Metabolic Responses of the Toad *Bufo marinus* to Environmental Hypoxia: An Analysis of the Critical Po_2

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Abstract

Characteristics of the critical Po_2 have been investigated by analyzing both O_2 uptake rates and modes of energy production during hypoxic exposure in the toad Bufo marinus. *After preacclimatization to moderate hypoxia, this oxyregulator exhibits constant rates of O_2 consumption in a wide range of Po_2. Below 30–37 Torr, an increase in O_2 consumption coincides with the onset of anaerobic metabolism. Consequently, a Po_2 in this range is concluded to be equivalent to the critical Po_2. The early decrease in the levels of high-energy phosphates and the accumulation of large amounts of lactate in the kidney and, even more so, the heart indicate that limits in the O_2 supply to aerobic organs determine the hypoxia tolerance of the animal. The appearance of lactate in the plasma and the concomitant increase in O_2 consumption suggest that lactate may mediate this increase, thus eliciting a stress response.*

Introduction

Many amphibian species are well adapted to survive long periods of hypoxia. Some of them (e.g., *Bufo marinus;* Zug and Zug 1979) live in burrows and, thus, may actively restrict the supply of O_2. Others (e.g., *Xenopus laevis;* Boutilier and Lantz 1989) voluntarily undergo periods of hypoxia also during diving. Amphibians, therefore, may be most suitable for studying the changes in metabolic rate and gas-exchange parameters in response to decreasing ambient O_2 tensions.

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A complete picture of metabolic changes in response to hypoxia can be achieved if changes in both O_2 consumption and anaerobic metabolism are considered. For example, the ambient P_{O_2} may be determined below which the animals start to rely on an anaerobic metabolism. This approach allowed the establishment of a critical P_{O_2} (P_c) in the oxyconformer *Sipunculus nudus* (Pörtner, Heisler, and Grieshaber 1985). The P_c not only indicates the transition to anaerobic energy production but is also characterized by the accelerated decline in overall O_2 consumption and a reduction of O_2 uptake via the blood (coelomic fluid). Studies in oxyregulators would suggest that a critical P_{O_2} exists as well that is not only characterized by the transition from a regulatory pattern to oxyconformity but also by a transition to anaerobic energy production (e.g., Pelster, Bridges, and Grieshaber 1988).

In spite of the existence of numerous studies on gas exchange (sites) in hypoxic amphibians, a similar analysis of the P_c has not been performed. Therefore, this study analyzes how hypoxia affects the steady-state metabolic rate of the toad *B. marinus* and evaluates the P_c in this amphibian by correlating changes in the overall O_2 consumption and O_2 uptake from the blood with the transition to anaerobic energy production. This study was designed such that the following requirement was met: The lowest steady-state aerobic metabolic rate was achieved during long-term measurements of O_2 consumption. Stress effects and potential struggling were minimized by preadapting the animals to intermediate values of P_{O_2} before exposing them to low O_2 tensions. Lactate and succinate were evaluated in tissues and plasma as indicators of cytosolic and mitochondrial anaerobic energy production, respectively (Grieshaber, Kreutzer, and Pörtner 1988).

Material and Methods

Animals

Toads (*Bufo marinus,* 200–500 g) were obtained from a commercial dealer (Sullivan, Nashville). After shipment they were kept at room temperature and ambient light in large aquaria (0.4 m^3) with a bottom layer of sand. They were provided with ample water and fed on beef liver on a weekly basis. The animals were used after a period of starvation of at least 24 h.

Cannulation

For the evaluation of blood gas values, toads were cannulated both in the ischiadic artery of the right leg and the femoral vein of the left leg. Prior to cannulation the animals were anaesthetized in a pH-neutralized solution of MS-222 (1.5 g L^{-1}). Catheters were filled with heparinized (125 IU/mL)

Mackenzie saline (de la Lande, Tyler, and Pridmore 1962) before being inserted. The artery was cannulated occlusively in an upstream direction by means of a PE 60 catheter (Boutilier et al. 1979). The femoral vein was uncovered dorsally on the cranially oriented side of the thigh, close to the pelvic girdle. The nonocclusive cannulation used a PE 50 catheter (drawn to a fine tip with three added holes to prevent blockage), which was placed in an upstream position, tied to the wall of the vena with fine surgical suture, and sealed with cyanacrylate glue (Bostik no. 7432, Bostik GmbH, Oberursel, F.R.G.). Catheters were secured to the surrounding musculature with nylon sutures. The wounds were closed and sealed by use of nylon sutures and cyanacrylate glue.

Measurements of O_2 Consumption and CO_2 Release

For the calculation of O_2 consumption according to Withers (1977), the measurement of both O_2 and CO_2 was required. Gas exchange was measured by using Ametek Applied Electrochemistry (Pittsburgh) equipment: N-37M double-cell O_2 sensor and S-3AII O_2 analyzer, P61B CO_2 cell, and CD-3A CO_2 analyzer, and R-2 flow control. Oxygen readings were differential, whereas the measurement of CO_2 required repeated changes between calibration and sampling gases at regular intervals. Calibration gases and gases withdrawn from the animal chambers were led through glass columns filled with drierite before being fed into the sensors. All gases were prepared from pure nitrogen, CO_2, and O_2 by gas-mixing pumps (type 303/a-F, Wösthoff AG, Bochum, F.R.G.) and saturated with water at 20 ± 0.1°C. The room in which the experiments took place was controlled at 20 ± 1°C.

The influence of external disturbances and of the photoperiod on diurnal fluctuations in O_2 consumption was minimized by keeping the animal in a darkened chamber (volume: 2.97 L) containing 400 mL of dechlorinated tap water. Water replacement was possible through a drain with minimal disturbance of the animal. Toads were allowed to rest for 24 h in air delivered by gas-mixing pumps. Oxygen consumption was analyzed during a stepwise reduction of the O_2 partial pressure. Water was changed at the end of each measurement period, before equilibration with a new P_{O_2} was begun. In accordance with the results of Hutchinson and Kohl (1971), O_2 consumption was found to be stable at a low level during the morning, diurnal changes being minimized during long-term measurements and under hypoxia. Therefore, readings were taken during the morning as long as diurnal changes were present. The measurement period, usually, was started after 12–14 h of equilibration at a new ambient P_{O_2}. Animals that did not survive 24 h at 3% O_2 were omitted from the analyses.

Metabolite Studies

For the investigation of changes in metabolite levels under different degrees of hypoxia, single animals were put into darkened 4-L flasks containing 400 mL of dechlorinated tap water each. Gases were prepared and temperature was maintained as described above. After 24 h of normoxia, control samples were taken. Other animals were exposed to normoxia and then to moderate hypoxia (P_{O_2} = 75 Torr, 1 Torr = 133.3 Pa). The transition to severe hypoxia (one P_{O_2} value below 75 Torr) only occurred after 12 h of preacclimatization to moderate hypoxia (P_{O_2} = 75 Torr). At the end of control and experimental periods (24 h at all O_2 tensions except 14 Torr, where incubation lasted 6 h), blood samples were withdrawn anaerobically and analyzed for P_{O_2} with Radiometer (Copenhagen) equipment (thermostated to the incubation temperature of the animals, ±0.1°C). Blood was collected in cooled Eppendorf tubes and centrifuged immediately for 1 min at 1,000 *g*. The plasma fraction was used for the analysis of metabolites.

The water in the animal chamber was replaced with a pH-neutralized solution of 3 g MS-222 (Sigma) L^{-1} in dechlorinated tap water. After about 10 min the flask was opened and the immobilized animal easily removed. From the fully anaesthetized animal, the gastrocnemius muscle of the left leg and both sartorius muscles, the ventricle, and the kidneys were excised, and the animal was pithed. All tissue samples were freeze clamped with a pair of aluminum tongs precooled in liquid nitrogen (Wollenberger, Ristau, and Schoffa 1960). This procedure helped to eliminate a large fraction of the residual blood from the ventricle, which froze on the tissue surface and could then be easily removed. This was especially important in animals exposed to severe hypoxia, where, in accordance with a vasoconstriction in peripheral tissues (Armentrout and Rose 1971), the blood volume of the heart appeared to be enlarged.

Tissue samples were ground under liquid nitrogen with a porcelain mortar and pestle. Fractions of the tissue powder were subjected to extraction in ice-cold 0.6 M perchloric acid in accordance with Beis and Newsholme (1975) through the homogenization procedure of Pette and Reichmann (1982). The extract was centrifuged for approximately 1 min in an Eppendorf centrifuge at maximum speed. The supernatant was neutralized to pH 7.5–8 by titration with 5 M KOH (10% of the volume of perchloric acid) and a mixture of solid K_2CO_3/$KHCO_3$ (1/6, w/w) (Pörtner 1990).

Perchloric acid (3 M) was added to a final concentration of 0.6 M in toad plasma. After mixing on a Vortex mixer (Scientific Industries, Bohemia, New York) the precipitate was removed by centrifugation (about 1 min at maximum speed). Neutralization of the supernatant occurred as described above. The neutralized extracts of the plasma and/or the tissues were an-

alyzed for phosphocreatine, creatine, ATP, lactate, and succinate by using standard enzymatic procedures (Bergmeyer 1984). Inorganic phosphate was assayed as described by Pörtner (1990). All chemicals were purchased from Sigma, Saint Louis, Missouri.

Differences were tested for significance at the 5% level by using Student's *t*-test for paired and unpaired samples.

Results

Oxygen consumption rates ($\dot{M}O_2$) of *Bufo marinus* did not change significantly between 1.17 ± 0.06 μmol g^{-1} h^{-1} at normoxia and 1.04 ± 0.23 μmol g^{-1} h^{-1} at P_{O_2} = 37 Torr (fig. 1). At P_{O_2} = 21 Torr, however, $\dot{M}O_2$ increased significantly, 1.5-fold above the value observed at 37 Torr, reaching 1.54 ± 0.22 μmol g^{-1} h^{-1}. At P_{O_2} = 14 Torr, no steady-state rate of O_2 consumption

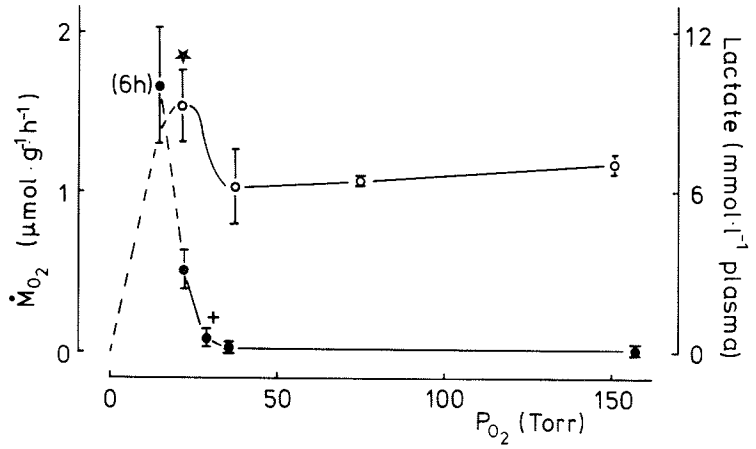

Fig. 1. Specimens of Bufo marinus *preadapted to mild hypoxia (P_{O_2} = 75 Torr) exhibited a more or less constant rate of O_2 consumption ($\dot{M}O_2$, ○) down to P_{O_2} values below 50 Torr. A steady-state increase was observed at P_{O_2} = 21 Torr. Below this P_{O_2} no steady-state rate of O_2 uptake was maintained (indicated by the dashed line). Lactate levels in the plasma (●) increased when the O_2 consumption rose. (The dashed line reflects that lactate levels were measured after 6 h at 14 Torr but after 24 h at all other O_2 tensions. Symbols and bars represent* $\bar{X}$ ± *SD,* n = *4 for O_2 consumption data, values of* n *for lactate levels as given in table 1; * indicates significant difference,* $P < 0.01$*, from animals exhibiting complete aerobiosis in hypoxia, P_{O_2} = 37 Torr, paired samples; + indicates onset of significant deviation from normoxic controls,* $P < 0.05$*, unpaired samples.)*

was maintained over 24 h. In accordance with the onset of a bradycardia and the decline in ventilation frequency found during transition to severe hypoxia by Boutilier and Toews (1977), O_2 consumption exhibited a steady decrease before the animals became asphyxic.

Starting at values of 64.7 ± 12.3 and 51.2 ± 7.5 Torr, respectively, both arterial and venous P_{O_2} (valid for the hindlimbs of the animals) decreased, the decrease being smaller between normoxia and an ambient P_{O_2} of 75 Torr (fig. 2). In the same range of P_{O_2}, the difference between arterial and ambient O_2 tensions fell from 93 to 31 Torr. Below 75 Torr (ambient) this difference decreased to values below 10 Torr. The arteriovenous P_{O_2} difference was also reduced when the ambient P_{O_2} fell. At oxygen tensions of 75 and 157 Torr, significant arteriovenous differences of 5.9 and 13.5 Torr were found, respectively. In the range of ambient P_{O_2} between 14 and 40 Torr, mean venous P_{O_2} was 2.6 Torr below arterial P_{O_2} (significantly different

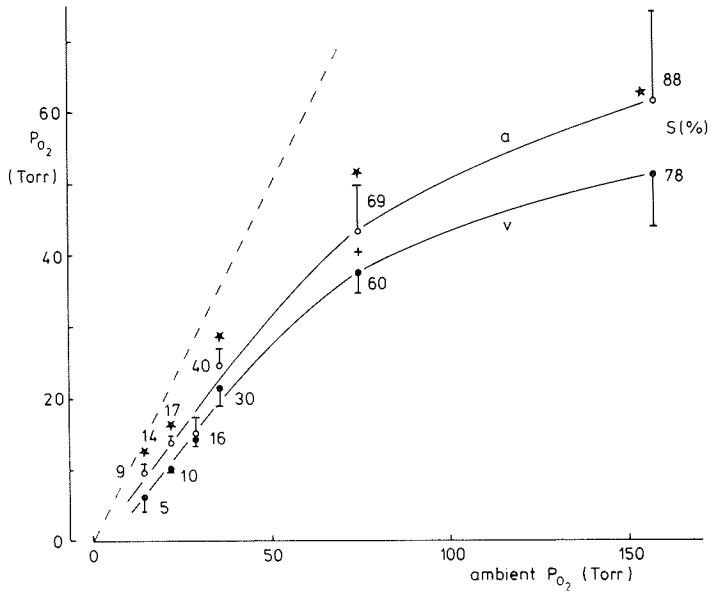

Fig. 2. Arterial (a, ○) *and venous* (v, ●) O_2 *tensions in* Bufo marinus *subjected to changes in ambient* P_{O_2}. *The dashed line is the line of equality for blood and ambient* P_{O_2}. *Approximate values of hemoglobin saturation* (S, *in %*) *were derived based on* O_2*-binding curves published by Boutilier et al. (1987) considering the Bohr coefficient given by Boutilier and Toews (1981) and pH values by H. O. Pörtner and D. P. Toews (unpublished data). (Symbols and bars represent* $\bar{X}$ ± *SD; for values of* n *see table 1;* * *indicates significant arteriovenous difference,* $P < 0.01$–0.05, *paired samples;* $^{+}$*indicates onset of significant deviation from normoxic controls,* $P < 0.01$–0.02, *unpaired samples; curves fitted by eye.)*

in most cases). Down to an ambient P_{O_2} of 35 Torr these differences in arterial and venous P_{O_2} represent a venous hemoglobin saturation by about 10% below arterial values. At lower O_2 tensions smaller oxygenation differences occurred.

Between ambient O_2 tensions of 35 and 29 Torr arterial plasma lactate increased slightly but significantly from 0.18 ± 0.02 mmol L^{-1} to 0.54 ± 0.32 mmol L^{-1} (fig. 1). Differences between arterial and venous lactate levels were always negligible. Exposure to 22 Torr led to an increase to 3.09 ± 0.71 mmol L^{-1}. Incubation at 14 Torr (asphyxia was not yet reached in most animals) caused lactate values to rise to 10.0 ± 2.2 mmol L^{-1} within 6 h. As evidenced by figure 1 the onset of lactate formation and the increase in O_2 consumption occurred in the same range of P_{O_2}. The increase in plasma lactate was accompanied by an increase in tissue lactate levels (fig. 3). Ventricular lactate at P_{O_2} = 22 Torr (6.5 ± 2.6 μmol g^{-1} fresh wt) was much greater than the respective values found in the skeletal musculature (1.4 ± 0.8 μmol g^{-1} fresh wt) and in the kidney (2.9 ± 0.5 μmol g^{-1} fresh wt). After 6 h at 14 Torr tissue lactate levels were even higher than those found after 24 h at 22 Torr. Again the highest values were found in the ventricle (8.4 ± 2.7 μmol g^{-1} fresh wt; fig. 3). The succinate content in the ventricle had increased significantly by 0.35 μmol g^{-1} fresh wt.

Table 1 and figure 4 demonstrate to what extent high-energy phosphates were affected by hypoxic exposure. The validity of all metabolite data presented for the various tissues from control and experimental animals depends on the assumption that the metabolic status of the tissues is, at most, min-

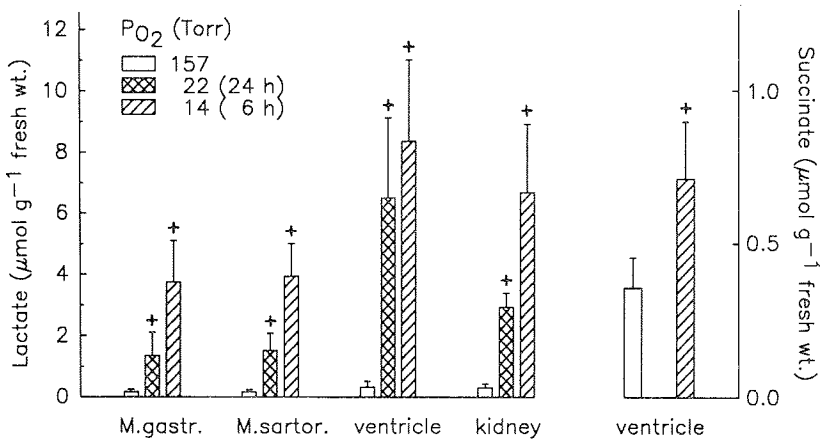

Fig. 3. Lactate formation was highest in aerobic tissues (e.g., ventricle and kidney). Succinate formation occurred in the ventricle. (Bars represent $\bar{X}$ ± *SD, for values of* n *see table 1;* + *indicates significant difference from normoxic controls,* $P < 0.01$–0.02, *unpaired samples.)*

TABLE 1
Levels of phosphocreatine (PCr), creatine (Cr), ATP, and inorganic phosphate (Pi) in various organs of the toad Bufo marinus *exposed to different levels of hypoxia* ($\mu mol\ g^{-1}$ *fresh wt*)

P_{O_2} (Torr)	PCr	Cr	ATP	Pi
M. gastrocnemius:				
157	17.3 ± 1.2	7.3 ± 1.4	3.4 ± .4	1.6 ± .3
22 (24 h)	14.3 ± 1.6*	9.4 ± .7*	3.3 ± .4	4.1 ± 1.3*
14 (6 h)	10.0 ± 2.3*	12.5 ± 2.0*	3.5 ± .2	5.6 ± 1.9*
M. sartorius:				
157	18.4 ± 2.1	7.7 ± 1.5	3.2 ± .4	1.9 ± .3
22 (24 h)	15.8 ± 1.0*	8.3 ± 1.0	2.7 ± .5	3.3 ± 1.3
14 (6 h)	12.1 ± 1.9*	11.7 ± 2.6*	3.1 ± .3	4.0 ± 1.5*
Ventricle:				
157	3.3 ± .9	3.8 ± 1.1	2.7 ± .4	2.3 ± 1.8
22 (24 h)	1.1 ± .4*	3.7 ± .5	2.7 ± .4	3.8 ± 1.0
14 (6 h)	.9 ± .3*	3.9 ± .8	1.5 ± .1*	3.8 ± 1.3
Kidney:				
157	.8 ± .1	1.0 ± .2	1.6 ± .2	3.0 ± .5
22 (24 h)	.3 ± .1*	1.1 ± .1	1.0 ± .2*	3.4 ± .3
14 (6 h)	.2 ± .1*	.9 ± .2	.9 ± .1*	3.2 ± .6

Note. Data are $\bar{X} \pm$ SD, $n = 5$, except for incubation under $P_{O_2} = 14$ Torr, where $n = 4$; * indicates significant difference from normoxic controls, $P < 0.01$–0.05, unpaired samples.

imally affected by the anaesthesia, excision, and extraction procedures. That this is actually true is supported by the ratio of phosphocreatine over inorganic phosphate levels found in the skeletal muscles under control conditions. Phosphocreatine is most sensitive as an indicator of metabolic stress since it is readily metabolized during O_2 deficiency and muscular activity, thus leading to the accumulation of inorganic phosphate. However, inorganic phosphate levels under control conditions were found close to those observed in ^{31}P-NMR studies of intact amphibian muscle (e.g., Tanokura and Yamada 1984). It can be concluded that the phosphorylation of creatine was close to maximum in samples obtained from control animals and was, thus, unaffected by the sampling or extraction procedure.

Adenosine 5′-triphosphate levels did not change significantly in the skeletal muscle tissues. An early significant drop occurred in the kidney at $P_{O_2} = 22$

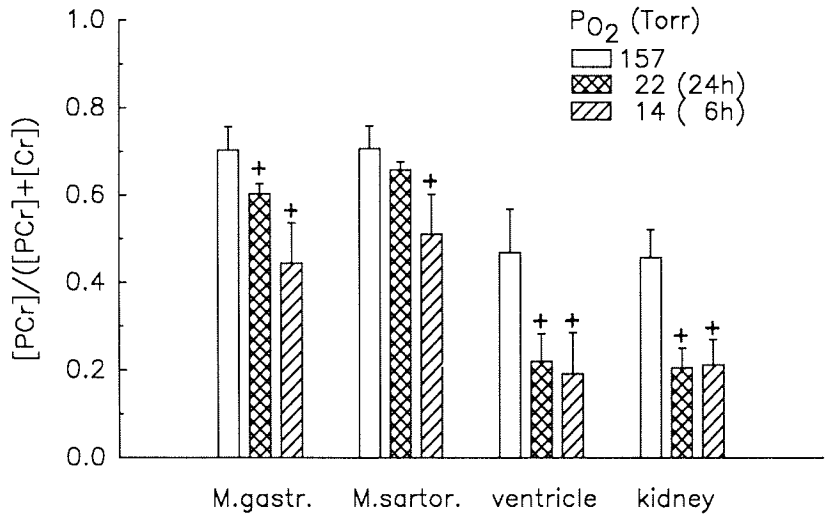

Fig. 4. The ratio of phosphocreatine to phosphocreatine + creatine contents, [PCr]/([PCr] + [Cr]), dropped to a larger extent in the aerobic (ventricle, kidney) than in the anaerobic organs (gastrocnemius and sartorius muscles). (Bars represent $\bar{X} \pm SD$, *for values of* n *see table 1; + indicates significant difference from normoxic controls,* $P < 0.01$, *unpaired samples.)*

Torr, whereas a drop in the ATP levels of the ventricle was only observed after 6 h at 14 Torr. The early drop in kidney ATP was correlated with the low phosphocreatine levels in this tissue under control conditions. In both heart and kidney the lowest phosphocreatine levels and the lowest values for the ratio of phosphocreatine/(phosphocreatine + creatine contents) (fig. 4) were already reached after 24 h at P_{O_2} = 22 Torr. Creatine levels remained constant during phosphocreatine depletion in both organs, suggesting that some creatine had been released into the blood.

The phosphocreatine stores were much higher in the skeletal muscles. In both gastrocnemius and sartorius muscles a slight drop was observed after 24 h at 22 Torr, which was significant only in the gastrocnemius. The decrease in phosphocreatine contents and in the ratio of [PCr]/([PCr] + [Cr]) was more prominent after 6 h at 14 Torr. In the muscle tissues the drop in phosphocreatine levels was mirrored by a concomitant significant increase in the creatine content. When phosphocreatine levels had fallen only slightly (at P_{O_2} = 22 Torr), a more or less equimolar increase in inorganic phosphate levels occurred. This was no longer true at P_{O_2} = 14 Torr, when, possibly owing to its release into the extracellular fluids, the increase in inorganic phosphate remained below the change in phosphocreatine contents (table 1).

Discussion

Oxygen Consumption and Mode of Energy Production

The response of *Bufo marinus* to declining O_2 tensions in the P_{O_2} range above 30 Torr was clearly that of an oxyregulating animal. Oxygen uptake was very likely maintained by increasing numbers of breathing periods (cf. Shelton and Boutilier 1982; Glass and Wood 1983; Kruhoffer et al. 1987), leading to a progressive reduction in the difference between arterial and ambient O_2 tensions (cf. fig. 2). However, the rising cost of ventilation did not significantly influence the regulated rate of O_2 consumption. The constant O_2 uptake rate was also reflected by a constant arteriovenous oxygenation difference in the hindleg that was maintained despite a decrease in the arteriovenous P_{O_2} difference (fig. 2). This finding is in accordance with the observation that the animals were still completely aerobic. Below an ambient P_{O_2} of 35 Torr, however, the oxygenation difference started to fall, indicating when O_2 gradients became too small for the maintenance of O_2 consumption (fig. 2). The onset of an anaerobic energy metabolism would compensate for a reduced O_2 uptake from arterial blood. This not only happened in peripheral tissues, where vasoconstriction further reduced the O_2 supply (Armentrout and Rose 1971), but also in the central aerobic organs (ventricle and kidney; fig. 3; table 1).

Below 30–37 Torr, O_2 consumption increased and, concomitantly, lactate accumulation started in the plasma. Rates of lactate accumulation were low in the skeletal muscles, even leading to tissue levels below those prevailing in the plasma. Lactate levels were highest in the kidney and, especially, the working heart. High lactate levels were also reported for the anoxic frog brain (Wegener, Michel, and Thuy 1986). Net lactate formation would lead to glycogen depletion in the respective tissue as described by Rose and Drotman (1967) for the amphibian heart. High rates of lactate formation in these and other aerobic tissues would indicate that part of the energy required for the maintenance of function during hypoxia was provided by use of anaerobic glycolysis. As a corollary, these organs may be responsible for a large fraction of the lactate found in the plasma.

Hypoxia led to the formation of succinate in the ventricle, indicating that mitochondria produced energy anaerobically (fig. 3). The phosphocreatine stores in the aerobic tissues were smaller than in the skeletal muscle and, therefore, were not able to sufficiently buffer ATP levels. The large drop in the ratio of [PCr]/([PCr] + [Cr]) was accompanied by a depletion of ATP. These processes reflect high energy requirements and may impair the long-term function of the aerobic organ under hypoxia. The observation that high-energy phosphates remained elevated in skeletal muscle but not in

kidney and heart supports the conclusion that the aerobic tissues set the limit for the whole-animal hypoxia tolerance.

Standard Metabolic Rate and Critical P_{O_2}

The lowest rate of O_2 consumption found during long-term measurements is equivalent to the standard metabolic rate (SMR; Beamish and Mookherjii 1964; Ultsch, Ott, and Heisler 1980). If SMR is monitored during a decline in ambient O_2 tension, the critical P_{O_2} may be assessed as the P_{O_2} below which oxyregulation ceases. This critical P_{O_2}, however, should also be characterized by the onset of anaerobic metabolism. Standard metabolic rate is then equivalent to the metabolic rate closest to the lowest aerobic metabolic rate (Grieshaber et al. 1988). The data now available for *B. marinus* suggest that the resulting critical P_{O_2} in this animal does not agree with the expectation that oxygen consumption must decline when anaerobic metabolism becomes involved. If priority is given to the onset of anaerobic metabolism as being crucial for characterizing the P_c, then the cessation of a regulatory pattern in (aerobic and total) metabolic rate coincides with an increase in O_2 consumption.

This analysis of the P_c differs from analyses in other studies. Ultsch et al. (1980) and Yeager and Ultsch (1989) assigned the term critical P_{O_2} to the P_{O_2} where the O_2 uptake rate decreases below the normoxic resting level. However, O_2 uptake may first rise before it finally falls. This critical P_{O_2} cannot even be determined based on figure 1 since, in the respective range of P_{O_2} (between 10 and 15 Torr, according to the dashed line in fig. 1), *B. marinus* was no longer able to maintain a constant rate of O_2 consumption. A value of zero was reached instead, after variable time periods within 24 h. For all of these reasons, no two-segment linear model (Yeager and Ultsch 1989) and no continuous two-phase regression (Nickerson, Facey, and Grossman 1989) are applicable to these data.

Bufo marinus (present study) and other oxyregulators (Teal and Carey 1967; Pamatmat 1978; Pelster et al. 1988) have been shown to maintain SMR down to P_{O_2} values below which anaerobiosis starts. If, with an unchanged physiological status, a decrease in the metabolic rate of an oxyregulating animal occurs before anaerobiosis begins (e.g., Boutilier et al. 1988), it is very likely that the metabolic rate observed at high O_2 tensions was not equivalent to SMR.

In *B. marinus,* aerobic and anaerobic metabolic rates increased concomitantly below the P_c. One possible explanation would be that the onset of anaerobic energy production was an important factor in eliciting the increase in metabolic rate. Hypoxic exposure is known to provoke a release of catecholamines in amphibians (Boutilier and Lantz 1989) or in fish (Butler,

Taylor, and Davison 1979; Boutilier et al. 1988). The start of anaerobiosis, possibly lactate formation and release by itself, could represent an alarm signal mediated, for example, by catecholamines. The resulting stress reaction is unlikely to cause severe struggling since phosphocreatine levels remained elevated in the skeletal muscles even after long-term exposure to hypoxia. However, the rise in metabolic rate could be linked to an increased awareness and nervousness of the animals (supported by video studies of S. C. Wood, personal communication, who found *B. marinus* to become restless after a stepwise transition to severe hypoxia). In fish acclimated to moderate hypoxia by long-term exposure, the increase in O_2 consumption at the P_c tends to be lower than in nonacclimated animals (Beamish 1964). Consequently, the degree of anaerobiosis and, as a corollary, the stress response may be minimized owing to hypoxia acclimatization. Further investigations must substantiate whether these assumptions really hold true.

In conclusion, the present approach of P_c analysis (cf. Pörtner et al. 1985) considers the essential metabolic transition phases during hypoxia in *B. marinus*. The P_c is reached at an ambient O_2 tension between 30 to 37 Torr (at 20°C). It is characterized by the onset of an anaerobic energy production, an increase in O_2 consumption, and a reduction of O_2 uptake by peripheral tissues from the blood.

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