Effects of Low pH and Aluminum on Ventilation in the Brook Trout (*Salvelinus fontinalis*)

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Brook trout (*Salvelinus fontinalis*) (acclimated to pH = 6.5, Ca^{2+} = 400 μequiv·L^{-1}), when exposed to acid (pH = 4.8, Ca^{2+} = 400 μequiv·L^{-1}) and Al (333 μg·L^{-1}), responded with a twofold increase in ventilation volume within the first 4 h of the challenge period (100 h). Increased ventilation stroke volume accounted for most of the change in ventilatory response; rate increased slightly. Although ventilation volume returned to prechallenge values by 6 h, coughing (flow reversal) and increased mucus production at the gills were notable throughout the challenge period. There were no significant changes in oxygen consumption or Pa_{O_2}, but hemoglobin oxygen content (micromoles per gram of hemoglobin) decreased by 20%. Arterial pH decreased as a result of both respiratory and metabolic disturbances. Exposure to acid (pH = 4.8, Ca^{2+} = 400 μequiv·L^{-1}) in the absence of Al resulted in similar initial changes in ventilation and blood acid–base status; however, ventilation remained elevated above the prechallenge values throughout the experiment (24 h). The transient increase and subsequent return of ventilation to prechallenge levels in the acid/Al-exposed fish suggests that Al interfered with the mechanism controlling the ventilatory response.

Des ombles de fontaine (*Salvelinus fontinalis*) acclimatés (pH = 6,5; Ca^{+2} = 400 μequiv/L) et exposés à un milieu acide (pH = 4,8; Ca^{+2} = 400 μequiv/L) et à de l'Al (333 μg/L) ont réagi par une augmentation double du volume de ventilation au cours des quatre premières heures de la période de provocation (100 h). Une augmentation du volume sistolique a expliqué la plus grande partie de la variation de la réaction ventilatoire, soit une légère augmentation du taux. Quoique le volume de ventilation soit revenu à sa valeur pré-provocation après 6 h, le renversement du débit et l'augmentation de la production de mucus au niveau des ouïes étaient évidents pendant toute la période de provocation. Aucune variation significative de la consommation d'oxygène ou du Pa_{O_2} n'a été observée, mais la teneur en oxygène de l'hémoglobine (micromoles par gramme d'hémoglobine) a diminué de 20 %. Des perturbations respiratoires et métaboliques ont entraîné une baisse du pH artériel. L'exposition à un milieu acide (pH = 4,8, Ca^{+2} = 400 μequiv/L) en absence d'Al a entraîné des variations initiales semblables dans le cas de la ventilation et de l'équilibre acidobasique du sang; toutefois, le taux de ventilation est demeuré élevé et supérieur aux valeurs pré-provocation pendant toute la durée de l'expérience (24 h). L'augmentation momentanée et le retour ultérieur du taux de ventilation au niveau pré-provocation chez les poissons exposés à un milieu acide et à l'Al portent à croire que l'Al perturbe le mécanisme qui contrôle la réaction ventilatoire.

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Trout hyperventilate in response to the combined effects of acid and Al (Muniz and Leivestad 1980; Rosseland 1980; Neville 1985). This observation was made by measuring opercular rate in unrestrained brown trout (*Salmo trutta*) and brook trout (*Salvelinus fontinalis*) (Muniz and Leivestad 1980; Rosseland 1980) or cough rate and mouth gape in juvenile rainbow trout (*Salmo gairdneri*) (Neville 1985). Ventilation volume was not measured in any of these studies. The response depends on the pH and Ca^{2+} concentration of the water. Below pH 4.5 and at Ca^{2+} concentrations less than 250 μequiv·L^{-1}, the combined effect of acid and Al was most severe (Rosseland 1980; Neville 1985). Mucus clogging of the gills and reduction of oxygen extraction from the water were also reported.

Blood data in accord with these findings have been reported by Wood et al. (1988b, 1988c) for brook trout exposed to a variety of acid, Ca^{2+}, and Al conditions. Severe respiratory and ionoregulatory disturbances were attributed to interaction of Al with the branchial epithelium. Gas exchange appeared to be greatly reduced, resulting in hypoxemia and hypercapnia in some treatment groups.

The effects on ventilation of acidification of water in the absence of Al are less clear. Dively et al. (1977) reported increases in mouth gaping and opercular expansion within 15–30 min and increased ventilation rate for the next 2–3 d of acid exposure. Neville (1985) reported similar ventilatory effects within the first hour of exposure of juvenile rainbow trout to

soft water at pH 4.5. However, Neville (1979) suggested that the initial increase in ventilation seen with acidification of water can be attributed to hypercapnia rather than an increase in plasma proton concentration. Janssen and Randall (1975) reported that a reduction in water pH to 5.0 caused a slow gradual increase in ventilation in rainbow trout, probably related to a slowly developing hypoxemia due to gill mucification. Wood et al. (1988b, 1988c), on the other hand, reported no significant disturbances in blood respiratory, acid–base, or ionoregulatory parameters in brook trout exposed to acid (pH 4.8) in the absence of Al.

The purpose of this study was to investigate the effects of acid versus acid plus Al on ventilation, oxygen uptake, blood respiratory gases, and acid–base status of brook trout acclimated to soft water. The effects of Al in the absence of acid could not be tested because Al is virtually insoluble in water at neutral pH. A Ca^{2+} level (400 μequiv·L^{-1}) towards the upper end of the softwater range was chosen because Wood et al. (1988b) found that higher Ca^{2+} exacerbated problems in respiratory blood gas regulation in Ontario brook trout under combined acid plus Al exposure. In light of the observations of Wood et al. (1988b, 1988c), it was hypothesized that the combined exposure to acid plus Al would greatly increase ventilation, both ventilation rate and stroke volume, and reduce the oxygen content of the blood, ultimately resulting in respiratory failure. The purpose of the acid-alone group was to clarify whether the ventilatory effects were attributable to the acid or the Al component of the challenge. Based on the results of Janssen and Randall (1975) and Wood et al. (1988b, 1988c), it was expected that there would be very little effect of acid on ventilation in the absence of Al. There have been no previous direct measurements of ventilation volume in fish subjected to acid/Al challenge.

Materials and Methods

Animal and Experimental Conditions

Experiments were conducted at the Fish Physiology and Toxicology Laboratory, Laramie, Wyoming (altitude = 2200 m). Brook trout weighing 150–350 g were acclimated for 10 wk to artificial soft water (pH = 6.5, Ca^{2+} = 400 μequiv·L^{-1}) at 11 ± 1°C. Details regarding the generation of the artificial soft water are given in Wood et al. (1988a). In brief, the water was prepared from hard well water by sediment filtration, NaCl softening, reverse osmosis, and separate bed deionization (Continental Water Systems, Denver, Colorado). Following adjustment to pH 6.5 with KOH and the addition of Na^+ (40 μequiv·L^{-1}) and Ca^{2+} (25 μequiv·L^{-1}) as chlorides, the water was thoroughly mixed and delivered to head tanks. From there it was distributed to 340-L fiberglass acclimation tanks at a rate of 1.9 L·min^{-1}. The Ca^{2+} concentration was adjusted upward to 400 μequiv·L^{-1} by addition of $CaCl_2$ via a marriott bottle to the acclimation tanks. Mixing was provided by aeration. Water pH was measured daily and other parameters (Ca^{2+}, Na^+, and Cl^-) weekly.

Fish were fed 1% body weight floating trout chow per day (Purina No. 5106) and solid wastes were removed by siphon daily. Photoperiod was adjusted biweekly to follow the natural cycle for Laramie, Wyoming.

Two groups of test animals were used: those challenged with acid and Al and those with acid in the absence of Al. A paired design was used with the prechallenge values for each fish acting as its own control.

The challenge consisted of either pH = 4.8, Al = 333 μg·L^{-1} for up to 100 h (series I) or pH = 4.8, Al = 0 μg·L^{-1} for 24 h (series II). Series II was conducted following completion of series I and was designed to test whether the initial increases in ventilation seen in series I were due to Al or simply the result of acidification of the water. Al (as $AlCl_3$) was added via marriott bottles to continuous-flow serial diluters and then to individual Plexiglas ventilation chambers (see below). The water pH was adjusted to 4.8 at the head tank by addition of H_2SO_4 using a Leeds and Northrup pH controller (model 7083).

Following the method outlined in Wood et al. (1988b), fish were anesthetized with MS-222 (ethyl *m*-aminobenzoate, 50 mg·L^{-1}; Sigma Chemical Co., St. Louis, Missouri). The water containing the anesthetic was adjusted to pH = 6.5 using 1 N NaOH and maintained at 11 ± 1°C using a glass cooling coil. Under anesthesia, fish were equipped with either caudal artery or vein catheters. Differentiation of successful arterial as opposed to venous catheterization was based upon observations of the blood pressure in the catheter following recovery from surgery. In addition, an oral membrane (to allow separation of inspired and expired water) was sutured around the mouth, as described by Davis and Cameron (1970). Each fish was placed in its own ventilation box which was supplied with acclimation water (pH = 6.5, Ca^{2+} = 400 μequiv·L^{-1}) at a flow rate of 0.5 L·min^{-1}. Except for a hole in the top of the box, strategically placed to allow observation of opercular movements, the boxes were covered in black plastic to reduce disturbance.

The box was identical to that illustrated by Davis and Cameron 1970). In brief, the principle of the measurement is that the oral membrane separates the inspired from the expired water, the hydrostatic levels of which are maintained at identical values by constant level overflows. The inspired chamber is served with an excess water inflow. Timed collection of water passing out the overflow of the expired chamber represents direct measurement of the fish's ventilation volume. A Plexiglas tube placed around the fish body provides light restraint and serves to thoroughly mix the expired water. A length of P.E. 160 was carefully positioned inside the restraining tube posterior to the opercula to sample the expired water for P_{O_2} determination. The water sample was obtained by siphoning (~0.5 mL·min^{-1}) into a 1-mL syringe with the plunger removed. The water was allowed to overflow the syringe by two to three volumes before the plunger was inserted and the syringe removed.

Following a 48-h recovery period, prechallenge ventilation rate (f_R, number per minute) was determined by observation of opercular movements, and ventilation volume ($\dot{V}_w$, millilitres per kilogram per minute) was measured by timed collection of water draining from the expired chamber of the ventilation box. Stroke volume (V_s, millilitres per kilogram per stroke) was calculated from ventilation volume and ventilation rate. Oxygen consumption (micromoles per kilogram per minute) was determined by the Fick method, using the difference in P_{O_2} between the water of the inspired chamber and mixed expired water from just behind the operculae, the ventilation volume, and an assumed oxygen solubility of 2.23 μmol·L^{-1}·$Torr^{-1}$ at 10°C (Dejours 1975).

After measuring the ventilatory parameters while in the acclimation water, an initial blood sample was drawn from the catheter using an heparinized, ice-cold, gas-tight Hamilton syringe. The blood sample (400 μL) was replaced by reinfusing the blood recovered from the P_{O_2} electrode (~100 μL) and

nonheparinized Cortland's saline (Wolf 1963). The blood was analyzed for arterial or venous O_2 tension (Pa_{O_2} or Pv_{O_2}), pH (pHa or pHv), total O_2 in whole blood (Ca_{O_2} or Cv_{O_2}), total CO_2 in true plasma (C_{CO_2}), hematocrit (Hct), and hemoglobin concentration ([Hb]).

A Radiometer G297/G2 electrode was used for the determination of pHa or pHv, and oxygen tension was measured with a Radiometer E5046 P_{O_2} electrode. Both electrodes were maintained at 11°C. C_{CO_2} was determined with a Corning 960 CO_2 analyzer. The true plasma was obtained from sealed, heparinized capillary tubes following centrifugation of whole blood at 5000 × *g* for 5 min. After Hct was read, the tube was broken at the plasma red cell interface and the plasma withdrawn with a Hamilton syringe for transfer to the Corning CO_2 analyzer.

Arterial CO_2 tension (Pa_{CO_2}) was calculated from the Henderson–Hasselbalch equation, using the $\alpha \cdot CO_2$ and pK values for trout at 11°C (Boutilier et al. 1984). True plasma bicarbonate concentration ($[HCO_3^-]$) was calculated using the formula

$$[HCO_3^-] = C_{CO_2} - (\alpha \cdot CO_2 \times Pa_{CO_2})$$

where C_{CO_2} is the total CO_2 content and ($\alpha \cdot CO_2 \times Pa_{CO_2}$) represents the dissolved fraction. In the instances where P_{CO_2} was measured, the method outlined by Boutilier et al. (1978) was employed using a Radiometer E5036 P_{CO_2} electrode.

[Hb] was determined colorimetrically using the cyanmethemoglobin method (Sigma kit No. 525). Mean cell Hb concentration (MCHC) was calculated as the [Hb]/Hct ratio (i.e. grams Hb per millilitre of red blood cells).

The oxygen content of whole blood was determined using a Lex-O_2-Con oxygen analyzer (Lexington Instruments Corp.). After conversion of the oxygen content from volume percent to millimoles per litre, the blood oxygen content was calculated by subtracting away the dissolved component, using the solubility coefficient of oxygen in human plasma (Boutilier et al. 1984). To express the content in terms of [Hb] (micromoles oxygen per gram of Hb), the oxygen content (micromoles per litre) was divided by [Hb] (grams per litre).

Following the prechallenge sampling period, in the series I experiments, acid/Al water was piped into the anterior portion of the ventilation chamber. Ventilation was measured 30 min into the challenge and then again immediately prior to blood sampling at 1, 2, 4, 8 (or 9), 24, 48, 72, and 96 h of exposure, if death did not occur earlier. In series II, the fish were challenged with acid alone and sampled in a similar manner; however, the challenge was terminated at the end of 24 h of exposure and the fish were returned to the acclimation conditions.

Treatment of Data

As discussed by Wood et al. (1988b, 1988c), it is difficult to accurately express group means when fish die at different times during the challenge. Therefore, in the case of the acid/Al group, the responses only during the first 20 h of the challenge have been portrayed as means ±1 SEM. For those parameters where there were significant effects, the individual data were plotted. However, for all parameters, the mean ±1 SEM of the prechallenge and final values are summarized, where the final values represent either the end of the challenge period or the last measurement prior to death. Since there were no mortalities in the acid-alone group, the mean values throughout the 24-h challenge period are plotted. In both groups a paired design was used; therefore, a Student's two-tailed *t*-test was employed for comparison between the prechallenge and intervening or final values. In all cases, statistical significance implied $p \leq 0.05$.

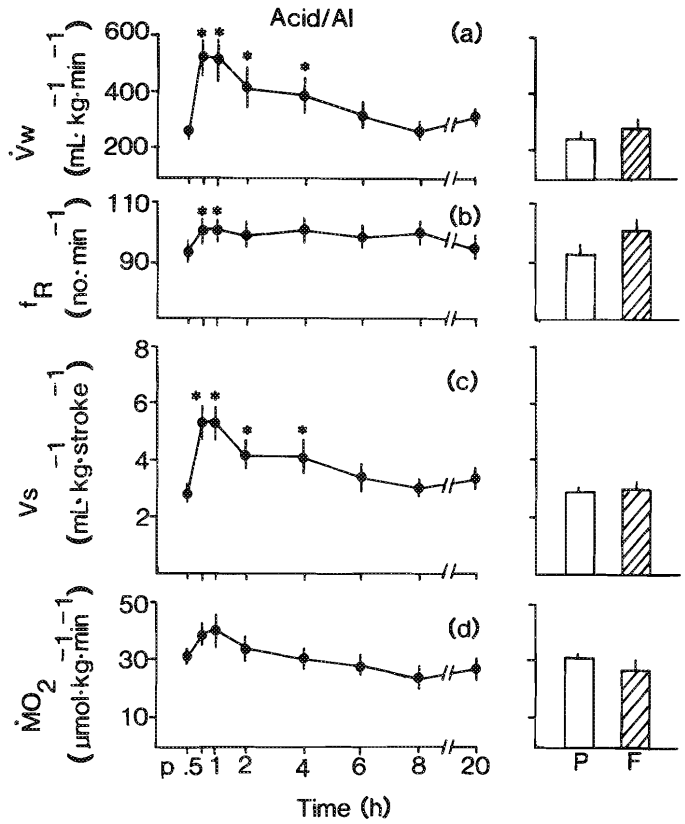

FIG. 1. Respiratory responses in brook trout challenged with acid (pH 4.8) and Al (333 μg·L^{-1}) in flowing water at 11°C following acclimation to pH 6.5 (Ca^{2+} = 400 μequiv·L^{-1} in both acclimation and challenge water). (a) Ventilation volume, $\dot{V}_w$; (b) ventilation rate, f_R; (c) ventilation stroke volume, V_s; (d) oxygen consumption, $\dot{M}_{O_2}$. The prechallenge values are indicated at "P" and by open bars. The final values (F, cross-hatched bars) were taken at 100 h or just prior to death. All points are means ±1 SEM (n = 11). Statistical significance ($p \leq 0.05$) between the prechallenge and challenge values is indicated by an asterisk.

Results

Of 11 fish used in series I (acid/Al challenge), seven survived 20 h of acid/Al challenge and five were alive at 50 h. Only two survived past 60 h; of these, one died at 74 h whereas the other remained alive at the termination of the experiment at 100 h. This pattern of death and survival time was similar to that reported by Wood et al. (1987c) for identically challenged Wyoming brook trout which had been similarly cannulated but not fitted with oral membranes.

There was a significant increase in the ventilation volume ($\dot{V}_w$) within the first 30 min of exposure to pH 4.8 and 333 μg Al·L^{-1} (from 258 to 516 mL·kg^{-1}·min^{-1}, Fig. 1). $\dot{V}_w$ remained above the prechallenge value for 4 h following the introduction of the acid/Al water. From 6 h to the end of the experiment, there was no significant difference in the mean $\dot{V}_w$ compared with the prechallenge period. Despite an increase in coughing frequency and mucus production in all fish, only three individ-

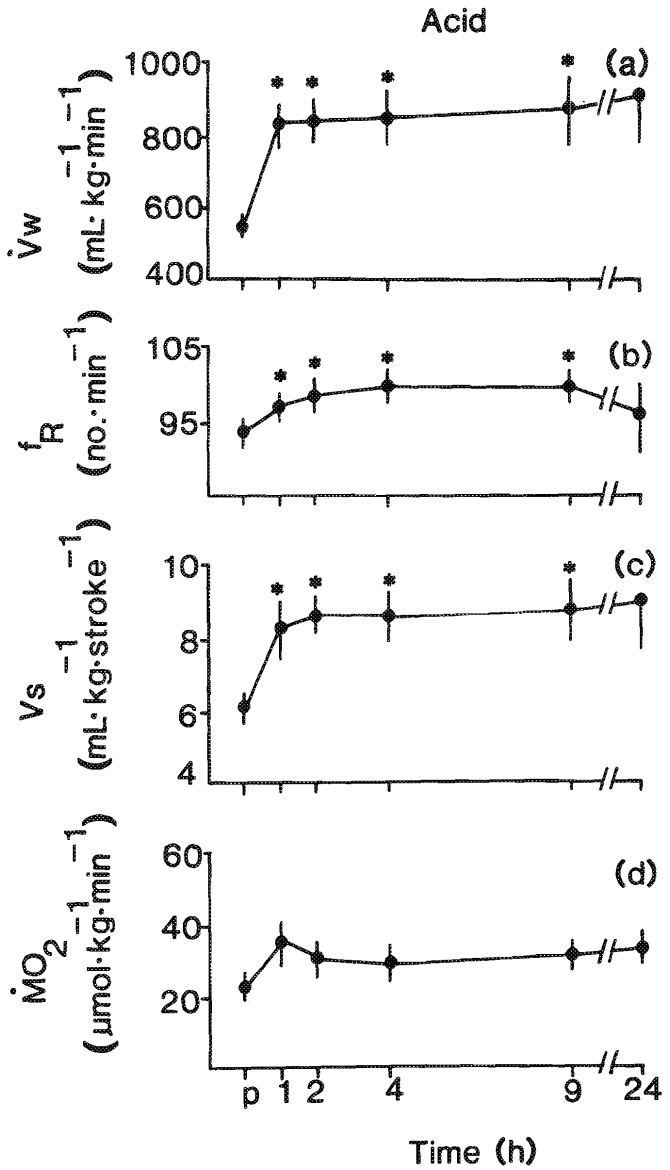

FIG. 2. Respiratory responses in brook trout challenged with acid (pH 4.8) in flowing water at 11°C after acclimation to pH 6.5 (Ca^{2+} = 400 μequiv·L^{-1} in both acclimation and challenge water), (a) Ventilation volume, $\dot{V}_w$; (b) ventilation rate, f_R; (c) ventilation stroke volume, V_s; (d) oxygen consumption, $\dot{M}_{O_2}$. The prechallenge values are indicated at "P." All points are means ± 1 SEM (n = 12). Statistical significance ($p \leq 0.05$) between the prechallenge and challenge values is indicated by an asterisk.

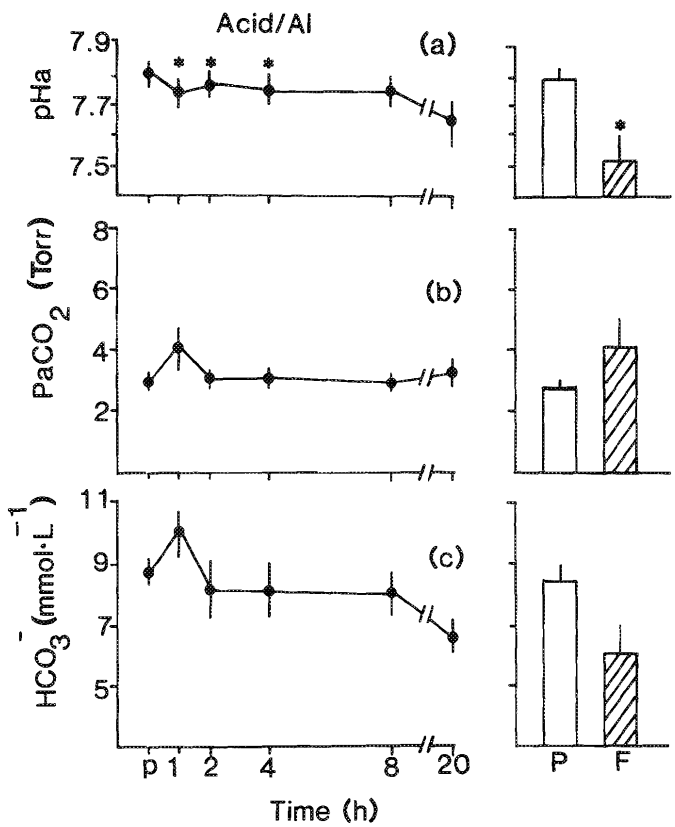

FIG. 3. Changes in the blood acid–base status of brook trout challenged with acid (pH 4.8) and Al (333 μg·L^{-1}) in flowing water at 11°C after acclimation to pH 6.5 (Ca^{2+} = 400 μequiv·L^{-1} in both acclimation and challenge water). (a) Arterial pH, pHa; (b) arterial CO_2 tension, Pa_{CO_2} (calculated); (c) plasma bicarbonate concentration, HCO_3^-. All points are means ± 1 SEM (n = 7). Other details as in legend to Fig. 1.

uals showed an increase in $\dot{V}_w$ prior to death, and as indicated in Fig. 1a, there was no significant difference between the average prechallenge and final $\dot{V}_w$.

The increase in $\dot{V}_w$ seen within the first 4 h can be mainly attributed to increased ventilation stroke volume (V_s, Fig. 1c). There was a rapid increase in V_s within the first 30 min of exposure to the acid/Al mixture (2.87–5.37 mL·kg^{-1}·stroke^{-1}). V_s remained elevated for 4 h and then declined to prechallenge levels. Opercular rate (f_R) was significantly elevated during the same time period (Fig. 1b); however, the relative increase (about 8% above preexposure values) was far less than that for V_s (about 85% increase). There was no significant difference between the prechallenge and the final f_R or V_s (Fig. 1b and 1c).

Similar trends in ventilation were seen in the series II (acid-exposed) fish (Fig. 2). V_s and $\dot{V}_w$ increased approximately 1.5-fold within the first hour, while there was little change in f_R. Unlike the acid/Al group, V_s and $\dot{V}_w$ did not return to prechallenge values for the remainder of the measurement period (24 h). Also, f_R continued to increase to 100·min^{-1} by 4 h, which was significantly higher than the prechallenge mean. The fact that the prechallenge values for $\dot{V}_w$ and V_s were significantly greater in the acid group than in the acid/Al group may reflect the difference in the body size of the two groups. The average weight of the acid/Al fish was 292 ± 17 g (SEM) and that of the acid-alone fish was 168 ± 7 g (SEM). The size difference was due to lack of availability of larger fish when the acid-alone group was tested.

Oxygen consumption ($\dot{M}_{O_2}$) tended to increase upon initial exposure to both acid and acid/Al (Fig. 1d and 2d), but the means were not significantly different from their respective prechallenge values. There was no significant difference between the average initial and final $\dot{M}_{O_2}$ values in the acid/Al fish (Fig. 1d).

Arterial pH decreased slightly, but significantly, from 7.80 to 7.74 over the first 4 h of acid/Al challenge (Fig. 3a). Although, as seen in Fig. 3a, there was a large drop in the average pHa between the prechallenge and final measurement (7.80 to 7.53), not all individuals experienced the same severity of acidosis prior to the terminal sample (Fig. 4a). Other than the slight increase in Pa_{CO_2} in one fish within the first hour

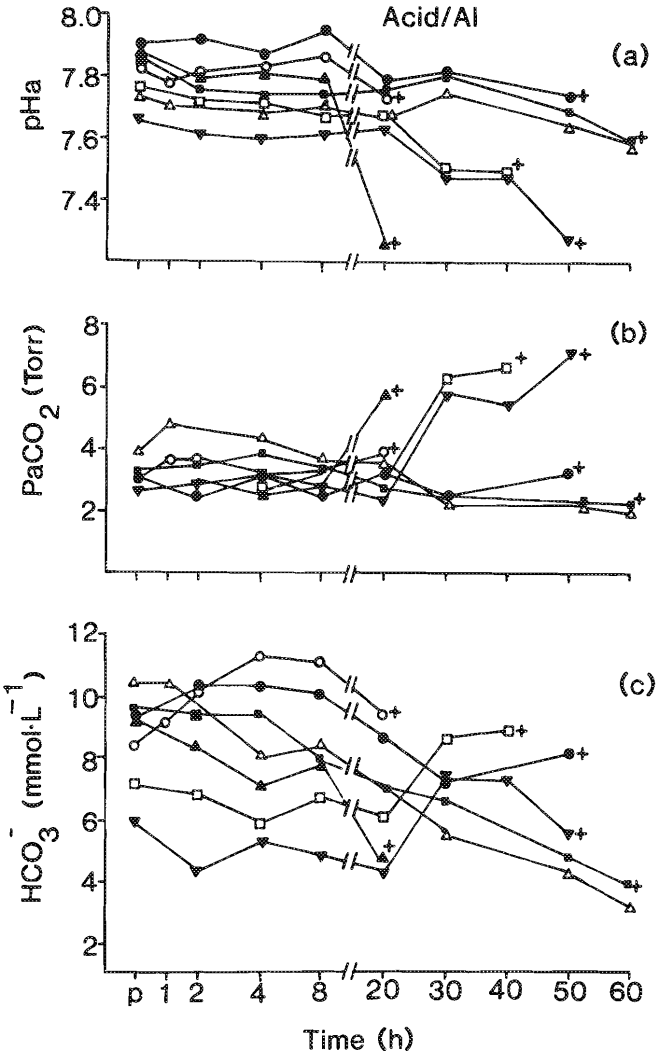

FIG. 4. Changes in the blood acid–base status of individual brook trout challenged with acid (pH 4.8) and Al (333 μg·L^{-1}) in flowing water at 11°C after acclimation to pH 6.5 (Ca^{2+} = 400 μequiv·L^{-1} in both acclimation and challenge water). (a) Arterial pH, pHa; (b) arterial CO_2 tension, Pa_{CO_2} (calculated); (c) plasma bicarbonate concentration, HCO_3^-. The prechallenge values are indicated at "P." The plus signs indicate time of death. Only one fish survived to 100 h; therefore, only the first 60 h of exposure are plotted.

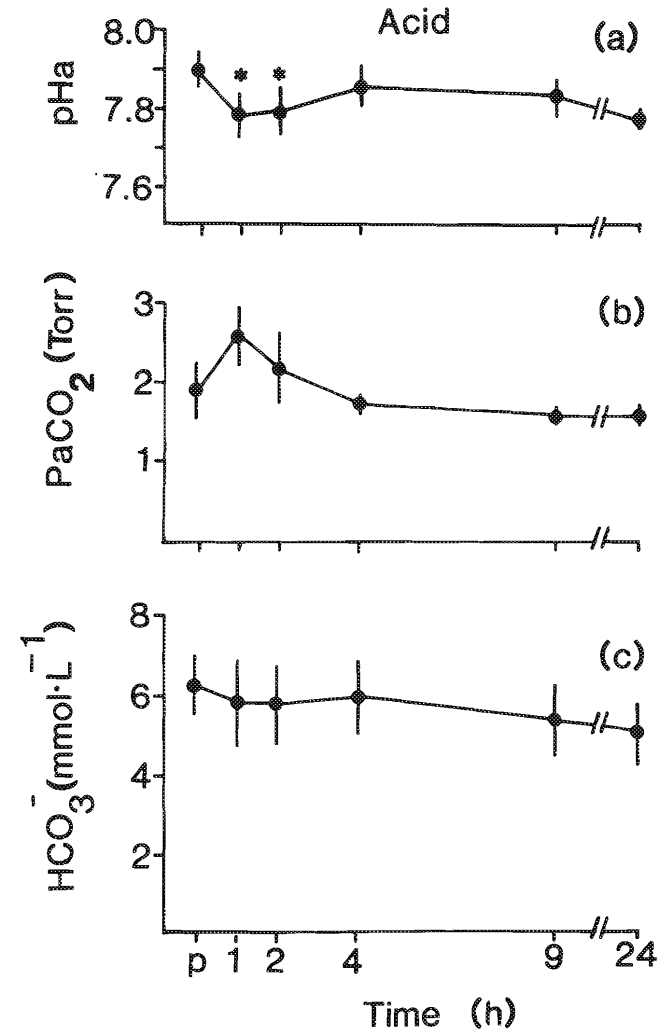

FIG. 5. Changes in the blood acid–base status of brook trout challenged with acid (pH 4.8) in flowing water at 11°C after acclimation to pH 6.5 (Ca^{2+} = 400 μequiv·L^{-1} in both acclimation and challenge water). (a) Arterial pH, pHa; (b) arterial CO_2 tension, Pa_{CO_2} (measured); (c) plasma bicarbonate concentration, HCO_3^-. All points are means ± 1 SEM (n = 12). Other details as in legend to Fig. 2.

(Fig. 4b), there were no significant changes in either Pa_{CO_2} or true plasma [HCO_3^-] during the experiment (Fig. 3b and 3c). Between 20 and 60 h there was a general trend toward a drop in [HCO_3^-] and an increase in Pa_{CO_2} (i.e. combined metabolic and respiratory acidosis), but individual variability was substantial (Fig. 4b and 4c).

There was a mild acidosis evident within the first 2 h of exposure to acid in the absence of Al (Fig. 5a, 5b, and 5c), pHa falling from 7.89 to 7.78. Coincidental with the drop in pHa was an increase in Pa_{CO_2} (measured) at 1 h (from 1.89 to 2.56 Torr, n = 4). By the second hour of the acid challenge, Pa_{CO_2} had returned to 2.17 Torr, which was not significantly different from the prechallenge value. No changes in plasma [HCO_3^-] were seen.

The changes in blood oxygen tension and oxygen content, expressed in terms of [Hb], were not dramatic in either study (Fig. 6, 7, and 8). In fact, rather than falling, Pa_{O_2} tended to rise initially in both groups, although the changes were not significant; Fig. 7a demonstrates that at least half of the animals in series I (acid/Al) did experience a drop in Pa_{O_2} before death. However, when averaged in with the other fish, there was no significant reduction in Pa_{O_2} at the time of the terminal sample (Fig. 6a). Pa_{O_2} of the fish challenged with acid alone was not different from the prechallenge mean throughout the experiment (Fig. 8a).

Pv_{O_2} was measured only in the series I experiments (Fig. 6a). Although there appeared to be a mild reduction in Pv_{O_2} over the course of 8 h (19.2 to 10.8 Torr), the changes were not significant.

There was a significant decrease in Ca_{O_2}, which fell from a prechallenge value of 5.2 to 2.6 mmol·L^{-1} in the acid/Al group prior to death (Fig. 6c and 7c). This represents a 50% decrease in oxygen content. However, when expressed per unit of Hb, the content fell from 66.8 to 54.6 μmol·g Hb^{-1}, an 18% decrease, which was significant, nonetheless. A similar reduction in Ca_{O_2} (millimoles per litre) was observed in the acid–alone group, yet the content per gram of Hb remained unchanged.

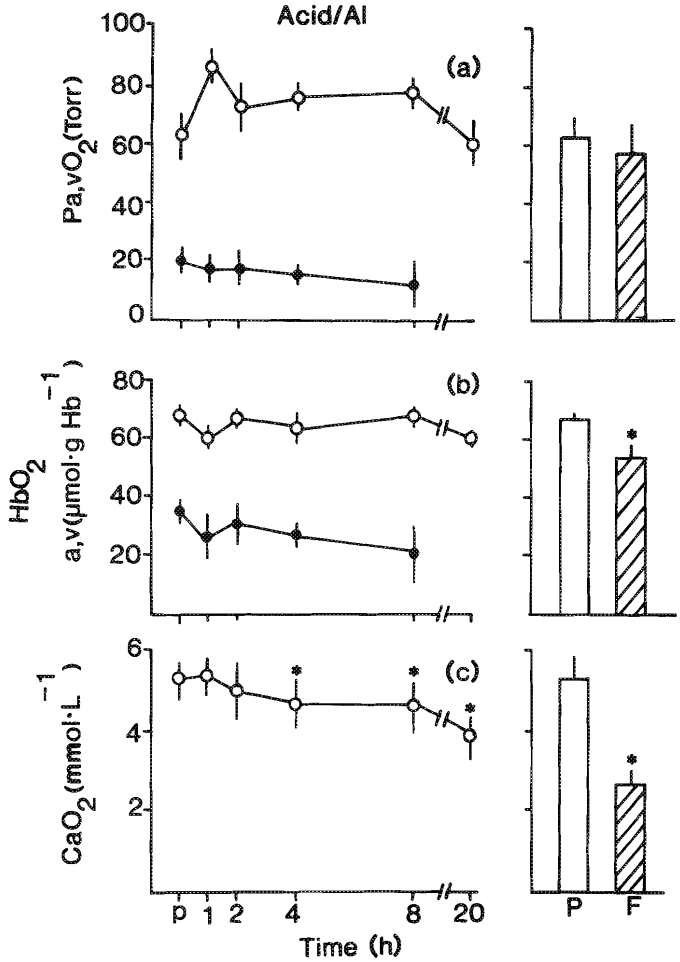

FIG. 6. Blood oxygen tension and content of brook trout challenged with acid (pH 4.8) and Al (333 $\mu g \cdot L^{-1}$) in flowing water at 11°C following acclimation to pH 6.5 Ca^{2+} = 400 $\mu equiv \cdot L^{-1}$ in both acclimation and challenge water). (1) Arterial (open circles) and venous (closed circles) oxygen tension, Pa,v_{O_2}; (b) arterial (open circles) and venous (closed circles) hemoglobin oxygen concentration, Hb_{O_2} a,v; (c) arterial blood oxygen content, Ca_{O_2}. All points are means ± 1 SEM (n = 7). Other details as in legend to Fig. 1.

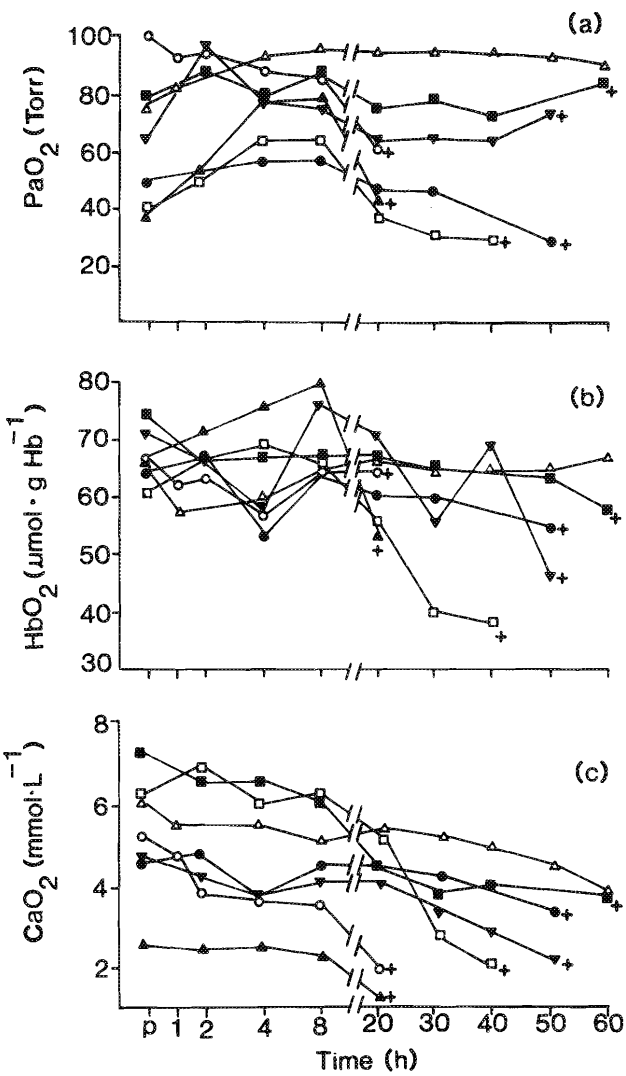

FIG. 7. Blood oxygen tension and content of individual brook trout challenged with acid (pH 4.8) and Al (333 $\mu g \cdot L^{-1}$) in flowing water at 11°C after acclimation to pH 6.5 (Ca^{2+} = 400 $\mu equiv \cdot L^{-1}$ in both acclimation and challenge water). (a) Arterial oxygen tension, Pa_{O_2}; (b) arterial hemoglobin oxygen concentration, Hb_{O_2}; (c) arterial blood oxygen content, Ca_{O_2}. Other details as in legend to Fig. 4.

Almost every individual showed a decrease in MCHC prior to death during the acid/Al challenge, with the result that the final average MCHC was significantly less than the prechallenge value (0.216 vs. 0.260 $g \cdot mL^{-1}$, respectively; Fig. 9c). [Hb] and Hct were reduced due to sampling, but not by the same amount (Fig. 9a and 9b). In the acid-challenged fish, there was a 30% decrease in [Hb] and Hct due to sampling, but there was no significant reduction in MCHC (Fig. 10).

Discussion

The initial increase in ventilation seen in this study in response to acid/Al supports the results of earlier investigators (Rosseland 1980; Neville 1985). However, the subsequent return to prechallenge values after 4 h and the general lack of a ventilatory increase prior to death were unexpected considering the respiratory disturbances reported by Wood et al. (1988b) under similar conditions. Even when considering individual fish, there was no apparent trend toward an increase in ventilation in response to the combined acid/Al stress, disregarding the initial burst of ventilatory activity. This was especially surprising, since all fish in the acid plus Al treatment exhibited visual signs of respiratory distress such as an increase in coughing frequency and mucus production. This finding illustrates the value of direct measurements of $\dot{V}_w$ and suggests that the stressed fish may have been unable to achieve increased net ventilatory flow because of metabolic limitations, increased gill resistance due to mucus, flow reversal due to coughing, or some combination of these factors. In the case of three fish, substantial increases in ventilation rate and stroke volume were noted 20–30 h before death; however, this was not the trend for most. Those fish showing the ventilatory increase were also the ones that experienced the most dramatic decrease in Hb oxygen content (micromoles per gram of Hb) and Pa_{O_2} (Fig. 7).

The immediate increase in ventilation seen in all acid- and acid/Al-treated fish at 0.5 and 1 h of exposure may have been the result of an increase in the P_{CO_2} of the water at the front end of the ventilation chamber. Although not directly measured in the acid/Al fish, the average Pa_{CO_2}, calculated from pHa and HCO_3^-, was slightly elevated (from 3 to 4 Torr) at 1 h. A

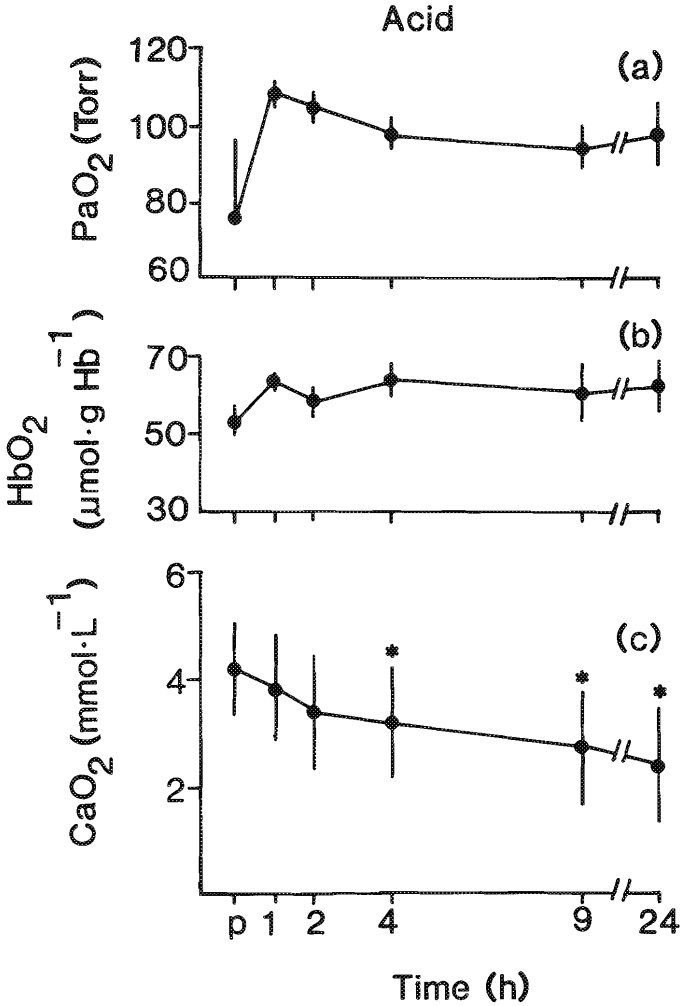

FIG. 8. Blood oxygen tension and content of brook trout challenged with acid (pH 4.8) in flowing water at 11°C after acclimation to pH 6.5 (Ca^{2+} = 400 μequiv·L^{-1} in both acclimation and challenge water). (a) Arterial oxygen tension, Pa_{O_2}; (b) arterial hemoglobin oxygen concentration, Hb_{O_2}; (c) arterial blood oxygen content, Ca_{O_2}. All points are means ±1 SEM (n = 3). Other details as in legend to Fig. 2.

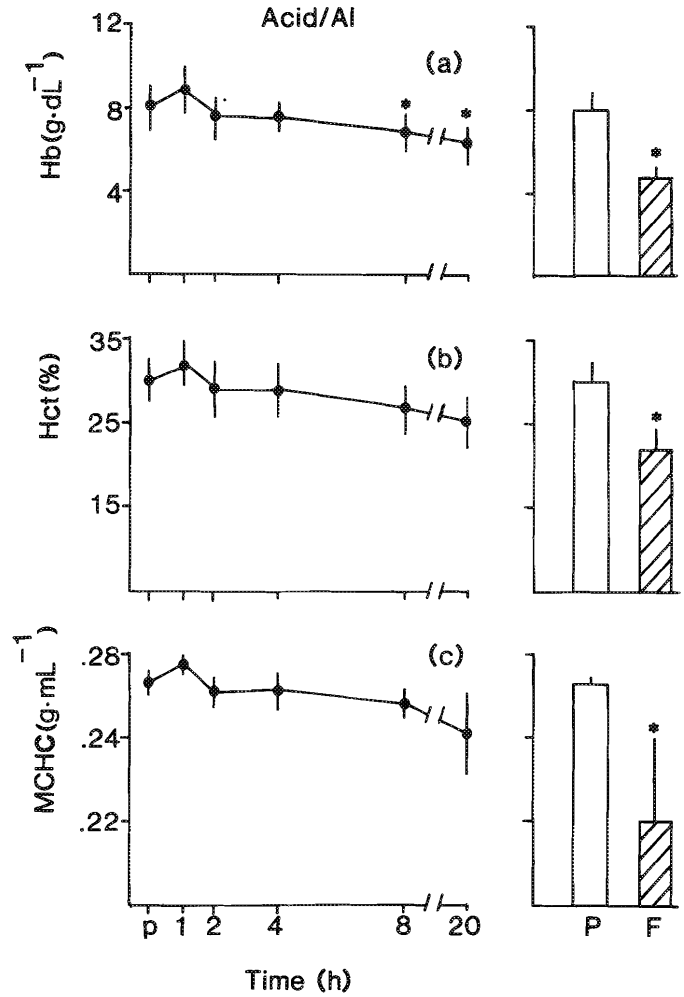

FIG. 9. Hematological responses of brook trout challenged with acid (pH 4.8) and Al (333 μg·L^{-1}) in flowing water at 11°C following acclimation to pH 6.5 (Ca^{2+} = 400 μequiv·L^{-1} in both acclimation and challenge water). (a) Hemoglobin concentration, Hb; (b) hematocrit, Hct; (c) mean cell hemoglobin concentration, MCHC. All points are means ±1 SEM (n = 7). Other details as in legend to Fig. 1.

similar increase in Pa_{CO_2} was directly measured in the acid-exposed fish. Neville (1979) found that an increase in inspired P_{CO_2} from less than 1.5 to 2.2 Torr was enough to significantly raise ventilation. Similar findings were reported by Janssen and Randall (1975). During the initial hour of acid or acid/Al exposure of our experiments, pH = 4.8 water was unavoidably mixed with the preceding pH = 6.5 water in the inspired chamber of the ventilation box. The water had a carbonate alkalinity of 2.4 mg·L^{-1} at pH = 6.5 which was reduced to less than 0.01 mg·L^{-1} when acidified to pH = 4.8. The free CO_2 released during addition of the pH = 4.8 water may have raised the inspired P_{CO_2} to a level sufficient to stimulate ventilation. The field studies reported by Rosseland (1980) and Muniz and Leivestad (1980) support the idea that initial ventilatory bursts are due to transient increases in water P_{CO_2}. In Rosseland's study there was an initial burst in ventilation that was followed by a decline. Therefore, the conditions reported in our study are not at all unlike the situation that one might expect during an episodic acid load in the natural environment.

Since hyperventilation persisted at least 9 h (and perhaps longer) at pH = 4.8, why did $\dot{V}_w$ return to control levels after only 4 h exposure to pH = 4.8 plus Al? We cannot rule out some sort of alleviating effect of Al on the stimulation of ventilation caused by external acidity. Neville (1985) noted that short-term increases (but not long-term increases) in f_R and ventilatory "gape" were attenuated by the presence of Al at pH = 4.0. However, it seems more likely that mucus clogging, coughing reversals of flow, or energetic limitations caused by Al toxicity may have depressed V_s and therefore $\dot{V}_w$ in our fish.

The initial acid–base disturbances were similar in both treatments; a slight decrease in pHa was observed within the first 2–4 h. The only major reduction in plasma pH occurred shortly before death in the acid/Al-treated fish. In some fish the respiratory component (increased Pa_{CO_2}) predominated, while in others the metabolic component (depressed $[HCO_3^-]$) was paramount. Because of this variability, terminal $[HCO_3^-]$ and Pa_{CO_2} were not significantly different from the controls. In general, our results compare favorably with those of Wood et al. (1988c) who also noted the development of a severe acidosis shortly before death in Wyoming brook trout acclimated to the same conditions (pH = 6.5, Ca^{2+} = 400 μequiv·L^{-1}) and exposed to the same acid/Al regime (pH = 4.8, Ca^{2+} = 400 μequiv·L^{-1}, Al = 333 μg·L^{-1}) used in this study. In addition, these investigators reported a substantial increase in plasma lac-

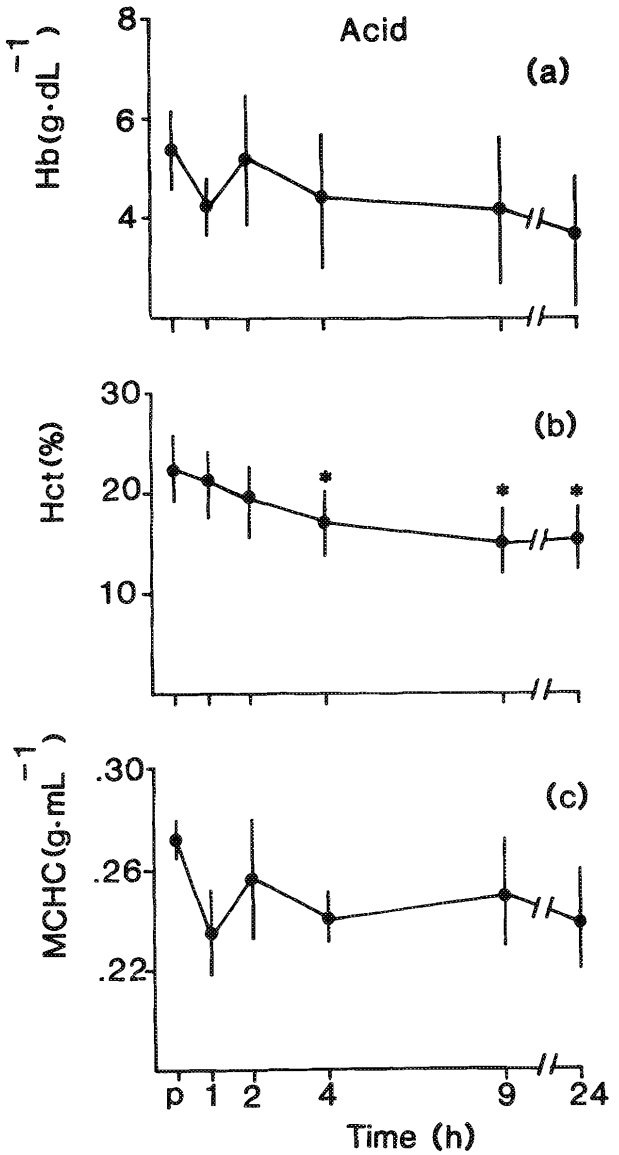

FIG. 10. Hematological responses of brook trout challenged with acid (pH 4.8) in flowing water at 11°C after acclimation to pH 6.5 (Ca^{2+} = 400 μequiv·L^{-1} in both acclimation and challenge water). (a) Hemoglobin concentration, Hb; (b) hematocrit, Hct; (c) mean cell hemoglobin concentration, MCHC. All points are means ± 1 SEM (n = 4 for Fig. 10a and 10c and n = 8 for Fig. 10b). Other details as in legend to Fig. 2.

tate prior to death, which is indicative of metabolic acidosis. The two studies are also in agreement in showing severe reductions in MCHC during the acid/Al challenge, but not during the challenge to acid alone. This response is indicative of stress and probably reflects swelling of the red blood cells due to a combination of plasma dilution, acidosis, and catecholamine mobilization into the bloodstream, as previously discussed (Wood et al. 1988b, 1988c).

The effects of the acid-alone challenge upon blood oxygen tension were negligible, while the responses to acid/Al were rather variable. Initially, there appeared to be an increase in Pa_{O_2} in both treatment groups, probably as a result of the increase in ventilation. While there were no effects of the acid alone on Pa_{O_2} or Hb oxygen content (micromoles per gram of Hb), at least half of the fish in the acid/Al group exhibited a decrease in Pa_{O_2} prior to death, and virtually all the fish showed a decrease in Hb oxygen content. Only the latter was statistically significant, and averaged about 20%. Based on in vivo and in vitro oxygen dissociation curves for brook trout blood (R. L. Walker, C. M. Wood, and C. E. Booth, unpubl. results), the Bohr effect associated with the reduction in plasma pH was more important than decreased Pa_{O_2} in causing reduced Hb saturation. Reduced Hb oxygen content of this magnitude was unlikely the sole cause of death, but likely a contributing factor. It remains unclear why respiratory failure did not predominate, as seen in Ontario brook trout under identical conditions (Wood et al. 1988b).

Although the amount of oxygen carried per gram of Hb remained fairly constant, the total oxygen content decreased steadily during the experiment. The role of anemic hypoxemia cannot be ruled out as a factor affecting ventilation, since both [Hb] and Hct were reduced in both groups during the challenge periods, probably as the result of sampling (Fig. 9 and 10). Smith and Jones (1982) have demonstrated that an anemia sufficient to reduce Ca_{O_2} content by 32% can cause a 1.6-fold increase in ventilation in trout. In this study the oxygen content was reduced by a similar amount. Therefore, the fact that ventilation remained elevated in the acid-alone group may be attributed to the hypoxemia associated with sampling, at least in part.

Hypoxemia was also evident in the acid/Al fish prior to death, yet there was no ventilatory response. A general lack of hyperventilation in this group, despite a significant reduction in Ca_{O_2} and Hb saturation, suggests that Al is interfering with the mechanism controlling ventilatory response to hypoxemia. The mechanism of this interference remains to be elucidated.

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