J. exp. Biol. (1978), 73, 15–27
With 1 *figure*
Printed in Great Britain

CARBONIC ANHYDRASE (ACETAZOLAMIDE-SENSITIVE ESTERASE) ACTIVITY IN THE BLOOD, GILL AND KIDNEY OF THE THERMALLY ACCLIMATED RAINBOW TROUT, *SALMO GAIRDNERI**

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(*Received* 16 *May* 1977)

SUMMARY

1. Gill, kidney and blood levels of acetazolamide-sensitive esterase (carbonic anhydrase) activity were estimated at acclimation temperature and at a common temperature (25 °C) in rainbow trout acclimated to 2, 10 and 18 °C. Plasma levels of sodium, potassium and chloride were also examined for possible acclimatory variations.

2. Plasma sodium and chloride levels, and the sodium:chloride ratio were unaffected by thermal acclimation; potassium concentrations were significantly elevated at 18 °C.

3. Significant, but modest changes in renal and branchial carbonic anhydrase activity were observed under physiologically realistic incubation temperature conditions. Blood carbonic anhydrase activity was sharply elevated at higher acclimation temperatures.

4. The data are discussed in relation to the hypothesis that carbonic anhydrase in this relatively stenothermal freshwater salmonid, through its intimate association with the coupled HCO_3^-/Cl^- and $H^+ + NH_4^+/Na^+$ exchange systems may provide for relatively thermostable basal rates of sodium and chloride uptake from the medium and recovery from urine. The renal, and more notably the branchial (Na^+/K^+)-stimulated ATPase systems, and erythrocytic carbonic anhydrase may then serve primarily as high-temperature amplifiers of sodium and chloride recruitment respectively.

INTRODUCTION

In teleost fishes temperature-induced increases in oxygen demand prompt branchial cardiovascular-ventilatory responses which lead to substantial increases in ion depletion rates. The not insignificant urinary electrolyte losses observed in cold-adapted animals are sharply elevated at higher temperatures (Houston, 1973; Mackay, 1974), and this is accompanied by some change in branchial efflux rates (Maetz, 1972; Cameron, 1976). Freshwater-adapted salmonids are nevertheless able to stabilize plasma sodium and chloride levels over relatively broad temperature ranges (Gordon,

* Supported by National Research Council of Canada Operating Grant A6972 to A. H. Houston.

1959; Hickman *et al* 1964; Houston *et al.* 1968; Byrne, Beamish & Saunders, 1972). The most obvious compensatory responses open to these animals involve reduction in lamellar ionic permeabilities, and increased branchial absorption and/or renal recovery of electrolytes. In the latter instance increases in the activity of transport enzyme systems might be anticipated as an accompaniment of the thermoacclimatory process. Consistent with this, (Na^+/K^+)- and (HCO_3^+)-stimulated ATPase activities are elevated following acclimation to increased temperatures in goldfish *Carassius auratus* (Murphy & Houston, 1974) and rainbow trout *Salmo gairdneri* (McCarty & Houston, 1977). The extreme cold-sensitivity of the ATPases suggests, however, that the maintenance of ionic balance at reduced temperatures requires intervention of a more thermostable system. Accordingly, in the present study the effect of acclimation upon carbonic anhydrase (EC 4.2.1.1) activity in the gills, kidneys and blood of the rainbow trout has been investigated.

MATERIALS AND METHODS

Origin and maintenance of experimental animals

Yearling trout of both sexes ranging in weight from 214 to 412 g were obtained from a local supplier (Goossens Trout Farm, Otterville, Ontario) and maintained in the laboratory in Frigid Unit HT-700 recirculating refrigerated troughs. Supplementary dechlorinated water inflows sufficient to renew tank volume at least once daily were provided for each tank. Through the use of thermistor-equipped relays of local design and construction (J. Rustenberg, unpublished) water temperatures were held to within 0·5 °C of desired acclimation values (2, 10, 18 °C). Each tank was provided with a photoperiod hood in which illumination varied from 118 to 194 lx at the water surface. All groups were held on a 12 h light/12 h darkness photoperiod regime throughout acclimation periods of not less than 3 weeks. Water quality conditions were comparable to those described earlier (McCarty & Houston, 1977; Murphy & Houston, 1977).

Blood sampling and electrolyte determination

Specimens were stunned, and blood samples drawn by caudal puncture into syringes treated with ammonium heparin. Plasma was separated immediately by centrifugation to forestall compositional changes, and stored at −80 °C prior to analysis. Chloride levels were estimated using a Buchler–Cotlove chloridometer; sodium and potassium concentrations by means of a Unicam SP-90 AAS used in the emission mode.

Determination of carbonic anhydrase activity

Gill arches were removed from the right side of each specimen and immediately immersed in ice-cold 250 mM sucrose, 40 mM-tris-H_2SO_4 (pH 7·5) solution, as were kidney samples. Upon completion of sampling gill filaments (0·9–2·0 g fresh weight) were stripped and transferred to 19 volumes of fresh solution in iced homogenizer tubes (less 5 ml retained for rinsing purposes and subsequently added). Kidney samples (1·0–2·0 g fresh weight) were similarly treated, and 1·0 ml whole blood diluted 1:20. Following homogenization (7 strokes with a motor-driven Teflon pestle) preparations were centrifuged (500 ***g***, ~ 0 °C, 30 min), and the supernatant decanted into ice-cold vessels. Preliminary trials covering a wide range of ***g*** values

and spin durations indicated that the combination cited provided an effective compromise between activity and precision of determination.

Protein content was estimated by the modified Schacterle–Pollock modification of the Lowry procedure (Albro, 1975). The alkaline haematin method (Anthony, 1961) was employed for determination of blood and tissue haemoglobin content. Although this procedure is inappropriate for use with tissues of substantial myoglobin content, interference of this type was not obvious with gill and kidney samples.

Lindskog *et al.* (1971) have recently reviewed alternative methods for assay of carbonic anhydrase activity. That based upon the esterase activity of the system was employed using *p*-nitrophenyl acetate substrate. Although this reaction proceeds at a much slower rate than the primary CO_2 reaction ($\times 10^{-5}$, Armstrong *et al.* 1966) esterase and CO_2 activities exhibit parallel behaviour with respect to pH, inhibitors and other influencing factors (Lindskog *et al.* 1971). It is of interest, moreover, that values for activity, at least in the blood, were of the same order of magnitude as those recently reported by Haswell (1976) on the basis of a manometric assay.

The assay protocol employed in the study was adapted from those described by Armstrong *et* al. (1966), Duff & Coleman (1966) and Verpoorte, Mehta & Edsall (1967) using 0·1 ml volumes of preparation (200–500 mg non-haemoglobin protein). The final assay medium comprised: p-nitrophenyl acetate – 1·5 mM, tris-H_2SO_4 buffer (pH 7·5) – 50 mM. Acetazolamide was prepared in the same buffer. Inhibition was found to be essentially constant over a range of acetazolamide concentrations exceeding 0·2 μM, and a final concentration of 20 μM was used throughout.

All assays were carried out in duplicate at the appropriate acclimation temperature (2, 10, 18 °C) and at 25 °C, a temperature close to the upper incipient lethal of the rainbow trout. As was the case in earlier studies upon (Na^+/K^+)- and (HCO_3^-)-stimulated ATPase activities in this species (McCarty & Houston, 1977) this incubation procedure was adopted to provide a basis for relating activities at a physiologically realistic maximum temperature to those at which the system actually operates.

Reactions were initiated by substrate addition to enzyme-buffer preparations preincubated at the appropriate temperature. Following mixing preparations were promptly introduced into the thermoregulated 'microflowthrough' cell of a Bausch and Lomb Spectronic 700. Changes in absorbance at 348 nm were recorded on a Fisher Omniscribe recorder. For each preparation duplicate assays were carried out without inhibitor to ascertain total esterase activity. Assays were then conducted in duplicate with acetazolamide, and the difference between the two taken as 'carbonic anhydrase' activity, while appreciating that the term 'acetazolamide-sensitive esterase' activity is technically more appropriate.

Activity values (μM min^{-1}) are reported for fresh tissue, and as approximate specific activities. In the instance of blood, specific activity estimates were corrected for the contribution of haemoglobin to total protein. Branchial and renal preparations were then corrected for activity associated with blood on the basis of haemoglobin content. Approximately 70% of the total protein content of gill tissue samples was attributable to haemoglobin; the corresponding figure for kidney samples being about 55%. From 6 to 15% of the total carbonic anhydrase activity in branchial preparations appeared to be associated with blood. Much higher proportions of activity, 61–80%, could be accounted for in this manner in the case of kidney tissues.

Equations (1) and (2) below were used in the calculation of corrected blood, and gill and kidney carbonic anhydrase activities.

Blood: [rate, μM/min]/[total protein − haemoglobin], (1)

Gill, kidney;

$$\frac{\left(\text{total tissue rate}\right) - \left(\text{total blood rate} \times \frac{\text{tissue Hb}}{\text{blood Hb}}\right)}{\left(\text{total protein} - \text{haemoglobin}\right.} \quad (2)$$

Statistical analysis

Comparisons were based upon use of the single-classification analysis of variance carried out by the procedures described by Sokal & Rohlf (1969). All data were subjected to logarithmic or arc-sin transformation as appropriate and significance attributed to differences at the 0·05 level or better.

RESULTS AND DISCUSSION

Plasma electrolyte levels

Sodium and chloride concentrations and the sodium:chloride ratio did not vary significantly over the range of acclimation temperatures employed (Table 1), confirming earlier findings (Houston *et al.* 1968; McCarty & Houston, 1977), and supporting the view that rainbow trout are capable of essentially perfect compensation for the perturbing effects of temperature upon ionic status. It is of particular note that the relative concentrations of these ions were thermostable, for it has been generally accepted that separate uptake mechanisms operate for each ion, and that these are independently regulated (Maetz, 1974). The maintenance of proportionality seen in this and earlier studies argues for interdependent regulation. It would seem necessary, therefore, that there be some form of 'tight' communication between the systems, and this would require at least one component common to both.

Such compensation does not extend to potassium (Table 1). Direct relationships between acclimation temperature and plasma levels of potassium and the other less abundant plasma ions have been frequently reported (Houston, 1973), and are particularly prominent in animals sampled during the winter months (Houston *et al.* 1968), or following acclimation to reduced photoperiod (Murphy & Houston, 1977). Their basis is, however, not yet clear.

Carbonic anhydrase activity

Estimates of carbonic anhydrase activity per unit fresh tissue weight are summarized in Table 2, while Fig. 1 includes specific activity data, i.e. that corrected for haemoglobin protein and blood-related activity. As temperature and thermoacclimatory effects are comparable in both cases subsequent comments refer specifically to the corrected values of Fig. 1.

(1) *Blood.* When carbonic anhydrase assays were carried out at 25 °C significant differences in blood activity were observed between specimens acclimated to 2 °C, and those held at 10 and 18 °C. No significant variations were apparent between the 10 and 18 °C groups. Under *in vivo* incubation temperature conditions activity increased in near-linear fashion with acclimation temperature; the differences

Table 1. *Plasma electrolyte levels* (mM) *and concentration ratios in rainbow trout acclimated to* 2, 10 *and* 18 °C ($N = 15$). *Values given as mean* ± *one standard error of the mean, with range in brackets*

Acclimation temperature (°C)	Sodium	Potassium	Chloride	$\frac{\text{Sodium}}{\text{Chloride}}$ (Means)	$\frac{\text{Potassium}}{\text{Sodium}}$ (Means)
18	148·8 ± 1·63 (141·1 – 159·9)	2·8 ± 0·35 (1·0 – 5·7)	126·6 ± 1·84 (113·6 – 138·7)	1·16	0·012
10	152·8 ± 1·19 (144·2 – 159·9)	1·7 ± 0·52 (1·0 – 2·7)	129·2 ± 1·00 (120·2 – 135·6)	1·18	0·011
2	151·5 ± 0·82 (147·6 – 156·5)	1·9 ± 0·27 (0·7 – 4·8)	130·4 ± 1·7 (121·5 – 136·9)	1·18	0·019
Significance	NS	$P < 0·05$	NS		

Table 2. *Carbonic anhydrase activity* (μM min^{-1} g^{-1}, *fresh tissue weight*) *in rainbow trout acclimated to* 2, 10 *and* 18 °C ($N = 15$). *Values given as mean* ± *one standard error of the mean, with range in brackets*

Acclimation temperature (°C)	Carbonic anhydrase activity					
	Gill		Kidney		Blood	
	in vivo	25 °C	*in vivo*	25 °C	*in vivo*	25 °C
18	0·59 ± 0·04 (0·40 – 0·80)	0·70 ± 0·04 (0·48 – 0·96)	0·33 ± 0·02 (0·16 – 0·48)	0·37 ± 0·02 (0·24 – 0·48)	0·83 ± 0·06 (0·40 – 1·12)	0·97 ± 0·07 (0·56 ± 1·52)
10	0·77 ± 0·05 (0·56 – 1·12)	1·10 ± 0·06 (0·72 – 1·52)	0·26 ± 0·02 (0·16 – 0·40)	0·40 ± 0·04 (0·24 – 0·64)	0·58 ± 0·03 (0·40 – 0·80)	0·90 ± 0·05 (0·64 – 1·28)
2	0·76 ± 0·03 (0·64 – 0·96)	1·27 ± 0·06 (0·88 – 1·60)	0·16 ± 0·01 (0·12 – 0·20)	0·25 ± 0·02 (0·14 – 0·36)	0·44 ± 0·02 (0·32 – 0·56)	0·69 ± 0·04 (0·40 – 0·88)

observed between both 2 and 10 °C and 10 and 18 °C being highly significant ($P < 0·01$). If values obtained at 25 °C are some function of maximum activity under physiologically realistic temperature conditions, and those observed in assays conducted under acclimation temperature incubation conditions similarly reflect functional activity, an interesting relationship emerges. In this instance, as in earlier studies upon (Mg^{2+})-dependent and (Na^+/K^+)-stimulated ATPase activities in this species (McCarty & Houston, 1977), intersection is obtained at a value close to the upper incipient lethal for the rainbow trout. Comparable relationships also hold for gill and kidney carbonic anhydrase activities, and lend support to the view that previously suggested ionoregulatory involvements in the thermal death of fishes (Doudoroff, 1945; Brett, 1952) may be related to limitations upon maximum transport capacity (McCarty & Houston, 1977).

Mean Q_{10} values calculated for preparations assayed at acclimation temperature and 25 °C ranged from 1·21 to 1·32, and were generally consistent with previously published Q_{10} values for this enzyme (Davis, 1961). The coefficient relating activities estimated under *in vivo* temperature conditions (properly termed a thermal coefficient rather than a Q_{10}) had a mean value of 1·49 over the range 2–18 °C.

(2) *Gill.* A strikingly different change in activity with temperature was observed in branchial preparations. Under both *in vivo* incubation temperature conditions and

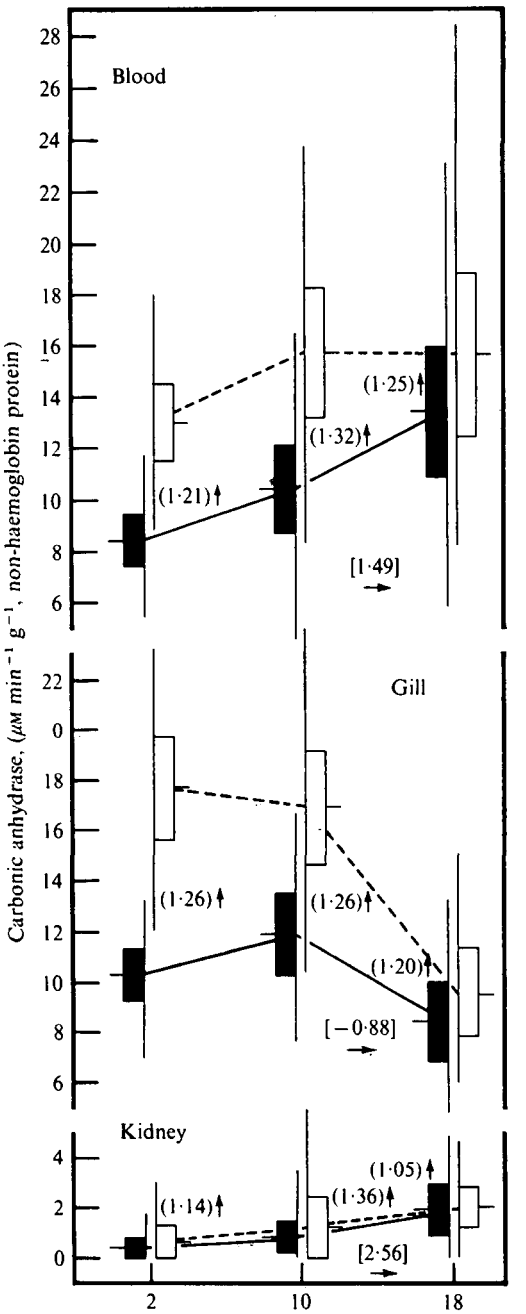

Fig. 1. Carbonic anhydrase activity (μM min^{-1} g^{-1} non-haemoglobin protein) in rainbow trout acclimated to 2, 10 and 18 °C. Values for gill and kidney have been corrected for blood-associated carbonic anhydrase. Cross-bar, mean; vertical bar, 95 % confidence interval; vertical line, range. Open bar, activity at 25 °C; solid bar, activity at acclimation temperature. Bracketed numbers, mean Q_{10} over range designated by arrows. Square brackets, mean thermal coefficient over range designated by arrows (N = 15).

at 25 °C the activities seen in specimens held at 2 and 10 °C did not differ significantly. At 18 °C, however, activity declined markedly ($P < 0.01$). Mean Q_{10} values were similar to those for blood preparations, ranging from 1·20 to 1·26. By contrast, the thermal coefficient relating activity at 2 °C to that at 18 °C was low and negative (−0·88).

(3) *Kidney*. As was the case with blood, renal carbonic anhydrase activity rose significantly ($P < 0.05$) following acclimation to higher temperatures. Mean Q_{10} values (1·05–1·36) were comparable to those observed with gill and blood preparations. The thermal coefficient relating *in vivo* activities between 2 and 18 °C was, on the other hand, substantially higher (> 2·5). As a consequence of the increased renal and decreased branchial activity observed under *in vivo* temperature incubation conditions the ratio of branchial-to-renal activity declined at higher acclimation temperatures from about 25 to less than 5. This argues that acclimation to elevated temperatures is associated with progressively greater involvement of carbonic anhydrase in the recovery of urinary electrolyte.

This tissue-specific change in carbonic anhydrase activity contrasts sharply with that previously reported for gill and kidney (Na^+/K^+)-ATPases. Acclimation to higher temperature resulted in proportionally greater increases in branchial than in renal (Na^+/K^+)-ATPase activity, and this suggested an increasingly more important role for the system in branchial sodium absorption under conditions of increased temperature (McCarty & Houston, 1977). Comparison of the specific activities of carbonic anhydrase and (Na^+/K^+) ATPase (McCarty & Houston, 1977) in the gills and kidneys of rainbow trout also suggests that the ratio of carbonic anhydrase activity to (Na^+/K^+) ATPase activity varies inversely with temperature in the gill, whereas the converse is true of the kidney.

Compensation for temperature effects

Two obvious means of compensating for the perturbing effects of increased temperature upon water-electrolyte balance are open to freshwater fishes: (1) reduction in lamellar permeability and (2) enhancement of branchial absorption and/or renal recovery of electrolytes. Evidence presently available suggests that the former response may be invoked in the case of some ions, but not in the instance of water. For example, the effects of temperature upon oxygen consumption, ventilatory flow and diffusional water influx are comparable (Evans, 1969; Isaia, 1972; Motais & Isaia, 1972), and this suggests that reductions in water permeability do not play a major role in the adaptive process. Compensation for water-loading is apparently achieved through substantial increases in urine output (Mackay & Beatty, 1968; Lloyd & Orr, 1969; Motais & Isaia, 1972). Although urinary ion concentrations tend to be reduced at higher temperatures (Houston, 1973; Mackay, 1974) – as might be anticipated from accompanying changes in renal carbonic anhydrase and (Na^+/K^+) ATPase activities (present study; McCarty & Houston, 1977) – overall urinary electrolyte losses are sharply elevated. There is, on the other hand, some indication that branchial electrolyte permeabilities may be adaptively reduced under these conditions for the effect of temperature on ion efflux rates is substantially less than that upon water influx (Maetz, 1972; Cameron, 1976). Nevertheless, the total rate of ion depletion rises and ionoregulatory compensation must include some means of increasing ionic uptake

Carbonic anhydrase and compensation in relation to sodium

There can be little question that (Na^+/K^+)-stimulated ATPase plays some role in the metabolism of sodium by fishes, particularly in seawater-adapted specimens (Maetz, 1974). It is, however, an extremely cold-sensitive system (Giles & Vanstone, 1976; Russell & Chambers, 1976). Recent studies involving assays conducted under physiologically realistic incubation temperature conditions suggest that it may serve primarily as a high-temperature sodium uptake amplifier in freshwater-adapted salmonids (McCarty & Houston, 1977). If this is the case at least one relatively cold-insensitive process is required if sodium balance is to be maintained at reduced temperatures. It is from this viewpoint that carbonic anhydrase is of particular interest for it is known to function in sodium absorption by rainbow trout (Kerstetter, Kirschner & Rafuse, 1970; Kerstetter & Keeler, 1976), and is characterized by both an unusually high turnover number and a low Q_{10} (Davis, 1961). Through provision of the hydrogen ion required to fuel H^+/Na^+ exchange, and for conversion of NH_3 to NH_4^+ prior to NH_4^+/Na^+ exchange, carbonic anhydrase could provide a relatively thermostable basal sodium uptake rate. Little change in gill or kidney activities would be expected following acclimation to higher temperatures, for sodium uptake could presumably be supplemented by thermal activation of the ATPase system. The results of the present study are quite consistent with the role postulated for carbonic anhydrase since little change in activity was observed in relation to acclimation temperature.

There are recent indications, moroever, that the changes in branchial (Na^+/K^+)-ATPase activity which accompany variations in environmental temperature may have a greater impact upon sodium absorption than might be anticipated from their actual magnitude. The freshwater-adapted rainbow trout possesses lamellar cells comparable to the 'chloride' cells of the seawater-adapted salmonid (Olson & Fromm, 1973), and widely regarded as sites of electrolyte transport. Such cells are characterized by 'tight' apical intercellular junctions, and an extensive network of blind-ended tubules which open baso-laterally and are therefore in communication with lamellar lacunae. Recent studies by Karnaky *et al.* (1976) have demonstrated the association of (Na^+/K^+) ATPase with this network in both freshwater- and seawater-adapted teleosts. It will be apparent that cells of this type have the morphological and transport enzyme characteristics required of a forward-flow solute transport system (Diamond, 1971), and Karnaky *et al.* (1976) have noted that they possess characteristics particularly appropriate to salt absorption from the medium. In a system of this type cold inhibition of the (Na^+/K^+)-ATPase system is functionally equivalent to reduction of solute input length in the tubular network, while activation at higher temperatures is comparable to an increase in this parameter. Simulation studies upon transport epithelia (Diamond, 1971) indicate that adjustments of this type lead to near-exponential changes in emergent solute concentration, and far outweigh the effects of variations in transport activity *per se*. If the teleostean branchial sodium absorption process operates at the cellular level as a forward-flow system, cold acclimation would, in effect, limit solute input to that which could be achieved as a consequence of carbonic anhydrase-based heterionic exchange. Thermal activation of (Na^+/K^+) ATPase, on the other hand, should compensate for the decline in branchial carbonic anhydrase activity observed at higher temperatures.

Carbonic anhydrase and compensation in relation to chloride

While chloride absorption by fish is known to involve HCO_3^-/Cl^- exchange many features of chloride metabolism and its regulation are unclear. The process satisfies several active transport criteria (Kerstetter & Kirschner, 1972), and a number of studies point to a membrane-associated, (SCN^-)-inhibited transport system sited at the apical epithelial surface of the gill (Motais & Garcia-Romeu, 1972; Kerstetter & Kirschner, 1972, 1974; Epstein, Maetz & de Renzis, 1973; deRenzis, 1975). The identity of this system, and its relation to HCO_3^-/Cl^- exchange are not clear. It does not appear to involve carbonic anhydrase (deRenzis, 1975) or, as earlier hypothesized, a (HCO_3^-)-stimulated analogue of the (Na^+/K^+) ATPase system (Kerstetter & Kirschner, 1974; Solomon *et al.* 1975; McCarty & Houston, 1977). Furthermore, the postulated membrane association of this system would, *a priori*, suggest involvement in transport only at relatively high temperatures.

There is substantial evidence that carbonic anhydrase participates in chloride as well as sodium exchange uptake (Maetz, 1974). However, the modest changes in branchial and renal activity which accompany acclimation suggest that some means must exist for supplementing chloride as well as sodium uptake at higher temperatures. The foregoing comments regarding active branchial transport systems not withstanding the hypothesis recently offered by Kerstetter & Kirschner (1972) in relation to chloride absorption by rainbow trout is of particular interest. Kerstetter & Kirschner suggest that a $HCO^-{}_3/Cl^-$ exchange mechanism driven by transepithelial diffusion of blood bicarbonate constitutes the principal means of chloride absorption in rainbow trout. If this is the case an increase in blood levels of carbonic anhydrase would be anticipated at higher temperature, and this was observed in the present study. Accordingly, increased erythrocyte carbonic anhydrase activity may provide the required amplification. It should be noted, however, that Milne & Randall (1976) have recently questioned the validity of the data upon which the Kerstetter–Kirschner hypothesis was developed, suggesting that the exchange capacity of the system may have been overestimated. However, support can be adduced from other studies. The generally reciprocal variations in plasma chloride and bicarbonate concentrations observed in rainbow trout following manipulation of the respiratory medium (Lloyd & White, 1967) can, for example, be interpreted as supporting the probability that a functional HCO_3^-/Cl^- exchange system, comparable to that observed in other freshwater species, exists in the trout as well. Furthermore, if erythrocyte-generated plasma bicarbonate is utilized to drive the exchange process it would not be unreasonable to expect that acclimation might be associated with some decrease in plasma bicarbonate and possibly pH as well. Both conditions have been reported in thermally acclimated freshwater-adapted salmonids (Rahn & Baumgardner, 1972; Randall & Cameron, 1973).

Relative roles of carbonic anhydrase and ion-stimulated ATPases in the maintenance of electrolyte balance in freshwater salmonids

There is some likelihood that carbonic anhydrase may play, in freshwater-adapted salmonids, the central ionoregulatory role which is commonly attributed to the (Na^+/K^+) ATPase system under marine conditions. Support for this speculation can,

for example, be adduced from the recent report by Kerstetter & Keeler (1976) that ouabain perfusion of *in situ* rainbow trout gill preparations has little effect upon sodium influx. Acetazolamide perfusion, however, significantly reduced the uptake of this ion. Some disagreement is seen between these findings and the earlier observations of Payan, Matty & Maetz (1975). Nonetheless, in the latter study acetazolamide influence upon sodium influx was substantially greater than that of ouabain.

Retrospectively, at least, this is not an unexpected finding. If the secondary esterase reaction proceeds at a rate which is only 10^{-5} that of the primary effect on CO_2 hydration (Armstrong *et al.* 1966) actual carbonic anhydrase activity probably exceeds that of the ATPase system. Taking this rate relationship between the primary hydration and secondary esterase reactions into account, and recognizing the pitfalls inherent in comparisons of *in vitro* enzyme assays it is worth noting that the results of the present and earlier studies (McCarty & Houston, 1977) suggest activity differentials which represent orders of magnitude. From a more abstract viewpoint the utilization of carbonic anhydrase in the achievement of reasonably thermostable plasma sodium and chloride levels is clearly consistent with the suggestion (Hochachka, 1973) that low-temperature adaptation (given saturation substrate concentrations) is most effectively achieved through utilization of systems having the high turnover number and low thermal dependency of carbonic anhydrase. Controlled amplification of uptake at higher temperatures could then be achieved using systems of the ion-activated ATPase type whose rates can be increased through thermal activation, and which are amendable to E–S affinity, lipid association and related forms of precise regulation.

In this context it is of interest that acclimation to higher temperatures was associated with significant increases in the carbonic anhydrase activities of blood and kidney whereas that of gill preparations actually declined. While several models will account for this differential effect it is noteworthy that in *Platichthys flesus* and *Anguilla anguilla* (Girard & Istin, 1975; Carter, Auton & Dando, 1975), as in several of the higher vertebrates (Lindskog *et al.* 1971), carbonic anhydrase iso-enzymes of markedly different activity have been observed. Moreover, variations in relative abundance and overall activity have been linked to variations in osmoregulatory requirements. Thus, there is some possibility that supplementation of chloride uptake at higher temperatures may involve selective, tissue-specific adjustments in isoenzyme complement during the acclimatory process, and this is presently under study.

Blood carbonic anhydrase activity

Finally, observations upon rainbow trout blood are of interest in relation to the recent report by Mashiter & Morgan (1975) that carbonic anhydrase activity could not be consistently detected in the blood of *Platichthys flesus*. Subsequent studies on this species (Carter *et al.* 1976), and *P. stellatus* (Haswell, 1976) indicated the presence of substantial activity, and Haswell has suggested that the *p*-nitrophenyl acetate assay used by Mashiter and Morgan may have been insufficiently sensitive. The findings of the present study indicate that this was not the case and point to an alternative explanation. The assay protocol employed by Mashiter & Morgan embodied the assumption that carbonic anhydrase distribution in the teleostrean erythrocyte corresponds to that in the mammalian red cell. Accordingly, assays were conducted

upon supernatants of haemolysed and centrifuged samples. The likelihood that carbonic anhydrase might be structurally associated was commented upon some years ago by Lindskog *et al.* (1971). Consistent with this, recent studies upon carbonic anhydrase distribution in rainbow trout erythrocytes (J. S. Smeda and A. H. Houston, unpublished observations) have demonstrated that most of the activity of haemolysed blood samples is associated with centrifugable material. Relatively little can be recovered from supernatants.

CONCLUSIONS

The observations of the present study, taken in context with earlier investigations of (Na^+/K^+) ATPase activities (Murphy & Houston, 1974; McCarty & Houston, 1977), are consistent with the view that branchial and renal carbonic anhydrase activities, through their effects on HCO_3^-/Cl^-, H^+/Na^+ exchanges and possibly conversion of NH_3 to NH_4^+ prior to NH_4^+/Na^+ exchange, may provide relatively thermostable basal rates of sodium and chloride uptake. Augmentation of recruitment at temperatures which promote increased electrolyte depletion appears to be largely associated with increased branchial (Na^+/K^+) ATPase and erythrocyte carbonic anhydrase activities.

The authors gratefully acknowledge the financial support provided by the National Research Council of Canada through Operating Grant AG972 awarded to the senior author, and the advice and assistance of our colleague J. S. Smeda.

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