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Anaerobic Metabolism, Gas Exchange, and Acid-Base Balance During Hypoxic Exposure in the Channel Catfish, *Ictalurus punctatus*

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ABSTRACT Gill ventilation, blood gas and acid-base values, $\dot{M}_{O_2}$, $\dot{M}_{CO_2}$ and the gas exchange ratio have been measured before, during, and after exposure to hypoxia in the channel catfish, *Ictalurus punctatus*.

I. punctatus maintains $\dot{M}_{O_2}$ at control levels to a P_{IO_2} as low as 60 mm Hg, through a profound branchial hyperventilation. Concomitantly, however, a lactic acidosis usually develops, indicating a significant anaerobic glycolysis. Both a metabolic acidosis and respiratory alkalosis occur in *Ictalurus* during hypoxic exposure, with the former usually predominating. $\dot{M}_{CO_2}$ doubles and the gas exchange ratio (R_E) increases from 0.8 at control levels to 2.0 at hypoxic levels, indicating that, in addition to anaerobic glycolysis, nonglycolytic pathways producing CO_2 also operate during hypoxic exposure.

Analysis reveals that at least half of the increased $\dot{M}_{CO_2}$ during hypoxic exposure is due strictly to lactate acidification of the tissue HCO_3^- pool, rather than from metabolic production of molecular CO_2. Thus, the actual respiratory quotient (R_Q) only rises to 1.5 during hypoxic exposure.

Within one hour of a return to air saturated water, a large lactate "flush" and severe plasma acidosis occur, and control levels for these and other values are not reachieved for 2–6 hours after hypoxic exposure.

Complete analysis of O_2 and CO_2 exchange in the catfish exposed to short-term hypoxia thus must consider both the time course of acid-base disturbance and the evolution of CO_2 from the acidified tissue bicarbonate pool.

Limitation of oxygen delivery to the tissues, whether due to the increased demands of exercise or the reduced supply during environmental hypoxia, is a physiological problem encountered at some time by almost every fish species. While extensive investigation has centered on adjustments and adaptations evoked to maintain oxygen delivery in the face of potential O_2 limitations, it is also generally acknowledged that various tissues of many fishes can sustain considerable levels of anaerobic metabolism by which the minimum energy requirements of the animal are alternatively satisfied.

Evidence for anaerobiosis is often presented in the form of increased blood, tissue, or urine levels of lactate, one of the major metabolic end-products of vertebrate anaerobic glycolosis. Thus, elevations in lactate during or following environmental hypoxia have been attributed to a progressive shift towards anaerobic glycolysis in many fresh water and marine fishes (Leifestád et al., '57; Heath and Pritchard, '65; Holeton and Randall, '67; Bandurski et al., '68; Caillouet, '68; Wittenberger, '68; Hunn, '69; Burton, '70a, b; Burton, '71; Burton and Spehar, '71; Hughes and Johnston, '78). However, nonglycolytic anaerobic pathways resulting in the evolution of CO_2 and end-products other than lactate, apparently also operate during hypoxic or anoxic exposure in fishes (see Blazka, '58; Burton and Spehar, '71; Hochachka and Somero, '73).

Increases in lactate and other anaerobic end-products in fish offer an indication that anaerobic metabolism is occurring—they cannot, however, provide quantitative information on the time course of development of anaerobiosis or on the anaerobic/aerobic metabolic balance during acute hypoxic expo-

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sure in fishes, because of the often very complex time course of the excretion of lactate and other end-products (Heath and Pritchard, '65; Wood et al., '77; Hughes and Johnston, '78; Wardle, '78). Another way in which insights into the aerobic/anaerobic balance could be gained, particularly in those fish where anaerobiosis results in CO_2 production, is by continuous measurement of the respiratory quotient (R_Q) of the fish under control and hypoxic conditions. However, a severe metabolic acidosis may accompany lactate liberation from the tissues during anaerobiosis (Hughes and Johnston, '78) and determination of R_Q simply on the basis of total CO_2 excretion is probably misleading for the following reason: Acidification of the bicarbonate pools of the body will cause the generation of molecular CO_2. Because the CO_2 solubility in water is high, it is likely that CO_2 liberated from the body HCO_3^- stores by tissue acidification will be excreted along with the CO_2 produced from both aerobic and anaerobic metabolism, particularly if a branchial hyperventilation accompanies hypoxia. Indeed, lactate acidification of the blood as a result of exercise has been shown to reduce CO_2 concentration in carp (Auvergnat and Secondat, '42), tench (Secondat and Diaz, '42), and rainbow trout (Black et al., '59).

During metabolic acidosis, then, it is essential that a distinction be made between the gas exchange ratio (R_E) or the *apparent* R_Q for metabolism, which will be a complex function of metabolism, gas exchange, and acid-base balance, and the true metabolic R_Q. The gas exchange ratio may be larger or smaller than the true metabolic R_Q, depending on acid-base state. Measurements of R_Q in water-breathing animals are rare, due to the difficulties of measuring small CO_2 changes in water, and we know of no study of systematic changes in R_Q related to aquatic hypoxia.

The intent of the present investigation thus was to examine the ventilatory and metabolic responses and acid-base balance of a fresh water teleost before, during, and after hypoxic exposure; and then 1) to determine to what extent total CO_2 elimination was actually the result of metabolic CO_2 production, as distinct from acidification of the bicarbonate pool; and 2) to examine the time course of, and especially recovery from, acidification by anaerobic end-products. The channel catfish, *Ictalurus punctatus,* was chosen for this investigation, as its anaerobic capabilities and plasma acidification during hypoxia have been well established (Burton, '71; Burton and Spehar, '71).

MATERIALS AND METHODS

Experiments were performed on 21 channel catfish, *Ictalurus punctatus,* weighing between 520 and 1069 g (mean weight 825 ± 145 g), and obtained from a commercial supplier. All experiments were carried out on fasting individuals at 18°C, and catfish were previously acclimated to this temperature for several days before use.

Branchial ventilation and gas exchange

Values for branchial ventilation and oxygen extraction, $\dot{M}_{O_2}$ and $\dot{M}_{CO_2}$, and blood gas data, pH and plasma lactate concentrations were measured in unanaesthetised, partially restrained catfish resting in a darkened plexiglass chamber, which provided for complete separation of inspired and expired water (Fig. 1). Before placing the catfish in the box, a rubber sleeve formed from the thumb of a latex surgical glove was carefully stitched over the mouth of the catfish, during MS 222 anaesthesia. By judicious placement of the glove around the lips, inspired water could be separated from expired water without apparently "loading" or otherwise impeding the buccal or opercular pumps. The opening of the rubber sleeve was stretched over a tube cemented into a plexiglass partition, which served to divide the chamber into a small anterior section (vol. 950 ml), and a larger posterior section containing the fish (vol. 2,700 ml, including volume of fish). Water entering the anterior chamber (500–1000 ml/min), which was in excess of the ventilatory requirements of the catfish (100–500 ml/min), spilled from the chamber via a fire-polished glass standpipe. All water pumped over the gills into the posterior chamber spilled out via a second, posterior standpipe, adjusted to the same height as the anterior one. The expired water was collected in a volumetric flask, giving a direct measurement of gill ventilation volume ($\dot{V}_g$).

A water-tight plexiglass lid covered the entire water surface, with the exception of two elevated water wells extending 3 cm above the lid level (Fig. 1), through which the standpipes were exposed to the atmosphere. Water collected as it flowed up into the anterior well was considered representative of inspired water. Minimum wash-out time for the anterior chamber was 1–2 min. The surface area exposed to room air in the posterior well was small, and the ventilatory flow through this well quite rapid (> 100 ml/min). It was thus assumed that water collected through the pos-

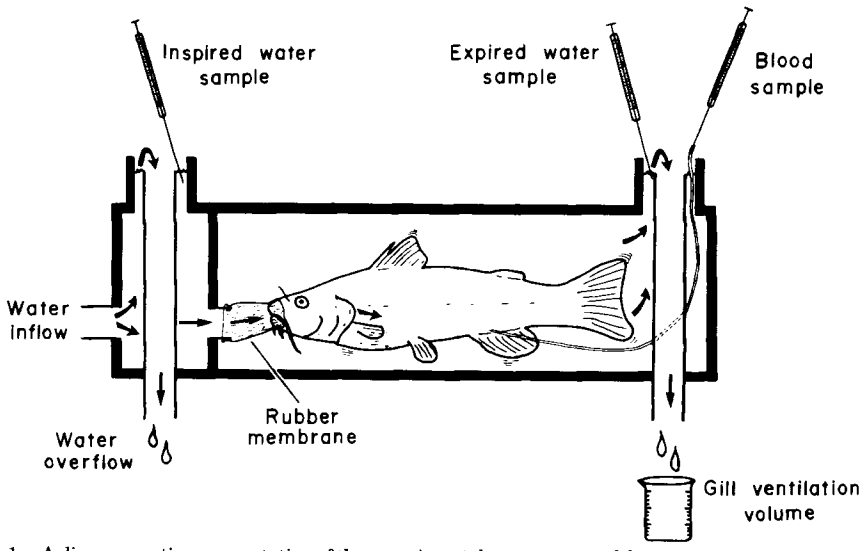

Fig. 1. A diagrammatic representation of the experimental apparatus used for most experiments. See text for details.

terior well was representative of expired water, once a steady-state flow and adequate turnover of water in the posterior chamber according to wash-out times had been achieved after any change in ventilatory state. Estimates of minimum wash-out time for the posterior chamber containing a 1 kg catfish with a $\dot{V}_g$ of 200 ml/min are 8–12 minutes, while with a hypoxic $\dot{V}_g$ of 300 ml/min, minimum wash-out time falls to 5–7 minutes. This box thus combined the advantages of a flow-through respirometry apparatus for $\dot{M}_{O_2}$ and $\dot{M}_{CO_2}$ determination, and van Dam type system (van Dam, '38) for monitoring gill ventilation.

Before placement in the experimental apparatus, the caudal artery or caudal vein of each catfish was nonocclusively cannulated, using a trochar insertion, with a PE 50 catheter filled with heparinized saline. Once in the chamber, the catheter from the fish was led out through the posterior water well, allowing undisturbed blood sampling.

Oxygen contents of inspired and expired water were determined by measuring the water P_{O_2} with a Radiometer pHM 71 blood gas analyzer and electrodes, and then multiplying by the solubility coefficient for O_2 in the water at 18°C. Carbon dioxide content was determined by a conductometric technique adapted from Maffly ('68). These data, along with ventilatory flow and body weight, permitted the calculation of $\dot{M}_{O_2}$, $\dot{M}_{CO_2}$, and the gas exchange ratio.

Ventilatory frequency was monitored visually by simply observing the prominent pulsation of the water meniscus in the posterior standpipe well, which was produced by branchial pumping.

Blood P_{O_2} and pH were measured with the Radiometer pHM 71 blood gas analyzer and appropriate electrodes, while blood O_2 content was measured with the method described in detail by Tucker ('67). Blood CO_2 content was determined with the conductometric apparatus referred to above. A commercially available assay (Sigma) was used to measure plasma lactate levels spectrophometrically.

The volumetric mixing technique of Edwards and Martin ('66) was used to construct blood oxygen dissociation curves in vitro for *I. punctatus*.

Intracellular pH and bicarbonate

The mean whole-body intracellular pH was measured using the DMO technique (Waddell and Butler, '59). Animals were cannulated in the caudal artery as described above, and allowed to recover overnight. An injection solution containing approximately 10 μCi of ^{3}H-labelled inulin or mannitol and 4 μCi of ^{14}C-labelled DMO (New England Nuclear) was infused slowly over a 2 to 4 min period. The solution generally contained ~ 20 μl of ethyl acetate and 100 μl of ethanol made up to 500 μl with Courtland saline. The infusion was rinsed in with another 0.3 ml of saline. Blood samples were then removed at regular intervals, when the following measurements were made: pH,

total plasma CO_2, and activity of tritium and ^{14}C. The inulin or mannitol data were used to calculate extracellular fluid volume (ECF) by calculating the zero time intercept of a regression of log (corrected ^3H dpm) versus time. Inulin space was routinely corrected upward by 7%, in order to express all results as mannitol space, since mannitol yields consistently larger values (Cameron, unpublished data).

Mean whole-body intracellular pH was calculated using the formula given by Heisler ('78), and ECF (mannitol) volume, and an ICF (intracellular fluid) volume obtained as the difference between ECF and total body water. The latter is 68.0 ± 1.0 g/kg (Cameron, unpublished observations), obtained by freeze-drying whole fish to constant weight. From values of intracellular pH (pH_i), plasma pH (pH_e), total plasma CO_2 (C_T), and the ECF and ICF volumes, both ECF and ICF bicarbonate pool sizes could be calculated, assuming a uniform P_{CO_2}. Intracellular HCO_3^- was calculated from the Henderson-Hasselbalch equation (1), which by rearrangement yields an expression for intracellular total CO_2 (C_{T_i}) (2).

$$pH = pK_1' + \log [(C_T/S_{CO_2}) - 1] \quad (1)$$

$$C_{T_i} = C_{T_e} \frac{1 + 10(pH_i - pK_2)}{1 + 10(pH_e - pK_2)} \quad (2)$$

Where pK_1' equals the pK for carbonic acid at 18°C (6.15), pK_2 was the value for DMO at plasma ionic strength and 18°C (6.20 from Heisler, '78), and S_{CO_2} equals dissolved CO_2 concentration.

Experimental protocol

$\dot{M}_{O_2}$, $\dot{M}_{CO_2}$, the gas exchange ratio, and ventilatory and blood gas/plasma variables were determined only in apparently undisturbed catfish which had been in the experimental apparatus at least overnight, and often for 24–36 hours. In addition, several unrestrained fish resting in nonrestrictive, darkened water chambers were examined. After this acclimation, baseline data were collected over 2–3 consecutive hours at an inspired P_{O_2} (P_{IO_2}) of 150–153 mm Hg until a steady state was reached. The P_{IO_2} was then reduced in a stepwise fashion to either approximately 105 mm Hg or 65 mm Hg. Data were then collected at about half-hour intervals until 2–3 consecutive readings indicated arrival at a new respiratory steady state. After this, the P_{IO_2} was then returned to air saturation and monitoring continued until all control baseline values were once again evident, following which the other level of hypoxia would be induced. Thus, after each period of hypoxic exposure catfish were allowed a complete recovery under normoxic conditions.

RESULTS

Steady-state values during normoxia and hypoxia

Gas exchange

Oxygen consumption and carbon dioxide production, gill ventilation and blood gases, pH and lactate were simultaneously measured during normoxia and two levels of hypoxia in eight *Ictalurus punctatus* fitted with the ventilation membrane.

Gill ventilation under control conditions, approximately 160 ml/kg/min, nearly doubled with a drop in P_{IO_2}, from 153 to 105 mm Hg, and was maintained at these elevated levels at P_{IO_2} levels of as low as 65 mm Hg (Fig. 2). The significant increase in $\dot{V}_g$ above control levels was mediated through a doubling in branchial stroke volume; ventilation frequency, approximately 65–70 beats/min, was not significantly different at the three inspired oxygen levels.

The percent utilization of oxygen (% U) at all oxygen levels was relatively constant at

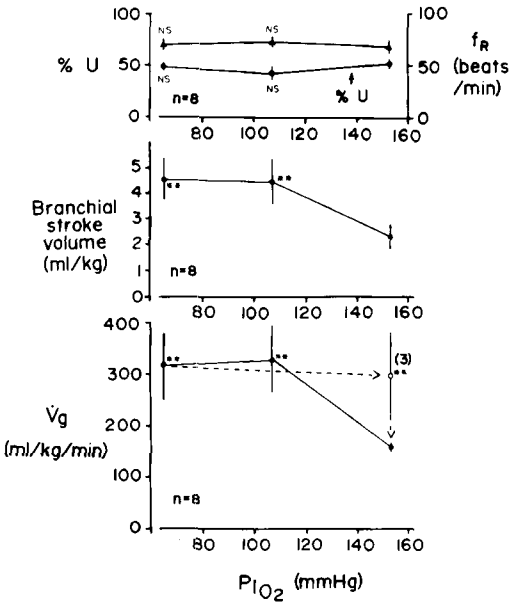

Fig. 2. Steady-state values for oxygen utilization (% U), branchial stroke volume, and gill ventilation volume ($\dot{V}_g$) during normoxic (control) and hypoxic conditions in *Ictalurus punctatus*. Data from 8 catfish are given, except where indicated. In this and all subsequent figures with P_{IO_2} as the abscissa, mean values ± 1 standard error are given, and the asterisks represent the significance level at which data points vary from control values (*, $P < 0.05$; **, $P < 0.01$). N.S. indicates no significant difference. The second, upper value for $\dot{V}_g$ at P_{IO_2} 153 mm Hg (open circle), which is arrived at along the dashed lines, represents a non-steady-state value in air saturated water measured following 1 hour of recovery after exposure to a P_{IO_2} of 65 mm Hg.

40–50%, and since gill ventilation increased at both hypoxic levels, the net effect was that steady-state $\dot{M}_{O_2}$, though showing a slight downward trend in some catfish, did not change significantly from the control values of about 25–30 ml/kg/h (Fig. 3). That is, adult *Ictalurus punctatus* is an oxygen "regulator." Shorter measurement periods would have indicated a different pattern, however, since most fish increased their $\dot{V}_g$ substantially during the first few minutes to one hour of hypoxia, only declining to a new steady state after 1–2 hr of hypoxic exposure (Fig. 4).

Arterial oxygen tension ($P_{a_{O_2}}$) was approximately 80 mm Hg at a $P_{I_{O_2}}$ of 153 mm Hg, falling to less than 20 mm Hg at an inspired P_{O_2} of 65 mm Hg. Over the same $P_{I_{O_2}}$ range, caudal venous P_{O_2} fell from 25 to 8 mm Hg (Fig. 5A). In order to interpret these changes in blood P_{O_2}, the in vitro respiratory properties of *I. punctatus* blood were examined. Oxygen capacity at a P_{O_2} of 153 mm Hg and a pH of 8.02 was 7.8 vol % ± 1.3 (N = 4), falling slightly to 7.4 vol % ± 1.1 (± sd) (N = 4) at pH 7.82. Hematocrit was 30% ± 6% (N = 8). Oxygen dissociation curves determined at 18°C with pooled whole blood samples from four *I. punctatus* are shown in Fig. 5B. P_{50} at a physiological pH during normoxia of 7.90 (se Fig. 6B) was about 20.1 mm Hg, and the Bohr shift (Δ log P_{50}/Δ pH) was comparatively large at −0.839.

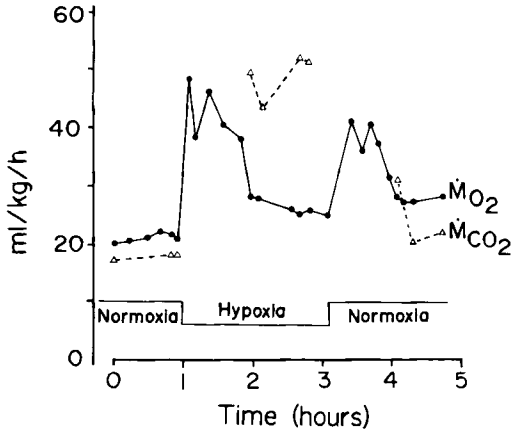

Fig. 4. Variations in oxygen uptake (solid circles) and carbon dioxide excretion (open triangles) during normoxia, hypoxic exposure, and the subsequent recovery period in a 520 g catfish.

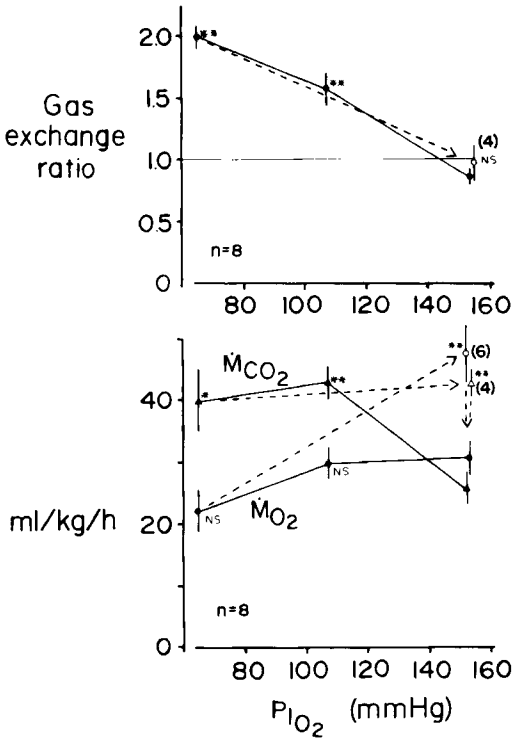

Fig. 3. Steady-state values for oxygen uptake ($\dot{M}_{O_2}$), carbon dioxide excretion ($\dot{M}_{CO_2}$), and the gas exchange ratio (R_E) during control conditions and hypoxic exposure. Also indicated by open symbols are transient values measured after a one hour recovery period from hypoxia as described in Fig. 2 and text.

Using these O_2-Hb dissociation curves and the calculated Bohr shift, arterial blood on the average was at least 95% saturated in vivo during normoxia, falling to only 20–30% saturation when $P_{I_{O_2}}$ was 65 mm Hg. Although caudal venous blood is not necessarily representative of mixed venous blood, arterial-venous oxygen content differences of approximately 2.4 vol % at air saturated $P_{I_{O_2}}$ levels, and 1.5 vol % at a $P_{I_{O_2}}$ of 65 mm Hg, are indicated.

Unlike $\dot{M}_{O_2}$, carbon dioxide excretion ($\dot{M}_{CO_2}$) increased significantly from about 25 ml/kg/h to 40–45 ml/kg/h at a $P_{I_{O_2}}$ of both 105 and 65 mm Hg, considerably exceeding $\dot{M}_{O_2}$ at these levels (Fig. 3). As a consequence, the gas exchange ratio, approximately 0.85–0.90 during normoxic conditions, increased progressively to a steady-state value of 2.0 at a $P_{I_{O_2}}$ of 65 mm Hg.

Water convection requirements for oxygen and carbon dioxide are given in Table 1. $\dot{V}_g/\dot{M}_{CO_2}$ increased only slightly during hypoxic exposure, while $\dot{V}_g/\dot{M}_{O_2}$ was nearly three times control levels at the lowest $P_{I_{O_2}}$. When the water convection requirement at each hypoxic level is "normalized" by multiplying by the percent O_2 saturation of the water, it can be seen that gill ventilation is adjusted during hypoxic exposure to maintain the mass transport of O_2 at near control levels (Table 1).

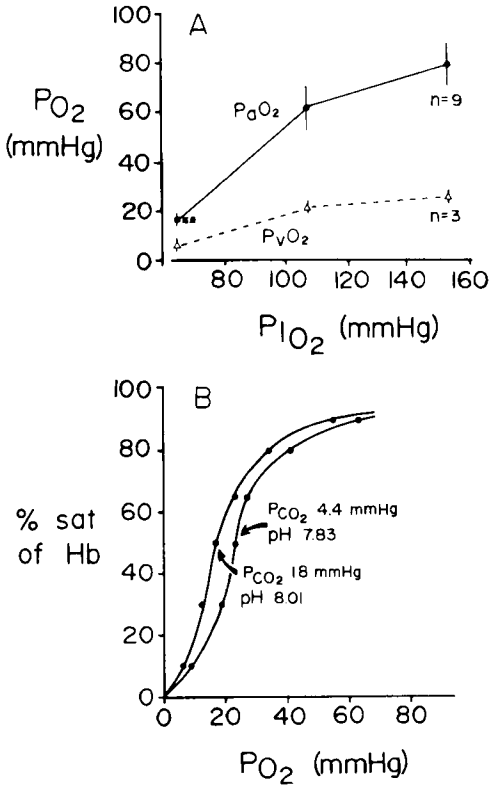

Fig. 5. A) Steady-state blood P_{O_2} in the caudal artery ($P_{a_{O_2}}$) and caudal vein ($P_{\bar{v}_{O_2}}$) during control conditions and hypoxic exposure in *Ictalurus punctatus*. B) Oxygen dissociation curves determined at 18°C with pooled whole blood samples from 4 *Ictalurus*. P_{CO_2} of the tonometered blood, as well as the pH measured at P_{50}, are indicated.

Acid-base balance

When the total blood CO_2 concentration values (C_T) were compared for ten fish (with and without respiratory membranes) during control (normoxic) and hypoxic periods, the hypoxic group was significantly ($P < 0.01$) lower. The decrease from 9.7 ± 1.3 mM to 7.5 ± 2.4 mM is somewhat misleading, however, since the C_T did not reach a steady state even after several hours. Rather, the hypoxic acidosis tended to gradually become more severe, and the consequent depletion of HCO_3^- stores greater as the hypoxia was continued.

Plasma lactate concentrations were normally quite low, about 2.5 mg%, and did not rise significantly at the intermediate level of hypoxia (Fig. 6A). At the low oxygen concentration, however, a highly significant four-fold rise in plasma lactate was apparent, not only for the eight fish which were membrane-fitted for respiratory studies, but also in an additional five fish which were not membrane-fitted. A moderate to severe plasma acidosis almost invariably accompanied the lactate increase, becoming more pronounced (pH_e = 7.62) at the lowest ambient oxygen level (Fig. 6B).

The mean whole-body intracellular pH (pH_i), determined from eight free catfish, was 7.394 ± 0.086, compared to mean plasma pH in these same fish of 7.883 ± 0.041. These eight fish were also subjected to hypoxia (65 mm Hg), but unfortunately only two of the eight responded with the typical lactate acidosis (Fig. 7). These two showed pH_i depressed by 0.2 to 0.4 units at greatly depressed values of pH_e, whereas the other six fish showed little change in either pH_i or pH_e (Fig. 7). The relationship of pH_e to pH_i is shown in Figure 8; the slope of all data taken together is not significantly different from one, although data points for some individual fish show a slope significantly less than one. This is to be expected, since the intracellular compartment is much better buffered than the extracellular compartment.

A summary of several aspects of the blood acid-base status of *I. punctatus* during hypoxia is provided by the "Davenport diagram" of Figure 9. During control conditions, blood C_T and pH_e were quite consistent from fish to fish, giving a mean value shown as the large solid dot. The diagonal straight line is the mean in vitro buffer line, determined from several fish, and the curved, numbered lines are P_{CO_2} isolines, or isopleths, derived from the Henderson-

TABLE 1. A summary of gas exchange, gill ventilation, and the water convection requirements in Ictalurus punctatus

$P_{I_{O_2}}$	$\dot{M}_{O_2}$	$\dot{M}_{CO_2}$	$\dot{V}_R$	Water convection requirement $\dot{V}_R/\dot{M}_{O_2}$	$\dot{V}_R/\dot{M}_{CO_2}$	
(mm Hg)	(ml/kg/h)	(ml/kg/h)	(ml/kg/h)	(ml H_2O/ml O_2 consumed)	(ml H_2O/ml CO_2 excreted)	$(\dot{V}_R/\dot{M}_{O_2})$ • % O_2 Sat. of H_2O
153	30.7	25.9	9,420	307	364	307
106	30.0	42.9	19,620	654	457	426
64	22.2	40.0	18,960	854	474	354

Mean values from 8 fish are given.

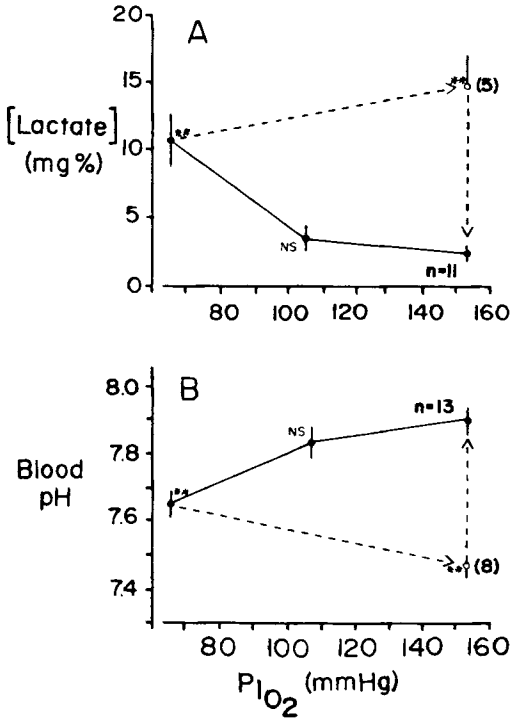

Fig. 6. Steady-state values of A) blood lactate concentration, and B) blood pH during control conditions and hypoxic exposure. Also indicated are the values measured 1 hour after return to air-saturated conditions from the lowest level of hypoxia.

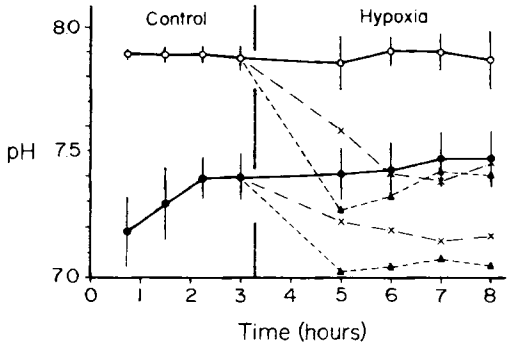

Fig. 7. Time course of intracellular and extracellular pH changes in 8 catfish before and during exposure to a P_{IO_2} of 60 mm Hg. Open circles represent mean values of pH_e ± 1 standard deviation for 6 catfish showing no acidosis during hypoxia, while individual symbols represent data points for two catfish exhibiting a hypoxic acidosis. Closed circles and the associated individual symbols give values of pH_i in these same fish.

Hasselbalch equation and a pK′ value of 6.154.

During hypoxia, all membrane-fitted fish showed a constant fall in plasma pH (Fig. 6B), as did two of eight catfish without membranes. Point H in Figure 9 represents the mean value for two membrane-fitted catfish in which C_T was monitored, while individual points (open circles) for C_T and pH_e during hypoxia for the fish without membranes (Figs. 7, 8) are also shown. The hypoxic disturbance for virtually all the fish can be characterized as a mixed metabolic acidosis and respiratory alkalosis. That is, the addition of lactic acid to the blood from anaerobic metabolism caused a metabolic acidosis, depressing the pH and C_T, whereas the hyperventilation (Fig. 2) tended to cause a reduction in P_{CO_2} of 0.5–1.0 mm Hg in most catfish, shifting values somewhat down and to the right on Figure 9.

Post-hypoxic recovery

Within minutes of a step-wise return to normoxic levels, very marked changes in most variables measured became evident. Particular attention was paid to the normoxic period between 30 and 90 minutes after exposure to the most severe level of hypoxia (65 mm Hg). Even though inspired P_{O_2} levels were at control levels, $\dot{V}_g$ remained elevated and % U was constant, and consequently $\dot{M}_{O_2}$ remained significantly elevated at levels nearly double control values (Figs., 3, 4). Unfortunately, only limited data on $\dot{M}_{CO_2}$ during this recovery period were gathered, but in the four catfish examined, $\dot{M}_{CO_2}$ declined only slowly to control levels during this recovery period.

A large lactate pulse into the blood also occurred during this recovery period, such that plasma lactate concentrations rose to their highest values of 14–15 mg% (a nearly sevenfold increase over control concentrations) within the first hour of the recovery period before subsequently slowly falling over the course of several hours to the low control levels (Fig. 6A). Associated with these extreme levels of plasma lactate was a marked acidosis, with pH falling to 7.4–7.5, and in two individuals to as low as 7.18 one hour after a return to air saturated P_{IO_2} levels. (Fig. 6B). Figure 10 shows the time course of plasma lactate and pH changes before, during, and after an unusually severe and prolonged hypoxic exposure. In this 704 g catfish, both the highest lactate concentrations and the lowest pH values occurred one hour after a return to normoxia, and even six hours later, a metabolic acidosis was still evident. Complete recovery to steady-state normoxic

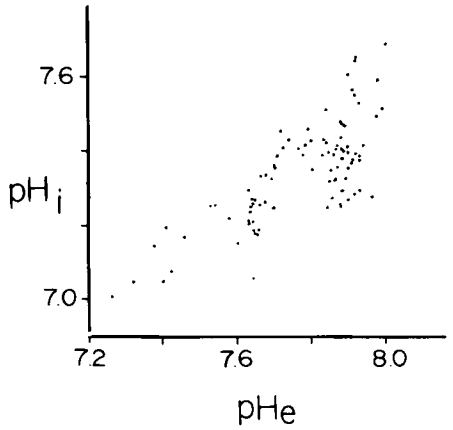

Fig. 8. Relationship between intracellular and extracellular pH in *Ictalurus punctatus*.

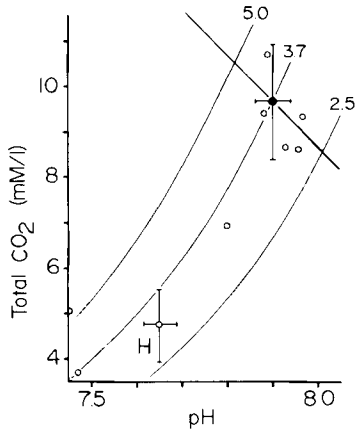

Fig. 9. Davenport diagram of acid-base adjustments to hypoxic exposure in *Ictalurus*. Total CO_2 was directly measured conductiometrically after acidification of a whole blood sample. P_{CO_2} isopleths have been drawn for 2.5, 3.7, and 5.0 mm Hg. The solid circle is composed of mean values ± 1 standard error from 16 catfish, both with and without membranes under normoxic conditions. Point H (open circle) presents mean values from the two membrane-fitted fish for which data were available during hypoxic conditions. Individual points are from catfish, without a membrane, exposed to hypoxia at a P_{O_2} of 60 mm Hg.

levels in this particular catfish was completed at least within 17 hours of a return to a P_{IO_2} of 153 mm Hg.

DISCUSSION

The channel catfish, *Ictalurus punctatus*, unlike the brown bullhead, *I. nebulosus*, is usually restricted to oxygenated river waters or deep ponds and lakes, where it maintains a comparatively low activity level. $\dot{M}_{O_2}$ values of approximately 30 ml/O_2/kg/h support the latter observation and are similar to values measured in other undisturbed, usually inactive bottom dwelling fishes (Grigg, '69; Burggren and Randall, '78). *I. punctatus* is clearly able to maintain oxygen uptake down to inspired P_{O_2} levels of at least 60 mm Hg (Fig. 3), which may approach the lowest oxygen levels naturally encountered by this species. A previous investigation of fingerling *I. punctatus* (Gerald and Cech, '70) indicated that this species was an oxygen "conformer," even though ventilatory adjustments were attempted during hypoxic exposure. Differences with the present study may have arisen from their use of 4–9 g fish, or possibly a restrictive apparatus interfering in some way with normal gas exchange.

We find that oxygen uptake in *I. punctatus* is maintained at control levels during hypoxic exposure, in part through a pronounced hyperventilation, a product largely of increased branchial stroke volume, and a maintained oxygen extraction from the ventilatory current. $P_{a_{O_2}}$ fell considerably and arterial blood was less than 50% saturated at the lowest levels of hypoxia, and although $P_{a_{O_2}}$ also decreased, tissue extraction and the a-v O_2 content difference fell overall. No cardiovascular variables were measured, but the fact that oxygen uptake at the gills was maintained in the face of a reduced a-v content difference, strongly indicates that gill perfusion rose to match the hyperventilation occurring during hypoxia. Matching of perfusion to ventilation in this fashion has been well documented for other fishes (Jones et al., '70; Cameron et al., '71).

The profound branchial hyperventilation in *Ictalurus punctatus* during hypoxic exposure must, of course, have resulted in a considerable energy expenditure by the skeletal muscles powering ventilation. Since $\dot{M}_{O_2}$ remained at control levels during hypoxia, this increased ventilatory effort could be sustained in one of several ways. For example, aerobic metabolism in tissues not involved in gill ventilation could have been reduced to preferentially maintain the limited oxygen delivery to active, aerobically respiring ventilatory muscles. Alternatively, the increased ventilatory effort may have been sustained through anaerobic glycolysis and other anaerobic pathways, with oxygen delivery diverted to hypoxia-sensitive tissues such as the nervous system and sense organs.

Whatever the body distribution of available O_2 taken up at the gills of *Ictalurus* during hypoxia, it is evident from 1) the highly elevated blood lactic acid concentration, 2) the increase in the gas exchange ratio, and 3) the post-hypoxic "repayment" of an "oxygen debt,"

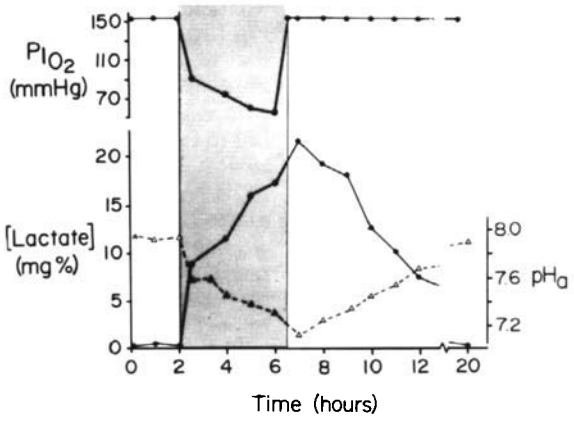

Fig. 10. Changes in blood pH (open triangles) and lactate concentration (solid circles) before, during, and after a particularly severe and long period of hypoxia in a 704 g *Ictalurus*.

that a substantial anaerobic contribution to the total energy production, from glycolysis and other undertermined pathways, occurs in *Ictalurus* during exposure to the lowest levels of oxygen.

A relatively severe plasma acidosis accompanied the lower hypoxia level in *I. punctatus* (Figs. 6, 7). Determination of intracellular pH values indicates that an extracellular (plasma) acidosis will be accompanied by a comparably severe intracellular acidosis (Fig. 7), since there was no obvious regulation of a constant pH_i.

Interestingly, these catfish also usually showed a decrease in blood P_{CO_2} of 0.5 to 1.0 mm Hg, indicating a small respiratory alkalosis antagonistic to the larger metabolic acidosis (Fig. 8). This may seem exceptional, since it has been argued that gill ventilation does not directly regulate plasma P_{CO_2} or pH in water-breathers (Randall and Cameron, '73; Cameron, '78). However, the observation that a doubling of ventilation causes less than 1 mm Hg change in $P_{a_{CO_2}}$, plus the close coupling of $\dot{V}_g$ to oxygen requirements (Table 1), lend support to the general idea that the control of ventilation is dominated by oxygen levels. Nonetheless, observations during the posthypoxic recovery period indicate a possible minor respiratory control mechanism in *Ictalurus,* involving plasma or tissue acidosis. Insufficient data on $P_{a_{CO_2}}$ during recovery were available for statistical analysis, but in two catfish so examined, $P_{a_{CO_2}}$ returned to control levels within 15 minutes of the return to normoxia, but $\dot{V}_g$ remained elevated for a further hour until pH_a had risen to control values. Cause and effect clearly remain to be demonstrated.

Several catfish, particularly those not fitted with membranes, exhibited the typical small respiratory alkalosis, but failed to develop a large metabolic acidosis. Although we cannot completely account for this discrepancy, we believe that the more marked metabolic acidosis of the membrane fitted fish can be attributed to occasional struggling and abortive swimming attempts during severe hypoxic exposure, which were rarely observed in fish without membranes. This form of what must be largely anaerobic glycolytic muscle activity under these conditions, probably contributed to the greater acidosis in these catfish. It also seems likely that 65 mm Hg is the lowest level at which O_2 regulation is maintained in *I. punctatus,* so small activity or stress differences at this hypoxic level may assume greater importance.

The more than doubling of the gas exchange ratio to a value of 2.0 at a P_{IO_2} of 65 mm Hg, indicates an active anaerobic but nonglycolytic metabolic pathway producing CO_2 during hypoxic exposure in *Ictalurus.* Several appropriate pathways, including pyruvate dehydrogenase and α-ketoglutarate dehydrogenase reactions, have been implicated in fishes (see Hochachka and Somero, '73). However, as cautioned earlier, a portion of this increased CO_2 excretion reflects acidification with lactate of the body bicarbonate pools, rather than excretion of molecular CO_2 produced from other anaerobic metabolic pathways. Sufficient data were available for two membrane-fitted catfish to calculate changes in the intra- and extracellular bicarbonate pool before and during hypoxic exposure, as well as to calculate $\dot{M}_{CO_2}$, R_E, and R_Q. Average values for these two ani-

mals are given in Table 2. Over a four hour period of hypoxic exposure, during which pH_a fell from 7.90 to 7.41, the total bicarbonate pool was reduced by approximately 35%, or about 31 ml/kg. This represents a molecular CO_2 excretion due strictly to lactic acid acidification of the extra- and intracellular bicarbonate pool of approximately 8.0 ml/kg/h. Thus, if a total increase in $\dot{M}_{CO_2}$ from all sources from 26 to 40 ml/kg/h is assumed to develop during hypoxic exposure (Fig. 3), then it can be readily calculated that only approximately 45% of the elevated $\dot{M}_{CO_2}$ during hypoxia is due to metabolically produced CO_2 from dehydrogenation of pyruvate, α-ketoglutarate, or other substrates. Thus, whereas the gas exchange ratio, R_E, of *Ictalurus punctatus* is about 2.00 at a P_{IO_2} of 65 mm Hg, the respiratory quotient, R_Q, is in fact only about 1.55. That acidification of body tissue of fishes reduces blood CO_2 levels to some extent has long been appreciated (Auvergnat and Secondat, '42; Secondat and Diaz, '42; Black et al., '59). However, the present investigation clearly has demonstrated that acidification of the bicarbonate pool must be a major consideration when determining respiratory gas exchange during hypoxia, activity, or transient temperature increases.

An additional, small source of CO_2 loss during hypoxic exposure may be a CO_2"washout" due to hyperventilation per se, either in the absence or presence of a metabolic acidosis. As evident in Figure 9, however, the nearly 50% fall in total CO_2 with only a ½ mm Hg decrease in P_{CO_2} in acidotic fish, in particular, would indicate that CO_2 washout from ventilation alone is a minor component of the overall change in acid-base balance. We have therefore not increased the complexity of the calculations by assuming a new P_{CO_2} steady state during hypoxia.

The period of recovery from hypoxia exposure is a telling and significant one, though receiving little attention in many previous studies on fishes. In *Ictalurus*, hyperventilation at hypoxic levels was maintained during a one hour recovery period following exposure to a P_{IO_2} of 65 mm Hg. $\dot{M}_{O_2}$ during this same period nearly doubled over any previous levels (Figs. 3, 4), indicating a classical "repayment" of an "oxygen debt" accumulated during hypoxic exposure. $\dot{M}_{CO_2}$ remained at high levels, but the immediate return of R_E to control values (Fig. 3) is misleadingly simple. Presumably the HCO_3^- stores must at some point in recovery begin to be replenished, but metabolic CO_2

TABLE 2. Changes in the intracellular (ICF) and extracellular fluid (ECF) pH, bicarbonate concentration, and bicarbonate pools during steady-state control conditions and after 4 hours of hypoxia (P_{IO_2} 65 mm Hg)

		ECF (vol = 23.4 ml/100 g tissue)		ICF (vol = 44.7 ml/100 g tissue)		Total HCO_3^- Pool (μEq)
	pH	7.90		7.38		—
Normoxia (P_{IO_2} = 153 mm Hg)	$[HCO_3^-]$ (μEq/ml)	11.05		3.50		—
	HCO^- pool (μEq)	2,753	+	1,664	=	4,417
	pH	7.41		7.11		—
Hypoxia (P_{IO_2} = 65 mm Hg)	$[HCO_3^-]$ (μEq/ml)	7.40		3.07		—
	HCO_3^- pool (μEq)	1,445	+	1,447	=	2,892
	Δ HCO_3^- pool over 4 hours			=		1,525 μEq

If acidified, Δ HCO_3^- = 34.2 ml molecular CO_2
= 8.5 ml CO_2/h
= 8.0 ml CO_2/kg/h

Also indicated is the reduction in the total bicarbonate pool during this period, and the volume of CO_2 produced assuming total acidification of HCO_3^- to molecular CO_2.

Data are mean values for two membrane-fitted catfish with a mean body weight of 1,068 g.

production (and excretion?) is high because of the upswing in $\dot{M}_{O_2}$. Compounding the situation during the recovery period was the often large flush of lactate into the plasma which followed hypoxic exposure. This produced a transient metabolic acidosis even more severe than during hypoxia, with blood pH sometimes transiently falling to as low as 7.18. In fact, a curious dilemma developed for us with regard to a few fish, in which they were approaching lethal pH levels during hypoxic exposure, yet to return them immediately to air saturated conditions would initiate the lactate flush and result in further fall in pH that would prove immediately lethal.

Caillouet ('68) made a preliminary study of the effects of anoxia on blood lactate and pH levels in disturbed *I. punctatus,* by completely removing catfish from water for very short (5–15 min) periods of time. Control blood lactate concentrations measured by Caillouet were 2–5 times those of the present study (probably caused by the disruption of cardiac puncture for blood sampling), but he nonetheless also reported that the highest lactate concentrations and lowest plasma pH levels were observed only after the exposed fish had been returned to water. The highest blood lactate concentrations in other fish species are often observed following, rather than during, hypoxia exposure or activity (Wood et al., '77; Hughes and Johnson, '78) and may indicate, as in mammals, that circulation to the anaerobically respiring tissues may be reduced during hypoxia (Black et al., '62; Wood and Randall, '73).

Wood et al. ('77) reported that, following exhausting activity in the starry flounder, *Platichthys stellatus,* there was a considerable temporal separation of an immediate respiratory acidosis and a later metabolic acidosis following within about 2–3 hours, perhaps to prevent a transient but major combined acidosis. Whether hypoxic exposure can be related to activity is equivocal, but our results are in agreement with respect to a delayed elimination of lactate from the tissues.

Finally, it is interesting to note that at the milder level of hypoxia (105 mm Hg), $\dot{M}_{CO_2}$ was clearly elevated above control levels, although lactate levels and pH values were not significantly different from those during normoxia (Figs. 3, 6). The elevation in CO_2 excretion would thus appear to be entirely of a metabolic, nonglycolytic origin, although how the end-products of this apparent "mild" anaerobiosis are metabolized (or even what they may be, if not lactate) requires further investigation.

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