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

II. RESPONSES TO TEMPERATURE CHANGE AND HYPERCAPNIA

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SUMMARY

Elevation of temperature from 20 to 30 °C, increased the pulmonary ventilation and pulmonary oxygen consumption (from 0·18 to 0·43 ml O_2.kg^{-1}.min^{-1}). The total CO_2 excretion also rose, but branchial ventilation and branchial oxygen consumption did not change significantly.

The blood pH dropped quickly when temperature was elevated, with a slope ($dpH/dT°$) of −0·015, but the OH^-/H^+ ratio did not change significantly. This change in pH resulted from an elevation in arterial P_{CO_2}, without any concomitant change in plasma HCO_3^-. Arterial P_{CO_2} *per se* was probably not actively regulated, but rose passively as a consequence of the gar's utilization of the lung for increased oxygen uptake, and the inefficiency of the lung in CO_2 exchange. The lung had an exchange ratio (R) of about 0·1.

Temperature change produced no significant alteration in the net acid excretion from either the kidney or the gills, despite an increased ammonia excretion rate at 30 °C. The urine formation rate was very low (74 μl.100 g^{-1}.h^{-1}) which imposed a limitation on the importance of the kidney in acid-base regulation.

Hypercapnia produced a respiratory acidosis which was partially compensated by an elevation in blood HCO_3^- after 24 h of exposure. The gill ventilation rose only slightly, and later fell as compensation proceeded. Air-breathing frequencies were not greatly affected by hypercapnia.

INTRODUCTION

As temperature changes, poikilothermic vertebrates regulate blood pH with a negative temperature slope of −0·015 to 0·020 pH/°C, which minimizes changes in protein ionization (Reeves, 1977). Air-breathing ectotherms make these changes by regulating arterial P_{CO_2} tensions (Jackson, Palmer & Meadow, 1974; Jackson & Kagen, 1976; Reeves, 1977), by changing ventilation rates and pulmonary O_2 utilization. Water breathers, unlike air breathers, have been found to manipulate blood HCO_3^- concentrations to regulate pH (Randall & Cameron, 1973). While the acid-base

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responses of air or water breathers have received considerable attention, little is known about how air-breathing fish regulate blood pH in the face of environmentally induced perturbations of their acid-base balance.

Air-breathing fish are particularly interesting because as transitional animals (with characteristics of both air and water breathers) they have two potential means for regulating acid-base balance. The high ventilatory requirements needed for O_2 transport in water-breathing fish, combined with the high solubility of CO_2 relative to O_2, preclude effective ventilatory control of P_{CO_2} without jeopardizing O_2 uptake (Rahn, 1966; Randall & Cameron, 1973). Air-breathing fish, however, are not exclusively dependent on their gills for supplying their O_2 demands, and may be able to regulate pH through ventilatory adjustments of blood P_{CO_2}. Rahn *et al.* (1971) found that venous P_{CO_2} increases in *Lepisosteus osseus* in response to an elevation of temperature, which supports this contention. Alternatively, P_{CO_2} may change somewhat, but the ultimate control of pH may be dependent on adjustments of blood HCO_3^- concentrations. In water breathers these changes in HCO_3^- occur via branchial and renal ion exchanges (Cameron, 1979; Heisler, 1981). Little is known about whole-animal acid or base efflux rates, except for studies on *Scyliorhinus stellaris* by Heisler, Weitz & Weitz (1976) and Heisler (1978). There are no published studies on the whole body, branchial or renal acid-base responses of air breathing fish to temperature change, but Cameron & Wood (1978) assessed renal function and acid-base balance in *Hopylerythrinus unitaeniatus* during post-operative acidosis and in response to hypercapnia.

This study is directed primarily towards determining how acid-base balance was regulated in an air breathing fish in response to two perturbations: temperature change and environment hypercapnia (aquatic). In water-breathing fish, both conditions require substantial transport of bicarbonate, outwards in the case of a temperature rise, and inwards in the case of hypercapnia. In the typical air-breather, temperature increase is handled by adjustment of pulmonary ventilation and hypercapnia by short-term hyperventilation and long-term renal acid excretion. Since it was by no means obvious which responses might be taken by an air-breathing fish, the responses of the spotted gar, including blood parameters, ventilation, renal output and branchial acid/base excretion, were assessed against the background outlined for strict air- and water-breathers.

METHODS

Animals

Spotted gar, *Lepisosteus oculatus*, weighing between 600 and 900 g were used in the study. The animals were caught by gill netting in the Guadelupe River in Texas, and kept in 1500 l tanks with recirculating oyster-shell filters at laboratory temperatures (18–23 °C).

Holding chambers

During the experiments the gar were held in darkened plexiglass chambers provided with a flow of dechlorinated tap water. The inflowing water temperature was

regulated at either 20 or 30 °C (±0·5 °C). The water could also be recirculated in the chambers. While recirculating, the water was pumped through two equilibration flasks, where it was oxygenated with atmospheric air. The recirculation system was used to measure the branchial acid excretion rates at 20 and 30 °C. The system was recirculated for only short periods of time in order to prevent the accumulation of ammonia and CO_2.

The system was used in its flow-through configuration for the hypercapnia experiments. The water was equilibrated either with air or 1% CO_2 in air.

Temperature-change experiments

Blood values

Dorsal aortic catheters were implanted in the gar for collection of arterial blood, and the blood obtained was analysed for pH, total CO_2 (C_T) and P_{CO_2} (surgery, recovery, and analytical techniques described by Smatresk and Cameron, this issue). Blood values were determined at 20 °C (twice, 24 h apart), and after a step change in the temperature to 30 °C at 1, 2, 4, 8, 24 and 48 h after the change. A final sample was obtained 24 h after the temperature was returned to 20 °C.

Respiration

At 30 °C the air-breathing interval and gill ventilation rates were determined using an opercular catheter and pressure transducer (Smatresk & Cameron, 1981). The $\dot{M}_{O_2}$ and $\dot{M}_{CO_2}$ were also both determined for air and water using the methods described in the preceding paper.

Urine measurements

A urinary catheter was implanted during surgery. The shape of the gar's cloacal aperture made the catheter difficult to secure. A purse-string suture secured a length of PE-60 tubing, with terminal perforations and a cuff, into the bladder. Of a number of operations, only seven of the preparations were patent before the temperature change, the remainder clogging or siphoning. Four of those still remained patent 48 h after the temperature change. Titratable acidity was one variable measured from urine collected. The urine was titrated back to the pH of the blood as determined during the collection interval. The urine formation rate was measured for each sample point. The urine ammonia concentration was measured and the total CO_2 (C_T) of the urine was measured. These variables allow the net H^+ ion excretion from the urine to be calculated (Hill, 1973) as $\dot{J}_{net}H^+ = TA + Amm. - HCO_3^-$. Ammonia was measured using the phenol-hypochlorite method (Solorzano, 1969). C_T was measured conductometrically and titratable acidity was measured with a titrator and autoburette assembly (Radiometer Copenhagen PHM 64, Autoburette ABU 11 and Titrator Assembly TTA 60). The net H^+ excretion was calculated as the sum of these variables. The urine formation was slow, and flux rates were determined for 24 h intervals throughout the experiment. Flux rates were measured for 2 or 3 days at 20 °C, and each day after the temperature changed to 30 °C.

Branchial acid excretion

The branchial acid excretion was determined by the changes of titratable acidity and ammonia in the recirculated water. The titratable acidity was measured by the changes in 2 ml aliquots of water (from the recirculation system) taken at hourly intervals and titrated to pH 4·0 with 0·01 M-HCl. Thus if there was a base output, an equivalent additional amount of acid would be necessary to reach an end point of pH 4·0. The titrations were made both with the autoburette system, and by hand with a microburette, with similar results. The TA flux rates were calculated hourly, from two aliquots, for the total water volume and per 100 g of fish weight, but are presented averaged over 3 h. This was the length of time the water was recirculated at each sample period except for one 4 h period immediately after the temperature change. TA flux rates were measured at 20 °C (24 h before the temperature change), and after the change at intervals of 0–1, 2–4, 5–8, 24–27 and 48–51 h and again 24 h after the return to 20 °C. Ammonia, measured by the same method used for urine ammonia, was measured at the same intervals and the ammonia efflux rates and TA flux rates were used to calculate the net acid flux ($\dot{J}_{net}H^+$ = TA + Amm.).

Hypercapnia

After the fish recovered (20 °C), blood withdrawn from the dorsal aorta was analyzed for pH, C_T, P_{CO_2} and P_{O_2}. The gill and lung ventilation rates were monitored in six fish at 40 min intervals. The water was then made hypercapnic with 1% CO_2 and air, the change in P_{CO_2} was completed in about 1 h. Blood variables and ventilation were measured 1, 2, 4, 8 and 24 h after the onset of hypercapnia on four fish. After 24 h the water was made normocapnic and measurements were made at 1 and 3 h.

RESULTS

Temperature-change experiments

Ventilation

Following the temperature change, the air-breathing frequency increased greatly, while the gill ventilation decreased slightly. These changes were both significantly different from the rates at 20 °C (*t* test, $P < 0.05$; Table 1). The oxygen consumption and CO_2 excretion from air and water for both 20 and 30 °C are shown in Table 1. The contribution of the lung to oxygen consumption is significantly greater at 30 °C, but branchial $\dot{M}_{O_2}$ is unchanged. The gill continues to be responsible for the majority of the CO_2 excretion. Total O_2 consumption increased by 63% at 30 °C while CO_2 excretion increased by 23%; both changes were statistically significant. The respiratory exchange ratio (R), was slightly lower at 30° than at 20 °C, but the difference was not statistically significant.

Blood acid-base balance

The time-course of the change in blood acid-base variables from 20 to 30 °C is shown in Fig. 1 (N = 13 at 20 °C and 48 h, N = 7 at the other points). From 20 °C

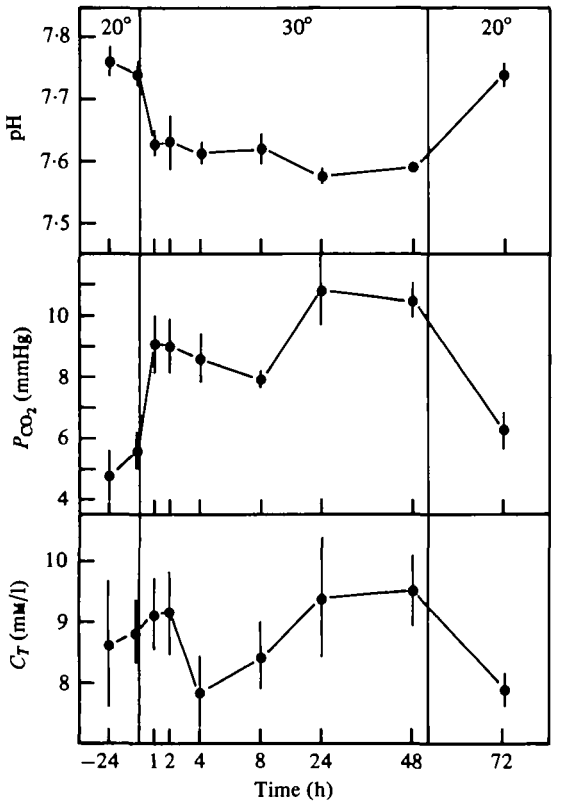

Fig. 1. The time course of changes of blood pH, P_{CO_2}, and C_T at 20 °C, after the change to 30 °C and on return to 20 °C.

Table 1. *A comparison of respiratory variables for spotted gar acclimated to* 20 °C, *and tested at* 20 °C *or after a change to* 30 °C

(Values given are the mean ±S.E., with the number of animals and total number of measurements given in parentheses. A single number in parentheses is the number of fish only.)

	20 °C	30 °C
Gill ventilation rate (bpm)	29·7 ± 1·1 (N = 6, 18)	28·8 ± 2·6 (N = 6, 18)
Air breathing interval (min)	approx. 1 hr (N = 10)	7·8 ± 2·8 (N = 4, 8)
Lung $\dot{M}_{O_2}$ (ml O_2.kg^{-1}.min^{-1})	0·18 ± 0·04 (N = 9)	0·43 ± 0·09 (N = 6)
Gill $\dot{M}_{O_2}$ (ml O_2.kg^{-1}.min^{-1})	0·25 ± 0·04 (N = 9, 27)	0·27 ± 0·02 (N = 6, 18)
Total $\dot{M}_{O_2}$ (ml O_2.kg^{-1}.min^{-1})	0·43 ± 0·05 (N = 9)	0·70 ± 0·08 (N = 6)
Lung $\dot{M}_{CO_2}$ (ml CO_2.kg^{-1}.min^{-1})	not measurable	0·05 ± 0·01 (N = 6)
Gill $\dot{M}_{CO_2}$ (ml CO_2.kg^{-1}.min^{-1})	0·47 ± 0·08 (N = 9, 27)	0·53 ± 0·07 (N = 6, 18)
Total $\dot{M}_{CO_2}$ (ml CO_2.kg^{-1}.min^{-1})	0·47 ± 0·08 (N = 9)	0·58 ± 0·07 (N = 6)
Respiratory Quotient	1·05 ± 0·08 (N = 9)	0·85 ± 0·05 (N = 6)

to 48 h after the temperature was elevated to 30°, the arterial P_{CO_2} rose from 5·6 ± 0·6 to 10·5 ± 0·5, pH fell from 7·74 ± 0·02 to 7·59 ± 0·01, both changes significant (paired t test $P < 0·05$). The arterial HCO_3^- concentration did not change significantly following the temperature elevation (from 8·8 ± 0·6 to 9·5 ± 0·6). Within 1 h after changing to 30 °C the blood pH dropped to 7·63 and P_{CO_2} rose to 9·1, but C_T did not

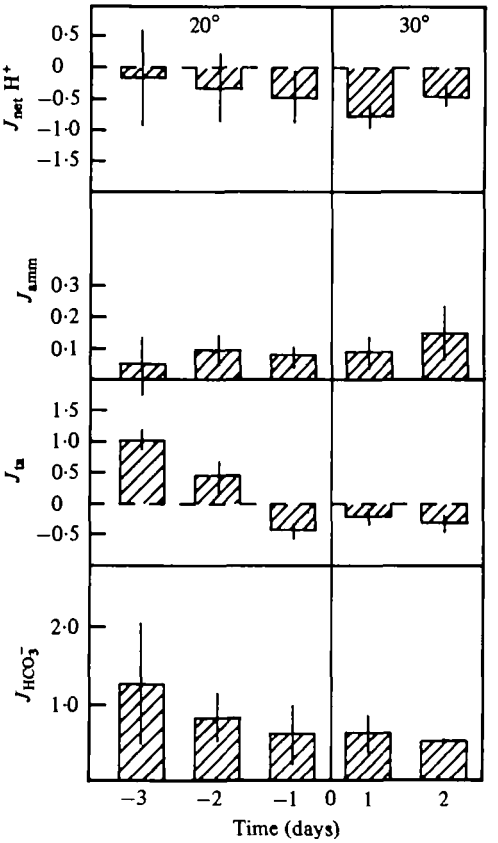

Fig. 2. Urine efflux of HCO_3^- ($J_{HCO_3^-}$), titratable acidity (J_{TA}), ammonia (J_{Amm}), and the net acid efflux ($J_{Net\ H^+}$) (all expressed as μequiv. $100\ g^{-1}.h^{-1}$). N = (3, 6) at −3 days, N = (5, 10) at −2 days, N = (7·14) at −1 day, N = (5, 10) at +1 day and N = (4, 8) at +2 days.

change significantly. The variables had stabilized by 24 h after the temperature change. When the animals were returned to 20 °C, the blood acid-base variables returned within 24 h to the initial levels. The *in vivo* pK at 20 °C was 6·265 and at 30 °C was 6·234, using the same CO_2 solubility coefficients determined earlier from *in vitro* blood work (Smatresk & Cameron, 1982*a*). $[OH^-]/[H^+]$ ratios, calculated from the pH values, were 19·2 at 20 °C and 21·2 at 30 °C (not significantly different). The extracellular dpH/dT was −0·015 in gar. The corresponding dpH/dT of neutral water between 20 and 30 °C is −0·016.

Urinary contribution acid/base excretion

Fig. 2 illustrates the changes in the net renal acid excretion. The urine formation rate at 20 °C was 0·074 ± 0·015 ml. $100\ g^{-1}.h^{-1}$ (N = 7); the average rate at 30 °C (24 h was 0·082 ± 0·069 ml. $100\ g^{-1}.h^{-1}$ (N = 4). The average change was not significant. Postoperative urinary flow (N = 7) was 0·167 ± 0·073 ml. $100\ g^{-1}.h^{-1}$, a statistically significant post-operative diuresis. At 20 °C there was a slight net base excretion. The numbers of fish sampled and the number of measurements at each point are shown in Fig. 2. Ammonia excretion into the urine was very low, titratable

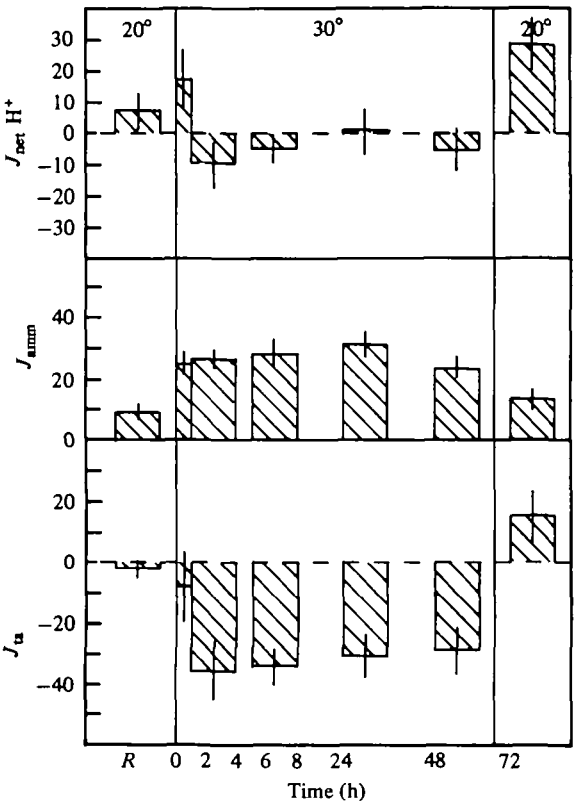

Fig. 3. Branchial efflux rates for titratable acidity (J_{TA}), ammonia (J_{Amm}), and net acid efflux ($J_{Net\ H^+}$) (all expressed as μequiv. 100 g^{-1}.h^{-1}). N = (7, 21) at R, N = (9, 27) at 30 °C and N = (6, 18) at 20 °C during recovery.

acidity was low, and bicarbonate excretion accounted for the small observed base excretion. When the temperature was raised to 30 °C, there were offsetting increases in TA and ammonia efflux, resulting in a slight elevation in net base excretion which was not significant. By 48 h after the temperature change, the net acid efflux was the same as the 20 °C levels; there was still a titratable base excretion, but bicarbonate efflux was reduced. There were no significant changes in any of the urine variables with temperature change. No catheters remained patent for the return to 20 °C. The resting renal base excretion accounted for less than 10% of the total whole animal acid-base flux.

Branchial acid/base excretion

Branchial acid-base transfer (Fig. 3) accounted for over 90% of the apparent whole body acid-base flux at 20 °C. At 20 °C there was a resting net acid excretion of 7·1 μequiv. 100 g^{-1}.h^{-1}. After the temperature had been changed, there was a transient elevation of net acid excretion that lasted for 1 h. This was followed by a net base excretion which appeared to stabilize by about 8 h after the temperature change. Because the net acid or base excretion was calculated as the sum of the ammonia

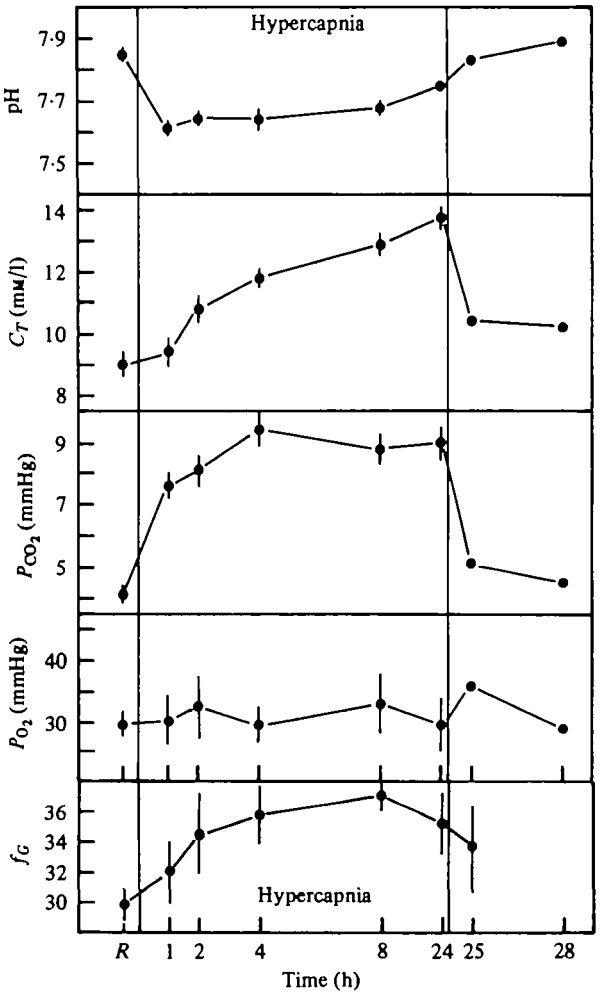

Fig. 4. Graph of the changes in pH, C_T, P_{CO_2}, P_{O_2}, and gill ventilation (f_G) in normocapnia (R), during hypercapnia and for a 4 h recovery period following hypercapnia.

efflux and the titratable acid efflux (positive or negative), the calculated net efflux could only be statistically significant if there was a significant difference between the two. The differences between NH_3 and TA efflux rates were not significant (t test $P > 0{\cdot}05$) except after the return to 20 °C, indicating that the branchial acid and base efflux rates were balanced. Both the ammonia and base efflux rates were higher at 30 °C.

Hypercapnia

Blood analysis

Shortly after the onset of hypercapnia ($P_{CO_2} \simeq 6$ torr) there was a fall in extracellular pH. This acidosis was accompanied by an elevation in P_{CO_2} and initially by only a slight elevation in C_T (Fig. 4). The acidosis was most severe 1 h after hypercapnia started and by 24 h of hypercapnia pH had recovered to within about 0·1 pH unit of resting values. P_{CO_2} stabilized after 4 h of hypercapnia, and C_T continued to rise

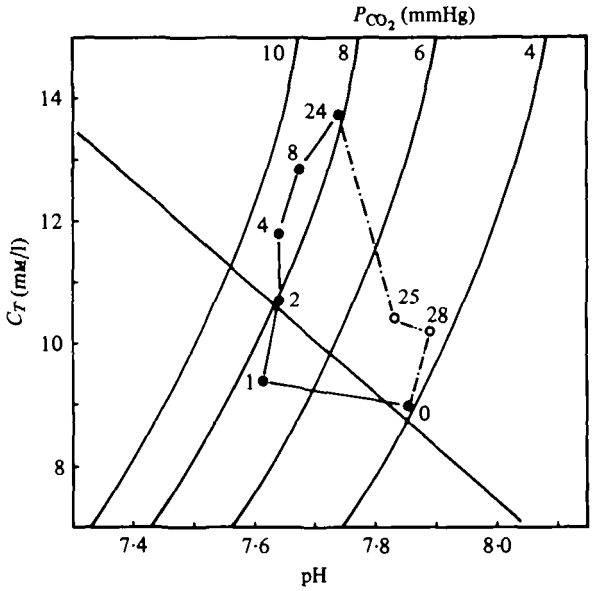

Fig. 5. Davenport diagram of the simultaneous changes in pH, C_T and the P_{CO_2} at rest (o), during hypercapnia (solid line and circles) and in recovery (dashed line, open circles).

Table 2. *Air-breathing frequency in breaths/h at rest, during hypercapnia and in recovery from hypercapnia for the four fish tested*

Fish no.	R	Hypercapnia 1 h	2 h	4 h	8 h	24 h	Recovery 1 h	3 h
81	0	0	0	1	4	8	0	0
82	0	2	2	2	3	4	0	0
83	1	2	1	5	20	0	0	0
84	0	0	0	0	0	0	0	0

throughout the experimental period. The changes in the blood acid-base variables are all significant (two-way ANOVA, with no replications; $P < 0{\cdot}05$). Blood P_{O_2} (Fig. 4) did not change significantly throughout the experiment. Blood acid-base variables were also monitored for two fish following the return to control conditions (normocapnia). The blood acid-base balance had been restored, except for a slightly elevated C_T, within 3 h after hypercapnia ceased.

A Davenport diagram (Fig. 5) was constructed using the *in vitro* blood buffer line and P_{CO_2} isopleths were calculated using an *in vivo* whole blood pK from resting fish of 6·227 calculated from the Henderson–Hasselbalch equation and an β_{CO_2} of 0·0509 (20 °C, 260 m-osmol). Because the Davenport diagram analyses changes in *in vivo* whole blood, the P_{CO_2} isopleths constructed from the 'functional' pK should fit the data more closely than would those calculated from an *in vitro* 'true plasma' pK. The diagram indicated that hypercapnia initially induced a mixed metabolic and respiratory acidosis. The respiratory acidosis remained, but was partially compensated by a metabolic alkalosis as time went on (elevation of C_T). The recovery from these conditions, after the hypercapnic period was over, was rapid and almost complete in 3 h.

Ventilation

Gill ventilation rose by about 20% during hypercapnia (Fig. 4). The differences in the gill ventilation rates among the fish accounted for the large standard errors, and a two-way ANOVA, with no repetitions, done on the raw data revealed no significant difference. Paired *t* tests on the differences between individual control values and experimental values at 4, 8 and 24 h of hypercapnia, however, were significant (i.e. all individual gar elevated their ventilation rate, $P < 0.05$).

The effects of hypercapnia on air breathing were more difficult to assess. The air-breathing frequency was determined during 1 h periods. At rest, only one fish took an air breath in that 1 h interval. Three of the four fish tested increased their air-breathing rates sometime during hypercapnia. Elevated air breathing frequency was always associated with increased activity of the fish. When the P_{CO_2} was returned to normal levels, none of the fish took an air breath in a 60 min interval. The data on air breathing for each of the fish tested are shown in Table 2.

DISCUSSION

The blood acid-base state

The change in blood pH in the spotted gar as temperature rose from 20 to 30 °C was very nearly the same as the change in the p*K* of water: −0·015 pH/°C for blood *v.* −0·016 for water in the same temperature range. Whether it is important to emphasise that such a change would maintain relative alkalinity at a nearly constant value, or that it would enforce a nearly constant fractional dissociation of imidazole groups (a-imidazole, Reeves, 1977), was not an issue in this study, especially since the two ideas predict nearly the same pH/temperature slope. Our observations are also consistent with earlier observations on a number of other, strictly water-breathing fishes (Garey, 1972; Randall & Cameron, 1973; Cameron, 1978). Further, Rahn *et al.* (1971) have observed a similar relationship in the long-nosed gar (*Lepisosteus osseus*) based on summer and winter measurements.

The acid/base changes observed in the blood in response to hypercapnia were similar to those reported for other, water-breathing fishes (i.e. there was a fairly rapid pH depression concomitant with the increase in P_{CO_2}, followed by a gradual rise in the blood bicarbonate concentration). This rise partially ameliorated the respiratory acidosis (cf. Cameron & Randall, 1972; Janssen & Randall, 1975; Cameron, 1980).

What was interestingly different about the response of the gar to temperature was that instead of a reduction in blood bicarbonate and constant P_{CO_2} at higher temperatures, the necessary reduction in blood pH was brought about by an increase in P_{CO_2}, at nearly constant CO_2 content. In this respect, then, the gar's response to temperature was like that of the typical air-breather (Jackson *et al.* 1974). The increase in P_{CO_2}, however, does not seem to result from an overall decrease in convection ratio (the ratio of ventilation to oxygen consumption), nor to a decrease of the gill ventilation relative to oxygen taken up there (Table 1). Increased P_{CO_2} appears to result instead from a decreased ratio of *gill* ventilation relative to *gill* excretion of CO_2, since the oxygen demand is met largely by switching to lung ventilation, but the lung appears ineffective in excreting CO_2.

It is of course impossible to say whether the gar's regulation of P_{CO_2} in this manner is an 'active' regulatory process, or whether it is simply a 'passive' and rather fortuitous result of the use of the lung at high temperatures to supply extra oxygen. Since the gar do not respond to acid/base disturbances like hypercapnia by significantly increasing ventilation, we tend to favour the latter view, and consider that the gar regulate ventilation of both the gill and the lung principally in response to oxygen demands, which results in the 'correct' P_{CO_2} due to the architecture of the two exchangers. We also point out that since the gar does regulate acid/base state in this manner during temperature change, no great change in the whole body bicarbonate content is expected, and no significant acid/base fluxes are predicted.

During the response to hypercapnia, there is clearly significant net transport of base into the body (or acid out of it), since the P_{CO_2} is artificially constrained to values above the ambient hypercapnia, and any change in ventilation would be ineffective in remedying the situation.

Whole-body acid-base exchanges

Consistent with the blood observations during the temperature change experiments, the data on whole-body acid/base exchange did not show any significant transport during either the increase to 30 °C or the return to 20 °C (Fig. 3). Regardless of the statistical significance, or lack thereof, the quantities transported in Fig. 3 are insignificant in relation to whole body pools. These results are somewhat at odds with those of Heisler *et al.* (1976) and Heisler (1978) from *Scyliorhinus stellaris*. Following a temperature increase in this elasmobranch, they observed a transient increase in net base excretion, accompanied by a rise in the total CO_2 in the blood, and also a rise in the P_{CO_2}. Although the contribution of ammonia was discounted in their study, published rates of ammonia excretion for elasmobranchs (Evans, Kormanik & Krasny, 1979; Goldstein & Forster, 1971) may indicate that a rise in ammonia excretion was accompanying the increase in apparent base efflux, which would mean that there was no net acid/base flux, as we observed for the gar (Fig. 3).

A further contrast between our work with the spotted gar and Heisler's results from *Scyliorhinus* is that since we found no significant change in CO_2 content, and no significant net acid/base flux from the fish during temperature change, we would not predict any large flux between the intra- and extracellular fluid pools. The increase in P_{CO_2} of the blood is doubtless reflected intracellularly, and would bring about changes in intracellular pH according to the physico-chemical buffer capacities of that compartment.

Urinary contribution to acid/base balance

The urinary flow rate is one potential indicator of the contribution of the kidneys to acid/base regulation, and the flow rate in the spotted gar was very low under all circumstances. Even the highest flow rate we measured, 1·7 $ml.kg^{-1}.hr^{-1}$ during post-operative diuresis, was far lower than the 3·0 $ml.kg^{-1}.h^{-1}$ reported for rainbow trout, 10·7 for goldfish, and 7·1 for carp (Hickman & Trump, 1970). The rate for gar was also much lower than the 3·8 ml/kg/h reported for another facultative air-breather *Hoplerythrinus unitaeniatus*, by Cameron & Wood (1978). The average rate for resting, recovered gar was only 0·74 ml kg^{-1} h^{-1}. With such a low-volume rate of flow, even

very high acid or base concentrations in the urine would not constitute a significant component of overall acid/base balance. Thus the rates of renal excretion were quite small in relation to the branchial component (cf. Figs. 2 and 3) and did not contribute to the temperature response. Having established that the rates were so low, the renal response to hypercapnia was not measured, but was likely also small.

The explanation for such low rates of urine flow was not directly investigated, but it seems quite likely that they are related to the reduction of the gill surface area, which is the greatest route of osmotic water influx in fish, and probably also to the extremely heavy armour of ganoid scales found in this species.

Ventilatory responses to hypercapnia and temperature change

The increase from 20 to 30 °C was naturally attended by a substantial increase in the oxygen demand of the animal, which was met entirely by increased pulmonary oxygen uptake, while neither branchial ventilation nor oxygen uptake changed significantly. The changes in CO_2 excretion were not quite as clear, possibly due in part to the greater determination error involved in CO_2 excretion measurements. there did appear, however, to be a real decrease in the respiratory quotient at the higher temperature, with the CO_2 continuing to be excreted almost entirely by the gills. The reduction in CO_2 excretion occurred in each fish studied, but due to the large experimental variability, the difference was not statistically significant.

There were some small changes in the gill and lung ventilation of the gar in response to increasing environmental P_{CO_2}. Hypercapnia initially produces a respiratory acidosis which apparently has little effect on the gill ventilation rate of most fish (Saunders, 1962; Dejours, 1975), but may stimulate ventilation in some fish (Janssen & Randall, 1975; Randall, Heisler & Drees, 1976). In the spotted gar the gill ventilation rate is slightly elevated initially, and appears to be stable or even decreasing 24 h after exposure. The temporary elevations in the rate of air breathing did not appear to be strongly correlated with any of the measurable variables. Hypercapnia, and the resulting pH drop, may cause increased irritability. The effects of hypercapnia may be from changes in the blood pH (or strong ion difference), from irritability, or from a reduction in blood O_2 capacity. The arterial P_{O_2} did not change significantly during hypercapnia, but the Root effect should have resulted in a significant reduction of blood O_2 content (an estimated reduction of 1 vol.% or about a 13% reduction in O_2 saturation). Such a reduction may have accounted for the initial changes in ventilation. Restoration of the plasma pH may also have partially reversed the adverse effects on blood O_2 capacity.

In general, air breathing is increased and branchial respiration changes little in other air-breathing fish exposed to hypercapnia. In *Trichogaster*, Burggren (1979) found that hypercapnia produced a sharply elevated air-breathing rate, as was found in *Piabucina* (Graham, Kramer & Pineda, 1977) and *Saccobranchus fossilis* (Hughes & Singh, 1971). Gill ventilation, when measured, fell slightly for these air breathers in hypercapnic water (Graham *et al.* 1977; Hughes & Singh, 1971). The wide range of responses shown by these air breathers, and by gar, suggests that some air-breathing fish may actively regulate P_{CO_2} by ventilation, under certain conditions, to maintain acid-base balance. The control of breathing in the spotted gar, however, seems on the whole far more closely tied to oxygen than to CO_2 (Smatresk & Cameron, 1982*b*).

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