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The effects of acid and acid/aluminum exposure on circulating plasma cortisol levels and other blood parameters in the rainbow trout, *Salmo gairdneri*

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Softwater ($Ca^{2+} = 50$, $Na^{+} = 50$ μequiv. l^{-1}) acclimated rainbow trout were fitted with chronic arterial catheters to allow for repetitive blood sampling. After 48 h recovery they were then exposed to either control (pH 6·5, Al = 0 μg l^{-1}), acid (pH 4·8, Al = 0 μg l^{-1}) or acid plus aluminum (pH 4·8, Al = 112 μg l^{-1}) conditions for 72 h. Parameters measured included blood glucose, lactate, haemoglobin, haematocrit and plasma Na^{+}, Cl^{-}, protein and cortisol.

Exposure to pH 4·8 alone caused no mortality, a moderate ionoregulatory disturbance and a transient elevation in plasma cortisol. All other parameters were not significantly different from controls. Addition of aluminum to this exposure caused 100% mortality with a mean survival time of only 27·0 h. There was a marked decrease in plasma ions, hyperglycemia, lactate accumulation, haemoconcentration, red cell swelling, and a sharp rise in plasma cortisol becoming greatly increased as the fish neared death. The mechanism of toxicity of acute acid/aluminum exposure, the role for cortisol under such conditions, and the validity of cortisol and glucose as indicators of stress in fish are discussed.

I. INTRODUCTION

The acidification of softwater lakes and streams such as those present in eastern North America and Scandinavia has been documented extensively (Beamish & Harvey, 1972; Leivestad *et al.*, 1976; Scheider *et al.*, 1979; Dillon *et al.*, 1984). Fish kills have been noted during periods of acid loading such as those seen during snowmelt or after a heavy rainfall (Leivestad & Muniz, 1976; Harvey, 1979; Harvey & Lee, 1982; Muniz, 1984; Marmorek *et al.*, 1985). The physiological effects of low pH exposures representative of episodic acid 'surges' (pH 4·0–4·5) in soft water have been studied extensively in salmonids (Muniz & Leivestad, 1980*a*; McWilliams, 1980; McDonald *et al.*, 1980; Wood & McDonald, 1982; McDonald, 1983*a*,*b*; McDonald *et al.*, 1983); this research has indicated that the primary toxic mechanism of pure acid exposure at this pH in soft water is ionoregulatory failure at the branchial surface, leading to secondary fluid volume disturbances, haemoconcentration, and circulatory collapse (see Wood, 1988, for a recent review).

However, many fish kills have been noted at higher pH levels than can be accounted for by studies concerning pure acid exposure alone (Schofield & Trojnar, 1980; Muniz & Leivestad, 1980*a*). Recently, the involvement of heavy metals in exacerbating the toxicity of low pH has been documented (reviews by Spry *et al.*, 1981; Campbell & Stokes, 1985; McDonald *et al.*, 1988). Aluminum has been implicated as one of the main elements responsible for this increased toxicity (Leivestad *et al.*, 1980; Muniz & Leivestad, 1980*a*,*b*; Grahn, 1980; Baker &

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Schofield, 1982; Dickson, 1983; Henriksen *et al.*, 1984). At low pH, inorganic aluminum is leached from the soils and bedrock, thus increasing aquatic aluminum concentrations. Aluminum appears to be maximally toxic to fish around pH 5·0.

Only a limited amount of work has been carried out on the physiological effects of acid plus aluminum stress (Muniz & Leivestad, 1980*a*,*b*; Rosseland, 1980; Stuarnes *et al.*, 1984; Neville, 1985; Witters, 1986; Wood, 1988). There appear to be at least two mechanisms of aluminum toxicity, the relative importance of which may vary with environmental pH and calcium levels. One mechanism appears to be an exacerbation of the acute ionoregulatory failure caused by acid stress alone, while the other mechanism seems to be an impairment of respiratory gas exchange at the branchial surface.

A primary response of all vertebrates to stress is the increased production of corticosteroids (Mazeaud *et al.*, 1977; Donaldson, 1981). The presence of a pituitary–interrenal axis has been proved conclusively for teleost fish, and the primary corticosteroid released is cortisol (Donaldson, 1981). Cortisol influences an array of physiological parameters, including carbohydrate and hydromineral balance, mobilization of amino and fatty acids from cellular stores, gluconeogenisis and plasma protein production. Increases in plasma cortisol levels and the often-associated rise in blood glucose concentration have been widely employed to assess the extent of stress in fish (for reviews, see Strange *et al.*, 1977; Mazeaud *et al.*, 1977; Wedemeyer & McLeay, 1981; Donaldson, 1981). Under acute pure acid exposure (pH 4·0–4·5), significant increases in both parameters have been documented within the first 24–48 h, but at higher pHs (>4·7), the responses took at least 4 days to develop, if they occurred at all (Mudge *et al.*, 1977; McDonald, 1983*b*; Lee *et al.*, 1983; Adams *et al.*, 1985; Brown *et al.*, 1984; Brown *et al.*, 1986*a*; Brown *et al.*, 1986*b*; Scherer *et al.*, 1986). To date, there has been no study on these parameters in fish under acid plus aluminum exposure.

The goal of the present study was to separate the physiological effects of aluminum from those of acid alone, with particular emphasis on the cortisol and glucose responses. Plasma sodium and chloride were measured as indices of ionoregulatory disturbance, haematocrit, haemoglobin and plasma protein as indices of fluid volume disturbance, and blood lactate as an index of oxygen uptake disturbance. The approach adopted was to examine the responses to acid alone and acid plus aluminum over the first three days of exposure at pH 4·8, where the acute effects of acid alone were expected to be small. The fish were fitted with chronic indwelling catheters to allow repetitive blood sampling with minimal disturbance. The experiments were conducted in artificial soft water resembling natural soft water in acid-threatened regions of eastern North America and Scandinavia. A relatively low level of aluminum ($112\,\mu g\,l^{-1}$) was employed, representative of concentrations documented during episodic acid surges in the wild (Christopherson *et al.*, 1984; Marmorek *et al.*, 1985).

II. METHODS

EXPERIMENTAL ANIMALS

Rainbow trout, *Salmo gairdneri* Richardson, (180–340 g) were obtained from Spring Valley trout farm, Petersburg, Ontario and held in flowing dechlorinated Hamilton city tap water (hard water: $Ca^{2+} = 1800$, $Na^{+} = 650\,\mu equiv.\,l^{-1}$). They were fed *ad libitum* with

commercial trout chow during this time. Fish were taken from this medium 14–24 days prior to experimentation and allowed to acclimate to flowing artificial soft water in large (350 l) darkened containers. Artificial soft water was prepared by reverse osmosis (Culligan Corp.) followed by readdition of Na^+ (NaCl) and Ca^{2+} ($CaCl_2$) to levels typical of acid-threatened softwater lakes and streams in eastern North America and Scandinavia (Spry *et al.*, 1981; Christopherson *et al.*, 1984; Dillon *et al.*, 1984). Acclimation water conditions were as follows: $Ca^{2+} = 52{\cdot}00 \pm 0{\cdot}61$ μequiv. l^{-1}, $Na^+ = 53{\cdot}80 \pm 0{\cdot}50$ μequiv. l^{-1}, temperature $= 14{\cdot}5 \pm 0{\cdot}5°$ C, pH $= 6{\cdot}53 \pm 0{\cdot}10$ (mean ± S.E.M.; $n = 119$). Trout were starved for 2 weeks prior to the start of the experimental period to minimize the effects of nutrition on circulating cortisol levels, and a constant 24-h light photoperiod was employed to minimize diurnal rhythms (Bry, 1982; Rance *et al.*, 1982).

TEST CONDITIONS

All experiments were conducted in a temperature-controlled ($14{\cdot}5 \pm 0{\cdot}5°$ C) continuous-flow water system. Artificial soft water from a 36-l common head tank supplied four darkened plexiglass chambers (2·6 l volume) in which cannulated fish were isolated. Each chamber received a flow of approximately $0{\cdot}5\,l\,kg^{-1}\,min^{-1}$ and each had separate aeration. Flow of water was unidirectional so that the water entered the box at the fish's head and left at the tail. Effluent was drained to waste. No metal parts were in contact with any part of the experimental set-up. Unidirectional flow without recirculation is necessary for experiments involving aluminum, in order to minimize the complex changes in speciation which can occur due to aging of the solutions, aluminum precipitation, organic complexation, and pH changes in the bulk water (LaZerte, 1984).

EXPERIMENTAL PROTOCOL

To allow repetitive blood sampling, trout were anaesthetized in acclimation water (MS-222; Sigma, 1:15 000, pH buffered back with KOH to pH 6·5). The trout were then surgically fitted with dorsal aortic cannulae filled with heparinized Cortland saline (Wolf, 1963; 50 iu ml^{-1} sodium heparin; Sigma) according to the method of Soivio *et al.* (1972). The fish were allowed to recover in the experimental chambers for at least 48 h prior to time zero sampling. All experiments were started between 07.30 and 09.00 hours to minimize any confounding effects of diurnal rhythms on cortisol levels. Care was exercised to disturb the fish as little as possible during both the recovery and experimental periods. Repetitive blood samples (550 μl) were taken at the following times: 0 (control), 1·5, 4, 7, 15, 24, 39, and 72 h after the start of the experimental exposures. Each sample was immediately replaced with an equal volume of non-heparinized Cortland saline (Wolf, 1963). The arterial blood samples drawn were analyzed for whole blood glucose, lactate, haematocrit, and haemoglobin and plasma sodium, chloride, protein and cortisol levels.

One control and two experimental conditions were examined.

(i) *Control*

Trout ($n = 13$) were exposed to acclimation media (pH 6·5) and blood sampling was performed as outlined in the protocol above. This experiment was performed to elucidate the effects of the sampling procedure on the various blood parameters.

(ii) *Acid alone*

Trout ($n = 12$) were exposed to nominal low pH 4·8 by titration of a head tank with 0·2 N H_2SO_4 (Radiometer PHM 82 pH meter with TTT80 autotitrator). Reverse osmosis had removed virtually all the bicarbonate from the water, and this, together with vigorous aeration in the head tank, ensured that there was no increase in P_{CO_2} accompanying acidification. The pH of the effluent water tended to rise as it passed through the fish chambers, probably due mainly to the production of NH_3 by the fish. Therefore, the incurrent pH was set slightly below the nominal pH to compensate for this increase. Measured mean pH in the boxes was $4{\cdot}82 \pm 0{\cdot}05$ ($n = 96$) as determined by a Radiometer PHM 82 pH meter using a Radiometer GK2401C electrode.

(iii) *Acid plus aluminum*

Trout ($n = 17$) were exposed to the same low pH as in procedure (ii) with the nominal addition of 100 $\mu g\,l^{-1}$ of aluminum [$AlCl_3(H_2O)_6$]. The aluminum was delivered to the head tank at a constant rate by a peristaltic pump from a concentrated (250 mg l^{-1}), acidic

(pH 4·0) stock solution. Vigorous aeration ensured complete mixing. Water samples were periodically taken during the experimental procedure and later analyzed for aluminum by flameless atomic absorption spectrophotometry using a Varian GTA-95 graphite tube atomizer. Mean measured aluminum concentration was $112 \pm 2{\cdot}53$ μg l^{-1} ($n = 63$).

Prior to the start of the experimental procedures, flow to the chambers was temporarily interrupted for a period of 0·5–1·5 h while the head tank was titrated down to pH 4·8. Simultaneously, an appropriate amount of the stock solution was added to bring the aluminum levels in the head tank quickly up to experimental levels. During this period, air saturation and pH 6·5 were maintained in the fish chambers.

ANALYTICAL TECHNIQUES

Blood samples were drawn from the dorsal aortic cannulae, using ice-cold Hamilton syringes pre-rinsed with heparinized saline. Haematocrit was immediately measured on an 80-μl sample, using ammonium heparinized haematocrit tubes after 5-min centrifugation at 5000 × **g**. Plasma [Na^+] was determined on duplicate 15-μl samples taken from the plasma present in the haematocrit tubes, diluted in 15 ml 0·2% HNO_3, and saved for later analysis on a Varian AA1275 atomic absorption spectrophotometer. Haemoglobin concentration was determined as cyanmethaemoglobin (Blaxhall & Daisley, 1973) by adding 20 μl of whole blood to 5 ml of Drabkins solution (*Sigma Bulletin No. 525*). Lactate was measured enzymatically (L-lactate dehydrogenase/NADH method; *Sigma Bulletin No. 340-UV*; Loomis, 1961) on 100-μl samples of whole blood which had been immediately deproteinized in 200 μl of ice-cold perchloric acid (8%) and centrifuged at 9000 × **g** for 2 min. Samples were stored at 4° C and later analyzed as per *Sigma Bulletin No. 340-UV*. Whole blood glucose levels were determined by the colourimetric o-toluidine method of Hyvarinon & Nikkita (1962; *Sigma Bulletin No. 635*); 70 μl of whole blood were added to 630 μl of ice-cold trichloroacetic acid and centrifuged for 2 min at 9000 × **g**.

The remainder of the blood sample was then centrifuged for 2 min at 9000 × **g**, the plasma was drawn off, and the blood cells discarded. Plasma chloride determinations were made by coulometric titration using a Radiometer CMT-10 chloride titrator. Plasma protein concentration was determined on 10-μl samples, using a Goldberg hand-held refractometer (American Optical; Alexander & Ingram, 1980). The remainder of the plasma sample was then frozen immediately in liquid nitrogen and stored at −80° C for later measurement of plasma cortisol levels. Cortisol levels were measured in duplicate by ^{125}I-radioimmunoassay using the 'immophase' RIA kit (Corning Medical) according to the manufacturer's instructions.

The mean cell haemoglobin concentration (MCHC) was determined by dividing the haemoglobin concentration by the haematocrit. This measure was used to assess the amount of red cell swelling present (Milligan & Wood, 1982).

STATISTICAL ANALYSIS

Data are reported as means ± S.E.M. (n). Significant differences ($P < 0{\cdot}05$) within each group were tested with Student's two-tailed t-test (paired design) using the time zero values for each fish as its own control. Comparisons between groups were tested by Student's two-tailed t-test (unpaired design, $P < 0{\cdot}05$). Significant values are indicated with an asterisk.

III. RESULTS

MORTALITY AND DATA ANALYSIS

All the fish in the control and acid-alone exposures survived throughout the 72-h experimental period, apart from the few which died by mishap (e.g. cannulae failure). Therefore, the means ± 1 S.E.M. (control, $n = 12$; acid alone, $n = 13$) are reported for all 72-h survivors from these groups at each sample time. However, the acid-plus-aluminum exposure proved to be highly toxic and none survived to 72 h; the fish succumbed at different times and the mean time of death was $27{\cdot}0 \pm 2{\cdot}9$ h ($n = 17$). Death was preceded by marked hyperventilation, coughing,

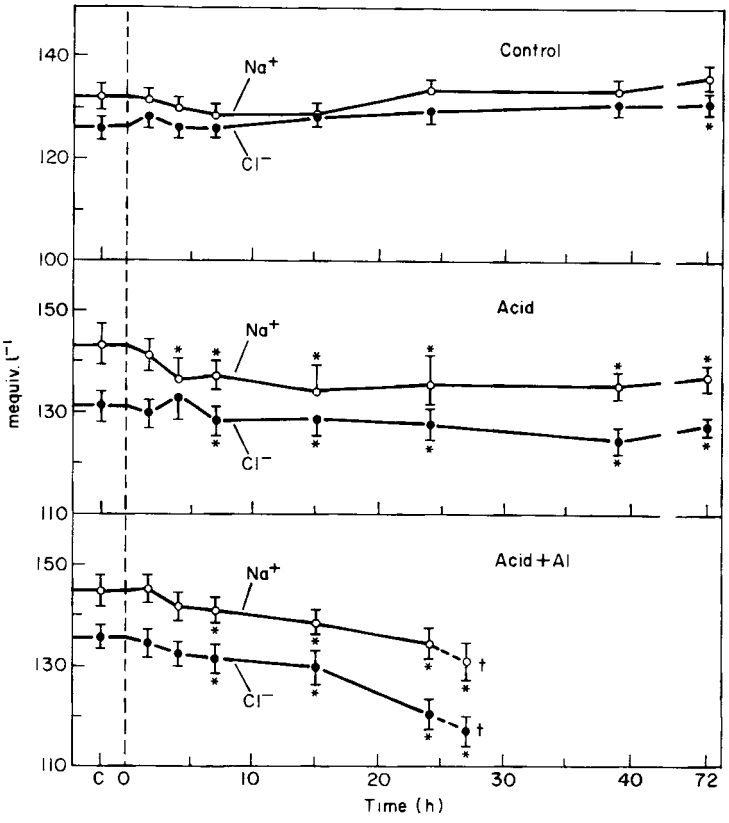

FIG. 1. Plasma sodium and chloride levels (means ± 1 S.E.M.) in the arterial blood of chronically cannulated rainbow trout exposed to three different conditions: control ($n = 13$), acid alone (pH = 4·82; $n = 12$) and acid plus aluminum (112 μg/l; $n = 12$) for the 24 h survivors. In addition, the mean ± 1 S.E.M. of the last measurement for all acid/aluminum-exposed fish taken prior to death ($n = 17$) was plotted at 27·0 h, the mean time of death. Asterisks indicate means significantly different ($P < 0·05$) from their respective initial means.

and discolouration of the skin. The means ± 1 S.E.M. of those fish surviving until 24 h ($n = 12$) were plotted to that point. In addition, the means ± 1 S.E.M. of the last measurements taken prior to death were plotted for all fish ($n = 17$) at 27·0 h. Thus the curves for the acid/aluminum-exposed trout represent the responses of the most resistant fish, while the terminal point represents the physiological state of the fish just prior to death.

PLASMA IONS (Fig. 1)

The only influence of serial sampling on major plasma electrolytes in the control group was a small rise in plasma [Cl^-] at 72 h, probably attributable to the effects of the saline replacement. Exposure to low-level acid alone (pH 4·82) resulted in a minor but significant decrease in both plasma [Na^+] and [Cl^-] over the first 7 h; thereafter the levels were more or less stable. The presence of 112 μg l^{-1} aluminum exacerbated this ionic disturbance, and electrolyte levels continued to decline until death ensued. However, the terminal concentration ([Na^+] = 131 ± 3·2, [Cl^-] = 117 ± 3·0 mequiv. l^{-1}, 17) were only about 10% below control levels.

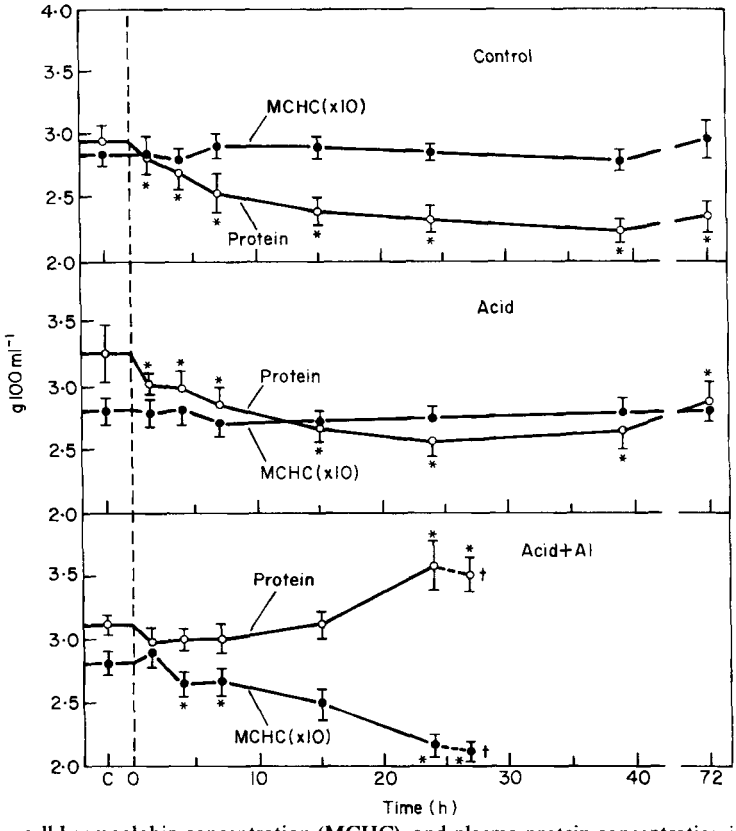

FIG. 2. Mean cell haemoglobin concentration (MCHC), and plasma protein concentration in the arterial blood of chronically cannulated rainbow trout exposed to three different conditions: control, acid alone and acid plus aluminum. Other details as in legend to Fig. 1.

HAEMATOLOGY AND PLASMA PROTEIN (Fig. 2, Table I)

Haematocrit, haemoglobin, and plasma protein levels fell in the control groups as a result of blood removal and subsequent saline replacement. However, MCHC remained constant, indicating an absence of red cell swelling or shrinkage. The relative fall in plasma protein was much less than in either haematocrit or haemoglobin, suggesting partial replacement of plasma protein by the fish over the time course of the experiment. There were no significant deviations from these patterns in the acid-alone exposure group. In contrast, there was evidence of a marked haemoconcentration and red cell swelling as a result of acid-plus-aluminum exposure. Haemoglobin fell to a much lesser extent, and haematocrit and plasma protein levels increased significantly after 15 h despite the influence of serial sampling. MCHC declined progressively, reaching a level 75% of the initial resting value immediately prior to death.

BLOOD GLUCOSE AND LACTATE (Fig. 3)

In the control group, blood glucose declined significantly, during the experiment, stabilizing after 15 h. This may have been due to the fact that, for reasons unknown, initial values were significantly higher than in the other two procedures.

TABLE I. Haematocrit and haemoglobin changes in chronically cannulated rainbow trout under control condition and during acid (pH 4·82) and acid-plus-aluminum exposure (Al = 112 μg l^{-1}) means ± 1 S.E.M.

	0 h	24 h	72 h	Terminal
Haematocrit				
Control	24·4	13·6*	12·0*	—
(n = 12)	±1·8	±1·1	±0·9	—
Acid alone	27·2	15·8*	13·6*	—
(n = 13)	±1·9	±1·6	±1·4	—
Acid/Aluminum	23·7	25·8	—	26·1 t
(n = 12)	±1·9	±1·8	—	±1·8
Haemoglobin				
Control	6·87	3·85*	3·46*	—
(n = 12)	±0·50	±0·31	±0·41	—
Acid alone	7·24	4·34*	4·05*	—
(n = 13)	±0·39	±0·43	±0·38	—
Acid/Aluminum	6·65	5·69*	—	5·77 t
(n = 12)	±0·57	±0·43	—	±0·58

*P < 0·05 relative to 0 h value.
t = last measurement prior to death in acid/aluminum exposure (n = 17).

Exposure to low level acid alone had no influence on blood glucose levels, but the presence of aluminum induced a rise, which was significant by 4 h, and reached a level 50% higher than the initial value immediately prior to death. Initial blood lactate concentrations were very low (< 0·2 mmol l^{-1}) in all groups. There was relatively little change in blood lactate concentrations in either the control or the acid-alone exposure groups, with only mild increases at 4–7 h. In contrast, the acid-plus-aluminum exposure group showed an immediate rise in lactate which continued to increase until death ensued. The mean terminal level was 4·85 ± 1·40 mmol l^{-1} (17), with concentrations in some fish exceeding 20 mmol l^{-1}.

PLASMA CORTISOL (Fig. 4)

Circulating plasma cortisol levels were not significantly altered from the time zero values in the control procedure although there was a slight trend to increasing values to the 7-h mark, thereafter decreasing as time between samples increased. In the acid-alone exposure group, the plasma cortisol became significantly elevated by approximately 70% at the 4-h mark, but by 15 h the levels had returned to resting values. Exposure to acid plus aluminum produced a much more rapid increase in plasma cortisol which was already significant at the first sample time (1·5 h). This response paralleled the response to acid stress until 15 h. Thereafter, as the fish neared death, the plasma cortisol was further elevated to almost five-fold higher than resting values, indicating a severe stress response.

IV. DISCUSSION

Acute exposure to a relatively low level of aluminum (112 μg l^{-1}) at pH 4·82 in artificial soft water proved extremely toxic to adult rainbow trout, with an average

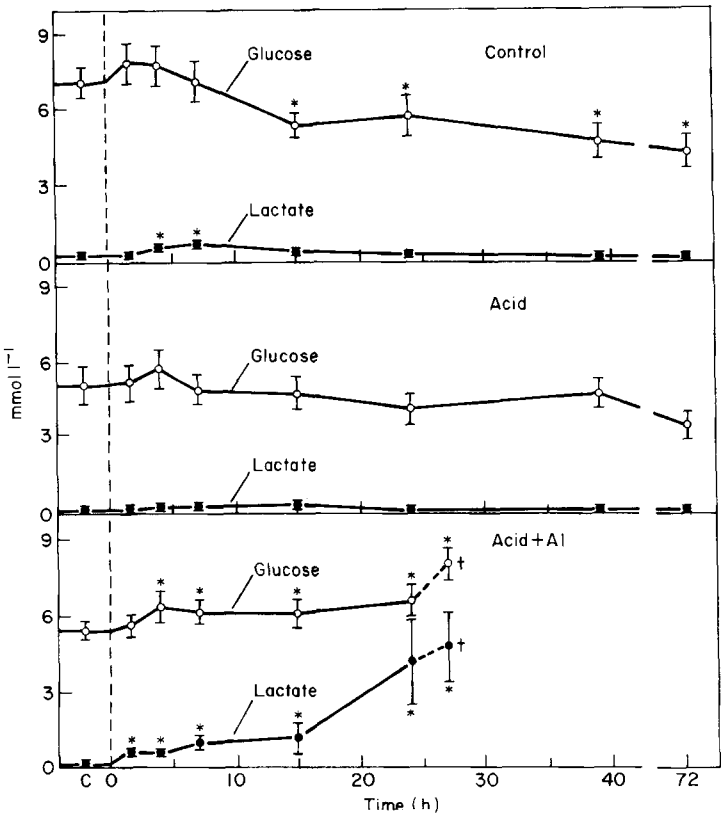

FIG. 3. Blood glucose and lactate concentrations in the arterial blood of chronically cannulated rainbow trout exposed to three different conditions: control, acid alone and acid plus aluminum. Other details as in legend to Fig. 1.

survival time of only 27·0 h. Acid exposure alone at this pH caused no mortality over 72 h. While it is recognized that *S. gairdneri* is the most sensitive of the salmonids (Grande *et al.*, 1978), the toxicity of aluminum appeared greater than in previous studies on this species (Neville, 1985; Orr *et al.*, 1986). The difference can be explained by the very low ionic concentrations (especially $Ca^{2+} = 52$ μequiv.l^{-1}) of the artificial soft water used in the present study (Brown, 1983; McDonald *et al.*, 1988) and by the fact that our flow-through exposure minimized complexation, ensuring that virtually all aluminum stayed in the toxic monomeric form (LaZerte, 1984). At pH 4·82, this monomeric aluminum would be about 50% Al^{3+} and about 50% various aluminum hydroxides (McDonald *et al.*, 1988).

The physiological responses to acid plus aluminum exposure included ionoregulatory disturbance, lactate accumulation, haemoconcentration, increased glucose levels, and greatly elevated cortisol levels. The effects of acid alone were much less severe, with only slight ionoregulatory disturbance and transiently elevated cortisol levels. There was no significant lactate accumulation, glucose elevation or haemoconcentration.

Ionoregulatory disturbance has been shown to be directly related to the pH to which the fish is exposed (Lee *et al.*, 1983; Giles *et al.*, 1984), with active branchial

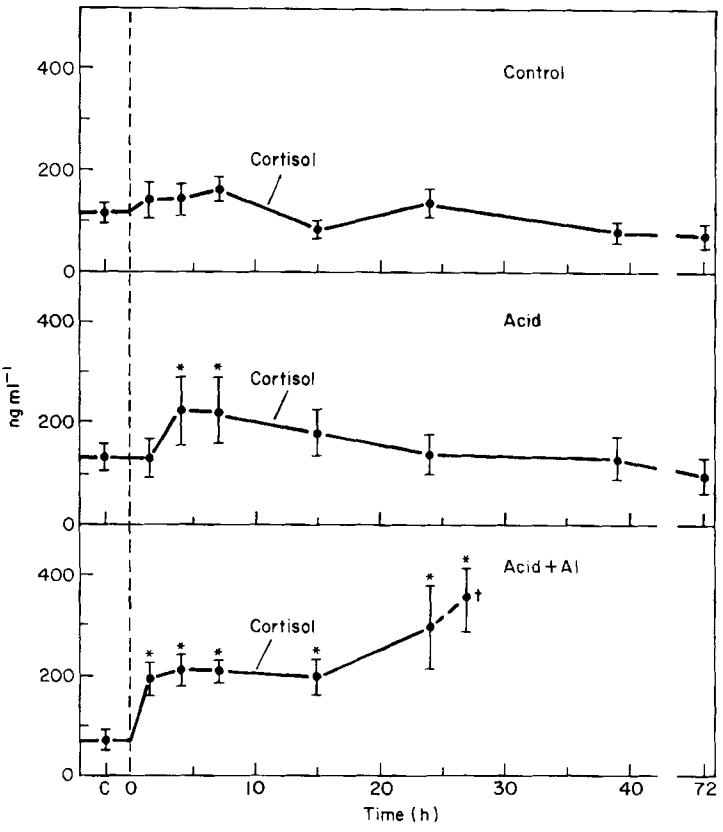

FIG. 4. Plasma cortisol concentration in the arterial blood of chronically cannulated rainbow trout exposed to three different conditions: control, acid alone and acid plus aluminum. Other details as in legend to Fig. 1.

ion influx being more sensitive than passive efflux (Wood, 1988). At pH 4·82, the small plasma $[Na^+]$ and $[Cl^-]$ depressions were consistent with the data of Lee *et al.* (1983) and Giles *et al.* (1984), and probably were caused mainly by a partial inhibition of influx. The exacerbation of ion loss by aluminum was in agreement with the results of Neville (1985) and Witters (1986). Extrapolating from findings on brook trout, *Salvelinus fontinalis*, (C. E. Booth, D. G. McDonald, B. P. Simons & C. M. Wood, unpubl.), the mechanism appears to be largely a stimulation of passive efflux, with a small contribution from increased blockade of influx, possibly due to an inhibition of the Na^+/K^+ ATPase pump (Stuarnes *et al.*, 1984). While probably a contributing factor, it is unlikely that ionoregulatory disturbance was the proximate cause of death, as terminal plasma $[Na^+]$ and $[Cl^-]$ depressions ($\simeq 10\%$) were less than those generally seen in rainbow trout ($\simeq 30\%$) dying of acid stress alone (Wood & McDonald, 1982; McDonald, 1983*a*; Wood, 1988). Recently, we have seen that rainbow trout under similar conditions of acid-plus-aluminum exposure exhibit severe decreases in arterial P_{O_2}, increases in arterial P_{CO_2} and combined respiratory and metabolic acidosis, while under acid exposure alone such problems were minimal (R. C. Playle, G. G. Goss & C. M. Wood, unpubl.). We suggest that inhibition of respiratory gas exchange, resulting

in hypoxemia, hypercapnia and acidosis, was a major cause of death in aluminum-exposed fish of the present study.

Blood lactate levels rose and MCHC fell in response to the acid-plus-aluminum exposure yet were not significantly altered by acid alone. The precipitation of $Al(OH)_3$ and other aluminum species may act as irritants on the gills of the fish, causing increased mucus accumulation on the gill lamellae (Muniz & Leivestad, 1980*a*; Baker & Schofield, 1982; Neville, 1985). At concentrations greater than the solubility limit of aluminum, the precipitation of $Al(OH)_3$ is very rapid, especially around pH 5·6 where aluminum solubility is minimal. Neville (1985) and Wood (1988) have suggested that the more alkaline local environment at the gill surface, due to acid uptake/base excretion and NH_3 production, acts to precipitate $Al(OH)_3$ onto the gill surface, causing increased mucus production. This increase in mucus thickness causes the diffusion distance between the water and the blood haemoglobin to increase. Since diffusion is inversely proportional to the square of the distance, a slight increase in diffusion distance rapidly impairs O_2 and CO_2 exchange. The significant increase in blood lactate strongly suggests a decreased O_2 delivery to the tissues and a concomitant rise in anaerobic metabolism. Haematocrit and haemoglobin levels were high enough to eliminate these parameters as a possible cause of the lactate accumulation. The marked red cell swelling (decreased MCHC) suggests plasma acidosis in addition to ionic dilution (Milligan & Wood, 1982).

Blood glucose levels have long been used as indicators of stress in fish (Hattingh, 1976; Donaldson, 1981; Wedemeyer & McLeay, 1981). It is generally thought that, under conditions of stress, hyperglycemia may provide additional energy during times of high metabolic need such as a ' fight or flight ' response. In addition, it may serve as a mechanism to maintain osmolarity in the face of declining plasma ion levels (McDonald, 1983*b*; Brown *et al.*, 1984). In the present study, blood glucose levels remained unchanged upon exposure to pH 4·82 alone for 72 h. These results are consistent with those obtained by other researchers (see Introduction) who found that blood glucose elevation took more than 4 days to occur at pHs $>4·7$, although it was rapid at pHs 4·0–4·5. However, the presence of aluminum at pH 4·82 caused blood glucose to increase within 4 h. This is consistent with the larger and more rapid elevations in plasma cortisol, the significant lactate build-up, and the greater ionoregulatory disturbance seen in this exposure group. These results support the theory that plasma cortisol mediates glycogenolysis and that the resultant hyperglycemia aids in regulation of plasma osmolarity.

It is known that cortisol is released from the interrenal tissue as a mechanism of coping with stress (Donaldson, 1981). However, the interpretation of plasma cortisol changes may be confounded by the stress of catheterization, by elevations caused by repetitive blood sampling (Brown *et al.*, 1986*a*) and by diurnal rhythms (Bry, 1982; Rance *et al.*, 1982). According to Brown *et al.* (1986*a*), the 48-h post-cannulation recovery period was sufficient to eliminate the first of the confounds, while the control experiment indicated that the latter two were not problems in the present study. The constant 24-h light photoperiod and starvation probably reduced any diurnal rhythms, while the absence of a sampling effect may be explained by the fact that cortisol levels were already rather high in the resting fish. This in turn may be due to the influence of soft water acclimation (Perry & Wood, 1985).

Plasma cortisol levels showed a transient increase under acid stress alone but had reverted to resting levels by the 24-h mark. This agrees with the recent findings of Barton *et al.* (1985) on *S. gairdneri* exposed to acid alone (pH 4·7–5·7) under similar conditions. Brown *et al.* (1984, 1986*a*,*b*) showed long-term increases in plasma cortisol under acid stress (pH 4·7–5·2), but this difference was not noticeable until approximately 8 days. However, because their first sampling was not until 24 h, the transitory increase seen in the present study would not have been noticed. Mudge *et al.* (1977) showed that acute exposure to pH 4·0 produced only transitory increases in blood cortisol, while other researchers have also reported short-term increases in blood cortisol under severe acid stress (Adams *et al.*, 1985). In interpreting these responses, it must be remembered that kinetics as well as concentration may vary, and that changes in cortisol turnover rates may be greater than indicated by simple concentration figures.

The presence of aluminum caused a similar but earlier initial rise in plasma cortisol compared to the acid exposure alone. However, rather than declining, this response was greatly exacerbated as the fish neared death. Similarly high plasma cortisol levels have been reported in other salmonids which were dying (Fagerlund, 1967; Strange *et al.*, 1977). The mobilization of cortisol under such stressors presumably has an adaptive advantage in aiding survival. In addition to its generally beneficial metabolic effects, cortisol may be of particular importance during acid and acid/aluminum stress in fighting ionic disturbances, as it has been shown to cause chloride cell proliferation on the branchial surface (Perry & Wood, 1985).

In summary, plasma cortisol concentration is a sensitive indicator of acute stress in the present study, with mildly stressed fish (pH 4·82) showing moderate increases, and highly stressed dying fish (pH 4·82, Al = 112 $\mu g\,l^{-1}$) showing large increases. However, the transitory nature of the response under mild stress makes the usefulness of this parameter doubtful in assessing longer-term, low-level stress. Donaldson (1981) suggests that this parameter may be particularly useful for determining stress levels in fish exposed to two or more sublethal stressors. On the basis of the results presented here, the use of cortisol as an indicator of lethal stress under condition such as acid (pH 4·82) plus aluminum (112 $\mu g\,l^{-1}$) is valid.

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