BLOOD GASES, AND EXTRACELLULAR/INTRACELLULAR ACID–BASE STATUS AS A FUNCTION OF TEMPERATURE IN THE ANURAN AMPHIBIANS *XENOPUS LAEVIS* AND *BUFO MARINUS*

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SUMMARY

Blood gases, and parameters of the extracellular and intracellular acid–base status, were measured in the anuran amphibians *Bufo marinus* and *Xenopus laevis* acclimated to temperatures of 10, 20 and 30°C for 12 days. Arterial P_{O_2} rose with temperature so that approximately constant oxygen saturation of the blood was maintained, a phenomenon explained on the basis of models for O_2 transport in animals with central vascular shunts and temperature-dependent shifts in O_2 equilibrium characteristics. Arterial plasma pH of both species varied inversely with temperature, the pH/temperature coefficient being not significantly different from that required for constant relative alkalinity or dissociation of imidazole. The change in plasma pH was brought about mainly by changes in P_{CO_2}, although plasma bicarbonate concentration also changed significantly. Intracellular pH/temperature relationships were found to be non-linear in most of the tissues. There was considerable variability among body tissue compartments and between the two species. These data confirm that the various tissue compartments in ectotherms maintain unique $\Delta pH/\Delta t$ relationships, and indicate that measurement of extracellular pH as a function of temperature is not a good indicator for alphastat-type, temperature-dependent, acid–base regulation.

INTRODUCTION

The early studies on the inverse relationship between extracellular pH and body temperature in ectothermic vertebrates (e.g. Robin, 1962; Rahn, 1967; Howell, Baumgardner, Bondi & Rahn, 1970; for further references see Heisler, 1986b) led to the formulation of two models: that of a constant relative alkalinity (Rahn, 1967) and the alphastat hypothesis (Reeves, 1972). These models were based on experimental

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findings which suggested that arterial plasma pH changed with temperature in a manner similar to that in neutral water ($\Delta pN/\Delta t = -0.019$ units $^\circ C^{-1}$ from 5 to 20 $^\circ C$; $\Delta pN/\Delta t = -0.017$ units $^\circ C^{-1}$ from 20 to 35 $^\circ C$; Weast, *Handbook of Chemistry and Physics*; see Heisler, 1986*b*). However, as more experimental data on individual animals became available, it was increasingly evident that the magnitude of changes in pH with temperature ($\Delta pH/\Delta t$) was quite variable, ranging from -0.008 to -0.021 in turtles (Robin, 1962; Malan, Wilson & Reeves, 1976), from -0.001 to -0.017 in lizards (Wood, Glass & Johansen, 1977; Wood & Moberly, 1970), from -0.011 to -0.021 in amphibians (Mackenzie & Jackson, 1978; Malan *et al.* 1976) and from -0.008 to -0.017 in fish (Walsh & Moon, 1982; Heisler, 1984*b*; for a complete tabulation of data for all orders of animals see Heisler, 1986*b*).

The alphastat hypothesis of Reeves (1972) predicts that the observed decrease in pH with increasing body temperature (Reeves, 1972; Malan *et al.* 1976) is based on adjustment towards a constant ionization of histidine–imidazole. As a central criterion of the model, Reeves proposed that pH adjustment was accomplished by regulation of ventilation and therefore P_{CO_2}. However, it was postulated that the bicarbonate concentration would remain constant because of the non-titration of non-bicarbonate buffers and the lack of any transmembrane or transepithelial, acid–base-relevant ion transfer. This type of pH regulation was claimed for both the extracellular and intracellular body compartments with pH/temperature coefficients ($\Delta pH/\Delta t$) close to the $\Delta pK/\Delta t$ of biological histidine–imidazole moieties [$\Delta pK_{Im}/\Delta t = -0.018$ to -0.024 units $^\circ C^{-1}$, depending on ligands and steric arrangement (Edsall & Wyman, 1958; see Heisler, 1986*b*)]. However, with constant bicarbonate concentration and semi-closed buffer system regulation (open only for CO_2, see Heisler, 1986*a*), constant imidazole dissociation can only be achieved with histidine–imidazole as the predominant non-bicarbonate buffer. The histidine–imidazole of haemoglobin is the predominant non-bicarbonate buffer of the extracellular space, but intracellular tissue buffering usually deviates considerably from this (Heisler & Neumann, 1980; Heisler, 1984*a*; see also Heisler, 1986*b*). Nevertheless, the magnitude of $\Delta pH_e/\Delta t$ is highly variable within and amongst different groups of ectotherms, with most data falling outside the predictions of the alphastat model (see Heisler, 1986*b*).

Considerably less is known about changes of intracellular pH with temperature, even though histidine–imidazole protein residues have been suggested as possible intracellular sensors for the regulation of the ratio of CO_2 production to ventilation and, thus, the regulated variable of the proposed system, P_{CO_2} (Rahn & Reeves, 1980). In fact, with amphibians and reptiles (the animals for which the alphastat hypothesis was originally conceived), intracellular pH as a function of temperature has been determined in only three species (Malan *et al.* 1976; Bickler, 1982; P. Neumann, G. M. O. Maloiy & N. Heisler, in Heisler, 1986*b*). In experiments on the turtle, *Pseudemys scripta*, $\Delta pH_i/\Delta t$ values in white muscle (-0.0186 units $^\circ C^{-1}$) and liver (-0.023 units $^\circ C^{-1}$) were not significantly different from the range of the $\Delta pK/\Delta t$ of imidazole compounds and of simultaneously measured blood

$(-0.0207 \text{ units} \,^{\circ}\text{C}^{-1})$, whereas $\Delta\text{pH}_i/\Delta t$ of heart $(-0.0122 \text{ units} \,^{\circ}\text{C}^{-1})$ was significantly lower (Malan *et al.* 1976). In parallel studies on the bullfrog, the $\Delta\text{pH}/\Delta t$ of mixed types of striated muscle $(-0.015 \text{ units} \,^{\circ}\text{C}^{-1})$ was not significantly different from that of arterial blood $(-0.0206 \text{ units} \,^{\circ}\text{C}^{-1})$. More recently, Bickler (1982) found that brain intracellular pH of the lizard, *Dipsosaurus dorsalis*, was maintained constant over an 18–35°C temperature range, whereas other tissue compartments conformed to the alphastat model at least in the preferred temperature range. As with the findings of Bickler (1982) for *Dipsosaurus* brain, the intracellular pH of *Varanus exanthematicus* white muscle $(\Delta\text{pH}/\Delta t = -0.005 \text{ units} \,^{\circ}\text{C}^{-1})$, heart $(\Delta\text{pH}/\Delta t = -0.003 \text{ units} \,^{\circ}\text{C}^{-1})$ and oesophagus $(\Delta\text{pH}/\Delta t = -0.002 \text{ units} \,^{\circ}\text{C}^{-1})$ changed little with changes in temperature (Neumann *et al.* in Heisler, 1986*b*). From these studies, data on intracellular pH in amphibians are available for only one muscle type in one species of Anura (Malan *et al.* 1976). This scarcity would be of little importance were it not for the primary role that the amphibian model has played in formulating the alphastat hypothesis (Reeves, 1972). Against this background we have examined the pH_i/temperature relationships of several tissue types in the aquatic anuran, *Xenopus laevis*, and in the semi-terrestrial toad, *Bufo marinus*.

MATERIALS AND METHODS

Specimens of *Bufo marinus* (mass 210–423 g, $N = 27$) and *Xenopus laevis* (mass 47–65 g, $N = 28$) were purchased from a commercial supplier (Charles D. Sullivan Co. Inc., Nashville, TN) and transported to Germany by airfreight. Animals were held in captivity for at least 1 month prior to experimentation and fed on crickets and chopped liver with vitamin supplements. Ten days before implantation of femoral artery cannulae (see Boutilier, Randall, Shelton & Toews, 1979; Boutilier, 1984) animals were acclimated to either 10, 20 or 30°C in thermostatically controlled aquaria or terraria ($\pm 1°$C). For cannulation, animals were anaesthetized by immersion in a 0·7 % solution of tricaine methane sulphonate (MS-222, Sigma) titrated to pH 7 with sodium bicarbonate. The entire operation including anaesthesia took approximately 1 h, whereupon the animals were returned to their respective acclimation temperature for a 48-h recovery period. The air and water phases of the experimental chambers were held to within $\pm 0·5$ °C of the acclimation temperatures.

Twelve hours before each experiment, $[^{14}\text{C}]$DMO (5,5-dimethyl-2,4-oxazolidine-dione) and $[^3\text{H}]$inulin were injected into the femoral artery for subsequent estimation of intracellular pH by use of the DMO distribution technique (Waddell & Butler, 1959). Blood samples (about 300 μl) were taken from the animals (four from *Bufo* and two from *Xenopus*) at their respective acclimation temperatures and analysed for plasma pH, total CO_2 concentration, P_{CO_2} and P_{O_2}. In the case of *Xenopus*, the samples were taken shortly after a breathing period at the surface (see Boutilier, 1984). Immediately after the last blood sample (which included an additional 300 μl for plasma isotope analysis) had been taken, the animals were swiftly killed by anaesthetic overdose and tissue samples removed for subsequent analysis of tissue water compartments and radioactivity. The gastrocnemius and

sartorius muscles from the non-cannulated limbs were dissected away, as were individual muscle bands associated with the pectoral area. Five samples (100–400 mg) from each skeletal muscle group of each animal were taken for analysis. Samples of heart tissue (50–150 mg) were taken from *Bufo* (five samples) and *Xenopus* (1–2 samples). Immediately after removal, tissues (and corresponding plasma samples) were weighed and then dried to a constant weight (at 110°C) before being prepared, by oxidation, for liquid scintillation counting.

Dried tissue samples and plasma samples were pressed into filter paper pills and combusted with a sample oxidizer (Packard Instruments, Model 306, modified) for analysis of [^{14}C]DMO and [^{3}H]inulin by liquid scintillation counting (Packard Instruments, Model 2660) (see Heisler, 1975, for details). The ratio of the extracellular fluid volume of muscle to the total muscle water, obtained from the concentrations of [^{3}H]inulin in muscle and plasma water, was taken to represent fractional extracellular volume (Q_{em}). Intracellular pH (pH_i) of muscle was determined by the transmembrane distribution of [^{14}C]DMO according to the equation:

$$pH = pK_{DMO} + \log\left\{\frac{[DMO]_i}{[DMO]_e}(10^{pH_e - pK_{DMO}} + 1) - 1\right\},$$

where $pK_{DMO} = 6.464 - 0.00874t$ (Albers, Usinger & Spaich, 1971). Intracellular DMO concentrations ([$DMO]_i$) were calculated according to the relationship:

$$[DMO]_i = \frac{[DMO]_m - [DMO]_e \times Q_{em}}{(1 - Q_{em})},$$

where $[DMO]_m$ and $[DMO]_e$ are the concentrations in the total muscle water and plasma water, respectively.

Measurements of blood pH, P_{CO_2} and P_{O_2} were carried out using Radiometer blood micro systems (BMS 3) calibrated at the respective temperatures with precision buffers (Radiometer S1500, S1510) and humidified gas mixtures (Wösthoff gas mixing pumps, Bochum, FRG). The total CO_2 concentrations of 50-μl samples of anaerobically obtained true plasma were determined using the electrode and cuvette method of Cameron (1971). Bicarbonate concentrations ([HCO_3^-]) in true plasma were estimated from the measured values of total CO_2 concentration ([CO_2]) and P_{CO_2}, using the equation:

$$[HCO_3^-] = [CO_2] - (\alpha CO_2 \times P_{CO_2}),$$

where αCO_2 is the solubility of carbon dioxide in plasma (in $mmol\,l^{-1}\,mmHg^{-1}$) (1 mmHg = 133.3 Pa) at the respective acclimation temperature (Heisler, 1984*b*, 1986*a*; molarity = 0.223 mol l^{-1}; note that the sign of the last line term of the αCO_2 formula is misprinted in Heisler, 1984, and should read '+').

Whole blood oxygen dissociation curves were constructed *in vitro* (at 10, 20 and 30°C) on blood samples obtained from cannulated animals at each of the respective temperatures. Samples of blood were pooled to achieve the required volumes and distributed equally to tonometers supplied with either air/CO_2 or N_2/CO_2 gas

mixtures (the CO_2 concentrations being adjusted at each temperature to approximate the corresponding levels of arterial P_{CO_2} seen *in vivo*). Blood samples of known O_2 saturation were prepared for P_{O_2} and pH measurement as described by Hicks, Ishimatsu & Heisler (1987), using the mixing method (Haab, Piiper & Rahn, 1960) detailed by Scheid & Meyer (1978).

Statistical significance of differences between variables was determined by application of Student's t-test at a level of $2P < 0.05$.

<div align="center">RESULTS</div>

The blood pH/temperature relationships ($\Delta pH_e/\Delta t$) in both *Xenopus laevis* and *Bufo marinus* were not significantly different from that of neutral water over the entire temperature range studied (10–30°C) (Fig. 1; Table 1). In both species the decreases in blood pH with increasing temperature were achieved primarily by increases in arterial P_{CO_2}; the changes in plasma $[HCO_3^-]$, although statistically significant, contributed less to the change in pH (Fig. 2).

The pH/temperature relationships of skeletal and cardiac muscle tissue compartments of *Xenopus laevis* and *Bufo marinus* are plotted in Fig. 3. Unlike arterial blood, $\Delta pH/\Delta t$ in the intracellular compartments varied considerably with temperature (Table 1) and was often markedly different from that of arterial blood. In *Bufo marinus*, for instance, $\Delta pH/\Delta t$ for skeletal muscle was lower in the temperature
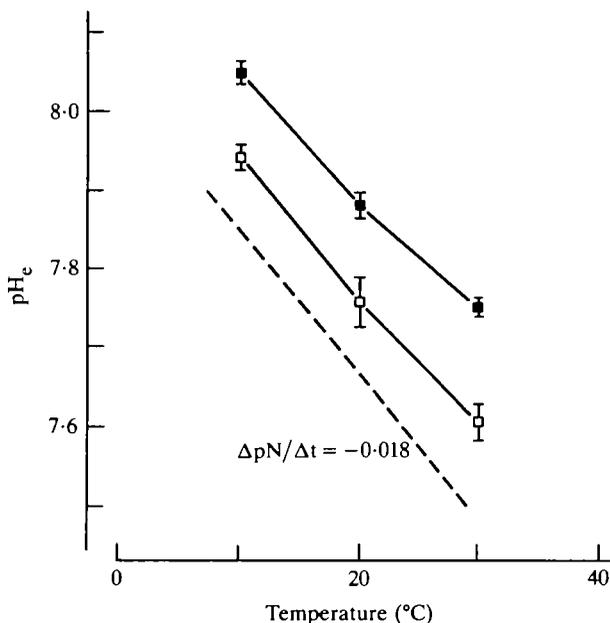

Fig. 1. Relationship between arterial plasma pH (extracellular pH, pH_e) and ambient temperature in *Xenopus laevis* (□, $\bar{x} \pm$ S.E., $N = 10$ at 10°C; $N = 8$ at 20°C; $N = 10$ at 30°C) and *Bufo marinus* (■, $\bar{x} \pm$ S.E., $N = 8$ at 10°C; $N = 10$ at 20°C; $N = 9$ at 30°C). Each N is the mean of two (*Xenopus*) or four (*Bufo*) independent measurements.

range 10–20 °C than at higher temperatures. However, the reverse was true for *Xenopus laevis* (Table 1). At any given temperature there were no significant differences in pH among the three groups of skeletal muscle in *Xenopus laevis*,
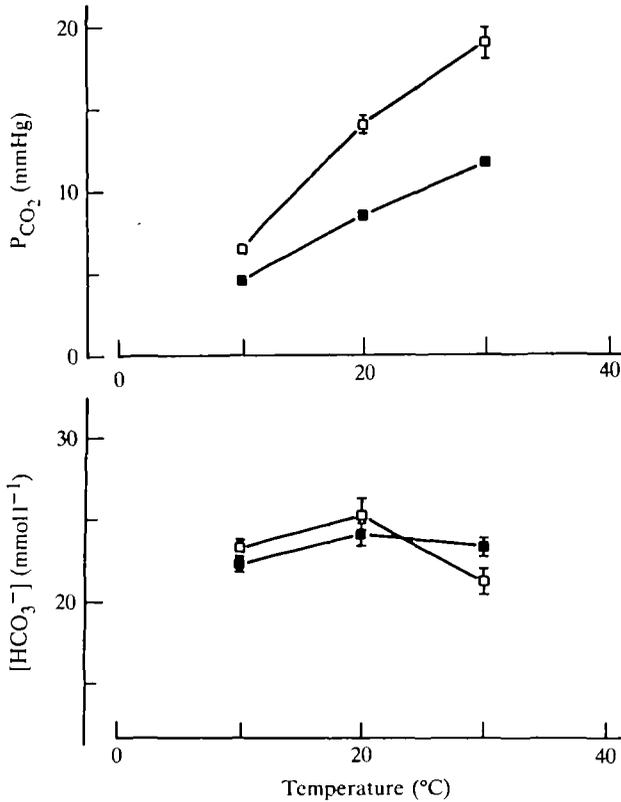

Fig. 2. Arterial plasma P_{CO_2} and bicarbonate concentration $[HCO_3^-]$ as a function of ambient temperature in *Xenopus laevis* (□) and *Bufo marinus* (■) ($\bar{x} \pm$ S.E., N as for Fig. 1; S.E. are sometimes covered by the points indicating the average).

Table 1. *pH changes with changes in body temperature* $(\Delta pH/\Delta t, units\,°C^{-1})$ *in body fluid compartments of* Bufo marinus *and* Xenopus laevis

| Species	Body fluid compartment	Temperature range		
		10–20 °C	20–30 °C	10–30 °C
Xenopus laevis	Arterial plasma	−0·018	−0·015	−0·017
	Sartorius muscle	−0·017	−0·014	−0·016
	Gastrocnemius muscle	−0·027	−0·007	−0·017
	Pectoral muscle	−0·018	−0·011	−0·014
	Heart muscle	0·000	−0·014	−0·007
Bufo marinus	Arterial plasma	−0·015	−0·014	−0·015
	Sartorius muscle	−0·014	−0·030	−0·022
	Gastrocnemius muscle	−0·011	−0·036	−0·023
	Pectoral muscle	−0·010	−0·017	−0·014
	Heart muscle	−0·024	−0·029	−0·026
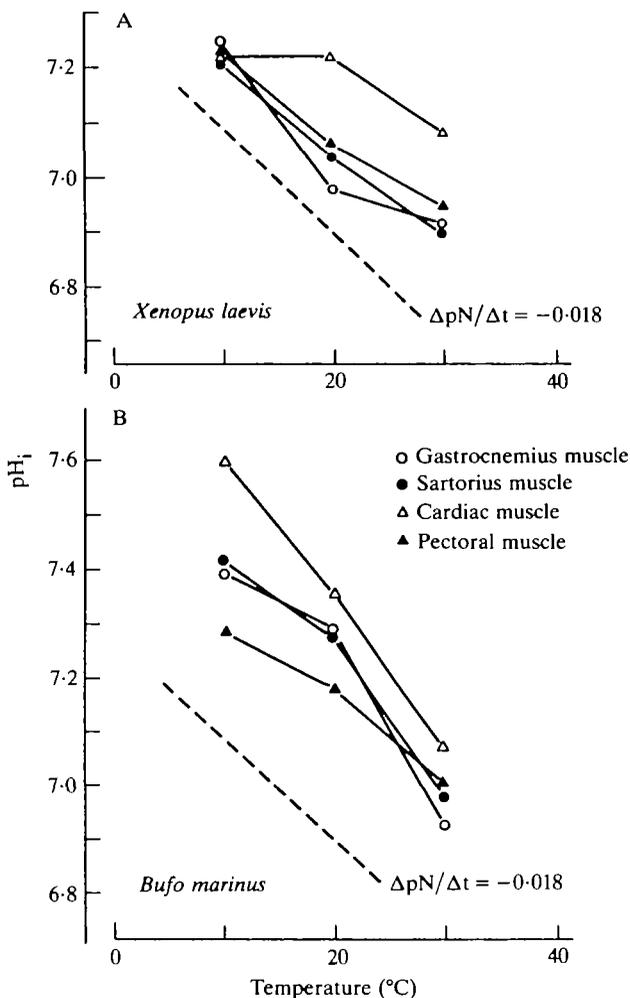
Fig. 3. Intracellular pH as a function of ambient temperature for three groups of skeletal muscle and for cardiac muscle of *Xenopus laevis* (A) and *Bufo marinus* (B) ($\bar{x} \pm$ s.e., which is presented in Table 3, has been omitted for clarity; N as for Fig. 1, each N is the mean of five independent measurements, except for heart muscle, 2, of *Xenopus*).

although pH in the pectoral muscle of *Bufo marinus* was significantly lower than in either the sartorius or gastrocnemius muscle at 10 and 20 °C (Fig. 3; Tables 2, 3). In both species, the pH of cardiac muscle was higher than that of skeletal muscle at any given temperature (Fig. 3). There were marked differences in the pH/temperature response of heart tissue between the two species. Cardiac muscle in *Bufo marinus* (Fig. 3) showed the largest change in pH with temperature of all the compartments studied (Table 1). In *Xenopus*, however, heart muscle pH was constant in the 10–20 °C temperature range, although in the 20–30 °C range the $\Delta pH_i/\Delta t$ of heart muscle tissue was not significantly different from that of arterial plasma (Table 1).

The values for the fractional water content of the tissues (F_{H_2O}) and extracellular space (Q_{em}), obtained using the inulin distribution technique, are presented in
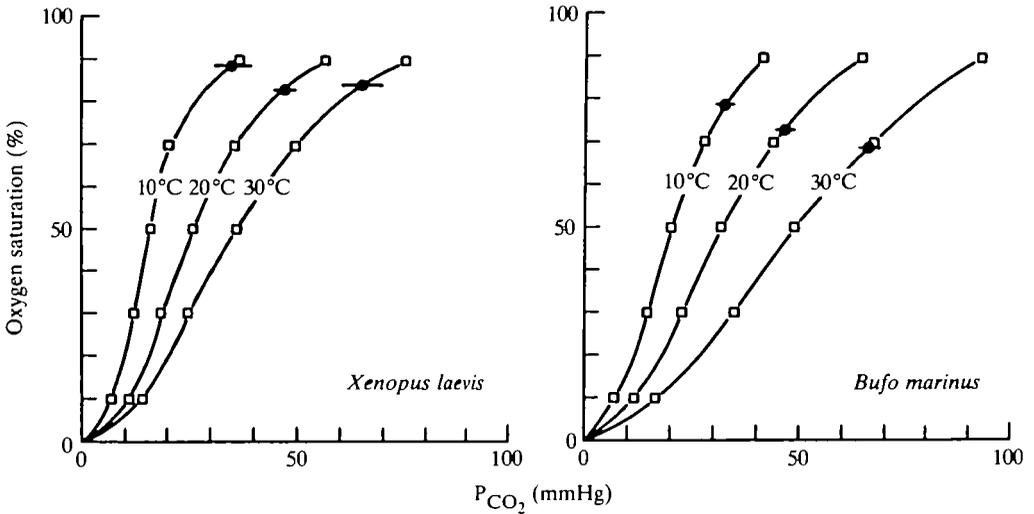
Fig. 4. Oxygen dissociation curves for whole blood of *Xenopus laevis* and *Bufo marinus* at 10, 20 and 30°C. Each curve was constructed *in vitro* from blood samples pooled from 2–3 animals acclimated for at least 96 h to the respective temperatures. Solid circles represent *in vivo* arterial blood P_{CO_2} values ($\bar{x} \pm$ S.E.) for the respective temperature.

Table 2. *Intracellular pH (pH$_i$), fractional water content (F$_{H_2O}$), and fractional extracellular volume (Q$_{em}$) of several skeletal muscle species and heart muscle of* Xenopus laevis

Parameter	Temperature (°C)	Sartorius muscle	Gastrocnemius muscle	Pectoral muscle	Heart muscle
pH$_i$	10	7·206 ± 0·026	7·243 ± 0·027	7·231 ± 0·025	7·217 ± 0·029
	20	7·033 ± 0·048	6·975 ± 0·047	7·054 ± 0·038	7·217 ± 0·045
	30	6·893 ± 0·045	6·908 ± 0·037	6·944 ± 0·036	7·075 ± 0·049
F$_{H_2O}$	10	0·785 ± 0·005	0·785 ± 0·003	0·790 ± 0·003	0·852 ± 0·007
	20	0·778 ± 0·008	0·776 ± 0·008	0·756 ± 0·008	0·813 ± 0·007
	30	0·786 ± 0·008	0·788 ± 0·008	0·788 ± 0·010	0·813 ± 0·007
Q$_{em}$	10	0·145 ± 0·005	0·159 ± 0·008	0·229 ± 0·006	0·223 ± 0·011
	20	0·155 ± 0·008	0·163 ± 0·010	0·240 ± 0·006	0·299 ± 0·026
	30	0·162 ± 0·005	0·145 ± 0·003	0·241 ± 0·009	0·280 ± 0·014

Values are $\bar{x} \pm$ S.E.

Tables 2 and 3. There was no significant effect of temperature on either measurement in either species.

Arterial blood P_{O_2} increased with temperature in both species. These Pa_{O_2} values were obtained during periods when the animals were ventilating their lungs (*Bufo marinus*) or shortly after a breathing episode (*Xenopus laevis*). Whole blood oxygen equilibrium curves, determined *in vitro* for both species, are shown in Fig. 4 at each of the acclimation temperatures examined in the present study. P_{CO_2} levels of the

Table 3. *Intracellular pH (pH_i), fractional water content (F_{H_2O}), and fractional extracellular volume (Q_{em}) of several skeletal muscle types and heart muscle of* Bufo marinus

Parameter	Temperature (°C)	Sartorius muscle	Gastrocnemius muscle	Pectoral muscle	Heart muscle
pH_i	10	7·418 ± 0·033	7·389 ± 0·026	7·281 ± 0·046	7·596 ± 0·038
	20	7·274 ± 0·031	7·282 ± 0·027	7·178 ± 0·040	7·353 ± 0·049
	30	6·978 ± 0·049	6·924 ± 0·067	7·004 ± 0·034	7·068 ± 0·078
F_{H_2O}	10	0·805 ± 0·013	0·799 ± 0·006	0·815 ± 0·008	0·815 ± 0·006
	20	0·786 ± 0·007	0·779 ± 0·004	0·797 ± 0·003	0·808 ± 0·006
	30	0·791 ± 0·005	0·786 ± 0·003	0·805 ± 0·005	0·817 ± 0·004
Q_{em}	10	0·160 ± 0·016	0·111 ± 0·009	0·201 ± 0·018	0·225 ± 0·006
	20	0·202 ± 0·019	0·145 ± 0·016	0·223 ± 0·020	0·257 ± 0·023
	30	0·224 ± 0·021	0·149 ± 0·004	0·196 ± 0·029	0·287 ± 0·040

Values are $\bar{x}$ ± S.E.

equilibrating gas mixtures were set close to the values obtained *in vivo* at the respective temperatures (Fig. 2), resulting in a pH-induced shift to the right with increasing temperature. When the measured values of Pa_{O_2} at each acclimation temperature *in vivo* are plotted on their corresponding O_2 equilibrium curve (Fig. 4) it is evident that arterial blood O_2 saturation remains relatively constant over the entire temperature range.

DISCUSSION

The changes in arterial blood P_{O_2} with temperature in *Xenopus* and *Bufo* occur at a relatively constant arterial blood O_2 saturation (Fig. 4). This can be explained on the basis of existing models for O_2 transport in animals with cardiovascular shunts (Wood, 1982, 1984; Wood & Hicks, 1985). Arterial desaturation during air breathing results from systemic venous admixture with oxygenated pulmonary venous blood (right to left intraventricular shunt; Shelton, 1976; Shelton & Boutilier, 1982). Under these conditions, the arterial P_{O_2} of a mixture of pulmonary and mixed venous blood becomes a function of the resulting O_2 saturation (Wood, 1982). The rightward shift of the oxygen dissociation curves with rising temperature (Fig. 4) results, therefore, in an increase in arterial P_{O_2} with temperature. Within the temperature range examined in the present study, the arterial O_2 saturation of *Xenopus* (during or shortly after a breathing episode) was maintained virtually constant, whereas in *Bufo* a small but progressive desaturation occurred as temperature increased (Fig. 4). Such effects have been previously demonstrated in a number of reptiles (Wood, 1982; Glass, Boutilier & Heisler, 1983; Wood & Hicks, 1985) and in two amphibians (Wood, 1982; Kruhøffer, Glass, Abe & Johansen, 1987). Although the underlying mechanisms of such phenomena are yet to be determined, the collected data imply that the common denominator of oxygen homeostasis in ectotherms may be O_2 saturation and/or content, rather than partial

pressure (Glass *et al.* 1983; Wood & Hicks, 1985). Intraventricular shunting and venous mixture has less of an effect on CO_2 partial pressures owing to different characteristics of the CO_2 dissociation curves.

The arterial blood pH values of *Bufo marinus* and *Xenopus laevis* vary inversely with temperature ($\Delta pH/\Delta t = -0.015$ and -0.017, respectively; Fig. 1; Table 1, range 10–30°C). These values are similar to those reported for the arterial blood of other amphibian species (Howell *et al.* 1970; Reeves, 1972; Burggren & Wood, 1981; Moalli, Meyers, Ultsch & Jackson, 1981; Hicks & Stiffler, 1984; Kruhøffer *et al.* 1987). The changes in arterial pH with temperature in the present study are brought about primarily through adjustments in Pa_{CO_2}, though small but significant changes in plasma bicarbonate concentrations with temperature were also observed (Fig. 2). In all instances, it appears that the arterial blood pH of amphibians varies inversely with temperature by a specific constant from the pH of neutral water, or that the animals maintain a constant relative alkalinity (Rahn, 1967). The compiled data on arterial blood of amphibians also fit the alphastat hypothesis of Reeves (1972). The biological importance of this hypothesis rests, however, on the assumption that the pH values of intracellular compartments also change with temperature, more or less in parallel with that of arterial blood (constant relative acidity).

The pH_i/temperature relationships for skeletal and cardiac muscle in the present study do not follow the patterns predicted by the alphastat model (Fig. 3; Table 1). Previous measurements of intracellular pH in amphibians are restricted to one study on *Rana catesbeiana* (Malan *et al.* 1976) in which the pH_i of striated muscle (apparently combinations of gracilis, sartorius and gastrocnemius) was determined as a function of temperature by the DMO method. Malan *et al.* (1976) found $\Delta pH_i/\Delta t$ in striated muscle (-0.0152 units °C^{-1}) to be not significantly different from that of arterial blood (-0.0206 units °C^{-1}). In our study, average $\Delta pH_i/\Delta t$ values for the range 10–30°C varied in different groups of striated muscle from -0.014 to -0.023 for *Bufo marinus* and from -0.014 to -0.017 for *Xenopus laevis* (Table 1), the differences being even larger for smaller temperature intervals. $\Delta pH/\Delta t$ tended to be lower at lower temperatures in *Bufo*, whereas the reverse was true for *Xenopus laevis*. These data suggest that intracellular pH may vary with temperature in a non-linear fashion, a conclusion also reached by Walsh & Moon (1982) on the basis of data obtained by acid–base/temperature studies in the eel. Similar non-linearities can also be found for the extracellular fluid of reptiles (for references see Heisler, 1986b). They are, however, not universal. For example, the $\Delta pH/\Delta t$ of arterial plasma of both species is more or less constant, as is $\Delta pH_i/\Delta t$ of *Bufo* heart muscle and *Xenopus* sartorius muscle. In all of the other intracellular body compartments, however, there is a remarkable difference in $\Delta pH/\Delta t$ between the lower and higher temperatures (Fig. 3; Table 1). This is particularly pronounced in heart muscle of *Xenopus*, where pH_i is held constant over the 10–20°C range, but falls by 0.14 pH units between 20 and 30°C (Fig. 3; Tables 1, 2). Similar observations were made in eel heart muscle (Walsh & Moon, 1982). These data indicate that mechanisms other than simple physicochemical dissociation processes are responsible for the fine adjustment of pH with changes of temperature.

Accordingly, there is no good *a priori* reason to assume that the pH/temperature relationship of any body compartment should follow a linear pattern.

Over the past few years, discussions about pH/temperature relationships in ectotherms and the validity of the alphastat hypothesis have become reduced to arguments about the relative magnitude of linearly interpreted $\Delta pH/\Delta t$ slopes. The present data on intracellular pH changes with temperature support data from the literature on non-amphibian ectotherms, showing a wide range of physiological $\Delta pH_i/\Delta t$ values in various body compartments (see Heisler, 1986b). These data also show that temperature-related intracellular acid–base regulation is relatively independent of extracellular acid–base regulation in amphibians, and clearly indicate that individual compartments may maintain unique pH levels and unique $\Delta pH/\Delta t$ relationships (see Fig. 3). The alphastat model, with adjustment of only organismic P_{CO_2}, cannot account for regulatory patterns such as these. Although less restricted than in fishes (see Heisler, 1984b), adjustment of P_{CO_2} in intermittently breathing animals is limited by the discontinuity of gas exchange (Boutilier & Shelton, 1986; Toews & Boutilier, 1986), and the resulting isothermal variability in arterial blood P_{CO_2} is likely also to be transmitted to intracellular body compartments. Intracellular pH regulation is, accordingly, passively affected by changes in P_{CO_2} and the temperature-dependent buffering characteristics, which are quite variable among species and among different tissues in individual species (see Heisler, 1986a,b). The active regulatory process, however, has to be sought in transmembrane and transepithelial ion transfer mechanisms. It should be emphasized that even concordance of $\Delta pH/\Delta t$ with $\Delta pK/\Delta t$ values of imidazole moieties (a relatively rare coincidence) (for references see Heisler, 1986b) provides little support for the alphastat model, if production of acid–base-relevant ions (by metabolic and buffering processes) and transfer of such ions across the borders of the respective compartments are neglected. Future studies must not, therefore, be limited to descriptions of pH/temperature coefficients, but should focus on detailed evaluation of more indicative features of acid–base regulation.

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