Temperature-dependent shift of pH_i in fish white muscle: contributions of passive and active processes

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Van Dijk, P. L. M., I. Hardewig, and H. O. Pörtner. Temperature-dependent shift of pH_i in fish white muscle: contributions of passive and active processes. *Am. J. Physiol.* 272 (*Regulatory Integrative Comp. Physiol.* 41): R84–R89, 1997.—This study was designed to determine the mechanisms causing temperature-induced pH shifts in the white muscle of the marine teleost *Zoarces viviparus*. The white musculature undergoes an intracellular acidification with increasing body temperature at a slope of the pH-temperature relationship equal to -0.016 ± 0.003 U/°C. This is in good accordance with the overall relationship between the change in pK and the change in temperature of the intracellular proteins, which was determined to be -0.013 ± 0.001 U/°C. Thus the dissociation state of muscle proteins is kept fairly constant in white muscle of *Zoarces viviparus*. The passive component of the observed pH shift, which is due to the physicochemical response of the intracellular buffers to temperature change, accounts for only 35% of the pH transition. Ventilatory adjustment of intracellular P_{CO_2} does not contribute to the temperature-induced shift of intracellular pH (pH_i) in *Zoarces viviparus*. Therefore, the remaining 65% of pH adjustment must be ascribed to ion exchange mechanisms. The nonbicarbonate buffer value amounted to 34.4 ± 2.3 meq·pH^{-1}·kg cell water^{-1} at 12°C and decreased slightly but not significantly with temperature. On the basis of our data we calculated that a removal of 0.52 mmol base equivalents·kg cell water^{-1}·°C^{-1} was necessary to shift pH_i to its new steady state.

acid-base regulation; intracellular pH; *Zoarces viviparus*

THE EFFECTS OF TEMPERATURE on acid-base parameters in vitro as well as in vivo have been studied since the beginning of this century (e.g., Ref. 1). Investigations by Rosenthal (29) showed that cooling of mammalian blood samples in a closed system in vitro, i.e., at constant total CO_2 (C_{CO_2}) content, results in a linear increase of blood pH by -0.0147 U/°C. The same effect of temperature changes on blood pH has been observed in air-breathing poikilotherms in vivo (28). Surprisingly, in the open system of the living animal, blood P_{CO_2} showed the same behavior as in the closed system in vitro; P_{CO_2} increased exponentially with rising temperature and falling pH such that C_{CO_2} content remained constant (28). In the intracellular compartment a similar, inverse relationship between temperature and pH as in blood was first observed in striated toad muscle and later in several other vertebrate and invertebrate tissues (cf. Ref. 25).

In his frequently cited paper, Reeves (24) discussed the similarity between the effect of temperature on intra- or extracellular pH and on the pK values of histidine residues ($\Delta pK_{Im}/\Delta T$ is approximately -0.018 U/°C) where pK_{Im} is the change in pK of imidazole in relationship to the change in temperature (T). He proposed the imidazole alphastat hypothesis, which holds that poikilotherms regulate the pH of their body fluids so that the dissociation state of imidazole groups (α-imidazole) is maintained despite changes in body temperature. That same idea had already been discussed as early as 1927 by Austin and co-workers (1) in their study of temperature effects on the acid-base status of alligators. Direct evidence for alphastat regulation has recently been obtained by ^1H-nuclear magnetic resonance spectroscopy on newts (*Notophthalmus viridescens*). The determination of the fractional dissociation of carnosine, a major imidazole compound in the white muscle of these animals, showed that this parameter remains nearly constant despite changes in body temperature. This was achieved by a shift of intracellular pH (pH_i) by -0.015 U/°C (14).

According to Reeves (24), two mechanisms are involved in the adjustment of the new steady-state pH after temperature change: the physicochemical response of the buffer components in the body fluids due to changes of their pK with temperature and changes in P_{CO_2} at constant C_{CO_2} regulated by adjustment of the ventilatory rate. A relative decrease of the ventilation rate at higher temperatures would cause an increase in P_{CO_2} and, therefore, an acidification of intra- and extracellular compartments. Indeed, model calculations by Reeves and Malan (26) showed that in some air-breathing poikilotherms those two effects are sufficient to cause the observed changes in pH_i, with temperature indicating that no ion exchange mechanisms are involved in the adjustment of pH to a new steady-state value. However, the experimental verification of the model calculations appears warranted.

Water-breathing fish show the same inverse relationship between intra- and extracellular pH and body temperature as observed in air breathers (5, 6). In water-breathing animals, the scope for the adjustment of the ventilatory rate as a mechanism for acid-base regulation is limited by the viscosity of the medium on one hand and the low solubility of oxygen in water on the other hand. Thus P_{CO_2} does not show the exponential increase at constant C_{CO_2} that is observed in air-breathing animals. Rather the change of P_{CO_2} with temperature in fish is variable between species. Generally, P_{CO_2} rises only slightly with rising temperature, whereas the C_{CO_2} content decreases (cf. Ref. 5). Heisler (11) showed that the changes in bicarbonate concentrations observed in dogfish after temperature change are caused by ion exchange processes between body compartments and the environmental water and that this active ion movement contributes a significant extent to the temperature-induced pH shift. On the basis of the above-mentioned studies it is now generally accepted that, unlike what has been postulated for air-breathing

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animals, in water breathers three components participate in the shift of pH_i to a new steady state after temperature change: *1*) physicochemical response of the intracellular ternary buffer system, *2*) changes in P_{CO_2} by respiratory response, and *3*) uptake or removal of acid-base equivalents by active ion transport or by metabolic processes.

Model calculations have been used to quantify the relative contributions of the different processes that are involved in the pH adjustment after shifts of body temperature (5, 11, 26). Some of those calculations, however, were based on assumptions concerning the temperature coefficients of the p*K* values of nonbicarbonate buffers (β_{NB}). The p*K*-temperature relationship ($\Delta pK/\Delta T$) for imidazole buffers is especially difficult to determine, because it depends on local charge configurations in the environment of the imidazole group as well as on ionic strength. $\Delta pK/\Delta T$ values may vary between −0.016 and −0.024 U/°C for histidine and free imidazole compounds (12), whereas an even larger variability, from −0.010 to −0.051 U/°C, has been reported for histidine residues in proteins (27).

This is the first study that directly quantifies the contribution of the passive physicochemical response of the intracellular buffer system to the adjustment of pH_i after temperature changes in fish. So far, such an approach has been available for neither air- nor water-breathing animals.

MATERIAL AND METHODS

Incubation experiments. Eelpouts *Zoarces viviparus* (L.) were caught from brackish water areas (salinity 2.5%) in the German Wadden Sea. Before experimentation, fish were kept for at least 3 wk in an aquarium with water of the same salinity at 12°C and a daily light period of 12 h. Ad libitum feeding with shrimps was terminated 7 days before experimentation. Experiments were performed in the winter of 1994–1995. The fish (mean mass 14.5 ± 8.4 g; n = 25) were transferred to individual chambers placed in a thermostated water bath. Each chamber contained 1,600 ml of constantly aerated seawater. After an acclimation period of 3 days at 12.0 ± 0.2°C, the temperature was lowered stepwise to 9.0, 6.0, 3.0, and 0.0 ± 0.2°C. Each incubation period at one temperature lasted 24 h. Thereafter, the fish were anesthetized by the addition of 0.24 g MS-222 (Sigma) and subsequently biopsied. White muscle tissue was freeze-clamped immediately and stored in liquid nitrogen until analysis. Concentrations of phosphocreatine and ATP were determined enzymatically according to the method of Heinz and Weissler (2). In 12°C-acclimated control fish, phosphocreatine and ATP levels were 17.8 ± 2.12 and 3.62 ± 0.41 µmol/g fresh wt, respectively, indicating a relatively undisturbed energy status of the tissue samples.

Determination of pH_i and P_{CO_2}. pH_i and P_{CO_2} in white muscle tissue were determined according to Pörtner et al. (22). For determination of in vivo pH_i values at different incubation temperatures, tissue samples were ground to a fine powder under liquid nitrogen and suspended in a 150 mmol/l KF, 6 mmol/l Na_2 nitrilotriacetic acid (NTA) solution. Both KF and NTA serve as inhibitors, largely reducing the rate of metabolic reactions that influence homogenate pH. After brief centrifugation (≤15 s) the pH of the supernatant was measured with a thermostated capillary pH electrode (G297/G2, Radiometer Copenhagen, Denmark) that had been calibrated at the particular incubation temperature of the fish.

To determine the passive component of the temperature-dependent adjustment of pH_i, ground muscle samples of five fish acclimated to 12.0°C were divided into five aliquots and measured at 12.0, 9.0, 6.0, 3.0, and 0.0°C, respectively. The same protocol was followed with white muscle homogenates of fish acclimated to 12°C that had been dialyzed for 24 h against 160 mM KCl (molecular cutoff of the dialysis tubing = 12,000–14,000 Da) to remove virtually all low molecular weight compounds. This approach was adopted to determine the apparent $\Delta pK/\Delta T$ of the titratable groups of muscle protein.

C_{CO_2} content of the homogenates was measured in triplicate by gas chromatography (100 AGC, Hach Carle, Loveland, CO). Calibration was performed by using 1 and 2 mmol/l bicarbonate standard solutions. Intracellular P_{CO_2} and bicarbonate concentrations were evaluated applying the Henderson-Hasselbalch equation. Values for the solubility of CO_2 and apparent dissociation constant under physiological conditions were calculated according to Heisler (12), assuming ionic strength = 0.16 mol/l, Na^+ concentration = 0.02 mol/l, molarity of dissolved species = 0.23 mol/l, and protein concentration = 220 g/l. The fractional water content of white muscle tissue and the fraction of extracellular water were assumed to be 0.79 and 0.11, respectively (13).

Determination of intracellular buffer values. The β_{NB} of white muscle tissue was determined according to Pörtner (21). Briefly, ~1 g of ground tissue was suspended in 5 vol of 540 mM KF and 10 mM NTA in a thermostated tonometer. The homogenate was equilibrated with different mixtures of CO_2 in air (1, 0.5, and 0.1% CO_2) for at least 20 min before samples were taken for the analysis of pH and C_{CO_2} as described above. Free inorganic phosphate was determined enzymatically according to Pörtner (21). Calculated values for ΔHCO_3^- and ΔpH between different equilibration steps were used to determine the β_{NB} capacity. This value was corrected for the influence of elevated phosphate concentrations in the homogenate (see Ref. 21). Intracellular free phosphate was assumed to be 1.0 µmol/g fresh wt in resting animals (G. van den Thillart, personal communication).

Statistics. Statistical differences between regressions were calculated by analysis of covariance. Analysis of variance followed by a Bonferroni-Dunn post hoc test was used to compare β_{NB} values at different temperatures.

RESULTS

A rise in body temperature caused a linear drop of the pH_i by −0.016 ± 0.003 U/°C (mean ± SE; n = 25) in white muscle of *Zoarces viviparus* (Fig. 1*A*). C_{CO_2} content of the tissue fell linearly with rising temperature, and, accordingly, HCO_3^- concentrations decreased with a slope of $\Delta HCO_3^-/\Delta T = -0.27\ mmol \cdot l^{-1} \cdot {}^\circ C^{-1}$ (Fig. 2, *A* and *B*). Tissue P_{CO_2} was not strongly correlated with temperature (r = 0.552, n = 25) but tended to fall with rising body temperature.

To estimate the overall $\Delta pK/\Delta T$ value for the intracellular proteins, we determined the passive pH changes of dialyzed homogenates of white muscle tissue with changing temperature. Hitzig et al. (14) showed that a solution of carnosine, a histidine-containing dipeptide, changed its pH with temperature according to the $\Delta pK/\Delta T$ of the imidazole moiety. After removal of all low molecular weight compounds, the muscle homogenates exhibited a pH-temperature relationship of $\Delta pH/\Delta T$ = −0.013 ± 0.001 U/°C (mean ± SE; n = 4; Fig. 1*B*),

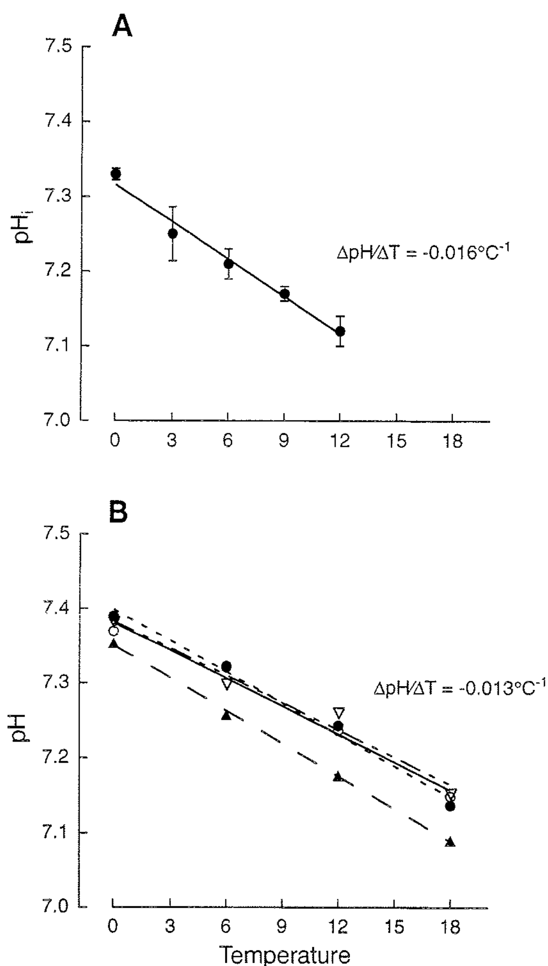

Fig. 1. *A*: relationship between intracellular pH (pH_i) and body temperature in white muscle from *Zoarces viviparus* (means ± SE; n = 5; r = 0.8361). *B*: apparent p*K*-temperature relationship ($\Delta pK/\Delta T$, as measured by $\Delta pH/\Delta T$) of the high molecular weight fraction of *Zoarces viviparus* white muscle tissue. Different symbols and lines represent the response to temperature of the dialyzed muscle homogenates of different fish.

which is not significantly different from the $\Delta pH/\Delta T$ observed in the intracellular compartment of white muscle in vivo.

The passive component of pH adjustment after temperature change was quantified by measuring the pH_i of tissue samples from 12°C-acclimated fish at different temperatures. The homogenate method for the determination of pH_i (22) allows the fast adjustment of the tissue homogenate to different temperatures and the subsequent measurement of pH as it would result from passive change with temperature, with no biochemical reactions or ion exchange being involved. The mean slope of the pH-temperature relationship obtained for each individual fish was −0.006 ± 0.001 U/°C (mean ± SE; n = 5; Fig. 3), which was significantly different from the slope obtained in white musculature in vivo. At an intracellular P_{CO_2} of 0.55 kPa this shift in pH with temperature would result in a passive decrease of intracellular HCO_3^- levels by −0.06 mmol·l cell water^{-1}·°C^{-1} compared with −0.27 mmol·l cell water^{-1}·°C^{-1} observed in vivo.

The β_{NB} of the white muscle tissue decreased from 24.2 ± 1.0 meq/kg fresh wt at 12°C to 20.3 ± 1.5 meq/kg fresh wt at 0°C (mean ± SE, n = 5; Fig. 4). Although these changes are not significant, the data show a tendency for buffer capacity to decrease with falling temperature.

DISCUSSION

Alphastat regulation. The change of pH_i in white muscle tissue of *Zoarces viviparus* with changing body temperature ($\Delta pH/\Delta T$ = −0.016 U/°C) is similar to results obtained in white muscle of the dogfish *Scyliorhinus stellaris* ($\Delta pH/\Delta T$ = −0.018 U/°C; Ref. 13) and the catfish *Ictalurus punctatus* ($\Delta pH/\Delta T$ = −0.015 U/°C; Ref. 7). According to Reeves (24), pH-temperature relationships with a slope of $\Delta pH/\Delta T$ = −0.015 to −0.020 U/°C result in a constant protonation of histidine residues. However, the calculation of the dissociation state of imidazole ($\alpha_{imidazole}$) is based on the temperature coefficient of the p*K* of imidazole. We determined

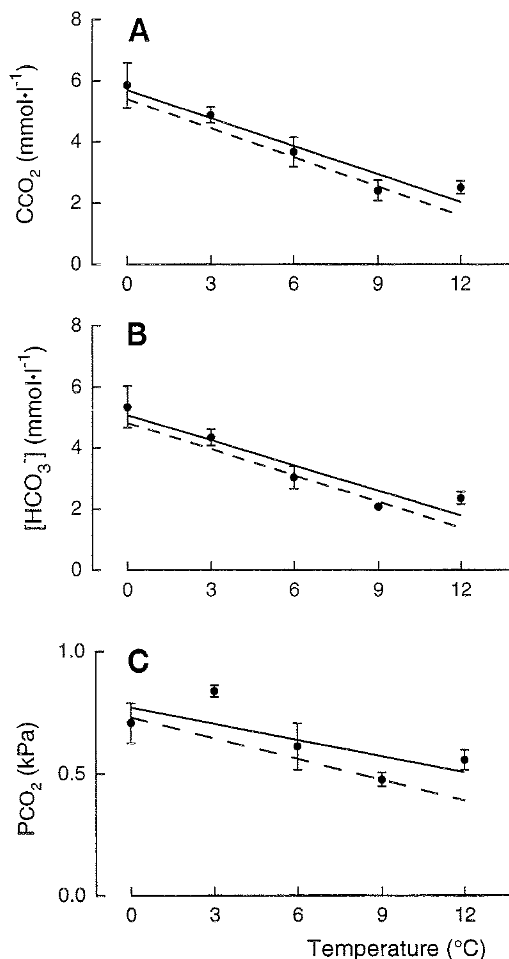

Fig. 2. Relationship between acid-base parameters and body temperature in white muscle of *Zoarces viviparus*. As outlined in the text, values can be regarded as intracellular concentrations (mmol/l cell water) or intracellular partial pressure. Conservative estimates of values corrected for influence of extracellular compartment are given by dashed lines. *A*: total CO_2 (C_{CO_2}; means ± SE; n = 5, r = 0.7806). *B*: HCO_3^- concentration ($[HCO_3^-]$; means ± SE; n = 5, r = 0.7793). C: CO_2 partial pressure (P_{CO_2}; means ± SE; n = 5, r = 0.552).

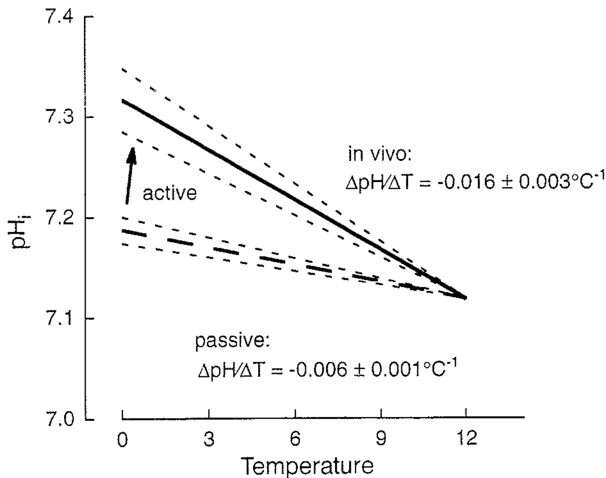

Fig. 3. Contribution of passive physicochemical response of intracellular buffer system to pH_i adjustment. Solid line, pH-temperature relationship in vivo; long-dashed line, pH-temperature relationship in vitro; short-dashed lines, SE of slopes. Difference between slopes quantifies active processes occurring in vivo.

an apparent $\Delta pK/\Delta T$ value for the intracellular proteins of the white muscle of *Zoarces viviparus* of -0.013 ± 0.001 U/°C, which is somewhat lower than the values given by Reeves (24). Because the pH-temperature relationship in vivo shows only a slightly (not significantly) higher slope (-0.016 ± 0.003) mean $\alpha_{imidazole}$ will increase from 0.83 to 0.84 with a temperature change from 12 to 0°C (assuming an absolute pK value of 6.5 at 12°C; Ref. 3). Hence the mean dissociation state of histidine residues of muscle proteins remains nearly constant with temperature change in *Zoarces* white muscle.

Passive mechanisms. Tissue samples of fish acclimated to 12°C showed a passive decrease of pH_i with rising measuring temperature at a slope of -0.006 ± 0.001 U/°C. This effect of temperature on homogenate pH is due to the physicochemical response of the intracellular buffers, and it represents the passive component of the temperature-induced pH shift in vivo.

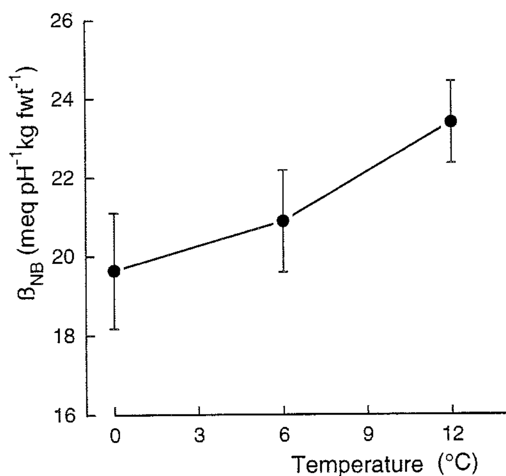

Fig. 4. Nonbicarbonate buffering capacity (β_{NB}) of white muscle tissue of *Zoarces viviparus* at different temperatures (means ± SE; $n = 5$); fwt, fresh wt.

The discrepancy between the passive $\Delta pH/\Delta T$ slope and the pH-temperature relationship determined in vivo (-0.016 ± 0.003) indicates that the adjustment of pH_i is largely generated by active processes, such as changes in intracellular P_{CO_2} or ion exchange mechanisms. It should be noted that our approach may lead to a slight overestimation of the passive temperature response of the tissue. The tissue homogenates can be regarded as a closed system with no exchange of ions or CO_2 with the environment. Equilibration and measurement of the samples at 0°C, therefore, result in a slight decrease in P_{CO_2} from 0.55 kPa at 12°C to 0.42 kPa at 0°C. For the quantification of the passive response of the intracellular components to temperature change, the intracellular compartment should be modeled as a semiclosed system, allowing for the exchange of CO_2. This could be achieved by the equilibration of the homogenate at constant P_{CO_2} during temperature adjustment. Long-term equilibration, however, would be accompanied by the hydrolysis of phosphorylated compounds, which would significantly influence pH. Therefore, this approach was not considered suitable. Owing to the high β_{NB} of the tissue, however, the effect of the occurring decrease in homogenate P_{CO_2} on pH is minimal. Graphic analysis of the data in a pH/bicarbonate diagram (not shown) revealed that only ~10% (-0.0007 U/°C) of the observed passive temperature response is caused by the changes in P_{CO_2}.

Surprisingly, the passive pH changes of the protein fraction of the tissue are much more pronounced than that of the crude homogenate (-0.013 and -0.006 U/°C, respectively). This difference in temperature sensitivity may be ascribed to low molecular weight components with low $\Delta pK/\Delta T$ values present in the crude homogenate. This could be phosphate ($\Delta pK/\Delta T = -0.003$ U/°C; Ref. 21) and bicarbonate ($\Delta pK/\Delta T = -0.006$ U/°C; Ref. 5). However, both buffers occur only in relatively low concentrations. Elevated phosphate concentrations in our tissue samples due to hydrolysis of phosphorylated compounds can be excluded because ATP and phosphocreatine concentrations were in the range of that reported for resting fish (see MATERIAL AND METHODS). The influence of Mg^{2+} complexation by F^- and NaNTA on the temperature sensitivity of the homogenate has been estimated and can be considered minimal.

The possible contribution of ventilatory adjustments of intracellular P_{CO_2} to the temperature-induced shift of pH_i was evaluated by determination of total tissue CO_2 content and calculation of P_{CO_2} and bicarbonate levels. Given that the fraction of the extracellular water in total tissue water is very small in fish white muscle (11% in dogfish, Ref. 13; and only 7% in catfish; Ref. 7), the values of total tissue CO_2 can be regarded as a good approximation of intracellular concentrations. We carried out model calculations to estimate the influence of extracellular CO_2 on the values for tissue CO_2, assuming extracellular P_{CO_2} = intracellular P_{CO_2} in resting fish (31) and a moderate rise in the fraction of extracellular water with increasing temperature (23). The resulting more conservative estimates of intracellu-

lar Cco_2, HCO_3^-, and Pco_2 are represented by the dashed lines in Fig. 2. Our data show that *Zoarces* does not follow the constant Cco_2 model that has been proposed for air breathers (24). Cco_2 content clearly drops with rising temperature. No data are published to date on the effect of temperature on the intracellular CO_2 concentrations in fish. Data on the extracellular compartment show a decline of Cco_2 with increasing body temperature, whereas extracellular Pco_2 rises to various extents in different fish species (cf. Refs. 4, 5). In *Zoarces viviparus* tissue, Pco_2 does not show a clear pattern in response to temperature change. However, on the basis of our data we can exclude an increase of Pco_2 at higher temperature, which would contribute to the adjustment of pH_i to lower values. On the contrary, there may even be a slight decrease of Pco_2 with rising temperature. From our data we conclude that, in *Zoarces viviparus*, the regulation of ventilatory rate does not a play a role in the adjustment of pH_i after temperature change.

Active mechanisms. The difference in slope between in vitro and in vivo measurements (see Fig. 3) must, therefore, be due to the removal of acid-base equivalents from the intracellular compartment. The absolute amount of nonrespiratory proton equivalents required to explain the discrepancy between in vivo and in vitro data can be calculated according to the formula

$$\Delta H^+_{nonresp} = -\beta_{NB} \cdot \Delta pH - \Delta HCO_3^- \quad (1)$$

where ΔpH and ΔHCO_3^- are the differences in slope of the $\Delta pH/\Delta T$ and $\Delta HCO_3^-/\Delta T$ relationship between in vitro and in vivo measurements, β_{NB} is the nonbicarbonate buffer value, and $H^+_{nonresp}$ is the nonrespiratory proton equivalent. β_{NB} of white muscle tissue of *Zoarces viviparus* ranges between 24.2 ± 2.3 $meq \cdot pH^{-1} \cdot kg$ wet wt^{-1} at 12°C and 20.3 ± 3.3 $meq \cdot pH^{-1} \cdot kg$ wet wt^{-1} at 0°C. Most buffer values of fish muscle reported in the literature are significantly higher than this. Castellini and Somero (9) determined values between 42 and 52 $meq \cdot pH^{-1} \cdot kg$ wet wt^{-1} in white muscle of different benthic fish species. Buffer values for fish white muscle given by Heisler (Ref. 11; 45–49 $meq \cdot pH^{-1} \cdot l$ cell $water^{-1}$) are also higher than those determined in this study. These high values were obtained either by fixed acid titration or by homogenate equilibration technique, which may lead to an overestimation of the buffer value due to uncontrolled hydrolysis of phosphorylated compounds as discussed by Pörtner (21), Pörtner et al. (22), and Kinsey and Ellington (18). The buffer capacity of *Zoarces* white muscle showed a slight decrease with decreasing temperature, although this was not significant. Other studies reveal the same trend but, because of small temperature effects, this trend has sometimes been overlooked (17, 34). In white muscle of trout, β_{NB} decreased from 49.2 $\Delta HCO_3^-/\Delta pH$ at 18°C to 47.1 $\Delta HCO_3^-/\Delta pH$ at 5°C (17). In white muscle of the notothenoid *Pagothenia borchgrevinki*, buffer capacity amounts to 60.8 ± 6.3 $meq \cdot pH^{-1} \cdot kg$ wet wt^{-1} at 37°C but only 50.1 ± 3.0 $meq \cdot pH^{-1} \cdot kg$ wet wt^{-1} at 0°C (34). The effect of temperature on the buffering capacity of proteins has recently been investigated in purified solutions of human hemoglobin (8). A temperature-dependent rise in buffer capacity could be partly attributed to an increased participation of NH_2-terminal α-amino groups in the buffering process at higher temperatures.

The calculation of net H^+-equivalent ion transfer was conducted on the basis of a mean β_{NB} capacity of 22.0 $meq \cdot pH^{-1} \cdot kg$ wet wt^{-1}, which corresponds to 31.3 $meq \cdot pH^{-1} \cdot kg$ cell $water^{-1}$. The observed discrepancy between in vitro and in vivo data can be ascribed to a transfer of 0.52 mmol protons/kg cell water into the intracellular compartment, with a temperature increase of 1°C (see *eq. 1*). This is in the same order of magnitude as calculated by Heisler (12) for red muscle of the dogfish *Scyliorhinus stellaris* (ΔH^+ = 0.75 $mmol \cdot kg$ cell $water^{-1} \cdot °C^{-1}$). The value calculated for dogfish white muscle was much lower (ΔH^+ = 0.03 $mmol \cdot kg$ cell $water^{-1} \cdot °C^{-1}$). In the same species, a transfer of ~8 mmol H^+/l cell water into the extracellular compartment occurs after strenuous exercise (data obtained from Ref. 15). Hence, a temperature drop by 10°C may cause ion transport to an extent that is comparable to that occurring after strenuous exercise. This indicates that acid-base adjustment after temperature change requires a substantial movement of ions across the cell membrane, which is associated with considerable metabolic costs. The accumulation or removal of acid-base equivalents from the intracellular compartment does not necessarily have to be achieved exclusively by ion exchange mechanisms. In recent years evidence has accumulated that metabolism itself may contribute to the regulation of acid-base balance (10, 20, 33). In case of a decrease in body temperature, a relative increase of reactions producing base equivalents would favor an alkalinization of the intracellular milieu. This could be achieved by a switch from oxidation of carbohydrates to degradation of fatty or amino acids (20). In fact, Jones and Sidell (16) showed that, in the striped bass *Morone saxatilis*, fatty acids are preferentially oxidized during cold acclimation.

In conclusion, our data have shown that the adjustment of pH_i after temperature change in white muscle tissue of the eelpout *Zoarces viviparus* occurs by ~35% through the passive physicochemical response of intracellular buffers. Respiratory adjustment of tissue Pco_2 does not contribute to pH_i regulation. Therefore, the remaining 65% must be ascribed to active ion transport mechanisms or metabolic production or removal of acid-base equivalents. The processes leading to the transition of pH_i after temperature change are completed within 12–24 h in fish muscle (12). One should be aware, however, that steady-state values of pH_i are always more alkaline than predicted from an equilibrium distribution of hydrogen ions across the cell membrane and are maintained by ongoing acid extrusion. Therefore, the maintenance of pH_i at a new steady state requires a permanent shift in set points of the acid-base-relevant ion exchangers, even in air-breathing organisms, where the transition of pH_i after temperature change may not rely on ion transport mechanisms.

Perspectives

Alphastat regulation of pH_i in response to temperature change may serve to maintain structure and function of enzymes over a wide temperature range. However, absence of alphastat regulation is observed in several organisms (4, 32) and has been interpreted as an adaptive strategy to inactivate enzymes in the cold to support metabolic depression during winter when food supply may be limited (32). A comparative study on crustaceans suggests that the relationship between active and passive contributions to acid-base regulation varies among species (30). Active pH adjustment may be more pronounced in species exposed to large temperature fluctuations in their environment, allowing for a flexible response to temperature change (30). In the eurythermic eelpout *Zoarces viviparus*, active processes contribute to a large extent to pH adjustment. A comparison with stenothermic Zoarcids, e.g., from Antarctic regions, may provide more evidence for the adaptational value of a large active component in the pH adjustment of pH_i.

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REFERENCES

1. **Austin, J. H., F. W. Sunderman, and J. B. Camack.** The electrolyte composition and the pH of serum of a poikilothermous animal at different temperatures. *J. Biol. Chem.* 72: 677–685, 1927.
2. **Bergmeyer, H. U., J. Bergmeyer, and M. Grassl** (Editors). *Methods of Enzymatic Analyses* (3rd ed.). Weinheim, Germany: Verlag Chemie, 1983, p. 507–513.
3. **Burton, R. F.** The role of imidazole ionization in the control of breathing. *Comp. Biochem. Physiol. A Comp. Physiol.* 83A: 333–336, 1986.
4. **Butler, P. J., and N. Day.** The relationship between intracellular pH and seasonal temperature in the brown trout *Salmo trutta*. *J. Exp. Biol.* 177: 293–297, 1993.
5. **Cameron, J. N.** Acid-base status of fish at different temperatures. *Am. J. Physiol.* 246 (*Regulatory Integrative Comp. Physiol.* 15): R452–R459, 1984.
6. **Cameron, J. N.** Acid-base homeostasis: past and present perspectives. *Physiol. Zool.* 62: 845–865, 1984.
7. **Cameron, J. N., and G. A. Kormanik.** Intracellular and extracellular acid-base status as a function of temperature in the freshwater channel catfish, *Ictalurus punctatus*. *J. Exp. Biol.* 99: 127–142, 1982.
8. **Castaing, M., E. Bursaux, and C. Poyart.** Effect of carbamination on the buffering power of purified human hemoglobin A solutions at two temperatures. *Eur. J. Biochem.* 121: 573–578, 1982.
9. **Castellini, M. A., and G. N. Somero.** Buffering capacity of vertebrate muscle: correlations with potentials of anaerobic function. *J. Comp. Physiol.* 143: 191–198, 1981.
10. **Hardewig, I., H. O. Pörtner, and M. K. Grieshaber.** Interactions of anaerobic propionate formation and acid-base status in *Arenicola marina*: an analysis of propionyl-CoA carboxylase. *Physiol. Zool.* 67: 892–909, 1994.
11. **Heisler, N.** Role of ion transfer processes in acid-base regulation with temperature changes in fish. *Am. J. Physiol.* 246 (*Regulatory Integrative Comp. Physiol.* 15): R441–R451, 1984.
12. **Heisler, N.** Comparative aspects of acid-base regulation. In: *Acid-Base Regulation in Animals*, edited by N. Heisler. Amsterdam: Elsevier, 1986, p. 3–47.
13. **Heisler, N., P. Neumann, and G. F. Holeton.** Mechanisms of acid-base adjustment in dogfish (*Scyliorhinus stellaris*) subjected to long-term temperature acclimation. *J. Exp. Biol.* 85: 89–98, 1980.
14. **Hitzig, B. M., W. Perng, T. Burt, P. Okunieff, and D. C. Johnson.** ^{1}H-NMR measurement of fractional dissociation of imidazole in intact animals. *Am. J. Physiol.* 266 (*Regulatory Integrative Comp. Physiol.* 35): R1008–R1015, 1980.
15. **Holeton, G. F., and N. Heisler.** Contribution of net ion transfer mechanisms to acid-base regulation after exhausting acivity in the larger spotted dogfish (*Scyliorhinus stellaris*). *J. Exp. Biol.* 103: 31–46, 1983.
16. **Jones, P. L., and B. D. Sidell.** Metabolic responses of striped bass (*Morone saxatilis*) to temperature acclimation. II. Alterations in metabolic carbon sources and distributions of fiber types in locomotory muscle. *J. Exp. Zool.* 219: 163–171, 1982.
17. **Kieffer, J. D., S. Currie, and B. Tufts.** Effects of environmental temperature on the metabolic and acid-base responses of rainbow trout to exhaustive exercise. *J. Exp. Biol.* 194: 299–317, 1994.
18. **Kinsey, S. T., and W. R. Ellington.** Interspecific comparisons of capacity for regulation of intracellular pH in molluscan muscle. *Physiol. Zool.* 68: 26–42, 1995.
19. **Milligan, C. L., and C. M. Wood.** Intracellular and extracellular acid-base status and H^+ exchange with the environment after exhaustive exercise in the rainbow trout. *J. Exp. Biol.* 123: 93–121, 1986.
20. **Pörtner, H. O.** The importance of metabolism in acid-base regulation and acid-base methodology. *Can. J. Zool.* 67: 3005–3017, 1989.
21. **Pörtner, H. O.** Determination of intracellular buffer values after metabolic inhibition by fluoride and nitrilotriacetic acid. *Respir. Physiol.* 81: 275–288, 1990.
22. **Pörtner, H. O., R. G. Boutilier, Y. Yang, and D. P. Toews.** Determination of intracellular pH and PCO_2 after metabolic inhibition by fluoride and nitrilotriacetic acid. *Respir. Physiol.* 81: 255–274, 1990.
23. **Prosser, C. L., W. Mackay, and K. Kato.** Osmotic and ionic concentrations in some Alaskan fish and goldfish from different temperatures. *Physiol. Zool.* 43: 81–89, 1970.
24. **Reeves, R. B.** An imidazole alphastat hypothesis for vertebrate acid-base regulation: tissue carbon dioxide content and body temperature in bullfrogs. *Respir. Physiol.* 14: 219–236, 1972.
25. **Reeves, R. B.** Alphastat of intracellular acid-base state? In: *Circulation, Respiration, and Metabolism*, edited by R. Gilles. Berlin: Springer Verlag, 1985, p. 414–423.
26. **Reeves, R. B., and A. Malan.** Model studies of intracellular acid-base temperature responses in ectotherms. *Respir. Physiol.* 28: 49–63, 1976.
27. **Roberts, G. C. K., D. H. Meadows, and O. Jardetzky.** Nuclear magnetic resonance studies of the structure and binding sites of enzymes. VII. Solvent and temperature effects on the ionization of histidine residues of ribonuclease. *Biochemistry* 8: 2053–2056, 1969.
28. **Robin, E. D.** Relationships between temperature and plasma pH and carbon dioxide tension in the turtle. *Nature Lond.* 195: 249–251, 1962.
29. **Rosenthal, T. B.** The effect of temperature on the pH of blood and plasma in vitro. *J. Biol. Chem.* 173: 25–30, 1948.
30. **Sartoris, F. J., and H. O. Pörtner.** Temperature dependence of ionic and acid-base regulation in boreal and arctic *Crangon crangon* and *Pandalus borealis*. *J. Exp. Biol.* In press.
31. **Tang, Y., H. Lin, and D. J. Randall.** Compartmental distributions of carbon dioxide and ammonia in rainbow trout at rest and following exercise, and the effect of bicarbonate infusion. *J. Exp. Biol.* 169: 235–249, 1992.
32. **Walsh, P. J., and T. W. Moon.** The influence of temperature on extra and intracellular pH in the American eel, *Anguilla rostrata* (Le Sueur). *Respir. Physiol.* 50: 129–140, 1982.
33. **Walsh, P. J., and T. P. Mommsen.** Intracellular pH regulation and metabolic interactions in hepatic tissues. *J. Exp. Zool.* 263: 176–185, 1992.
34. **Wells, R. M. G., G. Summers, L. A. Beard, and G. C. Grigg.** Ecological and behavioural correlates of intracellular buffering capacity in the muscles of antarctic fish. *Polar Biol.* 8: 321–325, 1988.