Effects of Long-Term Preexposure to Sublethal Concentrations of Acid and Aluminum on the Ventilatory Response to Aluminum Challenge in Brook Trout (*Salvelinus fontinalis*)

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Adult brook trout (*Salvelinus fontinalis*; 200–300 g) were preexposed for 10 wk to 75 μg $Al^{3+} \cdot L^{-1}$, pH = 5.2, in soft water (25 μequiv $Ca^{2+} \cdot L^{-1}$) and controls to the same conditions without Al^{3+}. When challenged with a lethal dose of Al^{3+} (333 $\mu g \cdot L^{-1}$) at the same pH (5.2) and Ca^{2+} (25 $\mu equiv \cdot L^{-1}$), the control fish demonstrated a twofold increase in ventilation volume and ventilatory stroke volume within the first two hours, an increase in $Paco_2$, and a decrease in pHa and hemoglobin O_2 saturation. These effects were not seen in the group chronically preexposed to sublethal Al, indicating that some acclimation had occurred. Although the prechallenge Pao_2 and $Paco_2$ were the same in the two groups, the arterial pH, plasma [HCO_3^-], and hemoglobin O_2 saturation of the Al-preexposed fish were significantly below those of the control fish, suggesting that the acclimation was achieved at some cost. Possible mechanisms are discussed.

Pendant 10 sem, on a exposé des ombles de fontaine *Salvelinus fontinalis* adultes pesant de 200 à 300 g à 75 μg $Al^{+3} \cdot L^{-1}$ en eau douce de pH 5,2 (25 μequiv $Ca^{2+} \cdot L^{-1}$). Les ombles témoins ont été exposés aux mêmes conditions en l'absence d'Al^{+3}. Lorsque mis en présence d'une dose létale d'Al^{+3} (333 $\mu g \cdot L^{-1}$) en milieu de même pH (5,2) et concentration de Ca^{+2} (25 $\mu equiv \cdot L^{-1}$), ces derniers ont montré une augmentation par un facteur de deux du volume de ventilation et du volume de l'amplitude de pulsation ventilatoire en deçà des deux premières heures d'exposition, une augmentation de la teneur en $Paco_2$ ainsi qu'une baisse du pHa et du niveau de saturation en O_2 de l'hémoglobine. Ces incidences n'ont pas été relevées dans le groupe pré-exposé de façon chronique à une teneur sublétale d'Al, ce qui indique qu'une certaine acclimatation a eu lieu. Quoique les teneurs en Pao_2 et $Paco_2$ avant l'exposition étaient les mêmes dans les deux groupes, le pH artériel, la concentration de HCO_3^- du plasma et la saturation en O_2 de l'hémoglobine chez tous les poissons pré-exposés à l'Al étaient nettement inférieurs aux valeurs relevées chez les poissons témoins. Ceci laisse supposer que l'acclimatation s'est effectuée aux dépens de certains processus. Les auteurs traitent de mécanismes pouvant expliquer ce phénomène.

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The combined effect of acid and aluminum on ventilation has been documented in brown trout (*Salmo trutta*) (Muniz and Leivestad 1980; Rosseland 1980), rainbow trout (*Oncorhynchus mykiss*) (Neville 1985; Malte and Weber 1988; Playle and Wood 1989), and brook trout (*Salvelinus fontinalis*) (Rosseland 1980; Walker et al. 1988). Although a variety of combinations of water pH, Al, Ca, and temperature was tested among these studies, the general conclusion is that exposure of fish naive to acid pH and Al in lethal combinations results in hyperventilation, mucus clogging of the gills, and eventual death which is caused at least in part by respiratory impairment. Typical internal symptoms of this respiratory distress are a drop in arterial O_2 tension, a reduction in hemoglobin O_2 content, a rise in arterial CO_2 tension, an accumulation of lactate, and a decrease in arterial pH as a result of the combined respiratory and metabolic acidosis (Neville 1985; Malte and Weber 1988; Wood et al. 1988b; Walker et al. 1988; Playle et al. 1989).

However, it now appears that prior exposure to sublethal concentrations of acid/Al can alleviate the respiratory distress and reduce the mortality associated with exposure to higher concentrations of these toxicants. Orr et al. (1986) found that prior exposure of rainbow trout fingerlings to sublethal concentrations of Al (87–154 $\mu g \cdot L^{-1}$) at pH = 5.2 for 2–3 wk significantly increased the LC50 of Al at the same pH. Wood et al. (1988a, 1988c) found that adult brook trout preexposed

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to Al = 150 $\mu g \cdot L^{-1}$ for 10 wk at pH = 5.2 were able to maintain electrolyte balance and blood respiratory parameters when challenged with Al = 333 $\mu g \cdot L^{-1}$ at pH = 4.8, a combination which proved lethal to fish lacking previous Al exposure (i.e. Al naive). In fact, there were no mortalities among the Al-preexposed group over the 72-h challenge period, while the Al-naive fish all died (Wood et al. 1988c). The evidence to date strongly suggests that acclimation occurs to Al alone, and not to low pH, during long-term exposure to sublethal acid/Al conditions (Wood et al. 1988a, 1988c; Audet and Wood 1988). Although the actual mechanism of acclimation has not been elucidated, Wood et al. (1988a, 1988c) speculated that gill ligand attraction for Al might be reduced during acclimation which in turn would minimize Al deposition on the gills and prevent mucification and inflammation during Al challenge.

Extended periods of exposure to sublethal acid/Al have been reported to cause thickening of the gill epithelium leading to increased water to blood diffusion distance (Chevalier et al. 1985; Tietge et al. 1988; Karlsson-Norrgren et al. 1986a, 1986b). Nevertheless, the observations of Wood et al. (1988a, 1988c) clearly indicated that there were no chronic abnormalities of arterial O_2 or CO_2 tensions or hemoglobin O_2 content as a result of the exposure and negligible disturbances of these parameters during more severe acid/Al challenge. Indeed, the implications are that pulses of acid and Al, seen when the snowpack melts in the spring, may be less severe with regards to fish survival than previously thought, considering the effects of prior exposure to sublethal acid/Al.

Nothing is yet known of the effects of Al acclimation on ventilatory responses. Would the long-term preexposure of adult brook trout to sublethal concentrations of acid and Al alleviate the acute hyperventilatory response (Walker et al. 1988) to a more severe Al challenge? In light of the previous discussion, we anticipated that acute respiratory distress would not occur in the acid/Al-preexposed fish and that mortality would be substantially reduced in response to the challenge.

Materials and Methods

Experimental Animals and Conditions

Experiments were performed at the Fish Physiology and Toxicology Laboratory, Laramie, WY (altitude = 2200 m), which provided facilities for continuous exposure of adult fish to defined pH, Ca^{2+}, and Al conditions in flowing artificial soft water. In brief, the artificial soft water was prepared from hard well water by sedimentation filtration, NaCl softening, reverse osmosis, and separate bed deionization (Continental Water Systems, Denver, CO). The pH was adjusted to 6.5 with KOH, Na^+ (40 $\mu equiv \cdot L^{-1}$) and Ca^{2+} (25 $\mu equiv \cdot L^{-1}$) were added as chlorides, and the water was thoroughly mixed and then delivered to head tanks where the pH was lowered to 5.2 with H_2SO_4. All pH adjustments were made with Leeds and Northrup pH controllers (pH analyzer/controller model No. 7083). The required levels of $AlCl_3$ were added via Mariotte bottles. From there, water was distributed directly to 340-L fiberglass fish holding tanks at a rate of 1.9 $L \cdot min^{-1}$ and then to waste.

Adult brook trout (200–300 g), obtained from Cline's Trout Farm (Boulder, CO), were exposed for 10 wk to either acidified soft water (pH 5.2, Ca^{2+} = 25 $\mu equiv \cdot L^{-1}$) or acidified soft water containing Al (pH = 5.2, Ca^{2+} = 25 $\mu equiv \cdot L^{-1}$, Al^{3+} = 75 $\mu g \cdot L^{-1}$) at 11 ± 1°C. Fish were fed 1 floating trout chow (Purina No. 5106) at 1% body weight $\cdot d^{-1}$ and solid wastes were removed by siphon daily. Dissolved O_2 was maintained above 60% air saturation. Photoperiod was adjusted biweekly to follow the natural cycle for Laramie, WY.

Following the 10-wk exposure period, fish from both holding conditions (Al naive, n = 6; Al preexposed, n = 10) were fitted with ventilation masks and arterial catheters (see below). Both groups were then challenged for 72 h with the same acidified soft water (pH = 5.2, Ca^{2+} = 25 $\mu equiv \cdot L^{-1}$) to which Al^{3+} = 333 $\mu g \cdot L^{-1}$ was added. This concentration of Al in the challenge condition was previously shown to be lethal to brook trout acclimated to pH = 6.5 and pH = 5.2 in the absence of Al (Wood et al. 1988a, 1988c).

Ventilation and Blood Measurements

Prior to the challenge, each fish was equipped with a caudal artery cannula and a latex ventilation mask under anesthesia using the methods outlined in Wood et al. (1988b), Davis and Cameron (1971), and Walker et al. (1988). Fish were anesthetized with MS-222 (ethyl-*m*-aminobenzoate, 50 $mg \cdot L^{-1}$; Sigma Chemical Co., St. Louis, MO) using the soft water from the appropriate acclimation tank. Immediately after surgery, each fish was placed in its own ventilation box which was supplied with the appropriate acclimation water at a flow rate of 0.5 $L \cdot min^{-1}$. Inspired P_{O_2} was maintained above 120 torr (1 torr = 133.322 Pa), P_{CO_2} less than 1 torr, and temperature at 11 ± 1°C. The ventilation boxes were covered in black plastic to reduce disturbance, except for a hole in the top of the box strategically placed to allow observation of opercular movements.

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. O_2 consumption (micromoles per kilogram per minute) was determined by the Fick method, using the difference in P_{O_2} between inspired water and mixed expired water from just behind the opercula, the ventilation volume, and an assumed O_2 solubility of 2.23 $\mu mol \cdot L^{-1} \cdot torr^{-1}$ at 11°C (Dejours 1975).

After measuring the ventilatory parameters while the fish was in the acclimation water, we drew an initial blood sample from the cannula using a heparinized, gas-tight Hamilton syringe. The blood sample (400 μL) was replaced by reinfusing blood from the P_{O_2} electrode (about 100 μL) and nonheparinized saline (Wolf 1963). The blood was analyzed for arterial O_2 tension (Pa_{O_2}), arterial pH (pHa), total O_2 in whole blood (vol% O_2), total CO_2 in true plasma (C_{CO_2}), hematocrit (Hct), hemoglobin concentration ([Hb]), plasma lactate concentration, and plasma Cl^- concentration.

Following the prechallenge sampling period, acid/Al water (pH = 5.2, Ca^{2+} = 25 $\mu equiv \cdot L^{-1}$, Al^{3+} = 333 $\mu g \cdot L^{-1}$) was piped into the anterior portion of the ventilation chamber. Ventilation was measured 1 h into the challenge and then again immediately prior to blood sampling at 2, 4, 8, 24, 48, and 72 h of exposure, if death did not occur earlier. This sampling regime was chosen because of the acute nature of the respiratory and acid–base response to a similar acid/Al challenge reported by Walker et al. (1988). At each blood sampling time, the suite of measurements outlined above was performed.

Analytical Methods

Water samples were taken from the acclimation tanks for measurement of pH (daily) and Ca^{2+}, Na^+, Cl^-, and Al (weekly). Na^+ and Ca^{2+} were measured on a Perkin-Elmer model 2380 atomic absorption spectrophotometer. Al was measured with an associated high-temperature graphite furnace (Perkin-Elmer model HGA-400). Using the method of LaZerte (1984), Al was speciated into total, monomeric, and inorganic monomeric. Cl^- levels were measured using a Dionex ion-exchange chromatograph (model 2110) with an AS 40 column.

A Radiometer G297/G2 electrode was used for the determination of pHa, and O_2 tension was measured with a Radiometer E5046 P_{O_2} electrode. Both electrodes were maintained at 11°C. Total CO_2 was determined with a Corning 960 CO_2 analyzer using true plasma obtained from sealed heparinized capillary tubes following centrifugation. Hct was read before the plasma was removed. Arterial CO_2 tension (Pa_{CO_2}) and true plasma bicarbonate ($[HCO_3^-]$) were calculated as outlined in Walker et al. (1988).

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

The O_2 content of whole blood was determined with a Lex-O_2-Con oxygen analyzer (Lexington Instruments Corp.). After conversion of the O_2 content from volume percent to millimoles per litre, the O_2 content per unit Hb (micromoles O_2 per gram of Hb) was calculated by subtracting the dissolved component (Boutilier et al. 1984) and dividing by the measured [Hb].

Plasma Cl^- was measured coulometrically with a Radiometer CMT 10 chloridometer. Lactate determination was performed with a YSI model 27 industrial analyzer (Yellow Springs Instruments).

Treatment of Data

Data have been reported as means ± 1 SEM (n) for both groups at each of the sampling times. For all parameters, the mean ± 1 SEM (n) 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. Differences among means were assessed by one-way analysis of variance. Student–Newman–Keuls multiple comparison tests were performed to establish specific differences among means when the F value indicated significance (Zar 1984). In all cases, a criterion of $p \leq 0.05$ was employed for statistical significance. Estimates of median survival times (LT50's) were computed by log-probit analysis of time-percent effect data. Nomographic methods outlined in Litchfield (1949) and Litchfield and Wilcoxon (1949) were used to compute 95% confidence limits for the log-probit regressions. LT50's were considered significantly different when the confidence limits did not overlap.

Results

Exposure Conditions

There was good agreement between the intended and measured water conditions of the acclimation tanks (Table 1). Variation in Na and Cl concentrations was attributed to cyling of the water-softening and deionizing systems. Speciation of Al showed that almost all the Al was available to the fish in the monomeric form, but only about 15–20% of the monomeric was inorganic, probably due to complexation with food or fecal material in the water. Although the speciation of Al was not monitored in the challenge condition, the flow of freshwater over the gill and out the posterior of the ventilation box with each opercular movement precluded the possibility of complexation with organic waste material.

Effects of Al Challenge on Ventilation and O_2 Consumption ($\dot{M}_{O_2}$)

There was approximately a twofold increase in the mean $\dot{V}_w$ of the Al-naive fish within 1 h of the start of the challenge (Fig. 1A). $\dot{V}_w$ continued to increase to about 2.5-fold of the prechallenge $\dot{V}_w$ at 2 h and remained significantly elevated through the first 8 h of the challenge. By 24 h, one third of the Al-naive fish had died, and $\dot{V}_w$ of the survivors was not significantly different from the prechallenge value. Only one of the six Al-naive fish survived to 48 h.

The elevation of $\dot{V}_w$ was largely attributable to a 2- to 2.5-fold increase in $\dot{V}_s$ (Fig. 1B). As with $\dot{V}_w$, $\dot{V}_s$ remained elevated during the first 8 h of the challenge. At 24 h, $\dot{V}_s$ was still significantly greater than that seen during prechallenge. There was also a gradual rise in f_R over the first 8 h of the challenge, although the maximum increase was only 12% (Fig. 1C).

The ventilatory parameters of the Al-preexposed fish were not significantly different from those of the Al-naive fish prior to challenge (Fig. 1; Table 2), and in contrast with the Al-naive fish, there were no significant changes in $\dot{V}_w$, $\dot{V}_s$, or f_R during the challenge period. The survival rate was slightly improved in that 4 of the 10 challenged fish survived to 48 h and 2 were still alive at 72 h. All three ventilation parameters showed a slight increase between 24 and 48 h, but the means were not statistically significant from the prechallenge values.

There was no statistically significant change in $\dot{M}_{O_2}$ in either group throughout the challenge period (Fig. 1D).

Effects of Al Preexposure and Al Challenge on Acid–Base Status and Blood Gases

With regard to the acid–base status of the two groups (Fig. 2), both the prechallenge pHa (7.69 versus 7.93) and true plasma $[HCO_3^-]$ (7.6 versus 11.6 mmol·L^{-1}) were substantially and significantly lower in Al-preexposed trout than in Al-naive fish. Calculated Pa_{CO_2} was also slightly elevated in the Al-preexposed fish compared to the Al-naive group (3.6 versus 3.1 torr). The prechallenge plasma lactate values were virtually identical in the two groups (2.9 versus 3.1 mmol·L^{-1}).

The Al challenge caused a significant reduction in pHa of the Al-naive fish which dropped from 7.93 to 7.80 within the first hour and then to 7.56 at 24 h (Fig. 2A). Although true plasma $[HCO_3^-]$ (Fig. 2B) showed a similar decrease, only the 24-h mean was significantly less than the prechallenge mean (5.4 versus 11.6 mmol·L^{-1}). There was also a significant increase in the Pa_{CO_2} (calculated) at 2 h of the challenge (Fig. 2C), but Pa_{CO_2} returned to prechallenge levels by 4 h and remained there for the balance of the challenge period. The average plasma lactate concentrations over the 24-h period were not significantly different from the prechallenge value (Fig. 2D). However, a comparison of the mean terminal lactate concentration and the prechallenge value (Table 2) revealed that there was a significant increase in plasma lactate (6.2 versus

TABLE 1. Intended exposure conditions and measured water chemistry (means and ranges) during the 10-wk acclimation period.

Intended			Measured						
					Al ($\mu g \cdot L^{-1}$)				
pH	Ca^{2+} ($\mu equiv \cdot L^{-1}$)	Al ($\mu g \cdot L^{-1}$)	pH	Ca^{2+} ($\mu equiv \cdot L^{-1}$)	Total Al	Monomeric	Inorganic monomeric	Na^{+} ($\mu equiv \cdot L^{-1}$)	Cl^{-} ($\mu equiv \cdot L^{-1}$)
5.2	25	0	5.09 (4.7–5.6)	27.1 (22–31)	0.6 (<0.1–2.5)	2.2 (<0.1–8.5)	—	51.8 (47–60)	51.4 (43–70)
5.2	25	75	5.21 (4.9–5.7)	26.8 (23–32)	66.3 (38–104)	59.3 (47–73)	10.7 (3–20)	47.8 (43–53)	54.1 (49–58)

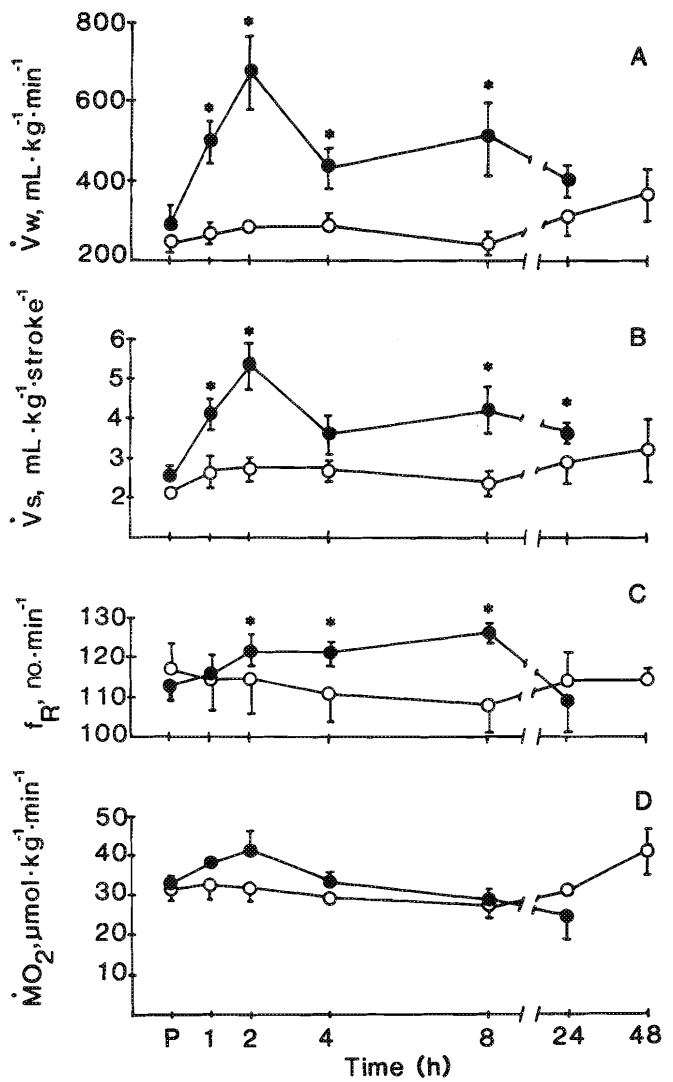

FIG. 1. (A) Ventilation volume ($\dot{V}_w$), (B) ventilatory stroke volume ($\dot{V}_s$), (C) ventilation rate (f_R), and (D) O_2 consumption ($\dot{M}O_2$) of Al-naive (closed circles) and Al-preexposed (open circles) brook trout prior to (P) and during acid/Al challenge (pH = 5.2, Al = 333 $\mu g \cdot L^{-1}$). Values are means ± 1 SEM. The number of observations diminished during the experiment due to deaths (n ranged from 6 to 4 for Al-naive and from 10 to 4 for Al-preexposed fish). *Significant difference from the respective prechallenge mean in each group ($p \leq 0.05$).

3.1 $mmol \cdot L^{-1}$) in the Al-naive fish just before death, together with significant decreases in pHa and $[HCO_3^-]$ as noted above.

As with the ventilatory parameters, there were no further significant changes in the acid–base status of the Al-preexposed fish in response to the Al challenge. Plasma pHa, $[HCO_3^-]$, Pa_{CO_2}, and lactate showed only minor fluctuations from the prechallenge values (Fig. 2). However, the mean terminal sample pHa and $[HCO_3^-]$ were significantly less than the prechallenge means, although lactate was not affected (Table 2).

Prechallenge levels of Pa_{O_2} were identical in the two groups, but the prechallenge arterial O_2 content (vol% O_2), the O_2 content per unit Hb (Hb_{O_2}), and MCHC were all significantly higher in the Al-naive fish (Fig. 3A). The difference probably reflected Bohr and Root effects and erythrocyte swelling associated with the lower pHa values for the Al-preexposed trout (Fig. 2A).

Overall there were no significant changes in Pa_{O_2} of either group of fish (Fig. 3A). Average P_{O_2}'s were maintained between 50 and 60 torr (the inspired P_{O_2} was about 120 torr at this altitude). The Al-naive fish experienced a significant drop in Hb_{O_2} at 2 h (Fig. 3C), corresponding with the reduction in arterial pH and the increase in P_{CO_2} at this time (Fig. 2). As with Pa_{CO_2}, the reduction in Hb_{O_2} was only transient, as the mean values at 4 h were not significantly different from the means for the prechallenge period. This transient decrease in Hb_{O_2} was not seen in the Al-preexposed trout (Fig. 3C). Hb, Hct (data not shown), and vol% O_2 (Fig. 3B) declined at 24 h in the Al-naive fish due to the combined effects of red blood cell loss through repetitive blood sampling and acidosis. Despite identical blood sampling in the Al-preexposed group, these fish experienced no significant decline in these parameters during challenge. No significant changes in MCHC were observed in either group in the individual sampling periods during challenge, but the terminal MCHC was significantly reduced relative to the prechallenge value in the Al-preexposed group (Table 2).

Effects of Al Preexposure and Al Challenge on Plasma Chloride and Mortality

Due to a shortage of plasma, the only plasma inorganic ion measured was Cl^- (Table 2). There was no significant difference between the prechallenge means, but the terminal samples from both groups were significantly less than their prechallenge values.

From the log-probit analysis of mortality, LT50's for the Al-naive and Al-preexposed fish were not significantly different at 32 and 36 h, respectively.

Discussion

The acclimation conditions chosen here were similar to those used by Orr et al. (1986), Wood et al. (1988b), and McDonald

TABLE 2. Initial (prechallenge) and terminal values (means ± 1 SEM (n)) during challenge (pH = 5.2, Al = 333 $\mu g \cdot L^{-1}$) for various ventilatory and blood parameters of brook trout preexposed to either pH = 5.2, Al = 0 (Al naive) or pH = 5.2, Al = 75 $\mu g \cdot L^{-1}$ (Al preexposed). *Significant difference from the respective prechallenge mean; **significant difference between group means prior to challenge ($p \leq 0.05$).

	Al naive		Al preexposed	
Parameter	Initital	Terminal	Initial	Terminal
pHa	7.929 ± 0.036 (5)	7.665* ± 0.074 (5)	7.687** ± 0.029 (9)	7.494* ± 0.044 (8)
$[HCO_3^-]$ ($mmol \cdot L^{-1}$)	11.6 ± 1.2 (5)	7.0* ± 0.9 (5)	7.6** ± 0.9 (9)	4.8* ± 0.5 (7)
P_{CO_2} (torr)	3.1 ± 0.4 (5)	3.4 ± 0.3 (5)	3.6 ± 0.4 (9)	3.6 ± 0.3 (5)
P_{O_2} (torr)	52.1 ± 6.6 (4)	39.3 ± 10.5 (4)	53.3 ± 8.1 (7)	71.5 ± 5.7 (7)
Hb_{O_2} ($\mu molO_2 \cdot g\ Hb^{-1}$)	63.7 ± 1.4 (4)	45.5 ± 9.9 (4)	54.2** ± 2.5 (6)	50.8 ± 4.0 (6)
MCHC ($g \cdot mL^{-1}$)	0.250 ± 0.005 (5)	0.226 ± 0.028 (5)	0.223** ± 0.012 (8)	0.183* ± 0.006 (7)
$\dot{V}_w$ ($mL \cdot kg^{-1} \cdot min^{-1}$)	287.5 ± 37.5 (6)	418.2 ± 84.2 (6)	250.0 ± 25.7 (10)	300.0 ± 57.0 (9)
$\dot{V}_s$ ($mL \cdot kg^{-1} \cdot stroke^{-1}$)	2.55 ± 0.36 (6)	3.62 ± 0.59 (6)	2.19 ± 0.24 (10)	2.76 ± 0.55 (8)
f_R ($no. \cdot min^{-1}$)	113 ± 3 (6)	114 ± 7 (6)	117 ± 7 (10)	116 ± 3 (8)
$\dot{M}_{O_2}$ ($\mu mol \cdot kg^{-1} \cdot min^{-1}$)	32.7 ± 3.2 (6)	27.6 ± 5.4 (6)	32.1 ± 2.6 (10)	24.3 ± 3.2 (9)
Lactate ($mmol \cdot L^{-1}$)	3.1 ± 0.52 (5)	6.2* ± 0.85 (5)	2.9 ± 0.36 (9)	4.4 ± 0.85 (9)
Cl^- ($mmol \cdot L^{-1}$)	114 ± 4.7 (5)	99.5* ± 4.6 (5)	110.9 ± 2.9 (10)	94.5* ± 3.0 (10)

et al. (1991), who clearly demonstrated the ameliorative effects of prior exposure to sublethal concentrations of Al on the response to lethal Al challenge. Al concentrations used during the challenge period closely matched the field conditions during acid pulses following melt of the snowpack (Schofield 1977; Harvey and McArdle 1986). The time span of the study was limited to 72 h, which was considered sufficient to observe any lethal effects of the Al challenge reported by Harvey and McArdle (1986) and Wood et al. (1988a, 1988c).

The acute hyperventilation (Fig. 1A) during challenge (pH = 5.2, Al = 333 $\mu g \cdot L^{-1}$) exhibited by Al-naive fish (preexposed to pH = 5.2, Ca^{2+} = 25 $\mu equiv \cdot L^{-1}$) was virtually identical to the response to a slightly different acid/Al challenge (pH = 4.8, Al = 333 $\mu g \cdot L^{-1}$) of adult brook trout naive to both acid and Al (preexposed to pH = 6.5, Ca^{2+} = 400 $\mu equiv \cdot L^{-1}$) reported in our previous study (Walker et al. 1988). In that investigation, interpretation of the hyperventilatory response was complicated by changes in water pH (and transiently P_{CO_2}) as well as water Al during the challenge. In the present study, this was not a problem because water pH was maintained unchanged at 5.2, and there was no disturbance of inspired P_{CO_2}. The hyperventilatory response was clearly caused by Al alone. Similar increases in ventilation in response to combined acid plus Al challenge in soft water have been reported in other salmonids (Rosseland 1980; Neville 1985; Malte and Weber 1988).

The fact that the decrease in the mean arterial Hb_{O_2} and increase in P_{CO_2} were only transient and that the mean values for P_{O_2} remained unchanged in the Al-naive fish suggests that impairment of respiratory gas exchange was relatively minor in the present study. The relative stability of the blood O_2 and CO_2 tensions may be the result of the hyperventilation. In general, the blood gas responses were similar to those of our previous study (Walker et al. 1988).

It is likely that the ventilatory response was a direct result of the action of Al on the gill. It is known that Al acts as an irritant, causing an increase in gill mucus secretion as reported by Muniz and Leivestad (1980), Mallant (1985), and Walker et al. (1988). In addition to a general irritation response, clogging of the interlamellar spaces with Al/mucus precipitate may have occurred, although gill mucus and Al content were not analyzed.

The ventilatory parameters of the Al-preexposed fish (pH = 5.2, Al = 75 $\mu g \cdot L^{-1}$) remained unchanged (Fig. 1) despite the Al challenge (pH = 5.2, Al = 333 $\mu g \cdot L^{-1}$). It is also important to note that these parameters were not significantly different from the prechallenge mean ventilation volume and rate for Al-naive fish. Thus, the work reported here is the first evidence that the ventilatory component of the toxic response to Al can be eliminated through long-term preexposure. How does one explain the complete lack of ventilatory response when the Al-preexposed fish were challenged with lethal levels of Al? Both groups experienced little disturbance in blood gases; therefore, the lack of one in the Al-preexposed fish is probably not the explanation. From previous work of Karlsson-Norrgren et al. (1986a, 1986b), Tietge et al. (1988), and Mueller et al. (1991), one might expect that long-term preexposure would result in a proliferation in mucus cells on the branchial epithelium. However, this does not necessarily imply increased mucus accumulation on the gills during challenge in the Al-preexposed fish. Indeed the reverse may well be true. McDonald et al. (1991) noted that the gill content of sialic acid, an important component of mucus, decreased during the initial stages of acclimation to sublethal Al in juvenile brook trout. This decrease coincided with a removal of Al from the gills. Gill sialic acid content decreased again during more severe Al challenge. McDonald et al. (1991) speculated that an increased turnover and sloughing rate of branchial mucus was an important part of the defence mechanism seen in Al-acclimated fish, for it would minimize the net accumulation of Al on the gills. In juvenile rainbow trout preexposed to sublethal Al, Reid et al. (1991) identified another mechanism which would reduce branchial Al accumulation during challenge, specifically a reduction in the cation binding affinity of the gill surface for Al and in the ability of Al to inhibit gill Ca^{2+} binding.

We therefore speculate that functional and/or compensational changes in the gill surface occurring as a result of preexposure minimized the net accumulation of Al on the gills during challenge. This minimized gill irritation, perhaps in combination with a desensitization of the inflammatory response as a

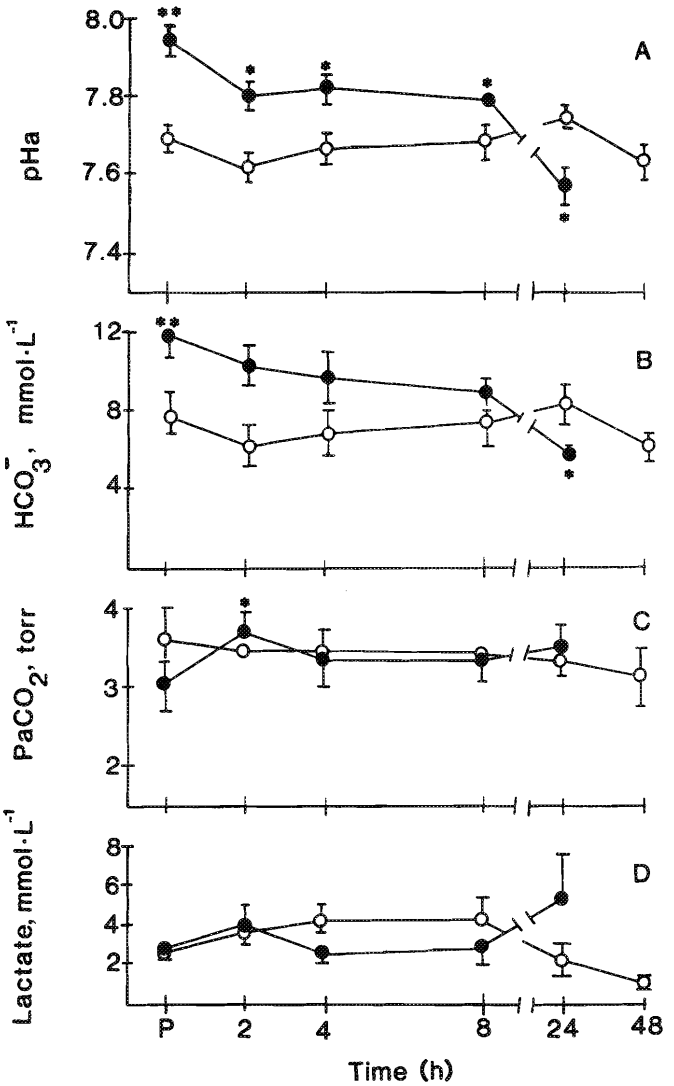

FIG. 2. (A) Arterial pH (pHa), (B) true plasma [HCO_3^-], (C) calculated arterial P_{CO_2} ($PaCO_2$), and (D) plasma [lactate] of Al-naive (closed circles) and Al-preexposed (open circles) brook trout prior to (P) and during acid/Al challenge (pH = 5.2, Al = 333 $\mu g \cdot L^{-1}$). Values are means ± 1 SEM. The number of observations diminished during the experiment due to deaths (*n* ranged from 5 to 3 for Al-naive and from 9 to 3 for Al-preexposed fish). *Significant difference from the respective prechallenge mean; **significant difference between group means prior to challenge ($p \leq 0.05$).

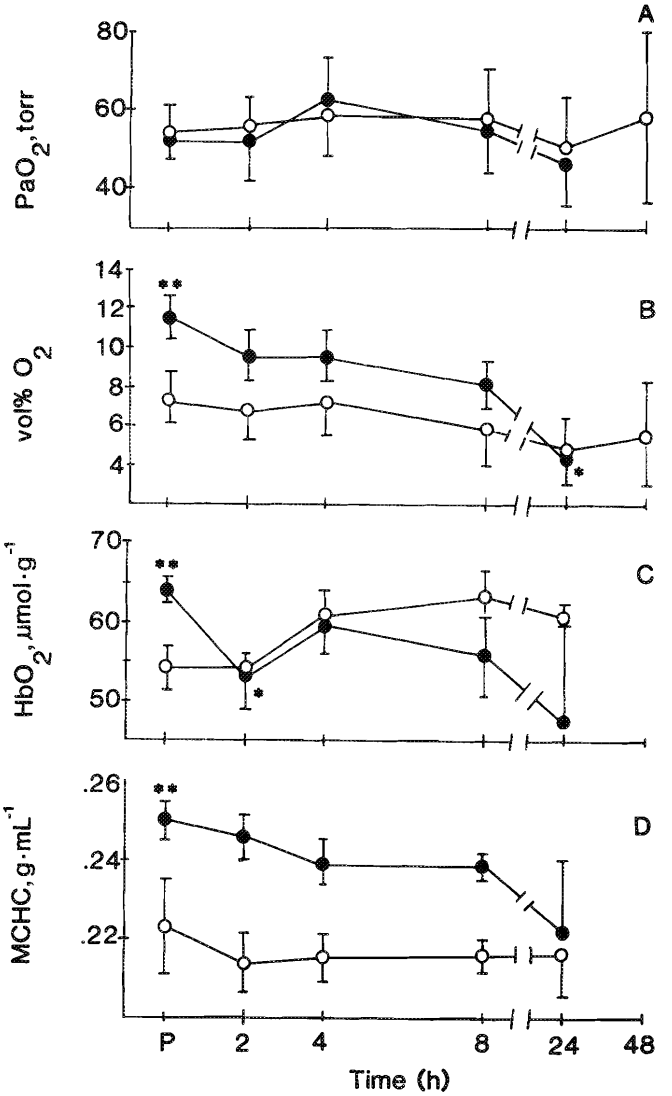

FIG. 3. (A) Arterial P_{O_2} (PaO_2), (B) vol% O_2, (C) hemoglobin O_2 content (HbO_2), and (D) mean cell hemoglobin concentration (MCHC) of Al-naive (closed circles) and Al-preexposed (open circles) brook trout prior to (P) and during acid/Al challenge (pH = 5.2, Al = 333 $\mu g \cdot L^{-1}$). Values are means ± 1 SEM. The number of observations diminished during the experiment due to deaths (*n* ranged from 5 to 3 for Al-naive and from 8 to 3 for Al-preexposed fish). *Significant difference from the respective prechallenge mean; **significant difference between group means prior to challenge ($p \leq 0.05$).

result of chronic exposure. The reduced irritation would explain the absence of a hyperventilatory response.

Comparing the prechallenge blood parameters of the Al-naive and preexposed fish, it is apparent that preexposure to Al had no significant effect on either PaO_2 (Fig. 3B) or $PaCO_2$ (Fig. 2C), but altered the resting acid–base status (decreased pHa, [HCO_3^-]; Fig. 2A, 2B) and lowered the resting arterial oxygen content (Fig. 3B), HbO_2 (Fig. 3C), and MCHC. We anticipate from previous histological studies (Chevalier et al. 1985; Karlsson-Norrgren et al. 1986a, 1986b; Tietge et al. 1988; Mueller et al. 1991) that preexposure to Al would cause a thickening of the branchial water-to-blood diffusion distance. This would result from general hyperplasia and proliferation of mucus and chloride cells on the lammellar epithelium. As a consequence, despite the maintenance of PaO_2 and $PaCO_2$ under these conditions, reduced blood O_2 content and HbO_2 occur probably due to Bohr and Root effects associated with the lowered plasma pH and [HCO_3^-]. In other words, PaO_2 and $PaCO_2$ are maintained but the O_2 carrying capacity, CO_2 carrying capacity, and buffer capacity of the blood are all reduced. As $\dot{M}O_2$ was unaffected (Fig. 1D), either cardiac output must be greater or the venous O_2 reserve must be reduced in these Al-preexposed fish.

The challenge ultimately resulted in significant acid–base and ionic disturbances in both groups of fish as revealed in the terminal samples (Table 2). Although the responses of the Al-naive fish to Al challenge reported here were similar to those reported by Wood et al. (1988c), there were some discrepancies regarding the responses of the Al-preexposed fish, in par-

ticular the decrease in plasma [Cl^-] and the deterioration of the blood acid–base status seen here as a result of the challenge. In addition, Wood et al. (1988a, 1988c) and McDonald et al. (1991) both reported substantial improvements in survivorship of brook trout preexposed to sublethal levels of Al when challenged with a lethal combination of acid/Al. The present study demonstrated much smaller and nonsignificant differences with regard to LT50. We suspect that a major factor in these discrepancies was the combined stresses of masking and cannulation in the present study.

With regards to the original hypotheses, although we were not able to unequivocally demonstrate an increase in survivorship to Al challenge as a result of sublethal Al preexposure, some ameliorative effects were apparent, most notably the elimination of hyperventilation during severe Al challenge.

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