

## BLOOD ACID–BASE REGULATION DURING ENVIRONMENTAL HYPEROXIA IN THE RAINBOW TROUT (*Salmo gairdneri*)

CHRIS M. WOOD and ERIC B. JACKSON

*Department of Biology, McMaster University, Hamilton, Ontario, L8S 4K1 Canada*

**Abstract.** Blood acid–base balance, blood gases, respiration, ventilation, and renal function were studied in the rainbow trout during and following sustained environmental hyperoxia ( $P_{\text{I}\text{O}_2} = 350\text{--}650$  Torr). Animals were chronically fitted with dorsal aortic cannulae for repetitive blood sampling, oral membranes for the measurement of ventilation, and bladder catheters for continuous urine collection. Hyperoxia caused a proportional increase in arterial  $\text{O}_2$  tension and a stable 60% reduction in ventilation volume ( $\dot{V}_w$ ), the latter mainly due to a decrease in ventilatory stroke volume.  $\text{O}_2$  consumption exhibited a short-term elevation. Arterial  $\text{CO}_2$  tension ( $\text{Pa}_{\text{CO}_2}$ ) rose within 1 h, causing an immediate drop in arterial pH ( $\text{pH}_a$ ), and continued to increase gradually thereafter, reaching a value 2–4 $\times$  the normoxic control level after 96–192 h. Compensation of the associated acidosis by the accumulation of  $[\text{HCO}_3^-]$  in the blood plasma started within 5–6 h, and was complete by 48 h. Thereafter, further compensation occurred simultaneously with the gradual rise in  $\text{Pa}_{\text{CO}_2}$ . The kidney played an important active role in this compensation by preventing excretion of the accumulated  $[\text{HCO}_3^-]$ . Upon reinstitution of normoxia,  $\text{Pa}_{\text{CO}_2}$  dropped to control levels within 1 h, and restoration of blood acid–base status by reduction of  $[\text{HCO}_3^-]$  had commenced by this time. A complete return to control values occurred within 20 h. During hyperoxia, an experimental elevation of the depressed  $\dot{V}_w$  above control normoxic levels caused only a minor and transient reduction in  $\text{Pa}_{\text{CO}_2}$  and no change in  $\text{pH}_a$ , but injection of branchial vasodilator 1-isoprenaline (10  $\mu\text{mol/kg}$ ) produced a large drop in  $\text{Pa}_{\text{CO}_2}$  and rise in  $\text{pH}_a$ . It is concluded that the rise in  $\text{Pa}_{\text{CO}_2}$  during hyperoxia is mainly due to internal diffusive and/or perfusive limitation associated with branchial vasoconstriction, rather than to external convective limitation associated with the decreased  $\dot{V}_w$ .

Acid–base balance	Renal function
Environmental hyperoxia	<i>Salmo gairdneri</i>
Isoprenaline	Ventilation

The effects of environmental hypoxia on aquatic animals have been studied extensively, but there are relatively few reports of the influence of environmental hyperoxia on water breathers. These include Peyraud and Serfarty (1964; carp),

Eclancher (1972; trout), Dejours (1972, 1973, 1975; carp, tench, goldfish, and trout), Randall and Jones (1973; trout), Truchot (1975; crabs), Dejours and Beekenkamp (1977; crayfish), Bornancin, DeRenzis and Maetz (1977; eels), Dejours, Toulmond and Truchot (1977; a variety of marine fish) and Jouve and Truchot (1978; crabs). Most of these studies have focussed on the role of  $O_2$  in ventilatory control. The general conclusion has been that hyperoxia markedly depresses ventilation despite an accompanying rise in blood  $P_{CO_2}$  and decrease in blood pH, thereby demonstrating the pre-eminence of  $O_2$  in setting the ventilatory drive in water-breathers. Herein lies a fundamental difference from air-breathers, where  $CO_2$  sets the main ventilatory drive. However there remain a number of unanswered questions with respect to the influence of hyperoxia on  $CO_2$  and acid-base regulation in aquatic organisms:

(i) The cause of the increase in blood  $P_{CO_2}$  and resultant fall in blood pH during hyperoxia is unknown. All workers who have observed this phenomenon have offered an essentially mammalian interpretation – *i.e.*, that it is a classical respiratory acidosis directly caused by the decrease in ventilatory convection. However, there exists no proof for this contention. Recently, Haswell, Perry, and Randall (1978), on the basis of experiments with an artificially perfused whole gill preparation of the trout, have offered an alternative explanation: that hyperoxia reduces the transfer factor for  $CO_2$  in the gills, perhaps by decreasing effective lamellar surface area. This would constitute an internal diffusive and/or perfusive limitation on  $CO_2$  excretion.

(ii) Complete compensation of the blood pH depression has never been reported in any of the hyperoxia studies, even those employing very long-term exposures (*e.g.*, Dejours and Beekenkamp, 1977; Bornancin, DeRenzis, and Maetz, 1977). At best, partial compensation was observed (*e.g.*, Dejours, 1973, 1975; Truchot, 1975). This is surprising in view of the fact that a comparable acidosis caused by environmental hypercapnia is fully compensated within a few days by the accumulation of plasma  $[HCO_3^-]$  (*e.g.*, Janssen and Randall, 1975; Randall, Heisler and Drees, 1976).

(iii) The extent and time course of restoration of blood acid-base and  $CO_2$  status following hyperoxia are uncertain. Both Truchot (1975) and Dejours and Beekenkamp (1977) observed a rapid fall in blood  $P_{CO_2}$  upon reinstitution of normoxia, but the latter reported that blood acid-base state remained disturbed for at least 2 months.

(iv) The mechanism(s) responsible for the partial compensation of hyperoxic acidosis are unknown. Acid-base regulation in aquatic organisms is traditionally attributed to  $Na^+$ /acid and  $Cl^-$ /base exchanges at the gills (*cf.* Cameron, 1978). However Bornancin, De Renzis and Maetz (1977) have documented a large increase in  $Cl^-$  influx with no change in  $Na^+$  influx during long-term hyperoxic compensation in the eel. This is exactly opposite the expected result if branchial ion exchanges were involved in the observed accumulation of  $[HCO_3^-]$  in the blood plasma. Recently we have shown that the kidney plays an important role in acid-base regulation in teleost fish (Wood and Caldwell, 1978; Cameron and Wood, 1978;

Kobayashi and Wood, 1980). A possible renal contribution to hyperoxic composition cannot be ruled out.

The present study on the freshwater rainbow trout (*Salmo gairdneri*) focussed on the above questions, utilizing direct measurements of ventilation, respiration, blood gas tensions, blood acid-base status, and renal function. The animals were surgically fitted with chronic dorsal aortic cannulae, urine collection catheters, and ventilation masks, allowed to fully recover, and then subjected to a number of different experimental treatments under normoxia and hyperoxia.

## Materials and methods

### I. EXPERIMENTAL ANIMALS

Rainbow trout (100–300 g) were acclimated in flowing dechlorinated freshwater for at least 2 weeks prior to experimentation. Water temperature ranged from 9.0° to 17.0°C at different times of the year, but acclimation and experimental temperatures never differed by more than 1°C. In all experiments, operations were performed under 1:10,000 MS-222 anaesthesia and the fish allowed to recover in darkened individual chambers for 24–72 h in normoxic water before any measurements were taken. Water flow to each chamber exceeded 300 ml/min and was obtained from a countercurrent exchange column bubbled with either air or O<sub>2</sub> to produce respectively normoxic (P<sub>I</sub>O<sub>2</sub> = 140–175 Torr) or hyperoxic water (P<sub>I</sub>O<sub>2</sub> = 350–650 Torr). P<sub>I</sub>CO<sub>2</sub> was approximately 1.5 Torr (absolute range 1.3–1.9 Torr) and unaffected by normoxia or hyperoxia. Whenever possible, blood was returned to the fish after analysis together with sufficient Cortland saline (Wolf, 1963) to replace any lost volume. Haematocrits were normally between 15 and 35%. Animals exhibiting haematocrits below 6% were excluded from the analysis because of possible anaemia-induced acid-base disturbances (Wood, McMahon, and McDonald, 1979, and unpublished results).

### II. EXPERIMENTAL SERIES

(i) Series I examined the influence of hyperoxia on blood gas and acid-base regulation. Trout were fitted with dorsal aortic catheters only (Smith and Bell, 1964) and placed in 2-L rectangular chambers which confined but did not physically restrain the fish. After control measurements under normoxia, the animals were subjected to 4 days of hyperoxia. Blood samples were drawn at time 0 (control) and 1, 5, 24, 48, 72, and 96 h during hyperoxia, and analyzed for Pa<sub>O</sub><sub>2</sub>, Pa<sub>CO</sub><sub>2</sub>, pH<sub>a</sub>, and Ca<sub>CO</sub><sub>2</sub>. Temperature = 15.0 ± 1.5°C, N = 7.

(ii) Series II studied the possible role of the kidney in the compensation of hyperoxic acidosis, and served to substantiate the results of series I at a different

temperature. It also provided information on changes in urine flow. This parameter is considered representative of net branchial water entry in freshwater teleosts (Wood and Randall, 1973) and might well reflect a change in the diffusive permeability of the gills during hyperoxia. Trout were starved for at least 7 days to remove the influence of diet on renal acid output (Wood and Caldwell, 1978). The animals were then fitted with dorsal aortic and urinary bladder cannulae (Wood and Randall, 1973), and allowed to recover for the 36-h period needed to permit stabilization of urinary acid excretion (Wood and Caldwell, 1978). The urinary catheters drained by a siphon of 7 cm  $H_2O$  into covered vials, allowing continuous urine collection. Two 12-h control collections were taken together with a control blood sample under normoxia. Four days of hyperoxia were then instituted. The same blood sampling regime as in series I was employed. Urine was collected over successive 12-h intervals, each collection being analyzed separately. Blood samples were assayed for  $pH_a$  and  $Ca_{CO_2}$ , and urine samples for total acid content. Temperature =  $9.0 \pm 0.5^\circ C$ ,  $N = 6$ .

(iii) Series III was designed to provide further information on changes in branchial water permeability and to assess the alterations in blood gas and acid-base status which occur after a return to normoxia. Animals were prepared in an identical manner to those of series II. Control blood samples and urine collections were taken during normoxia. Eight days of hyperoxia were then instituted, during which time urine collections were made every 12 h, but no blood samples were drawn. At 192 h, a blood sample was taken, and then normoxia was reimposed. Blood samples were taken at 1, 6 and 20 h after the return to normoxia, together with two 12-h urine collections. Blood samples were analyzed for  $Pa_{O_2}$ ,  $Pa_{CO_2}$ ,  $pH_a$ , and  $Ca_{CO_2}$ ; urine samples were assayed only for volume. Temperature =  $16.0 \pm 1.0^\circ C$ ,  $N = 5$ .

(iv) Series IV investigated the influence of hyperoxia on ventilation and respiratory performance. Trout were fitted with oral membranes and then placed in ventilation collection boxes. The methodology was identical to that developed by Davis and Cameron (1970). After control observations under normoxia, 4 days of hyperoxia were imposed with measurements at 1, 5, 24, 48, 72 and 96 h. Each set of measurements consisted of several determinations of  $PI_{O_2}$  (from directly in front of the fish's mouth),  $PE_{O_2}$  (from the rear of the mixing chamber),  $\dot{V}_w$  (by overflow) and  $f_R$  (by visual observation). Multiple determinations of  $f_R$  were carried out so as not to give undue weight to occasional periods of apnoea.  $VS.R$  was estimated as  $\dot{V}_w/f_R$ , and  $\dot{M}_{O_2}$  calculated by the Fick principle. Temperature =  $14.0 \pm 2.0^\circ C$ ,  $N = 8$ .

(v) Series V tested whether external convective limitation associated with decreased ventilation was responsible for the effects observed during hyperoxia. It also served to substantiate the results of series I, II, and IV. Fish were fitted with both oral membranes and dorsal aortic catheters and placed in the ventilation boxes. Control blood samples, and respiration and ventilation measurements were taken under normoxia and then at 6, 24, and 48 h during hyperoxia, by which time

the fish were judged to be in a relatively stable hyperoxia-adjusted condition. The water level in the anterior section of the ventilation box was then elevated to create a buccal head relative to the opercular chamber, thereby artificially elevating  $\dot{V}_w$  (*cf.* Jones and Schwarzfeld, 1974). The aim was to restore the control normoxic level of ventilation, but a precise re-setting of  $\dot{V}_w$  was made difficult by the fish's ability to adjust its own gape. Hyperoxia was maintained throughout this treatment, and further measurements were taken at 2, 24, and 48 h during forced ventilation. Blood samples were assayed for  $P_{aCO_2}$ ,  $pH_a$ , and  $Ca_{CO_2}$ . Temperature =  $14.0 \pm 1.0^\circ\text{C}$ ,  $N = 5$ .

(vi) Series VI tested whether internal diffusive and/or perfusive limitation associated with lamellar vasoconstriction was responsible for the effects observed during hyperoxia. Fish were fitted with dorsal aortic catheters, control blood measurements were taken, and then hyperoxia was instituted. After 6 days of continuous hyperoxia, another blood sample was drawn, and then  $10 \mu\text{mol/kg}$  of 1-isoprenaline ( $1.0 \text{ ml/kg}$  of a  $10 \text{ mM}$  solution of 1-isoprenaline bitartrate (Sigma) in Cortland saline) was rapidly injected *via* the dorsal aortic catheter followed by a  $1 \text{ ml/kg}$  saline wash. Isoprenaline, a selective  $\beta$ -adrenergic agonist, is a potent branchial vasodilator in trout (Wood, 1974, 1975). Blood samples were drawn at 1, 5, 10, 15, 20, and 30 min after injection, and three of the fish were sampled again at 24 h. As a control, an additional three fish were subjected to the identical experiment under normoxia. Blood samples were analyzed for  $P_{aCO_2}$ ,  $pH_a$ , and  $Ca_{CO_2}$  at control, preinjection, and 15 min and 24 h post-injection times. Because of the limitation of electrode response time, only  $pH_a$  was determined on the 1, 5, 10, 20, and 30 min post-injection samples. Three fish (bearing oral membranes) from series V were also used in the present experimental series after completion of the forced ventilation studies; these animals gave similar results to the others, and their data were combined in the overall analysis. Temperature =  $15.0 \pm 2.0^\circ\text{C}$ ,  $N = 11$ .

### III. ANALYTICAL TECHNIQUES

Blood samples were handled anaerobically in Hamilton syringes.  $P_{O_2}$ ,  $P_{CO_2}$ , and pH levels in blood and water were determined using Radiometer microelectrodes thermostatted to the experimental temperature.  $P_{CO_2}$  measurements at the low temperatures and  $CO_2$  levels of fish are difficult. In order to increase accuracy, thin silicone rubber membranes were used on the  $P_{CO_2}$  electrode. The system was calibrated to an arbitrary scale at close to maximum gain on the Radiometer PHM 71 MK 2 analyzer. Each sample was bracketed by calibration standards (humidified gas mixtures of known  $P_{CO_2}$ ) in the range of the experimental values (1–10 Torr). A response time of 10 min was employed with sample replacement at 8 min as recommended by Boutilier *et al.* (1978).  $P_{CO_2}$  levels were not measured at temperatures below  $12.0^\circ\text{C}$  (*i.e.*, series II) because of excessive electrode response

time. Blood samples for  $\text{Ca}_{\text{CO}_2}$  determinations were centrifuged in sealed, heparinized microhaematocrit tubes (Radiometer) at  $5000 \times g$  for 4 min. The haematocrit was read directly from the tube which was then broken to allow aspiration of the plasma into a Hamilton syringe. Total  $\text{CO}_2$  content of the plasma was determined by the method of Cameron (1971). Plasma  $\text{HCO}_3^-$  levels were calculated as  $\text{Ca}_{\text{CO}_2} - \alpha\text{CO}_2 \cdot \text{Pa}_{\text{CO}_2}$ . Where  $\text{Pa}_{\text{CO}_2}$  levels were not measured directly (series II), and also for comparative purposes (series I), they were calculated by the Henderson-Hasselbalch equation using values of  $\text{pK}'$  and  $\alpha\text{CO}_2$  tabulated in Severinghaus (1965).

As in mammalian renal physiology (Hills, 1973), total urinary acid output was calculated as urinary  $[\text{NH}_4^+ + \text{TA (titratable acid)} - \text{HCO}_3^-] \times \text{urine flow rate}$  (*cf.* Kobayashi and Wood, 1980).  $[\text{NH}_4^+]$  was measured colorimetrically (Solorzano, 1969), and  $[\text{TA} - \text{HCO}_3^-]$  was determined as a single value in the double titration procedure recommended by Hills (1973). A micro-electrode thermostatted to the experimental temperature was employed, the titrants were 0.02 N HCl and 0.02 N NaOH, and the final end point of the titration was taken as the mean pHa recorded during the urine collection period.

Each animal served as its own control, and all results were analyzed by means of the paired Student's two-tailed *t*-test ( $P < 0.05$ ). All data are presented as means  $\pm$  1 standard error (N), where N equals the number of fish contributing to a mean. N numbers tended to decline in the later stages of some experiments due to cannula failure or low haematocrit.

## Results

### I. SERIES I

The imposition of hyperoxia caused pronounced and persistent changes in arterial blood gas tensions (fig. 1). Within 1 h,  $\text{Pa}_{\text{O}_2}$  rose from  $110 \pm 5(7)$  Torr to  $312 \pm 36(7)$  Torr in approximate proportion to the rise in  $\text{PI}_{\text{O}_2}$  (from  $\approx 160$  to  $\approx 410$  Torr), and remained significantly elevated at this level for the entire 96 h (fig. 1A).  $\text{Pa}_{\text{CO}_2}$  increased within 1 h, rising from  $2.51 \pm 0.06(7)$  Torr to  $4.34 \pm 0.28(7)$  Torr (fig. 1B). This significantly higher  $\text{Pa}_{\text{CO}_2}$  persisted throughout the hyperoxic period and tended to increase gradually with time. Absolute values of  $\text{Pa}_{\text{CO}_2}$  as measured directly were consistently lower than those calculated by the Henderson-Hasselbalch equation, but the two determinations showed similar trends with hyperoxia. Possible reasons for such discrepancy have been detailed by Reeves (1977). Overall, there were no significant changes in pHa (fig. 1C) though there was a tendency for this parameter to fall (from  $7.828 \pm 0.015(7)$  under normoxia to  $7.773 \pm 0.034(7)$  at 5 h hyperoxia). This was followed by a return to control levels with an overshoot on day 2. Plasma  $[\text{HCO}_3^-]$  steadily increased during hyperoxia, reaching a value approximately twice the control by day 4 ( $8.50 \pm 0.48(7)$  mmol/L in normoxia to  $15.66 \pm 1.41(4)$  mmol/L at 96 h hyperoxia, fig. 1D).

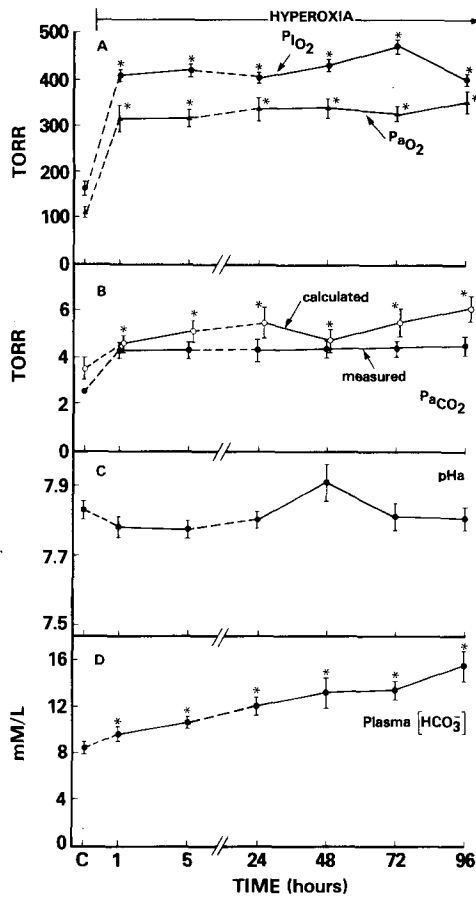


Fig. 1. Changes in: (A)  $P_{tO_2}$  and  $P_{aO_2}$ ; (B)  $P_{aCO_2}$ , measured directly, and calculated by the Henderson-Hasselbalch equation; (C) pHa; and (D) plasma  $[HCO_3^-]$  in rainbow trout of series I during four days of environmental hyperoxia. C = control measurements under normoxia. Means  $\pm$  1 SE \* = significantly different ( $P < 0.05$ ) from normoxic control. N = 7 at C, 1, and 5 h; N = 5 at 24, 48, and 72 h; N = 4 at 96 h.  $T = 15.0 \pm 1.5^\circ\text{C}$ .

Therefore the data clearly showed that  $\text{CO}_2$  retention (*i.e.*, increase in  $P_{aCO_2}$ ) was associated with hyperoxia in trout. The actual acidosis caused by this  $P_{aCO_2}$  rise was small and transitory as it was quickly compensated by the build-up of plasma  $[HCO_3^-]$ . The overall effect was that of a fully compensated respiratory acidosis.

## II. SERIES II

Despite the lower temperature ( $9.0 \pm 0.5^\circ\text{C}$  *versus*  $15.0 \pm 1.5^\circ\text{C}$ ), the blood data from this experiment showed very similar trends to those of series I. In particular,

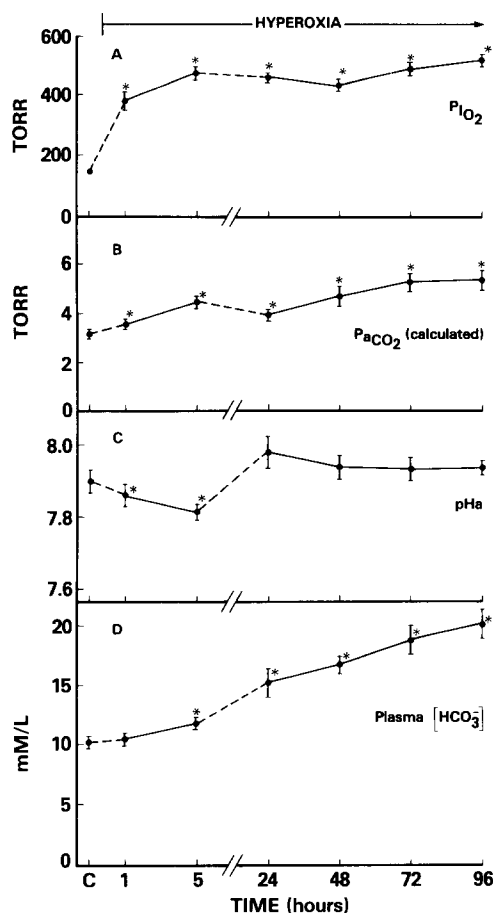


Fig. 2. Changes in: (A)  $P_{tiO_2}$ ; (B)  $P_{aCO_2}$  calculated by the Henderson-Hasselbalch equation; (C) pHa; and (D) plasma  $[HCO_3^-]$  in rainbow trout of series II during four days of environmental hyperoxia.

C = control measurements under normoxia. Means  $\pm$  1 SE. N = 6 throughout.  $T = 9.0 \pm 0.5^\circ\text{C}$ .

hyperoxia caused a progressive increase in  $P_{aCO_2}$  (calculated; fig. 2B) and initial fall in pHa (fig. 2C) which was fully compensated by the accumulation of plasma  $[HCO_3^-]$  (fig. 2D). There was again a slight overshoot in pHa, this time at 24 h (fig. 2C). Plasma  $[HCO_3^-]$  increased progressively from  $10.28 \pm 0.51(6)$  mmol/L under normoxia to  $20.12 \pm 1.39(6)$  mmol/L after 96 h of hyperoxia (fig. 2D), thereby fully compensating for the gradual rise in  $P_{aCO_2}$  (fig. 2B). Minor quantitative differences from series I (slightly higher  $HCO_3^-$  levels and pHa) are attributable to the lower experimental temperature (Randall and Cameron, 1973).

Under normoxia, total renal acid effluxes were extremely uniform at a value close to zero (*i.e.*, no net acid or base output; fig. 3A). However, hyperoxia caused a great increase in variability. Total renal acid output rose markedly in two fish (but at different times), remained essentially unchanged in two others, declined



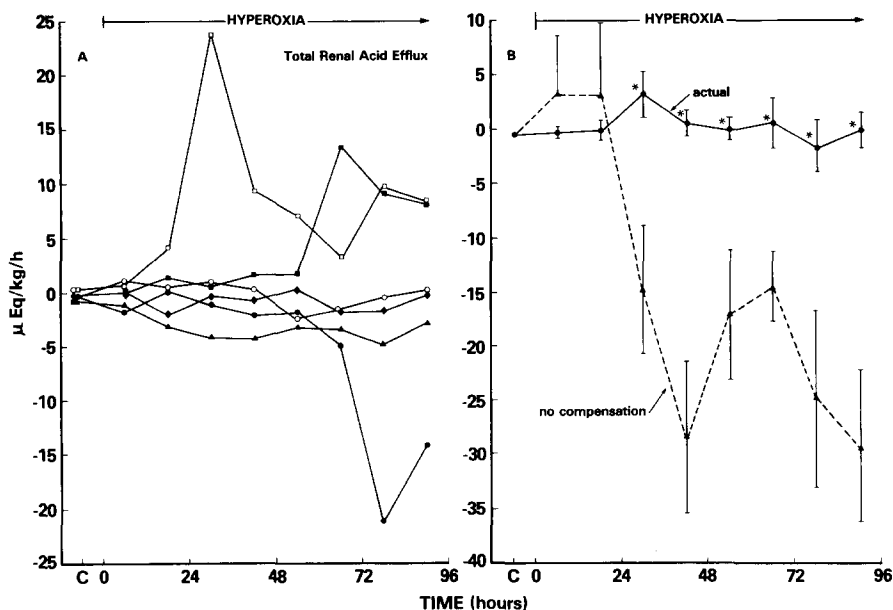


Fig. 3. Changes in total renal acid efflux (urine flow  $\times$   $[\text{TA} - \text{HCO}_3^- + \text{NH}_4^+]$ ) during four days of environmental hyperoxia in the six rainbow trout of series II. Values are plotted at the midpoints of the 12 h collection periods. In (A), individual values are shown to illustrate the variability of the response. In (B), the values are presented as means  $\pm$  1 SE and compared with the acid effluxes expected if there had been no renal compensation. The method of calculation for the 'no compensation' points is outlined in Results. C = control measurements under normoxia. There were no significant changes in the actual renal acid efflux throughout the experimental period. \* = significantly different ( $P < 0.05$ ) from expected 'no compensation' value at that time.  $N = 6$  throughout.  $T = 9.0 \pm 0.5^\circ\text{C}$ .

slightly in the fifth, and showed a marked decrease after 60 h in the sixth (fig. 3A). Overall, there were no significant changes (fig. 3B).

Nevertheless in all six fish, the net renal acid excretions which were seen during hyperoxia were a great deal larger than those which would have occurred if there had been no renal compensation, as illustrated by the 'no compensation' line in fig. 3B. For each fish, the net rate of proton secretion by the renal tubule cells during normoxia was calculated as the  $\text{HCO}_3^-$  filtration rate ( $\text{GFR} \times \text{measured plasma}[\text{HCO}_3^-]$ ) plus the measured net acid excretion rate ( $[\text{TA} - \text{HCO}_3^- + \text{NH}_4^+] \times \text{urine flow rate}$ ). The GFR was conservatively estimated as  $1.5 \times$  the urine flow rate (Hickman and Trump, 1969). This normoxic rate of proton secretion was applied to the  $\text{HCO}_3^-$  filtration rate ( $1.5 \times \text{urine flow rate} \times \text{measured plasma}[\text{HCO}_3^-]$ ) at each hyperoxic interval to predict the net rate of renal acid excretion in the absence of renal compensation. It is quite apparent from Fig. 3B that in the absence of renal compensation,  $\text{HCO}_3^-$  added to the blood plasma over the hyperoxic period would have been excreted at the kidney, resulting in highly negative urinary acid effluxes and a failure of blood pH regulation. Renal com-

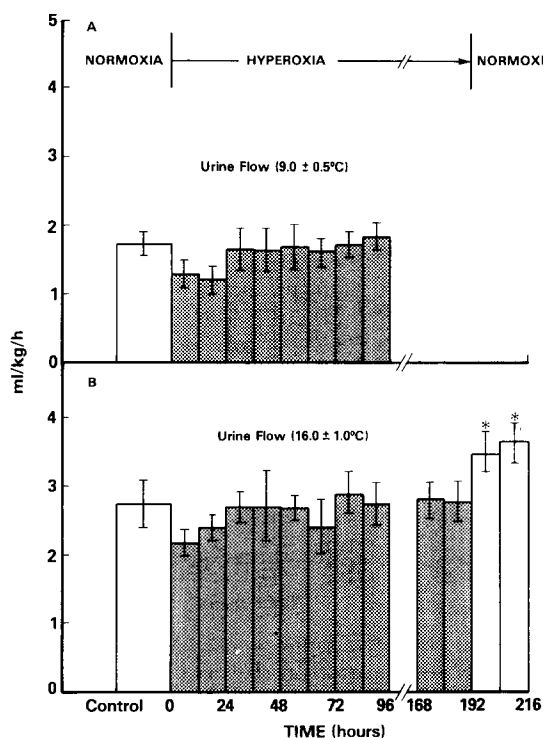


Fig. 4. Changes in urine flow rate during hyperoxic exposure in rainbow trout. Means  $\pm$  S.E. (A) series II,  $T = 9.0 \pm 0.5^\circ\text{C}$ ,  $N = 6$  throughout, no significant changes. (B) series III,  $T = 16.0 \pm 1.0^\circ\text{C}$ ,  $N = 5$  until 72 h,  $N = 4$  thereafter. Urine flow was essentially constant in the period between 96 and 168 h (not shown). At 192 h, the re-imposition of normoxia caused significant increases (\*,  $P < 0.05$ ) in urine flow over the following two 12 h periods. There were no other significant changes.

pensation became effective after 24 h and maintained urinary acid excretion significantly above the 'no compensation' level for the remainder of the hyperoxic period. This adjustment was also manifested in the  $\text{NH}_4^+$  component of renal proton excretion, which rose significantly from  $0.7 \pm 0.2(6) \mu\text{equiv/kg/h}$  under normoxia to  $1.8 \pm 0.4(6) \mu\text{equiv/kg/h}$  after 4 days of hyperoxia.

Urine flow rate was measured as an estimate of branchial water entry. Urine flow tended to decrease during the first 24 h of hyperoxia and then returned to control levels (fig. 4A), but the changes were not significant ( $0.20 > P > 0.10$ ).

### III. SERIES III

It was thought that the non-significant decrease in urine flow seen at the start of hyperoxia in series II (fig. 4A) might be more clearly expressed at a higher temperature where branchial permeability is reputedly greater (MacKay and Beatty, 1968). However in this series at  $16.0 \pm 1.0^\circ\text{C}$ , the changes at the start of hyperoxia, while similar to those of series II (fig. 4B), were again not significant ( $0.10 > P > 0.05$ ).

After the first 24 h, urine flow remained relatively stable at the control rate for the ensuing 7 days of hyperoxia. The return to normoxia at the end of day 8 caused significant increases in urine flow over the following 24 h (fig. 4B).

The other objective of series III was to examine the time course and extent of changes in blood acid-base and  $\text{CO}_2$  regulation which occur upon a return to normoxia. After 8 days of hyperoxia,  $\text{Pa}_{\text{CO}_2}$  had risen almost threefold from  $3.43 \pm 0.20(4)$  Torr to  $9.31 \pm 0.28(4)$  Torr (fig. 5B), and this was fully compensated by a proportional rise in plasma  $[\text{HCO}_3^-]$  from  $9.11 \pm 0.49(4)$  mmol/L to  $23.61 \pm 1.97(4)$  mmol/L (fig. 5D), resulting in no significant change in pHa (fig. 5C).  $\text{Pa}_{\text{O}_2}$  was also significantly elevated (fig. 5A), as in series I (fig. 1A). The return to normoxia caused a rapid decline in  $\text{Pa}_{\text{CO}_2}$ ; by 1 h it had returned to the normoxic control level (fig. 5B), as had  $\text{Pa}_{\text{O}_2}$  (fig. 5A). This fall in  $\text{Pa}_{\text{CO}_2}$  caused a dramatic rise in pHa from  $7.820 \pm 0.009(4)$  to  $8.060 \pm 0.055(4)$  (fig. 5C), because plasma  $[\text{HCO}_3^-]$ , while significantly lower than the hyperoxic level, remained well above the normoxic control value (fig. 5D). Plasma  $[\text{HCO}_3^-]$  and pHa were still elevated at 6 h, but had completely returned to normoxic control levels by 20 h (fig. 5C,D).

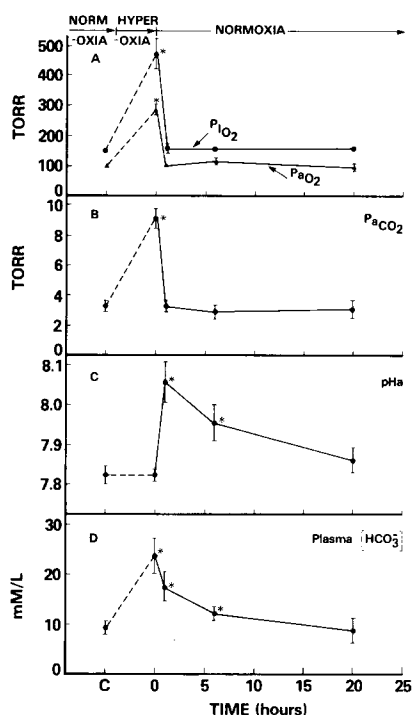


Fig. 5. Changes in (A)  $\text{P}_{\text{IO}_2}$  and  $\text{P}_{\text{aO}_2}$ ; (B)  $\text{P}_{\text{aCO}_2}$ , measured directly; (C) pHa; and (D) plasma  $[\text{HCO}_3^-]$  in rainbow trout of series III during the re-institution of normoxia after 8 days of continuous hyperoxia. C = control measurements under normoxia. 0 = measurements after 8 days of hyperoxia taken immediately prior to the re-institution of normoxia. Means  $\pm$  ISE. \* = significantly different ( $P < 0.05$ ) from normoxic control. N = 4 throughout. T =  $16.0 \pm 1.0^\circ\text{C}$ .

## IV. SERIES IV

$\dot{V}_w$  decreased dramatically from  $270 \pm 31(8)$  ml/kg/min under normoxia to  $131 \pm 19(6)$  ml/kg/min after 1 h of hyperoxia (fig. 6C). Thereafter,  $\dot{V}_w$  remained stable at about 40% of the control level for the following 4 days of hyperoxia. Ventilation rate (fR) varied slightly over the course of the experiment, but was only significantly lower than the normoxic control at 24 h hyperoxia (fig. 6D). The amplitude of respiratory movements declined greatly during hyperoxia, to the extent that they could not be visually detected at times. Measurements with impedance recording techniques indicated that occasional periods of real apnoea did occur during hyperoxia (C.M. Wood, unpublished results). Therefore these apparent apnoeic periods were averaged (as fR = 0) into the overall visual measurements. If these values had been excluded from the analysis, it is unlikely that there would have been any significant changes in fR. In view of this relative constancy of fR, virtually all of the reduction in  $\dot{V}_w$  was due to decreases in VS.R. For example, VS.R declined significantly from  $2.84 \pm 0.38(8)$  ml/kg/stroke under normoxia to  $1.38 \pm 0.22(6)$  ml/kg/stroke at 1 h hyperoxia, while fR decreased only slightly ( $92.1 \pm 6.1(8)$  versus  $81.2 \pm 8.8(6)$  breaths/min, NS).

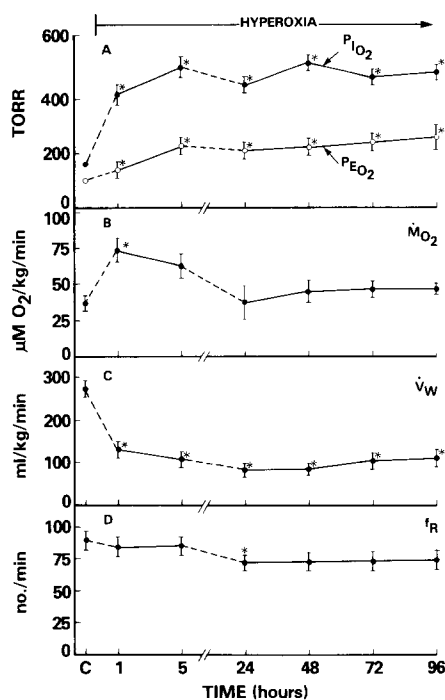


Fig. 6. Changes in (A)  $P_{tO_2}$  and  $P_{E_{tO_2}}$ ; (B)  $\dot{M}_{O_2}$ ; (C)  $\dot{V}_w$ ; and (D) fR in rainbow trout of series IV during four days of environmental hyperoxia. C = control measurements under normoxia. Means  $\pm$  1SE. \* = significantly different ( $P < 0.05$ ) from normoxic control. N = 6–8 until 48 h; N = 5–6 thereafter.

T =  $14.0 \pm 2.0$  °C.

The decline in  $\dot{V}_w$  was more than balanced by an increase in absolute extraction (*i.e.*,  $PI_{O_2} - PE_{O_2}$ ; fig. 6A) which caused a significant rise in  $\dot{M}_{O_2}$  at 1 h hyperoxia (fig. 6B). However  $\dot{M}_{O_2}$  thereafter declined and was not significantly different from the control normoxic level for the remainder of the hyperoxic exposure. Relative  $O_2$  extraction [ $Ew_{O_2} = (PI_{O_2} - PE_{O_2})/PI_{O_2} \times 100\%$ ] rose from  $43.2 \pm 5.1\%$ (8) in normoxia to  $66.8 \pm 2.6\%$ (8) at 1 h hyperoxia, followed by a stabilization at about 54% for the next 4 days. The convection requirement for water ( $\dot{V}_w/\dot{M}_{O_2}$ ) fell from 7.3 L/mmol  $O_2$  in normoxia to about 2.2 L/mmol  $O_2$  over the whole hyperoxic period. Therefore these complex alterations in ventilation during hyperoxia produced only a transitory disturbance of  $\dot{M}_{O_2}$ . The long term constancy of  $\dot{M}_{O_2}$  indicated that the increased  $Pa_{CO_2}$  was unlikely to have been caused by a greater rate of  $CO_2$  production by the fish.

## V. SERIES V

The first 3 days of this experiment confirmed the blood acid-base results of series I and II and the ventilatory and respiratory findings of series IV in fish fitted with both dorsal aortic catheters and ventilation collection masks.  $\dot{V}_w$  declined during hyperoxia (fig. 7A) in a comparable manner to series IV (fig. 6C); again decreases in  $V_{S,R}$  were almost totally responsible for the phenomenon. The normoxic control level of  $\dot{M}_{O_2}$  ( $32.6 \pm 4.7 \mu\text{mol } O_2/\text{kg}/\text{min}$ ) was not significantly altered by 6, 24, or 48 h hyperoxia. The changes in blood acid-base status during hyperoxia were actually more marked than in previous series.  $Pa_{CO_2}$  doubled by 6 h (fig. 7B) and continued to increase gradually until 48 h. This caused a highly significant fall in pH<sub>a</sub> which remained depressed at 24 h (fig. 7C). However by 48 h, a 2.5-fold increase in plasma  $[HCO_3^-]$  had returned pH<sub>a</sub> to the control normoxic level (fig. 7D). These more pronounced changes probably reflected the higher level of hyperoxia employed (520–650 Torr versus 350–530 Torr in previous series).

At 48 h, the ventilatory flow was artificially increased by raising the buccal head in order to test whether external convective limitation (due to decreased  $\dot{V}_w$ ) was responsible for the increase in  $Pa_{CO_2}$  occurring during hyperoxia. The original aim was to restore  $\dot{V}_w$  to the normoxic control levels, but the actual flows attained were significantly higher than these by about 50% (fig. 7A). Even in the face of this large increase, the effect on  $Pa_{CO_2}$  was relatively small (fig. 7B). Two hours after the imposition of high  $\dot{V}_w$ ,  $Pa_{CO_2}$  had fallen significantly from  $6.86 \pm 0.94(5)$  Torr to  $5.30 \pm 0.96(5)$  Torr, but the latter was still a great deal higher than the normoxic control value,  $2.82 \pm 0.26(5)$  Torr. There were no significant changes in pH<sub>a</sub> (fig. 7C) or plasma  $[HCO_3^-]$  (fig. 7D). After 1 and 2 days on continuous high  $\dot{V}_w$  (*i.e.*, 72 and 96 h respectively),  $Pa_{CO_2}$  had returned to the hyperoxic control value of 48 h (fig. 7B). These results clearly indicate that external convective limitation plays only a small role in the  $CO_2$  retention of hyperoxia.

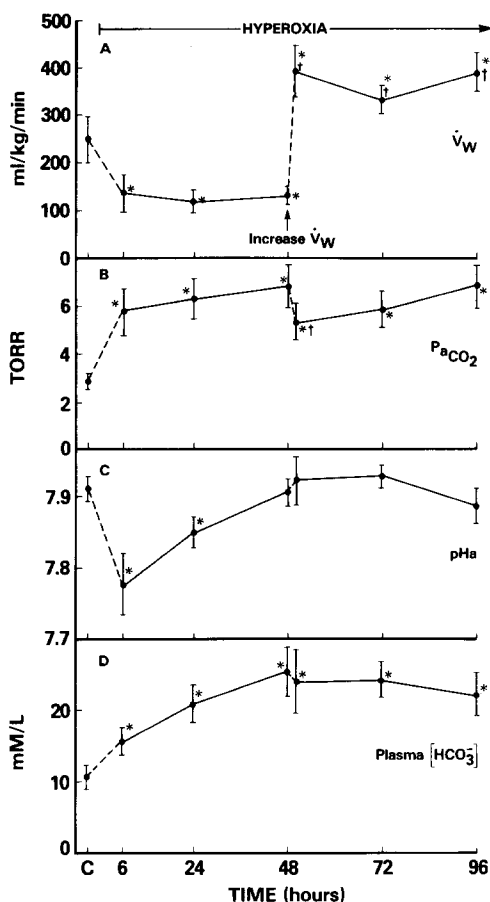


Fig. 7. The effect of an artificial increase in  $\dot{V}_W$  above the normoxic control level during continuous environmental hyperoxia in the rainbow trout of series V.  $\dot{V}_W$  was elevated after 48 h hyperoxia by raising the buccal head in the ventilation collection box. (A)  $\dot{V}_W$ ; (B)  $P_{aCO_2}$ , measured directly; (C) pH<sub>a</sub>; and (D) plasma  $[HCO_3^-]$ . C = control measurements under normoxia. Means  $\pm$  1 SE. \* = significantly different ( $P < 0.05$ ) from normoxic control; † = significantly different ( $P < 0.05$ ) from 48 h hyperoxic value. N = 5 throughout. T =  $14.0 \pm 1.0$  °C.

The artificial elevation of  $\dot{V}_W$  increased  $\dot{M}_{O_2}$  in all fish (from  $39.7 \pm 8.9(5)$   $\mu\text{mol O}_2/\text{kg}/\text{min}$  at 48 h hyperoxia to  $87.5 \pm 25.2(5)$   $\mu\text{mol O}_2/\text{kg}/\text{min}$  at 2 h post-elevation but because of the great variability in the data, the change was not significant ( $0.20 > P > 0.10$ ). By 24 h post-elevation,  $\dot{M}_{O_2}$  ( $33.5 \pm 8.0(5)$   $\mu\text{mol}/\text{kg}/\text{min}$ ) had returned to the pre-elevation level. Thus the convection required for water ( $\dot{V}_W/\dot{M}_{O_2}$ ) increased only slightly from 3.0 at 48 h hyperoxia to 4.5 at 2 h post-elevation, but by 24 h post-elevation had reached 9.9, close to the normoxic control value of 7.7.

## VI. SERIES VI

This experiment tested the alternative hypothesis, that the rise in  $\text{Pa}_{\text{CO}_2}$  during hyperoxia was due to an internal limitation caused by lamellar vasoconstriction. After 6 days of hyperoxia,  $\text{pH}_a$  had been restored to the normoxic control value (fig. 8A) in the face of an almost 3-fold rise in  $\text{Pa}_{\text{CO}_2}$  (fig. 8B) by a proportional increase in plasma  $[\text{HCO}_3^-]$  (fig. 8C). Injection of  $10 \mu\text{mol/kg}$  of 1-isoprenaline, a potent branchial vasodilator, caused a dramatic increase in  $\text{pH}_a$  which became significant 5 min after infusion (fig. 8A). The maximum effect was seen at 15 min, by which time  $\text{pH}_a$  had risen from  $7.802 \pm 0.024(8)$  to  $8.054 \pm 0.050(8)$ . A pronounced and significant drop in  $\text{Pa}_{\text{CO}_2}$ , from  $9.31 \pm 1.03(8)$  Torr to  $5.28 \pm 1.01(8)$

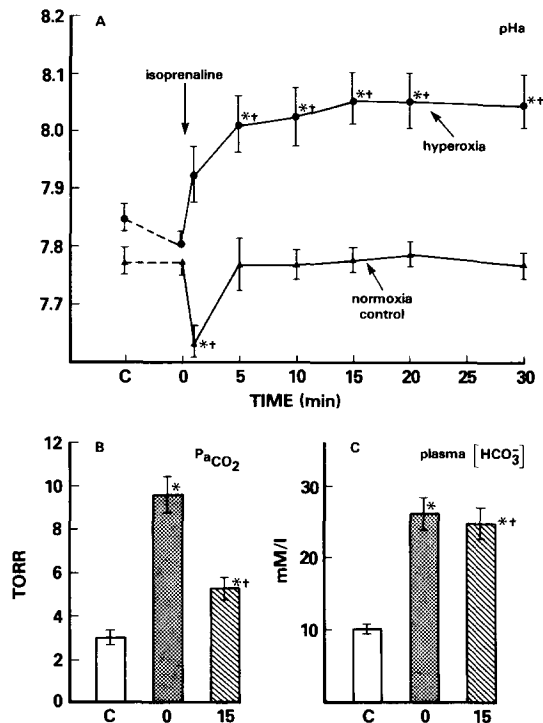


Fig. 8. The influence of a dorsal aortic injection of  $10 \mu\text{mol/kg}$  of 1-isoprenaline on: (A)  $\text{pH}_a$ ; (B)  $\text{Pa}_{\text{CO}_2}$ , measured directly; and (C) plasma  $[\text{HCO}_3^-]$  in the rainbow trout of series VI during continuous environmental hyperoxia (●). The results of an identical experiment performed under continuous normoxia in a control group (▲) are also shown in (A). There were no significant changes in the control group in (B) and (C) (not shown). C = control measurements under normoxia; 0 = measurements after 6 days of hyperoxia, taken immediately prior to the injection of isoprenaline; 15 = measurements taken 15 min after the injection of isoprenaline. Means  $\pm$  SE. \* = significantly different ( $P < 0.05$ ) from C value; † = significantly different ( $P < 0.05$ ) from 0 value. N = 8 throughout in the experimental hyperoxic group (●); N = 3 throughout in the control group (▲) under continuous normoxia.  $T = 15.0 \pm 2.0^\circ\text{C}$ .

Torr at 15 min (fig. 8B), was entirely responsible for the change in pHa. However,  $P_{aCO_2}$  remained significantly above the normoxic control value,  $3.07 \pm 0.22(8)$  Torr. Plasma  $[HCO_3^-]$  fell slightly, but the change was only that expected from the decline in  $P_{aCO_2}$ . In the 3 fish monitored 24 h after injection, all parameters had returned to the hyperoxic pre-injection level. Administration of  $10 \mu\text{mol/kg}$  of 1-isoprenaline to the 3 control fish under continuous normoxia produced only a very short-lived pHa depression (fig. 8A) attributable to the low pH (6.4) of the isoprenaline-saline solution.  $P_{aCO_2}$  and plasma  $[HCO_3^-]$  (not shown) were unaffected at 15 min post-injection in this control group. These results clearly indicate that internal diffusive and/or perfusive limitation plays a major role in the  $CO_2$  retention of hyperoxia.

## Discussion

In agreement with several previous studies (see Introduction), the present investigation has shown that environmental hyperoxia causes a marked decrease in  $\dot{V}_w$ , increase in  $P_{aCO_2}$ , and associated fall in pHa in the rainbow trout. However we now present a number of completely new findings which answer some of the questions raised in the Introduction:

(i) The  $CO_2$  retention appears to be largely due to an internal diffusive and/or perfusive limitation at the gills; external convective limitation plays only a small role.

(ii) The depression of pHa by hyperoxia is completely compensated within 48 h by the accumulation of plasma  $[HCO_3^-]$ .

(iii) The blood acid-base and  $CO_2$  changes occurring during hyperoxia are completely reversed within 20 h of the re-institution of normoxia.

(iv) The kidney plays an important role in the compensation of hyperoxic acidosis, though other unknown sites must also be involved.

The results of series V (fig. 7) and VI (fig. 8) showing the marked importance of internal diffusive/perfusive limitation and minor importance of external convective limitation contradict the explanation given by all previous hyperoxia studies on whole animals (see Introduction). However these findings support the interpretation offered by Haswell, Perry and Randall (1978). Using an artificially perfused trout gill, these workers showed that high perfusate  $P_{O_2}$  levels increased branchial vascular resistance and inhibited branchial  $CO_2$  excretion. As in the present study, the effects were reversed by isoprenaline. This vasoconstrictory influence of high  $P_{O_2}$  on the gills is opposite to its influence on the lungs of air-breathers (Comroe, 1974). As with the influence of  $P_{O_2}$  on ventilation, this again indicates the singular importance of  $O_2$  in setting the respiratory strategy of water-breathers. Isoprenaline, a synthetic catecholamine, is a selective  $\beta$ -adrenergic agonist and powerful branchial vasodilator (Wood, 1974, 1975) which probably acts like other  $\beta$ -stimulating catecholamines to increase the extent of lamellar perfusion (Holbert, Boland, and



Olson, 1979), thereby increasing branchial permeability to non-electrolytes (*e.g.*, gases, water) (Isaia, Maetz and Haywood, 1978; Wood, McMahon and McDonald, 1978). At the dose used here ( $10 \mu\text{mol/kg}$ ), l-isoprenaline exerts a relatively long-lasting cardiovascular effect *in vivo* (30–90 min), comprising a decrease in branchial vascular resistance and increase in  $\dot{V}_{S,H}$  with only minor changes in mean blood pressure levels afferent and efferent to the gills (Wood and Shelton, 1980; C. M. Wood, unpublished results). In the eel (Peyraud-Waitzenegger, 1979), isoprenaline is reported to increase ventilatory activity, but no rise in  $\dot{V}_w$  was observed in the three trout fitted with oral membranes in the present study.

The isoprenaline effect, a drop in  $P_{a_{CO_2}}$  of  $4.04 \pm 0.70(8)$  Torr, was extremely pronounced (fig. 8A,B), but did not result in a complete return of  $P_{a_{CO_2}}$  to normoxic levels (fig. 8B). This may mean either that part of the lamellar vasoconstriction was resistant to isoprenaline, at least at the dose used here, or that the degree of  $P_{a_{CO_2}}$  elevation which persisted was due to true external convective limitation. With regard to the former, Haswell, Perry, and Randall (1978) noted that isoprenaline at  $10^{-5}$  M, a concentration which provides maximum branchial vasodilation (Wood, 1974), only partially reversed the effects of hyperoxia on  $CO_2$  excretion in a perfused gill preparation where external convective limitation was unimportant. However, in support of the latter explanation, it is interesting that the amount of  $P_{a_{CO_2}}$  elevation (re normoxic levels) which persisted after isoprenaline,  $2.19 \pm 0.88(8)$  Torr (fig. 8B), was very similar to the drop in  $P_{a_{CO_2}}$ ,  $1.56 \pm 0.40(5)$  Torr (fig. 7B), which occurred when  $\dot{V}_w$  was artificially raised above normoxic levels. Partitioning on this basis would suggest that internal diffusive/perfusive limitation is 2–3 $\times$  as important as the external convective limitation during hyperoxia.

One criticism that can be levelled at the results of series V (fig. 7) is that artificial elevation of  $\dot{V}_w$  may not have duplicated an endogenous elevation of  $\dot{V}_w$  by the fish itself. For example, the procedure could increase the relative dead space ventilation and/or raise the cardiac output (*cf.* Davis and Cameron, 1970). However, the fact that  $\dot{V}_w$  was elevated to 1.5 $\times$  normoxic levels and that  $P_{a_{CO_2}}$  was only minimally affected at both 2 h post-elevation (when  $\dot{V}_w/\dot{M}_{O_2}$  was little altered) and at 24 h post-elevation (when  $\dot{V}_w/\dot{M}_{O_2}$  had returned to the normoxic control level) ameliorates this criticism.

At present, it is impossible to quantitatively predict the effect of external convective limitation at the gills on  $P_{a_{CO_2}}$ . Simple calculations (*e.g.*, Rahn, 1966) suggest that the 60% decrease in  $\dot{V}_w$  seen during hyperoxia (fig. 6C) should cause a 2.5-fold rise in  $P_{E_{CO_2}} - P_{I_{CO_2}}$ . However, this does not take into account the unknown nature of the  $CO_2$  dissociation curve of branchial water. More importantly, the relationship between  $P_{a_{CO_2}}$  and  $P_{E_{CO_2}}$  is unknown. It must be remembered that our measurements are reflective of equilibrium conditions of the  $CO_2/HCO_3^-$  system which are obtained in the measuring electrodes. There is no guarantee that such equilibria are ever achieved within the animal. Nevertheless, the present results, in agreement with unpublished experiments of Randall and Cameron cited by

Camaron and Polhemus (1974), do indicate that convective limitation is of minor importance in setting  $P_{aCO_2}$ , at least over the range of  $\dot{V}_w$ 's studied here.

The urine flow data (fig. 4) only partially support the concept of lamellar vasoconstriction and diffusive/perfusive limitation during hyperoxia. If urine flow is considered indicative of branchial permeability (*cf.* Wood and Randall, 1973), then the decrease at the start of hyperoxia (though non-significant) and the increase upon the re-institution of normoxia support the theory, but the return of urine flow to control values during long-term hyperoxia does not. Clearly other factors may come into play, such as variation of the drinking rate or dissociation of branchial water and  $CO_2$  permeabilities.

In fig. 9, the results of series I (fig. 1) and III (fig. 5) have been combined in a Davenport diagram (Davenport, 1974) to illustrate the time course and extent of hyperoxic compensation. The slope of the buffer line ( $\beta = -10.3$  slykes) was calculated from the mean haematocrit (25.7%), using the relationship of McDonald, Höbe and Wood (1980). This plot clearly shows that the compensation of hyperoxic

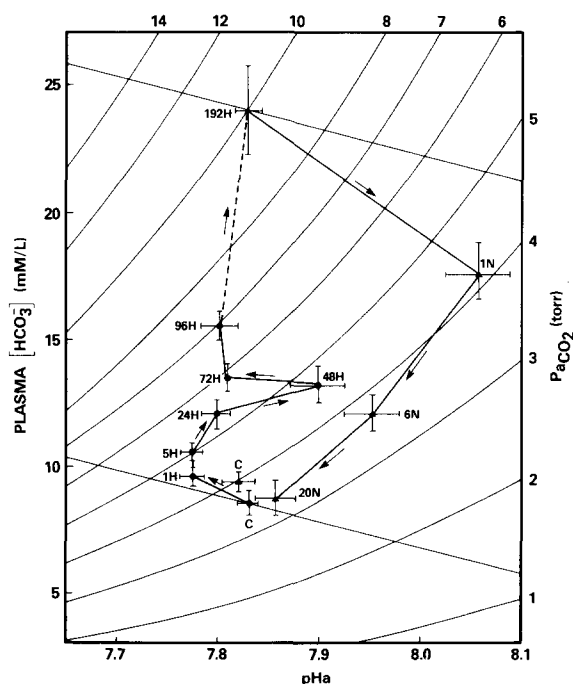


Fig. 9. Davenport diagram display of sequential changes in blood acid-base status during environmental hyperoxia and the re-institution of normoxia in the rainbow trout of series I (●) and III (▲). C = control measurements under normoxia; H = measurements under hyperoxia; N = measurements after the re-institution of normoxia; numbers = hours after introduction of hyperoxia or normoxia. The buffer lines through the C and 192H points were calculated from the mean haematocrit (25.7%).

Means  $\pm$  1SE. For further details, see legends of figs. 1 and 5.

acidosis started almost immediately, there being a slight increase in plasma  $[\text{HCO}_3^-]$  above the buffer line by 1 h, and a significant rise by 5 h. Similar plots for series II and V confirmed that significant compensation commenced within 5–6 h. Compensation was complete within 48 h, in some cases with evidence of temporary over-compensation. After this,  $\text{Pa}_{\text{CO}_2}$  continued to rise gradually with time but was compensated by a simultaneous accumulation of plasma  $[\text{HCO}_3^-]$ . A return to normoxia produced an even faster readjustment. By 1 h, plasma  $[\text{HCO}_3^-]$  was significantly below the buffer line, and had returned to control values by 20 h.

These results are entirely in agreement with the responses of rainbow trout to environmental hypercapnia. Janssen and Randall (1975) reported a complete compensation of hypercapnic acidosis within 48–72 h, and a complete restoration of control acid–base status (over an unspecified time course) after a return to normocapnia. In a comparable study Eddy *et al.* (1977) defined a very similar time course (20 h) to that seen in the present investigation for the restoration of control values. The reasons for the discrepancies between the present results and those of all previous hyperoxia studies in the extent of compensation and its reversibility (see Introduction) are unknown. Perhaps the simplest explanation is that of species difference, the rainbow trout being a more rapid and accurate regulator of blood pH than are other aquatic organisms studied. This in turn may reflect the great importance to blood  $\text{O}_2$  transport of normal acid–base state in this highly active animal.

The contribution of the kidney to the correction of hyperoxic acidosis in the trout is illustrated by fig. 3B. The 'no compensation' line in this figure was calculated using the mammalian model of renal acid excretion and  $\text{HCO}_3^-$  re-absorption (Hills, 1973; Davenport, 1974). All available evidence indicates that the trout kidneys behave like the mammalian in terms of acid–base regulation (Wood and Caldwell, 1978; Kobayashi and Wood, 1980).

After 24 h of hyperoxia, urinary acid excretion in the absence of renal compensation would have been highly negative (net base excretion) due to the higher  $\text{HCO}_3^-$  filtration rate. The latter, in turn, would be due to the accumulation of  $\text{HCO}_3^-$  in blood plasma as a compensation for  $\text{Pa}_{\text{CO}_2}$  elevation. Since net renal acid excretion remained unchanged, an increase in  $\text{H}^+$  secretion by the renal tubule cells during hyperoxia must have effected greater  $\text{HCO}_3^-$  reabsorption. Otherwise  $\text{HCO}_3^-$  would have been passively lost in the urine as quickly as it was built up in the blood, and the observed net accumulation of plasma  $[\text{HCO}_3^-]$  responsible for pH<sub>a</sub> regulation would not have been possible. The stimulus for the increased  $\text{H}^+$  secretion may have been the rise in  $\text{Pa}_{\text{CO}_2}$ , as in the mammal, and the observed increases in urinary  $\text{NH}_4^+$  excretion may have been a manifestation of this phenomenon (Hills, 1973; Davenport, 1974). In summary, while the kidney may not have played the dominant role in actually adding  $\text{HCO}_3^-$  to the blood, its action was of great significance in preventing excretion of accumulated plasma  $\text{HCO}_3^-$ . In this sense renal compensation made an active and significant contribution to the overall adjustment.

The large inter-animal variability in the renal response to hyperoxia (fig. 3A) is noteworthy. Similar variability has been seen in the kidney's contribution to the correction of hypercapnic acidosis in the rainbow trout (C. M. Wood, unpublished results). The explanation may be a varying balance in the relative contributions of renal and extra-renal mechanisms in different animals. The nature of these extra-renal mechanisms, which on average are responsible for most of the actual accumulation of  $\text{HCO}_3^-$  in the blood, is unknown. Acid excretion *via* ion exchanges at the gills (Cameron, 1978) seems the most likely possibility, but the only study on these exchanges during hyperoxia directly opposes this idea (Bornancin, DeRenzis and Maetz, 1977; see Introduction). Mobilization of  $\text{HCO}_3^-$  from extravascular compartments of the animal (ICF, bone) therefore warrants particular attention in future studies. Such mechanisms are already known to be of importance in acid-base adjustments in elasmobranchs (Randall, Heisler and Drees, 1976; Heisler, 1978).

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### References

- Bornancin, M., G. DeRenzis and J. Maetz (1977). Branchial  $\text{Cl}^-$  transport, anion stimulated ATPase and acid-base balance in *Anguilla anguilla* adapted to freshwater: effects of hyperoxia. *J. Comp. Physiol.* 117: 313-322.
- Boutilier, R. G., D. J. Randall, G. Shelton and D. P. Toews (1978). Some response characteristics of  $\text{CO}_2$  electrodes. *Respir. Physiol.* 32: 381-388.
- Cameron, J. N. (1971). Rapid method for determination of total carbon dioxide in small blood samples. *J. Appl. Physiol.* 31: 632-634.
- Cameron, J. N. and J. A. Polhemus (1974). Theory of  $\text{CO}_2$  exchange in trout gills. *J. Exp. Biol.* 60: 183-194.
- Cameron, J. N. (1978). Regulation of blood pH in teleost fish. *Respir. Physiol.* 33: 129-144.
- Cameron, J. N. and C. M. Wood (1978). Renal function and acid-base regulation in two Amazonian erythrinid fishes: *Hoplias malabaricus*, a water-breather, and *Hoplerethrinus unitaeniatus*, a facultative air-breather. *Can. J. Zool.* 56: 917-930.
- Comroe, J. H. (1974). *Physiology of Respiration*. 2nd Ed. Chicago, Yearbook Medical Publishers Inc.
- Davenport, H. W. (1974). *The ABC of Acid-Base Chemistry*. 6th Edn. Chicago, The University of Chicago Press.
- Davis, J. C. and J. N. Cameron (1970). Water flow and gas exchange at the gills of rainbow trout, *Salmo gairdneri*. *J. Exp. Biol.* 54: 1-18.

- Dejours, P. (1972). Action des changements de  $P_{O_2}$  et de  $P_{CO_2}$  de l'eau sur la ventilation et l'équilibre acide-base du sang chez quelques poissons téléostéens. *J. Physiol. (Paris)* 65: 386A.
- Dejours, P. (1973). Problems of control of breathing in fishes. In: *Comparative Physiology: Locomotion, Respiration, Transport, and Blood*, edited by L. Bolis, K. Schmidt-Nielsen and S.H.P. Maddrell. Amsterdam and New York, North Holland/American Elsevier, pp. 117-133.
- Dejours, P. (1975). *Principles of Comparative Respiratory Physiology*. Amsterdam, North-Holland.
- Dejours, P. and H. Beekenkamp (1977). Crayfish respiration as a function of water oxygenation. *Respir. Physiol.* 30: 241-251.
- Dejours, P., A. Toulmond and J.P. Truchot (1977). The effect of hyperoxia on the breathing of marine fishes. *Comp. Biochem. Physiol.* 58A: 409-411.
- Eclancher, B. (1972). Action des changements rapides de  $P_{O_2}$  de l'eau sur la ventilation de la truite et de la tanche. *J. Physiol. (Paris)* 65: 397A.
- Eddy, F.B., J.P. Lomholt, R.E. Weber and K. Johansen (1977). Blood respiratory properties of rainbow trout (*Salmo gairdneri*) kept in water of high  $CO_2$  tension. *J. Exp. Biol.* 67: 37-47.
- Haswell, M.S., S.F. Perry and D.J. Randall (1978). The effect of perfusate oxygen levels on  $CO_2$  excretion in the perfused gill. *J. Exp. Zool.* 205: 309-314.
- Heisler, N. (1978). Bicarbonate exchange between body compartments after changes of temperature in the larger spotted dogfish (*Scyliorhinus stellaris*). *Respir. Physiol.* 33: 145-160.
- Hickman, C.P., Jr. and B.F. Trump (1969). The kidney. In: *Fish Physiology*, Vol. I (W.S. Hoar and D.J. Randall, editors), New York, Academic Press Inc.
- Hills, A.G. (1973). *Acid-Base Balance*. Chemistry, Physiology, Pathophysiology. Baltimore, The Williams and Wilkins Co.
- Holbert, P.W., E.J. Boland and K.R. Olson (1979). The effects of epinephrine and acetylcholine on the distribution of red cells within the gills of the channel catfish (*Ictalurus punctatus*). *J. Exp. Biol.* 79: 135-146.
- Isaia, J., J. Maetz and G.P. Haywood (1978). Effects of epinephrine on branchial non-electrolyte permeability in rainbow trout. *J. Exp. Biol.* 74: 227-237.
- Janssen, R.G. and D.J. Randall (1975). The effects of changes in pH and  $P_{CO_2}$  in blood and water on breathing in rainbow trout, *Salmo gairdneri*. *Respir. Physiol.* 25: 235-245.
- Jones, D.R. and T. Schwarzfeld (1974). The oxygen cost to the metabolism and efficiency of breathing in trout (*Salmo gairdneri*). *Respir. Physiol.* 21: 241-254.
- Jouve, A. and J.P. Truchot (1978). Influence de l'oxygénation de l'eau sur la consommation d'oxygène et la ventilation branchiale du crabe, *Carcinus maenas* (L.). *C.R. Acad. Sci. Paris. Ser. D.* 286: 331-334.
- Kobayashi, K. and C.M. Wood (1980). The response of the kidney of the freshwater rainbow trout to true metabolic acidosis. *J. Exp. Biol.* 84: 227-244.
- Mackay, W.C. and D.D. Beatty (1968). The effects of temperature on renal function in the white sucker fish, *Catostomus commersoni*. *Comp. Biochem. Physiol.* 26: 235-245.
- McDonald, D.G., H. Höbe and C.M. Wood (1980). The influence of calcium on the physiological responses of the rainbow trout, *Salmo gairdneri*, to low environmental pH. *J. Exp. Biol.* (In press).
- Peyraud, C. and A. Serfaty (1964). Le rythme respiratoire de la carpe (*Cyprinus carpio*, L.) et ses relations avec le taux de l'oxygène dissous dans le biotope. *Hydrobiologia* 23: 165-178.
- Peyraud-Waitzenegger, M. (1979). Simultaneous modifications of ventilation and arterial  $P_{O_2}$  by catecholamines in the eel, *Anguilla anguilla* L: participation of  $\alpha$  and  $\beta$  effects. *J. Comp. Physiol.* 129: 343-354.
- Rahn, H. (1966). Aquatic gas exchange: Theory. *Respir. Physiol.* 1: 1-12.
- Randall, D.J. and J.N. Cameron (1973). Respiratory control of arterial pH as temperature changes in rainbow trout, *Salmo gairdneri*. *Am. J. Physiol.* 225: 997-1002.
- Randall, D.J. and D.R. Jones (1973). The effect of deafferentation of the pseudobranch on the respiratory response to hypoxia and hyperoxia in the trout (*Salmo gairdneri*). *Respir. Physiol.* 291-301.

- Randall, D.J., N. Heisler and F. Drees (1976). Ventilatory response to hypercapnia in the larger spotted dogfish *Scyliorhinus stellaris*. *Am. J. Physiol.* 230: 590-594.
- Reeves, R.B. (1977). The interaction of body temperature and acid-base balance in ectothermic vertebrates. *Ann. Rev. Physiol.* 39: 559-586.
- Severinghaus, J.W. (1965). Blood gas concentrations. In: Handbook of Physiology. Section 3. Volume 2, edited by W.O. Fenn and H. Rahn. Washington, DC., American Physiological Society. pp. 1475 - 1487.
- Smith, L.S. and G.R. Bell (1964). A technique for prolonged blood sampling in free swimming salmon. *J. Fish. Res. Bd. Can.* 21: 1775-1790.
- Solorzano, L. (1969). Determination of ammonia in natural waters by the phenolhypochlorite method. *Limnol. Oceanogr.* 14: 799-801.
- Truchot, J.P. (1975). Changements de l'état acide base du sang en fonction de l'oxygénation de l'eau chez le crabe, *Carcinus maenas* (L.). *J. Physiol. (Paris)* 70: 583-592.
- Wolf, K. (1963). Physiological salines for freshwater teleosts. *Prog. Fish. Cult.* 25: 135-140.
- Wood, C.M. and D.J. Randall (1973). The influence of swimming activity on water balance in the rainbow trout (*Salmo gairdneri*). *J. Comp. Physiol.* 82: 257-276.
- Wood, C.M. (1974). A critical examination of the physical and adrenergic factors affecting blood flow through the gills of the rainbow trout. *J. Exp. Biol.* 60: 241-265.
- Wood, C.M. (1975). A pharmacological analysis of the adrenergic and cholinergic mechanisms regulating branchial vascular resistance in the rainbow trout (*Salmo gairdneri*). *Can. J. Zool.* 53: 1569-1577.
- Wood, C.M. and F.H. Caldwell (1978). Renal regulation of acid-base balance in a freshwater fish. *J. Exp. Zool.* 205: 301-307.
- Wood, C.M. and F.H. Caldwell (1978). Renal regulation of acid-base balance in a freshwater fish. *J. Exp. Zool.* 205: 301-307.
- Wood, C.M., B.R. McMahon and D.G. McDonald (1978). Oxygen exchange and vascular resistance in the totally perfused rainbow trout. *Am. J. Physiol.* 234: R201-R208.
- Wood, C.M. (1978). *Biol.* 82: 139-162.
- Wood, C.M. and G. Shelton (1980). Cardiovascular dynamics and adrenergic responses of the rainbow trout *in vivo*. *J. Exp. Biol.* 87: 247-270.