J. exp. Biol. (1980), 85, 99–110
With 1 figure
Printed in Great Britain

THE ROLE OF PHYSICO-CHEMICAL BUFFERING AND OF BICARBONATE TRANSFER PROCESSES IN INTRACELLULAR pH REGULATION IN RESPONSE TO CHANGES OF TEMPERATURE IN THE LARGER SPOTTED DOGFISH (*SCYLIORHINUS STELLARIS*)

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(*Received* 4 *July* 1979)

SUMMARY

In order to evaluate the contributions of physico-chemical buffering to the adjustment of intracellular pH in response to changes of temperature in tissues of dogfish (*Scyliorhinus stellaris*), the CO_2 equilibration method for the determination of intracellular buffer values was modified to yield data for the mathematical simulation of the intracellular compartments as closed buffer systems, and for the calculation of transmembrane bicarbonate transfer *in vivo*.

The respective buffer values of imidazole-like and phosphate-like buffer substances were estimated to be about 39 and 11 in white muscle, 21 and 18 in red muscle and 27 and 10 mequiv/(pH.l cell water) in heart muscle. In white muscle, the observed changes of intracellular pH can be explained by physico-chemical buffering and changes of P_{CO_2}. In red muscle and heart muscle considerable amounts of bicarbonate have additionally to be transferred across the cell membrane to achieve the temperature-dependent variations of pH observed *in vivo*.

INTRODUCTION

The regulation of intracellular pH (pH_i) in response to changes of temperature is still not entirely understood. Several mechanisms are possible (Heisler, Neumann & Holeton, 1980). Of these, only change of P_{CO_2} and changes of intracellular buffer pK values are favoured by Reeves & Malan (1976) as being exclusively responsible for the pH_i adjustment in frog and turtle. In dogfish, however, it has been shown that net bicarbonate transfer between intracellular and extracellular body compartments and the environmental sea water is also important (Heisler, 1978). It has been further shown that even moderate hypercapnia at low acclimation temperature is compensated by appropriate bicarbonate concentration changes, to attain the same $\Delta pH/\Delta t$ relationship as in normocapnic animals (Heisler *et al.* 1980).

The present study assesses the relative contributions of the three mechanisms which are involved in the intracellular pH regulation in dogfish when temperature is

changed. This was achieved by determining intracellular buffer values for dogfish, and then modelling the intracellular compartment as a physico-chemical buffer system, as for frog and turtle (Reeves & Malan, 1976).

Physico-chemical buffering properties can only be determined in a closed system, where bicarbonate, H^+ or OH^- exchange with other compartments can be excluded (Heisler & Piiper, 1971). Such conditions can be achieved by application of the CO_2-equilibration technique of homogenates, which has previously been used for the determination of total intracellular buffer values at fixed temperature (Heisler & Piiper, 1971). The methodology, however, has to be modified by introduction of temperature, as well as P_{CO_2}, as a variable. The resultant pH and bicarbonate changes can then be utilized for a mathematical simulation of either the intracellular compartment modelled as a closed buffer system, or of the transmembrane bicarbonate exchanges *in vivo*.

METHODS

Theoretical considerations

Two different approaches can be used to evaluate the obtained data sets of Δt, ΔpH and $\Delta[HCO_3]$ values:

(A) This first approach describes the behaviour of the intracellular buffer system via the determination of the buffer values of the predominant intracellular non-bicarbonate buffers. These are the imidazole-like buffers, with a fairly high temperature coefficient of their pK values ($\Delta pK/\Delta t \sim -0.02$ U/°C), and the phosphate-like buffers, with a low temperature coefficient ($\Delta pK/\Delta t \sim -0.002$ U/°C). The intracellular compartment can be modelled as if it was a closed buffer system with no transfer of non-volatile substances across the cell membrane, or the bicarbonate transfer due to changes of temperature *in vivo* can be calculated.

The buffer value (β) of the non-bicarbonate buffer of a binary buffer system is defined at constant temperature as the amount of H^+ ions bound, or bicarbonate produced, in a unit of volume per unit change in pH when P_{CO_2} changes (see Heisler & Piiper, 1971):

$$\beta = \frac{\Delta[H^+]_{bound}}{\Delta pH} = \frac{-\Delta[HCO_3^-]}{\Delta pH}. \quad (1)$$

This definition is only valid if the position of the buffer titration curve, expressed by the pK value, is constant with respect to the pH scale. However, when temperature is changed the titration curve is shifted. The shift of pK per unit temperature change ($\Delta pK/\Delta t$) is an expression of the ionization heat and has a constant value for each individual buffer. Under these conditions H^+ ions are bound only according to the change in pH in excess of the change in pK. Therefore the divider of (1) has to be extended by the term ΔpK when temperature is variable during the measuring process:

$$\beta = \frac{\Delta[H^+]_{bound}}{\Delta pH - \Delta pK} = \frac{-\Delta[HCO_3^-]}{\Delta pH - \Delta pK}. \quad (2)$$

The amount of bicarbonate produced by the buffering process is then:

$$\Delta[HCO_3^-] = \beta(\Delta pK - \Delta pH). \quad (3)$$

This equation can be extended to a ternary buffer system consisting of bicarbonate and two non-bicarbonate buffers (indices I and II) with different temperature coefficients of their pK's:

$$\Delta[HCO_3^-]_{I+II} = \beta_I(\Delta pK_I - \Delta pH) + \beta_{II}(\Delta pK_{II} - \Delta pH). \quad (4)$$

If temperature is changed in such a buffer system by a known value successively in combination with two different changes in P_{CO_2} (see Procedure), then the resultant two different pH and bicarbonate concentration changes can be used to apply (4) twice:

$$\Delta[HCO_3^-]_1 = \beta_I(\Delta pK_I - \Delta pH_1) + \beta_{II}(\Delta pK_{II} - \Delta pH_1), \quad (5)$$

$$\Delta[HCO_3^-]_2 = \beta_I(\Delta pK_I - \Delta pH_2) + \beta_{II}(\Delta pK_{II} - \Delta pH_2). \quad (6)$$

When the ΔpK values of the two non-bicarbonate buffers are known, then these two equations can be utilized to determine the buffer values β_I and β_{II} of the individual buffers by solving (6) for β_{II}, substituting into (5), and rearrangement:

$$\beta_I = \frac{\Delta[HCO_3^-]_1(\Delta pK_{II} - \Delta pH_2) - \Delta[HCO_3^-]_2(\Delta pK_{II} - \Delta pH_1)}{(\Delta pK_I - \Delta pK_{II})(\Delta pH_1 - \Delta pH_2)}, \quad (7)$$

$$\beta_{II} = \frac{\Delta[HCO_3^-]_2 - \beta_I(\Delta pK_I - \Delta pH_2)}{\Delta pK_{II} - \Delta pH_2}. \quad (8)$$

On the basis of these buffer values the intracellular compartment of the original tissues can be remodelled as a closed buffer system. Then all changes in intracellular bicarbonate concentration have to be attributed to buffering of CO_2 by the non-bicarbonate buffers. Thus the following equation is valid for a temperature transition from temperature a to temperature b:

$$[HCO_3^-]'_b = [HCO_3^-]_a + \Delta[HCO_3^-]_{NB}, \quad (9)$$

where

$[HCO_3^-]_a$ = concentration of intracellular bicarbonate determined *in vivo* at temperature a,

$\Delta[HCO_3^-]_{NB}$ = concentration of bicarbonate produced by CO_2 buffering of the intracellular non-bicarbonate (NB) buffers during the transition from temperature a to b and from P_{CO_2} *in vivo* at a to P_{CO_2} *in vivo* at b,

$[HCO_3^-]'_b$ = concentration of bicarbonate in the closed buffer system at temperature b.

$[HCO_3^-]'_b$ can be expressed in terms of pH and P_{CO_2}, and $\Delta[HCO_3^-]_{NB}$ can be substituted according to (4); (9) then becomes:

$$\frac{10^{pH'_b}.\alpha.P_{CO_2}}{10^{pK}} = [HCO_3^-]_a + \beta_I(\Delta pK_I - \Delta pH') + \beta_{II}(\Delta pK_{II} - \Delta pH'), \quad (10)$$

where

pH'_b = pH of closed buffer system at temperature b,

α and pK = respective constants of the Henderson–Hasselbalch equation of the bicarbonate buffer system at b,

$\Delta pH'$ = difference between intracellular pH at a, and pH'_b,

P_{CO_2} = P_{CO_2} determined *in vivo* at b.

Equation (10) can be solved numerically for pH'_b by an iteration program, or by graphical analysis. If the intracellular compartments were open buffer systems, then the pH changes induced by temperature changes could be affected by movements of bicarbonate, H^+ or OH^- across the cell-membrane. In this case, the adjustment of intracellular bicarbonate would be due both to titration of non-bicarbonate buffers and to transmembrane bicarbonate transfer:

$$[HCO_3^-]_b = [HCO_3^-]_a + \Delta[HCO_3^-]_{NB} - \Delta[HCO_3^-]_{i\rightarrow e}, \qquad (11)$$

where $[HCO_3^-]_b$ = *in vivo* determined intracellular bicarbonate concentration at b, $[HCO_3^-]_{i\rightarrow e}$ = bicarbonate concentration difference due to intracellular/extracellular transfer. Substituting $\Delta[HCO_3]_{NB}$ according to (4), the bicarbonate transfer is:

$$\Delta[HCO_3^-]_{i\rightarrow e} = [HCO_3^-]_a - [HCO_3^-]_b + \beta_I(\Delta pK_I - \Delta pH_i) + \beta_{II}(\Delta pK_{II} - \Delta pH_i), \qquad (12)$$

where ΔpH_i = *in vivo* determined change of intracellular pH on changing temperature from a to b.

(B) The second approach uses a similar principle, but by-passes the calculation of intracellular buffer values. The term $\Delta[HCO_3]_{NB}$ in (9) and (11) is substituted by the following equation; the linear regression of $\Delta[HCO_3]$ and ΔpH values determined in the homogenates multiplied by the dilution factor d (see calculations):

$$\Delta[HCO_3^-]_{NB} = d(e + c.\Delta pH). \qquad (13)$$

In order to calculate the pH in the closed intracellular buffer system, (pH'_b), (13) can be substituted into (9). The expression of $[HCO_3^-]'_b$ in terms of pH and P_{CO_2} then gives:

$$\frac{10^{pH'_b}.\alpha.P_{CO_2}}{10^{pK}} = [HCO_3^-]_a + d(e + c.\Delta pH'). \qquad (14)$$

This equation again can be solved numerically for pH'_b by an iteration program or graphical analysis.

Considering the intracellular compartment as an open buffer system the transmembrane bicarbonate exchange is (substitution of (13) into (11)):

$$\Delta[HCO_3^-]_{i\rightarrow e} = [HCO_3^-]_a - [HCO_3^-]_b + d(e + c.\Delta pH_i). \qquad (15)$$

Procedure

Series A: Varied temperature

Samples of white muscle, red muscle, and heart muscle of *Scyliorhinus stellaris* (body weight: 0·3–3 kg) were frozen in liquid nitrogen, powdered with mortar and pestle and diluted with dogfish Ringer solution (Table 1) by a factor of about 3 (for

Table 1. *Composition of the dogfish Ringer solution used for suspension of the homogenized tissues* (*mM*)

Na^+	316·5	HCO_3^-	5
K^+	5	Cl^-	328
Ca^{2+}	5	$H_2PO_4^-$	0·5
Mg^{2+}	2·5	Lactate	3

Urea 360. Glucose 2 g/l.

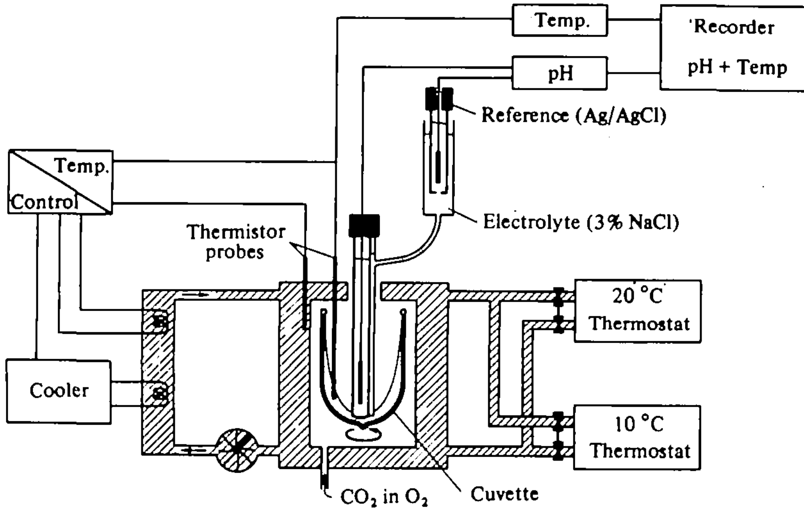

Fig. 1. Experimental apparatus. Tissue homogenates were equilibrated at 10 or 20 °C with several different levels of P_{CO_2} in an intermittently rotating cuvette. Temperature was quickly changed by complete exchange of the water in the temperature-regulating jacket and then held constant at the desired temperature to ±0·1 °C by a double circuit analogue thermostat. The pH in the homogenate was recorded continuously by means of double electrolyte bridge electrodes (see text for details of the procedure).

details of the procedure see Heisler & Piiper, 1971). This homogenate was equilibrated in an intermittently rotating cuvette (Fig. 1) at 10 and 20 °C (±0·1 °C) with gases of known P_{CO_2} values (consisting of only CO_2 and O_2) according to the following protocol:

(*a*) Equilibration at 10 °C with a known P_{CO_2}. The pH of the homogenate was adjusted by addition of either HCl or $NaHCO_3$ to a value falling into the range of pH_i values determined *in vivo* for the respective tissue (Heisler, Weitz & Weitz, 1976*b*; Heisler *et al.* 1980).

(*b*) Subsequent equilibration at 20 °C with a different P_{CO_2} which was sufficient to produce a change in pH (ΔpH) of −0·04 to −0·08 in comparison with the value measured at the end of equilibration period *a*.

(*c*) Re-equilibration with the P_{CO_2} used during equilibration period *a*.

This equilibration sequence (procedure 1) was repeated twice with different P_{CO_2} values in order to produce ΔpH's of −0·15 to −0·22 (procedure 2) and of −0·27 to −0·33 (procedure 3). The pH of the homogenate was recorded continuously during all equilibrations with one of two single-unit double electrolyte bridge electrodes.

Each electrode was used for measurements at only one temperature with temperature-controlled precision phosphate buffers adjusted to the same ionic strength as the Ringer solution used for dilution of the muscle powder. Equilibration with each gas mixture was continued (20–40 min) until the pH was either constant or varied only slightly at a constant rate due to metabolic release of H^+ ions. Complete equilibration was checked by frequent measurements of P_{CO_2} in the homogenate.

Series B: Constant temperature

In a separate series of experiments, equilibration procedures similar to those described above, but at constant temperature, were performed in order to determine the total buffer value of the non-bicarbonate buffers (for details of the method see Heisler & Piiper, 1971).

Calculations

In about 60% of the experiments a small but continuous release of H^+ caused a progressive small fall in pH, even when CO_2 equilibration was complete. In order to eliminate any influence of this unspecific pH fall on the obtained results, the pH values measured after period *b* and *c* (pH_{mb} and pH_{mc} respectively) were corrected using the rate of unspecific pH change during the last 5 min ($\Delta pH_u/5$) of each equilibration period as follows:

$$pH_b = pH_{mb} + \frac{\Delta pH_{ub}}{5} . t_b, \tag{16}$$

$$pH_c = pH_{mc} + \frac{pH_{ub}}{5} . t_b + \frac{pH_{uc}}{5} . t_c, \tag{17}$$

where t_b and t_c represent the length of time, and pH_b and pH_c the corrected pH values of the respective periods. The pH value measured after period *a* was used directly. If, after the described correction, small differences still existed between pH_a and pH_c (0·002–0·004, in some cases up to 0·008 pH units), the arithmetic mean of these two (pH_{ac}) was taken.

The buffer value of imidazole-like and phosphate-like compounds (β_{ic}) in the intracellular compartment of each tissue was calculated from the respective homogenate buffer value (β_{hom}) and the dilution factor (d):

$$\beta_{ic} = \beta_{hom} . d \text{ (mequiv/pH.l cell water)}, \tag{18}$$

$$d = \frac{W_t . F_{H_2O} + W_R}{W_t . F_{H_2O} . (1 - Q_e)}, \tag{19}$$

where W_t = the weight of the pulverized tissue sample, F_{H_2O} = the fractional water content of the tissue, Q_e = the fractional extracellular space, W_R = the amount of Ringer solution added to the pulverized tissue sample. F_{H_2O} and Q_e were adopted from Heisler *et al.* (1976*b*).

Bicarbonate concentrations were calculated from the Henderson–Hasselbalch equation using α_{CO_2} and pK' values reported for dogfish plasma (Pleschka & Wittenbrock, 1971; Albers & Pleschka, 1967). Values for plasma were chosen because of the

similarity in ionic strength and protein concentrations in plasma and homogenate. The pH and corresponding bicarbonate differences of each equilibration procedure are:

$$\Delta pH = pH_{ac} - pH_{b}, \quad (20)$$

$$\Delta[HCO_3^-] = [HCO_3^-]_{ac} - [HCO_3^-]_{b}. \quad (21)$$

Three different ΔpH and $\Delta[HCO_3^-]$ values were obtained in each experiment according to the equilibration procedures 1, 2 and 3. Each combination of two pairs of ΔpH and $\Delta[HCO_3^-]$ (equilibration procedures 1 and 2, 2 and 3, 1 and 3) was used to calculate the homogenate buffer values of imidazole-like and phosphate-like compounds according to (7) and (8) applying mean $\Delta pK/\Delta t$ values of −0·021 U/°C and −0·002 U/°C for biological imidazole and phosphate compounds respectively (Edsall & Wyman, 1958).

The behaviour of pH in the intracellular compartments as closed buffer systems, and the bicarbonate transfer between the intracellular compartments as open buffer systems and the extracellular space were calculated on the basis of equations (10), (12), (14) and (15) using P_{CO_2}, pH_i and $[HCO_3]$ values reported by Heisler *et al.* (1980).

RESULTS

The buffer values determined for juvenile (0·3–1 kg) and adult specimens (1–3 kg) did not show any trend to be different from each other so the mean values of all studied specimens were calculated and are presented in Table 2. The total buffer values, both those determined as the sum of imidazole-like and phosphate-like buffer values according to the method described in this paper and those determined at constant temperature (method adopted from Heisler & Piiper, 1971) are fairly similar and somewhat smaller than values determined in mammalian and bird species using in principle the same technique (Heisler & Piiper, 1971; Lai, Attebery & Brown, 1973; Lykkeboe & Johansen, 1975).

Table 2. *Buffering properties in intracellular compartments of the Larger Spotted Dogfish* (Scyliorhinus stellaris)

(Data are given as mean ± S.D., n = number of experiments (three determinations/experiment). β_{im} and β_{ph} are the respective buffer values of imidazole-like and phosphate-like compounds. β_{tot} = the total buffer value measured at constant temperature (mequiv/pH.l cell water). All buffer value determinations were carried out in the pH range of 7·1–7·5 for white and heart muscle, and of 7·2–7·6 for red muscle.)

Series A: temperature variable

	β_{im}	β_{ph}	β_{im}/β_{ph}	$\beta_{im}+\beta_{ph}$	n
White muscle	38·9 ± 6·3	10·6 ± 1·7	3·67 ± 0·48	49·5 ± 7·9	7
Red muscle	20·5 ± 3·4	17·9 ± 3·7	1·15 ± 0·25	38·4 ± 5·5	6
Heart muscle	27·1 ± 5·0	10·4 ± 2·1	2·16 ± 0·58	37·5 ± 7·0	6

Series B: temperature constant

	β_{tot}	n
White muscle	52·3 ± 4·2	10
Red muscle	36·1 ± 5·0	7
Heart muscle	39·0 ± 6·1	5

Table 3. *Changes of intracellular pH with temperature* ($\Delta pH_i/\Delta t$) *in tissues of adult dogfish specimens* in vivo (*Heisler* et al. 1980) *and in the intracellular compartments modelled as closed buffer systems* (*see text*)

	In vivo		Closed buffer system	
$\Delta P_{CO_2}/\Delta t$	Normal	Reversed	Normal	Reversed
White muscle	−0·018	−0·018	−0·017	−0·014*
Red muscle	−0·031	−0·028	−0·013**	−0·008**
Heart muscle	−0·007	−0·005	−0·016**	−0·013**

Values are significantly different from the respective values *in vivo*: * $P < 0{\cdot}05$, ** $P < 0{\cdot}005$.

The contribution of imidazole-like and phosphate-like buffering to the total buffer value is different in the three muscle types. In white muscle, imidazole-like buffering contributes about 80% to the total buffer value. The observed ratio for β_{im}/β_{ph} of about 3·7 is very similar to the concentration ratio for imidazole and phosphate compounds of 4, calculated for frog intracellular muscle compartments by Reeves & Malan (1976). While in white muscle the contribution of phosphate-like buffering is relatively small, it is larger in the other two muscle types, especially in red muscle, where about half (47%) of the total buffer value has to be attributed to phosphate-like buffering.

The intracellular compartments have been modelled as closed buffer systems according to (10) and (14). The changes of intracellular pH that would occur under these conditions when temperature is changed are summarized in Table 3. The $\Delta pH_i/\Delta t$ values calculated for red muscle and for heart muscle are significantly different from the corresponding values determined *in vivo* with both normal and with increased P_{CO_2} at low acclimation temperature (reversed $\Delta P_{CO_2}/\Delta t$), but for white muscle they are different only with hypercapnia at low temperature.

The net bicarbonate transfer between intracellular and extracellular compartments of the studied tissues occurring *in vivo* after temperature is changed by 10 °C are listed in Table 4. The transferred amounts are considerable in comparison with the normal intracellular concentration of about 1 mM, and are significantly different from zero for heart muscle and for red muscle. In white muscle the difference is only significant with increased P_{CO_2} at low temperature.

DISCUSSION

Methodology

The mathematical treatment of the obtained data according to both approaches 'A' and 'B' is based on a linear correlation between the amount of buffered H^+ ions and the change in pH. The linear relationship was chosen as the best approximation to the obtained correlation of $\Delta[HCO_3]$ and ΔpH values determined in the individual experiments. The non-bicarbonate buffer curve in the muscle tissues of dogfish is similarly linear, as in the intracellular compartments of human erythrocytes (Dill, Edwards & Consolazio, 1937). This quasi-linearity can be explained by the overlapping sigmoid buffer curves of several buffer groups in different stereometric positions in larger molecules, which have different p*K* values according to their position and chemical modifications.

Both approach 'A' and approach 'B' (see Theoretical Considerations) are equivalent in their final description of the closed intracellular buffer systems and of the bicarbonate transfer *in vivo*. The main advantage of approach 'A' is that the obtained data allow the simulation of the intracellular compartment for every chosen temperature change, whereas approach 'B' is limited to application with the temperature step imposed during the experimental procedure. Additionally, approach 'A' yields buffer values for the imidazole-like and phosphate-like buffers. The calculations of these values, however, are fairly sensitive to mis-estimates of the chosen mean ΔpK values.

The error introduced by such mis-estimates is dependent on the relative buffer value of the respective buffer group. Considering the range covered by the ΔpK values of different imidazole-like and phosphate-like buffer substances (Edsall & Wyman, 1958) and the determined relative buffer values, mis-estimates of about $\pm 20\%$ have to be considered possible, even when the similarity between the total buffer values determined at constant temperature and the values of $(\beta_{im}+\beta_{ph})$ suggests that the obtained values fit quite well the actual conditions.

While mis-estimates of the ΔpK values have considerable influence on the calculated buffer values, the calculations of $\Delta pH_i/\Delta t$ for the closed buffer systems (Table 3) and of the bicarbonate transfers (Table 4) are completely independent of the ΔpK values according to the applied mathematical treatment. They are only influenced by the α_{CO_2} and pK values used as constants for the bicarbonate calculations by means of the Henderson–Hasselbalch equation. Application of the values for dogfish plasma to the non-diluted intracellular compartments may result in a maximal error of 5%, a value that does not influence the significance of the data presented in Tables 3 and 4.

While all sources of error discussed above cannot significantly affect the results presented in this paper, autolysis of ATP and creatine phosphate (CP) in the muscle sample and later in the homogenate would affect the quantitative conclusions of this paper considerably. Decomposition of ATP and CP would release fairly large amounts of inorganic phosphate, which is a good buffer in the physiological pH range. As CP, and ATP in the presence of Mg^{2+}, hardly buffer in the physiological pH range, this mechanism would result in an overestimate of the buffer value for phosphate-like substances. But even the assumption of a maximal overestimate would not change the qualitative conclusions of this paper: even with a phosphate buffer value of zero the $\Delta pH/\Delta t$ values for the closed buffer system in red muscle and heart muscle would still be significantly different in the same direction from the *in vivo* values as those given in Table 3. Also the bicarbonate transfers for these tissues would be significantly different from zero in the same direction as those reported in Table 4.

It is, however, unlikely that significant autolysis can take place during the applied procedure. The muscle samples were deep frozen precipitously in liquid nitrogen within 20 s from the start of the excision and then kept frozen until the muscle powder was diluted with Ringer solution. During the equilibration procedure any decomposition of CP or ATP would have been recorded as a considerable rise in homogenate pH measured at the end of equilibration C in comparison with pH_a (see Procedure), resulting from the release of OH^- ions (for CP: 0·5 mmol OH^-/mmol CP decomposed, Meyerhoff & Lohmann, 1928). As we have not observed such alkaline pH shifts in our experiments and as the hypoxic time of the tissues can be considered to be only a very few seconds, we believe that no significant decomposition of ATP or

Table 4. *Bicarbonate transfer from intracellular to extracellular compartment* ($\Delta[HCO_3^-]_{i\rightarrow e}$) *after a temperature change from* 10 *to* 20 °C *in dogfish tissues, calculated on the basis of buffering properties determined in this study and* in vivo *measured* pH_i *and* P_{CO_2} (*Heisler* et al. 1980)

$\Delta P_{CO_2}/\Delta t$	$\Delta[HCO_3^-]_{i\rightarrow e}$ (mequiv/l. cell water)		
	Normal		Reversed adult
	Juvenile	Adult	
White muscle	1·8	0·3	2·3*
Red muscle	7·0**	7·5**	8·2**
Heart muscle	−3·1**	−3·7**	−3·3**

Values are significantly different from zero: * $P < 0.05$, ** $P < 0.005$.

CP has affected the reported results, especially as our phosphate-like buffer values appear rather low with respect to inorganic phosphate concentrations measured in fish muscle tissues (i.e. muscle of resting trout: 44·5 mmol/kg fresh tissue, Tomlinson, Geiger & Kay, 1965).

Physiological significance

White muscle appears to be the only one of the studied tissues in which the intracellular pH is adjusted after changes of temperature more or less without the necessity of bicarbonate being transferred across the cell membrane. The $\Delta pH/\Delta t$ value in the closed intracellular buffer system is at normal environmental P_{CO_2} almost the same as observed *in vivo* (see Table 3). When the $\Delta P_{CO_2}/\Delta t$ value is reversed by hypercapnia at low acclimation temperature, however, the closed buffer system $\Delta pH_i/\Delta t$ is significantly smaller than *in vivo* and a significant amount of bicarbonate has to be transferred across the cell membrane to achieve the *in vivo* steady state value (Table 4). In the other two studied tissues, red muscle and heart muscle, the closed buffer system $\Delta pH_i/\Delta t$ values differ by a factor of 2 from the *in vivo* conditions. The amount of bicarbonate that has to be transferred to attain the *in vivo* equilibrium is in heart muscle about 3 times as large as the normal intracellular content, and in red muscle about 7 times as large. It is quite evident on the basis of the data summarized in Tables 3 and 4 that net bicarbonate transfer processes play an important role for the pH_i adjustment in dogfish after temperature changes. Bicarbonate transfer, however, appears to be not a special mechanism for acid-base regulation after temperature changes, since it has been shown to be significantly involved in acid-base regulation under various conditions: in hypercapnia at constant temperature in mammalian tissues (Heisler & Piiper, 1972; Strome, Clancy & Gonzalez, 1976), dogfish tissues (Heisler *et al.* 1976*a*) and teleost fish tissues (Heisler *et al.* 1978), after exhausting exercise in mammalian and amphibian tissues (Benadé & Heisler, 1978) and in dogfish muscle tissues (Holeton & Heisler, 1978). These accumulated data suggest that net bicarbonate (or H^+ or OH^-) transfer is similarly widespread among species and tissues as the transport system for H^+, OH^- or bicarbonate across the cell membrane serving the maintenance of the steady state intracellular/extracellular bicarbonate distribution. It may even be that both transfers are mediated by the same regulatory

mechanism which is modulated with changing setpoint values under various conditions. The extent, however, to which net bicarbonate movements are actually involved in acid-base regulation may be quite variable according to the contributions of other mechanisms.

Apparently the importance of this mechanism is smaller in the two air-breathing species frog and turtle. When temperature is changed in these animals, the intracellular pH is adjusted by changes of buffer p*K* values and changes in P_{CO_2} (Reeves & Malan, 1976). As the p*K* values change almost immediately with temperature and as P_{CO_2} appears to be adjusted by changes of ventilation, at least in turtles, in less than 1 h (Jackson & Kagen, 1976), the deviation of the intracellular pH from the steady state values for the new temperature can be expected to be small and of only short duration (Reeves & Malan, 1976). Therefore, this type of regulation appears to be rather advantageous. But water breathing animals have no choice of mechanism, for they are fairly limited in the adjustment of P_{CO_2}, as a result of the low oxygen content of their breathing medium. Bicarbonate transfer is a relatively slow method of acid-base adjustment in response to temperature change (Heisler, 1978) but this disadvantage is mitigated by the fact that the extent and the rate of temperature changes most fish normally encounter are small in comparison with those faced by air breathing ectotherms.

CONCLUSION

The present results have shown that in dogfish the changes of intracellular pH in response to changes of temperature can be explained on the basis of physico-chemical buffering and changes of P_{CO_2} only in white muscle under normal conditions. In red muscle and heart muscle, and also in white muscle when $\Delta P_{CO_2}/\Delta t$ is reversed by hypercapnia at low acclimation temperature, the pH_i is predominantly and probably ultimately regulated by means of intracellular/extracellular bicarbonate transfer processes.

The authors gratefully acknowledge the support by the Stazione Zoologica at Naples.

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