

Why do fish die after severe exercise?

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Trout fitted with dorsal aortic cannulae were subjected to 6 min of intensive exercise and monitored over the following 12 h recovery period. Delayed mortality was $\approx 40\%$; the majority of deaths occurred 4–8 h post-exercise. Surviving fish exhibited a short-lived haemoconcentration reflected in increased haematocrit, haemoglobin, plasma protein, Na^+ and Cl^- levels; an extended rise in plasma $[\text{K}^+]$; a quickly corrected respiratory acidosis; and a more prolonged metabolic acidosis in concert with a rise in blood lactate. Dying fish exhibited very similar trends except for a significantly greater metabolic acidosis, lower plasma $[\text{Cl}^-]$, and the apparent accumulation of an unknown anion in the blood prior to death. Cardiac failure did not occur. Blood metabolic acid levels, while elevated, were only $\sim 50\%$ of peak lactate anion levels and well within the normal range of tolerance, as were all other changes observed in the blood of non-survivors. The hypothesis that post-exercise mortality is due to excessive 'lactic acid' accumulation in the blood is discounted. It is suggested that intracellular acidosis may be the proximate cause of death.

I. INTRODUCTION

It is known that severe exercise may result in the death of fish several hours after the end of exercise. This finding is of importance to fisheries management, for it may render pointless the legislated return of trawled or angled fish to the wild. There is much literature on the subject, the earlier aspects of which have been reviewed by Black (1958). Black reached no firm conclusion as to the physiological cause of death, but emphasized the possible role of high blood lactic acid levels. In a review of the more recent literature, Bennett (1978) supported that idea, concluding that 'acidosis associated with lactic acid diffusion into the blood is probably the important factor'. However, in none of the studies cited to support this concept have blood lactic acid levels actually been measured; instead, the concentration of the lactate anion alone has been determined.

In mammals, lactate anions and metabolic protons (i.e. 'base deficit') are found in the bloodstream in approximately equimolar amounts after exercise, so lactate anion levels are representative of 'lactic acid' levels (Visser *et al.*, 1964; Keul *et al.*, 1967). Certainly, this is not always the case in poikilotherms. A number of recent reports (Piiper *et al.*, 1972; Wood *et al.*, 1977; McDonald *et al.*, 1979; McDonald *et al.*, 1980a; Boutilier *et al.*, 1980; Wood & Randall, 1981) show that discrepancies may exist at any time between blood lactate and metabolic proton levels in these animals. The lactate anion alone is relatively non-toxic (Jonas *et al.*, 1962), so it is necessary to measure blood metabolic acid levels after exercise

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to prove that 'lactic acid' is the cause of mortality. This can only be done by analysing the complete acid-base status of the blood and applying the appropriate physico-chemical principles (Davenport, 1974; Woodbury, 1974; Wood *et al.*, 1977; McDonald *et al.*, 1979). A second problem with previous studies is that caudal or cardiac puncture sampling was used; the physiological disturbance associated with these methods is sufficient to render blood acid-base data meaningless, and can perturb blood lactate and ionic parameters.

We have employed a chronic cannulation approach to make a detailed analysis of the changes in blood acid-base, electrolyte, haemoglobin, and lactate status following severe exercise in the rainbow trout (Turner *et al.*, in press). Considerable post-exercise mortality occurred in these experiments, as well as in similar trials examining the interactions between water hardness, environmental acid stress, and exercise responses (Graham *et al.*, in press). The present report compares the changes seen in dying fish with those in survivors in order to clarify the cause of post-exercise mortality in the trout. The results cast some doubt on the 'blood lactic acid' hypothesis.

II. MATERIALS AND METHODS

EXPERIMENTAL PROCEDURE

Rainbow trout, *Salmo gairdneri* Richardson (200–400 g) were obtained from Spring Valley Trout Farm, Ontario, and acclimated to $14.5 \pm 1.5^\circ \text{C}$ in either hard ($\approx 140 \text{ mg l}^{-1}$ as CaCO_3 ; $n=13$) or soft ($\approx 14 \text{ mg l}^{-1}$ CaCO_3 ; $n=11$) dechlorinated water. *Ad libitum* feeding with commercial trout pellets (Martin Feed Mills) was suspended at least 7 days prior to experimentation. To allow repetitive blood sampling without disturbance to the animal, dorsal aortic cannulae were implanted while the fish were under 1 : 15 000 MS-22 (Sigma) anaesthesia (Smith & Bell, 1964; Holeton & Randall, 1967). The cannulae were filled with Cortland saline (Wolf, 1963) containing 50 iu ml^{-1} ammonium heparin (Sigma). Following surgery, the fish were allowed to recover for a minimum of 24 h in darkened acrylic chambers ($=4 \times 7 \times 30 \text{ cm}$) flushed with air-equilibrated acclimation water at $300\text{--}450 \text{ ml min}^{-1}$.

Severe exercise was imposed by releasing the fish into a circular 500-l tank (diameter = 91 cm) filled with acclimation water and then chasing vigorously for 6 min with a blunt prod. By the end of the 6 min, all trout seemed incapable of further burst performance, but some continued to swim slowly around the tank. This condition probably represented an exhaustion of the largely glycolytic white muscle, while the continued slow activity was supported by red muscle (Johnston, 1977). Each fish was returned to its individual chamber and monitored for the following 12 h.

Samples (600 μl) of whole blood were anaerobically withdrawn into ice-cold gas-tight Hamilton syringes via the dorsal aortic catheter. Samples were taken at rest (control), immediately after exercise (0 h), and at 0.5, 1, 2, 4, 8 and 12 h (if death did not ensue earlier). The blood removed at each time was replaced with an equal volume of Cortland saline. Blood samples were analysed for pH, total CO_2 content (whole blood and true plasma), haematocrit, haemoglobin concentration, lactate concentration, and plasma levels of sodium, potassium, chloride and total protein.

In one experiment, arterial blood pressure during the post-exercise period was monitored by connecting the dorsal aortic catheter to a Narco RP-1500 pressure transducer. The signal was amplified by a Gilson IC-MP pre-amplifier and displayed on an ICT-5H polygraph.

ANALYTICAL METHODS

Arterial blood pH (pHa) was determined by injecting a 40 μl aliquot into a Radiometer micro-electrode (type E5021) thermostatted to the experimental temperature and connected to a Radiometer PHM 71 or 72 acid-base analyser. Total CO_2 (CaCO_2) was

measured on 50 μl samples of true plasma and whole blood using the method of Cameron (1971) and a Radiometer P_{CO_2} electrode (type E5036). True plasma was obtained by centrifuging 80 μl whole blood in sealed, heparinized microhaematocrit tubes (Radiometer) at 5000 g for 5 min. The haematocrit was read directly from the tube which was then broken to allow anaerobic aspiration of the plasma into a Hamilton syringe for transfer to the Cameron chamber. Total blood [haemoglobin] was measured colorimetrically by the cyanmethaemoglobin method (Blaxhall & Daisley, 1973) using 20 μl samples and Sigma reagents. Lactate was measured on 100 μl of whole blood immediately deproteinized in 200 μl of ice-cold 8% HClO_3 (w/v) and then centrifuged at 9000 g for 3 min. The supernatant was analysed enzymatically for L-lactate employing Sigma reagents (Sigma, 1977).

The remainder of the blood sample was centrifuged at 9000 g for 2 min to separate plasma (~200 μl). Total plasma protein was determined with an American Optical Goldberg refractometer (Alexander & Ingram, 1980). Plasma concentrations of sodium and potassium were appropriately diluted and measured against known standards using flame photometry (EEL Mark II). Swamping was employed to eliminate the interfering effect of Na^+ on K^+ emission. Chloride was determined by coulometric titration (Radiometer CMT10).

CALCULATIONS

Arterial CO_2 tension (Pa_{CO_2}) was calculated using the Henderson-Hasselbalch equation (Albers, 1970) in the following form:

$$\text{Pa}_{\text{CO}_2} = \frac{\text{Ca}_{\text{CO}_2}}{\alpha\text{CO}_2 \times (1 + \text{antilog } \text{pHa} - \text{pk}')} \quad (1)$$

where Ca_{CO_2} was measured in true plasma and values of αCO_2 and pk' at the appropriate temperature were taken from Severinghaus (1965) and Albers (1970) respectively. Bicarbonate concentrations in whole blood and true plasma were calculated by:

$$[\text{HCO}_3^-] = \text{Ca}_{\text{CO}_2} - (\alpha\text{CO}_2 \times \text{Pa}_{\text{CO}_2}) \quad (2)$$

using the appropriate measurements of Ca_{CO_2} . The concentration of metabolic H^+ ions (ΔH_m^+) added to the whole blood over any period (time 1–2) was calculated by the equation of McDonald *et al.* (1980b):

$$[\Delta\text{H}_m^+] = [\text{HCO}_3^-]_1 - [\text{HCO}_3^-]_2 - \beta(\text{pHa}_1 - \text{pHa}_2) \quad (3)$$

which derives from principles described in Davenport (1974), Woodbury (1974), Wood *et al.* (1977), and McDonald *et al.* (1979). In this equation, values of $[\text{HCO}_3^-]$ are for whole blood, and β , the non-bicarbonate buffer capacity of whole blood, is estimated from the blood [haemoglobin] (Hb) at time 2 using the relationship determined *in vitro* by Wood *et al.* (1982):

$$\beta = -1.073 [\text{Hb}] - 2.48. \quad (4)$$

The total blood metabolic acid concentration at any time was calculated by summing the $[\Delta\text{H}_m^+]$'s, signs considered, for each period from the rest sample onwards. The calculation was performed for whole blood rather than plasma because lactate was also determined in whole blood. Mean cell haemoglobin concentration (MCHC—i.e. g Hb ml^{-1} erythrocytes) was estimated from the haemoglobin and haematocrit (Ht) measurements:

$$\text{MCHC} = \frac{[\text{Hb}]}{\text{Ht}}. \quad (5)$$

TREATMENT OF DATA

The focus of this report is the comparison of various blood parameters between fish suffering delayed mortality after exercise and those which survived throughout the 12 h recovery period. Two sets of experiments were performed, one in hard water and one in soft water. The responses seen in the two groups were similar, as were post-exercise mortalities (five of 13 in hard water, five of 11 in soft water). The few differences attributable to water hardness have been described in detail by Graham *et al.* (in press). For simplicity,

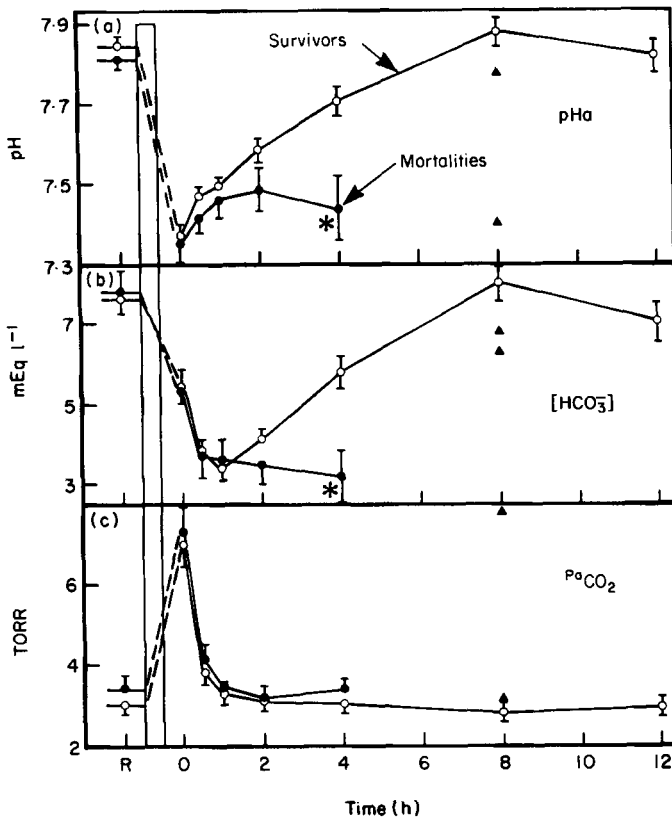


FIG. 1. Changes in (a) arterial blood pH; (b) arterial true plasma bicarbonate concentration and (c) arterial blood CO₂ tension after 6 min of severe exercise in surviving (O, $n = 14$) and non-surviving trout (●, $n = 9$ until 2 h, $n = 7$ at 4 h and $n = 2$ at 8 h, the latter shown as individual points—▲). Means ± 1 S.E.M. R = pre-exercise control value (rest); bar indicates exercise period; * signifies differences between surviving and non-surviving groups at the same sample times ($P \leq 0.05$).

data from the two series have been combined in the present report, and comparisons made only between surviving and non-surviving trout. This treatment has no qualitative effects on the conclusions of the study, and negligible quantitative effect.

The data (means $(\bar{x}) \pm$ S.E.M.) for the 14 surviving fish are plotted at each sample time in the Figures. In the 10 non-survivors, the majority (five) died between 4 and 8 h post-exercise, two between 2 and 4 h, and two between 8 and 12 h. Data from one fish which succumbed immediately after exercise were eliminated because this could not be considered a 'delayed mortality'. Means ± 1 S.E.M. for the non-survivors are plotted with $n = 9$ until 2 h, and $n = 7$ at 4 h, while the values for two fish still alive at 8 h are shown individually. The significance of differences ($P \leq 0.05$) between means in the surviving and non-surviving groups were tested until the end of 4 h by Student's unpaired two-tailed t -test.

III. RESULTS

Six minutes of severe exercise in the rainbow trout caused a delayed mortality of about 40% over the next 12 h, the majority of which occurred between 4 and 8 h post-exercise. This mortality cannot be attributed to the blood sampling regime because similar regimes have been applied to resting trout in a variety of

studies with negligible disturbance of physiological parameters and negligible mortality. Furthermore, in none of the parameters measured (Figs 1–5) were there any significant differences in the rest (control) values between surviving and non-surviving fish. Thus pre-exercise variations in these parameters cannot be considered either causative or predictive of delayed post-exercise mortality.

Initial changes after exercise in three standard parameters of blood acid-base status were identical in surviving and non-surviving fish (Fig. 1). Arterial pH underwent a sharp decline at 0 h [Fig. 1(a)] in concert with an equally rapid tripling of P_{aCO_2} [Fig. 1(c)], while plasma $[HCO_3^-]$ manifested a slower fall which continued until 1 h [Fig. 1(b)]. Subsequent changes in P_{aCO_2} were the same in the two groups, the values returning to resting levels by 1 h, but the same was not true of pHa and HCO_3^- . Arterial pH rose more slowly in the non-survivors and actually declined again slightly at 4 h, at which time the difference between the two groups became significant [Fig. 1(a)]. Similarly HCO_3^- continued to fall until 4 h in the non-survivors, in contrast to the return towards resting levels in the survivors [Fig. 1(b)]. Note however, that the two non-survivors still alive at 8 h showed relatively high plasma $[HCO_3^-]$'s.

Blood lactate varied in a similar pattern in the two groups, rising rapidly after the first 0.5 h and then more slowly to a peak at 2 h, followed by a slow decline thereafter [Fig. 2(a)]. The mean post-exercise levels were slightly higher in non-survivors, but none of the differences were significant. Calculated blood metabolic acid levels increased to the same extent as blood lactate levels immediately after exercise [0 h; compare Figs 2(a) and 2(b)] in both groups. However, metabolic acid concentrations then stabilized at ≈ 7 mEq l^{-1} over the next hour [Fig. 2(b)], while lactate levels continued to rise to 13–16 mEq l^{-1} at 2 h [Fig. 2(a)]. In other words, the actual concentration of 'lactic acid' in the blood was only about 50% of the blood lactate anion level over the period. Metabolic acid levels were identical in the two groups until 1 h, but thereafter declined more slowly in the non-survivors, a difference which became significant by 4 h [Fig. 2(b)]. However, the two non-survivors still alive at 8 h showed almost complete correction of their metabolic acid loads [Fig. 2(b)], despite the persistence of high blood lactate loads [Fig. 2(a)].

Plasma $[Na^+]$ increased by about 12% in the 0–1 h period after exercise and then slowly returned to resting levels by 8 h [Fig. 3(a)]. There were no significant differences in this parameter at any time between surviving and non-surviving trout. Chloride concentrations also increased after exercise, but to a slightly lesser extent ($\sim 9\%$) and then proceeded to fall below resting values at 4 and 8 h before returning to normal at 12 h [Fig. 3(b)]. This secondary decline in Cl^- was more pronounced in the non-survivors, a difference which was significant at both 2 and 4 h. Plasma $[K^+]$ followed a very different trend from the other two ions, increasing by about 70% immediately after exercise, declining slightly at 0.5 h, and then continuing to rise to approximately twice resting values at 2–4 h [Fig. 3(c)]. At this time, mean plasma K^+ levels were somewhat higher in the non-survivors, but the differences were not significant. Resting levels were not restored until 12 h in the survivors.

Plasma protein rose by about 12% in the 0–0.5 h period after exercise, and then proceeded to fall below resting levels during subsequent recovery [Fig. 4(a)], the latter presumably reflecting the influence of repetitive blood sampling. There

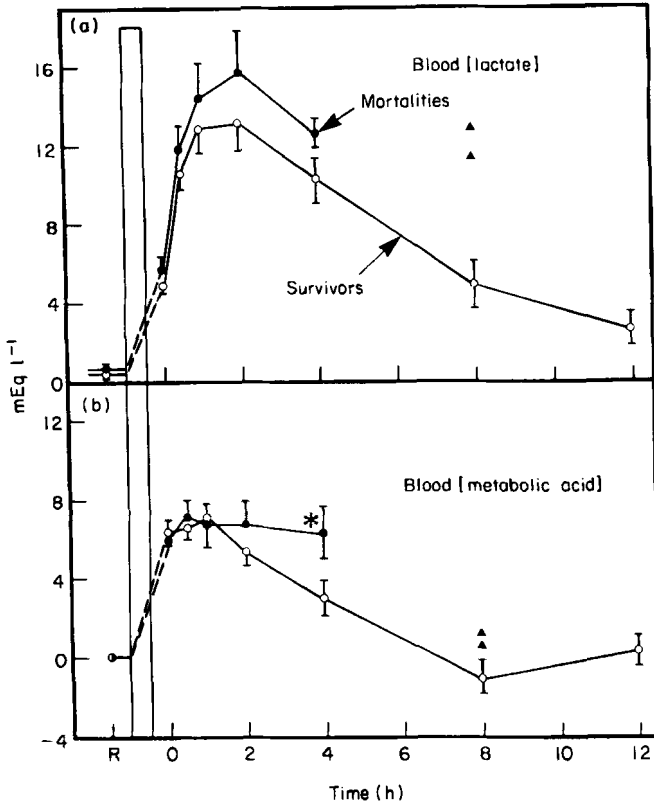


FIG. 2. Changes in (a) whole blood lactate concentration; and (b) whole blood metabolic acid concentration after 6 min of severe exercise in surviving and non-surviving trout. Other details as in legend of Fig. 1.

were no differences between survivors and non-survivors in this parameter. Blood haemoglobin increased over the same period by approximately 18%, and then declined below resting values with progressive blood sampling [Fig. 4(b)]. The effect of sampling was apparently more pronounced on haemoglobin than on plasma protein [cf. Fig. 4(a), (b)]. Again there were no significant differences between the two groups. Mean red cell haemoglobin concentration fell slightly after exercise in both groups [Fig. 4(c)], an effect correlated with a rise in haematocrit (~25%; data not shown) greater than that in haemoglobin (~18%). There were no significant differences between survivors and non-survivors in haematocrit or MCHC at any time.

With experience, it became possible to predict with reasonable accuracy which fish were going to die during the recovery period, by visual observation. Dying fish typically lost balance 1–2 h prior to death and manifested an increasingly rapid but more shallow ventilation rate. Eventually ventilation ceased with the mouth closed, without any struggling by the fish. The death was therefore different from that seen in hypoxia where fish struggle violently prior to death, show periodic gasping, and die with the mouth agape. Dorsal aortic blood pressure was measured in one of the fish dying between 4 and 8 h to see if cardiac failure was the cause. While the heart rate tended to slow and became irregular in a typical

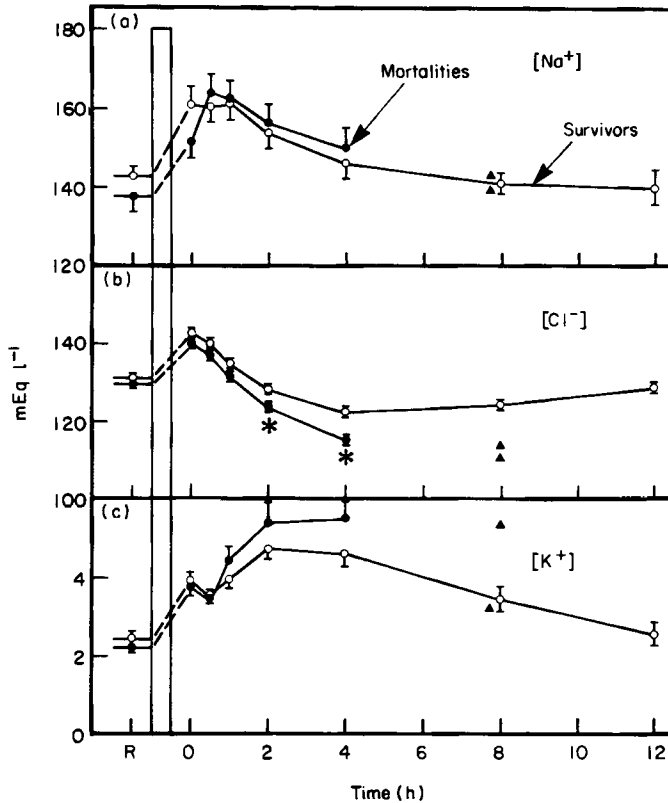


FIG. 3. Changes in (a) plasma sodium concentration; (b) plasma chloride concentration; and (c) plasma potassium concentration after 6 min of severe exercise in surviving and non-surviving trout. Other details as in legend of Fig. 1.

vagal bradycardia (Wood & Shelton, 1980) prior to the cessation of breathing, there was no evidence of cardiac failure. Indeed a strong heart beat and a dorsal aortic blood pressure within the normal range (20–40 cm H₂O) were maintained for at least 15 min following the cessation of ventilation.

IV. DISCUSSION

The physiological changes occurring during recovery from severe exercise in surviving trout have been discussed and analysed in detail by Turner *et al.* (1982), and only major points are here reviewed. The post-exercise acidosis [Fig. 1(a)] is initially due to both a substantial PaCO₂ accumulation [Fig. 1(c); respiratory acidosis] and a release of metabolic protons into the blood from the white muscle [Fig. 2(b); metabolic acidosis]. After 0.5 h, the acidosis is almost entirely metabolic as PaCO₂ is returned to normal, while high blood metabolic acid levels persist. The metabolic acidosis is reflected in the loss of plasma HCO₃⁻ [Fig. 1(b)]. During burst activity, anaerobic metabolism has built up massive concentrations of lactate (≈45 mEq kg⁻¹), and presumably equimolar levels of protons, in the large white muscle mass. However, the effluxes of lactate and protons from the muscle into the blood appear to be functionally separate events. Net proton

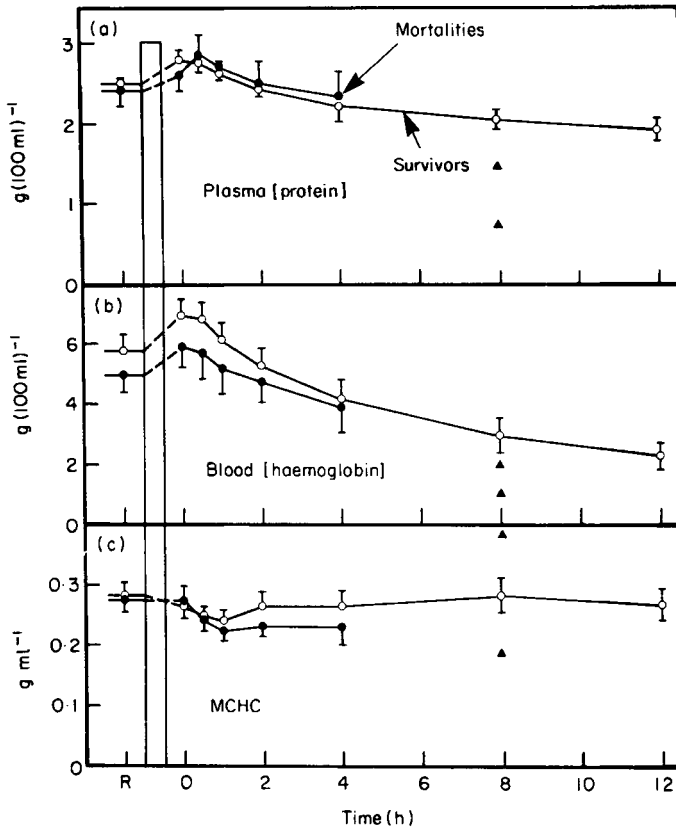


FIG. 4. Changes in (a) plasma protein concentration; (b) whole blood haemoglobin concentration; and (c) mean red blood cell haemoglobin concentration after 6 min of severe exercise in surviving and non-surviving trout. Other details as in legend of Fig. 1.

movement is inhibited by the low post-exercise blood pH, thereby preventing a possibly fatal blood acidosis, while lactate efflux continues. Blood metabolic acid stabilizes at the immediately post-exercise level [Fig. 2(b)], while blood lactate continues to rise to twice that value by 2 h [Fig. 2(a)]. At this time, blood 'lactic acid' level is therefore only about one half the lactate anion concentration. Subsequently, metabolic processes remove the protons and lactate from the blood at similar rates resulting in a very slight alkalosis by 8 h. While the differential release of lactate and protons from the muscle to the blood is physiologically significant, experiments with a perfused trout trunk preparation (Turner & Wood, unpubl. data) suggest that over 80% of the post-exercise lactate and proton load in the white muscle mass is never released into the blood, but is slowly removed by metabolic processes *in situ*. The post-exercise rise in several blood constituents (Na^+ , Cl^- , plasma protein, haemoglobin; Figs 3, 4) is mainly reflective of a water shift out of the plasma and into the cellular compartment due to the osmotic gradient created by this intracellular lactate accumulation. Erythrocyte recruitment and swelling, as evidenced by the fall in MCHC [Fig. 4(c)] also contribute to the red cell response. The pronounced and prolonged rise in plasma K^+ [Fig. 3(c)] is thought to reflect K^+ extrusion from muscle cells in response to the intracellular acidosis.

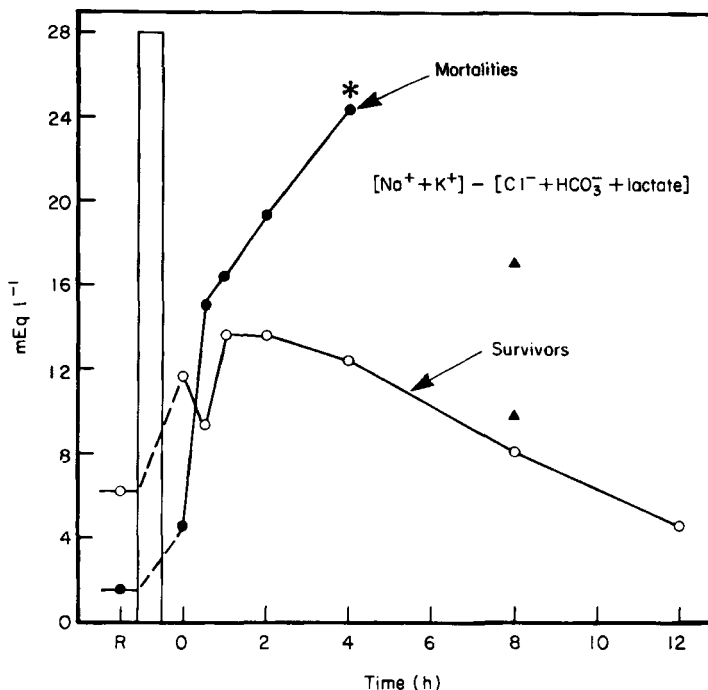


FIG. 5. Changes in the mean levels of 'unmeasured anion', i.e. the sum of plasma [sodium plus potassium] minus [chloride plus bicarbonate plus lactate] concentrations after 6 min of severe exercise in surviving and non-surviving trout. Means only. Other details as in legend of Fig. 1.

The most obvious difference between surviving and non-surviving fish was the pattern of post-exercise blood acidosis (Fig. 1) from about 2 h onwards. At that time, the acidosis was almost totally metabolic in nature, and the lower pH_a and HCO₃⁻ levels in non-survivors resulted entirely from the higher blood metabolic acid loads [Fig 2(b)]. This effect occurred despite small differences in blood lactate concentrations [Fig. 2(a)], illustrating the danger of equating lactate anion levels to 'lactic acid' levels. However, we believe it unlikely that extracellular acidosis was the cause of death. Firstly, at time 0 h, the fish had already survived a more severe blood acidosis of both respiratory and metabolic origin [Fig. 1(a)]. Indeed, blood metabolic acid levels had stabilized at about the 0 h level from then onwards [Fig. 2(b)] while respiratory acid levels declined [Fig. 1(c)]. Secondly, the two non-surviving fish still alive at 8 h had virtually eliminated their blood metabolic acid loads by this time [Fig. 2(b)], despite the persistence of high blood lactate levels [Fig. 2(a)], yet died anyway. Thirdly, we have shown that rainbow trout, subjected to environmental acid stress (water pH=4.0-4.5) survive 4-10 days with *sustained* depressions in blood pH (7.2-7.4) and elevations in blood metabolic acid loads (5.7-10.9 mEq l⁻¹) equal to or greater than these seen in fish dying 2-12 h after exercise (McDonald *et al.*, 1980*b*; McDonald & Wood, 1981; Wood & McDonald, in press).

Bouck & Ball (1966) noted changes in blood haemoglobin and plasma protein in trout dying as a consequence of angling stress, but the present data (Fig. 4) provide no evidence that these are involved in the key toxic mechanism of action.

Wydoski *et al.* (1976) described complex disturbances in plasma osmolality and chloride levels following angling stress in trout. Marked ionic disturbances were also seen in the present study (Fig. 3); however, again it is doubtful that these effects contributed to mortality. Changes in plasma $[\text{Na}^+]$ were identical in survivors and non-survivors [Fig. 3(a)]. Potassium levels were elevated to a somewhat greater extent in dying fish than in the survivors [Fig. 3(c)], and we initially suspected that this might have caused cardiac failure. However, the persistence of a strong heart beat during and following the cessation of ventilation in a dying fish argues against this, as does the fact that much higher levels of K^+ ($\approx 10 \text{ mEq l}^{-1}$ v. 5.5 mEq l^{-1}) are required to stop the heart in mammals (Guyton, 1981). Chloride concentration was significantly lower in dying fish than in survivors from 2 h onwards [Fig. 3(b)]. Even in survivors $[\text{Cl}^-]$ declined significantly below resting values at 4 and 8 h, the depression being approximately equivalent to the rise in blood lactate at that time and reflecting the constraints of electroneutrality (Turner *et al.*, in press). Chloride levels seen in dying fish ($110\text{--}120 \text{ mEq l}^{-1}$) were well within the normal range of variation. Indeed under environmental acid stress, where ionoregulatory failure is the key toxic event, trout generally survive until plasma $[\text{Cl}^-]$ falls to about 90 mEq l^{-1} (Wood & McDonald, 1982).

The significance of the lower plasma $[\text{Cl}^-]$ in dying fish is that it indicates the presence of an unknown anion building up to relatively high concentrations in the blood prior to death. By the constraints of electroneutrality, total anions in the system must equal total cations. Our measurements of Na^+ , K^+ , Cl^- , HCO_3^- and lactate comprise the major charged components of plasma except for protein (Oh & Carroll, 1977). The sum $[\text{Na}^+ + \text{K}^+] - [\text{Cl}^- + \text{HCO}_3^- + \text{lactate}^-]$ should therefore estimate the unmeasured negative charge, which is mainly due to plasma proteins in healthy resting animals (Adrogué *et al.*, 1978). The results of that calculation are shown in Fig. 5. Standard errors are large ($\pm 25\%$), reflecting the summated errors of five different determinations, and therefore have been omitted for clarity. Nevertheless, there is a clear difference in trend between surviving and dying animals. Values increased moderately after exercise and returned to normal by 12 h in survivors, but in non-survivors rose dramatically to $24.7 \pm 3.3 \text{ mEq l}^{-1}$ at 4 h, a figure twice as high as that for survivors at this time ($12.40 \pm 3.7 \text{ mEq l}^{-1}$; $P < 0.05$). This cannot be explained by a difference in protein between the two groups [Fig. 4(a)]; indeed the greater acidosis at that time in dying fish [Fig. 1(a)] would tend to reduce rather than increase the (unmeasured) negative charge on plasma protein (Adrogué *et al.*, 1978). Various explanations are theoretically possible (Oh & Carroll, 1977), but the simplest is that the anion of another organic acid(s) besides lactic is appearing in the blood after exercise, and building up to much higher levels in dying than surviving fish. We suggest this may be a keto acid due to excessive fat metabolism, or one of the novel end products of anaerobic metabolism (e.g. succinate) which can be produced by fish tissue (Johnston, 1975*a, b*; Smith & Heath, 1980).

Beggs *et al.* (1980) have recently employed a cannulation approach to study angling stress in muskellunge, *Esox masquinongy*. While a full blood acid-base analysis was not performed, they suggested that blood metabolic acid levels were probably greater than those of the lactate anion, a discrepancy opposite to that of the present study. Relative to surviving animals, dying muskellunge exhibited greater acidosis, apparently of metabolic origin, greater plasma $[\text{K}^+]$ and only

moderately greater blood lactate levels. Beggs *et al.* (1980) concluded that none of these effects were great enough to be the proximate cause of death, a conclusion identical to that of the present study.

What then is the cause of delayed post-exercise mortality? Having discounted a number of factors in the blood, we suggest that the key toxic event may occur in the intracellular compartment. Possibly, intracellular acidosis is the critical factor, for it is in the white muscle cells that lactic acid is actually produced, and our perfusion studies suggest that over 80% of these protons are never released, but rather removed by metabolism *in situ* (Turner & Wood, unpubl. data). Other organic acids may also be produced intracellularly, and the higher blood levels of unknown anion in dying fish (Fig. 5) suggest that this process may occur to a greater extent prior to death. Proton movement into the blood, while small, seems to be a function of the ICF to ECF pH gradient (Turner & Wood, unpubl. data), so the slightly greater metabolic acid levels in the blood of dying fish [Fig. 2(b)] may be indicative of a much greater intracellular acidosis. The challenge of future experiments to prove this hypothesis lies in sampling the intracellular compartment after exercise without affecting the ultimate survival or death of the animal.

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