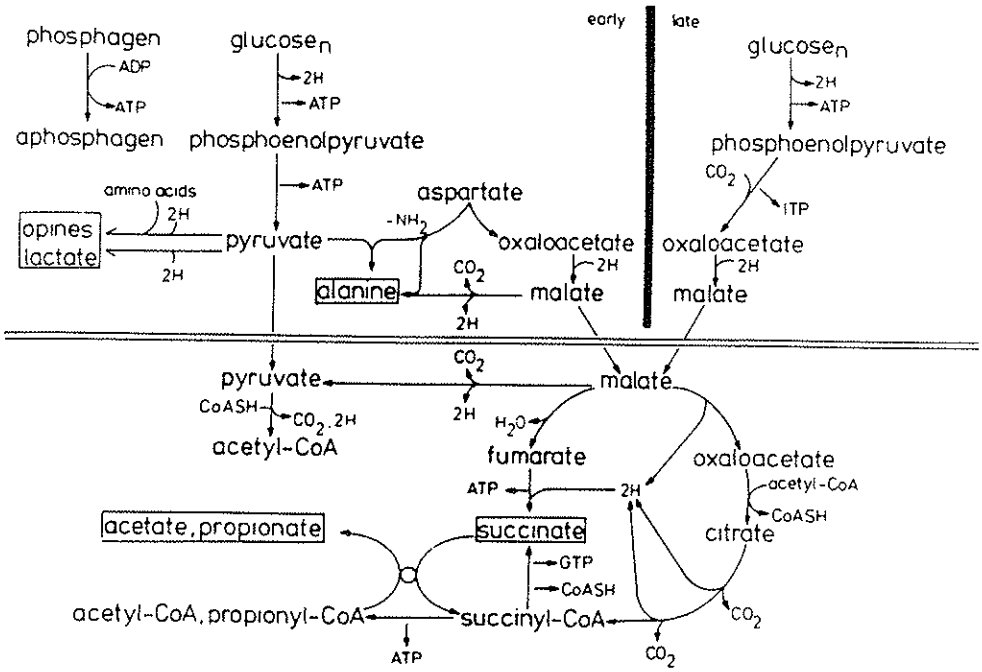

Figure 1. Anaerobic metabolic pathways in marine invertebrates (annelids, molluscs and sipunculids), starting from aspartate and glycogen in the early phase and solely from glycogen in the late phase of anaerobiosis. Important intermediary and end products, which accumulate during anaerobiosis, are outlined in boxes (adopted from Grieshaber *et al.* (25)).

III. Critical P_{O_2}: Oxyregulators and Oxyconformers

A. Oxyregulators

The concept of a critical P_{O_2} was introduced to characterize the oxygen tension below which an oxyregulating animal is no longer able to maintain its rate of oxygen consumption independent of the ambient oxygen tension but exhibits a decreasing rate of oxygen uptake (25). Recent available studies, which include an analysis of anaerobic processes at different oxygen tensions, demonstrate that this critical P_{O_2} is not only characterized by the transition from an oxygen independent to an oxygen dependent pattern of oxygen consumption, but also by the onset of an anaerobic energy metabolism. Thus the failure of oxyregulation as well as the onset of anaerobiosis coincide (*cf.* Figures 2,3) (70,52).

Figure 3 demonstrates that the rate of oxygen uptake remains constant down to a P_{O_2} of 30 Torr. Below this value an increase instead of a decrease in oxygen consumption coincides with the onset of lactate accumulation in the plasma and in the tissues (predominantly found in aerobic organs like the kidney or the working ventricle). Succinate is found in the toad ventricle. The onset of anaerobiosis is linked to an increased release of CO_2 indicating the metabolic titration of bicarbonate stores and a drop in the arteriovenous oxygenation difference which had been constant above this

point (Figure 4). The change in the difference between arterial and venous oxygen content had also been monitored during environmental hypoxia in the invertebrate oxyregulator, *Carcinus maenas*. A drop in this difference occurs close to the ambient P_{O_2} where the oxygen consumption starts to fall progressively (43,69).

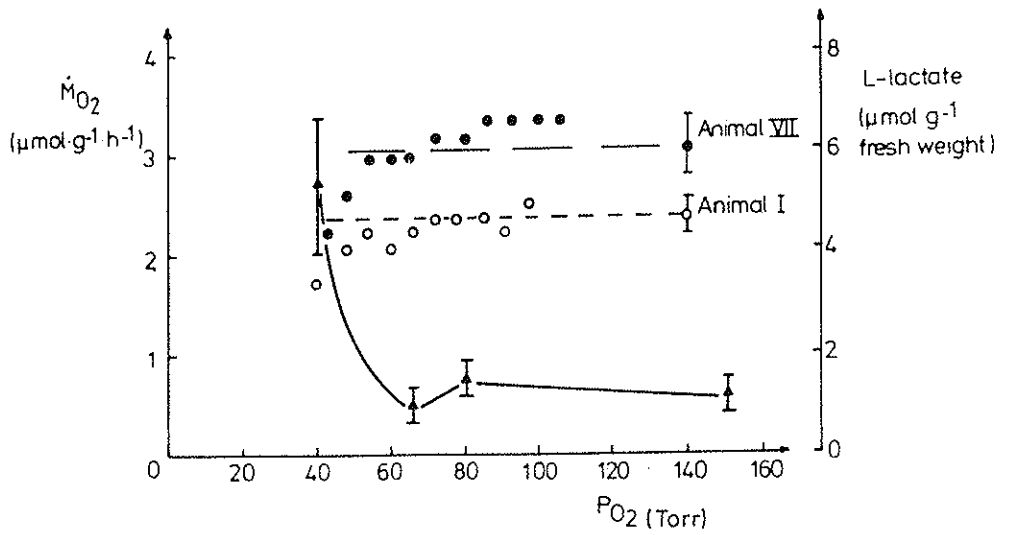

Figure 2. Oxygen consumption of the marine teleost *Lumpenus lampretaeformis* shown for two individual animals in relation to ambient P_{O_2}. A comparison with lactate levels in white muscle tissue (triangles) suggests that lactate formation starts with the onset of a decrease in oxygen consumption, thus characterizing the critical P_{O_2} (for further explanations see text, modified from Pelster *et al.* (51)).

The transient increase in oxygen consumption has been recorded not only in other lower vertebrates (like in fish) but also, among invertebrates, in the shrimp *Palaemon adspersus* and has so far been neglected in critical P_{O_2} determinations (6,48). However, if the critical P_{O_2} is defined as the P_{O_2} below which an oxygen independent animal is no longer able to maintain a regulated, constant rate of oxygen consumption this may also imply an increase instead of a decrease in oxygen consumption. Concomitantly, homeostatic mechanisms fail to ensure complete aerobiosis. A Pc analysis based on oxygen consumption changes alone would neglect the additional anaerobic ATP supply and, thus, would be inaccurate. The change in oxygen uptake, the failure of oxygen provision via the blood and the simultaneous accumulation of lactate are, therefore, indicative of the critical P_{O_2}.

The onset of anaerobic energy production may be an important factor in eliciting the increase in oxygen consumption in the mentioned species. Hypoxic exposure is known to cause a release of catecholamines in amphibians and in fish (7,8). It is entirely conceivable that lactate release into the plasma could be the initial signal reporting an insufficient oxygen supply of a tissue to neuronal structures thereby eliciting a release of catecholamines. Rapid pH-driven release of lactic acid from the severely hypoxic tissue into the blood may be a precondition for this "hormonal" function (55).

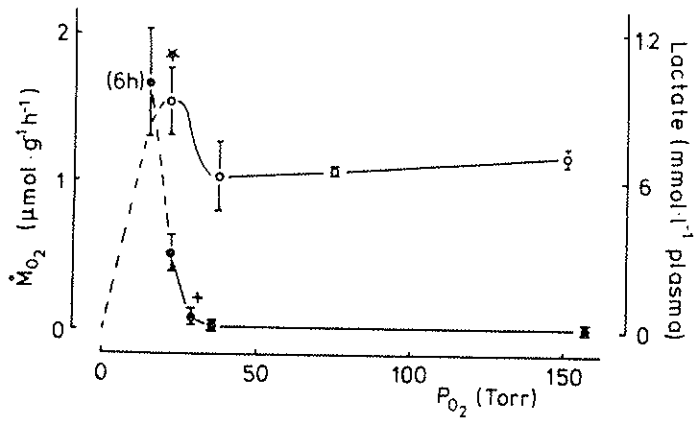

Figure 3. Specimens of *Bufo marinus* preadapted to mild hypoxia (P_{O_2} = 75 Torr) exhibit a more or less constant metabolic rate down to P_{O_2} values below 50 Torr. A steady state increase in oxygen consumption (open symbols) at P_{O_2} = 21 Torr coincides with the onset of lactate accumulation in the plasma (closed symbols) and is, thus, indicative of the critical P_{O_2}. The broken line indicates that, below this P_{O_2} no steady state rate of oxygen uptake is maintained (adopted from Pörtner *et al.* (52), * denotes a significant change from animals exhibiting complete aerobiosis in hypoxia).

It is important in this context that Steffenson (this volume) could eliminate the increase in oxygen consumption at the Pc in rainbow trout during overnight measurements. In other fish species (brook trout, *Salvelinus fontinalis*; carp, *Cyprinus carpio*, and goldfish, *Carassius auratus*) acclimated to moderate hypoxia by long term exposure, the increase in oxygen consumption at the Pc was found to be reduced (6). Long term preacclimation and the measurement during the typical resting period (early morning) did, however, not eliminate this response in *Bufo marinus*. The increase in the rate of oxygen consumption may, perhaps, only occur in animals which are alert and responsive to the onset of lactate formation. Animals which are alert may, on the other hand, have a higher metabolic rate and tend to reach the Pc at higher oxygen tensions. In support of this, the data by Ott *et al.* (49) would suggest that an elimination of this response is linked to a shift of the Pc to lower values.

B. Oxyconformers

Oxygen dependence of oxygen uptake has been described many times in the literature without considering whether this dependence includes anaerobic energy production or not, *i.e.* whether this oxyconformity occurs below the critical P_{O_2}. For the sake of clarity these two cases must be distinguished:

1. Oxyconformity including anaerobiosis. It is based on a failure or shut-down of the oxygen transport system to the mitochondria. This type of oxyconformity occurs in both oxyregulators and aerobic oxyconformers below the critical P_{O_2}.
2. Oxyconformity without anaerobiosis. It represents the inability of an animal or tissue to maintain a constant rate of oxygen consumption. Oxygen consumption declines with

falling ambient P_{O_2}, but the animals must rely on anaerobiosis only at very low oxygen tensions. These aerobic oxyconformers can clearly be distinguished from aerobic oxyregulators which maintain a constant and entirely aerobic metabolic rate above the Pc. However, it must be ensured that the finding of an oxygen dependent mode of energy turnover cannot be explained by changes in spontaneous activity or by stress phenomena elicited during the experiments (see section VI, for a discussion of the standard metabolic rate, and Figure 15).

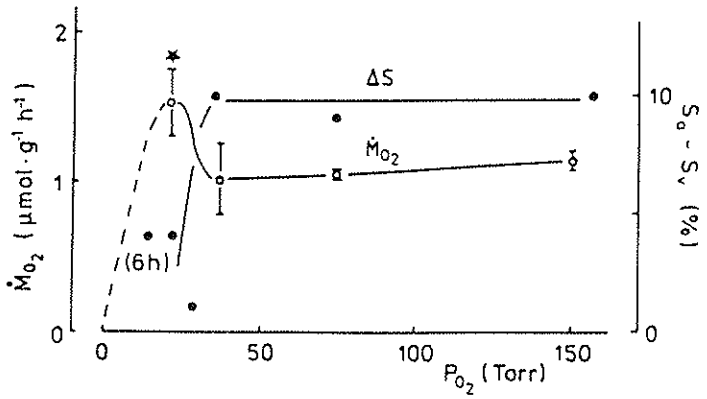

Figure 4. Difference in haemoglobin oxygen saturation between arterial (a, sciatic artery) and venous (v, sciatic vein) blood and oxygen consumption (*cf.* Figure 3) of the toad *Bufo marinus* exposed to graded levels of hypoxia (based on data from Pörtner *et al.* (52)).

As a corollary, only aerobic oxyconformers should be considered as "true" oxyconformers. In these oxyconformers the start of anaerobiosis is particularly important to characterize the critical P_{O_2} since unequivocal changes in other physiological parameters (*e.g.* oxygen uptake or blood gas transport), may not always occur (*cf.* Figure 14).

Detailed investigations of changes in oxygen consumption, total energy expenditure, and the P_{O_2} in the coelomic fluid were performed in the peanut worm *Sipunculus nudus* (54,31). All investigated parameters decreased linearly with declining ambient P_{O_2} until a critical P_{O_2} was reached (between 20 and 70 Torr, depending on the size of the animals) which was characterized by an enhanced decline in overall oxygen consumption. Below this point the P_{O_2} gradient between coelomic fluid and ambient medium was more progressively reduced indicating a falling oxygen uptake via the coelomic fluid owing to insufficient diffusion gradients. Most importantly, however, a compensatory transition to anaerobic energy production occurred as indicated by the accumulation of succinate (Figure 5).

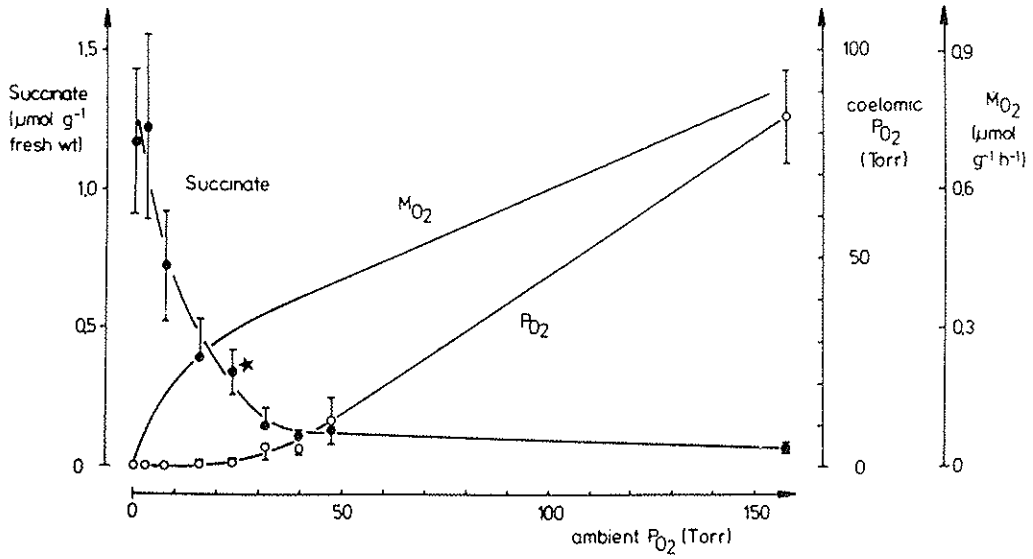

Figure 5. Small specimens of *Sipunculus nudus* (9 g body weight) are oxyconforming in a wide range of ambient P_{O_2} without being anaerobic. Below the critical P_{O_2}, which is characterized by the enhanced decline in oxygen consumption, inner tissues (introvert retractor muscles) start an anaerobic energy metabolism in both the cytosol and the mitochondria (succinate values shown for 24 h of incubation at 15°C, modified from Pörtner *et al.* (54). A star indicates the onset of a significant deviation from normoxic controls.

The observation of oxyconformity above the Pc is in accordance with findings in other marine invertebrates. *Scoloplos armiger*, a thin polychaete worm, exhibits a critical P_{O_2} close to 16 Torr, but increases its rate of oxygen consumption at higher oxygen tensions (30). Detailed data on oxygen consumption and the mode of energy provision are also available for the lugworm, *Arenicola marina*. The rate of oxygen consumption is constantly reduced when the ambient P_{O_2} falls below 120 Torr but anaerobiosis commences only at P_{O_2} values below 50 Torr (Figure 14) (61,72). At high P_{O_2} values below or close to normoxia an independent rate of oxygen consumption is observed in *Arenicola marina* and *Scoloplos armiger*, but could not be found in *Sipunculus nudus* (see below).

A wide range of aerobic oxyconformity has also been postulated for two fish species, the bluegill, *Lepomus macrochirus* and the brown bullhead, *Ictalurus nebulosus*, based on a comparison of the anaerobic threshold with Pc determinations adopted from different literature studies (32). It must be emphasized, however, that the critical P_{O_2} and the anaerobic threshold are variables (*cf.* Table 1) and, in order to avoid the erroneous finding of a discrepancy between the two in otherwise oxyregulating specimens, should only be compared in animals which are in the same physiological state and have been subjected to identical or very similar experimental conditions.

Table 1
Factors affecting standard metabolic rate (SMR) and/or the critical P_{O_2} of transition to anaerobiosis (Pc_M in Figure 12, partly based on Herreid's review (33)).

hypoxia acclimatization	(Pc)
state of nutrition	(SMR, Pc)
state of development	(SMR, Pc)
temperature	(SMR, Pc)
blood pigment content function	(Pc)
stress	(SMR, Pc)
salinity	(SMR, Pc)
ventilation	(SMR, Pc)
body size	(SMR, Pc)
locomotory activity	(Pc)

IV. Oxygen Consumption of Isolated Organs and Cells

A. Organs.

The principles of oxyconformity, oxyregulation and the critical P_{O_2} can also be applied to analyses of oxygen consumption in isolated organs and cells and the question arises to what extent the respective findings are comparable with those obtained in whole animal studies. For the oxyconformer *Sipunculus nudus* the oxygen dependence found for the intact animal could, for example, be confirmed for isolated, denervated muscle preparations (53). However, oxygen consumption of isolated organs has in most cases been studied in tissue preparations from mammals, all of which can be classified as typical oxyregulators.

Oxygen consumption in relation to ambient P_{O_2} has been investigated in the liver of neonatal lambs (15). Oxygen metabolism is regulated until P_{O_2} falls below 10 to 15 Torr, but seems to cease rapidly when some oxygen is still available. These data prove the transition from an oxygen independent to an oxygen dependent mode of energy provision in the liver.

An estimate of the pattern of oxygen utilization and lactate formation in the canine brain demonstrates that oxygen provision in this organ remains below the critical P_{O_2} at arterial oxygen tensions between 30 and 40 Torr. Oxygen consumption is less at 30 Torr than at 40 Torr and at these P_{O_2} values anaerobiosis contributes to energy production as judged from lactate formation (37).

A distinct threshold for the onset of an anaerobic energy metabolism in the perfused isolated dog heart has been demonstrated by the work of Wiesner *et al.* (79). These authors reported tissue levels as well as arterio-venous concentration differences of anaerobic key metabolites and oxygen of a myocardial region which was progressively

underperfused by occluding a side branch of the left circumflex or the left anterior descending coronary artery of the heart as well as by controlled underperfusion. The rate of oxygen uptake and steady state levels of succinate and lactate remained constant until a perfusion rate of less than 1 $ml \cdot min^{-1}$ was reached. Below this value the tissue levels of both metabolites increased whereas oxygen consumption was reduced. At the same perfusion rate lactate and succinate were released from the myocardium. Thus a perfusion rate of 1.0 $ml \cdot min^{-1}$ seems to reflect a critical oxygen supply of the rat heart.

A recent study in the oxyregulator *Bufo marinus* shall be analyzed in more detail since it allows to integrate the changes in blood gas transport into this general picture and also since, at a first sight, some of its results appear to deviate from the traditional understanding of the critical P_{O_2} and, as such, may underline the importance of a concomitant analysis of anaerobic metabolic events and oxygen consumption in critical P_{O_2} determinations (52,56).

Investigations on the rat lung lead closer to the relationship between intracellular P_{O_2} and the mode of energy production. Since the lung parenchyma is essentially a passive conduit for delivery of oxygen from the alveoli to the tissue, the alveolar P_{O_2} should reflect the P_{O_2} of the parenchyma as long as steady state conditions prevail. Fisher *et al.* (18) evaluated the redox state of the isolated rat lung. The ratios of the redox couples lactate/pyruvate, glycerol 3-phosphate/dihydroxyacetone phosphate and glutamate/2-oxyglutarate remained more or less constant until the alveolar P_{O_2} decreased to less than 1 Torr. Graded hypoxia elicited a significant accumulation of lactate when the P_{O_2} fell below 7 Torr with some indications of a limited onset of lactate production at 35 Torr and below (17). Unfortunately, Fisher and Dodia did not record the rate of oxygen consumption in these experiments but the critical P_{O_2} can be anticipated between 0.7 and 7 Torr. These data would already suggest that intracellular P_{O_2} reaches very low values before an anaerobic metabolism sets in.

In an elegant study Kreutzer and Jue recently measured the intracellular oxygenation state of the isolated rat heart using the ^1H nuclear magnetic resonance signal of the proximal histidine NH proton of deoxymyoglobin (39). This signal appears when the myoglobin becomes deoxygenated and can be directly used to monitor the intracellular oxygenation state of the heart. Using this signal and the P_{50} of the myoglobin, the mean intracellular P_{O_2} can be calculated and related to the ambient conditions (extent of ischaemia or ambient P_{O_2}) and to the metabolic status of the cell. Neither ATP nor creatine phosphate change significantly until the flow rate is reduced below 1.0 $ml \cdot min^{-1}$, but the ^1H signal of deoxy-myoglobin appears already at a flow rate of 2.0 and 1.5 $ml \cdot min^{-1}$. Calculation of the intracellular P_{O_2} using a P_{50} of 1.5 Torr for myoglobin demonstrates that the degradation of ATP and creatine phosphate commences below an intracellular P_{O_2} of approximately 1.5 Torr.

B. Cells.

The question arises, to what extent are changes in the rate of oxygen consumption in the whole animal or isolated organ depending on a central response or reflect modifications at the cellular level. Recent investigations of the oxygen dependence of metabolism in isolated cells allow to answer this question for both oxyconformers and oxyregulators. In particular the question can be addressed whether an oxygen dependent metabolism is only due to the fact that oxyconforming animals usually lack highly

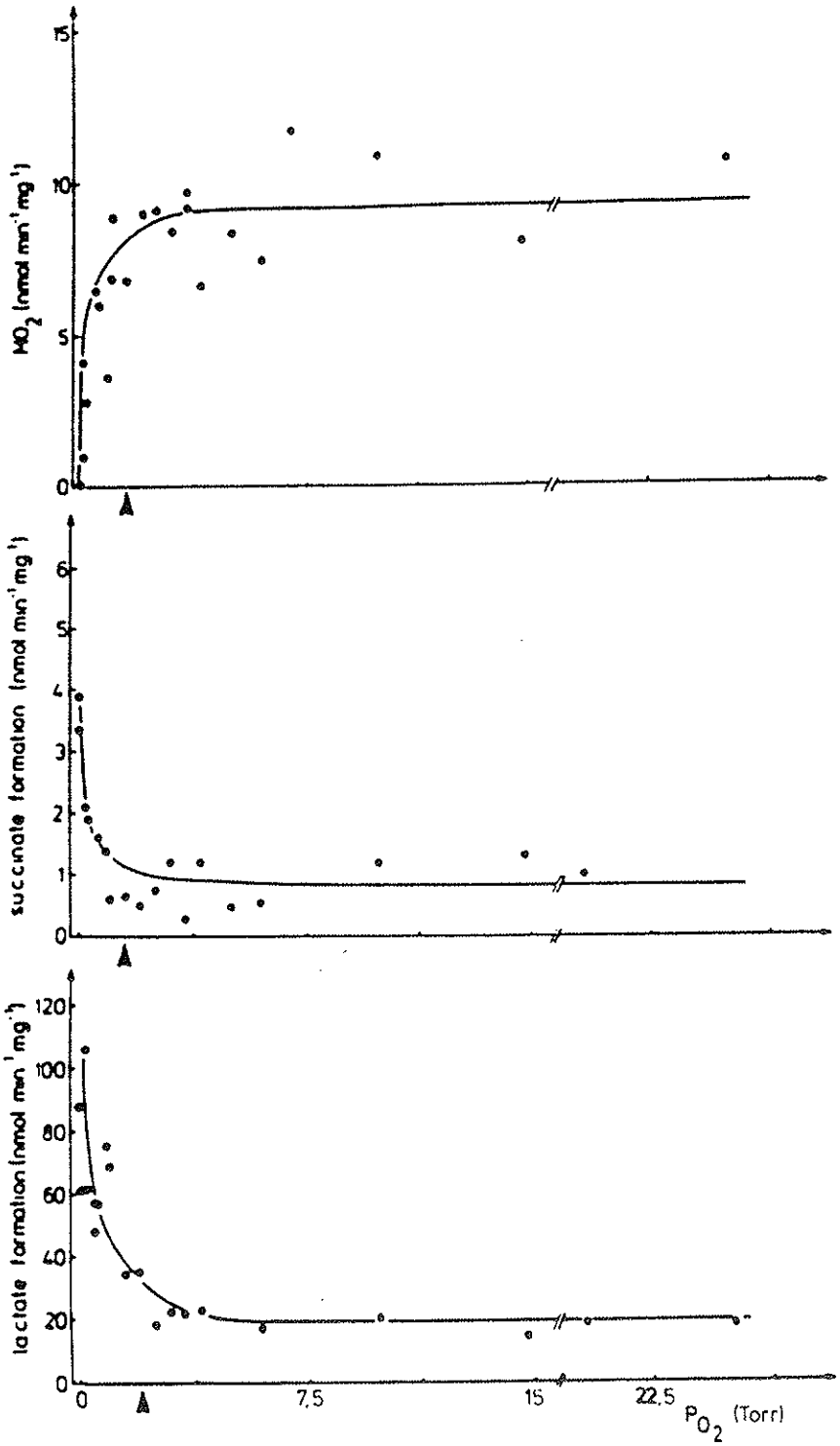

Figure 6. Oxygen consumption and the rates of succinate and lactate formation of isolated rat heart myocytes under different steady state oxygen partial pressures at 37°C (adapted from Riefke *et al.* (60)).

specialized respiratory organs and circulatory systems and, thus, are unable to transport oxygen rapidly enough to the metabolizing tissues. If only systemic factors are responsible for limiting oxygen supply already at moderate hypoxia, then isolated cells from oxygen independent species should exhibit a constant rate of oxygen consumption down to a critical P_{O_2}. If the same holds true for oxygen conformers isolated cells should not reflect the mode of oxygen consumption of the intact animals and should be oxygen independent because the factors causing diffusion limitation of oxygen may be eliminated.

In addition, an analysis of metabolism in relation to ambient P_{O_2} in isolated cells can also provide a rough estimate of O_2 gradients between the medium and the mitochondrial compartment.

De Groot *et al.* (27) reported oxygen consumption data of isolated rat hepatocytes in an incubation system which allows to keep the ambient P_{O_2} constant at any chosen level down to very low partial pressures (46). The respiratory rate of the isolated hepatocytes is almost P_{O_2} independent between 2 to 100 Torr. At P_{O_2} values below 2 Torr there is a rapid decrease in the rate of oxygen uptake. A half-maximal value is reached at 0.7 Torr. Since *in vitro* measurements of the P_{50} of cytochrome oxidase led to values between 0.02 and 0.2 Torr, (28,68) it is very likely that an insufficient oxygen supply to mitochondria is responsible for the observed drop in oxygen consumption (36). Energy supply becomes inadequate and a half-maximal drop in ATP content is reached at a P_{O_2} of 1.4 Torr. However, anaerobic metabolism compensates for some of the energy deficit since, at the same oxygen level, cells start to release L-lactate (26).

Similar results were obtained with isolated myocytes of rat hearts incubated in an oxystat system (Figure 6) (60). The rate of oxygen consumption was constant down to a value of 1.5 Torr and decreased steeply below this value. Succinate release started at 1.5 Torr whereas lactate production became enhanced at P_{O_2} values slightly above this value.

As a corollary, isolated hepatocytes and cardiomyocytes from a mammalian oxyregulator keep their rate of oxygen consumption independent of ambient P_{O_2}. Only at very low oxygen levels is there a marked reduction in the rate of oxygen consumption which coincides with the onset of an anaerobic metabolism. In both cell types the oxygen gradient between outside the cell and the mitochondrial compartment is less than 2 Torr. In addition, measurements of the critical intracellular P_{O_2} with the proton NMR technique and estimates of the critical P_{O_2} for the individual cell are very close arguing for minimal diffusion gradients from the cell membrane to mitochondria for both rat liver and heart (for a potential modulation of this gradient by a redistribution of mitochondria in hepatocytes see Jones et al (36).

Finally, oxygen consumption was investigated in coelomic cells of the oxyconformer *Sipunculus nudus* and in obliquely striated myocytes isolated from the body wall of the same species (Figures 7,8) (59). Under steady state conditions, maintained by an oxystat system, the rate of oxygen consumption of all cell types fell at a linear rate with decreasing ambient P_{O_2}. Similar data were obtained in a closed system thus confirming earlier data on the oxygen dependence of coelomic cells (53). Therefore, the pattern of oxygen consumption in this oxyconforming animal is not different between isolated cells and the intact animal. Obviously, the oxygen dependence of metabolism above the

critical P_{O_2} is not related to respiratory and circulatory factors but primary regulation occurs at the cellular level.

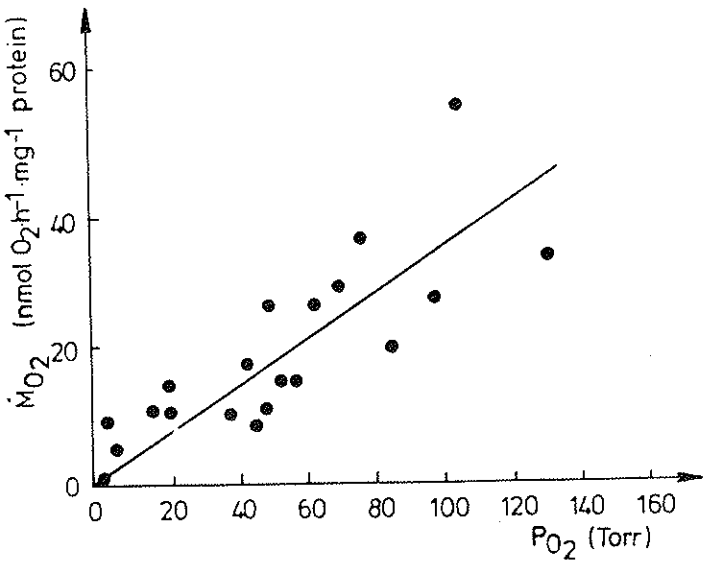

Figure 7. Oxygen consumption of cells isolated from the coelomic fluid of *Sipunculus nudus*, measured at 15°C in an oxystat system (see text (59)).

V. Intracellular P_{O_2} and Cellular Oxygen Consumption

For an explanation of the differences between oxyregulators and oxyconformers one important aspect to consider is the modulation of mitochondrial metabolism in response to declining oxygen tensions, when substrate concentrations (mainly ADP, inorganic phosphate, oxygen and redox equivalents) might change (50). In oxyregulating cells the redox ratio ([NADH]/[NAD]) shifts to higher values and the phosphorylation potential ([ATP]/[ADP][P_i]) decreases in order to maintain a constant rate of ATP production (80,82). These changes even begin at oxygen tensions far above the critical P_{O_2} and compensate for the falling oxygen levels. In two studies on oxygen consumption in the oxyconformers *Arenicola marina* and *Sipunculus nudus*, a significant change in adenylate or phosphagen concentrations in the range of aerobic oxyconformity could not be detected and, thus, changes in the levels of free ADP or inorganic phosphate are most unlikely to occur (54,61). The drop in oxygen consumption is obviously not compensated for by an increase in the ratio [NADH]/[NAD] which is likely to happen already during hypoxia above the Pc (82).

The question arises whether the changing rate of oxygen consumption observed above the Pc could be explained as being caused by fluctuating oxygen supply to mitochondria. What is the range of intracellular P_{O_2} in the tissue of oxyconformers and oxyregulators and how does it influence oxygen consumption? If the findings of Sugano *et al.* and Chance (11,68) can be generalized for both oxyregulators and oxyconformers, mitochondria only require a P_{O_2} of below 0.2 Torr to saturate their oxidative capacity

(see above). The minimum intracellular P_{O_2} for maximum cytochrome turnover in red mammalian muscle was found to be below 0.5 Torr (21). These are the intracellular P_{O_2} values below which compensatory changes in phosphorylation potential and redox status fail to maximize oxygen uptake.

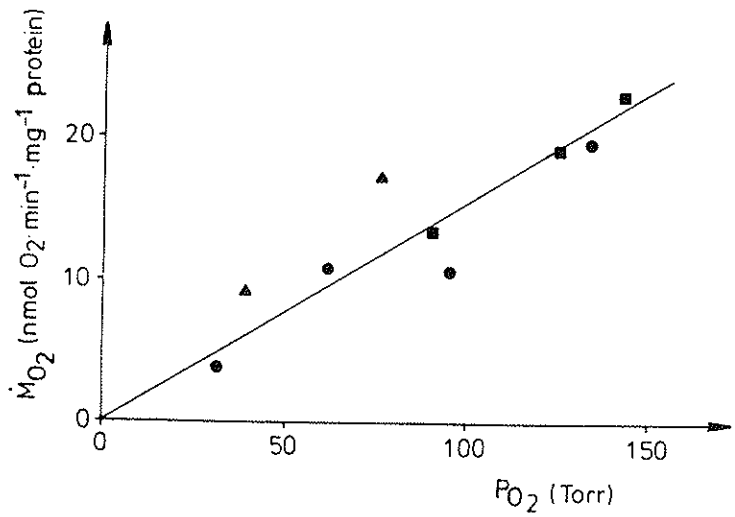

Figure 8. Oxygen consumption of obliquely striated muscle cells isolated from the body wall musculature of *Sipunculus nudus* measured at 15°C in an oxystat system (see text). Symbols characterize different cell preparations (59).

In the investigated range of aerobic oxyconformity in *Sipunculus nudus* (Figure 5) the ambient P_{O_2} varies between *e.g.* 30 to 155 Torr, whereas coelomic fluid P_{O_2} values change between 3 and 64 Torr and the rate of oxygen consumption is definitely oxygen dependent, although mitochondrial oxygen demand should be saturated (54). In contrast, if the intracellular P_{O_2} should indeed remain below 0.5 Torr a drastic diffusion resistance for oxygen in the tissue must be anticipated. In the cell the major (but small) diffusion gradient is assumed to be built up across the cytosol (see above), and it is unlikely that the O_2 transport properties of this medium are modified to the required extent. Vasoconstriction as a means to reduce oxygen supply to the tissues can be excluded as a mechanism in some oxyconformers, *e.g. Sipunculus nudus*, since no circulatory system exists. Consequently, the fluctuations in oxygen metabolism above the Pc are very likely not related to a reduction in intracellular P_{O_2} below 0.5 Torr. These considerations are in accordance with the conclusion that the regulation of the metabolic rate in an aerobic cell happens on the side of ATP consumption and is not oxygen limited above a critical oxygen level (13,81,82). This situation changes, however, if the rate of oxygen consumption is uncoupled from the rate of energy turnover. In these cases, which will be discussed in more detail below (section VIII), the rate of oxygen uptake may directly depend upon the cytosolic oxygen concentration even above the critical P_{O_2}.

As a corollary, the view that the critical P_{O_2} of an individual cell, an organ and an intact animal characterizes the oxygen tension below which diffusion limitation for oxygen will affect the mode of energy production, fits these considerations much better.

Diffusion limitation very likely leads to the onset of anaerobiosis below the Pc. The Pc increases with the organizational complexity from the cellular level to the whole animal because mechanisms causing additional limitations of oxygen provision become involved: (1) In a ***cell*** diffusion limitation of oxygen in the membrane and the cytoplasm together with the rate of mitochondrial respiration and, perhaps, the clustering of mitochondria in areas of high energy needs define the point at which the oxygen concentration finally becomes limiting and anaerobiosis commences. (2) In a ***tissue*** capillary or coelomic P_{O_2} is the key parameter in determining whether oxygen provision is sufficient for the individual cell. The maintenance of the pressure head for sufficient oxygen supply depends upon the rate of perfusion, the density of the capillaries and on blood oxygen transport (29). (3) For the whole ***organism*** the required pressure head will also depend upon the structures and functions of the gas exchange organ and the circulatory system, and upon the O_2 affinity of the pigment and its regulation.

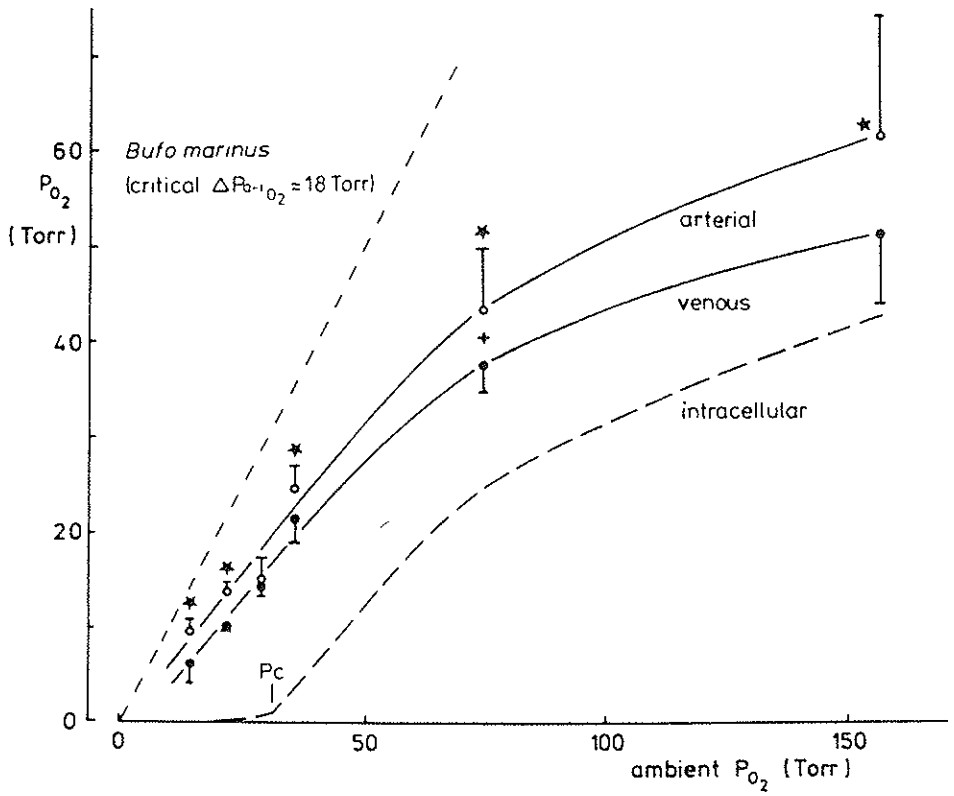

Figure 9. Changes in arterial, venous, and intracellular oxygen tensions in the toad *Bufo marinus* exposed to graded levels of hypoxia (at 20°C). The critical P_{O_2} difference between arterial blood and the intracellular compartment (of the "critical" tissue, see text) gives the pressure head required for the maintenance of oxygen flow (based on Pörtner *et al.* (52)).

On the basis of these considerations an estimate can be made for the changes in intracellular P_{O_2} of the "critical" tissues in the intact animal (Figures 9,10). The

"critical" tissue can be defined as being the first to rely on anaerobic mechanisms in declining oxygen tension because Pi_{O_2} reaches the critical P_{O_2} of mitochondria. In the oxyregulator *Bufo marinus*, the extent of lactate accumulation observed in organs like the working heart or the kidney suggests that one of these could be the first to suffer from anaerobiosis. At the Pc the critical difference between the intracellular P_{O_2} of this tissue and arterial P_{O_2} amounts to about 18 Torr. Above the Pc, the metabolic rate and those parameters reflecting the oxygen supply to a tissue appear to remain unchanged in the resting animal. The respective parameters include the difference between arterial and venous oxygenation (Figure 4), blood flow and hematocrit (83). As a corollary the pressure head of 18 Torr should remain more or less unchanged in the range of P_{O_2} above the Pc thus reflecting constant oxygen flux to the resting tissue. Accordingly, mean Pi_{O_2} varies between close to 0 and 45 Torr in the whole range of constant oxygen uptake from the Pc up to normoxia and is always below the measured venous oxygen tensions (Figure 9). Similarly, a high variability of extracellular tissue P_{O_2} with values always below the respective venous oxygen tensions was found in mammalian skeletal muscle (29).

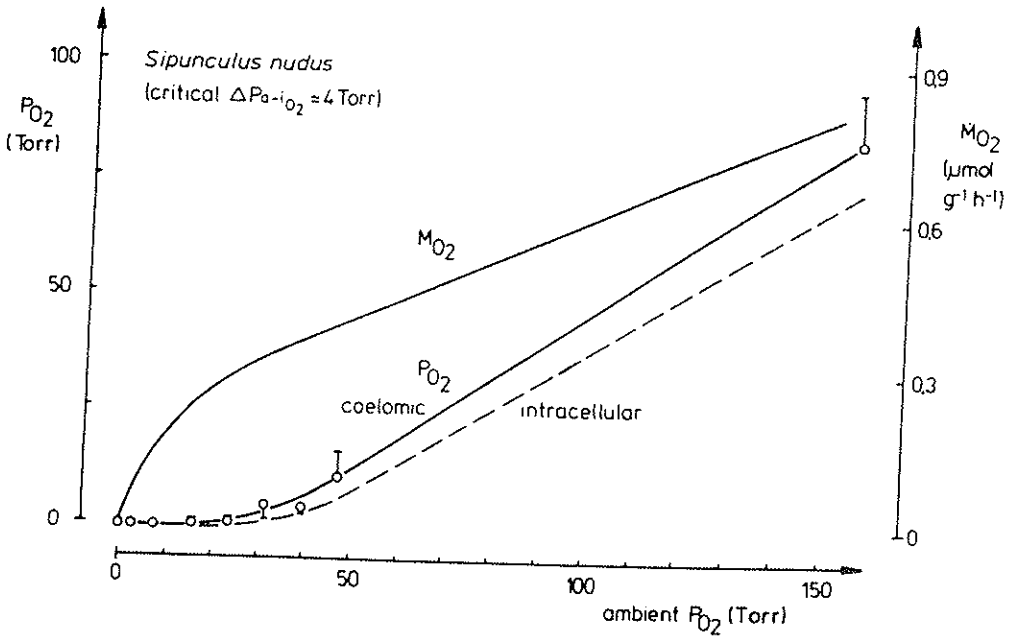

Figure 10. Changes in oxygen consumption (M_{O_2}) and coelomic and intracellular P_{O_2} values in the intertidal worm, *Sipunculus nudus* (at 15°C) exposed to graded levels of hypoxia. The critical P_{O_2} difference between coelomic fluid and the intracellular compartment (of the critical tissue, see text) starts from about 4 Torr at the critical and increases at higher ambient oxygen tensions owing to the rising oxygen consumption (based on Pörtner *et al.* (54)).

In the oxyconformer *Sipunculus nudus* the "critical" tissue exclusively relies on oxygen provision via the coelomic fluid. This is true for the introvert retractor muscles which are the first to show an accumulation of succinate (54). At the Pc, the difference

between intra- and extracellular P_{O_2} amounts to about 5 Torr (Figure 10). This P_{O_2} difference increases with the overall rate of oxygen consumption and Pi_{O_2} varies between close to 0 and 72 Torr in the range of aerobic oxyconformity up to normoxia. Whereas cellular oxygen consumption remained constant in the regulator *Bufo marinus* despite of an increase in cellular P_{O_2}, the increase in Pi_{O_2} in the oxyconformer *Sipunculus nudus* is paralleled by an increase in oxygen consumption.

VI. Standard Metabolic Rate

The above approach to evaluate critical P_{O_2} values in oxyconformers and oxyregulators may be extended towards a general concept which permits to explain similarities and differences in their responses to declining oxygen tensions. To find the lowest possible rate of aerobic metabolism would allow for such a comparison. The standard metabolic rate (SMR) is defined as the lowest rate of oxygen consumption for oxyregulators, "which would be obtained when all organs were absolutely at rest" (5,40). The data available for several oxyregulators suggest that this rate is maintained down to the critical P_{O_2} below which anaerobiosis starts (see above) (25).

SMR is evaluated by extrapolation to zero activity (6,45). For this extrapolation anaerobic metabolism must be excluded as an energy source during the different degrees of activity. If only the influence of locomotory activity is considered by the extrapolation the calculated rate of oxygen consumption may still include a varying influence of ventilation. This may be the reason why in squid, "standard" rates were higher than resting rates, possibly owing to higher ventilatory efforts in active specimens (47). In an alternative approach, Ultsch *et al.* (64) evaluated the lowest rate of oxygen consumption found during long term measurements (see also Hughes *et al.* (34)). A similar approach was used for *Bufo marinus*, thereby excluding the influence of spontaneous activity and diurnal variations on the metabolic rate (52). This analysis permits to reliably assess the critical P_{O_2} valid for the respective standard metabolic rate (see Table 1). As a corollary, standard metabolic rate (SMR) can be defined as the "minimum metabolic rate during aerobiosis excluding locomotory or excess muscular activity." If these precautions are not considered, SMR may be overestimated. In some cases a depressing effect of hypoxia on the level of spontaneous activity may lead to the evaluation of an apparent discrepancy between the onset of a decrease in oxygen consumption and the actual critical P_{O_2} (8).

SMR and, as already discussed by Herreid, the critical P_{O_2} will not only vary between species but may also fluctuate in the same individual depending upon the ontogeny of the animal and the acclimatization to environmental factors (Table 1) (33). For example, long term acclimatization to hypoxia may occur, such that the critical P_{O_2} and SMR may be different for animals under hypoxia as compared to those exposed to higher oxygen tensions (4).

In oxyconformers, oxygen consumption curves cannot give unequivocal access to the lowest rate of a completely aerobic metabolism. In addition, uncertainty prevails whether all animals classified as oxyconformers in the literature are "true" (aerobic) oxyconformers or can be seen as oxyregulators with a high Pc. Future analyses must also show whether some, especially vertebrate oxyconformers may be identified as oxyregulators under more adequate experimental conditions (*e.g.* Ultsch *et al.* (74), *cf.*

Figure 14 for an analysis in *Mytilus edulis*). In addition, apparent "true" oxyconformity may be elicited by an oxygen dependence of spontaneous activity.

In some marine invertebrates the study of mitochondrial and cytosolic mechanisms of anaerobic energy production may provide some evidence whether the analysis is influenced by elevated energy requirements like during excess muscular activity (see Section I and Figure 11). The long term incubation of *Sipunculus nudus* below the Pc demonstrated that succinate accumulation occurred before the opines were formed as glycolytic end products. Consequently, anaerobic glycolysis became involved only when the oxygen supply fell to an extent which did no longer allow (all of) the mitochondria to produce energy by aerobic means. Since muscular activity may lead to pyruvate and lactate or opine accumulation when mitochondria are still performing aerobically, this observation presents strong evidence that the animals exhibited their minimal aerobic metabolic rate at the critical P_{O_2} (14,38). Thus, the metabolic rate at the Pc can be seen to represent the standard metabolic rate (SMR) of both oxyregulating and oxyconforming organisms, organs and cells.

Unfortunately, such an analysis is not possible in animals which rely on a circulatory system or on anaerobic glycolysis as the main anaerobic metabolic pathway (see Section I). If, for example, a circulatory system exists, the working contractile element(s) (*i.e.* the heart) may cause early lactate formation. In addition, if glycolysis is involved in homeostatic mechanisms glycolytic end product formation may become (one of) the first signal(s) to indicate the onset of anaerobic energy production in declining oxygen tension as discussed for *Bufo marinus* (Figure 3) (76). Activity monitoring and long term analysis of oxygen uptake may then be more adequate to evaluate SMR.

VII. Two Critical Oxygen Tensions?

Herreid postulated that a critical P_{O_2} should exist for both oxyconformers and oxyregulators, above which the rate of oxygen metabolism remains constant (33). However, based on the above definition of SMR it is evident that this constant rate of oxygen consumption does not necessarily equal the minimum aerobic or standard metabolic rate (called resting metabolism by Herreid). In "true" oxyconformers like in *Arenicola marina* or in *Scoloplos armiger* two critical P_{O_2} values can be distinguished: a Pc_M, which indicates the shift to anaerobiosis (M for a metabolic change in the mode of energy production), and a higher Pc_R which is characterized by the transition from oxyregulation towards oxyconformity (R for a respiratory change which is not linked to a change in the mode of energy production) (61,62). In *Sipunculus nudus*, however, the existence of a high critical P_{O_2} (Pc_R) could not yet be confirmed. In the polychaete *Scoloplos armiger* Pc_R was found to be variable. Pre-spawning animals collected in April exhibit a higher rate of oxygen consumption than those collected in June (post-spawning). In pre-spawning animals oxygen consumptions starts to decrease soon after the ambient P_{O_2} is reduced below normoxic values, but in post-spawning specimens oxygen consumption only decreases below an ambient P_{O_2} of 60 Torr. However, Pc_M remains unchanged between pre- and post-spawning animals.

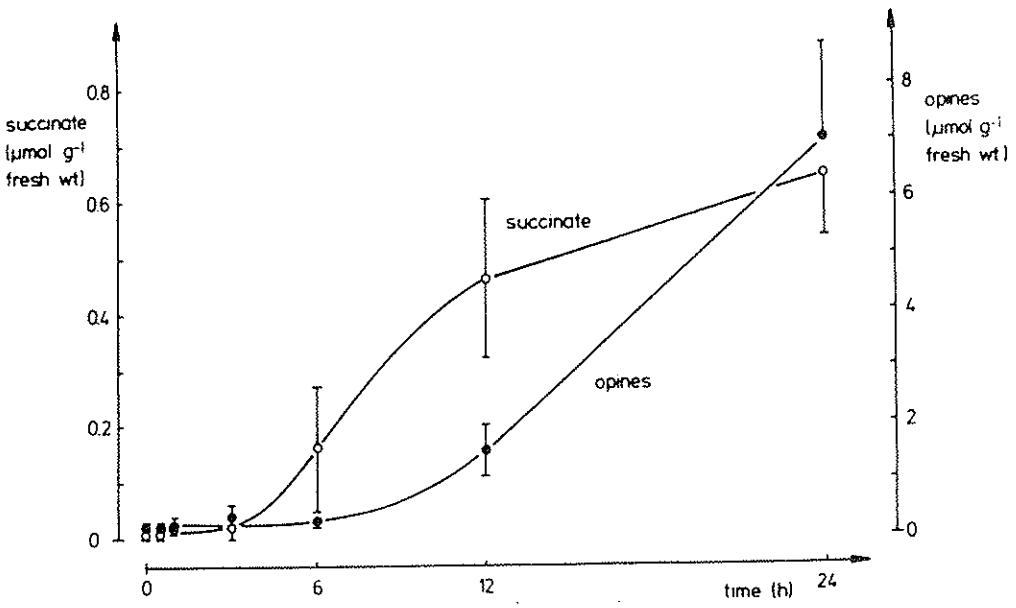

Figure 11. At hypoxia below the critical P_{O_2} (P_{O_2} =7.5 Torr) cytosolic anaerobic energy production in *Sipunculus nudus* begins after the onset of mitochondrial anaerobiosis. The fact that cytosolic anaerobiosis only starts in declining oxygen tension when mitochondrial metabolism is no longer exclusively aerobic, indicates that the metabolic rate is minimal under the experimental conditions applied (modified after Pörtner *et al.* (54)).

A summary of the different patterns discussed for regulators and conformers leads to a simple model (see Table 2 and Figure 12) (5). Generally, the oxygen consumption at Pc_M is equivalent to the standard metabolic rate considering that a potential increase in oxygen uptake below the Pc may be a response linked to the onset of anaerobic metabolism. The model developed here does not state that SMR or Pc_M should be in the same order of magnitude in oxyregulators or oxyconformers. As stated above, these values are variable between species depending upon the level of organization and the workload imposed by homeostatic mechanisms. SMR and Pc are also variable in an individual animal according to its physiological state under the prevailing endogenous and exogenous conditions (see Table 1). Gnaiger (see also this volume) pointed out that animals show different abilities to extract and consume oxygen in a very low range of ambient P_{O_2} (below Pc_M), the good "microxic regulators" being able to sustain a high percentage of aerobic ATP turnover (23). In the context of the present model this would only partly be equivalent to a reduction of the critical P_{O_2} (Pc_M) to low values, which may be achieved by highly efficient oxygen extraction and transport, but may also be based on a low standard metabolic rate. Below Pc_M, the ability to continue efficient oxygen extraction may also vary. However, long term measurements must show whether the animals are able to sustain a steady state rate of oxygen uptake specific for any certain P_{O_2} below the Pc. *Sipunculus nudus* was able to do so over a period of 24 h

(31). In *Bufo marinus* an elevated rate of oxygen consumption was maintained for 24 h below the Pc at P_{O_2}=21 Torr, but in the range of ambient P_{O_2} below this value, long term incubation demonstrated that the animals were not able to maintain a constant rate at all, but gradually fell into asphyxia (Figure 3) (52). More long term analyses are required to evaluate whether good "microxic" regulation just represents the ability to sustain homeostatic mechanisms longer than others under non-steady state conditions in hypoxia.

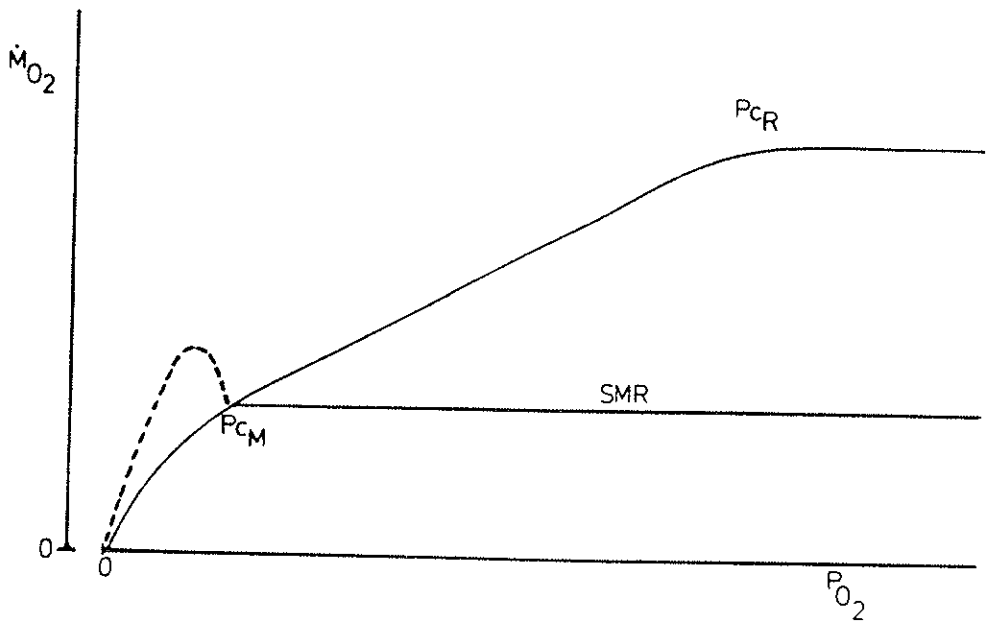

Figure 12. Graphical presentation of the model used for the comparison of the physiological responses of oxyconforming and oxyregulating animals in declining oxygen tensions. For the sake of clarity, oxygen consumption rates and critical P_{O_2} values are compared in absolute terms, neglecting the fact that standard metabolic rates are variable between species and individuals. An increase in the oxygen consumption of oxyregulators at low P_{O_2} values occurs below Pc_M (For the definition of terms see Table 2 (57)).

Evidently, oxyregulators are able to maintain a low metabolic rate (SMR) at P_{O_2}-values higher than Pc_M, whereas the oxygen consumption of "true" oxyconformers increases above Pc_M. These animals show a second critical P_{O_2} (Pc_R), above which the elevated rate of oxygen uptake remains constant.

The question arises, whether the increase or, generally, the scope of oxygen consumption values observed above Pc_M in oxyconformers reflect an insufficient ability to control energy expenditure or may be caused by other oxygen consuming processes (see below). Obviously, the rate of oxygen consumption observed at Pc_R is not equivalent to the standard metabolic rate of an oxyconforming animal and does not reflect the basic energy requirements under resting conditions.

Figures 13 to 15 illustrate some examples, of how this general model can be applied considering the contribution of anaerobic metabolism to the metabolic heat output. Based on data by Pörtner (53), Pörtner *et al.* (54), and Hardewig *et al.* (31) a quantitative interpretation is possible for *Sipunculus nudus* considering a reduction in anaerobic heat dissipation below 30 % of the rate expected from the oxygen consumption found at normoxia (Figure 13). The drop in oxygen consumption below Pc_M is partly compensated for by anaerobic energy production. In this species the anaerobic heat could be explained by the anaerobic metabolic processes (see Figure 1) on a quantitative basis. A so-called "anoxic gap" which leaves a large fraction of the anaerobic heat dissipation unexplained (*e.g.* in *Lumbriculus variegatus* or *Mytilus edulis*) (24,64) could not be confirmed for *Sipunculus nudus* (31).

Table 2
Definition of terms used for modelling the physiological responses of oxyregulators and oxyconformers in declining oxygen tensions (see Figure 12).

Term	Definition
Pc_M	critical P_{O_2}, below which anaerobiosis start
Pc_R	critical P_{O_2} of constant aerobic metabolic rate
SMR	standard metabolic rate = minimum metabolic rate in complete aerobiosis (at Pc_M), excluding locomotory or excess muscular activity
aerobic oxyregulator	able to maintain SMR above Pc_M, $Pc_M = Pc_R$
aerobic oxyconformer	regularly exceeds SMR above Pc_M, $Pc_R > Pc_M$

In a depiction for *Arenicola marina*, which is also valid for another polychaete worm, *Scoloplos armiger*, no correlated change in the rate of oxygen consumption decline and the onset of anaerobic ATP production is evident. A clear difference exists, however, between Pc_M and Pc_R (Figure 14) giving the range of "true" (aerobic) oxyconformity.

Other literature studies seem to provide data conflicting with the model. Famme *et al.* and Hammen (16,30), by using calorimetry, found a high degree of anaerobic in excess over aerobic heat dissipation at P_{O_2} values close to normoxia in oxyconforming *Mytilus edulis* or *Crassostrea virginica*. However, if the work by Bayne (3) is considered it becomes evident that *Mytilus* can be both oxyregulator or oxyconformer depending on its physiological state (stress level). Since other authors have found oxyregulation in larval and adult *Crassostrea virginica* the situation might be the same in this species (65,78). In accordance with the model the following solution arises (Figure 15): In regulating animals anaerobic energy production is likely to start below the critical P_{O_2} (Pc_M = 50 Torr). The transition from oxyregulation to oxyconformity implies a shift of Pc_M to higher values, *i.e.* these animals become anaerobic

oxyconformers even at high oxygen tensions. Some energy is saved by the reduction in ventilation but not enough to keep Pc_M low. Anaerobic energy production has to compensate for the energy gap down to a threshold value of P_{O_2} (may be former Pc_M), below which the overall metabolic regulation ceases and the metabolic rate declines. This pattern of metabolic regulation in the two bivalves appears to be independent of the mode of energy production and leads to the interesting assumption that some external oxygen sensors and not the aerobic nature of metabolism might be decisive in regulating the metabolic rate of the animals. There are several other reports in the literature where a switch from oxyregulation to oxyconformity is described (*e.g.* toadfish, or lobster) without considering whether anaerobiosis becomes concomitantly involved or not (66,74). Future work must show whether all of these examples, in accordance with the above model, mean an upward shift of Pc_M of an oxyregulator or, more unlikely, whether a separation of Pc_M and Pc_R occurs transforming an oxyregulator into a "true" (aerobic) conformer.

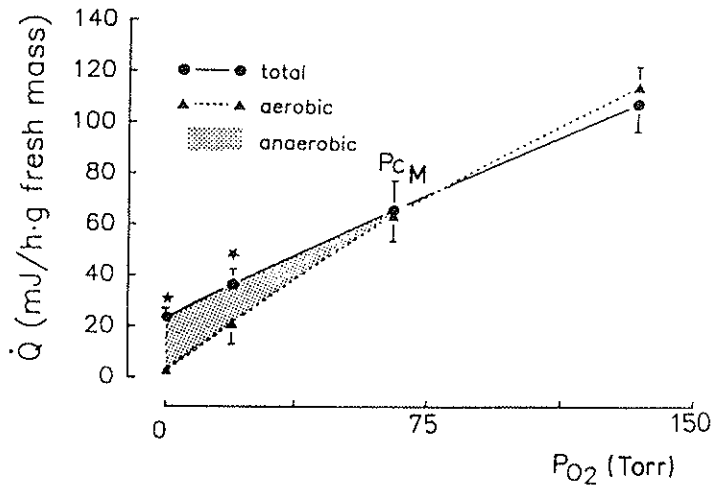

Figure 13. Comparison of the rates of heat dissipation ($\dot{Q}$) at different oxygen tensions as determined indirectly (by an analysis of oxygen consumption and metabolite levels) and directly (by calorimetry) in large specimens (40 g body weight) of the oxyconformer *Sipunculus nudus*. Asterisks indicate a significant difference. An enhanced drop in oxygen consumption below Pc_M is only partially compensated for by an anaerobic energy production. Total heat dissipation ($\dot{Q}$) continues to fall (for further explanations see text, modified after Hardewig *et al.* (31)).

VIII. Open Questions and Conclusions

The present analysis strongly supports the conclusion that the regulation of oxygen consumption at the cellular level is primarily related to a regulation of energy consumption and not to a regulation of energy production. The question results, why the metabolic rate remains close to SMR in an oxyregulator, when the ambient P_{O_2} rises above Pc_M, whereas an oxyconformer appears unable to maintain SMR. Therefore, the

background of Pc_R and of the elevated rate of oxygen consumption needs to be investigated.

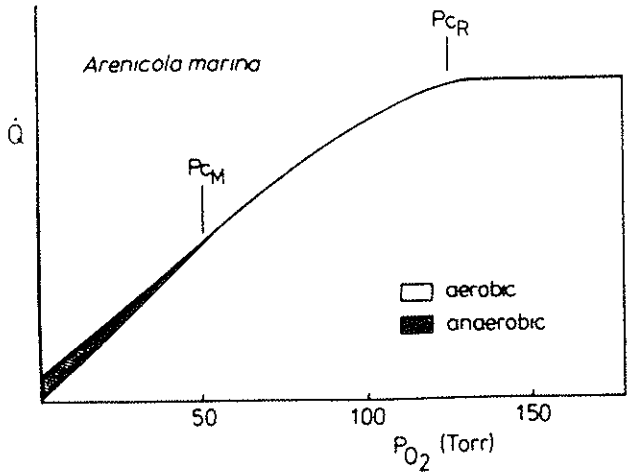

Figure 14. Semiquantitative illustration of the changes in aerobic and anaerobic heat dissipation (Q) in *Arenicola marina*. A difference prevails between Pc_M and Pc_R, the P_{O_2} range between these partial pressures indicating the range of aerobic oxyconformity (for further explanations see text, based on Schöttler *et al.* (61), Toulmond (31), and Toulmond and Tchernigovtzeff (32)).

One might speculate that in aerobic oxyconformers the increase in the rate of oxygen uptake does not necessarily reflect an increase in metabolic rate. This would mean a partial uncoupling of oxygen consumption and energy production. Such a mechanism is likely to exist in another group of oxyconformers, among parasitic helminths. The presence of cytochrome o causes a large fraction of the oxygen consumption to be independent of the cytochrome oxidase reaction (Figure 16). It leads to hydrogen peroxide formation and may be involved in oxygen detoxification (2,9). Cytochrome o is not found in oxyregulating parasites (2). This mechanism, which still needs to be investigated in many free living oxyconformers (*cf.* Mendis and Evans (44)), may consume excess oxygen in the animal's environment for detoxification. This makes sense since the aerobic oxyconformers considered so far live in oxygen-poor environments where excess oxygen could be removed by this mechanism.

The cytochrome o reaction is linked to partial uncoupling of the respiratory chain with a P/O ratio of 1 (12). An uncoupling of the rate of oxygen consumption from the actual metabolic rate results, which is not detectable by calorimetry (*cf.* Figures 13, 14). Both oxygen consumption measurements and calorimetry will lead to an overestimate of the metabolic rate, the actual rate possibly being much less than suggested by the degree of oxyconformity (*cf.* Figure 16). If these assumptions really hold true then aerobic oxyconformity could be identified as being a relict among primitive metazoa adapted to life in oxygen-poor environments like the hydrogen sulfide layer of marine and fresh water sediments.

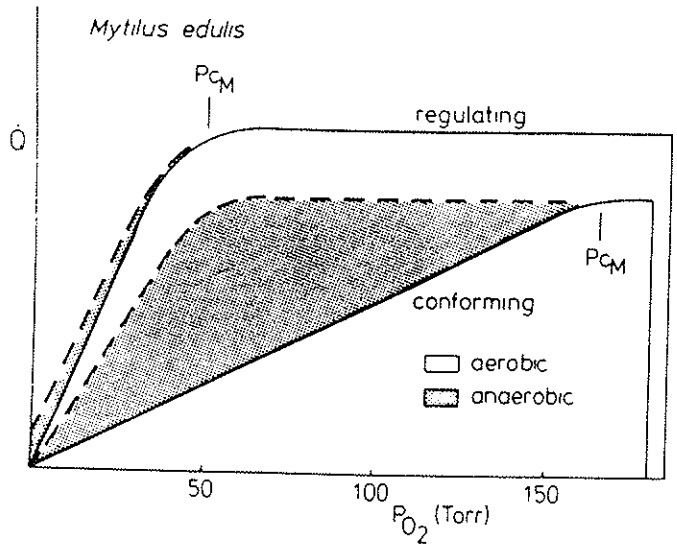

Figure 15. In *Mytilus edulis*, the transition from oxyregulation to oxyconformity very likely means a shift of Pc_M to higher values (see text). During hypoxia, anaerobic energy production has to compensate for the energy gap down to a P_{O_2} (perhaps the former Pc_M of the regulating animal), below which the overall metabolic rate declines (based on Bayne (3), and Famme *et al.* (16)).

Dwelling in the sediment may lead to a change from oxyconformity to oxyregulation in these animals which depend on their ventilatory activity for oxygen provision. They could, thereby, minimize the extent to which oxygen detoxification by cytochrome o is necessary.

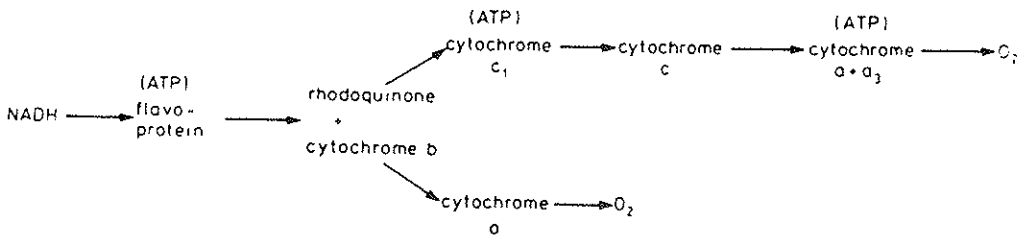

Figure 16. Simple scheme of the respiratory chain showing the sites of oxygen consumption and ATP synthesis. Oxygen consumption via cytochrome o, as known for oxyconforming endoparasites, leads to a partial uncoupling of oxygen consumption and ATP synthesis (modified after Cheah (12)).

The results obtained with *Arenicola marina* dwelling in artificial (glass) burrows are somewhat contradictory. For example, complete oxyregulation was found above an ambient P_{O_2} of 22 Torr (at 16°C), when the animals were irrigating a U-shaped tube which resembles their natural burrow (41). In animals dwelling in a simple tube, this type of regulation was not present and aerobic oxyconformity was found up to a P_{O_2} of 120 Torr (Figure 13) (73). Future experiments must show whether *Arenicola* is able to "regulate" the amount of oxygen pumped into its burrow. Ventilatory regulation of oxygen provision appears possible based on epidermal oxygen sensors in this species (71).

With the present review we have tried to draw a picture of the interrelationships between oxygen availability, oxygen consumption and metabolic rate, which tries to encompass many of the experimental findings in vertebrates and invertebrates and which could provide the framework for a useful comparison of oxyregulating and oxyconforming animals. We know that some of our ideas are hypothetical and hope that these will stimulate future research to prove or disprove what we feel are unifying principles in determining the relationship between oxygen availability and metabolism.

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