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AERIAL CO_2 EXCRETION IN THE OBLIGATE AIR BREATHING FISH *TRICHOGASTER TRICHOPTERUS*: A ROLE FOR CARBONIC ANHYDRASE

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SUMMARY

Total oxygen uptake and carbon dioxide excretion and their partitioning between gills and aerial exchange organs have been measured in an obligate air breathing fish, the blue gourami (*Trichogaster trichopterus*). Measurements were made during normal bimodal breathing and in air-exposed fish. $\dot{M}_{O_2}$ increased during air exposure, but the aerial exchange organs or 'labyrinth' of *Trichogaster* could assume a highly effective CO_2 excretion in the absence of branchial gas exchange. Hence, aerial $\dot{M}_{CO_2}$ was greatly increased during air exposure, and the labyrinth gas exchange ratio increased to 0·8 from the value of 0·1 evident during bimodal gas exchange.

The large aerial CO_2 excretion in *Trichogaster*, unobtainable in many air breathing fish, was unaffected by the injection of carbonic anhydrase, but was seriously disrupted in the presence of this enzyme's potent inhibitor, acetazolamide. *In vitro* assay for carbonic anhydrase demonstrated the labyrinth epithelium of the gourami to be rich in this enzyme. It is concluded that highly effective CO_2 excretion from the labyrinth organs during air exposure in *Trichogaster* results from dehydration of plasma bicarbonate to molecular CO_2 at the catalysed rate; carbonic anhydrase for this purpose being present in the labyrinth epithelium. The distribution of carbonic anyhdrase in the tissues of other air breathing fishes is determined, and its implications to aerial CO_2 excretion discussed.

INTRODUCTION

The pattern of CO_2 excretion in Teleost fish is quite different from that in mammals, in that plasma bicarbonate is dehydrated within the respiratory epithelium rather than within erythrocytes, erythrocytic carbonic anhydrase being unavailable for plasma bicarbonate dehydration (Haswell & Randall, 1976, 1978). Plasma bicarbonate enters the Teleost gill epithelium where high levels of carbonic anhydrase catalyse the formation of molecular CO_2, which then readily diffuses into the water ventilating the gills.

Accessory gas exchange organs have evolved in numerous groups of fish in order to

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obtain oxygen from air. The air breathing organs may be modified swimbladders, pharyngeal cavities and even the stomach and intestine (see Johansen, 1970; Munshi, 1976; Singh, 1976 for reviews). However, most often these organs are utilized mainly for oxygen uptake, as indicated by their typical gas exchange ratios of 0·1–0·4. The gills are thus retained as the major route for CO_2 excretion, with the method of aquatic excretion presumably being identical to that in aquatic fish. Randall, Farrell & Haswell (1978) found that in *Hoplerythrinus*, a characid fish having a respiratory air bladder, most of the CO_2 in the blood is lost to the water upon passage through the gills. Since the air bladder is perfused with efferent branchial blood now low in CO_2, only a small P_{CO_2} gradient exists from blood to gas in the bladder, and hence aerial CO_2 excretion is minor compared to branchial excretion. Upon removal from water, *Hoplerythrinus* is deprived of its gills for CO_2 exchange, blood P_{CO_2} levels rise and so CO_2 excretion into the air bladder rises. However, aerial CO_2 excretion is still low and insufficient to prevent the development of a hypercapnic acidosis. Randall *et al.* (1978) found that artificially increasing the CO_2/HCO_3^- reaction velocity in *Holperythrinus* blood by infusion of bovine carbonic anhydrase caused a significant rise in the gas exchange ratio of the swimbladder during air exposure and greatly reduced or alleviated entirely the hypercapnic acidosis usually associated with removal from water in this fish. These results indicate that not only is the carbonic anhydrase in erythrocytes in this air breathing fish unavailable for plasma bicarbonate dehydration as in strictly aquatic Teleosts, but also that the epithelium of the modified gas bladder of *Hoplerythrinus* is incapable of catalysing plasma bicarbonate dehydration.

Burggren (1979) has investigated bimodal gas exchange in the blue gourami *Trichogaster trichopterus*, also an obligative air breather. In hypercapnic water with access to air the gas exchange ratio of *Trichogaster*'s aerial exchange organ can exceed 1·1, a value far in excess of those reported for other air breathing fish under a wide variety of conditions. The aerial gas exchanger or so-called labyrinth organs of *Trichogaster* and other Anabantidae are embryonically derived from the first and second gill arches and remain intimately associated with branchial structures in adults (Henninger, 1907; Munshi, 1968, 1976). It is therefore possible that the labyrinth epithelium of *Trichogaster*, like that of the gills, contains carbonic anhydrase available to greatly enhance the rate of dehydration of plasma HCO_3^- to molecular CO_2. The present investigation was designed to clarify the role of carbonic anhydrase in both aquatic and aerial CO_2 excretion in *Trichogaster*, and to determine the presence or absence of carbonic anhydrase in the aerial gas exchange organs and other tissues of additional species of bony fishes.

METHODS

Experiments were performed on 19 adult blue gouramis, *Trichogaster trichopterus* (mean mass 8·10 ± 1·50 g). All experiments on intact animals were carried out at 27 ± 1/2 °C on fish which had been fasting for at least one day. Tissue analyses were also performed on 5 rainbow trout (*Salmo gairdneri*), 3 carp (*Cyprinus carpio*), 5 blue gouramis (*Trichogaster trichopterus*), 4 walking catfish (*Clarias batrachus*), and 4 African reed fish (*Calamoichthys calabancus*); the last three fish being bimodal breathers. All fish were either reared in the laboratory or obtained from local suppliers.

Oxygen uptake and carbon dioxide elimination in the gills (aquatic exchange) and the labyrinth organs (aerial exchange) were measured in an apparatus described in detail in a companion investigation (Burggren, 1979). Briefly, gouramis were placed in a vessel containing both air-equilibrated water and air, the two respiratory media in contact only through a very small breathing hole. After a 24 h acclimation period in the apparatus, the vessel was sealed and the fish allowed to continue bimodal respiration. By recording changes in P_{O_2} and P_{CO_2} of both gas and water during a standard 1 h measurement period, both aquatic and aerial $\dot{M}_{O_2}$ and $\dot{M}_{CO_2}$ in μM gas/g/h could be calculated (see Burggren, 1979). After several control measurements during bimodal respiration, the container was drained of water, the fish was loosely covered with a moistened tissue to prevent desiccation, and the vessel, now containing only humidified air, was sealed. Subsequent changes in P_{O_2} and P_{CO_2} of the gas in the vessel resulting from strictly aerial gas exchange were then measured over several consecutive 1 h periods. After air exposure, the apparatus was partially filled with air-equilibrated water, and monitoring of $\dot{M}_{O_2}$ and $\dot{M}_{CO_2}$ in bimodally breathing fish was continued.

Another similar series of experiments on five fish were designed to determine to what extent *Trichogaster* respires cutaneously when air exposed. Each fish was placed in the bottom of a rubber condom, with just the mouth and head extending from a small slit cut in the condom tip. The condom was sealed just beyond the end of the fish's tail, forming a closed gas bag around the body, but leaving the mouth and opercular slits unenclosed. After a known volume of air was injected into the previously collapsed bag, the fish was placed in the respirometer chamber containing only humidified air, and left to respire for a 1 h period. At the end of this period, gas was sampled both from within the chamber at large and from within the gas bag covering the body, and the P_{O_2} and P_{CO_2} of these samples measured. The condom rubber was impermeable to gases, and relatively large gas partial pressure gradients were maintained between the gas in the bag and the gas in the respirometer chamber. These data then allowed the calculation of the partitioning of oxygen uptake and carbon dioxide excretion between the labyrinth organs and the skin in totally air exposed gouramis.

The effects of bovine carbonic anhydrase and the carbonic anhydrase inhibitor, acetazolamide (Diamox, Lederle), on $\dot{M}_{O_2}$ and $\dot{M}_{CO_2}$ and their partitioning between gills and labyrinth organs were measured in two groups of six fish each, one group were always in air-equilibrated water with access to air, and the other group were totally air exposed. After obtaining a series of control values during bimodal respiration, each fish in the first group was intraperitoneally injected with 1000 W.A. units (1350 enzyme units, E.U., see Results) of bovine carbonic anhydrase in a carrier volume of 10 μl Cortland saline. Aerial and aquatic $\dot{M}_{O_2}$ and $\dot{M}_{CO_2}$ was determined over a 1 h period 2–3 h after carbonic anhydrase injection. Fish were then injected with 20 μg/g body weight of acetazolamide in a carrier volume of 10 μl Cortland saline, and aquatic and aerial $\dot{M}_{O_2}$ and $\dot{M}_{CO_2}$ once again determined 2–3 h after injection. In the second group of fish $\dot{M}_{O_2}$ and $\dot{M}_{CO_2}$ were determined first under control conditions during total air exposure. Fish were then injected with bovine carbonic anhydrase and returned to water for 2 h to allow complete rehydration, after which the fish were exposed to air and $\dot{M}_{O_2}$ and $\dot{M}_{CO_2}$ measured again. The fish were then injected with

acetazolamide, returned to water for another 2 h, then $\dot{M}_{CO_2}$ measured during air exposure. All drug doses and rates of administration were exactly the same in both groups of fish.

Direct intravascular injection of drugs was not possible due to the small size of the fish, so an intraperitoneal administration route was necessary. Bovine carbonic anhydrase is a large molecule and it is possible that it might move only very slowly between body compartments. A series of experiments were performed on rainbow trout in which bovine carbonic anhydrase was injected intraperitoneally, and the plasma was analysed at 30 min intervals for carbonic anhydrase activity. A large carbonic anhydrase flux into blood plasma occurred within 30 min of injection. Plasma activity levels peaked 2 h after injection and activity was still detectable after 24 h. It was thus assumed that intraperitoneal injection of carbonic anhydrase also raised plasma activity after 1–3 h in *Trichogaster*. Acetazolamide is readily absorbed and distributed to body tissues (Maren, 1967).

Labyrinth ventilation rate was determined in four *Trichogaster* during both bimodal respiration and during total air exposure. Copper wire electrodes (30 gauge) implanted externally in the skin on the underside of the buccal cavity were attached to a Biocom 2991 Impedance Convertor, whose output in turn was directed into a Fisher Recordall chart recorder. Ventilation of the suprabranchial chamber was easily discernible from branchial ventilation, the former producing much larger impedance changes across the recording electrodes.

Gill tissue, whole blood, swimbladder and aerial gas exchange organs from two aquatic breathing and three air breathing fish species were analysed for carbonic anhydrase activity. Fish were first anaesthetized in MS 222. The heart was exposed and blood drawn by cardiac puncture. The ventral aorta was then cannulated and the entire circulation perfused with copious amounts of heparinized Cortland saline to flush all red blood cells and plasma from the circulation. Gill arches, the swimbladder (if present) with gas gland removed, and the aerial exchange organs (if not the swimbladder) were dissected free, and homogenized in distilled water with a trace of Triton Tx-100. These homogenates were centrifuged at 5000 rev./min for 5 min, and the supernatant, along with the lysed blood samples, were analysed for total protein concentration with an Accu-stat (Clay-Adams) total protein analyzer. They were then assayed manometrically for carbonic anhydrase activity as previously described in detail (Haswell & Randall, 1976). Carbonic anhydrase activity, defined in enzyme units (E.U.) where 1 enzyme unit represents sufficient carbonic anhydrase to double the uncatalysed rate of CO_2 evolution from bicarbonate (Haswell & Randall, 1976), was expressed both on a per gram tissue and a per gram protein basis.

Significance levels of all data were assessed with Students' t test, and a fuducial level of $p < 0{\cdot}05$ was chosen for differences of means.

RESULTS AND DISCUSSION

Air exposure and gas transfer

All *Trichogaster* examined could survive 4–6 consecutive h of exposure to air, a situation alien to this fish in its natural environment. However, skin necrosis developed

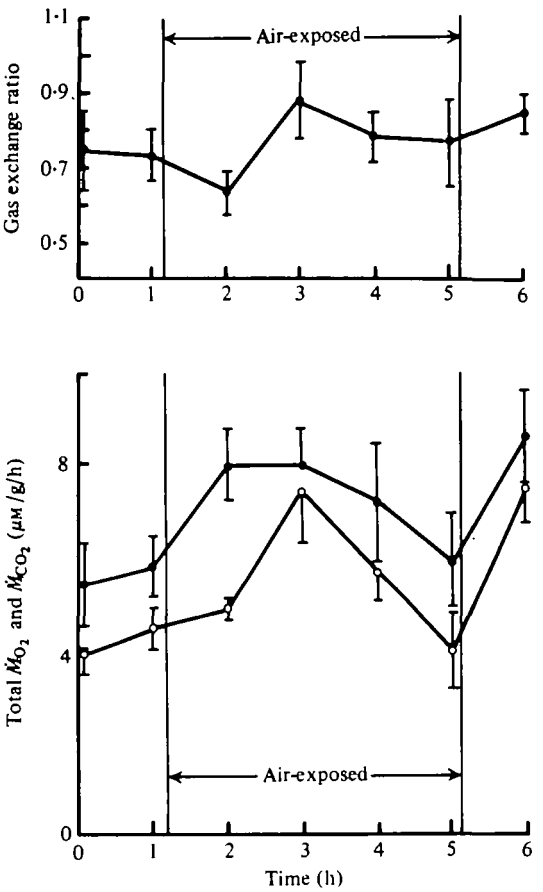

Fig. 1. Total oxygen uptake (closed symbols) and carbon dioxide (open symbols) and the overall gas exchange ratio before, during and after air exposure in *Trichogaster trichopterus*. Mean values ± 1 S.E. derived from experiments on six fish are presented.

unless the fish were kept in humidified air, or could lie on a moist substrate, both of which were provided during the present experiments.

Fig. 1 presents total $\dot{M}_{O_2}$ and $\dot{M}_{CO_2}$ as well as the overall gas exchange ratio for 6 *Trichogaster* before, during and after a 4 h period of air exposure. Most fish showed few locomotory movements not associated with surfacing for air breathing when in water. Although their laterally compressed body and small, delicate fins allowed little in the way of effective terrestrial locomotion, every gourami initially became active and attempted to move about the respiration chamber upon air exposure. Consequently, total $\dot{M}_{O_2}$ increased from approximately 4·9 μM/g/h up to 7·8 μM/g/h within 1 h of removal from water (Fig. 1).

In air exposed gouramis only 12% ± 11% of $\dot{M}_{O_2}$ and 12% ± 5% of $\dot{M}_{CO_2}$ ($\bar{X}$ ± S.D., $n = 5$) could be attributed to cutaneous gas exchange. Thus, nearly 90% of all gas exchange during air exposure was the result of the labyrinth organs in the suprabranchial chamber. It is possible that the gills of air-exposed *Trichogaster* made some

very small contribution to gas exchange. However, all branchial ventilatory movements soon stopped upon removal from water, and there appears to be no structural modifications of the gills which could act to prevent their collapse during air exposure. Under these conditions almost all of the total O_2 uptake and CO_2 excretion had to occur via the labyrinth organs. Not surprisingly, then, air ventilation of the suprabranchial chamber increased from 12 ± 3 breaths/h during bimodal breathing to 98 ± 39 breaths/h ($\bar{X} \pm$ S.D., $n = 4$) at the end of 1 h of air exposure. Four hours after removal from water, $\dot{M}_{O_2}$ and $\dot{M}_{CO_2}$ had returned to control levels, as the fish became less active and presumably began to acclimate to exposure.

Trichogaster is among the few air breathing fishes which can sustain an elevated $\dot{M}_{O_2}$ during air exposure. In *Saccobranchus* and *Neoceratodus* oxygen uptake clearly falls during air exposure (Lenfant, Johansen & Grigg, 1967; Hughes & Singh, 1971). Conflicting reports on $\dot{M}_{O_2}$ exist for *Clarias* (Singh & Hughes, 1971; Jordan, 1976), the former study reporting a decrease in oxygen uptake during air exposure. Removal from the water is accompanied by a small fall in oxygen uptake in *Anabas*, which taxonomically is closely related to *Trichogaster*, even in individuals which are hyperventilating their suprabranchial chamber with air (Hughes & Singh, 1970). In this Anabantid oxygen uptake by the labyrinth organs appears to have important limitations. Among the air-breathing fishes examined to date, only the lungfish *Protopterus* (Lenfant & Johansen, 1968), *Amphipnous* (Lomholt & Johansen, 1974), and possibly *Clarias* appear to be able to match aerial oxygen uptake performance with *Trichogaster*.

CO_2 excretion in *Trichogaster* increased in similar proportions to oxygen uptake during air exposure, but maximum aerial $\dot{M}_{CO_2}$ developed only approximately 2 h after removal from water (Fig. 1). Consequently, the overall gas exchange ratio for *Trichogaster* initially fell slightly from 0·77 to 0·65, but returned to control levels after 3 h of air exposure. The gas exchange ratio of the labyrinth organs showed a concomitant large rise from 0·18 to 0·79 (Table 1). *Trichogaster*, unlike almost all other air breathing fish for which data is available, can maintain $\dot{M}_{CO_2}$, and even eventually increase this factor above control levels when metabolic rate increases during air exposure. In *Anabas*, *Clarias* and *Saccobranchus*, for example, total $\dot{M}_{CO_2}$ falls during air exposure to a half or less of those values evident during bimodal breathing (Hughes & Singh, 1970; Singh & Hughes, 1971; Hughes & Singh, 1971; Randall *et al.* 1978). However, with the exception of *Trichogaster*, in the air breathing fishes examined the maximum sustainable aerial CO_2 elimination during total air exposure falls far short of matching metabolic CO_2 production, and in the bladder breather *Hoplerythrinus*, for example, results in a severe hypercapnic acidosis after removal from water (Randall *et al.* 1978).

Clearly, under experimental conditions the labyrinth organs of *Trichogaster* can assume at least 90% of the total gas transfer burden, even when a large elevation of metabolic rate develops. Burggren (1979) has shown that this situation is approached when the contribution to gas exchange by the gills of the gourami is severely reduced by ventilation with hypoxic or hypercapnic water; the present experiments demonstrate this to be the ease when effective branchial gas transfer is mostly eliminated (and cutaneous exchange is miminal) during total air exposure in the gourami.

Why CO_2 excretion lagged behind O_2 uptake in the first hour of air exposure is not clear. An increase in inspired water P_{CO_2} (and hence blood P_{CO_2}) serves as a potent stimulation to labyrinth ventilation (Burggren, 1979). Perhaps the small rise in bl[illegible]

Table 1. *Effects of carbonic anhydrase and acetazolamide on gas transfer during normal bimodal breathing and in air-exposed* Trichogaster.

(Mean values ± 1 S.D. are given. $N=6$. Significance levels where mean values are different from control values are indicated by asterisks. $^{*}0{\cdot}025 < P < 0{\cdot}05$, $^{**}P < 0{\cdot}025$.)

	Control	After carbonic anhydrase	After acetazolamide
	Bimodal breathing		
Total $\dot{M}_{O_2}$	7·9 ± 2·9	9·6 ± 3·3	8·7 ± 1·7
% $\dot{M}_{O_2}$ from gills	65 ± 11	60 ± 12	71 ± 6
% $\dot{M}_{O_2}$ from lab.	36 ± 11	40 ± 12	30 ± 6
Total $\dot{M}_{CO_2}$	6·5 ± 2·4	8·7 ± 3·4*	3·6 ± 0·8*
% $\dot{M}_{CO_2}$ from gills	92 ± 2	93 ± 2	88 ± 5*
% $\dot{M}_{CO_2}$ from lab.	8 ± 2	7 ± 2	12 ± 5*
R gills	1·57 ± 0·44	1·49 ± 0·56	0·54 ± 0·53*
R lab.	0·18 ± 0·08	0·16 ± 0·04	0·18 ± 0·07
R overall	0·88 ± 0·18	0·89 ± 0·17	0·43 ± 0·10*
	Air-exposed		
Total $\dot{M}_{O_2}$	14·3 ± 4·9	13·8 ± 4·9	13·6 ± 3·6
Total $\dot{M}_{CO_2}$	10·7 ± 2·8	11·4 ± 4·8	7·1 ± 2·6*
R overall	0·79 ± 0·11	0·82 ± 0·14	0·43 ± 0·10**

P_{CO_2} which probably developed in the first hour of air exposure as the gas exchange ratio fell slightly was initially insufficient to stimulate changes in labyrinth ventilation and/or perfusion necessary for augmented CO_2 excretion.

Upon return to water most fish showed considerable exploratory swimming. Both $\dot{M}_{O_2}$ and $\dot{M}_{CO_2}$ were initially higher than pre-exposure levels, though the gas exchange ratio didn't change significantly from control levels.

Carbonic anhydrase and gas transfer

The large capacity for CO_2 excretion by the labyrinth organs of *Trichogaster* led us to investigate whether aerial CO_2 excretion was dependent in some part upon the catalysis by carbonic anhydrase in labyrinth epithelium of plasma bicarbonate through to readily excreted molecular CO_2. This route is of paramount importance in branchial excretion of CO_2 in fish (Haswell & Randall, 1976, 1978).

Total $\dot{M}_{CO_2}$ following the injection of carbonic anhydrase into bimodally breathing fish was elevated by approximately 20% even after 2 h. This was presumably from the handling incurred during drug injection, since saline injection produced similar increases in $\dot{M}_{O_2}$ (Table 1). Hence, carbonic anhydrase injection produced no significant change in overall gas exchange ratio in these fish. The distribution of CO_2 excretion between gills and labyrinth organs was also totally unaffected in the presence of carbonic anhydrase, and gas exchange ratios of these specific organs remained unchanged from control levels (Table 1). Neither total $\dot{M}_{O_2}$ nor $\dot{M}_{CO_2}$ were changed from control levels after carbonic anhydrase injection into the group of gouramis exposed to air. The overall gas exchange ratio, effectively the respiratory quotient for the labyrinth organs in view of the very limited cutaneous gas exchange, was thus very similar in both groups of fish after carbonic anhydrase injection (Table 1).

If CO_2 excretion into either water or air in bimodally breathing gouramis, or into

air in those fish removed from water, was in any way limited by the velocity of the plasma bicarbonate dehydration reaction, then the injection of bovine carbonic anhydrase should have altered carbon dioxide elimination. Those changes in *Trichogaster* which did occur in the transfer of oxygen and carbon dioxide, however, were consistent with those occuring simply after the injection of saline alone. This indicates that either (1) the dehydration of plasma bicarbonate was already catalysed by carbonic anhydrase in control fish and so was never rate limiting, or (2) the reaction was not previously catalysed, but even at catalysed rates was not important to CO_2 excretion.

Experiments were designed to further test the involvement of carbonic anhydrase and plasma bicarbonate dehydration in CO_2 excretion in *Trichogaster* by inhibiting activity of this enzyme with its potent inhibitor, acetazolamide. As with the injection of both carbonic anhydrase and saline, $\dot{M}_{O_2}$ was elevated after acetazolamide injection into bimodally breathing fish (Table 1). However, total $\dot{M}_{CO_2}$ was nearly halved after acetazolamide injection. The role of the gills in CO_2 excretion relative to the labyrinth organs decreased by a significant but small amount (from 92% to 88% of total excretion), but this combined with the sharp reduction in branchial $\dot{M}_{CO_2}$ and increase in $\dot{M}_{O_2}$ produced a large fall in the gas exchange ratio of the gills (Table 1). Aerial $\dot{M}_{CO_2}$ during bimodal breathing was also reduced by acetazolamide, but not the gas exchange ratio of the labyrinth. Levels of oxygen uptake and its distribution between gills and labyrinth in *Trichogaster* during bimodal respiration were unaffected by acetazolamide.

These results indicate that relegation of bicarbonate dehydration to the uncatalysed reaction rate had a profound effect on total $\dot{M}_{CO_2}$, with the greatest impairment of CO_2 elimination occurring at the gills during bimodal breathing (Table 1). This is not surprising since during normal bimodal breathing in *Trichogaster* approximately 90% of total CO_2 excretion is into the water (Burggren, 1979; Table 1). Branchial excretion of CO_2 in fishes originates from plasma bicarbonate entering the gill epithelium and dehydrated to molecular CO_2 at the catalysed rate by epithelial carbonic anhydrase (Haswell & Randall, 1978). Even though blood P_{CO_2} will undoubtedly increase after acetazolamide injection because of the documented CO_2 retention (Table 1), the excretion of plasma CO_2 via the gills still appears to be limited, for the gas exchange ratio of the gills still falls after carbonic anhydrase inhibition. Any rise in blood P_{CO_2} after acetazolamide injection will also increase the blood–air CO_2 gradient in the labyrinth organs, presumably increasing the movement of molecular CO_2 into the labyrinth. Yet $\dot{M}_{CO_2}$ of the labyrinth also falls considerably after inhibition of carbonic anhydrase, thus leading us to suggest that the dehydration of plasma bicarbonate to molecular CO_2 at the catalysed rate, probably occurring in the epitheliun of the gas exchange organ, is an important component of aerial as well as aquatic CO_2 excretion in bimodally breathing gouramis. It is not impossible that the gourami may be capable of utilizing red cell carbonic anhydrase for the dehydration of plasma bicarbonate. Unfortunately, the extremely small blood volumes of the gourami prevented us from experimentally dismissing this possibility, but a red blood cell carbonic anhydrase available to the plasma would be a situation contrary to that in any of a diverse range of teleost fish yet examined (Haswell, unpublished).

To assess the involvement of carbonic anhydrase in CO_2 excretion from the labyrinth, both in the absence of a concomitant branchial CO_2 excretion and when a lar

rial gas transfer could potentially occur, the effect of acetazolamide was examined in the group of air exposed fish. Both total $\dot{M}_{CO_2}$ and the gas exchange ratio of the labyrinth fell significantly in the presence of acetazolamide, indicating a progressive accumulation of CO_2 in the body compartments. Acetazolamide injection in *Trichogaster* was often accompanied by severe loss of equilibrium after 4–6 h, even when fish were returned to air-equilibrated water, and in one instance proved fatal after 12 h.

The gas exchange ratio of the labyrinth organs of *Trichogaster* after the inhibition of carbonic anhydrase, 0·43, now lay within the range of values reported for other air breathing fishes when removed from water (see Singh, 1976; Randall *et al.* 1978). CO_2 excretion from the air bladder of *Hoplerythrinus* during air exposure is, in fact, limited by the uncatalysed rate of the dehydration of plasma bicarbonate, for the injection of bovine carbonic anhydrase results in a doubling of the bladder gas exchange ratio and a reduction in the P_{CO_2} gradient from blood to bladder gas (Randall *et al.* 1978). It is doubtful, on the basis of the evidence these authors present, that carbonic anhydrase is present in any quantity in the air bladder of this fish.

Tissue distribution of carbonic anhydrase

In order to test the hypothesis that the considerable CO_2 excretion potential of the labyrinth organs of *Trichogaster* relates to labyrinth epithelium containing active carbonic anhydrase involved in bicarbonate dehydration, and that this enzyme may not be present in the aerial exchange organs of other air breathing fish, total protein concentrations and enzyme activity of carbonic anhydrase from tissues of three air breathing fish as well as two aquatic species were determined (Table 2). Carbonic anhydrase is present in large but varying amounts in the lysed blood samples and gill tissues of all species examined. No correlation between protein or enzyme concentration of gills or blood and aquatic or aerial breathing was evident. *Salmo*, *Cyprinus*, and *Trichogaster* all have swimbladders used for buoyancy control and/or hearing but not for respiration. No carbonic anhydrase activity could be detected in the bladder of the gourami, slight activity was present in the rainbow trout bladder, while considerable activity was evident in carp bladder.

Calamoichthys, like *Hoplerythrinus* which was shown to have a limited potential for aerial CO_2 excretion (Randall *et al.* 1978), utilizes modified swimbladders for aerial respiration. Carbonic anhydrase activity even at low levels was never detectable in either of the paired respiratory bladders from four of these fish examined (Table 2). *Trichogaster* and *Clarias* use a labyrinth organ and branchial arborizations and 'fans', respectively, for aerial respiration (see Munshi, 1976). Most importantly, in both species these structures arise embryonically from the gill arches. In the adult, though they extend up into a suprabranchial chamber which is ventilated with air, these aerial exchange organs are still intimately associated with the gills. The aerial gas exhange organs as well as gills in both *Clarias* and *Trichogaster* have a very high carbonic anhydrase activity on a gram–protein basis, greater than blood activity for both fish, and comparable to or exceeding that of the gills of trout, for example (Table 1). In the bladder breather *Calamoichthys* (and on the basis of indirect physiological evidence, probably *Hoplerythrinus*), the aerial exchange organ is almost totally devoid of this enzyme. Although only a few species of fish have been examined, there appears to be a tive correlation between the presence of carbonic anhydrase in the aerial exchange

Table 2. *Protein concentrations and carbonic anhydrase enzyme activity in selected tissues of five fishes.*

(Mean values ± 1 S.D. are given.)

Species	*n*	g prot/100 ml	E.U./g tissue	E.U./g protein
		Whole blood		
Salmo gairdneri	5	21 ± 6	68 ± 23	319 ± 47
Cyprinus carpio	3	19 ± 2	222 ± 121	1148 ± 570
Trichogaster trichopterus	5	17 ± 2	267 ± 40	1564 ± 258
Clarias batrachus	4	17 ± 3	282 ± 57	1660 ± 258
Calamoichthys calabancus	4	21 ± 8	63 ± 41	289 ± 131
		Gill		
Salmo gairdneri	5	6 ± 1	62 ± 8	972 ± 186
Cyprinus carpio	3	6 ± 1	43 ± 6	817 ± 269
Trichogaster trichopterus	5	9 ± 4	74 ± 32	826 ± 125
Clarias batrachus	4	7 ± 1	100 ± 32	1385 ± 327
Calamoichthys calabancus	4	8 ± 3	95 ± 17	1584 ± 1140
		Bladder (non-respiratory)		
Salmo gairdneri	5	6 ± 2	2 ± 1	25 ± 18
Cyprinus carpio	3	3 ± 2	6 ± 4	184 ± 59
Trichogaster trichopterus	5	3 ± 2	n.d.	n.d.
		Air breathing organ		
Trichogaster trichopterus	5	7 ± 4	31 ± 24	396 ± 128
Clarias batrachus	4	18 ± 4	54 ± 8	315 ± 111
Calamoichthys calabancus	4	7 ± 2	n.d.	n.d.

n.d. = not detectable.

organ and its derivation from branchial tissue, which is rich in this enzyme. While the non-respiratory swimbladder is not necessarily devoid of carbonic anhydrase, this enzyme is lacking in the bladder of those fish examined which uses the bladder for aerial respiration (*Calamoichthys*, *Hoplerythrinus*) (Table 2; Randall *et al.* 1978).

The present study has demonstrated that the ability of *Trichogaster* to maintain high levels of aerial CO_2 excretion is related to significant levels of labyrinth carbonic anhydrase, presumably catalysing plasma bicarbonate in the labyrinth much as proposed for branchial excretion in teleosts. The aerial exchange organs of the air breathing catfish *Clarias batrachus* also contain high levels of carbonic anhydrase, and so it might be anticaipted that there is also a large potential for aerial CO_2 excretion in this fish. *Clarias* voluntarily makes terrestrial sojourns (Das, 1927; Smith, 1945), and so selection pressures to evolve effective aerial CO_2 excretion would seem to be greater in *Clarias* than in the strictly aquatic *Trichogaster*. It is perhaps surprising, then, that Singh & Hughes (1971) report that the gas exchange ratio of air exposed *Clarias* only reaches about 0·5, well below that value evident in *Trichogaster* when removed from water (Table 1). However, their measurement was made at some unspecified time during 5–6 h exposure to air, and we have shown for *Trichogaster* that the gas exchange ratio changes considerably with time during air exposure (Fig. 1). Moreover, Singh & Hughes (1971) report that air-exposed *Clarias* in a closed respirometer over a 5½–6 h period produced a fall in P_{O_2} of 55 mmHg and a concomitant rise in P_{CO_2} of 45 mmHg, indicating a respiratory quotient over the entire experimental period of approximately 0·8, a value very similar to that measured in the present investigation for *Trichog*[redacted].

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