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THE EFFECT OF INCREASED AMBIENT CO_2 ON ARTERIAL CO_2 TENSION, CO_2 CONTENT AND pH IN RAINBOW TROUT

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INTRODUCTION

Aquatic animals, particularly in fresh water, may encounter water with CO_2 levels above that observed in air. If Pa_{CO_2} in teleost fish is dependent upon conditions for diffusion across the gill epithelium and the ventilation/perfusion ratio ($\dot{V}_G/\dot{Q}$ ratio), then Pa_{CO_2} will always be above ambient. The only way Pa_{CO_2} can be regulated in the face of a rise in ambient water P_{CO_2} is to reduce the P_{CO_2} difference between inspired water and arterial blood. The capacity for such regulation is small. Pa_{CO_2} in fish in air-equilibrated water is only of the order of 2–3 mmHg (Randall, 1970). In mammals the difference between ambient air and arterial blood P_{CO_2} can be adjusted by altering ventilation. Increases in $\dot{V}_G$ above resting level in rainbow trout did not cause any change in arterial pH and total CO_2 content (Randall and Cameron, 1972*b*). Fish therefore appear unable to regulate Pa_{CO_2} in the face of a rise in ambient P_{CO_2} by changes in ventilation.

A rise in Pa_{CO_2} will result in a fall in blood pH unless there is a concomitant rise in blood [HCO_3^-]. Regulation of arterial blood pH would be similar in this instance to that observed by Randall & Cameron (1972*a*). They reported that trout regulate arterial blood pH as temperature changes by altering plasma [HCO_3^-] levels. Pa_{CO_2} did not change with temperature.

Fish exposed to water of high CO_2 content may therefore regulate Pa_{CO_2} by a reduction of the P_{CO_2} difference between water and arterial blood. However, the capacity to regulate this parameter is limited, and in the face of large changes in ambient P_{CO_2}, Pa_{CO_2} must increase. The rise in Pa_{CO_2} will be associated with a fall in blood pH or a rise in blood [HCO_3^-] levels. The object of this study was to determine the relationship between arterial blood pH and Pa_{CO_2} in the fact of an increase in ambient P_{CO_2}.

MATERIALS AND METHODS

All experiments were carried out on rainbow trout obtained from Sun Valley Trout Farm, Chilliwack, B.C., Canada. The fish weighed between 200 and 300 g, and were maintained in outdoor tanks at a water temperature of 12 ± 2 °C. All experiments were carried out at 13 ± 1 °C.

For all *in vivo* experiments the fish were anaesthetized with MS 222 and a cannula

was implanted in the dorsal aorta as described by Smith & Bell (1964). Recovery for 24–48 h was allowed before any measurements were made. Variations in blood pH other than those resulting from changes in Pa_{CO_2} are probably due either to transient increases in blood lactate levels or to diuretic imbalance of the animal due to handling and anaesthesia. To avoid the former source, animals were kept in blackened chambers, and disturbance from the investigator was minimized. For the latter, papers by Houston and co-workers (1969, 1971 *a*, *b*) indicate that the major part of post-operative trauma has disappeared after 24 h recovery, and that 24 h levels of most measured parameters were not significantly different from 72 h values.

Measurements of pH and Pa_{CO_2} were made with a Radiometer PHM 71 acid-base analyzer, using thin silicone rubber membranes on the CO_2 electrode and calibration gas mixtures supplied by Wösthof gas mixing pumps. Total CO_2 measurements were made using an electrode and cuvette system described by Cameron (1971).

In vitro measurements of CO_2 dissociation curves and pH *v.* P_{CO_2} curves were made after equilibration of blood samples in a temperature-controlled shaker bath. Gas mixtures were passed through water saturators, and then into tonometers holding the blood.

Most measurement errors are small; total CO_2 values are estimated to be accurate to $\pm 2\%$ (Cameron, 1971), and pH measurement errors are probably about 0·01 pH unit. However, the measurements of P_{CO_2} are undoubtedly less precise, since the electrode response time is slow at 13 °C. The necessary increase in meter gain to measure very low Pa_{CO_2} values induces instability errors. In order to minimize this source of error, calibration was performed before *and* after each measurement. Still, error is likely to be about $\pm 20\%$. Even so, the conclusions of the work are not affected.

Water used in the experiments came from two sources: water low in CO_2 came from a de-chlorinated city water system, and water high in CO_2 from a well and concrete reservoir system. The P_{CO_2} and total CO_2 content of the former were below detection limits, and averaged 2·5 torr, 1 mM respectively, for the latter. Detection limit for P_{CO_2} was approx. 0·3 torr, and for total CO_2 approx. 0·2 mM/l.

Experimental procedure was to maintain the fish in low-CO_2 environments for at least a day or two, while the normal levels of pH, P_{CO_2}, etc., were measured, then to transfer them to the water of high CO_2. After at least 24 h at high $P_{I(CO_2)}$ the various parameters were re-measured.

Unless otherwise indicated, all means are given plus or minus standard error, and 5% was taken as the limit of statistical significance.

RESULTS

In Table 1 results are shown for six fish that were held first at low $P_{I(CO_2)}$ and then at the high $P_{I(CO_2)}$. Also shown are results for an additional four fish held only at the high $P_{I(CO_2)}$. For each fish, the figure in any column represents the mean of all measurements for that fish under those conditions, and is an average of from 2–10 separate measurements. Grand means are given at the bottom of the columns plus or minus one standard error. Also shown is the haematocrit for each fish; haematocrit tended to drop somewhat over the experimental period, due to sampling.

For the low-CO_2 group, ambient CO_2 was assumed to be zero in the calculation of

Table 1. *Changes in arterial pH, CO_2 tension and CO_2 content caused by transfer from low to high environmental CO_2*

(Figures in columns are means of all measurements for that particular animal; grand means are given plus or minus standard error. CO_2 tensions are in torr, and total CO_2 in mM/l.)

Fish no.	Pa_{CO_2}	$P_{I(CO_2)} - Pa_{CO_2}$	pH	Total CO_2	Haematocrit (%)	Treatment
1	1·77	1·77	7·99	4·91	27·8	Low CO_2
2	1·31	1·31	8·08	4·52	20·6	
3	1·78	1·78	8·05	5·49	18·3	
4	1·80	1·80	7·99	5·05	18·5	
5	2·13	2·13	7·99	5·11	17·8	
6	1·91	1·91	7·99	4·50	23·7	
						< 0·3 torr
Mean ± S.E.	1·78 ± 0·11	1·78 ± 0·11	8·02 ± 0·02	4·93 ± 0·15	21·1 ± 1·61	
1	3·57	1·37	7·89	10·95	20·8	High CO_2
2	—	—	7·98	10·84	14·8	
3	—	—	7·87	9·09	14·6	
4	4·36	2·16	7·94	11·48	17·2	
5	4·29	2·09	7·89	11·70	15·5	
6	5·43	3·23	7·92	12·63	12·5	
7	2·92	0·70	7·96	8·42	21·4	
8	2·85	0·63	7·98	7·45	25·3	
9	2·90	0·68	7·90	7·28	19·1	
10	3·55	1·33	7·94	9·82	21·1	
						~ 2·5 torr
Mean ± S.E.	3·73 ± 0·32	1·52 ± 0·32	7·93 ± 0·01	9·97 ± 0·59	18·2 ± 1·26	
t-value	5·05	0·66	4·56	6·50	1·41	
P	< 0·01	ns	< 0·01	< 0·01	ns	

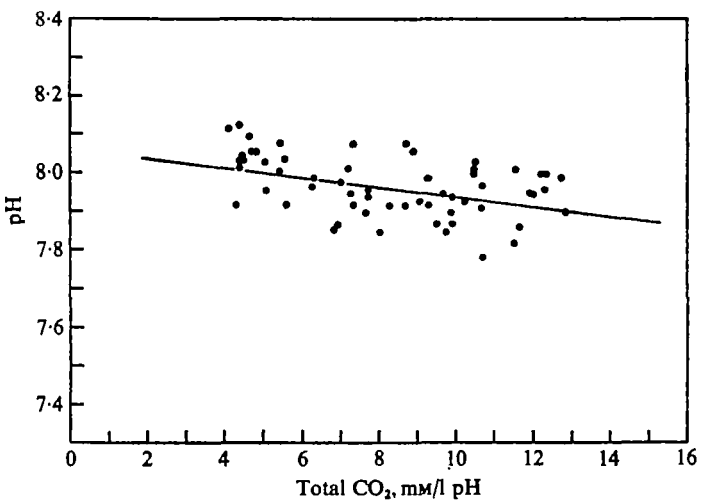

Fig. 1. Combined data on arterial pH *v.* total CO_2 for the fish analysed in Table 1. The line is a least-squares regression: pH = 8·06 − 0·013 [ΣCO_2], where ΣCO_2 is in mM/l.

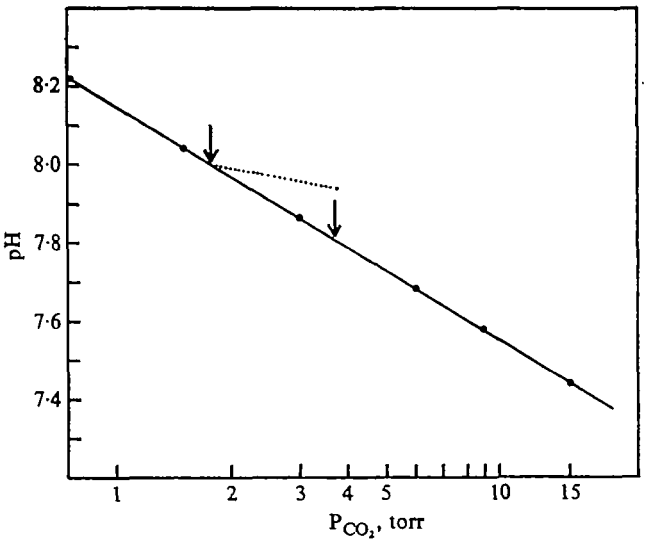

Fig. 2. Typical *in vitro* pH *v.* P_{CO_2} curve; slope determined from ten different curves. See text for explanation of arrows. Dotted line shows observed pH change *in vivo* as Pa_{CO_2} is increased.

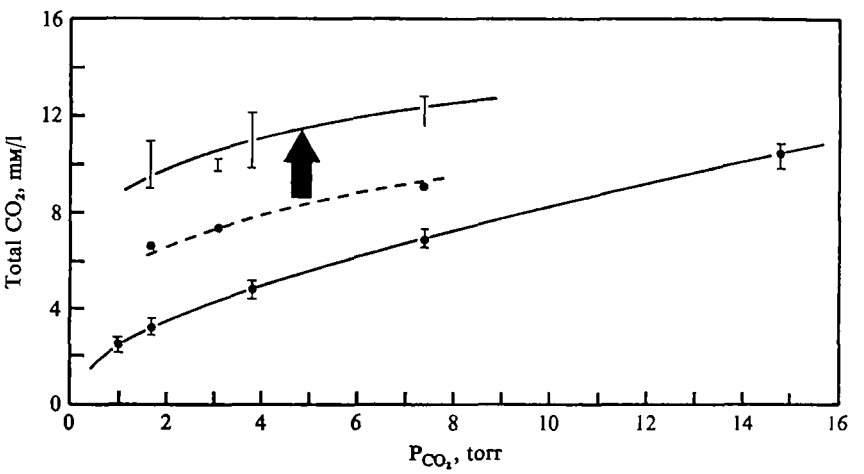

Fig. 3. Dissociation curves determined *in vitro* at 13 °C for whole blood. Lowest curve is an average for four fish, triplicate determinations for each. Upper curves show change as $P_{I(CO_2)}$ is increased.

$P_I - Pa$. However, if the water is assumed to have been air-saturated, it would have had a P_{CO_2} of 0·22 torr, and the gradient would then be 1·56 torr, virtually identical with the high-CO_2 group.

The four fish tested only in a high-CO_2 environment (nos. 7–10) had somewhat lower total CO_2 and a lower gradient from blood to water. However, their Pa_{CO_2} and total CO_2 values were still significantly higher than those of the control fish and were not separated in the analysis. Part of this difference may be due to the shorter length of observation for the fish tested only in high CO_2 (see Discussion below).

Although the pH data given in Table 1 show a significant drop in pH at the highe

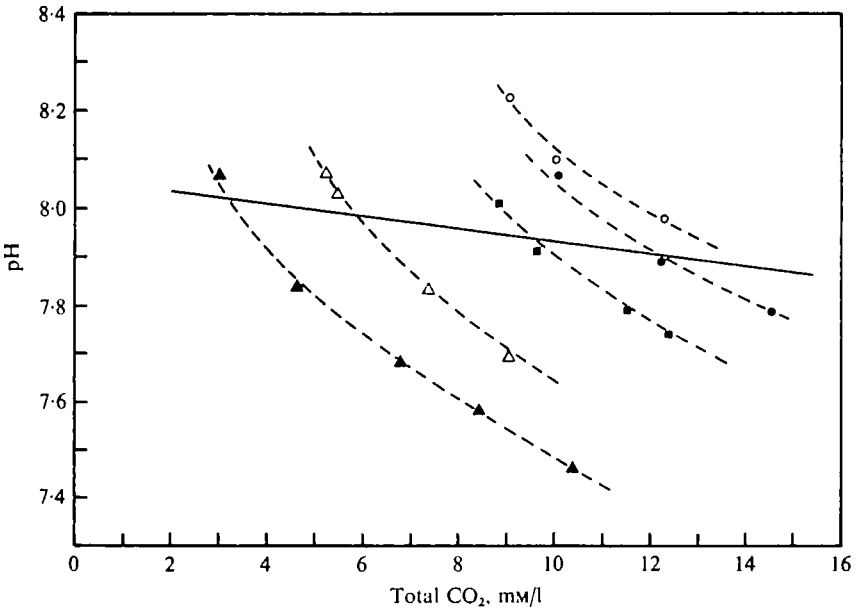

Fig. 4. *In vitro* curves for pH *v.* ΣCO_2 shown superimposed on regression line for *in vivo* pH changes. Curves on left determined at low CO_2, those on the right at high CO_2.

ambient CO_2 tensions, the data are shown in more detail in Fig. 1. Linear regression analysis yielded the following relationship:

$$[pH] = 8{\cdot}06 - 0{\cdot}013[\Sigma CO_2]$$

where ΣCO_2 is expressed in mM/l. This relationship will vary with temperature and is for trout at 13 °C. The regression was significant at the 1% level. One can now calculate the pH change that would be expected due to the increase in Pa_{CO_2} at constant bicarbonate and compare it to the pH change actually observed. Fig. 2 shows a typical pH *v.* P_{CO_2} curve, with slope calculated from curves determined for ten different fish *in vitro*. From this curve, the pH change expected due to Pa_{CO_2} increase from 1·78 to 3·73 torr (arrows on Fig. 2) is seen to be about 0·19 pH units. From the regression equation of pH *v.* CO_2 the observed pH change is 0·06 pH units (dotted line, Fig. 2). In other words, although the pH did drop in response to increased $P_{I(CO_2)}$, and Pa_{CO_2}, the change was only about one-third of what it would have been without adjustment of the bicarbonate and total CO_2 (Table 1).

The effect of increased total CO_2 on the CO_2 dissociation curve is shown in Fig. 3. The lower curves were determined *in vitro* from fish held in normal water, and the upper ones were determined from fish held for varying periods in high CO_2. The arrow indicates the direction of the shift in dissociation curve.

The effect of the changes in dissociation curve and pH are shown in Fig. 4, where the regression line for pH change *v.* total CO_2 *in vivo* is shown, along with curves of pH *v.* total CO_2 determined *in vitro* for some of the fish in Table 1. Without changes in the dissociation curve, a shift to the right along the total CO_2 axis would cause a pH change following any one of the curves down and to the right.

DISCUSSION

The results indicate that rainbow trout do not adjust the P_{CO_2} difference between arterial blood and water in the face of an increase in ambient P_{CO_2}. In fact, Pa_{CO_2} increased in proportion to the change in ambient P_{CO_2} such that Pa_{CO_2} is always about 2 mmHg above ambient. The rise in Pa_{CO_2} with an increase in ambient P_{CO_2} did not result in a large change in arterial blood pH. Blood bicarbonate levels were adjusted to regulate blood pH. These results support the more general conclusion that arterial blood pH in aquatic vertebrates is adjusted by changes in plasma bicarbonate rather than in Pa_{CO_2}. Also ventilation in aquatic animals is not a mechanism by which Pa_{CO_2} and therefore arterial pH is regulated (Randall & Cameron, 1972*a*,*b*).

Two further papers add support to the conclusions of the present work. Lloyd & White (1967) and Lloyd & Jordan (1964) describe increases in the blood plasma bicarbonate following acclimation to high levels of CO_2. They rightly conclude that the purpose of the observed increase is to maintain pH. They also observe that there is a concomitant decrease in the plasma chloride concentration. The changes in bicarbonate and chloride were of approximately the same magnitude and were reversed when the animals were restored to water of low P_{CO} Further support for a HCO_3^-/Cl^- exchange in fish has been presented by Maetz & Romeu (1964) and Dejours (1969). Fish transferred to water of very low chloride content show a marked reduction in CO_2 excretion immediately following transfer. Maetz & Romeu (1964) were able to alter chloride movements across the gill by altering the CO_2 gradient across the gills. We suggest that in freshwater the fish links chloride influx to HCO_3^- efflux, thus replacing chloride lost by diffusion and excreting a portion of the metabolically produced CO_2 as bicarbonate. Upon transfer to an environment high in CO_2, bicarbonate/chloride exchange is reduced, chloride efflux continues, plasma chloride falls and the anion deficit is made up by bicarbonate as CO_2 accumulates in the blood. Bicarbonate/chloride exchange may even be reversed on occasion. Dejours (1969) observed a net uptake of CO_2 when fish were transferred to water of very low chloride content.

Profound difficulties are experienced when trying to speculate about the mechanisms of CO_2 excretion and pH regulation in salt water, granted that the above description of chloride–bicarbonate exchange in freshwater is accurate. Presumably chloride uptake in salt water would be an expensive and non-adaptive method of getting rid of metabolic CO_2. Furthermore, excretion of bicarbonate by the kidney seems unlikely in view of the much reduced urine flow in marine teleosts, and the lack of particularly high concentrations of bicarbonate in the urine. An altered carbonic anhydrase system may provide much faster conversion time of bicarbonate to CO_2 at the gill, facilitating diffusive CO_2 loss. Salt water fishes obviously offer an opportunity for much new research.

Lloyd & White (1967) measured the time course of bicarbonate/chloride exchange upon exposure to high CO_2. Bicarbonate and chloride levels change slowly, the process approaching completion within 24 h. Regulation of arterial pH is therefore a slow process in fish and accounts for the wide range of recorded values for arterial pH in fish at any one temperature (e.g. Houston, 1971, table 1). Activity, or any other change in the animal that results in an increase in the hydrogen ion concentration in th

blood will cause a prolonged fall in arterial blood pH (Piiper & Baumgarten, 1969).

Eddy & Morgan (1969) observed no change in blood oxygen capacity or haemoglobin concentration in fish acclimated to high CO_2. Although a reduction in capacity may have occurred initially due to the Root effect, accumulation of bicarbonate in the blood presumably negated any change in arterial blood pH as a result of a rise in Pa_{CO_2}. Blood oxygen capacity was therefore not affected by the prolonged change in Pa_{CO_2}.

SUMMARY

1. The effect of exposure of rainbow trout to high and low levels of environmental (water) CO_2 tension on blood CO_2 and pH was studied.

2. An increase in environmental CO_2 caused a rise in the arterial CO_2 tension (Pa_{CO_2}), but the blood-to-water gradient (P_I–Pa) remained about the same.

3. Changes in arterial pH were small – about one-third of what would be predicted from the change in Pa_{CO_2} if plasma bicarbonate remained constant.

4. There was a corresponding increase in the total CO_2 content of the blood which involved an upward shift of the CO_2 dissociation curve, and readjustment of the buffering to maintain pH.

5. The results are consistent with the hypothesis that blood CO_2 and pH levels are regulated via a chloride–bicarbonate exchange mechanism, rather than by ventilation and diffusive washout of gaseous CO_2.

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