J. exp. Biol. (1977), 67, 37–47
With 5 figures
Printed in Great Britain

BLOOD RESPIRATORY PROPERTIES OF RAINBOW TROUT (*SALMO GAIRDNERI*) KEPT IN WATER OF HIGH CO_2 TENSION

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(*Received* 1 *September* 1976)

SUMMARY

1. Blood O_2 transport and acid-base balance were studied at 20 °C in rainbow trout (*Salmo gairdneri*) which had been kept in water of high CO_2 content (15 mmHg) for at least a week. Also the blood gas chemistry of fish rapidly entering or leaving the hypercapnic environment was studied.

2. Fish entering high CO_2 water suffered a sharp decrease in blood pH which significantly reduced O_2 transport by the blood, but after a few hours considerable compensation was achieved.

3. After at least a week in high CO_2 water, trout showed elevated plasma bicarbonate and P_{CO_2} levels, and a decrease in plasma chloride, while pH was about 0·1 pH unit below the level for control fish. Oxygen transport by the blood was marginally reduced.

4. Hypercapnic fish rapidly entering fresh water showed a sharp increase in blood pH and a decrease in blood P_{O_2}. These parameters regained normal values after a few hours but plasma bicarbonate and chloride levels took much longer to regain control concentrations.

5. Acid-base balance in hypercapnic fish is discussed with particular reference to the role of the branchial ion exchanges.

INTRODUCTION

Recent studies have described acute and short-term effects of hypercapnia on respiratory function in fish (Lloyd & White, 1967; Eddy & Morgan, 1969; Cameron & Randall, 1972). In these studies the fish were subjected to high CO_2 for periods of less than 48 h. The effects of longer-term exposure to CO_2 have been investigated rarely and often in relation to its toxicity (Alabaster, Herbert & Hemens, 1957; Lloyd & Jordan, 1964). Janssen & Randall (1975) studied respiratory function in trout exposed to 8 mmHg P_{CO_2} for up to 3 days.

Elevated levels of CO_2 may occur in fresh water for several reasons. The breakdown of organic matter in polluted water may produce up to 50 ppm free CO_2 (Hynes, 1960) while the discharge of acid into water of high carbonate content may significantly increase CO_2 tension (Doudoroff & Katz, 1950). Ground waters usually

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contain significant quantities of CO_2, the amount depending on the geographical location. Finally the metabolism of fish produces CO_2. This is normally of little importance but when fish are densely stocked and supplied with recirculating ground water (as is the case in some fish farms), CO_2 tensions of around 20 mmHg are not uncommon.

One of the objectives of the present study was to investigate respiratory properties of blood in rainbow trout which had been exposed to high CO_2 for at least 1 week. Experiments were also carried out to study the rate at which fish accommodate changes in environmental CO_2 after transfer from freshwater to high CO_2 water and the reverse.

METHODS

Rainbow trout (*Salmo gairdneri*) weighing 500–12000 g were obtained from the trout farm at Forsögdambruget, Bröns, Denmark, and held indoors in 750 gal freshwater tanks, temperature 20 ± 1 °C. Oxygen saturation of the water was maintained at near 100% by aeration and the water was replaced by a fresh dechlorinated supply at approximately 50 gal/h. A number of the trout were transferred to a similar tank containing water with a carbon dioxide tension of about 15 mmHg obtained by metering CO_2 gas into the tank's air supply. Normally each tank contained about 15 fish. The fish remained in this tank to acclimatize for at least a week before being used for experiments. During this period the P_{CO_2} of the water remained reasonably constant and rarely fell below 13 mmHg. The fish seemed to suffer no ill effects from this treatment and appeared to be as healthy and active as those in CO_2-free fresh water.

Fish selected for an experiment were anaesthetized with MS 222 (Sandoz) and then either the dorsal aorta or the ventral aorta was cannulated as indicated by Holeton & Randall (1967). They were allowed to recover for at least 24 h in small experimental tanks which were supplied with either fresh water or high CO_2 water. Houston *et al.* (1973) suggest that at least 24 h are required for recovery from the effects of surgery and anaesthesia. These experimental tanks were designed so that the fish could move backwards and forwards to a small extent, but were too narrow to allow the fish to turn round. The walls and cover were opaque so that the fish were not unduly disturbed by external visual stimuli. Also the design of the tank allowed retrieval of the free end of the cannula with minimum disturbance of the fish. In most cases the processes of blood sampling and flushing the cannula with fresh heparinized saline produced no visible reaction from the fish.

In the transfer experiments fish were lifted by hand from a freshwater tank to an adjacent CO_2 tank, or the reverse. With practice it was possible to effect the transfer in 2–3 s without disturbing the fish at all. Cameron (1976) working with Arctic grayling describes a similar stress-free method of transfer.

Blood samples (normally 0·3–0·5 ml) were analysed for P_{O_2}, P_{CO_2} and pH using a Radiometer PHM71 Mk 2 meter connected to a BMS3 electrode assembly. Blood equilibration was carried out using a Radiometer BMS2 Mk 2 supplied by gas mixing pumps (Wörsthoff) which could be adjusted to deliver air/N_2 mixtures containing up to 30 mmHg P_{CO_2}. Thus O_2–Hb equilibrium curves could be constructed at known P_{CO_2} and pH values. Oxygen capacity and content were determined using

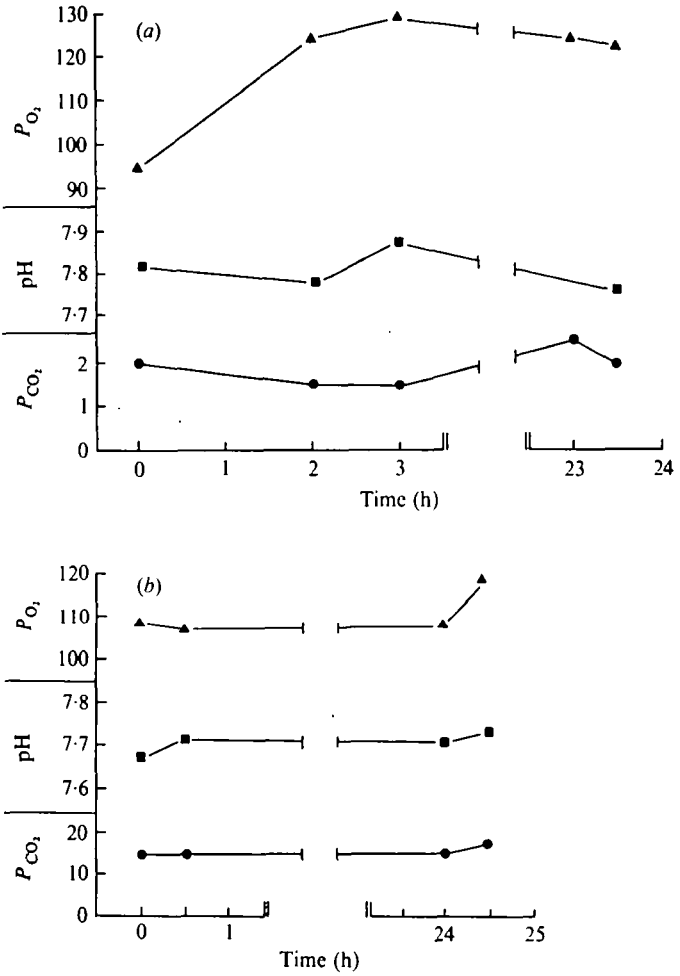

Fig. 1. Arterial P_{O_2}, P_{CO_2} and pH for rainbow trout kept in fresh water (*a*) or in water containing about 15 mmHg P_{CO_2} for at least one week (*b*). Results are for single fish and are typical of results obtained from a total of six hypercapnic and four control fish.

a Lex-O_2-con oxygen analyser (Lexington instruments). Chloride was determined using a Radiometer CMT10 chloride titrator. Normally 3–4 samples were drawn from the fish during the course of the day and a further one the next morning.

In a second series of experiments blood was withdrawn from both groups of fish by heart puncture and this was then used for a number of *in vitro* determinations. Nucleoside triphosphate (NTP) was determined by the Sigma enzymic method (St Louis, U.S.A.), and haemoglobin was estimated spectrophotometrically after quantitative dilution and haemolysis of the blood in 0·1 M Tris buffer, pH 7·5, using the extinction coefficients of human haemoglobin (Antonini & Brunori, 1971).

Table 1. *Blood respiratory characteristics of rainbow trout kept in freshwater (controls) and for a week or more in high CO_2 water, average 15 mmHg P_{CO_2} (hypercapnic fish)*

	Control	Hypercapnic
	Arterial	
P_{O_2}	117 ± 3·5 (16)	103·4 ± 2·9 (14)*
P_{CO_2}	2·03 ± 0·17 (16)	15·04 ± 0·33 (13)*
pH	7·76 (16)	7·66 (15)*
HCO_3^-	3·78 ± 0·3 (15)	23·16 ± 1·56 (11)*
H_2CO_3	0·09 ± 0·01 (15)	0·69 ± 0·02 (10)*
Cl^-	120·4 ± 1·64 (14)	102·5 ± 1·48 (20)*
	Mixed venous	
P_{O_2}	31 (2)	35·8 ± 8·64 (5)
P_{CO_2}	3·25 (2)	13·3 ± 0·96 (6)*
pH	7·67 (2)	7·65 (6)
HCO_3^-	5·2 (2)	18·9 ± 1·37 (6)*
H_2CO_3	0·15 (2)	0·6 ± 0·04 (6)*

* Significantly different at 0·002.

RESULTS

Rainbow trout which had been living in high CO_2 water for a week or more showed a number of changes in their blood gas characteristics when compared to normal fish. The main differences were that hypercapnic fish showed a lower pH value, and higher values for P_{CO_2}, HCO_3^-, H_2CO_3 + dissolved CO_2 (Fig. 1, Table 1).

Analysis of haemoglobin and total nucleoside triphosphate revealed similar concentrations for each group. The NTP/Hb ratio for hypercapnic fish was 1·06 while that for normal fish was 1·03. The O_2–Hb equilibrium data has been arranged to present the curves at constant pH (Fig. 3) and individual values for P_{50} and the Root effect are shown in Fig. 2(*a*) and (*b*).

O_2–Hb equilibrium curves show that at similar pH values the Root effect was larger in hypercapnic fish, and blood oxygen affinity of those fish was lower, while values of n from Hill equation plots were about the same for both groups (Fig. 2*a*, *b*). The O_2–Hb equilibrium curves (Fig. 3) together with the Root effect data (Fig. 2*a*) were used to determine percent O_2 saturation of arterial and venous blood for both groups of fish (Table 2). Here it can be seen that O_2 transport is disturbed immediately after transfer from fresh water to high CO_2 water (or vice versa). However, in each case the situation is almost normal again after a period of about 6 h (Figs. 4, 5).

The response of fish when transferred from fresh water to high CO_2 water, or the reverse, could be broadly divided into two phases. First, the initial response lasting about an hour, and then the recovery phase lasting up to 24 h. When first transferred to high CO_2 water the fish showed a sharp drop in blood pH, a rapid increase in P_{CO_2} and a slow rise in bicarbonate. Blood P_{O_2} was little altered, but the drop in arterial pH would have resulted in a decrease in arterial oxygen content via the Root effect (Table 2). After about 20 h all values had reached new equilibrium values; notably blood pH had levelled off about 0·1 pH unit below the normal value (Fig. 1).

Fish transferred from high CO_2 water to fresh water showed an immediate increase in blood pH and rapid decreases in P_{O_2} and P_{CO_2}. However, blood HCO_3^- showed a much more gradual decrease (Fig. 5). The high arterial blood pH values shown shortly

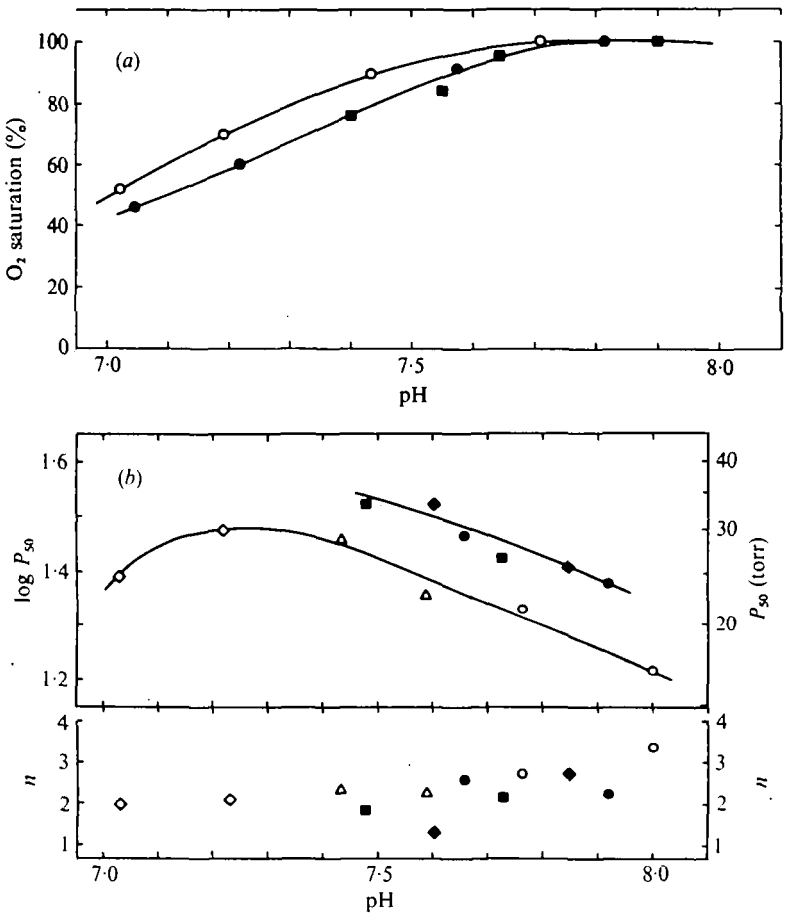

Fig. 2. *Salmo gairdneri*. (*a*) The Root effect in normal fish (open symbols) and hypercapnic fish (closed symbols). (*b*) Half saturation oxygen tension, P_{50}, and Hill's coefficient, n, as a function of pH in normal fish (open symbols) and hypercapnic fish (closed symbols). Different symbols refer to individual fish.

after transfer indicate that arterial blood became fully O_2 saturated at comparatively low P_{O_2} tensions, the Root effect being absent and the Bohr effect tending to shift the dissociation curve significantly to the left (higher blood O_2 affinity).

DISCUSSION

Oxygen transport by the blood

(*a*) *Fish entering high CO_2 water*

These fish show a sharp decrease in blood pH (Fig. 4) which has several effects on blood oxygenation.

(i) Even though P_{O_2} remains at around 100 mmHg, arterial saturation is reduced to around 70% (Table 2, Fig. 2*a*) and arterial blood carries less O_2 per unit volume.

(ii) The Bohr effect operates to increase blood O_2 loading and unloading tensions.

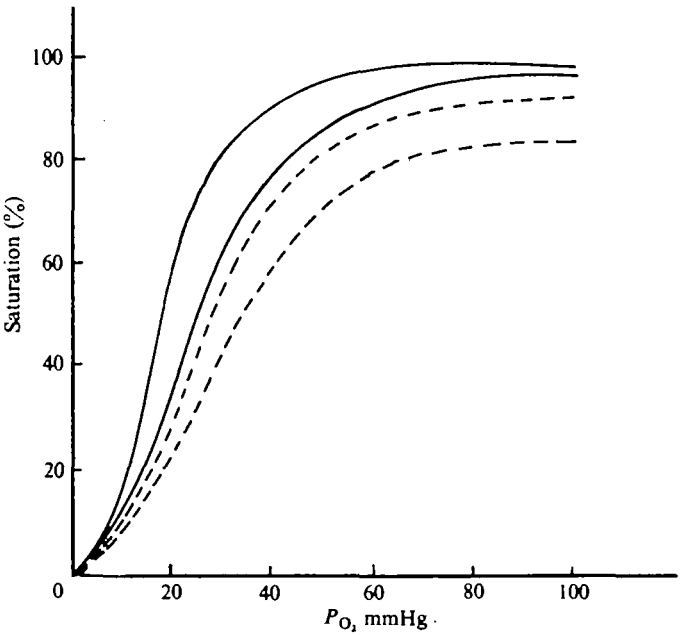

Fig. 3. O_2–Hb binding curves of rainbow trout blood at 20 °C. Solid lines, pH 7·9; broken lines, pH 7·5. In each case the curve displaced to the left (indicating greater O_2 affinity) is for control fish, and the curve to the right is for hypercapnic fish. The curves are based on data from three hypercapnic and three control fish.

Thus, at any given tissue P_{O_2}, more O_2 can be unloaded, but for fish entering high CO_2 water this advantage is greatly reduced because, compared to the controls (Table 2), they have lower venous O_2 tensions.

(iii) Because of (i) and (ii) above, efficiency of O_2 uptake by the blood and O_2 removal from the water is decreased. Other factors such as changes in gill ventilation and perfusion may change, but the net effect is that the blood becomes a less efficient medium for O_2 transport.

The fish attempts to remedy this situation by adjustments to circulatory, ventilatory and other systems which would normally increase O_2 delivery to the tissues. However, increased gill ventilation as observed by Janssen & Randall (1975) and Eddy (1976) is unlikely to improve blood oxygenation to any great extent. Thus during the initial period of hypercapnia the fish will have a diminished scope for increasing its O_2 uptake and therefore its activity. However, after 6 h there is some compensation and after 24 h this is almost complete (Figs. 1, 4).

(*b*) *Physiological adaptation to the hypercapnic environment*

After at least 24 h, hypercapnic swimming performance of salmon is little impeded (Dahlberg, Surnway & Duodoroff, 1968), plasma HCO_3^- of rainbow trout is increased (Lloyd & White, 1967) and blood O_2 carrying capacity is improved (Eddy, 1976). In the present experiments blood gas data suggest that compensation is incomplete even in fish which had been hypercapnic for more than 2 weeks. Arterial pH is consistently lower than normal by about 0·1 pH unit (Fig. 1, Table 1), although the data of

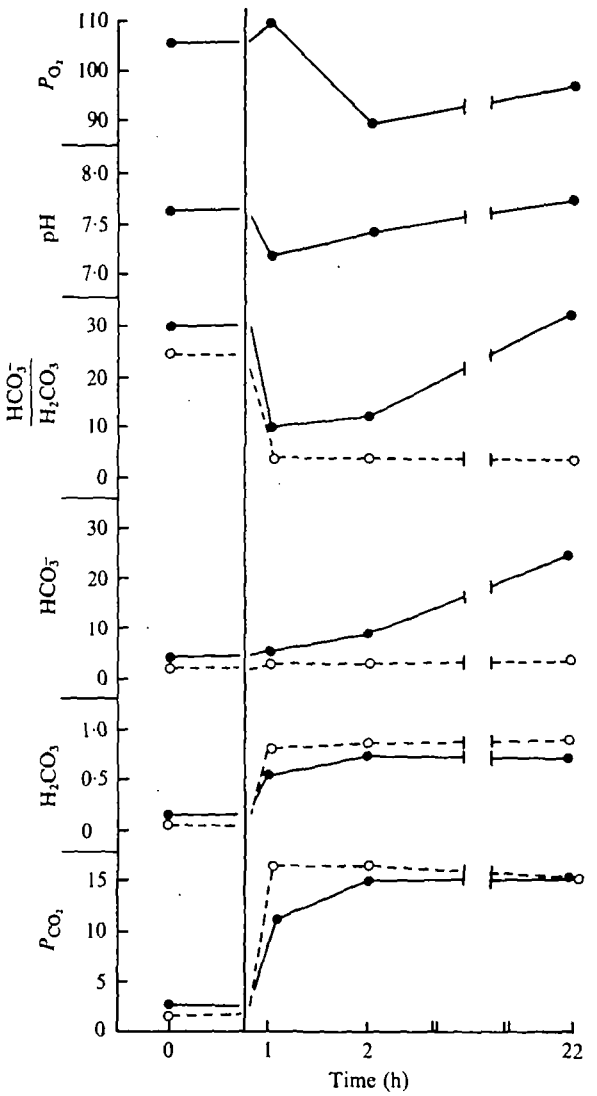

Fig. 4. Blood gas physiology of a rainbow trout rapidly transferred from fresh water to high CO_2 water (approx 15 mmHg P_{CO_2}). Open symbols refer to water while solid symbols refer to arterial blood.

Janssen & Randall (1975) indicate that this parameter has regained normal levels after 3 days hypercapnia. In their experiments on rainbow trout lower levels of CO_2 were used (8 mmHg compared to 15 mmHg) and it is reasonable to assume that trout are less able to adjust to higher levels of CO_2. Secondly their experiments were conducted at a lower temperature, 9 °C compared to 20 °C.

Analysis of red cell contents reveals a number of interesting points. First it is known that increased cellular ATP will increase the Root effect (Weber & de Wilde, 1975) and increased cellular ATP is often associated with decreased intraerythrocyte pH (Wood & Johansen, 1973), a condition likely to exist in hypercapnic trout. Analysis of

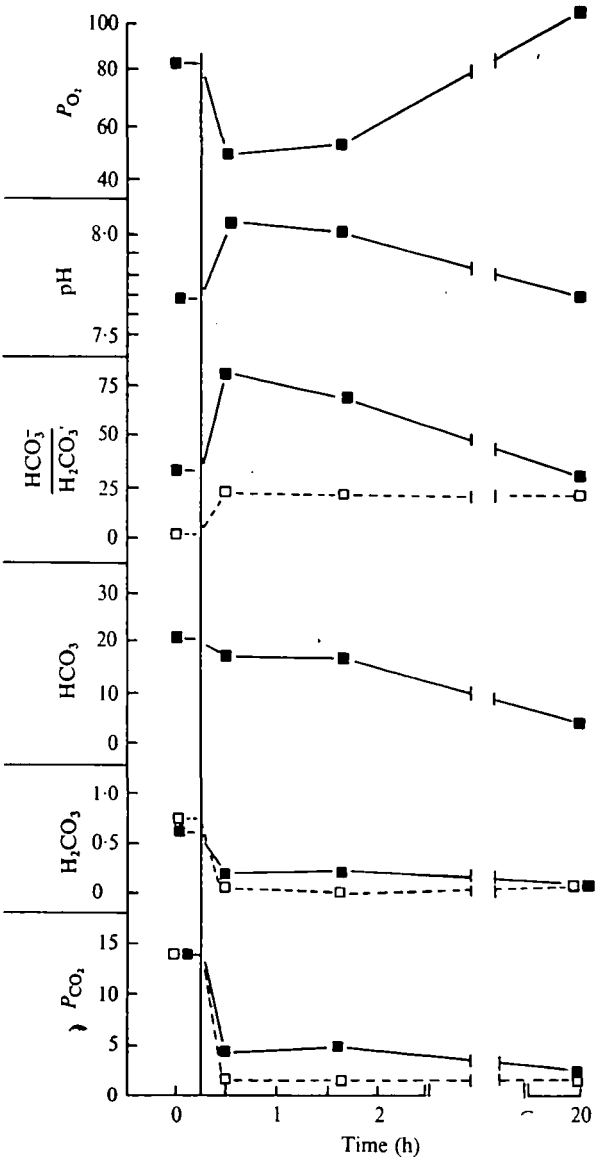

Fig. 5. Blood gas physiology of a rainbow trout rapidly transferred from high CO_2 water (approx. 15 mmHg) to fresh water. Open symbols refer to water while solid symbols refer to arterial blood.

erythrocyte nucleoside triphosphates, which in trout are 90% ATP and 10% GTP (Lykkeboe, personal communication) indicate that their concentrations are approximately equal in both normal and hypercapnic fish, and an increase in the Root effect from this cause seems unlikely in hypercapnic trout. Thus the increased Root effect observed in hypercapnic trout may be explained by decreased intraerythrocytic pH (Schaefer, Messier & Morgan, 1970; Eddy, 1976) or perhaps a direct CO_2 effect (Riggs, 1970). Both Börjeson & Höglund (1976) and Eddy & Morgan (1969) report a reduction in the Root effect in CO_2 adapted salmonids, which disagrees with the

Table 2. In vivo O_2 *transport by rainbow trout blood when the fish was transferred from fresh water to high* CO_2 *water* (*a*) *and from high* CO_2 *water to fresh water* (*b*)

(*a*) Fresh water to 15 mmHg P_{CO_2} in the water

	Fresh water		15 min high CO_2		6+ hours high CO_2	
	Art.	Ven.	Art.	Ven.	Art.	Ven.
P_{O_2}	117	33	110	21	103	35
Sat. (%)	95	70	62	25	95	68
pH	7·76	7·67	7·20	6·98	7·76	7·65
P_{CO_2}	2	3·5	11	14	15	—

(*b*) High CO_2 to fresh water

	High CO_2 water		15 min fresh water		6+h fresh water	
	Art.	Ven.	Art.	Ven.	Art.	Ven.
P_{O_2}	88	54	54	35	117	35
Sat. (%)	95	80	95	90	95	70
pH	7·66	7·65	8·03	7·95	7·76	7·67
P_{CO_2}	13	13	6	7	2	3·3

Arterial values are means for three fish while mixed venous values are for a single fish in each case.

present results (Fig. 2); this topic requires further investigation. Decreased O_2 affinity of hypercapnic fish when expressed at constant pH, rather than constant P_{CO_2} (Eddy & Morgan, 1969), could also be accounted for by lower intracellular pH values, but this factor appears to have a marginal influence on O_2 transport.

(*c*) *Fish leaving the hypercapnic environment*

CO_2 rapidly diffuses out of the gills leaving the blood alkaline with pH values of pH 8·0 or more and low CO_2 tensions. Similar pH values were obtained by HCO_3^- injection (Janssen & Randall, 1975) but this procedure tends to increase P_{CO_2} as well. In the present experiments the Root effect is without influence on arterial blood (Fig. 2*a*) while the Bohr effect significantly increases blood O_2 affinity such that full arterial O_2 saturation occurs at the observed low arterial P_{O_2} values of around 50 mmHg (Fig. 5, Table 2). Hence the O_2 unloading tension is also decreased and from the few data available for venous blood this appears to be the case (Table 2). Normal blood pH values are resumed after 12–24 h (Fig. 5) and the rate limiting step appears to be removal of HCO_3^- from the plasma.

Acid-base balance. After a few minutes in high CO_2 water the carbonic acid content of the blood increased from 0·09 to 0·69 mM (Table 1). (For convenience carbonic acid will be used to refer H_2CO_3 and dissolved CO_2 together.) The HCO_3^- concentration required to restore blood pH to around pH 7·7 can be calculated to be about 25 mM using the Henderson–Hasselbalch equation (values for pK' and CO_2 solubility from Severinghaus, Stupfel & Bradley, 1956), and after 20 h hypercapnia this is indeed observed (Table 1). Cameron (1976) suggests that this HCO_3^- originates from retained metabolic CO_2; however, each equivalent of CO_2 retained as HCO_3^- will generate an equivalent of H^+ and displace approximately the same amount of Cl^- from the blood plasma (Lloyd & White, 1967, and Table 1). Thus over 24 h hypercapnia the trout increases the bicarbonate concentration of the extracellular fluid

by about 20 mM and similar amounts of Cl^- and H^+ will need to be removed. The possible mechanisms underlying these changes will now be examined.

Obviously the difference between the rates of acid excretion (Na^+/H^+ and Na^+/NH_4^+ exchange) and base retention (HCO_3^-/Cl^- exchange) (Maetz, 1971, 1973) will determine blood pH at any instant. Values from the literature for trout in fresh water indicate that Na influx usually exceeds Cl^- influx by a significant amount (Kerstetter & Kirschner, 1972; Lahlou *et al.* 1975) and this may be related to the species' carnivorous diet which would lead to an excess of metabolic acid. In Arctic grayling transferred to high CO_2 water Cameron (1976) observed an increase in Na^+ influx while Cl^- decreased, giving an increased capacity for acid excretion, and, assuming a 1:1 exchange, 66 h would be needed to remove the H^+ generated by retention of respiratory CO_2. Thus the branchial ion exchanges appear to be mechanisms capable of maintaining body Na^+, H^+ and Cl^- concentrations at the correct levels under normal conditions, but are not well adjusted to deal rapidly with large amounts of acid such as are generated during hypercapnia.

It is of interest that in hypercapnic dogfish (Heister, Weitz & Weitz, 1976) blood pH is stabilized by HCO_3^- entering the extracellular fluid both from the sea water and from the cellular compartment. Uptake of HCO_3^- from the water by reversal of the HCO_3^-/Cl^- exchange was observed in goldfish by Dejours (1969). The role of these mechanisms in acid-base balance requires further study.

In hypercapnic trout the fall in plasma Cl^- (Table 1) could be achieved if the efflux exceeded the rate of active uptake, and this was observed to be the case for hypercapnic grayling (Cameron, 1976). But even in fresh water both Na^+ and Cl^- showed negative net uptakes and it is possible that this is a normal state in Arctic grayling (and other fish) the ionic balance being made good in the diet.

Hypercapnic fish entering fresh water immediately lose about 0·5 mM-H_2CO_3 from the blood and this is sufficient to increase the plasma HCO_3^-/H_2CO_3 ratio from 30 to about 100 (Fig. 5), and blood pH to pH 8·0 or more. If blood pH is restored to normal levels then about 20 mM HCO_3^- needs to be removed from the extracellular fluid together with significant quantities of H^+ which have accumulated in the body cells. If the bicarbonate space of a 1 kg fish is assumed to be 30% then $20 \times 0{\cdot}3 = 6$ mM-HCO_3^- has to be removed. If the HCO_3^-/Cl^- exchange operates at about 330/μM kg^{-1} h^{-1} (Kerstetter & Kirschner, 1972) then 6/0·33 = 18 h will be required. Plasma HCO_3^- probably decreases more rapidly than this because H^+ released from the cellular compartment will convert HCO_3^- to CO_2 which then diffuses rapidly from the gills. The rate at which Cl^- is restored to the plasma will depend upon the difference between passive efflux and active uptake. Thus if 6 mM of Cl^- are required and the passive efflux is 200 μM kg^{-1} h^{-1} (Kerstetter & Kirschner, 1972), then it will take 6/(0·33–0·2) = 46 h. Lloyd & White (1967) noted in hypercapnic fish transferred to fresh water that plasma Cl^- returned to normal levels more slowly than did plasma HCO_3^-.

In conclusion it is worth mentioning that trout are remarkably tolerant to changes in blood pH. Changes of 0·5 pH or more were recorded in some experimental fish and these were tolerated without undue ill effects.

This work was supported by the Danish Natural Science Research Council. Skilled technical assistance from Sonja Kornerup and Winnie Heidemann is acknowledged.

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