Do Rainbow Trout (*Salmo gairdneri*) Acclimate to Low pH?

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Adult rainbow trout (*Salmo gairdneri*) previously exposed to long-term sublethal acid stress (3 mo, pH 4.8) in artificial soft water (Ca^{2+} = 50, Na^+ = 50, Cl^- = 100 $\mu eq \cdot L^{-1}$) were challenged with acute severe acid exposure (4.5–5 h, pH 4.0). Their response in terms of whole-body ionic exchanges and blood chemistry was compared with that of trout that had no previous history of acid exposure (naive fish). Acute pH 4.0 exposure caused significant ionoregulatory disturbances in both acid-preexposed and naive fish. Rates of net Na^+ and Cl^- body losses were twice as large in acid-preexposed fish as in naive fish. The two groups showed similar slight net uptake of acidic equivalents. However, the dynamics of acid–base exchange differed, especially with regard to ammonia excretion which was elevated in acid-preexposed fish and inhibited in naive fish. A larger decrease in plasma Na^+, red blood cell swelling, and the maintenance of high-stress indicators (elevated plasma glucose and ammonia excretion, depressed osmolality) confirmed that osmo-ionoregulatory disturbances were more intense in acid-preexposed fish than in naive fish. Thus, long-term sublethal acid exposure did not improve but rather significantly decreased the ability of rainbow trout to respond to more severe acid stress. We conclude that acclimation to acid stress does not occur in rainbow trout.

Des truites arc-en-ciel adultes (*Salmo gairdneri*), préalablement exposées à un stress acide sous-létal de longue durée (3 mo, pH 4,8) en eau douce à faible teneur ionique (Ca^{+2} = 50, Na^+ = 50, Cl^- = 100 $\mu eq \cdot L^{-1}$), ont été exposées pour quelques heures à un pH acide plus sévère (stress acide aigü, 4,5–5 h, pH 4,0). Leur réponse, en termes d'échanges ioniques corporels et de paramètres sanguins, a été comparée à celle de truites n'ayant jamais connu de conditions acides. Une exposition aigüe à pH 4,0 a causé une perturbation des mécanismes iono-régulateurs chez les poissons pré-exposés ainsi que chez ceux non pré-exposés à l'acidité. Les taux de pertes nettes de Na^+ et de Cl^- ont été deux fois plus élevés chez les poissons pré-exposés à l'acidité. Une légère entrée nette d'équivalents acide a été observée chez les deux groupes mais la dynamique des échanges acide-base s'est avérée être différente pour chacun d'eux. Ainsi l'excrétion d'ammoniaque a été accrue chez les truites pré-exposées à l'acidité alors qu'elle était inhibée chez celles non pré-exposées. La chute plus prononcée de Na^+ plasmatique, le gonflement des globules rouges et le maintien d'un taux élevé d'indicateurs de stress (glucose plasmatique et excrétion d'ammoniaque élevés, osmolalité abaissée) ont confirmé que les truites pré-exposées à l'acidité connaissaient des perturbations plus importantes au niveau osmo-iono-régulation que celles non pré-exposées. L'exposition à un stress acide sous-létal prolongé n'a donc pas amélioré, mais décru, la capacité de la truite arc-en-ciel à répondre à un stress acide plus sévère. Nous concluons qu'il n'y a pas eu d'acclimatation à l'acidité chez la truite arc-en-ciel.

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Salmonids collected from naturally acidified soft water show greater physiological resistance to acid challenge in the laboratory (McWilliams 1980a, 1982, 1983; Brown 1981), but it is unclear whether this results from acclimation, or simply from natural selection, for acid tolerance is a heritable trait (Leivestad et al. 1976; Swarts et al. 1978). In general, the results of laboratory tests on acclimation have been negative or equivocal, but few have been performed under environmentally realistic water chemistry conditions (discussed in McWilliams 1980a, 1980b; Wood 1988).

In the accompanying paper (Audet et al. 1988), we have described the physiological effects on rainbow trout (*Salmo gairdneri*) of long-term sublethal acid exposure (3 mo at pH 4.8) in artificial soft water (ASW) closely duplicating water chemistry in the field. Following severe initial disturbances in ionoregulatory and related functions, most ionoregulatory parameters stabilized after the first few weeks at a new steady state. However, there was no indication of recovery back to control levels. The goal of the present study was to test whether this preexposure conferred increased tolerance when the fish were challenged with more severe acidity, which would constitute evidence for true acclimation. To test this hypothesis, we compared the response of ASW-acclimated rainbow trout held in circumneutral pH conditions (pH 6.5; naive fish) with trout exposed to long-term sublethal acid conditions (3 mo, pH 4.8, acid-preexposed fish) when both groups were submitted to acute, potentially lethal acid conditions (4.5–5 h, pH 4.0). These experimental conditions may simulate field conditions in some acidified streams where fish are chronically exposed to sublethal pH levels, with sudden brief pulses to acute lethal levels due to snowmelt or rainstorm runoff. Whole-body ion fluxes and related physiological parameters were examined to

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assess whether either the nature or the magnitude of responses to acute acid stress was different.

Methods

This study was performed concurrently with that of the accompanying paper (Audet et al. 1988) which provides complete information on fish holding, experimental protocols, and analytical methodology (unidirectional fluxes of Na^+ and Cl^-, net fluxes of Na^+, Cl^-, K^+, Ca^{2+}, ammonia, titratable acidity, acidic equivalents, plasma levels of Na^+, Cl^-, K^+, Ca^{2+}, glucose, osmolality, blood concentrations of hemoglobin, hematocrit, mean cell hemoglobin concentration (MCHC), and epaxial muscle water content). All fish were acclimated to ASW ($[Na^+]$ and $[Ca^{2+}]$ = 50, $[Cl^-]$ = 100 $\mu eq \cdot L^{-1}$) for 2–3 mo (pH 6.5 ± 0.3) prior to experiment. The temperature and photoperiod were kept constant at 15 ± 1°C and 24 h of light, respectively.

In the experiments reported here, two groups of adult rainbow trout (200–300 g) of both sexes were submitted to acute severe acid stress: 4.5–5 h in pH 4.0 (±0.1) ASW, acidified with H_2SO_4. Such exposure is invariably lethal within 0.5–3 d in previously unexposed trout. One group had been previously exposed to long-term sublethal acid conditions (3 mo at pH 4.8, as described by Audet et al. 1988) and were called "acid-preexposed fish." The other group was kept under circumneutral conditions (pH ≈ 6.5) prior to challenge with acute lethal acid exposure and were called "naive fish." Suitable control data prior to challenge for each group (i.e. acid-preexposed fish at pH 4.8; naive fish at pH 6.5) were furnished by the experiments described in Audet et al. (1988).

Fluxes were measured every hour from water samples taken during 4 consecutive h and final calculations based on the average of the four measurements. The total exposure time to pH 4.0 was close to 5 h taking into account the time to set up stable pH 4.0 conditions in the flux boxes, the addition of radioisotopes, and the 0.5 h left for the mixing of radioisotopes (see Audet et al. 1988). The fish were then individually anaesthetized and a terminal blood sample drawn by caudal puncture.

In additional sampling experiments (blood and tissue chemistry), individually labelled fish were transferred at time 0 to a 450-L tank, similar to the holding tank but supplied with ASW at pH 4.0. The fish were sampled after 4.5–5 h at pH 4.0. No significant differences within treatment groups were found for blood parameters taken from fish exposed in this manner versus those exposed in the flux boxes (t-test, $p \leq 0.05$), so the data were pooled. All samples were taken at times between 1330 and 1700. As in Audet et al. (1988), all the experiments were not done at the same time but controls were always run for each experimental group.

Statistics

The comparisons of control data between the two groups (i.e. acid-preexposed fish at pH 4.8; naive fish at pH 6.5) were drawn from Audet et al. (1988) (ANOVA, $p \leq 0.05$, followed by Tukey–Kramer test of comparison of means, $\alpha = 0.05$ (Sokal and Rohlf 1981)). All other statistical comparisons were done by t-test ($p \leq 0.05$). Data were tested for normality and for homogeneity of variances (Kolmogorov–Smirnov test and F_{max} test, respectively). All the data were normally distributed but some groups showed heterogeneous variances. In such cases, the t-test was then used with appropriate modifications (Sokal and Rohlf 1981).

Results

Ion Exchanges

Prior to acute acid exposure, net flux rates of the two major electrolytes, Na^+ and Cl^-, were similar in the two groups, although both J_{in} and J_{out} were significantly lower in the acid-preexposed fish (Fig. 1a, 1b). Whether or not the fish had been preexposed to sublethal acidity, acute exposure to pH 4.0 resulted in large net losses of Na^+ and Cl^- (Fig. 1a, 1b), consequent to an almost total inhibition of influx (J_{in}) and a tremendous increase of efflux (J_{out}). However, in acid-preexposed fish, the increase of J_{out} was almost twice that of the naive fish. This resulted in significantly larger net body Na^+ and Cl^- losses in the former. Net body K^+ losses also increased greatly during acute exposure in both acid-preexposed and naive fish (Fig. 1c); the increase was greater in the former, but the difference was not significant. We did not observe any significant disturbances of net Ca^{2+} flux in either group, the overall mean of net Ca^{2+} flux after 5 h at pH 4.0 being -11.1 ± 6.9 $\mu eq \cdot kg^{-1} \cdot h^{-1}$ (N = 22).

Similar net acidic equivalent uptake was observed in the two groups of fish prior to acute acid exposure (Fig. 1d). However, the titratable acidity and ammonia exchanges underlying these net acidic equivalent fluxes were very different in acid-preexposed and naive fish. Thus the elevated ammonia and titratable acidity fluxes caused by the pH 4.8 exposure persisted when acid-preexposed fish were submitted to pH 4.0. In contrast, this exposure resulted in a significant decrease of ammonia and no change in titratable acidity flux in naive fish. As a result, net acidic equivalent uptake increased in the naive fish during the pH 4.0 exposure whereas no changes occurred in the dynamics of acidic equivalent exchange in the acid-preexposed fish.

Blood and Tissue Chemistry

Control levels of plasma Na^+ and Cl^- were substantially lower in the acid-preexposed fish (Fig. 2a, 2b). A further drop in plasma Na^+ was observed following acute lethal acid exposure in acid-preexposed fish but not in naive fish (Fig. 2a). In acid-preexposed fish, the decrease in the plasma Na^+ concentration attributable to the pH 4.0 exposure was three times greater than the one observed in naive fish. Plasma Cl^- concentration decreased by approximately the same magnitude in the two groups (Fig. 2b). A slight increase in plasma Ca^{2+} (Fig. 2c) and a slight decrease in plasma K^+ (Fig. 2d) were observed in naive fish, while no changes occurred in acid-preexposed fish. Nevertheless, acid-preexposed and naive fish presented similar Ca^{2+} and K^+ plasma concentrations after 5 h of pH 4.0 exposure.

Prior to acute acid exposure, plasma osmolality (Fig. 3a) was significantly lower and plasma glucose (Fig. 3b) and protein levels (Fig. 3c) higher in the acid-preexposed trout. Despite the greater loss of plasma electrolytes (mainly Na^+; Fig. 2a) at pH 4.0 in the acid-preexposed group, there was no significant change in plasma osmolality in these fish, while in naive fish, plasma osmolality decreased (Fig. 3a). The response may have been obscured by greater variability in the preexposed group. Plasma glucose and plasma protein concentrations increased in naive fish in contrast with acid-preexposed fish for which no such effects were observed (Fig. 3b, 3c). However, plasma glucose and protein concentrations were already elevated to significantly higher levels as a result of acid preex-

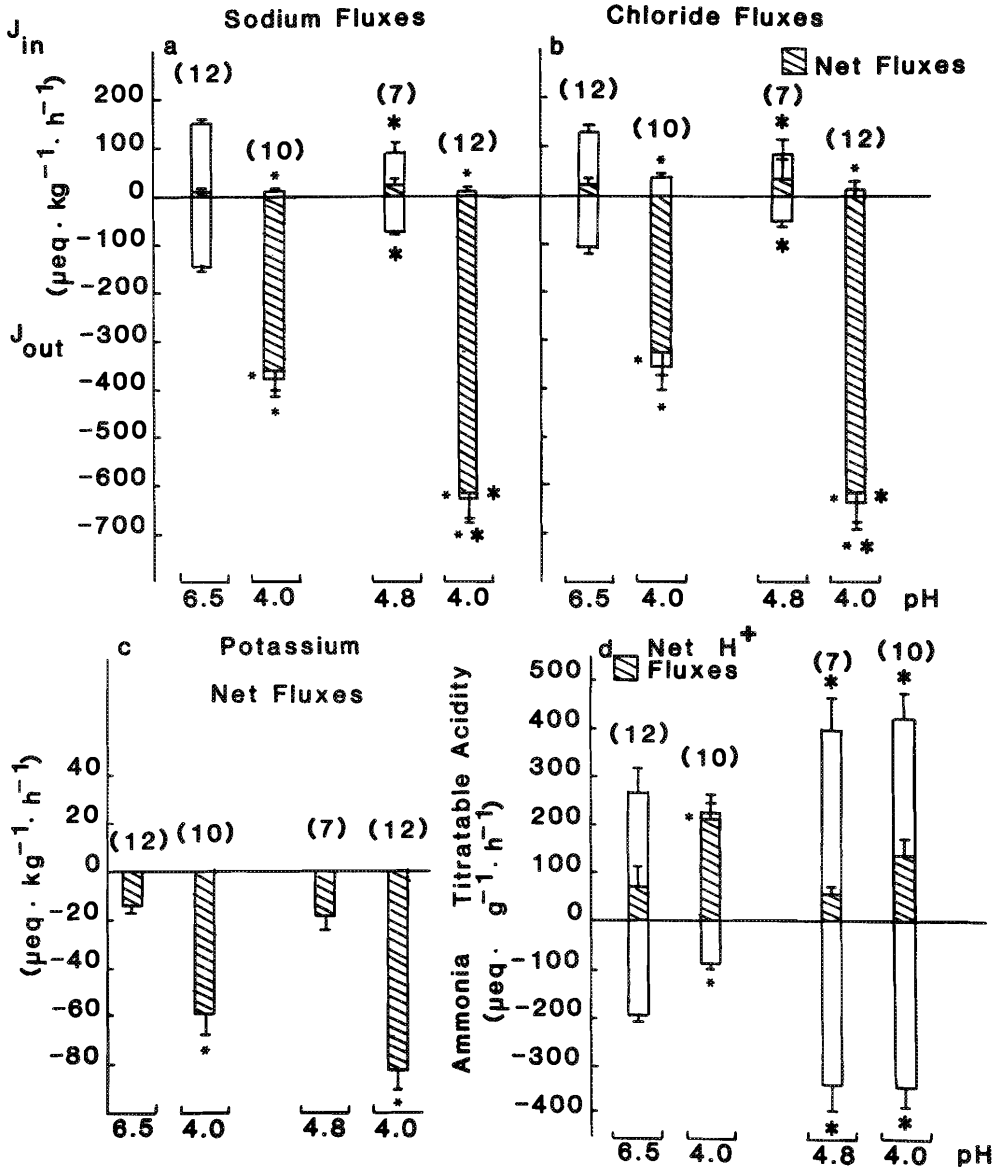

FIG. 1. Effects of acute pH 4.0 exposure on whole-body ion fluxes in naive and acid-preexposed rainbow trout. (a) Unidirectional and net sodium fluxes; (b) unidirectional and net chloride fluxes; (c) net potassium fluxes; (d) net fluxes of acidic equivalents, ammonia, and titratable acidity. Within each panel, from left to right the columns represent sodium fluxes in naive fish tested at pH 6.5 (column 1), in naive fish challenged with pH 4.0 (column 2), in fish after 3 mo of pH 4.8 exposure (column 3), and in fish preexposed for 3 mo to pH 4.8 and challenged with pH 4.0 (column 4). The small asterisks represent significant effects ($p \leq 0.05$) of pH 4.0 exposure in naive fish (column 1 vs. column 2) or in acid-preexposed fish (column 3 vs. column 4). The large asterisks represent significant effects ($p \leq 0.05$) of long-term sublethal acid exposure (column 1 vs. column 3) or significant differences ($p \leq 0.05$) between the two groups challenged with pH 4.0 (column 2 vs. column 4). Numbers in parentheses represent the number of fish in each group.

posure. No significant changes in water content in epaxial white muscle, as a result of pH 4.0 exposure, were detected in either acid-preexposed or naive fish (Fig. 3d). However, the combined effects of long-term sublethal and acute lethal exposure significantly increased muscle water content relative to that of naive fish exposed to pH 4.0 conditions.

Acute pH 4.0 exposure did not further affect the already elevated hemoglobin concentration in acid-preexposed fish, while hemoglobin concentration doubled in naive fish (Fig. 4a). There was also a significant 29% increase of the hematocrit in naive fish, while a similar (22%) but not significant trend was observed in acid-preexposed fish. Nevertheless, final hematocrit level after pH 4.0 exposure was not different between naive and acid-preexposed fish. The overall effect of these changes in hemoglobin concentration and hematocrit level was a 15% decrease of the MCHC after acute pH 4.0 exposure in acid-preexposed fish compared with a 60% increase in naive fish (Fig. 4c).

Discussion

The present study indicated that long-term sublethal acid exposure did not improve the physiological ability of softwater-

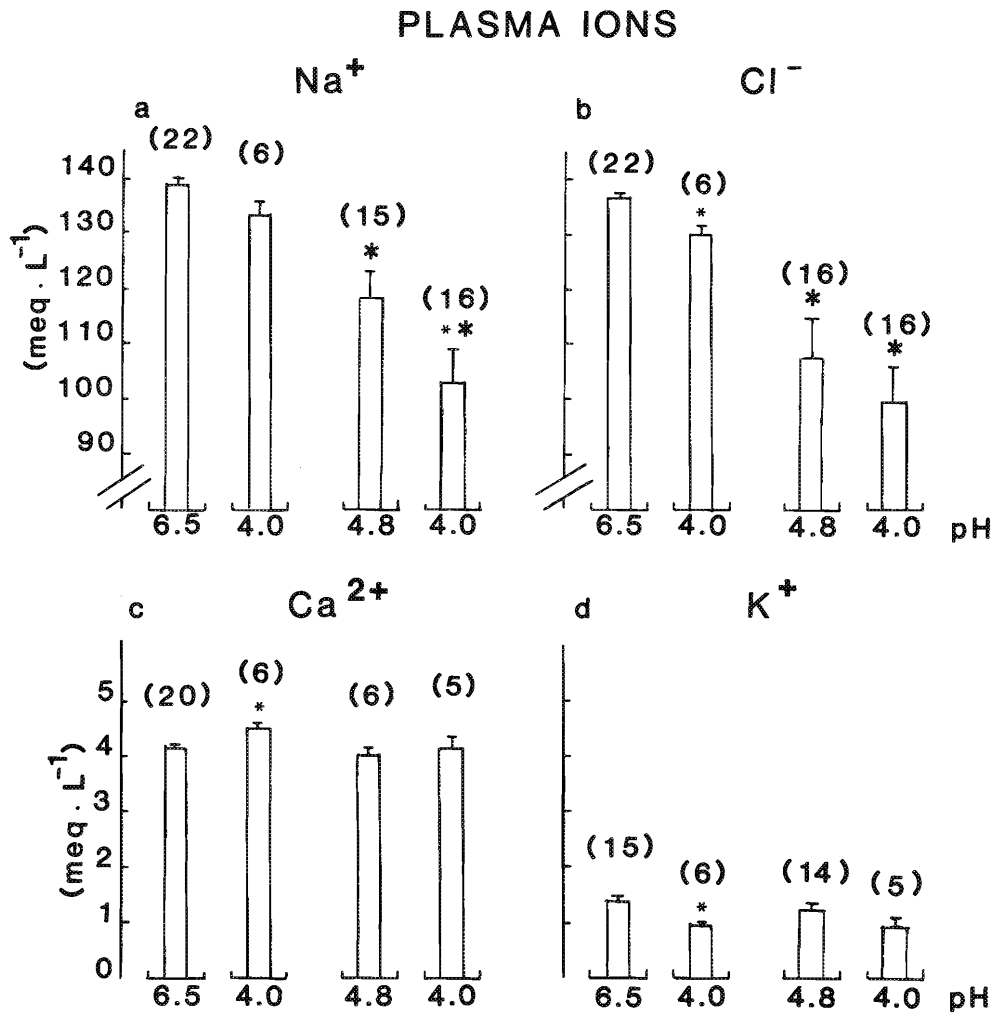

FIG. 2. Effects of acute pH 4.0 exposure on plasma ions in naive and acid-preexposed rainbow trout. (a) Plasma Na^+; (b) plasma Cl^-; (c) plasma Ca^{2+}; (d) plasma K^+. See legend to Fig. 1 for other details.

acclimated rainbow trout to respond to acute lethal acid exposure. For both groups, we observed large negative net fluxes of Na^+ and Cl^- resulting from inhibition of J_{in} and stimulation of J_{out}. Such observations are in agreement with earlier studies on lethal acid stress in softwater-acclimated rainbow trout (McDonald 1983b; McDonald et al. 1983; Höbe et al. 1984). However, the interesting and novel observation is that acid-preexposed fish lost their body ions twice as fast as naive fish, essentially due to a greater stimulation of J_{out}. This occurred despite the fact that plasma Na^+ and Cl^- levels were lower, and therefore the driving diffusive gradients from blood to water were reduced.

Thus, preexposure clearly rendered the fish more, rather than less, sensitive to a more severe challenge. Acclimation did not occur.

The stimulation of J_{out} in fish submitted to acid stress is thought to result from an increase in gill membrane permeability following leaching of Ca^{2+} away from the paracellular channels (McWilliams 1983; McDonald 1983a; Marshall 1985). Prolactin is thought to oppose this effect and reduce gill membrane permeability to water and ions (reviewed by Nicoll 1981). In the accompanying paper (Audet et al. 1988), we proposed that chronic prolactin mobilization might explain the maintained reductions in J_{out}^{Na+} and J_{out}^{Cl-} seen during 3 mo of exposure to pH 4.8. In tilapia, Wendelaar Bonga et al. (1984, 1987) reported massive degranulation of prolactin cells during the first few hours following exposure to pH 4.0. If the long-term sublethal exposure in some way decreased the ability of the fish to either mobilize further prolactin (e.g. through exhaustion of stores or synthetic capacity) or respond to prolactin (e.g. desensitization of receptors during acute exposure), then this might explain the greater Na^+ and Cl^- effluxes in the preexposed fish. Alternatively, the chronic response may have damaged some other mechanisms (e.g. mucus secretion) involved in resistance to the "permeabilizing" effect of acute, severe acid exposure. Undoubtedly, more work is needed to resolve this question.

The other physiological disturbances resulting from acute exposure to pH 4.0 can be viewed as direct consequences of the massive rates of ion loss from the gills. Indeed, the measured changes in plasma ions were very close to those predicted by the measured flux rates and previous estimates of internal Na^+ and Cl^- pools in trout (Wood and McDonald 1982; McDonald 1983b). The effects of such rapid ion losses have been discussed in detail previously (McDonald 1983b; Wood and McDonald 1982; Wood 1988), so will not be dealt with here. However, there were two important qualitative differences between the responses of naive fish and acid-preexposed fish which deserve comment.

Firstly, in naive fish, acute exposure to pH 4.0 caused a 50% drop in ammonia excretion whereas in acid-preexposed fish,

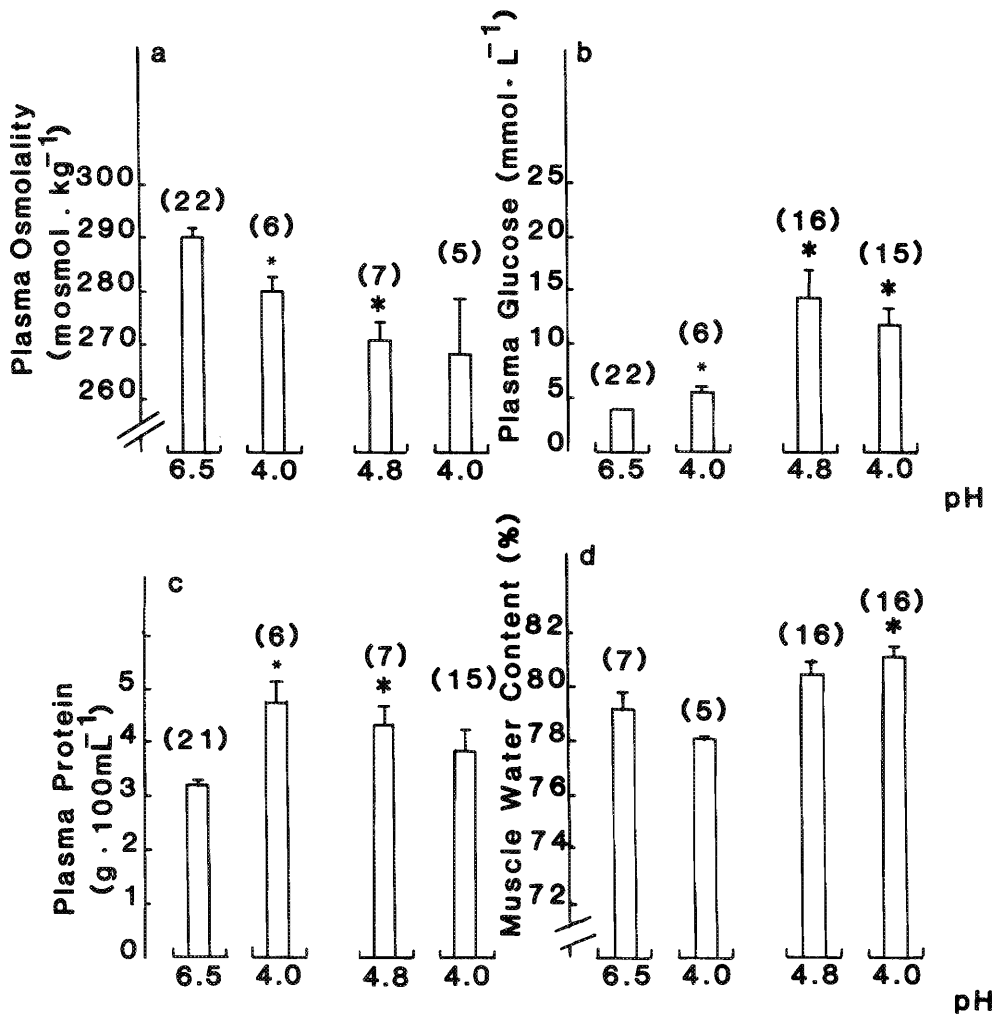

FIG. 3. Effects of acute pH 4.0 exposure on osmotic parameters in naive and acid-preexposed rainbow trout. (a) Plasma osmolality; (b) plasma glucose; (c) plasma protein; (d) water content in epaxial white muscle. See legend to Fig. 1 for other details.

ammonia efflux remained at chronically elevated levels. Wright and Wood (1985) found a similar drop in ammonia excretion of naive fish exposed to pH 4.0 in hard water and related it to an inhibition of J_{in}^{Na+}, i.e. a blockade of Na^+/NH_4^+ exchange. A similar explanation is resonable for the naive fish of the present study because the drop in ammonia efflux was apparently equimolar in the inhibition of J_{in}^{Na+} ($\approx$ 130 $\mu eq \cdot kg^{-1} \cdot h^{-1}$). In the acid-preexposed trout, J_{in}^{Na+} was already partially inhibited by chronic exposure, and ammonia production rates were chronically elevated (Audet et al. 1988). These fish probably relied on NH_3 diffusion to a greater extent; any inhibition of the small remaining Na^+/NH_4^+ component would have been masked by the increased NH_3 efflux along the improved diffusion gradient at pH 4.0.

Secondly, acute exposure of acid-preexposed fish to pH 4.0 did not cause the classic hemoconcentration evidenced by increased hemoglobin and plasma protein concentrations in the naive fish (see Milligan and Wood 1982 for a full discussion of this response), although the red blood cells swelled, as evidenced by a fall in MCHC. The explanation is unclear, but it should be noted that the pre-exposed fish already manifested chronic increases in hemoglobin and plasma protein. The capacity for further response may have been limited.

Few previous studies have compared the responses to more severe acid stress between fish preexposed to acid conditions and naive fish. Falk and Dunson (1976) compared survival time to pH 3.1–3.5 in brook trout (*Salvelinus fontinalis*) but did not find that preexposure to sublethal acidity improved the response of fish. Studies done on the same species by Wood et al. (1988a, 1988b) showed physiological evidence of acclimation to combined acid *plus* aluminium stress after preexposure (10 wk) to sublethal pH (5.2) and aluminum (150 $\mu g \cdot L^{-1}$). However, there was no evidence that preexposure to sublethal pH (5.2) alone conferred greater tolerance, i.e. the acclimation appeared to be to aluminium and not to acidity. These results are therefore in general accord with those of the present study.

In conclusion, acclimation did not occur. No improvements of the ability to respond to acute lethal acid stress were observed in rainbow trout chronically preexposed to sublethal acid stress in soft water. Moreover, larger rates of Na^+ and Cl^- efflux, faster decreases in plasma Na^+, and greater red blood cell swelling showed that they were in fact more sensitive to acute lethal acid stress than naive fish. These results, in association with maintained low osmolality and high levels of glucose and ammonia excretion (stress indicators), indicated that these fish were in poor physiological condition. The death of fish previously exposed to pH 4.8 is therefore expected to occur faster under lethal acid exposure. In the field, we predict that fish chronically exposed to pure acid stress in soft water will be more sensitive to episodic acid surges (e.g. rainstorm and snow-

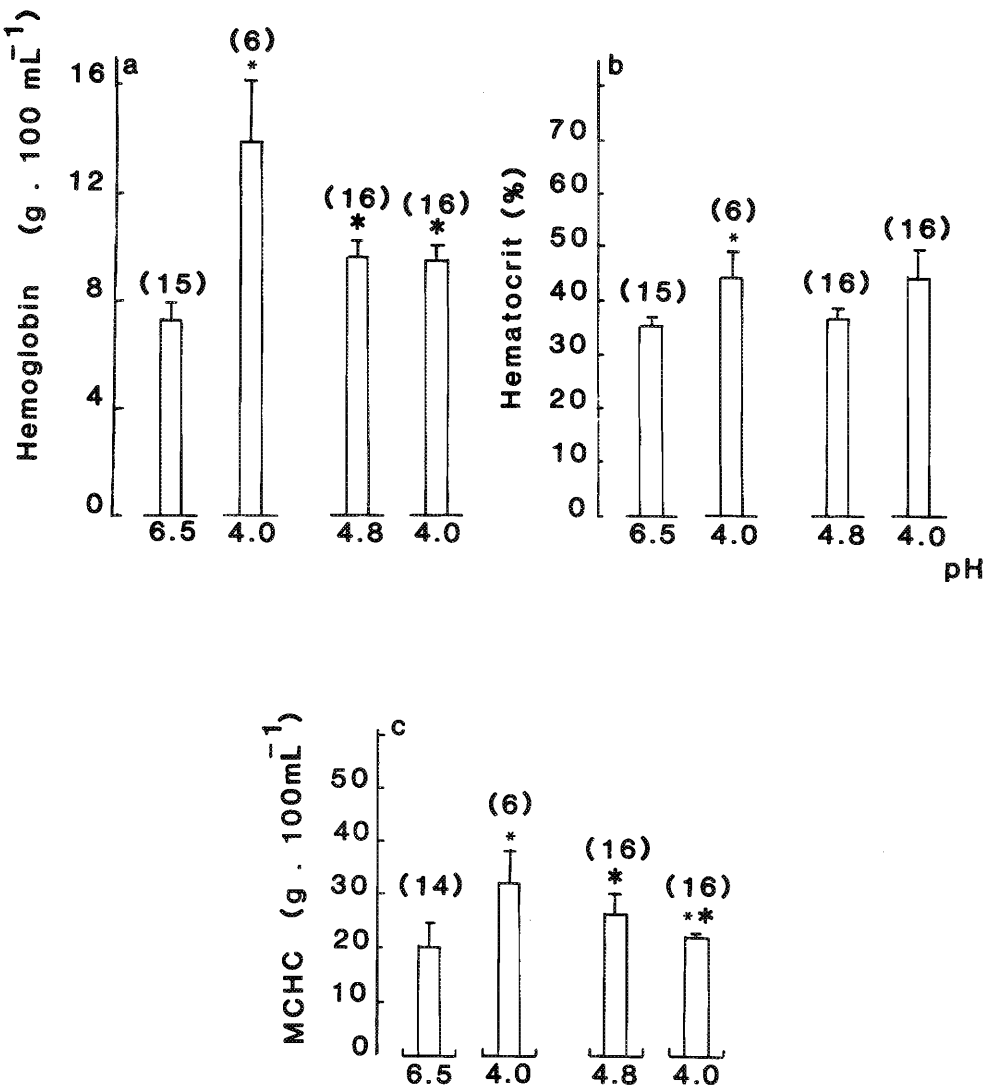

FIG. 4. Effects of acute pH 4.0 exposure on hematology in naive and acid-preexposed rainbow trout. (a) Blood hemoglobin concentration; (b) hematocrit level; (c) MCHC. See legend to Fig. 1 for other details.

melt runoff) than predicted by laboratory trials with naive fish. However, this interpretation may not apply when a genetic basis for acid tolerance has been favoured by natural selection, or when metals are simultaneously involved.

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