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RESPIRATION AND ACID-BASE PHYSIOLOGY OF THE SPOTTED GAR, A BIMODAL BREATHER

I. NORMAL VALUES, AND THE RESPONSE TO SEVERE HYPOXIA*

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SUMMARY

In normally aerated water, at 20 °C, gar accounted for 42% of their $\dot{M}_{O_2}$ from their lungs, while in hypoxic water ($P_{O_2} \simeq 12$ torr) their entire $\dot{M}_{O_2}$ was from the lung, and O_2 was lost through the gills. CO_2 excretion in both normoxia and hypoxia was primarily via the gills.

Lung ventilation increased 1150%, accompanied by an elevation of pulmonary perfusion from 5·9 to 12·1 $ml \cdot kg^{-1} \cdot min^{-1}$ in hypoxia, which accounts for the enhanced pulmonary $\dot{M}_{O_2}$. Cardiac output increased from 31 to 40·5 $ml \cdot kg^{-1} \cdot min^{-1}$ and systemic perfusion was maintained in hypoxia.

The difference in acid-base status between pre- and post-branchial blood (P_{CO_2}, pH and total CO_2), changed only slightly during hypoxia, but the oxygen difference reversed. Normal dorsal aortic (DA) P_{O_2} was 23·8 torr, ventral aortic (VA) 20·3; during aquatic hypoxia the mean values were 21·9 and 22·6, respectively. Blood pressure rose in both the VA and DA in hypoxia but the branchial vascular resistance did not change. The oxygen transfer factor did not change significantly between normoxia and hypoxia.

Anatomical studies of the gill microvasculature revealed a reduced and channelized lamellar circulation. No respiratory shunt pathways were found around the lamellae.

The physiological and anatomical data indicated that the gar did not change lamellar perfusion or use shunt pathways to avoid hypoxic O_2 loss.

INTRODUCTION

In normally aerated water, air-breathing fish may utilize both their gills and their air-breathing organs, in varying proportions, for gas exchange (Johansen, 1970; Singh, 1976). As aquatic oxygen tensions decrease, the dependence on aerial gas exchange increases, although the gills generally remain the site for CO_2 excretion (Singh, 1976; Rahn & Howell, 1976). In many air-breathing fish the circulation of the 'lung' is in series with the gills and parallel to the tissues (Satchell, 1976). Thus, oxygenated pulmonary blood and deoxygenated systemic blood are mixed in the

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venous return pathway and must pass through the gills to supply the tissues. In severely hypoxic water this could jeopardize tissue O_2 delivery. As this mixed blood transits the gills the fish could actually lose the oxygen that had been gained from its lung.

It has been suggested by Satchell (1976) and Smith & Gannon (1978) that to avoid hypoxic oxygen loss, an air-breathing fish may shunt blood-flow past the gill exchange surfaces. In some air-breathing fish, such as the lungfish (Johansen, Lenfant & Hanson, 1968*a*) and *Hoplyerythrinus unitaeniatus* (Smith & Gannon, 1978; Farrell, 1978), the branchial vasculature is modified to direct blood flow into the lung. In hypoxic water these fish can increase pulmonary perfusion to gain O_2 and may simultaneously reduce their branchial O_2 efflux because they are effectively reducing lamellar perfusion.

Despite the speculation on the importance of branchial shunts in preventing O_2 loss, there have been few actual measurements of O_2 transport across the gills of air breathers in severely hypoxic conditions. Of particular interest is how (or if) hypoxic O_2 loss is avoided in air-breathing fish that retain functional gills and have no apparent modification of their branchial vasculature. Branchial shunting during hypoxia could adversely affect air-breathing fish that use their gills for CO_2 excretion, as well as O_2 transport. If blood bypassed the lamellar exchange surface to any significant degree it could upset the blood acid-base balance by reducing CO_2 excretion.

Lepisosteus oculatus, the spotted gar, a holostean fish, is a facultative air-breathing fish, as is the longnose gar, *L. osseus* (Rahn *et al.* 1971). The perfusion of lung in gar is derived from the dorsal aorta and numerous pulmonary arteries (Potter, 1927), rather than efferent branchial arteries. The gills of gar are reduced, but function in both O_2 uptake and CO_2 excretion (Potter, 1927) and show no remarkable vascular modifications (Landolt & Hill, 1975). The gar must depend on aerial respiration in severely hypoxic water (McCormack, 1967), and because of the admixture of pulmonary and systemic blood in their heart, face the problem of hypoxic O_2 loss.

In this study we have examined a number of respiratory and cardiovascular variables from gar, in normoxic and hypoxic water, in an attempt to outline the basic physiological changes that accompany air breathing. We were specifically concerned with determining perfusion patterns, blood gas transport (including acid-base balance), and branchial O_2 transport, emphasizing the problems of branchial O_2 loss and the importance of shunting in hypoxia. The gill microvasculature was also studied to identify any lamellar or branchial shunt pathways.

MATERIALS AND METHODS

Animals

Spotted gar, *Lepisosteus oculatus*, were collected throughout the year by gill netting from the Guadalupe River (Hog Bayou) in Texas. Specimens weighing between 580 and 1400 g were used in this study. The gar were maintained in 1500 l tanks with recirculating oyster-shell filters at laboratory temperatures (19–23 °C).

Animal preparation

Gar were prepared for surgery using benzocaine (ethyl-p-aminobenzoate) dissolved in alcohol at a concentration of 1 g benzocaine in 10 l H_2O (1:10000). The gar were ventilated continuously during surgery with a 1:15000 benzocaine solution. Dorsal and ventral aortae were catheterized with PE-50 tubing using a 20-guage canine catheter sleeve and needle assembly to insert the cannulae. The catheters were filled with heparinized Cortland saline and plugged.

Opercular and buccal pressure catheters were constructed of PE-160 tubing and secured using the method described by Randall, Holeton & Stevens (1967).

Pulmonary catheters of PE-50 were inserted into the lung via the pneumatic duct. The inserted ends were slit for approximately 2 cm to ensure easy gas withdrawal.

Following surgery the animals were revived in darkened plexiglass chambers provided with a continuous flow of dechlorinated tapwater and an air space. A stripping column was used to control the gas tension of the water flowing into the chambers. The water temperature during the experiments varied between 18 and 21 °C. The fish were allowed a minimum recovery period of 24 h after surgery, and experimental samples were collected after arterial blood pH was judged to be stable. Fish that exhibited high gill ventilation rates and air-breathing frequencies or high activity levels were allowed additional recovery time.

Analytical techniques

Blood P_{O_2} was measured with a thermostatted microelectrode (Radiometer) and meter (Radiometer PHM-71 Acid Base Analyser). Blood P_{CO_2} and pH were also measured with thermostatted microelectrodes (Radiometer). The P_{O_2} and P_{CO_2} electrodes were connected in series and blood was injected as a series of three aliquots to ensure gas equilibration (Boutiller *et al.* 1978). After measurement the blood was injected back into the fish. Total CO_2 (C_T) was determined conductometrically using a method modified from Maffly (1968). This method allowed an accurate measurement of C_T to be made on 40 μl of blood. O_2 content was determined using the micro method of Tucker (1967) on 20 μl of whole blood.

The P_{O_2} of the water was measured using the same electrode and methods employed for measuring the P_{O_2} of blood. Water C_T was measured using a modification of the blood measurement of C_T on 100 μl sample of water. The P_{O_2} and P_{CO_2} of air were also measured with microelectrodes.

Gill ventilation frequency (f_G) was measured by the pressure excursions made in the opercular cavity. The catheters were kept full of saline and were connected to pressure transducers (Hewlett Packard) which in turn were coupled via an amplifier to a high-frequency response recorder (Gulton TR 222). An air breath causes a large pressure excursion, allowing the rate of air breathing (f_L) to be continuously monitored. An estimate of the change in gill ventilation was gained by measuring the pressure differential between the buccal and opercular cavities using a differential pressure transducer (Hewlett Packard 267BC).

Blood pressure was measured in the dorsal and ventral aortae catheters (PE-50), which were shortened and attached to PE-160, to reduce dampening of the pressure

pulse. Pressures were monitored with a transducer (HP 267BC) and recorder (TR 222). Heart rate could also be monitored in this fashion.

Blood flow was measured by the retention of 25 μm diameter unlabelled microspheres. The principle employed was the same as that used with radioactive tracer microsphere (Wagner *et al.* 1969; Cameron, 1974), but instead of measuring the specific radioactivity of the spheres the actual number of spheres was counted. The spheres were recovered by digesting tissue samples in strong base (20% KOH), centrifuging, rinsing and filtering them through a Nytex nylon mesh. The spheres were then counted in a Coulter counter. The percentage of the total number of spheres injected, recovered from a given tissue, was assumed to be directly proportional to the percentage of the blood flow received by the organ.

Anatomical studies

Fish were anaesthetized with benzocaine to prepare for methyl methacrylate corrosion casts. The ventral aorta was transected and catheterized with PE-160. The gills were then perfused with a heparinized isosmotic (270 m-osmol) NaCl solution that had been deoxygenated by bubbling with N_2. After the saline perfusion the vascular space was filled with a mixture of Murakami's plastic, as described by Gannon (1978), that had been prepolymerized to a viscosity of approximately 40–50 cP. The infusion pressure was 40 cm H_2O. After the plastic had filled the entire vascular system the cannula was occluded and the cast was placed in a 50 °C saline bath to reduce the hardening time. Tissue corrosion was done first in water, then in 20% KOH. When the tissue was digested the casts were rinsed in distilled water and examined under a dissecting light microscope. The branchial microvasculature was further studied under a scanning electron microscope after gold sputter coating. A total of eight fish were filled and five of the preparations were suitable for SEM study.

In vitro *blood work*

Oxygen dissociation curves were done separately on six fish, using blood withdrawn from the dorsal aorta. The heparinized whole blood was equilibrated with either N_2 or air in flasks immersed in a temperature-controlled shaker bath at 20 °C. Mixtures of saturated and unsaturated blood of 0, 10, 25, 50, 75, 83, 90 and 100% saturation were then made (Edwards & Martin, 1966) at 1% and 2% CO_2. Blood P_{O_2} was measured twice at each point, thus at each point $N = 6$ (number of fish), 12 (total number of determinations).

Oxygen content was also measured at 100% saturation at 20° ($N = 8$, 16) using the micro O_2 content method of Tucker (1967) at 1% and 2% CO_2. No significant haemolysis was noted in any of the experiments.

CO_2 combining curves were measured at 20 °C in oxygenated whole blood at 3·9, 7·6 and 15·2 torr P_{CO_2} ($N = 11$, 22) and the same mixtures in deoxygenated blood $N = 6$, 12). At each P_{CO_2} mixture the pH and C_T were determined twice. The non-bicarbonate buffer slope was calculated as a linear least-square regression equation. A functional pK value for whole blood was calculated using the Henderson–Hasselbalch equation as follows:

$$pK = pH - \log\left(\frac{C_T}{a.P_{CO_2}} - 1\right)$$

using a solubility coefficient (α) derived from a blood osmolarity of 265 m-osmol at 20 °C (average osmolarility determined on a Wescor vapour-pressure osmometer; $\alpha = 0{\cdot}0509$ mM $CO_2 \cdot l^{-1}$). The functional pK may be used to calculate *in vivo* P_{CO_2} values and is not the same as the pK of the plasma.

In vivo *blood properties*

In normally aerated water ($P_{O_2} \simeq 150$ torr) paired samples were withdrawn from the dorsal and ventral aorta within 20 s of each other; 0·7 ml of blood was withdrawn from each. For each sample the pH, C_T, P_{CO_2} and P_{O_2} were determined. The blood lost (approximately 0·1 ml/sample) was replaced with Cortland saline and injected with the recovered blood back into the vessel it was withdrawn from. These paired samples were drawn immediately after a breath, half-way through the air-breath interval, and at the end of an air breath. The anticipated air-breath duration was estimated from previous intervals in the same animals. In animals with exceptionally long air-breathing intervals ($\simeq$ 1 h) three samples were withdrawn at 15 min intervals. The blood-sampling procedure was repeated, but with the water made hypoxic (12 torr P_{O_2}) using intervals of 1, 2, 4, 6 and 8 min following each air breath.

Ventilation and respiration

In normoxia the gill ventilation and air-breathing frequencies (f_G, f_L) were determined from 2 h recordings ($N = 10$) and from 1 h recordings in hypoxia ($N = 10$). Gill ventilation volume ($N = 8$, 24) was also determined in both normoxia and hypoxia.

Lung P_{O_2} and P_{CO_2} were determined on 2 ml samples withdrawn from the lung in normoxia and hypoxia at several intervals throughout the air-breathing cycle. After the sample a fish was allowed to recover for several breaths, or 30 min. End expired volume and gas contents were also determined. The expired gas was trapped under a thin plastic sheet floated on the water surface. This method of gas collection prevented an inhalation and disturbed the fish, so collections were spaced at least 2 h apart. This method appeared to disturb the resting condition of the fish and the values obtained were probably more representative of a routinely active fish than a resting fish. In both normoxia and hypoxia, $\dot{M}_{O_2}$ was measured from the water by measuring the inflowing and outflowing water (P_{O_2} P_{I,O_2} and P_{E,O_2}) and water flow rate (V_w). The $\dot{M}_{O_2}$ was calculated by the Fick principle:

$$\dot{M}_{O_2} = (P_{I,O_2} - P_{e,O_2}) \cdot \beta_{W,O_2} \cdot V_w$$

where β_{W,O_2} was the O_2 solubility coefficient for the water. The oxygen uptake from the air was monitored by sealing the air space and measuring the reduction of P_{O_2} in the space.

$\dot{M}_{CO_2}$ into the water was also measured in normoxia and hypoxia using the Fick principle:

$$\dot{M}_{CO_2} = (C_{T_I} - C_{T_E}) \cdot V_w$$

and the CO_2 excretion into the air was measured by the increase of the P_{CO_2} in the sealed air space.

Blood flow and blood pressure

Gar implanted with dorsal aorta catheters were injected with 25 μm diameter microspheres in 0·1 ml of dextran and Cortland saline. Several 0·1 ml aliquots of the mixture were injected in the same fashion into test vials for counting, so that the total dose of spheres could be estimated. The spheres were injected exactly 1 min following an air breath in either normoxia or hypoxia. The animals were sacrificed 4 min after the injection and the spheres were recovered and counted (as previously outlined). Lung blood flow was measured in normoxia ($N = 6$) and hypoxia ($N = 6$).

Cardiac output ($\dot{Q}$) was calculated by the Fick principle:

$$\dot{Q} = \frac{\dot{M}_{CO_2}}{(C_{T_{DA}} - C_{T_{VA}})} \cdot 100.$$

from a difference in total CO_2.

Blood pressure and heart rate were recorded in fish during normoxia and hypoxia from the dorsal aorta and the ventral aorta. The position of the VA catheter was determined by autopsy following the experiment. Blood pressure recordings were also obtained from the ventricle and conus arterious.

RESULTS

Anatomy

The general circulation is shown schematically in Fig. 1. The 3rd and 4th gill arches share a common afferent and efferent supply. The pulmonary circulation was provided by a number of small segmental arteries arising from the dorsal aorta and proceeding along the length of the lung. The entire cardiac output must pass through the gills. Blood in the dorsal aorta may pass either to the lung, and back to the heart via the posterior cardinals, or to the systemic venous blood. On a gross level the gar had no circulatory provisions for bypassing its gills.

The methyl methacrylate corrosion casts of the gills revealed that the pattern of microcirculation (Fig. 2) was similar to that of other fish (Vogel, 1978; Richards & Fromm, 1969; Randall, 1970). The blood that passes through the respiratory pathways, and ultimately goes to the dorsal aorta, comprises the arterioarterial vasculature. The arterioarterial system (Fig. 2, bottom hemibranch) is supplied by the afferent branchial artery, and passes via the afferent lamellar arteriole to the secondary lamellae, where branchial gas exchange occurs. The efferent primary artery collects the arterial blood and drains into the efferent branchial artery, which in turn leads to the dorsal aorta. The lamellar circulation was quite different from the conventional teleost scheme. A scanning electron micrograph (SEM) of a corrosion cast of the lamellar vascular space with associated filamental circulation is shown in Fig. 3. The lamellar circulation is channelized in the gar. The lamellar casts showed supporting ridges, which may function as pillar cells, and also had enlarged basal channels (15–20 μm diameter). The capillary channels were about 10 μm in diameter.

The remaining gill vasculature (Fig. 2) constitutes the arteriovenous system. The arteriovenous vasculature consists of an extensive system of anastomoses, sinuses and veins whose circulation ultimately returns to the heart (Fig. 2, top hemibranch). The

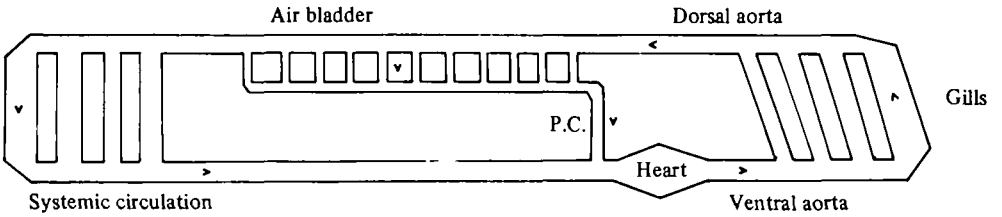

Fig. 1. A schematic representation of the gross vascular pathways of spotted gar, from vascular corrosion casts.

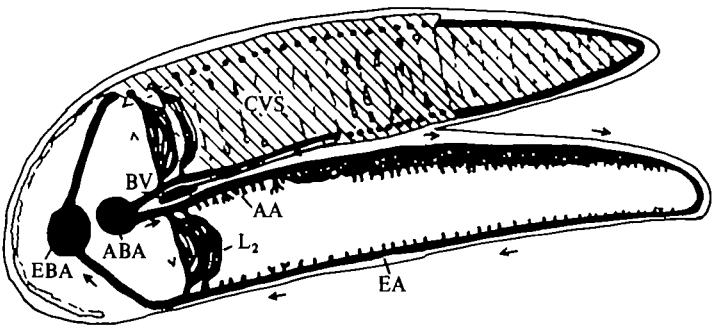

Fig. 2. Schematic of gill vasculature in spotted gar, drawn from observations and micrographs of gill corrosion casts. The bottom hemibranch illustrates the arterioarterial vasculature which consists of the afferent branchial artery (ABA), the afferent primary artery (AA) from which arises a diffuse anastomoses and the secondary lamellar circulation (L_2). The arterialized blood goes to the dorsal aorta via the efferent primary artery (EA) and efferent branchial artery (EBA). The arteriovenous portion of the vasculature is represented in the top hemibranch by the central venous sinus (CVS) and the branchial vein (BV).

major feature of the system is the central venous sinus (CVS) which is drained by the branchial vein which returns blood to the heart. We have not yet been able to determine the origin of the CVS. There are some small connections to the afferent filamental anastomoses, but it seems unlikely that these represent a significant blood supply.

In vitro *blood properties*

Oxygen dissociation curves for whole gar blood at 20 °C, at 1 % (N = 6, 12) and 2 % CO_2 (N = 5, 10) are shown in Fig. 4. The magnitude of the Bohr effect was $-0{\cdot}5$ ($\Delta \log P_{50}/\mathrm{pH}$), changing the P_{50} from 24·1 torr at 1–30·4 torr at 2 % CO_2. There O_2 content at 100 % saturation was $10{\cdot}78 \pm 0{\cdot}33$ vol % (ml/100 ml) (N = 8, 16) at 1 % CO_2 and at 2 % CO_2 was $9{\cdot}40 \pm 0{\cdot}31$ vol % (N = 8, 16). The reduction of content at 2 % CO_2 was significant (paired t test $P < 0{\cdot}05$), indicating a significant Root effect.

The calculations for the pK of gar whole blood are shown for oxygenated and deoxygenated blood in Table 1. The two buffer curves at each P_{CO_2} are shown in

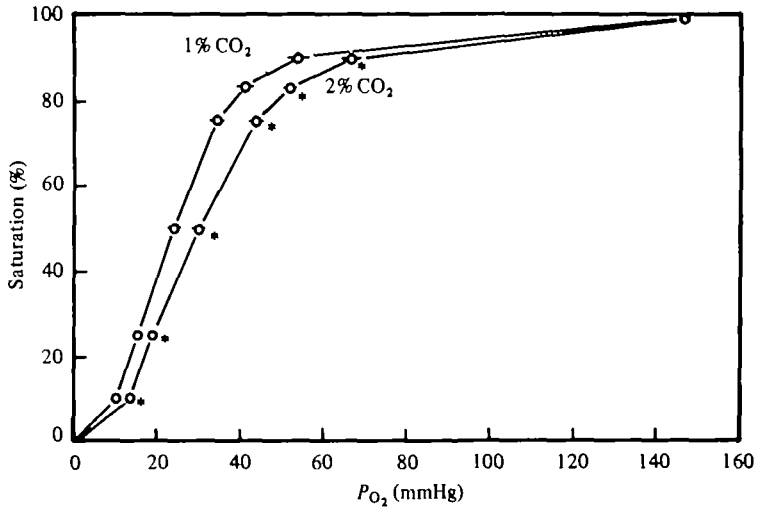

Fig. 4. Oxygen dissociation curves for gar blood at 20 °C with 1 % and 2 % CO_2, an asterisk indicates the value is significantly different than the 1 % value (paired t test, $P < 0.05$). Bars are ±S.E. around mean.

Table 1. *Calculated* pK *values for gar whole blood at* 20 °C, *oxygenated and deoxygenated*; *calculations discussed in text*

Oxygenated		Deoxygenated	
P_{CO_2} (torr)	pK ± S.E.	P_{CO_2} (torr)	pK ± S.E.
3·91	6·263 ± 0·012	3·91	6·293 ± 0·018
7·63	6·278 ± 0·012	7·63	6·300 ± 0·014
15·23	6·297 ± 0·012	15·23	6·350 ± 0·005

Fig. 5. The regression equation of the oxygenated blood was $Y = 75{\cdot}69 - (8{\cdot}76)X$ with a correlation coefficient of 1·00 and for deoxygenated blood was $Y = 51{\cdot}03 - (5{\cdot}62)X$ with a correlation coefficient of 0·98. The non-bicarbonate buffer value of the oxygenated blood, 8·76 slykes, was unexpectedly higher than that for deoxygenated blood, 5·62 slykes.

Ventilation and respiration

The changes in gill and lung ventilation between normoxia and hypoxia are shown in Table 2, along with the tidal volume and end-expired gas concentrations. The elevation of the air-breathing frequency and reduction of gill ventilation in hypoxia were significantly different (t test, $P < 0.05$) than the normoxic rates. The rate of gill ventilation (Fig. 6*c*) did not change significantly over the course of an air breath. The elevation of tidal volume in hypoxia was significant (t test, $P < 0.05$) and gill ventilation volume decreased significantly (paired t test, $P < 0.05$) by 62·9 ± 8·6 % ($N = 8, 24$) in hypoxia.

The P_{O_2} in the lung drops slowly but significantly in normoxia while there was no significant change in the P_{CO_2} of the lung ($N = 9, 36$) over a 30 min interval (Fig.

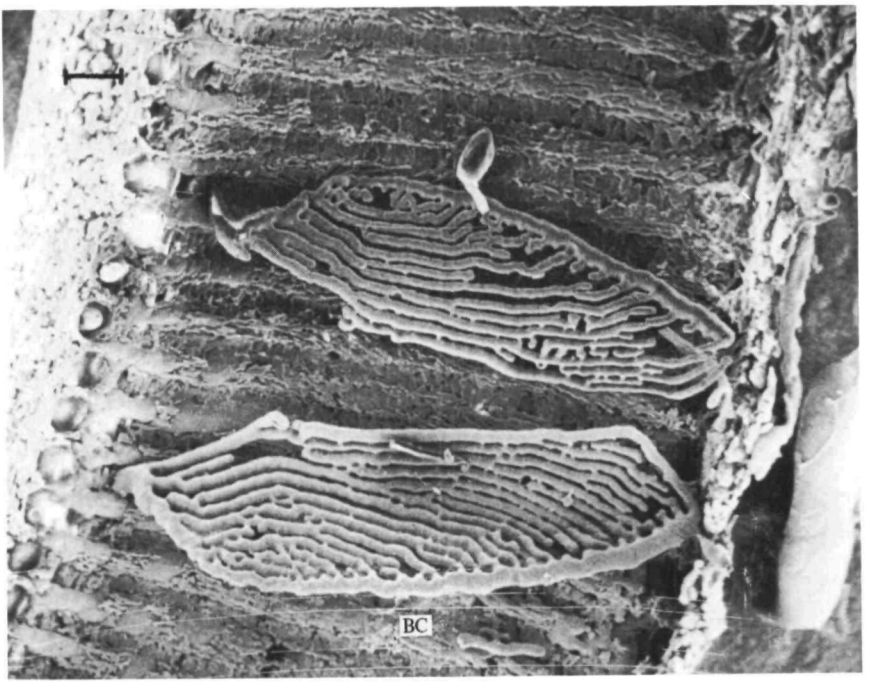

Fig. 3. A scanning electron micrograph of a vascular cast showing two lamellae placed on their sides to reveal their basal channels (BC). The calibration bar = 50 μm.

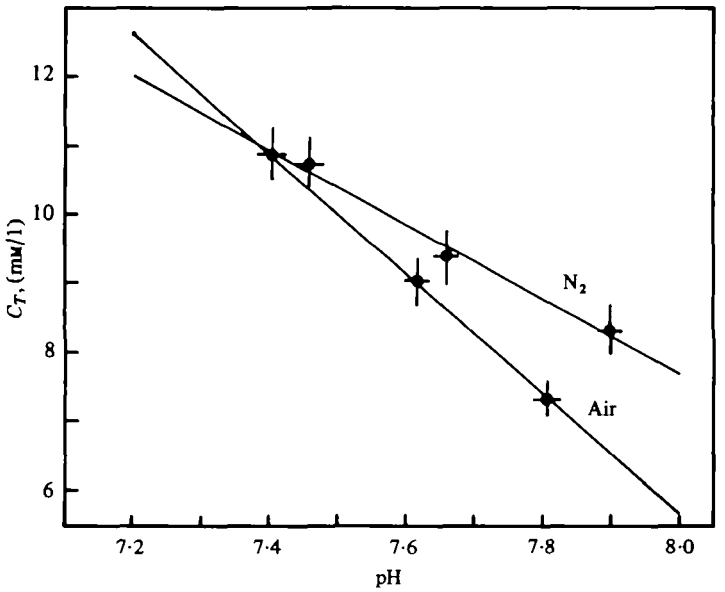

Fig. 5. Buffer curves for gar whole blood at 20 °C, using oxygenated blood (N_2). Bars are one standard error around the mean.

Table 2. *A comparison of respiratory variables and heart rate in normoxia and hypoxia*

(Values given ± 1 S.E. N = number of animals, total number of measurements.)

	Normoxia	Hypoxia
Gill ventilation rate (bpm)	35·3 ± 2·0 (N = 10, 20)	21·0 ± 2·1 (N = 10, 50)
Air-breathing interval (min)	Approx. 1 h	7·2 ± 2·2 (N = 10, 50)
Tidal volume (ml/kg)	24·9 ± 2·9 (N = 4, 8)	35·2 ± 1·4 (N = 8, 16)
End-expired P_{O_2} (torr)	51·7 ± 6·3 (N = 5, 10)	45·5 ± 5·1 (N = 5, 10)
End expired P_{CO_2} (torr)	6·6 ± 0·8 (N = 8, 16)	10·8 ± 1·0 (N = 7, 14)
Heart rate (bpm)	32·9 ± 1·0 (N = 8, 16)	35·9 ± 0·8 (N = 11, 44)

6*d*). The reduction of P_{O_2} was significant (two-way ANOVA, no replications, $P < 0{\cdot}05$). In hypoxia (Fig. 6*d*), the P_{CO_2} dropped much more rapidly and P_{CO_2} rose in the lung by 1 torr during the air breathing interval. The changes of both P_{O_2} and P_{CO_2} in hypoxia were significant (two-way ANOVA, no replications, $P < 0{\cdot}05$). The partitioning of M_{O_2} and M_{CO_2} in air and water are shown in Fig. 7. In normoxia the majority of O_2 was obtained by the gills, while all the CO_2 was excreted into the water. In hypoxia, the lung was responsible for the entire $\dot{M}_{O_2}$, and a small amount of O_2 gained by the lungs was lost by the gills into the water (approximately 10%). The gills continued to be the primary source for CO_2 excreted into the air. The respiratory quotient was 1·05 ± 0·10 in normoxia and 1·00 ± 0·10 in hypoxia. There were no significant differences between the total $\dot{M}_{O_2}$, $\dot{M}_{CO_2}$ or R.Q. between normoxia and hypoxia.

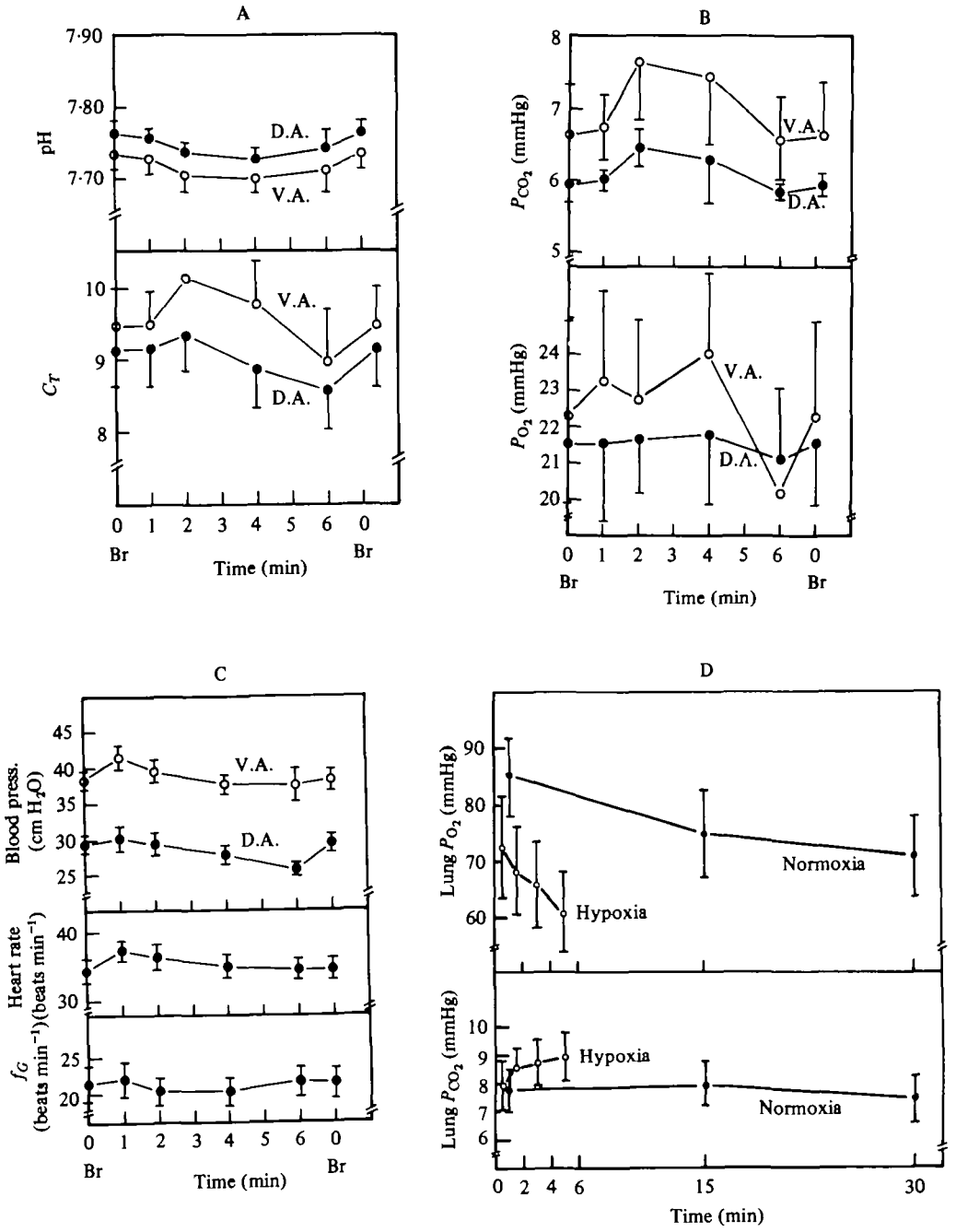

Fig. 6. The changes during the course of an air breathing interval in (A) pH and total CO_2 (C_T) in the dorsal aorta (DA) and ventral aorta (VA); (B) P_{CO_2} and P_{O_2} in the dorsal aorta (DA) and ventral aorta (VA), and (C) blood pressure (DA and VA), heart rate and the gill ventilation rate (f_G), and (D) lung P_{O_2} and P_{CO_2} in normoxia and hypoxia. Br = air breath, the vertical bars are 1 S.E.M.

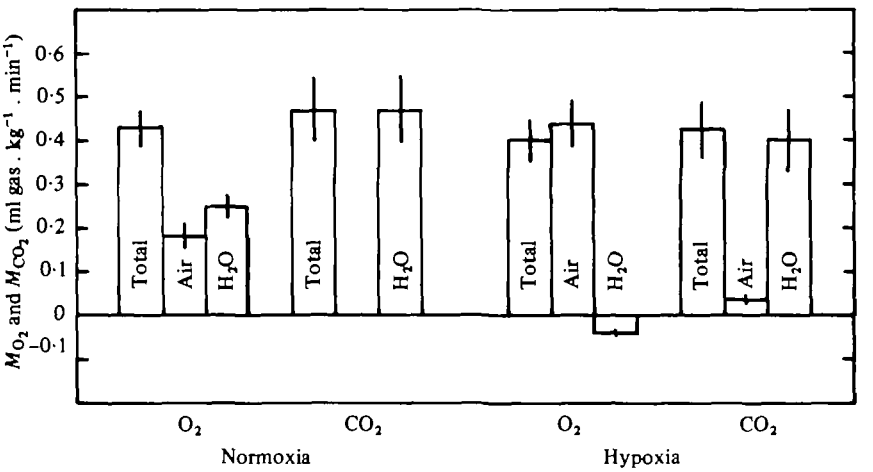

Fig. 7. The partitioning of oxygen consumption (M_{O_2}) and CO_2 excretion (M_{CO_2}) into the air and water (H_2O) and the total M_{O_2} or M_{CO_2} in both normoxia (left side) and hypoxia (right side). Vertical bars are ± 1 S.E.M.

Table 3. *A comparison of blood pH, total CO_2 (C_T), P_{CO_2}, and P_{O_2} from the dorsal aorta (DA) and the ventral aorta (VA) and the DA–VA difference (Diff.)*

(These are average values made at *all* points of the air breathing cycle for an overall comparison of normoxia and hypoxia. Values given ± 1 S.E., N = total number of determinations. The asterisk indicates that the hypoxic value is significantly different than the normoxic value (t test, $P < 0.05$).

	Normoxia			Hypoxia		
	VA	DA	Diff.	VA	DA	Diff.
pH	7·752 ± 0·020 (N = 16)	7·783 ± 0·016 (N = 19)	+0·031	*7·718 ± 0·009 (N = 37)	*7·747 ± 0·008 (N = 37)	+0·029
C_T	10·04 ± 0·37 (N = 16)	9·36 ± 0·32 (N = 16)	−0·68	*9·50 ± 0·24 (N = 36)	*9·06 ± 0·22 (N = 36)	−0·44
P_{CO_2}	6·6 ± 0·4 (N = 16)	5·6 ± 0·2 (N = 19)	−1·0	7·2 ± 0·3 (N = 38)	6·3 ± 0·2 (N = 37)	−0·9
P_{O_2}	20·3 ± 1·4 (N = 19)	23·8 ± 1·5 (N = 19)	+3·5	22·6 ± 1·3 (N = 40)	*21·9 ± 0·8 (N = 40)	*−0·7

Table 4. *Microsphere retention (expressed as a percentage of the total number injected into the ventral aorta) in gill arches 1–4 of two fish in normoxia and two fish in hypoxia: the number should correspond to the percentage of the cardiac output supplying each gill*

Fish no.	Arch 1	Arch 2	Arch 3	Arch 4
		Normoxia		
12	27·5	36·1	22·8	13·6
13	31·8	29·8	19·3	19·1
		Hypoxia		
14	25·5	22·9	22·2	29·3
15	28·3	29·0	25·5	17·1

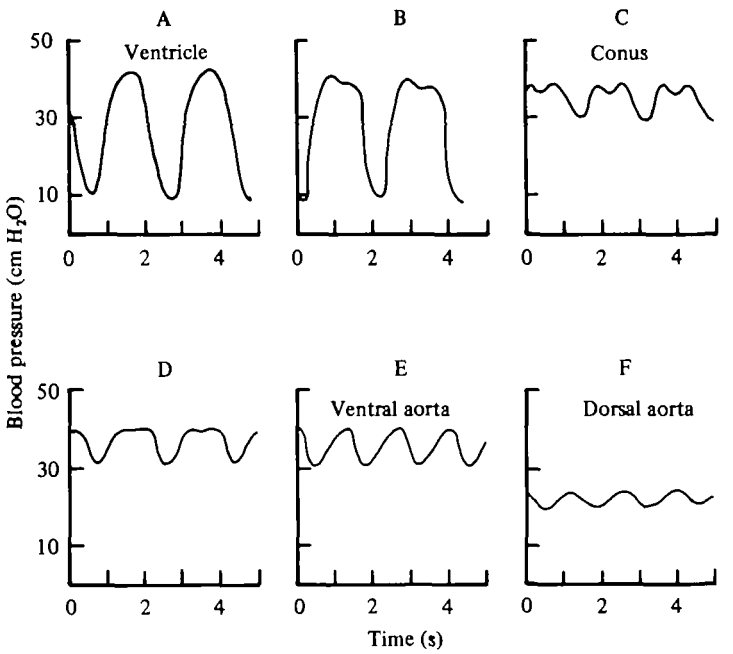

Fig. 8. Tracings of blood pressure recordings taken from (A) ventricle, (B) between the ventricle and conus, (C) *conus arteriosus*, (D) at the junction of the conus and ventral aorta, (E) ventral aorta, and (F) dorsal aorta.

In vivo *blood properties*

A comparison of blood gas and acid-base variables in normoxia and hypoxia is shown in Table 3 from both the ventral aorta and dorsal aorta. These are values for paired samples drawn simultaneously from the DA and VA. The difference between DA and VA was significant (paired t test, $P < 0{\cdot}05$) for all variables except P_{O_2} in hypoxia. In hypoxia, pH and C_T in both DA and VA, and the P_{O_2} in the DA were significantly lower than their normoxic values. The changes in blood variables during the course of an air-breathing interval in hypoxia are shown in Figs. 6(*a*) and (*b*). The blood changes during the air breath were not significant (two-way ANOVA, no replications, $P < 0{\cdot}05$), but the change in pH was significant if the data were normalized to initial values for each fish.

Blood flow and blood pressure

The change in heart rate from normoxia to hypoxia is shown in Table 2. While the heart rate was only slightly elevated in hypoxia, the cardiac output, calculated from the branchial $\dot{M}_{CO_2}$ and the difference in C_T across the gills, increased from 31·0 to 40·5 ml·kg^{-1}·min^{-1} in hypoxia. In normoxia, 19 ± 3% (N = 6) of the total number of microspheres injected into the DA were recovered from the lung, rising significantly in hypoxia to 30 ± 5% (N = 6). Assuming that the microspheres were distributed evenly with the cardiac output distribution, the fraction of the cardiac output to the lung rose from 19% of 31 ml·min^{-1}·kg^{-1} in normoxia, or 5·9 ml·min^{-1}·kg^{-1}, to 30% of 40·5 ml·min^{-1}·kg^{-1}, or 12·1 ml·kg^{-1}·min^{-1}, in hypoxia. Th

systemic blood flow ($\dot{Q}$ = lung blood flow) changed only slightly from 25·1 ml·kg^{-1}·min^{-1} in normoxia to 28·4 ml·kg^{-1}·min^{-1} in hypoxia. The microsphere distribution to the different gill arches was also determined in normoxia (N = 2) and hypoxia (N = 2) to see if there were any large changes in blood flow to the various arches (Table 4). Based on this small body of data there appear to be no large changes in the blood flow distribution between the four gill arches.

In normoxia the systolic VA blood pressure was 32·06 ± 1·53 and systolic DA blood pressure was 23·56 ± 1·36 cm H_2O (N = 18). In hypoxia VA blood pressure rose to 38·90 ± 0·75 cm H_2O (N = 45) while DA pressure rose to 28·82 cm H_2O (N = 51), both being significantly higher than normoxic pressures (t test, $P < 0.05$). The average pressure drop across the gills increased significantly from 8·5 cm H_2O in normoxia to 10·1 cm H_2O in hypoxia, although this elevation was proportional to the elevation in the VA and DA blood pressures. The changes in blood pressure and heart rate during the course of an air breath are shown in Fig. 6*c*; none of the changes were statistically significant (two-way ANOVA, no replication). Blood-pressure traces taken from the ventricle through to the dorsal aorta are shown in Fig. 8.

DISCUSSION

Respiration and ventilation

In normoxic water the spotted gar behaves much like *Neoceratodus* (Johansen, Lenfant & Grigg, 1967) or *Amia* (Randall *et al.* 1981) and other 'facultative' air breathers. The lung acts primarily to supplement the animal's O_2 uptake, while the gills are the exclusive site for CO_2 excretion. The most conspicuous responses to hypoxia occur in the lung. The pulmonary ventilation volume increases 1150% during hypoxia. The gar maintains its resting $\dot{M}_{O_2}$ via lung O_2 uptake, despite a small loss of O_2 through gills in hypoxia, and there is no measureable metabolic cost involved in switching to air breathing. The reduction of gill ventilation and gill ventilation volume may help to reduce O_2 loss slightly. Despite the elevated lung ventilation and reduced gill ventilation in hypoxia, CO_2 excretion is still primarily a function of the gills. The resultingly low arterial P_{CO_2} assures a small diffusion gradient for CO_2 between the arterial blood and lung gas, which in turn explains the low lung R values (0·10) in the gar. The Haldane effect (Fig. 5) may facilitate pulmonary CO_2 excretion slightly. The P_{CO_2} in the dorsal aorta was generally lower than the lung P_{CO_2} by about 1 torr in both normoxia and hypoxia. This may be a result of off-loading of CO_2 from the blood in the presence of the high levels of O_2 in the lung. Due to the nature of the pulmonary venous return, postpulmonary blood P_{CO_2} could not be measured.

Blood gas transport

Oxygen

An overview of blood O_2 transport and blood flow is shown in Fig. 9 for both normoxia (A) and hypoxia (B), which incorporates both measured and calculated values for O_2 saturation, blood flow and $\dot{M}_{O_2}$. In normoxia the small change in blood O_2 saturation through the gills is probably a result of the small lamellar surface area: This leads to a low arterial percentage saturation in normoxia. During hypoxia, when

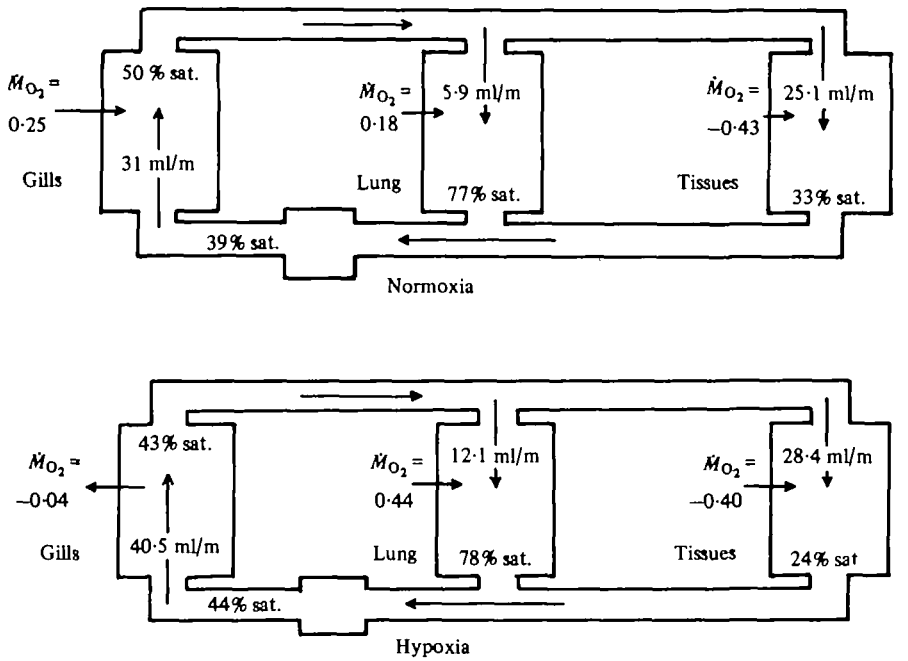

Fig. 9. A model of oxygen transport in the spotted gar including the oxygen transported ($\dot{M}_{O_2}$), blood O_2 saturation (Sat) and cardiac output (given in ml.min^{-1}) across the gills, lung and tissues in the normoxia (top) and hypoxia (bottom).

there is no branchial O_2 uptake, the arterial O_2 saturation is low because saturated pulmonary and unsaturated venous blood are mixed in the VA. The gar, however, has a moderately high blood O_2 capacity (11 vol. %) compared to most water breathers (Randall, 1970), which helps to compensate for the low arterial saturation and may help to prolong the interval between air breaths (Johansen, 1970).

During both normoxia and hypoxia the pulmonary blood O_2 saturation is the same, but increased cardiac output and an increased volume of oxygenated blood from the lung helps to maintain tissue O_2 delivery in hypoxia. Gas transport studies on lungfish (Johanson, Lenfant & Hanson, 1968*a*) and *Amia* (Randall *et al.* 1981) suggest that similar elevations of lung perfusion and cardiac output occur in these fish while air breathing. In *Neoceratodus* (Johansen *et al.* 1968*a*) there was also a large increase in the O_2 saturation of pulmonary blood during hypoxia, unlike the gar.

CO_2 transport

Air-breathing fish normally maintain higher blood P_{CO_2} levels than do water breathers. At 20 °C, however, gar are primarily water breathers and CO_2 excretion is still primarily from the gills, even while they are air breathing frequently in hypoxia. As a result gar maintain low arterial P_{CO_2} compared to obligate air breathers. This has apparently precluded the need for the high blood CO_2 combining capability or large buffer capacity which is seen in *Protopterus* (Lenfant & Johansen, 1968), *Electrophorus* (Johanson *et al.* 1968*b*) and other obligate air breathers. The gar does exhibit a moderate Haldane effect, which, along with the Root and Bohr effects, appears more

frequently in water breathers and facultative air breathing fish than in obligate air breathers (Johansen, 1970).

Deoxygenated blood is normally a better buffer than oxygenated blood (Albers, 1970), but we found just the reverse in gar. The difference between the slopes may be spurious, as the sample points are only significantly different at 3·9 torr P_{CO_2}. It is possible that anaerobic metabolism in the deoxygenated blood by the RBC's resulted in an accumulation of acid metabolites, thus decreasing the C_T as progressive determinations were made. Albers & Pleschka (1967) made similar observation on the CO_2 combining curves of elasmobranchs.

The changes in blood CO_2 transport through the gills between normoxia and hypoxia were small, as are the changes in CO_2 excretion rates. CO_2 excretion does not seem to be greatly affected by hypoxia, which probably indicates that there are no major changes in the gill ventilation/perfusion ratio in hypoxia. It is unlikely that the Bohr or Root effects contribute significantly to branchial O_2 uptake as a result of the small prebranchial and postbranchial changes in CO_2 and pH.

Cardiovascular adaptations to hypoxia

The channelling of the blood through the secondary lamellae is one of the most striking anatomical adaptations of the gar. This channelling represents a reduction in the surface area available for gas exchange even beyond the already reduced external lamellar area, and may help to reduce O_2 loss during aquatic hypoxia. Gannon (1977) found similar channelling of the lamellar vasculature of *Hoplyerythrinus*.

The elevation of the lung perfusion in hypoxia enabled gar to enhance pulmonary O_2 uptake, but if cardiac output had not also risen in hypoxia the systemic blood flow and O_2 transport would have been compromised (Fig. 9). An elevation in cardiac output in hypoxia and a subsequent elevation of pulmonary perfusion have also been observed for *Protopterus*, *Electrophorus*, *Neoceratodus*, *Lepidosiren* (Johansen *et al.* 1968*a*) and *Amphipnous* (Lomholt & Johansen, 1976). The mechanisms responsible for the redistribution of blood flow, however, may differ markedly among air breathing fish.

Farrell (1978) and Smith & Gannon (1978) observed that *Hoplyerythrinus* could direct blood flow to the 3rd and 4th gill arches, which shared a common efferent, and joined the pulmonary artery via the coeliac artery. This preferential shunting elevated lung blood flow and might also be expected to reduce lamellar perfusion in the other gill arches. The lungfish also preferentially perfuse specialized branchial arches and reduce total lamellar perfusion to increase pulmonary perfusion (Johansen *et al.* 1968*a*). Gar, unlike the lungfish or *Hoplyerythrinus*, have no single pulmonary artery or connexions between specific gill arches and the lung blood supply (except for the dorsal aorta). It is unlikely that a redistribution of branchial blood flow could greatly affect pulmonary perfusion in hypoxia. Several microsphere experiments, done to gain an estimate of the branchial blood flow distribution in normoxia and hypoxia, supported this conclusion. Based on these data the gar does not preferentially perfuse specific gill arches in either normoxia or hypoxia. Johansen *et al.* (1968*a*) noted a reduction in branchial vascular resistance across the gills of *Protopterus* associated with perfusion of the shunt pathways. In gar, both the pressure drop across the gills

and the cardiac output increase in hypoxia. The branchial vascular resistance (BVR) calculated simply as the ratio of the pressure drop across the gills/blood flow through the gills is the same in either normoxia or hypoxia for gar. This indicates that lamellar recruitment and perfusion probably do not change significantly between normoxia and hypoxia in gar. Thus the control of changes in pulmonary perfusion in gar is probably independent of changes in gill-arch perfusion. The pulmonary perfusion may be regulated by the numerous pulmonary arteries, but the specific vascular mechanism controlling lung perfusion in gar remains to be discovered.

Changes during an air breath

There were regular changes in most of the variables measured during the course of an air breath (Fig. 6*a–c*), but only the changes in pH and lung gasses proved to be statistically significant. The variability of the air-breathing intervals in different fish made normalization of these data difficult and contributed to the error. There are few other experiments on air breathing fish to compare with these data. Farrell (1978) observed that *Hoplerythrinus unitaeniatus* elevated heart rate after an air breath, and elevated lung perfusion but did not change cardiac output. The lungfish, on the other hand were observed to elevate heart rate, lung perfusion and cardiac output immediately after a breath (Johansen *et al.* 1968*a*). *Anabas* (Sing & Hughes, 1973) and *Synbranchus* (Johansen, 1966) show similar trends. While the changes in gar were not statistically significant, they reflect the same trends observed in these other air-breathing fish. Heart rate (Fig. 6*c*) rises after a breath then falls slowly, and blood pressures in both the DA and VA increase then slowly decrease (Fig. 6*c*), indicating that cardiac output may also be changing through the air-breathing interval. There are statistically significant changes in the pH from the DA and VA during an air breath (Fig. 6*a*) which are paralleled by changes in P_{CO_2} and C_T (Fig. 6*b*). These changes may be indicative of changing perfusion patterns between the lung and systemic circulation, but without further information on circulatory dynamics, they are difficult to interpret.

Branchial hypoxia O_2 loss

In the apparent absence of any lamellar shunting, or preferential gill perfusion, what limits O_2 loss from the gills during aquatic hypoxia? The transport of gas across the gills should be sensitive to changes in the surface area available for gas exchange, the water flow past the gills and the magnitude of the partial pressure gradient between blood and water.

Changes in the oxygen transfer factor (T_{O_2}), where ($T_{O_2} = \dot{M}_{O_2}/P_{O_2}$ (Randall, 1970), are usually indicative of changes in lamellar perfusion or the diffusion distance for O_2 (indicating changes in convection). Based on average measured $\dot{M}_{O_2}$ and P_{O_2} values in normoxia the T_{O_2} = 0·0025 $cm^3 \cdot min^{-1} \cdot torr^{-1} \cdot kg^{-1}$ and in hypoxia is 0·0031 $cm^3 \cdot min^{-1} \cdot torr^{-1} \cdot kg^{-1}$. These values are about half the calculated T_{O_2} for trout (Randall *et al.* 1967), which probably reflects the reduced lamellar surface area in gar. The reduction of ventilation volume in hypoxia should reduce the T_{O_2}, yet T_{O_2} is slightly enhanced during hypoxia, implying that there may be a slight elevation in the perfused lamellar surface area. These values, however, are estimates of the oxygen transfer and probably best serve to indicate that there are no large

differences between normoxia and hypoxia in the perfused lamellar surface area or diffusion distance. If there are no significant changes in the variables affecting T_{O_2} then any change in oxygen transport ($\dot{M}_{O_2}$) between normoxia and hypoxia must be a result of changes in the O_2 diffusion gradient between blood and water. Thus the small O_2 efflux in hypoxia is closely matched by the small reversed P_{O_2} gradient. A recent investigation of *Amia* (Randall *et al.* 1981) produced similar results; the T_{O_2} did not change between normoxia and hypoxia and there was no evidence for functional or anatomical shunting.

Spotted gar and *Amia* probably do not modify lamellar blood flow to any significant degree as part of their hypoxic response and both lose a small amount of O_2 through their gills in severely hypoxic environments. This O_2 loss appears to be inconsequential to the gar because the animal's O_2 demands are met in hypoxia, without an elevated metabolic cost (as estimated by $\dot{M}_{O_2}$). Thus, for the spotted gar there is no need for shunting, and the ancillary functions of the gill (e.g. CO_2 excretion and osmoregulation) are unaffected by hypoxia. The O_2 loss in any air breathing fish could be expected to be low, perhaps insignificantly so, in most hypoxic situations. Preferential gill arch perfusion, in fish where it has been observed may serve primarily as an adaptation to enhance pulmonary perfusion, and may not be a critical mechanism for preventing hypoxic O_2 loss.

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