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ANALYSIS OF ACID EXCRETION IN FISH AND ITS APPLICATION TO ACIDIC ENVIRONMENTS

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BRANCHIAL AND RENAL ACID AND ION FLUXES IN THE RAINBOW TROUT, *SALMO GAIRDNERI*, AT LOW ENVIRONMENTAL pH

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SUMMARY

Rainbow trout were exposed for 4 days to an environmental pH averaging 4·2, an exposure which resulted in a continuous net branchial influx of acid. The influx provoked a progressive depression in blood pH and [HCO_3^-], virtually complete by 48 h, and a marked increase in renal acid excretion, also complete by 48 h. The increase in the latter was sufficient to remove, at maximum, about half of the protons entering at the gills; those remaining were buffered in body fluids. The low pH exposure also impaired gill ion regulation as indicated by continuous net branchial losses of Na^+, Cl^- and K^+ and by a progressive decline in plasma Na^+ and Cl^- levels. Evidence is presented which indicates that there was a significant contribution by the intracellular compartment both to the total body ion losses and to the buffering of the body acid load.

INTRODUCTION

There is now an extensive literature on the interaction of low pH environments with the physiology of freshwater fish (see Fromm, 1980, for a recent review). Effects on normal blood physiology are particularly well known and disturbances to acid-base state (Packer & Dunson, 1970; Neville, 1979*a*; Packer, 1979; McDonald, Höbe & Wood, 1980), ion levels (Mudge & Neff, 1971; Leivestad & Muniz, 1976; Neville, 1979*b*; McDonald *et al.* 1980) and oxygen transport (Vaala & Mitchell, 1970; Packer, 1979) have now been reported. Less well understood are effects on the actual mechanisms of ionoregulation. Whole-body unidirectional Na^+ fluxes have been examined in acid environments (Packer & Dunson, 1970; McWilliams & Potts, 1978) but no attempt has been made to separate the branchial and renal components of these fluxes nor has the regulation of other major body ions (e.g. Cl^- and K^+) been assessed. Also largely unknown are the effects of acid environments on the mechanisms of acid-base regulation. Such regulation has been largely thought to occur at the gills via Na^+/proton and Cl^-/basic anion exchange mechanisms (cf. Maetz, 1974) but the recent demonstration (Wood & Caldwell, 1978) of a considerable renal competence in the excretion of an injected acid load suggests that the kidney may also play an important role. Thus in order to explore more fully these aspects of low pH exposure, we have undertaken a study of the effects of such exposure on branchial and renal function in the rainbow trout.

Katie Gilmour discusses the 1981 paper by Gord McDonald and Chris Wood entitled: 'Branchial and renal acid and ion fluxes in the rainbow trout, *Salmo gairdneri*, at low environmental pH.'

A copy of the paper can be obtained from http://jeb.biologists.org/content/93/1/101.full.pdf+html

The discovery in the late 1960s of 'dead' or dying lakes (Beamish and Harvey, 1972) nearly devoid of living animals and plants owing to the effects of acid precipitation spawned intense research into the effects of low pH environments on the physiology of freshwater fish. Over the next decade, an extensive body of literature documented the adverse effects of acid environments on acid–base status, ion levels and oxygen transport (Fromm, 1980). It became abundantly clear that acid exposure killed fish, but how acid killed fish, i.e. the underlying mechanisms and the proximate cause of mortality of acid-exposed fish, remained uncertain. Enter Chris Wood, a newly hired faculty member at McMaster University, Canada, and his collaborator, Gord McDonald. Wood and McDonald recognized the need for mechanistic studies of the ionoregulatory and acid–base disturbances of acid-exposed fish, and set out to generate the necessary data, adapting established experimental approaches to their needs. Their 1981 paper in *The Journal of Experimental Biology* (McDonald and Wood, 1981) showcased this new approach, yielding valuable insight into the mechanisms underlying acid–base disturbances in acid-exposed fish, as well as experimental methods for the measurement of acid–base fluxes that are still in use some 30 years later.

The rainbow trout used by McDonald and Wood were fitted with an arterial catheter, or cannula – to allow repetitive blood sampling without disturbing the fish – and then the blood samples were analyzed for acid–base status and ion concentrations. Crucially, the duo also recognized the value of analyzing the acid–base status and ion concentrations of the water in which the fish were held. By measuring changes in water ion concentrations and acid–base equivalents for fish held in a recirculating (i.e. closed) water system, McDonald and Wood were able to calculate net ion and acid–base fluxes, that is, values for the net exchange of ions and acid–base equivalents between the animal and its environment. In fish, both the gill and the kidney are key sites of ionic and acid–base regulation. Thus, McDonald and Wood fitted the fish with a bladder catheter for urine collection. Separate analyses of the collected urine and the water in which the fish were held allowed the relative contributions of the gill and kidney to net ion and acid–base fluxes to be distinguished. McDonald and Wood therefore were able to measure gains or losses of ions and acid across the gill and *via* the urine, and to assess the importance of these transfers to blood acid–base and ion status over time as the fish were exposed to acidic water (pH 4). In so doing, they identified the gill as the site of acid uptake and demonstrated an important role for the kidney in excreting the acid load gained *via* the gill. Renal acid excretion was not, however, sufficient to compensate for branchial acid gain, resulting in a depression of blood pH. They also reported that exposure to acid water promoted a continuous loss of ions across the gill, accounting for the well-known lowering of plasma ion levels in acid-exposed fish, and they suggested that fluid shifts triggered by ion losses might ultimately cause circulatory collapse and death, a hypothesis supported by subsequent studies (e.g. Milligan and Wood, 1982).

Their mechanistic approach to analyzing the effects of acid exposure on a freshwater fish is one reason that McDonald and Wood's 1981 paper has been cited over 225 times. Particularly during the 1980s, as researchers struggled to come to grips with the complexities of acid environments and their effects on fish – for example, the acutely lethal effects of acid 'surges' *versus* the sublethal effects of chronic acid exposure, the effects of low pH in hard *versus* soft water and the protective effects of Ca^{2+}, species differences in sensitivity to low pH, and the additive effects of exposure to heavy metals such as aluminium that frequently occurs in conjunction with acid exposure – the data and experimental approach outlined by

McDonald and Wood proved invaluable. Of particular importance was their use of the flux approach, because the convention prior to their work was to identify toxic effects through analysis of blood and/or tissue samples. By measuring changes in ions or acid–base equivalents in water, McDonald and Wood shifted the focus of attention to the physiological processes affected by acid exposure rather than the consequences of changes in these processes. Rather than having to wait for the toxic effects of low pH to accumulate over hours or days before manifesting as changes in blood pH or ion levels, the toxic effects of low pH were immediately apparent and could be followed over time as changes in the movement of acid–base equivalents or ions across the gills. Moreover, it is much easier to detect small changes in ion levels in water, where ion levels are very low (e.g. $[Na^+]<1\,mmol\,l^{-1}$), than in plasma, with its correspondingly higher ion levels (e.g. $[Na^+]\approx150\,mmol\,l^{-1}$). The much greater sensitivity of the flux approach over conventional methods allowed toxic effects to be detected earlier and with temporal resolution, facilitating the study of more subtle, sublethal effects of acid exposure.

The other major innovation provided by McDonald and Wood's 1981 JEB paper was a straightforward method for measuring net acid–base fluxes across the gill. Water samples collected at the beginning and end of a flux period were aerated to remove respiratory CO_2 and then titrated to an endpoint of pH 4.0 using HCl. The difference in the volume of titrant required to achieve the endpoint of pH 4.0 between the two samples is termed the titratable component of the net acid flux, and reflects the acid (when less acid titrant is added to the final water sample relative to the initial sample) or base (when more acid titrant is added to the final water samples) added to the water by the fish. The fish also loses protons in association with branchial ammonia excretion, because NH_3 combines with H^+ to give NH_4^+. This reaction occurs in the water passing over the gills as the fish excretes NH_3; NH_4^+ also may be excreted across the gill. Protons trapped in this fashion are not titratable, and so to capture the proton loss associated with ammonia excretion, water samples were also assayed for ammonia concentration and the net ammonia flux was calculated. The net branchial acid–base flux then could be determined as the sum of the titratable net flux and ammonia excretion. The measurement of net acid–base flux in this manner required little in the way of specialized equipment (a good pH meter/electrode and a precision burette for titration) and was relatively rapid, effectively allowing bulk analysis so that multiple flux periods or fish could be evaluated. It therefore contrasted favourably with the other approach available at the time [Δbicarbonate measuring system (Heisler, 1978)], which relied upon an elaborate setup that could accommodate only a single fish.

Over the decade following its introduction, McDonald and Wood's approach for measuring net acid–base flux across the gill was widely adopted. Indeed, it remains in common use now (e.g. Gilmour et al., 2012), 30 years later, because it is simple and accessible. A quick scrutiny suggests that over half the papers that cite their 1981 JEB publication do so with reference to this approach for measuring acid–base fluxes, and these citations are likely to continue to mount because no one has come up with a better way of measuring acid–base fluxes to water.

For relatively few papers can it be claimed that significant contributions have been made to both the development of a method and the advancement of the field, but this description certainly applies to McDonald and Wood's 1981 publication. This classic paper pioneered a method for measuring net acid–base fluxes that remains widely used. At the same time, the mechanistic analysis of the effects of acid water on ionic and acid–base regulation in a freshwater fish provided by the duo shifted the focus of such toxicology studies from chronicling a list of toxic effects to identifying mechanisms of toxicity. McDonald soon joined Wood on the faculty of McMaster University, and their collaboration flourished over many years, evolving from the effects of low pH, to the effects of heavy metals (in conjunction with low pH), to the effects of global warming, but in all cases maintaining a mechanistic focus. The experimental approaches laid out in 1981 by McDonald and Wood proved to be a highly effective blueprint for this exceptionally productive team!

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