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THE EFFECTS OF CALCIUM ON SODIUM FLUXES IN THE BROWN TROUT, *SALMO TRUTTA*, IN NEUTRAL AND ACID WATER

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It is well established that external calcium can have a marked effect on sodium fluxes in both marine and freshwater organisms (Potts & Fleming, 1971; Cuthbert & Maetz, 1972; Fleming, Nichols & Potts, 1974; Eddy, 1975; Carrier & Evans, 1976; Isaia & Masoni, 1976). In the few fish species which have so far been examined the effects of external calcium appear to be primarily on sodium efflux, which is reduced in the presence of calcium (Fleming *et al.* 1974; Eddy, 1975; Isaia & Masoni, 1976). The influence of calcium on sodium influx shows considerably more variation between species (Shaw, 1960; Sutcliffe, 1971; Eddy, 1975) making it difficult to propose a suitable mechanism to account for the observed effects.

More recently calcium has been recognized as an important factor influencing the survival of fish in acid waters, both in the laboratory (Brown, 1981) and in the field (Wright & Snekvik, 1978). Low pH has been shown to stimulate passive sodium efflux (Packer & Dunson, 1970, 1972; McWilliams & Potts, 1978) and inhibit sodium influx (Packer & Dunson, 1970; McWilliams & Potts, 1978; McWilliams, 1980) in freshwater salmonids. In the brown trout, *Salmo trutta*, increased sodium efflux results as a consequence of changes in the transepithelial gill potential (TEP), which becomes more positive (inside) at low pH (McWilliams & Potts, 1978). Calcium moderates the effect of low pH on this potential by changing gill permeability to Na^+, Cl^- and H^+ ions (McWilliams & Potts, 1978), which should in turn influence the rate of passive efflux of sodium from fish in such conditions.

Sodium fluxes in brown trout were measured as previously described (McWilliams & Potts, 1978) in unbuffered artificial media (approx. volume 40 l) containing 0·25 mM/l NaCl and 0·02 mM/l K_2SO_4.$Ca(NO_3)_2$ was added to give a range of calcium concentrations from 0 to 8·0 mM/l and pH adjusted to, and maintained at, pH 6·5 or pH 5·5 throughout each experiment using a pH-stat dispensing H_2SO_4 or KOH. The circulated water was aerated with Co_2-free air and there was no significant variation in pH during the course of each experiment.

At near-neutral pH (6·5) low calcium stimulates sodium efflux so that in 0 Ca^{2+} media the rate of sodium efflux is nearly 2% of the total body sodium content per hour. Body sodium content in the fish used was $32·5 \pm 1·6$ μequiv/g wet wt. (McWilliams & Potts, 1978). Sodium loss falls sharply as external calcium concentration

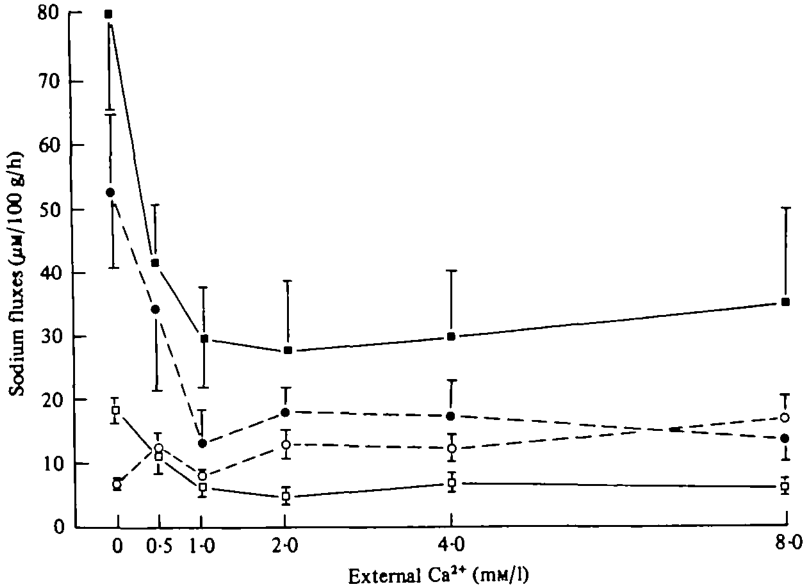

Fig. 1. The influence of external calcium on sodium ion influx and efflux at pH 6·5 and pH 5·5 in the brown trout (means ± 1 × S.E.). ○ -- ○, Influx pH 6·5; ● -- ●, efflux pH 6·5; □——□, influx pH 5·5; ■——■, efflux pH 5·5.

increases until at concentrations of 1·0 mM/l and above there is no further significant effect (Fig. 1). At low Ca^{2+} levels the TEP in brown trout becomes more negative (inside) as the gill permeability to sodium is increased (McWilliams & Potts, 1978). Calcium has the greatest influence on gill permeability at concentrations between 0 and 1·0 mM/l (McWilliams & Potts, 1978) so it would be expected that the effects on sodium efflux should also be confined to this concentration range. Under these circumstances the electrical gradient against which the active sodium pump operates is reduced and an increase in the rate of sodium transport might be expected. However, in low Ca^{2+} media an increase in the sodium permeability of the gill means that passive sodium efflux will increase (Fig. 1) and the pump must work faster to maintain sodium balance. Statistically ($P > 0·05$) calcium has no significant effect on sodium influx at pH 6·5 (Fig. 1) although there appears to be a gradual decrease in uptake rate as calcium concentration decreases. These results contrast with those of Cuthbert & Maetz (1972) and Eddy (1975), who found that at near-neutral pH sodium influx was stimulated by low external Ca^{2+}. In the present experiments uptake and loss of sodium were not significantly different ($P > 0·05$) at Ca^{2+} levels of 0·5 mM/l and above, and at Ca^{2+} concentrations of 1·0 mM/l and above there was no change in overall sodium turnover rate (Fig. 1).

In media of pH 5·5 sodium efflux rates are increased compared to those in near-neutral media (Fig. 1) although statistically the increase is only significant at 0 and 1·0 mM/l Ca^{2+} ($P < 0·05$), but the general effect of calcium on efflux rates is similar in both cases. Again, calcium has little effect on efflux rates from acid-exposed fish above a concentration of 1·0 mM/l (Fig. 1). This would suggest that Ca^{2+} is binding to a limited number of specific sites on the gill epithelium and that the binding affinities for calcium are similar in both neutral and acid media.

In acid-exposed fish low external Ca^{2+} elevates sodium influx (Fig. 1). The reasons why this phenomenon should only be observed in acid water are not clear, but if acid is leaching Ca^{2+} from the gill surface, effectively increasing the permeability of the gill to sodium (McWilliams & Potts, 1978) but to an extent in excess of that in neutral media, then as explained above, an increase in the rate of active sodium transport might be expected. Cuthbert & Maetz (1972) used chelating agents to remove calcium from the gills of goldfish (*Carassius auratus*) and recorded elevated sodium fluxes. They concluded from observations of sodium fluxes in fish subsequently placed in low-calcium media that the calcium lost from the gill surface was not easily replaceable. This may partly explain why elevated sodium uptake rates are observed in the brown trout only in acid, low-calcium media (Fig. 1).

At all calcium concentrations used in the present experiments the differences in gill potential between fish in water of pH 6·5 and pH 5·5 (McWilliams & Potts, 1978) would be insufficient to fully account for the apparent increases in the rate of passive sodium efflux. For example, in 0 Ca^{2+} media the shift in potential of +6 mV (McWilliams & Potts, 1978) is sufficient to increase the passive loss of sodium by a factor of 1·1 (Hodgkin & Horowicz, 1959) whereas the actual passive loss of sodium in 0 Ca^{2+} water is some 1·6 times faster in acid-exposed fish than from fish in near-neutral water (Fig. 1). Loss of Ca^{2+} from the gills in acid water could account for this descrepancy. Variations in the ability of gills to bind Ca^{2+} in media of a variety of calcium concentrations may explain some of these observed differences and also those between species with respect to the effect of external Ca^{2+} on sodium uptake rate in low Ca^{2+} media.

At 1·0 mM/l Ca^{2+} and above sodium influx rates are not affected by Ca^{2+} but overall sodium uptake rates are depressed ($P < 0·01$) compared to fish in near-neutral media. McDonald, Hobe & Wood (1980) recorded a negligible blood acidosis in acid exposed rainbow trout (*Salmo gairdneri*) in low-calcium media compared to high-calcium media and suggested an increased proton excretion rate, linked to sodium influx, in low-calcium media as a possible explanation. In such a case the activation of sodium transport by low Ca^{2+} must override the inhibition of sodium uptake by low pH. At pH 5·5 ($Ca^{2+} = 0·5$ mM/l) in brown trout sodium influx is inhibited (McWilliams & Potts, 1978) but in the present experiments at pH 5·5 (Fig. 1) sodium uptake rate is stimulated at low Ca^{2+} levels despite the low pH. McDonald *et al.* (1980) also recorded reduced plasma-sodium levels during acid exposure in low calcium water and suggested that the increase in sodium efflux in low pH media (Packer & Dunson, 1970; McWilliams & Potts, 1978) must be greater than the stimulation of sodium influx by low calcium to explain this. The data in Fig. 1 clearly demonstrate this to be so in *Salmo trutta*. In acid-exposed trout at 0 Ca^{2+} the net loss of sodium is some 2% of the body content per h.

The laboratory experiments of McDonald *et al.* (1980) suggest that in low-calcium water ion regulatory failure is the main toxic mechanism of low pH, an observation supported by field data (Leivestad & Muniz, 1976; Leivestad *et al.* 1976). However, it should be remembered that the fish observed in these field studies were adapted to low-calcium waters (< 0·025 mM/l). The data reported here suggest that the fish used, which were adapted to relatively high calcium levels (0·2–0·6 mM/l) have gills with a low binding affinity for calcium, as judged from the observation that external Ca^{2+} influences sodium efflux up to a concentration of 1·0 mM/l, a concentration many times that found in natural water liable to acidification. The question then arises whether fish living in low-calcium waters would have gills with a higher binding affinity for calcium. If this were so, the effects of lowered pH would perhaps not be so immediate as that suggested by laboratory data from fish from high Ca^{2+} environments. Until we have a better understanding of the role of calcium in these situations, particularly with respect to fish populations in acidified, low-calcium waters, care should be taken when extrapolating laboratory data to the situation in the field.

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