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INTERNAL HYPOXIA–HYPERCAPNIA IN TENCH EXPOSED TO ALUMINIUM IN ACID WATER: EFFECTS ON BLOOD GAS TRANSPORT, ACID–BASE STATUS AND ELECTROLYTE COMPOSITION IN ARTERIAL BLOOD

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SUMMARY

Tench exposed for 6 days to acid (pH 5) hard water ($[Ca^{2+}] = 3{\cdot}5\ mmol\ l^{-1}$) in the presence of aluminium ($2\ mg\ l^{-1}$) showed a rapid decrease (within 3 h) in dorsal aortic P_{O_2} and a simultaneous rise in P_{CO_2}. Arterial oxygen content and haemoglobin-oxygen ($Hb\text{-}O_2$) saturation decreased sharply, as did red cell [Hb], whereas blood haematocrit (Hct) and [Hb] increased. Red cell nucleoside triphosphate (NTP) content was reduced within 1 day through a selective reduction in guanosine triphosphate (GTP).

Although the rising P_{CO_2} caused an extracellular acidosis (to which lactic acid production and perhaps H^+ influx at the gills contributed), red cell pH increased compared to control values, mainly as a result of the decrease in $Hb\text{-}O_2$ saturation. Plasma $[Cl^-]$ declined, whereas $[HCO_3^-]$, $[K^+]$ and $[Ca^{2+}]$ increased. Ionic disturbances, however, were small compared to the changes in blood O_2 transport, which appeared to correlate with high ambient $[Ca^{2+}]$.

Tench exhibited a high tolerance to the acid–Al exposure and most of the above parameters showed partial recovery within 1–2 days. In some specimens, however, the exposure was lethal because of an obstruction of gill function, and arterial blood became almost completely deoxygenated and showed very low pH values and high lactate concentrations, attesting to deep internal hypoxia.

INTRODUCTION

Anthropogenic emission of industrial waste gases, such as sulphur dioxide (which is oxidized to sulphuric acid in the atmosphere), has increased the acidity of rain and led to an extensive environmental acidification of freshwater systems. The massive numbers of dead fish occasionally observed in such waters call for research elucidating the mechanisms and nature of physiological disturbances in acid-exposed fish.

Teleost fish exposed to acid water below pH 5 generally show extensive losses of body electrolytes and disturbances in the acid–base balance (Ultsch, Ott & Heisler, 1981; McDonald & Wood, 1981; Höbe, Wood & McMahon, 1984). The severity of

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these responses is modulated by the environmental calcium concentration, high ambient [Ca^{2+}] having a protective effect on salmonids (see review by McDonald, 1983). In extremely acid water (i.e. pH values below those usually recorded in nature) a hypoxic syndrome is manifested, shown by large decreases in arterial P_{O_2} and increases in lactate concentration (Ultsch *et al.* 1981; Hõbe *et al.* 1984).

Recent data have drawn attention to a role for aluminium in acid water toxicity. Acid rain solubilizes soil aluminium, increasing its concentration in natural waters. Thus large inputs of aluminium (reaching concentrations of 0·7–0·8 mg l^{-1}) are observed during heavy rain and when the snow melts in spring (Driscoll, Baker, Bisogni & Schofield, 1980; Dickson, 1983). Aluminium renders the water toxic at pH values that otherwise appear to be physiologically safe. The toxicity of aluminium-containing water appears maximal around pH 5 (Muniz & Leivestad, 1980; Baker & Schofield, 1982), which coincides with pH values at which fish deaths occur in nature.

Data on the physiological consequences of exposure to aluminium-containing, acid water in fish are scarce. Muniz & Leivestad (1980), however, reported an extensive depletion of plasma electrolytes and a reduction in venous P_{O_2} in *Salmo trutta* subjected to pH 5 in water of low Ca^{2+} but high Al content. In rainbow trout in aluminium-containing, acid (pH 5) water of high Ca^{2+} content, plasma [Na^+] and [Cl^-] values were unaffected, whereas arterial P_{O_2} drastically decreased before death, thus pointing to a hypoxic stress as the dominant cause of death in these circumstances (Malte, 1986).

This study focuses on this internal hypoxia by analysing the physiological effects of aluminium exposure in acid water of high Ca^{2+} content in a hypoxia-tolerant species, the tench, and records the influences on blood O_2 transport, acid–base status and ionic composition.

MATERIALS AND METHODS

Experimental animals

Tench (*Tinca tinca*), 900–1300 g, were collected from lakes at Gisselfeld, Zealand, Denmark, during May 1985 and maintained in 500 l tanks, with running, filtered, normoxic (P_{O_2} > 135 mmHg; 1 mmHg = 133·3 Pa) tap water at 15 °C, for at least 2 weeks prior to experimentation. The photoperiod was controlled at a 12 h light: 12 h dark rhythm.

Experimental protocol

The fish were surgically fitted with chronic dorsal aortic catheters (Soivio, Nyholm & Westman, 1975), while under mild MS 222 anaesthesia, and allowed to recover from surgery for 48 h in individual aquaria with 60 l normoxic water at pH 8·4.

Exposure to aluminium-containing, acid water (acid–Al exposure) was carried out in a large aquarium containing 450 l of tap water at 15 °C, with measured electrolyte concentrations of [Ca^{2+}] = 3·5 mmol l^{-1}, [Na^+] = 1·2 mmol l^{-1}, [Cl^-] =

1 mmol l^{-1} and a calculated increase in [SO_4^{2-}], due to titration to pH 5 with H_2SO_4, of 2·75 mmol l^{-1}. The water pH was monitored with a combined pH electrode and matching meter (Portamess 654, Knick) and kept at pH 5 (range: 4·95–5·3) by addition of 0·5 mol l^{-1} H_2SO_4. Aluminium was added (at −18 h) as $Al_2(SO_4)_3 \cdot 18H_2O$ to a concentration of 2 mg l^{-1} Al. Steady bubbling of air prior to the introduction of fish and during the entire exposure period ensured normoxic ambient conditions ($P_{O_2} > 135$ mmHg).

Blood samples (0·7 ml) were obtained through the catheters in the normoxic control situation, whereupon one or two fish were quickly (in <2 s) transferred to the large acid aquarium. This was achieved without noticeable disturbance (i.e. struggling or other visual stress symptoms) to the fish (also reflected in unchanged [lactate$^-$] at 3 h, see Fig. 6). The time of transfer was defined as time zero. Further blood samples (0·7 ml) were drawn at 3 h and subsequently once per day (24 h, 48 h, etc.).

The chemistry of aluminium in aquatic solutions is complex (see Burrows, 1977) and different species of aluminium compounds can be assumed to occur in the experimental water (as is the case in nature, see Driscoll *et al.* 1980). In the present experiments, which simulate a pulse input of aluminium with subsequent ageing, a slow formation of polymeric aluminium hydroxide precipitates is expected. Such precipitation was indeed visible at later stages of the experiments.

Six tench were subjected to acid–Al exposure until death or until the end of day 6. Five additional tench was examined during acute exposure only. Two further specimens were subjected to acid water (pH 5) in the absence of aluminium. A final series of eight tench was used to investigate the effects on sodium concentrations.

Measurements

Arterial P_{O_2} and blood pH (pH_e) were measured with Radiometer (Copenhagen) electrodes thermostatted at 15 °C in a BMS 3 electrode assembly connected to PHM 73 and 71 blood gas monitors and the signals were displayed on REC 80 recorders.

Blood haemoglobin (Hb) concentration was determined as cyanmethaemoglobin, and haematocrit (Hct) was measured by centrifugation in glass capillaries.

Dorsal aortic oxygen content (C_{O_2}) was measured according to the method of Tucker (1967). The Hb-O_2 saturation (S_{O_2}) was calculated from the C_{O_2} (after correction for dissolved O_2, see Christoforides & Hedley-Whyte, 1969) by division by the theoretical O_2 capacity determined from the Hb concentration. This is justified by the close agreement in tench between the measured O_2 capacity and that calculated from Hb content (Jensen & Weber, 1982).

The total content of nucleoside triphosphates (NTP) was assessed enzymatically (Sigma Bulletin no. 366-UV) and apportioned between ATP and GTP using thin-layer chromatography (Johansen, Lykkeboe, Weber & Maloiy, 1976). The red cell concentrations were calculated from the corresponding Hct values.

Red cell pH (pH_i) was measured with a BMS 3 pH electrode after gas-tight separation of red cells and three freeze–thaw treatments in liquid nitrogen.

Plasma bicarbonate concentrations and P_{CO_2} values were calculated from measurements of true plasma total CO_2 content (Cameron, 1971) and pH_e, using the Henderson–Hasselbalch equation and the appropriate pK_1' and αCO_2 values (see Jensen, 1986*b*).

Plasma lactate concentrations were assayed using the Boehringer–Mannheim lactate dehydrogenase method. Plasma chloride concentrations were determined by coulometric titration (Radiometer CMT 10) and plasma potassium, calcium and sodium concentrations by atomic absorption spectrophotometry (Perkin–Elmer 2380). Plasma sodium was also measured using a flame photometer (Instrumentation Laboratory 243).

Presentation of the results

Of the six animals subjected to prolonged acid–Al exposure, two died between day 3 and day 4 and two died at the end of day 6. Major differences between dying fish and surviving fish were limited to the last sample obtained from the dying fish. The data, accordingly, are presented as means ± S.E.M., without inclusion of the last sample from non-surviving fish. N thus is 6 until and including day 2, whereas it is 4 until day 5. This procedure improves clarity in the data without distorting the general trends in the changes. The values for the last samples obtained from dying fish are presented separately as means ± S.E.M. ($N = 4$) at the mean time (i.e. 4·5 days) at which they were drawn. Means and ranges of values for the two fish exposed to pH 5 without aluminium are shown for comparison.

Statistical significance of differences between normoxic control values and values obtained during acid–Al exposure were evaluated using Student's t-test (each fish serving as its own control).

RESULTS

Blood respiratory properties

Exposing tench to moderately acid water (pH 5) containing aluminium resulted in a rapid and drastic decrease ($P < 0{\cdot}01$) in the dorsal aortic oxygen tension (Fig. 1). As arterial P_{CO_2} rose in parallel (Fig. 6), a condition of internal hypoxia–hypercapnia was established. The rapidity and magnitude of these changes were subsequently verified in five additional tench (the characteristic acute responses were, therefore, observed in a total of 11 fish). The arterial P_{O_2} was at a minimum (mean 6·1 mmHg) on day 1, then increased slightly (to 9 mmHg on day 4). In the absence of aluminium, the arterial P_{O_2} remained around control values (Fig. 1).

In fish exposed to aluminium-containing, acid water the arterial oxygen content (C_{O_2}) and Hb-O_2 saturation (S_{O_2}) decreased ($P < 0{\cdot}01$) in parallel with P_{O_2} (Fig. 1). Minimum values were observed on day 1 followed by partial recovery during continued acid–Al exposure. All dying fish showed C_{O_2} and S_{O_2} values which were significantly ($P < 0{\cdot}05$ and $P < 0{\cdot}01$, respectively) lower than day 1 values.

Hct and blood [Hb] were marginally elevated at 3 h (not statistically significant) but increased strongly on day 1 ($P < 0{\cdot}01$ and $P < 0{\cdot}05$, respectively) of acid–Al

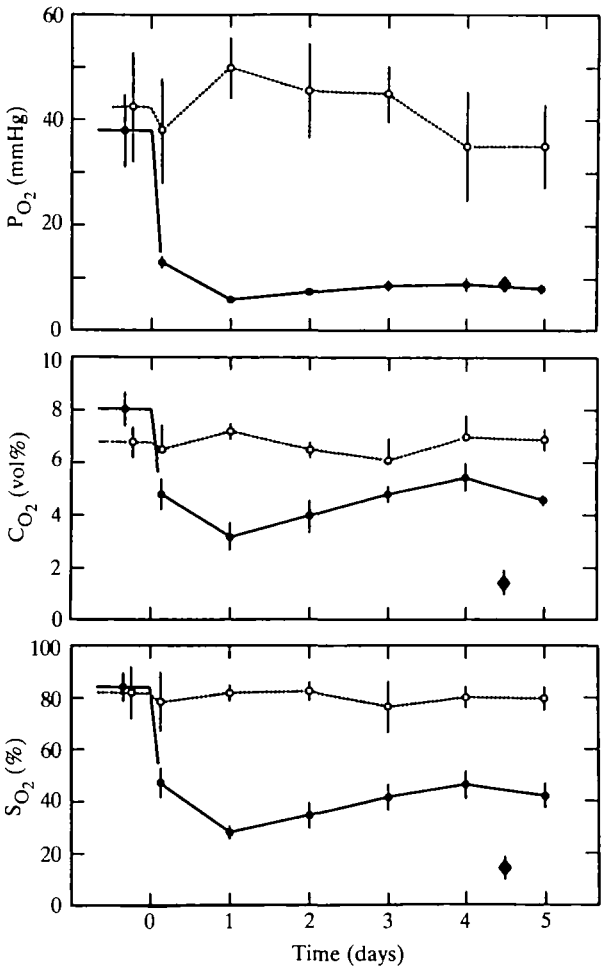

Fig. 1. Time-dependent changes of dorsal aortic oxygen tension (P_{O_2}), O_2 content (C_{O_2}, ml O_2 100 ml^{-1} blood) and Hb-O_2 saturation (S_{O_2}) in tench exposed to aluminium-containing, acid (pH 5) water (●), or acid (pH 5) water without aluminium (○). Values from the last blood sample obtained from fish that died during the acid–aluminium exposure are shown separately (◆) at the mean sampling time (i.e. 4·5 days). Acid–Al (or acid) exposure was started at time zero, after measuring the normoxic control values (which are plotted furthest to the left) at a water pH of 8·4.

exposure (Fig. 2). Hct subsequently fell slightly whereas blood [Hb] remained virtually constant. These changes are concomitant with a decrease in the red cell haemoglobin concentration, which was at a minimum on day 1 ($P < 0{\cdot}001$), then showed partial recovery towards control values (Fig. 2). The cellular [Hb] was low in dying fish. Upon exposure to acid water without Al, no major changes were observed in Hct or in [Hb].

In the absence of aluminium, acid exposure produced no change in the cellular NTP concentration or in the NTP/Hb ratio (Fig. 3). Acid–Al exposure, however, decreased both red cell [NTP] ($P < 0{\cdot}01$ on day 1 and day 2) and NTP/Hb ($P < 0{\cdot}05$

on day 2) (Fig. 3), the latter reflecting a decrease in the total cellular content of the nucleoside triphosphate cofactors. This decrease resulted from a selective reduction in GTP/Hb, while ATP/Hb remained constant (Fig. 3). ATP/Hb remained unchanged even in dying fish, which showed further decreases in the GTP/Hb ratio.

Blood acid–base and electrolyte status

Acid exposure in the absence of Al produced only a minor decrease in extracellular pH_e, which, however, was paralleled by a minor decrease in red cell pH_i. The pH_e decrease was larger during acid–Al exposure, attaining a minimum on day 1 ($P < 0{\cdot}02$) whereupon pH_e recovery was observed. Red cell values (pH_i), however, increased above normoxic control values until day 3 ($P < 0{\cdot}02$), then remained more or less constant (Fig. 4). In dying fish a large decrease in both pH_e and pH_i was observed.

Analysed in a pH_i–pH_e diagram (Fig. 5), the points from pure acid-exposed tench group around a regression line with $\Delta pH_i/\Delta pH_e \approx 0{\cdot}6$, which is in close agreement with the *in vitro* slope for oxygenated tench blood (Jensen & Weber, 1982). In tench

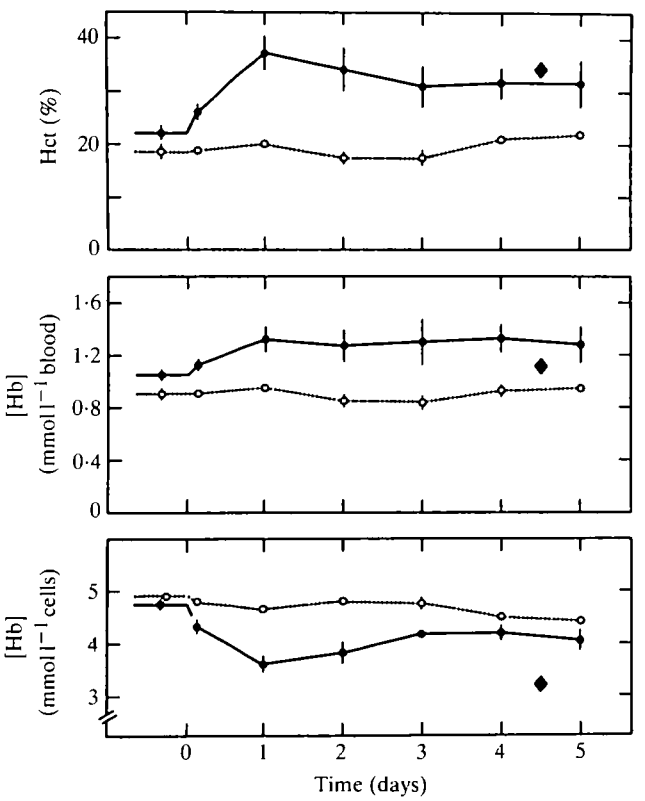

Fig. 2. Changes in haematocrit, and blood and red cell haemoglobin (Hb) concentrations in tench exposed to acid water in the presence or absence of aluminium. Symbols as in Fig. 1.

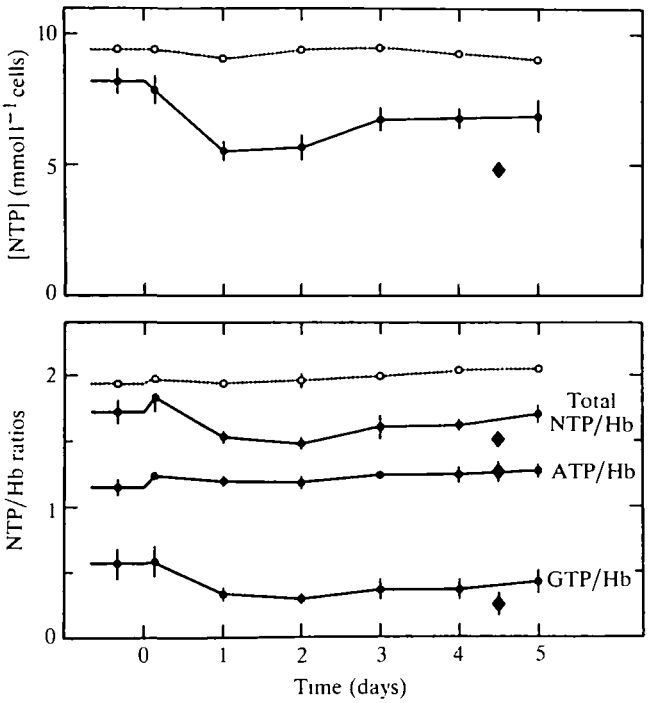

Fig. 3. Red cell nucleoside triphosphate (NTP) concentration and total NTP/Hb, ATP/Hb and GTP/Hb ratios in tench during exposure to acid water with or without aluminium. Symbols as in Fig. 1.

exposed to aluminium-containing, acid water a pronounced selective elevation of pH_i was seen (Fig. 5).

The arterial P_{CO_2} rose to a peak at 3 h ($P < 0{\cdot}001$) of exposure to acid–Al, then declined slightly, although it remained elevated compared to controls (Fig. 6). This rapid increase in P_{CO_2} did not occur in the absence of aluminium.

The plasma bicarbonate concentration changed only slightly upon pure acid exposure, whereas in the presence of aluminium it showed an initial increase ($P < 0{\cdot}05$), then stayed more or less unchanged until day 2 when a further increase ($P < 0{\cdot}02$) occurred (Fig. 6).

Plasma [lactate$^-$] remained low during acid exposure in the absence of Al. In acid–Al exposure [lactate$^-$] was unchanged at 3 h, then increased on day 1 and day 2 ($P < 0{\cdot}05$ and $P < 0{\cdot}02$, respectively), declined on day 3 and day 4, and rose again on day 5 (Fig. 6). The individual variation in [lactate$^-$] was, however, large. In dying fish high [lactate$^-$] and low [HCO_3^-] were observed (Fig. 6). The changes in extracellular acid–base status during acid–Al exposure are illustrated in a [HCO_3^-] *vs* pH_e diagram in Fig. 7 (see Discussion for interpretations).

Although plasma [Cl^-] was unaltered in acid exposure, a progressive decline ($P < 0{\cdot}02$) in [Cl^-] was observed during acid–Al exposure (Fig. 8). Plasma [K^+] and [Ca^{2+}], however, increased slightly during acid–Al exposure, reaching maximum values on day 2 ($P < 0{\cdot}02$ and not statistically significant, respectively; Fig. 8).

Sodium measurements initially proved unreliable due to malfunctioning of the atomic absorption determinations at the sensitivity (589 nm) necessitated by the small amounts of plasma available. To obtain information on the changes in $[Na^+]$, eight additional tench were divided into two groups and their blood was sampled after 3 days exposure to either acid (pH 5) or acid–Al water. At this stage the blood pH was identical in the two groups ($7{\cdot}91 \pm 0{\cdot}04$ *vs* $7{\cdot}93 \pm 0{\cdot}03$) as predicted by Fig. 4, while the plasma sodium concentration was $130{\cdot}2 \pm 3{\cdot}1\ mmol\ l^{-1}$ in the acid group (which is similar to normal normoxic values, unpublished results) and $125{\cdot}3 \pm 5{\cdot}2\ mmol\ l^{-1}$ in the acid–Al group. The 3·8% reduction seen in the acid–Al group was not significant, but identical to the percentage difference (also not significant) observed in plasma osmolality ($270{\cdot}8 \pm 4{\cdot}6\ mosmol\ l^{-1}$ *vs* $260{\cdot}0 \pm 7{\cdot}9\ mosmol\ l^{-1}$). Although the difference in plasma $[Cl^-]$ was smaller than predicted from Fig. 8, it was larger (6·3%; from 112 ± 3 to $105 \pm 3\ mmol\ l^{-1}$) than that in $[Na^+]$ and significant ($P < 0{\cdot}02$).

DISCUSSION

In the absence of aluminium a minor arterial acidosis (Fig. 4) appeared to be the only physiological disturbance in acid-exposed (pH 5) tench in water of high $[Ca^{2+}]$. The general lack of serious physiological effects corroborates findings for the closely related carp subjected to comparable environmental conditions (Ultsch *et al.* 1981).

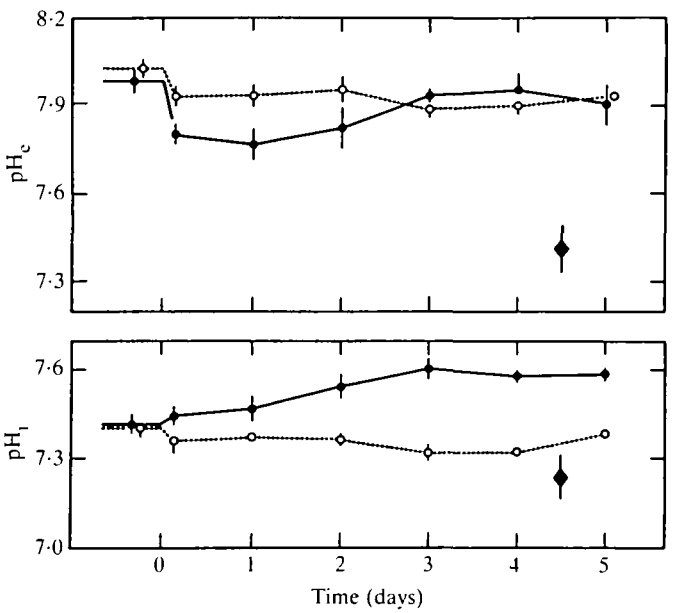

Fig. 4. Time-dependent changes in extracellular blood pH (pH_e) and red cell pH (pH_i) in tench exposed to aluminium-containing, acid water and in acid-exposed tench. Symbols as in Fig. 1.

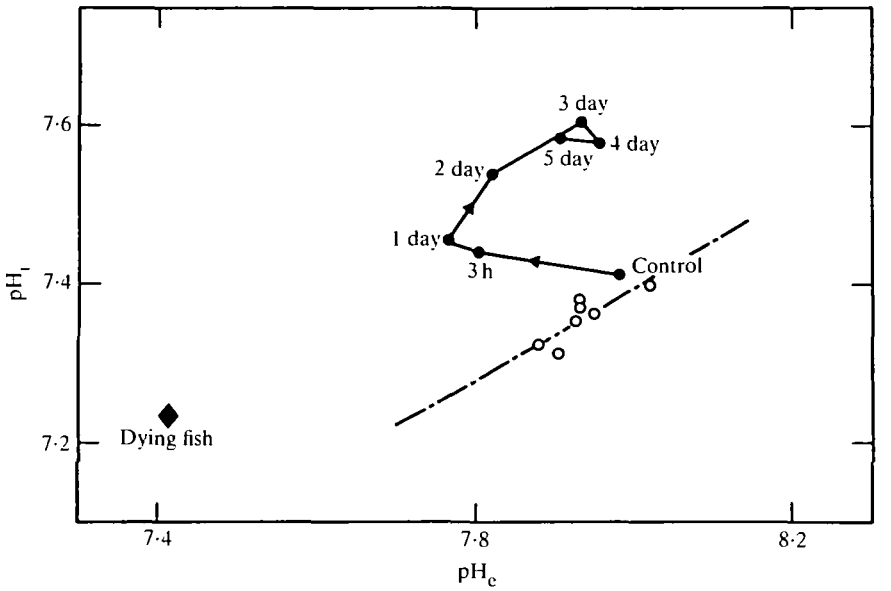

Fig. 5. Red cell pH (pH_i) *vs* plasma pH (pH_e) diagram, depicting the *in vivo* pH_i–pH_e relationship (mean values) in tench exposed to aluminium-containing, acid water (closed symbol at the time indicated for each point). The regression line through mean values for acid-exposed tench (open symbols) is shown. The mean pH_i–pH_e value for dying fish is shown separately.

The additional presence of aluminium, however, caused major perturbations, which became dominated by internal hypoxia–hypercapnia.

Internal hypoxia–hypercapnia

The simultaneous rapid decrease in arterial P_{O_2} (Fig. 1) and increase in P_{CO_2} (Fig. 6) in the face of a high external P_{O_2}, and visually observed increase in ventilatory stroke volume, suggest a rapid decrease in the gas exchange efficiency across the gills during acid–Al exposure. Large P_{O_2} decreases and P_{CO_2} increases also characterize exposure to very low and acute lethal pH values (Ultsch *et al.* 1981; Hōbe *et al.* 1984) and acute, lethal exposure to zinc (Spry & Wood, 1984). These changes have been ascribed to increased mucus secretion and increased water–blood diffusion distances caused by detachment of the secondary lamellar epithelium (see above references and Daye & Garside, 1976; Tuurala, 1983). Although a pH decrease to 5 in itself may have caused only a marginal response (Daye & Garside, 1976), the additional presence of aluminium might have strengthened it. Mucus clogging of the gills was, however, not observed in the early phases of the experiments. Also, in rainbow trout exposed in this laboratory to the same aluminium concentration and water quality, a simultaneous large decrease in P_{O_2} and increase in P_{CO_2} was observed only in the last blood samples taken before death, after the fish had been exposed for 30–100 h (Malte, 1986). The rapidity of the response in tench, together with the subsequent extended survival, thus makes it doubtful whether the changes can be ascribed to a rapid toxic action of aluminium on the gills, although

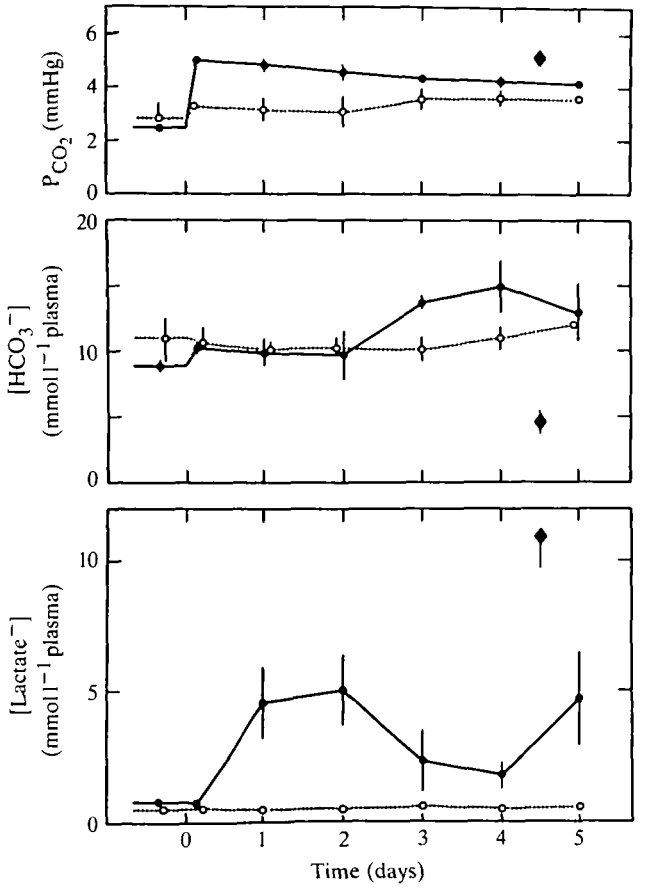

Fig. 6. Time-dependent changes in carbon dioxide tension (P_{CO_2}) and plasma concentrations of bicarbonate and lactate in acid-exposed tench and tench exposed to aluminium-containing, acid water. Symbols as in Fig. 1.

this cannot be excluded. Perhaps aluminium represents a noxious stimulus which causes a redistribution of blood flow in the gills, increasing blood flow through the basal channels of the secondary lamellae, and thus the diffusion distance. Alternatively, the high O_2-affinity blood of tench may be more sensitive to small changes in diffusion conductance across the gills than the low O_2-affinity blood of trout.

In dying fish, however, impairment of gill function was clearly visible, which is in accordance with the almost completely deoxygenated state of their arterial blood (Fig. 1). Macroscopic examination of these gills indicated mucus clogging, as reported for brown trout (Muniz & Leivestad, 1980), but also showed aluminium hydroxide precipitation on the gills. This is supported by Baker & Schofield's (1982) study, which showed that in the early life stages of brook trout and white suckers aluminium is most toxic at pH 5·2–5·4 at concentrations where it precipitates out of solution as $Al(OH)_3$, as indeed was observed in later stages of the present experiments.

Blood oxygen transport

The decrease in arterial P_{O_2} drastically decreases both arterial Hb-O_2 saturation and O_2 content (Fig. 1), although the latter was partially countered by an increase in maximal arterial O_2 capacity (i.e. increase in blood [Hb], Fig. 2). Thus the amount of oxygen transported to tissues per unit volume of blood fell sharply. This can only be compensated by an increase in cardiac output and/or a decrease in routine O_2 consumption. A decrease in routine O_2 consumption indeed rapidly follows subjection of tench to environmental hypoxia–hypercapnia (Jensen & Weber, 1985*a*). Rainbow trout, however, respond to acid–Al exposure by increasing standard O_2 uptake (Malte, 1986). Full compensation is apparently not achieved in tench, since the increase in plasma [lactate$^-$] (Fig. 6) reflects an increase in anaerobic energy production. Lactate concentrations, however, decline in parallel with the improvement of blood O_2 transport, as shown by increasing S_{O_2} and C_{O_2} values after day 1.

The recovery in arterial C_{O_2} and S_{O_2} results partly from the rise in P_{O_2}. Although this rise is small (from 6 to 9 mmHg), it improves S_{O_2} by 10% or more, as the P_{O_2} change coincides with the steep part of the O_2 equilibrium curves of tench blood (Jensen & Weber, 1982). In addition, the pH recovery (Fig. 4) increases Hb-O_2 affinity, and thus C_{O_2} and S_{O_2}, through the Bohr shift. In dying fish the Bohr (and

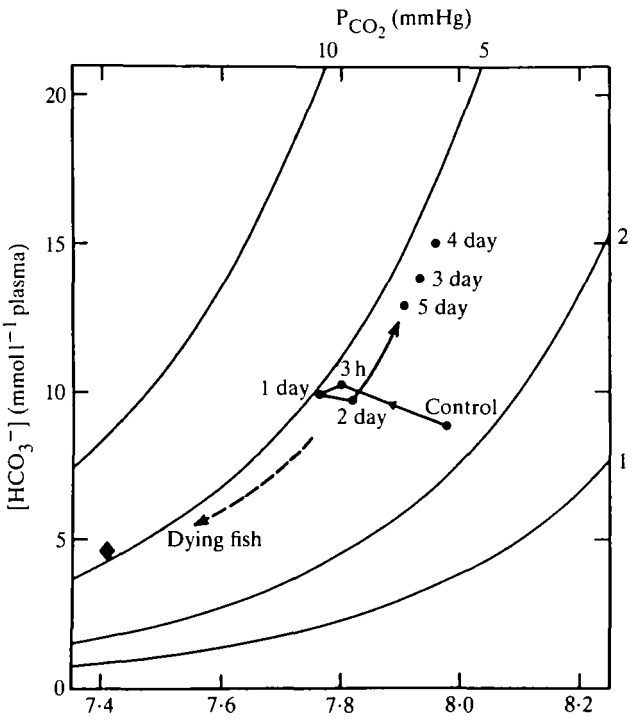

Fig. 7. Plasma $[HCO_3^-]$ *vs* plasma pH (pH_e) diagram including carbon dioxide tension (P_{CO_2}) isopleths and the *in vivo* changes in extracellular acid–base status in tench exposed to acid water containing aluminium.

Root) shifts exert the opposite effect, as obstruction of O_2 diffusion at the gills results in a severe lactacidosis (Figs 4, 6) which renders the blood almost completely deoxygenated (Fig. 1).

Red cell cofactors

The condition of internal hypoxia–hypercapnia triggers a reduction in the erythrocytic nucleoside triphosphate concentration and total NTP content. The decrease in NTP/Hb is manifested through a selective reduction in GTP/Hb, with ATP/Hb remaining constant (Fig. 3). This is exactly the same response as observed in tench subjected to environmental hypoxia–hypercapnia (Jensen & Weber, 1985*a*). As GTP has a stronger potential than ATP for improving Hb-O_2 affinity (see review by Weber, 1982), this change most probably contributes by favouring high arterial O_2 concentration.

Haematological changes

A sharp increase in Hct and blood [Hb] and a decrease in red cell [Hb] was a consistent response in tench exposed to aluminium-containing, acid water (Fig. 2), as it is in acid-exposed trout (Milligan & Wood, 1982). In trout (at pH 4·2 in hard

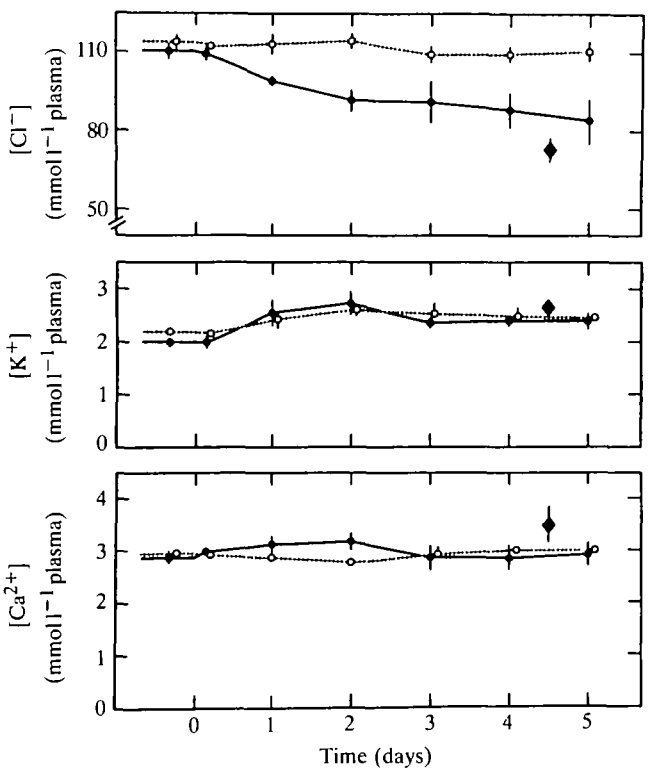

Fig. 8. Time-dependent changes of $[Cl^-]$, $[K^+]$ and $[Ca^{2+}]$ in dorsal aortic plasma of tench exposed to acid water with and without aluminium. Symbols as in Fig. 1.

water) these changes appear to be due to mobilization of Hb-poor red cells from the spleen, water shifts from extra- to intracellular compartments (due to plasma ion loss) and red cell swelling (see Milligan & Wood, 1982). These same mechanisms may apply to tench exposed to aluminium-containing, acid water. The decline in $[Cl^-]$ in tench, however, may partly be a consequence of HCO_3^- accumulation (see below), so that the acid-induced ion loss is smaller than in trout (in which both $[HCO_3^-]$ and $[Cl^-]$ decline; McDonald & Wood, 1981). Also, the contribution from red cell mobilization to the observed increases in Hct and [Hb] may be smaller in tench as judged from the haematological changes following environmental hypoxia–hypercapnia, exercise and adrenaline infusion (Jensen & Weber, 1985*a*; Jensen, 1986*b*). The present haematological changes resemble those observed during exposure to *environmental* hypoxia–hypercapnia both qualitatively and quantitatively, suggesting that internal hypoxia–hypercapnia exerts the dominating response, while contributions from other events (i.e. acid-water-induced ion loss, etc.) are small. It should also be noted that part of the red cell swelling (red cell [Hb] decrease) observed here can be explained by increases in the Donnan distribution ratios of diffusible ions across the red cell membrane with the decrease in S_{O_2}, NTP/Hb and pH_e (Jensen, 1986*a*, and unpublished data).

Extracellular acid–base status

The small extracellular acidosis in tench in acid water (Fig. 4) is probably the result of a net influx of H^+ (or loss of base) at the gills, as observed in trout (McDonald & Wood, 1981), carp (Ultsch *et al.* 1981) and white suckers (Hōbe *et al.* 1984), and this correlates with the increased H^+ gradient across the gills and the high H^+ permeability resulting from transepithelial potential changes (McWilliams & Potts, 1978). The larger arterial acidosis in tench exposed to aluminium-containing, acid water (Fig. 4) is related to the increase in P_{CO_2} (Fig. 6). The initial $[HCO_3^-]$–pH change (Fig. 7) occurs with an apparent *in vivo* non-bicarbonate buffer value, β, of 8 mmol l^{-1} pH $unit^{-1}$, which is in the range observed *in vitro* at constant S_{O_2} (F. B. Jensen, unpublished results). The initial acidosis may not, however, be entirely respiratory, as the parallel *in vivo* reduction in S_{O_2} at 3 h will elevate plasma $[HCO_3^-]$ through the Haldane effect (i.e. a metabolic alkalosis component). In the present case this appears to be counteracted by H^+ entry at the gills (i.e. a metabolic acidosis component), thereby producing an apparent *in vivo* β value which is close to that *in vitro*.

The rise in arterial P_{CO_2} stimulates HCO_3^- accumulation, as under environmental hypoxia–hypercapnia (Jensen & Weber, 1985*b*). This is shown by increased plasma $[HCO_3^-]$ values after day 2, after an apparent temporary balance between accumulated HCO_3^- and HCO_3^- washed out by metabolic protons from the lactic acid influx to the blood (see Fig. 6). Thus the $[HCO_3^-]$–pH values for day 2 to day 5 lie on the $P_{CO_2} = 4{\cdot}3$ mmHg isopleth (Fig. 7), reflecting the pH_e recovery. The HCO_3^- accumulation improves the buffering capacity for additional metabolic protons, and in this sense the initial development of internal hypoxia–hypercapnia is beneficial and may contribute to extend survival. Indeed, in acid-exposed rainbow

trout, the presence of a mild environmental (and thus also internal) hypercapnia improves tolerance to acid water and almost restores control pH (Neville, 1979). Rainbow trout exposed to aluminium-containing, acid water, however, do not respond with a rapid internal hypoxia–hypercapnia as tench do, and pH_e recovery is not observed (Malte, 1986). In severely affected acid–Al tench (i.e. those dying from strongly impeded gill function) internal anaerobic lactic acid production eventually reaches intolerable levels (Fig. 6) accompanied by declines in $[HCO_3^-]$ and pH_e, mainly along the P_{CO_2} 4·3 mmHg isopleth, although there is a contribution to the acidosis from a further elevation of P_{CO_2} (see Fig. 7).

Electrolyte changes

Acid exposure in salmonids results in large net losses of Na^+ and Cl^- across the gills, due to an inhibition of the active ion uptake and an increase in the diffusive ion efflux, these effects being greatest in water of low $[Ca^{2+}]$ (e.g. see McDonald, 1983). In aluminium-containing water at pH 5 similar responses are apparent when water $[Ca^{2+}]$ is low (Muniz & Leivestad, 1980), whereas in water with high $[Ca^{2+}]$ neither plasma $[Na^+]$ nor $[Cl^-]$ changes (Malte, 1986). In tench (at high water $[Ca^{2+}]$) plasma $[Na^+]$ appears to decrease only marginally, whereas plasma $[Cl^-]$ decreases significantly (Fig. 8) upon exposure to aluminium-containing, acid water. This decline in $[Cl^-]$ may result only partly from a disturbance of gill ion regulatory function, as a significant contribution could arise from the inverse relationship between plasma $[HCO_3^-]$ and plasma $[Cl^-]$ in hypoxic–hypercapnic tench (Jensen & Weber, 1985*b*). Further contributions might come from the increase in $[lactate^-]$ (Holeton, Neumann & Heisler, 1983). Also, it is doubtful whether the $[Cl^-]$ reaches critical levels during 96 h of exposure to aluminium-containing, acid water, as the decrease from control to day-4 values is only slightly above that during 48 h of environmental hypoxia–hypercapnia (Jensen & Weber, 1985*b*). Precise judgement of eventual ionoregulatory disturbances and their biological importance requires assessment of ion fluxes between fish and environment and the changes in intracellular ion pools. If, however, the permeability of the gills to ions is increased (McDonald, 1983) it is possible that blood shunting, limiting branchial ion loss, causes the internal hypoxia–hypercapnia.

The relative constancy of plasma $[Ca^{2+}]$ (Fig. 8) resembles that in acid-exposed trout (McDonald & Wood, 1981; Neville, 1979), although the small (not statistically significant) increase (approx. 10 % on day 2) in acid–Al tench is indicative of a water shift to tissues. The larger increase in plasma $[K^+]$ (approx. 36 % on day 2, Fig. 8) can be ascribed to K^+ efflux from the muscles (McDonald & Wood, 1981).

Red cell acid–base status

Red cell pH in acid–Al tench increases despite the decrease in extracellular pH (Fig. 4). This opposite response in acid–base status of the two blood compartments is the same as that observed in tench exposed to environmental hypoxia–hypercapnia (Jensen & Weber, 1985*b*). Recent results show that, at constant extracellular pH, red cell pH in tench increases markedly with a decrease in S_{O_2} values; this effect was also

found in acid–Al tench and can be accounted for by the Haldane effect and the buffer capacity of the haemoglobin (see Jensen, 1986*a*). This suggests an explanation for the relationships between pH_i and pH_e (Fig. 5). In acid-exposed tench, in which S_{O_2} is high and constant (Fig. 1), pH_i decreases along with pH_e with the same $\Delta pH_i/\Delta pH_e$ value as in oxygenated blood. In acid–Al tench, however, the effect of decreasing S_{O_2} (Fig. 1) upon pH_i overrides that of the decrease in pH_e, so that the pH_i and pH_e points lie above the normoxic relationship (Fig. 5). Following day 1, pH_i increases further, primarily due to the recovery in pH_e values. The concomitant recovery in S_{O_2} (Fig. 1) is not reflected in pH_i, since the major change of pH_i occurs in the range 100–50 % S_{O_2} (Jensen, 1986*a*). Overlapping effects might include contributions to pH_i from the NTP decrease (Wood & Johansen, 1973) and β-adrenergic swelling (Nikinmaa, 1982), although the latter, at least in normoxia, is absent in tench (Jensen, 1986*b*).

Tench respond to aluminium in acid, hard water with a rapid *internal* hypoxia–hypercapnia, a condition which tench tolerate in their natural habitats, where *environmental* hypoxia–hypercapnia frequently occurs. The acid–Al-induced internal hypoxia–hypercapnia appears to evoke an adaptive increase in bicarbonate buffering capacity towards protons. Considerable compensation is observed in perturbed physiological parameters, and survival appears strongly improved compared to trout. The physiological changes in tench exposed to aluminium-containing, acid water appear to be strictly analogous to those induced by environmental hypoxia–hypercapnia, the physiological conditions deteriorating only shortly prior to death. Ultimately death appears to be caused by $Al(OH)_3$ precipitation and mucus clogging on gills, leading to a severe hypoxic syndrome with insufficient O_2 delivery to the tissues and a pulse influx of lactic acid from the tissues to the blood, which exaggerates the hypoxic syndrome through the Bohr–Root shift of the blood.

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