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EFFECTS OF TEMPERATURE CHANGE ON ACID–BASE REGULATION IN SKIPJACK TUNA (*KATSUWONUS PELAMIS*) BLOOD

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Abstract—1. The effects of temperature change (*in vitro*) on acid–base balance of skipjack tuna blood were investigated.

2. By examining the relationship between blood pH and temperature (*in vitro*) under conditions of constant CO_2 tension (open system), it was observed that dpH/dT = −0.0131 U/°C.

3. This value falls well within the range of *in vivo* values reported for other ectothermic vertebrates, and is only slightly different than results obtained *in vitro* under conditions of constant CO_2 content (closed system; dpH/dT = −0.0165 U/°C).

4. It is concluded that changes in pH following temperature changes can be accounted for solely by the passive, *in vitro* behaviour of the chemical buffer system found in the blood, so that active regulatory mechanisms of pH adjustment need not be postulated for skipjack tuna.

INTRODUCTION

Tuna are extraordinary teleosts with respect to their ability to maintain muscle temperature above ambient temperature (1–11°C higher). The mechanism by which tuna conserve heat is by retention of metabolic heat through vascular counter-current heat exchangers or retia (see Carey *et al.*, 1971). Very steep thermal gradients develop within the body musculature and thus blood temperature varies during circulation through the systemic vasculature. Behaviour patterns of tuna also may lead to rapid temperature changes of the blood. Indeed, Dizon *et al.* (1978) have shown that extensive vertical migrations (surface to 273 m) are continuous features of the daylight activity pattern of skipjack tuna in areas where water temperature ranged from 25°C at the surface to less than 12°C at 270 m depth. These temperature changes also would appear to place extraordinary demands on acid-base regulatory systems.

In air-breathing ectotherms blood pH changes with a temperature slope of approx. −0.016 to −0.018 U/°C by ventilatory adjustments of P_{CO_2} (see review by Reeves, 1977). Generally, air-breathing ectotherms behave as constant CO_2 content (C_{CO_2}) systems or closed systems, *in vitro* and dpH/dT parallels the neutral pH of water (dpN/dT = −0.016U/°C, range 20–37°C; *Handbook of Chemistry and Physics*, 56th edn). Such pH changes allow maintenance of constant relative alkalinity (Rahn, 1967) or constant fractional dissociation of alpha imidazole groups on protein histidine residues (Reeves, 1977). Similar changes in pH with temperature have been observed in water-breathers (*Salmo gairdneri*, −0.017; Randall and Cameron, 1973; *Cancer magister*, −0.018; McMahon *et al.*, 1978; *Carcinus maenas*, −0.019; Howell *et al.*, 1973; −0.0162; Truchot, 1973; *Callinectes sapidus*, −0.015; Wood and Cameron, 1984). However, a significant number of aquatic species (primarily fish) display dpH/dT of blood slightly lower than dpN/dT such that constant relative alkalinity is not maintained (e.g. *Scyliorhinus stellaris*, Heisler, *et al.*, 1976; Heisler, *et al.*, 1980; *Ictalurus punctatus*, Cameron and Kormanik, 1982; *Cynoscion arenarius*, Cameron, 1978; *Anguilla rostrata*, Walsh and Moon, 1982). Also, the situation in water-breathers is complicated further because, depending on the species, they may behave as constant CO_2 tension (P_{CO_2}) systems (e.g. *Salmo gairdneri*, Randall and Cameron, 1973) or as a combination of the two types (i.e. changes in both P_{CO_2} and C_{CO_2}; see Heisler, 1980) following temperature changes. While acid–base adjustments following temperature changes take place rapidly in air-breathers (< 2 hr), they take as long as 24 hr in water-breathers. Such prolonged adjustments would seem ill-suited to the active lifestyle of the tuna which imposes the simultaneous acid–base challenges of temperature fluctuations and exercise. In the present study we have investigated blood acid–base changes in skipjack tuna blood during temperature changes. These experiments were designed to determine whether skipjack blood, *in vitro*, obeys the rule of constant relative alkalinity (i.e. dpH/dT = −0.016 U/°C such that the ratio of OH^-/H^+ remains constant, Rahn, 1967) under conditions of constant CO_2 content (closed system) as one would predict (Rosenthal, 1948; Reeves, 1972, 1976a, b), as well as to determine blood pH temperature depen-

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dence under conditions of constant CO_2 tension (open system) which may more closely resemble the situation in fish. In particular, the experiments test the possibility that acid–base adjustments following temperature changes occur passively.

MATERIALS AND METHODS

Skipjack tuna (*Katsuwonus pelamis*) were captured on hook and line by local fishermen and transported to Kewalo Research Facility (National Marine Fisheries Service, Southwest Fisheries Centre, Honolulu Laboratory) where all subsequent experiments were performed. Fish of either sex, weighing between 1.2 and 2.3 kg (mean wt = 1.7 kg ± 0.2 SE, $N = 10$) were maintained outdoors in large circular holding tanks and supplied with rapidly flowing seawater (temperature = 25°C). Animals were used within the first three days of capture and were not fed while in captivity.

Experimental protocol

In order to sample blood, a fish was netted and quickly injected intramuscularly with 0.2–0.4 ml of the neuromuscular blocking agent, gallamine triethiodide (Flaxedil). Upon cessation of swimming, the fish was transferred to an operating table, submerged in water and a tube was inserted into the mouth which allowed continuous irrigation of the gills with aerated seawater (10–12 l/min). One ml of sodium heparin (10,000 U.S.P. units) was injected into the ventral aorta and, following a five min mixing period, as much blood as possible (usually 30–50 ml) was withdrawn via "blind" ventral aortic or cardiac puncture. Blood was centrifuged in order to obtain plasma and to prepare blood of approximately 40% haematocrit (hct). Blood or plasma then was transferred to tonometer flasks (50 ml) and equilibrated with humidified mixtures of CO_2 in air (using flowmeters) in a temperature controlled water bath. Three different experiments were performed. In the first, the blood or plasma was warmed from 15° to 30°C in 5-degree increments every 60 min under conditions of constant P_{CO2} (approximately 1% CO_2 in air). pH and total CO_2 content (C_{CO2}) were determined at each temperature and from these values, P_{CO2} and the molar concentration of CO_2 (S_{CO2}; $\alpha CO_2 \times P_{CO2}$) were calculated. pH measurements were made with a Radiometer PHM-71 digital acid–base analyser and associated micro pH electrode at 25°C. C_{CO2} was determined according to the method of Cameron (1971). P_{CO2} and bicarbonate concentration (HCO_3^-) were calculated using a reorganization of the Henderson–Hasselbalch equation. pK′ values of carbonic acid were obtained from Severinghaus *et al.* (1956) and the solubility coefficients of CO_2 (α_{CO2}) were obtained from Albers (1970). In the second series of experiments, blood again was warmed in 5-degree increments, but under conditions of constant C_{CO2}, in sealed syringes. Temperature equilibration was slow in these experiments and 60 min was required to reach stable water and blood temperatures. pH and C_{CO2} were measured as described previously. Because of the long time blood remained sealed in syringes, it was possible that red blood cell (rbc) metabolism alone, might cause changes in pH and P_{CO2}. To test for the effect of rbc metabolism, blood was sealed in syringes for identical time periods as before, but at constant temperature (25°C). The results of these control experiments made it possible to correct the results obtained during the temperature change experiments for the effects of rbc metabolism. In the final series of experiments, blood was equilibrated first with 1% CO_2 (in air) at 15°C and pH and C_{CO2} were determined. After making these measurements, samples of blood were quickly warmed to 30°C (20 min) in sealed syringes and pH and C_{CO2} measured again. Open and closed system experiments were performed on blood obtained from different sets of animals ($N = 3$ in each case).

In the figures, variability of the data is indicated by ± 1 SE. Sample means have been statistically analysed using Student *t*-test and 5% was taken as the fiducial limit of significance.

RESULTS

The results of changing temperature under conditions of constant P_{CO2} (open system) in blood (hct ≃ 40%) and plasma are illustrated in Fig. 1. The effects of temperature change on pH and C_{CO2} for plasma and whole blood are strikingly different. In blood, pH fell as temperature increased with a slope of −0.0131 U/°C. In contrast, plasma pH increased with increasing temperature (dpH/dT = +0.004 U/°C). This increase in pH was a result of the ratio $[HCO_3^-]/S_{CO2}$ increasing from 26.7 at 15°C to 37.1 at 30°C and the change in pK′ being so small that the overall effect was an increase in pH. Similarly, blood C_{CO2} decreased greatly with increasing temperature ($dC_{CO2}/dT = -0.339$ mM/°C) whereas plasma C_{CO2} was affected only slightly ($dC_{CO2}/dT = -0.100$ mM/°C). The minor changes of P_{CO2} in both blood and plasma during conditions of supposed constant P_{CO2} were probably due to small variations in air or CO_2 flow which were undetectable using flowmeters as monitoring devices. In both cases, S_{CO2} decreased with increasing temperature and simply reflects the inverse relationship between αCO_2 and temperature.

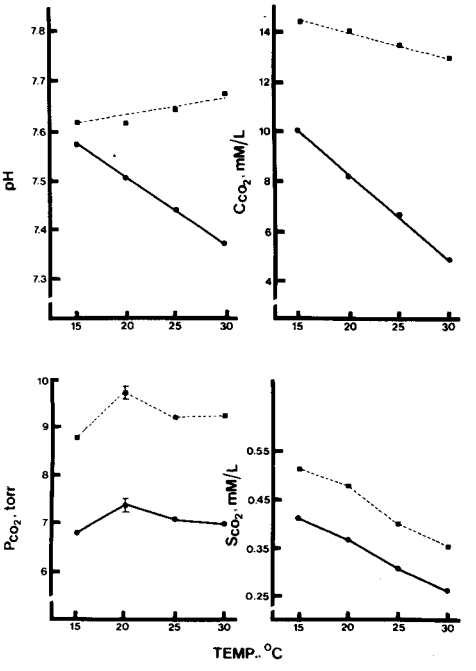

Fig. 1. The effect of temperature change on *in vitro* blood (hct ≃ 40) (●—●; $n = 3$) and plasma (■—■; $n = 2$) acid–base status under conditions of constant P_{CO2} (open system). S_{CO2} = the molar concentration of CO_2 ($\alpha_{CO2} \times P_{CO2}$). Where not shown, standard error bars lie within the data points. See text for further details.

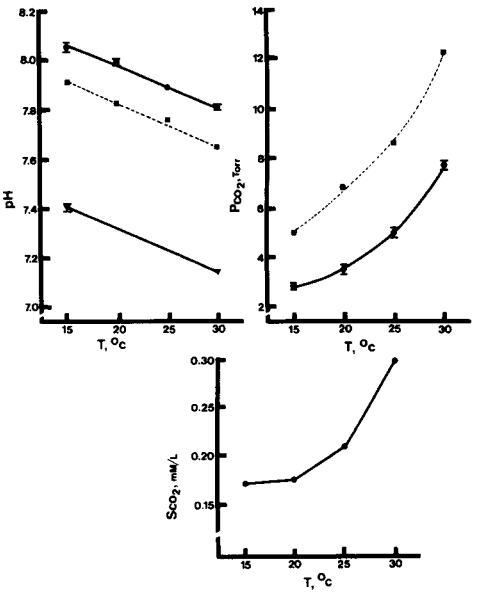

Fig. 2. The effect of temperature change on *in vitro* blood (●—●; $n=3$) and plasma (■—■; $n=2$) acid–base status under conditions of constant CO_2 content (closed system). S_{CO_2} = the molar concentration of CO_2 ($\alpha_{CO_2} \times P_{CO_2}$). All values have been corrected for the effects of metabolism with time (see text) except for blood subjected to rapid temperature change (▼—▼; $n=3$). Where not shown, standard error bars lie within the data points. Curves of P_{CO_2} vs T were fitted by eye.

Figure 2 summarises the effects of temperature change under conditions of constant C_{CO_2} (closed system). Unlike the open system experiments, blood (hct $\simeq$ 40%) and plasma were affected in a similar fashion; for plasma dpH/dT was -0.0162 U/°C and for whole blood dpH/dT was -0.0165 U/°C. This value for whole blood was obtained by correcting for the effects of metabolism, as described earlier. The effect of metabolism alone, caused pH to change with a slope of -0.0355 U/hr. Since temperature was changed every hour, the measured pH values were corrected by subtracting $0.0355 \times t$ (hr). To check the validity of this correction, samples of blood were warmed from 15 to 30°C as quickly as possible (20 min). In this instance pH changed with a temperature slope of -0.0174 U/°C, a value very similar to our corrected value of -0.0165 U/°C (Fig. 3). The slight difference between the temperature slopes was probably due to a small effect of metabolism during the 20 min it took to warm the blood 15°C. Indeed, if we correct the slope of the rapid temperature change as before, the resultant slope (-0.0168 U/°C) is almost identical to that obtained (-0.0165 U/°C) using the longer equilibration technique. In the constant content system, P_{CO_2} increased for both blood and plasma in a similar manner (blood; $dP_{CO_2}/dT = 0.32$ Torr/°C; plasma; $dP_{CO_2}/dT = 0.48$ Torr/°C). Correction of blood P_{CO_2} for the effect of metabolism was made using a correction factor of 1.0 Torr/hr. As expected, S_{CO_2} increased with temperature as a result of a large increase in P_{CO_2} compared to only a small decrease in α_{CO_2}.

DISCUSSION

Since the observations of Rahn (1967), a great body of evidence has been accumulated showing that blood pH in fish varies inversely with temperature with a slope ranging between -0.012 and -0.017 U/°C (see review by Reeves, 1977; Heisler, 1980). A notable exception is the blood of the American eel (*Anguilla rostrata*), which changes with a temperature slope of only -0.0076 U/°C (Walsh and Moon, 1982).

Alpha-imidazole is the most concentrated blood buffer and its dissociation constant is affected by temperature ($dpK/dT \simeq -0.017$ U/°C) in a similar manner as the blood of a great variety of ectothermic vertebrates (Reeves, 1977), although to a greater extent in fish (Heisler, 1980). It is clear, however, that regulation of the CO_2/HCO_3^- buffer system represents the principal physiological control of blood acid–base status. Since the pK′ of carbonic acid is only slightly affected by temperature ($dpK'/dT \simeq -0.005$), it is apparent when viewing the Henderson-Hasselbalch equation,

$$pH = pK' + \log \frac{(HCO_3^-)}{\alpha_{CO_2} \times P_{CO_2}},$$

that either the demoninator ($\alpha_{CO_2} \times P_{CO_2}$) or the numerator ($HCO_3^-$) must be adjusted following a temperature alteration in order to explain the observed changes in blood pH. Air-breathing ectotherms behave as constant CO_2 content systems and adjust P_{CO_2} by ventilatory changes (Reeves, 1977). In water-breathers, the high ventilation–perfusion ratio required for adequate oxygen uptake precludes ventilation as a means of regulating P_{CO_2}. Alternatively, water-breathers primarily manipulate bicarbonate levels to achieve the final adjustment of blood pH. Strictly speaking, water-breathers do not behave as constant CO_2 content systems or constant P_{CO_2} systems but as a combination of the two types, depending on the species in question (Cameron and Kormanik, 1982). What is clear however, is that generally, adjustment of (HCO_3^-) is the method by which fish manipulate blood pH following temperature changes. The adjustment of blood HCO_3^- levels is a slow process that likely involves manipulations of active ion-exchange mechanisms (see review of Heisler, 1980).

The results of the present study demonstrate, that in skipjack tuna, there is no need for gradual active regulation of blood bicarbonate levels following temperature changes. The blood pH change observed *in vitro*, under conditions of constant P_{CO_2} ($dpH/dT = -0.0131$ U/°C, Fig. 1) falls well within the range of values noted for other ectothermic vertebrates (Reeves, 1977) and is only slightly different than the pH change observed under conditions of constant C_{CO_2} ($dpH/dT = -0.0165$ U/°C, Fig. 2). The large decrease in pH with increasing temperature in the open system is a result of a pronounced reduction in blood C_{CO_2} (and hence HCO_3^-) compared to only a small decrease in S_{CO_2} (Fig. 1). That dpH/dT in the

open system was not closer to the closed system value may have been due to the fact that blood was equilibrated with 1% CO_2 (7.5 Torr). Clearly, similar changes in HCO_3^- concentration at physiological P_{CO_2} (2–3 Torr) would cause greater changes in pH.

Our results differ from a similar *in vitro* experiment performed on sand-trout (*Cynoscion arenarius*) blood (Cameron, 1978). In that study a temperature slope of only −0.0048 U/°C was observed, which is the value one would predict based solely on the effect of temperature on pK′, as discussed previously. Similarly, C_{CO_2} decreased only slightly as a result of the change in αP_{CO_2} ($dC_{CO_2}/dT = -0.05$ mM/°C) compared to $dC_{CO_2}/dT = -0.34$ mM/°C in the present study. Why does tuna blood act so differently than sand-trout blood? More specifically, why does bicarbonate decrease so greatly in tuna blood and only slightly in sand-trout blood? It is highly unlikely that the dissimilarities are related to differences in α_{CO_2} or pK′. We believe that the large reduction in C_{CO_2} as temperature increases, under conditions of constant P_{CO_2}, is caused by dissociation of haemoglobin (presumably histidine residues) which allows H^+ ions to combine with HCO_3^- and thereby removing HCO_3^- from the system as gaseous CO_2. This idea is supported by the fact that in plasma, under identical conditions, pH actually increased with increasing temperature. That this process does not occur in *Cynoscion* and presumably other fishes may be a reflection of the greater amount of haemoglobin present in tuna blood which would allow more protons to be released. Haemoglobin levels as high as 14–20 g/100 ml have been measured in skipjack blood (Klawe *et al.*, 1963; Perry and Daxboeck, unpublished observations) whereas most fish have haemoglobin levels in the area of 4–6 g/100 ml. Alternate possibilities are that tuna haemoglobin contains more histidine residues than in haemoglobins of other fish or that the temperature sensitivity of imidazole dissociation is somehow greater in tuna haemoglobin.

Given the results of the present study on skipjack tuna, it is not necessary to postulate extrinsic mechanisms that can add or subtract base to or from the system in response to temperature change, unlike the situation in other fish. Instead, the changes in pH and C_{CO_2} can be accounted for solely by the passive, *in vitro* behaviour of the chemical buffer system found in the blood. A similar passive mechanism has recently been identified by Wood and Cameron (1984) in blue crab haemolymph. The rapid nature of such a passive system clearly is advantageous to skipjack tuna considering they make frequent vertical migrations through waters of greatly varying temperature. Rapid pH changes would not be possible if active adjustment of blood bicarbonate levels was responsible for blood pH regulation.

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