The Journal of Experimental Biology 205, 641–650 (2002)
Printed in Great Britain © The Company of Biologists Limited 2002
JEB3839

Evolutionary determinants of normal arterial plasma pH in ectothermic vertebrates

Richard F. Burton*

Institute of Biomedical and Life Sciences, Thomson Building, University of Glasgow, Glasgow G12 8QQ, UK

*e-mail: R.F.Burton@bio.gla.ac.uk

Accepted 17 December 2001

Summary

Mean values of normal arterial pH in different species of fish, amphibians and reptiles at 15 and 25 °C, taken from the literature, are negatively correlated with arterial P_{CO_2} and plasma [Na^+]. At either temperature, the data accord with the hypothesis that extracellular acid–base homeostasis evolved to maintain an optimal pH at particular cell-surface sites that are similar in all species. These hypothetical sites bear fixed negative charges that attract H^+, but which are partially screened by Na^+; for the surface pH to be constant, the bulk interstitial pH should then vary inversely with [Na^+], as is the case. At the same time, the bulk interstitial fluid must be more acid than arterial plasma by an amount that increases with decreasing arterial P_{CO_2}. With allowance made for additional screening by Ca^{2+} and Mg^{2+}, the relevant cell-surface pH is probably approximately 6.2.

Key words: acid–base balance, arterial pH, plasma pH, interstitial pH, vertebrate, fish, amphibian, reptile, carbon dioxide tension, plasma [Na^+], alphastat.

Introduction

The regulation of extracellular pH implies that this has an optimum range. Normal arterial plasma pH (pHa) varies from one species to another, even when allowance is made for the important effect of temperature (see below), but the significance of the differences is unclear. Although pHa affects plasma proteins, it presumably matters most for its influence, through interstitial and cell-surface pH, on the ionization, and hence biological properties, of cell-surface molecules. The aim of the present study is to explore the possibility that the optimum pH at particular regions of the cell surface is similar (at any given temperature) in all heterothermic vertebrates. An alternative view might be that extracellular pH is more relevant to the homeostasis of intracellular pH, but the two are not rigidly linked, either in evolution or as acid–base balance changes in individual animals. Indeed, the cells in a single body may show diverse patterns of intracellular pH homeostasis (e.g. Heisler, 1986). Independent treatment of extracellular homeostasis is therefore appropriate.

That pH matters for its effects on ionization has long been orthodoxy, especially since the waning of the notion of relative alkalinity, or [OH^-]/[H^+] ratio (Rahn, 1967); this ratio is no easier to define in terms of activities than is pH (Covington et al., 1985), its use does not eliminate the difficulties inherent in comparing pH values at different temperatures, as suggested by Howell et al. (1970), and it has no known functional significance.

This paper is little concerned with temperature effects, but it is helpful to consider these briefly here – and temperature differences must be allowed for when comparing pH data. The ionization of any one chemical group varies with (pH–pK), where K is its dissociation constant and pK=–log$K$. The condition for constant ionization with varying temperature, T, is that $\Delta pH/\Delta T = \Delta pK/\Delta T$. In individual heterothermic vertebrates, pHa (as operationally defined) typically decreases *in vivo* with increasing temperature, with $\Delta pHa/\Delta T$ usually in the range –0.005 to –0.02 °C^{-1} (e.g. Robin, 1962; Howell et al., 1970; Reeves, 1972; Heisler, 1986; Ultsch and Jackson, 1996). This suggests that extracellular pH homeostasis relates to key ionizing groups with matching temperature-sensitivity, the most obvious candidates being protein imidazole groups since $\Delta pK/\Delta T$ values in these fall mostly in a similar range. Thus, $\Delta pK/\Delta T$ values for imidazoles in myoglobin are –0.01 to –0.02 °C^{-1} (Bhattacharya and Lecomte, 1997). Therefore, the temperature-dependence of pHa characteristic of a species may be largely such as to preserve the ionization state of key imidazole groups at cell surfaces.

This idea is suggestive of the much debated ‘imidazole alphastat hypothesis’ of Reeves (1972), but this does not refer specifically to cell-surface molecules other than those involved as sensors in pH homeostasis. An important issue here is whether the wide range of $\Delta pHa/\Delta T$ found in different species is compatible with a single optimum value of $\Delta pH/\Delta T$ at the hypothetical cell-surface sites. Regarding this, two points may be made. First, it is an important implication of this paper (see also Burton, 2001) that cell-surface pH is not uniquely related to pHa. Second, different studies on given species have sometimes yielded very diverse values for $\Delta pHa/\Delta T$, so that not all can be fully representative; Heisler (1986) discusses possible errors.

Just as temperature and pH may affect protein function, so too may salt concentration. Specific binding of Na^+ or Cl^- is sometimes involved, but more often relevant is screening of the protein fixed charges (Kao et al., 2000). A constant functional state of the protein may therefore be achieved, not only by matching pH to temperature, but by balancing pH and salt concentration. At given temperatures, negative correlations have been found between normal pHa and plasma $[Na^+]$ in both tetrapods and teleosts, but with pH generally higher in the fish for any given $[Na^+]$ (Burton, 1994, 1996). The proposed explanation for the correlations within either group (Burton, 1994) involves the maintenance of an optimum pH within a diffuse double layer that is associated with an excess of fixed negative charges on important, but unidentified, regions of cell membrane; these would attract protons, but be screened by other cations. If Na^+ and H^+ were the only significant cations involved, then the condition for surface pH to be invariant with bulk-phase extracellular $[Na^+]$ would be that local bulk-phase pH decreases with increasing $[Na^+]$, with $\Delta pH/\Delta\log[Na^+]=-1$. The relationships between pHa and plasma $[Na^+]$ within the tetrapods and within the teleosts each conform roughly to this value of –1, but need to be better quantified. The little-studied effects of salt concentration on the ionization equilibria of dissolved proteins, hence enzyme activity etc., can be much smaller (e.g. Burton, 1969; Kao et al., 2000), but proteins at the hypothetical sites may lie amongst separate fixed anionic groups such as phosphate. We return to the possible role of other cations in the Discussion.

Another reason for re-examining the pHa/$[Na^+]$ relationships is that Ultsch and Jackson (1996) failed to confirm them, although this was mainly because they were not then aware of the need to treat teleosts and tetrapods separately. What they did find is that pHa in freshwater teleosts and freshwater amphibians does tend to be higher, at a given temperature, than in marine or terrestrial species, which include teleosts, elasmobranchs and reptiles (in which $[Na^+]$ is generally higher). This suggested that a pHa/$[Na^+]$ relationship might at least apply to teleosts.

For a given temperature and $[Na^+]$, pHa seems to be approximately 0.2 unit higher in water-breathing teleosts, on average, than in air-breathing tetrapods (Burton, 1996). However, most cells are bathed by an interstitial fluid that should generally be more acid than arterial plasma. Thus, if the latter difference happens to be greater in water-breathers, the interstitial pH could actually be more nearly similar in the two groups than is pHa. In water-breathers, arterial and tissue CO_2 tensions (P_{CO_2}) tend to be much lower than in air-breathers (Rahn, 1966) (see below), so that CO_2 from the cells (or released from extracellular HCO_3^- by secreted acid) would have a larger proportionate effect on interstitial P_{CO_2}, thus enhancing the pH difference.

Thus put, the argument is simplistic, but more detailed modelling (Burton, 2001) suggests that the difference between interstitial and arterial pH for the whole body on average (DpH) could typically be approximately 0.1–0.2 unit more in water-breathing fish than in air-breathing tetrapods. Given certain assumptions, DpH may be estimated by relating CO_2 diffusion in one direction with O_2 diffusion in the other. The ratio of these diffusion rates, equal to the respiratory exchange ratio or respiratory quotient (R), depends on the relative solubilities (S) and diffusion coefficients (D) of the two gases and also on the tension differences between capillary blood and interstitial fluid. Relevant tensions are estimated from arterial and venous values (denoted by a and v respectively), with arbitrary low values assigned to the unknown mean interstitial P_{O_2}. For simplicity, P_{CO_2} and P_{O_2} are taken as changing uniformly along the capillaries. The model yields an estimate of mean interstitial P_{CO_2} and, hence, if the interstitial $[HCO_3^-]$ is modelled as being that of the arterial plasma, an estimate of mean DpH. This estimate, denoted D′pH, is given by:

$$D'pH = \log[A + (KRG)/Pa_{CO_2}], \quad (1)$$

where A is $[1+(Pv_{CO_2}/Pa_{CO_2})]/2$, K is $(S_{O_2}/S_{CO_2})\times(D_{O_2}/D_{CO_2})$ and G is the mean difference in P_{O_2} between interstitial fluid and capillary blood. For the data collated by Burton (2001), A is 1.01–1.40 and $(Pa_{O2}+Pv_{O2})/2$ varies from 16 to 81 mmHg with a mean of 51 mmHg (1 mmHg=0.133 kPa). How far the latter values match those for typical mean capillary P_{O_2}, as assumed for simplicity by Burton (2001), must depend on the oxygen dissociation curves, which are unavailable for most species. With interstitial fluid treated as a solution of NaCl, K is 0.036 at 15 °C and 0.040 at 25 °C (Burton, 2001). For plausible values of R and of mean capillary and interstitial P_{O_2}, representative values of KRG are thus likely to be approximately 1–3 mmHg. For any given value of KRG, equation 1 implies an inverse relationship between D′pH and Pa_{CO_2} that is steepest when Pa_{CO_2} is low, as it in water-breathers; DpH in air-breathing tetrapods should be generally lower than in water-breathers and less dependent on Pa_{CO_2}.

The assumption that $[HCO_3^-]$ is identical in interstitial fluid and arterial plasma implies that there is no net loss of acid or base equivalents from all the cells treated collectively. Net acid secretion, releasing CO_2 from interstitial HCO_3^-, would raise R and, hence, DpH. Then $[HCO_3^-]$ would become a factor in determining DpH, but the reduction in $[HCO_3^-]$ by the acid from the cells would be proportionately much less than the resulting elevation in P_{CO_2}. (In human extracellular fluid, for example, a 4 % reduction in $[HCO_3^-]$ from 25 to 24 mmol l^{-1} would nearly double P_{CO_2}.) Most tissues produce very much more CO_2 than titratable acid.

Adequate data on all the relevant variables in the model are available for few species (Burton, 2001), but the main determinant of D′pH is Pa_{CO_2}, and for this there are many more data. It is therefore appropriate to explore here the dependence of normal pHa, not just on $[Na^+]$, but on Pa_{CO_2} as well, without reference to variables for which data are scarce.

A correlation between pHa and Pa_{CO_2} is not demanded by the Henderson–Hasselbalch equation, for this relates pH not just to P_{CO_2} but to the $[HCO_3^-]/P_{CO_2}$ ratio. To consider data for 15 °C in Table 1 of Ultsch and Jackson (1996), in seven species of obligate water-breathing fish, Pa_{CO_2}, $[HCO_3^-]$ and pHa average 2.7±1.5 mmHg, 8.3±5.0 mmol l^{-1} and 7.91±0.06

respectively. In eight species of obligate air-breathing anphibians and reptiles, the respective means are 13.6±5.1 mmHg, 25.2±7.6 mmol l^{-1} and 7.80±0.14 (means ± S.D.). Given the variation in $P_{a_{CO_2}}$ and [HCO_3^-] within each group, it is not obvious, from these considerations alone, why these variables could not be matched to give the same pHa in every species.

There are several elements to the thesis of this paper: the existence of correlations amongst pHa, [Na^+] and $P_{a_{CO_2}}$; the undoubted effect of [Na^+] on pH in regions of fixed negative charge; the inevitable pH gradients between most interstitial fluid and arterial plasma that probably vary with $P_{a_{CO_2}}$; and the possibility that particular cell-surface structures require a similar local pH in all vertebrates. Although these ideas are integrated in a single quantitative thesis, they, like the multicomponent imidazole alphastat hypothesis, are not a single theory to be judged only as a whole.

Materials and methods

The nature of the data

In many vertebrates, pHa varies much more than in ourselves, even at constant temperature, and variability may increase in the context of particular normal activities and conditions. According to species, these might include, for example, diving, vigorous activity, hibernation and dehydration. Intermittent breathing, as in the turtle *Chelonia mydas*, causes pHa to fluctuate (Kraus and Jackson, 1980). Whether extracellular pH is usually more nearly optimal under non-stressful resting conditions or when special activity is called for is not known. Here, however, the pragmatic approach is adopted of using mean values obtained either in studies on the effect of temperature or for groups of animals used as experimental controls (not all of which actually purport to characterize normality). Two representative temperatures are emphasized here, 15 °C and 25 °C. For some species, pHa and $P_{a_{CO_2}}$ at one or other temperature were obtained by linear interpolation between measurements at other temperatures or from published regression equations. In other cases, data are used that were obtained within 3 °C of those two temperatures. Some data were read from graphs. All blood had been sampled from chronically implanted arterial cannulae.

The data vary in their suitability. Where several studies have been published, the most representative values were generally selected. Nevertheless, preference was usually given to sets in which all three variables were measured in the same study. In other cases, mean [Na^+] had to be taken from separate studies. Effects of temperature on [Na^+] are perforce largely ignored; in the steady state, these are generally small and inconsistent (Burton, 1986) so that uncertainties in this regard are unlikely to be of overriding importance here. (Errors in [Na^+] of 10 % result in errors of 0.04 in log[Na^+].) All species are represented for which full data were found; exclusion on the basis of too-strict criteria would increase both the risk of biased selection and the impact of any grossly inappropriate data that are included.

Data analysis and statistics

According to arguments in the Introduction, regulation of cell-surface pH implies regulation of pHa+N–DpH, where N is some function of [Na^+] (and possibly other cations also; see Discussion) and DpH is largely dependent on $P_{a_{CO_2}}$. The expression pHa+N–DpH should differ from the cell-surface pH by a constant amount which, with [Na^+] expressed in mmol l^{-1}, is likely to approach 4 units (see Discussion). On the assumption that the expression has a similar optimum in each species, analysis of the data on pHa, [Na^+] and $P_{a_{CO_2}}$ involves finding parameters relating to N and DpH that minimize variations in that expression. For N, a convenient function is log([Na^+]+n), where n is the parameter in question. This function was chosen because it is simple and because it can be interpreted in terms of screening by other cations (see Discussion). Since data on $P_{a_{CO_2}}$ alone do not suffice to calculate either DpH or D′pH, DpH is replaced by a new variable, D″pH. Like D′pH, this is based on equation 1, but it is a function only of $P_{a_{CO_2}}$ with all the other variables subsumed within three empirical parameters. Of these, m replaces KRG, so that D″pH is defined as $\log[A+(m/P_{a_{CO_2}})]$. The other two parameters relate A to $P_{a_{CO_2}}$ (see Results).

Since the parameters to be estimated are contained within log([Na^+]+n) and D″pH, multiple regression analysis cannot be used. In any case, no one variable is clearly 'dependent' in this evolutionary context: D″pH and pHa together determine surface pH (at a given [Na^+]) and need to be matched one to the other.

Irrespective of any basis in theory, log([Na^+]+n) and D″pH may be seen as convenient functions of [Na^+] and $P_{a_{CO_2}}$ that yield approximately linear relationships in the exploration of correlations. Analysis of the data in terms of these therefore obviates the need for more straightforward correlational analysis of untransformed data.

Because the heterogeneous data are unsuited to the calculation of statistical significance, correlation coefficients (r), of 0.6 or higher, are stated just for their descriptive value. Within some classes, e.g. the Amphibia, the small numbers of data and narrow ranges of [Na^+] and $P_{a_{CO_2}}$ make significant correlations unlikely.

Mean values taken from the literature are presented without indications of scatter. Other means are given ± 1 S.D.

Results

The data

Tables 1 and 2 show mean values of pHa and plasma [Na^+], and in most cases $P_{a_{CO_2}}$, at approximately 25 °C (34 data sets) and approximately 15 °C (30 data sets). Some data need special comment. Of those on [Na^+] in reptiles taken from the compilation of Minnich (1979), five are means of two or more of his tabulated values. For *Oncorhynchus mykiss* (Table 2), pHa and $P_{a_{CO_2}}$ are means based on 34 studies (Ultsch and Jackson, 1996), while [Na^+] is a mean based on five studies (Burton, 1996). The pHa of 7.82 given for *Bufo marinus* at 25 °C (Boutilier et al., 1987) is much higher than the 7.54

Table 1. *Plasma [Na^+], arterial pH (pHa) and arterial P_{CO_2} in vertebrates at approximately 25 °C*

Species	[Na^+] (mmol l^{-1})	pHa	Pa_{CO_2} (mmHg)	References
Alligator mississippiensis	141	7.50	17.5	Davies et al. (1982); Minnich (1979)
Iguana iguana	157	7.60	–	Wood and Moberly (1970); Minnich (1979)
Dipsosaurus dorsalis	164	7.69	14	Bickler (1981, 1984)
Sauromalus obesus	179	7.38	23	Crawford and Gatz (1974); Minnich (1979)
Varanus spp.	155	7.57	21.3	See text
Chelonia mydas	162	7.50	30.1	Kraus and Jackson (1980); Minnich (1979)
Caretta caretta (23.5 °C)	166	7.63	17.2	Lutcavage and Lutz (1991); Minnich (1979)
Trachemys scripta elegans	120	7.62	27.3	Jackson et al. (1974); Minnich (1979)
Chrysemys picta bellii	119	7.68	27	Glass et al. (1985); Minnich (1979)
Chelydra serpentina	135	7.69	32.5	Howell et al. (1970); Minnich (1979)
Gopherus polyphemus	137	7.61	22.9	Ultsch and Jackson (1996); Minnich (1979)
Coluber constrictor	156	7.55	19.7	Stinner et al. (1998)
Nerodia sipedon	159	7.61	–	Dean and Gratz (1983); Minnich (1979)
Pituophis melanoleucus	171	7.61	–	Stinner (1982); Minnich (1979)
Lapemis hardwickii	237	7.41	–	Seymour and Webster (1975); see text
Necturus maculatus	93	7.69	7.2	Stiffler et al. (1983)
Ambystoma tigrinum, adult	109	7.72	10.5	Stiffler (1991)
Siren lacertina	105	7.79	11.7	Heisler et al. (1982)
Amphiuma means	106	7.74	17.3	Heisler et al. (1982)
Cryptobranchus alleganiensis	89	7.75	7.2	Moalli et al. (1981)
Bufo marinus	111	7.82	10	Boutilier et al. (1987); Stinner and Hartzler (2000)
Bufo viridis	129	7.68	12.0	Katz (1980); Gordon (1962)
Rana catesbeiana	100	7.81	15.1	Howell et al. (1970); Stinner and Hartzler (2000)
Protopterus aethiopicus	101	7.60	26.4	DeLaney et al. (1977)
Lepisosteus platostomus	155	7.67	8.1	Smatresk and Cameron (1982); Urist et al. (1972)
Lepisosteus oculatus	154	7.73	6.8	Burleson et al. (1998); see text
Amia calva (24.5 °C)	138	7.77	5.5	Randall et al. (1981); Urist et al. (1972)
Electrophorus electricus (28 °C)	172	7.58	28.6	Garey and Rahn (1970); Mangum et al. (1978)
Ictalurus punctatus	142	7.79	2.8	Cameron and Kormanik (1982); Cameron (1980)
Cynoscion arenarius	219	7.74	2.5	Cameron (1978); Sulya et al. (1960)
Cyprinus carpio	131	7.95	3.9	Takeda (1990); Ultsch et al. (1981)
Negaprion brevirostris	307	7.72	–	Bushnell et al. (1982); Oppelt et al. (1966)
Scyliorhinus stellaris, juvenile (23 °C)	284	7.70	1.3	Heisler et al. (1976); see text
Scyliorhinus stellaris, adult (23 °C)	284	7.77	2.9	Heisler et al. (1976); see text

Where there are two references on a line, the first is for pHa and Pa_{CO_2} and the second is for [Na^+].
Temperatures other than 25 °C are as indicated.
1 mmHg=0.133 kPa.

plotted by Ultsch and Jackson (1996), but close to their tabulated mean of 7.814 based on 11 studies.

For *Dipsosaurus dorsalis* at 25 °C, Burton (1994) inappropriately plotted a mean venous pH (7.53) instead of the arterial mean of 7.69 (Bickler, 1981). Ultsch and Jackson (1996) used a much higher pHa of 7.87, since a value for [Na^+] (164 mmol l^{-1}), typical of normal values tabulated previously (Minnich, 1979), was available from the same source (Bickler, 1984). The most appropriate of those means is probably 7.69 (P. E. Bickler, personal communication), and that is the value in Table 1. There is some diurnal variation in this species (Bickler, 1986).

For '*Varanus* spp.', the pHa and Pa_{CO_2} are means for *V. niloticus* (Ishimatsu et al., 1988), *V. salvator* (Mitchell and Gleeson, 1985) and *V. exanthematicus* (Wood et al., 1981), all individually similar, and the value for [Na^+] is the mean of five separate means for *V. griseus* and *V. gouldii* (Minnich, 1979). In four other cases, [Na^+] is again taken as the mean for one or more related species. Thus, for the sea snake *Lapemis hardwickii* in Table 1, [Na^+] is for the closely related *Pelamis platurus*. Here, the combination of low pHa (7.41) and high [Na^+] (237 mmol l^{-1}, the mean of two means given previously) (Minnich, 1979) would be especially worth confirming for either of the species. Seymour and Webster (1975) give data on Pa_{CO_2} in *Lapemis hardwickii*, but no mean for use here. For *Lepisosteus oculatus* (Table 1), [Na^+] is the mean for four other species of *Lepisosteus* (Urist et al., 1972; Sulya et al., 1960). For *Myoxocephalus octodecimspinosus* (Table 2), [Na^+] is for the related *M. spinosus* (Oikari, 1975). For *Scyliorhinus stellaris* in both tables, there are separate entries for juveniles and adults (Heisler et al., 1976) since they differ markedly in their patterns of acid–base regulation; [Na^+] for

Table 2. *Plasma [Na^+], arterial pH (pHa) and arterial* P_{CO_2} *in vertebrates at approximately 15 °C*

Species	[Na^+] (mmol l^{-1})	pHa	Pa_{CO_2} (mmHg)	References
Alligator mississippiensis	141	7.68	11.8	Davies et al. (1982); Minnich (1979)
Dipsosaurus dorsalis (17.5 °C)	164	7.80	10	Bickler (1981, 1984)
Sauromalus obesus (16 °C)	179	7.54	11	Crawford and Gatz (1974); Minnich (1979)
Chelonia mydas	162	7.55	25.3	Kraus and Jackson (1980); Minnich (1979)
Trachemys scripta elegans	120	7.72	18.4	Jackson et al. (1974); Minnich (1979)
Chrysemys picta bellii	119	7.79	19.8	Glass et al. (1985); Minnich (1979)
Coluber constrictor	156	7.57	16.2	Stinner et al. (1998)
Amphiuma means	106	7.79	8.5	Hicks and Stiffler (1984); Heisler et al. (1982)
Cryptobranchus alleganiensis	96	7.90	4.2	Moalli et al. (1981)
Bufo marinus	103	7.96	7	Boutilier et al. (1987); Stinner and Hartzler (2000)
Rana catesbeiana	98	7.96	10.2	Howell et al. (1970); Stinner and Hartzler (2000)
Acipenser baeri	119	7.86	2.7	Nonnotte et al. (1993)
Amia calva	131	7.94	2.5	Gonzalez et al. (2001)
Ictalurus punctatus	142	7.92	1.9	Cameron and Kormanik (1982); Cameron (1980)
Cynoscion arenarius (18 °C)	219	7.83	2.1	Cameron (1978); Sulya et al. (1960)
Cyprinus carpio	131	7.86	3.5	Ultsch et al. (1981)
Catostomus commersoni (14 °C)	115	7.96	2.4	Wilkes et al. (1981)
Tinca tinca	130	7.98	2.7	Jensen and Weber (1987)
Myoxocephalus octodecimspinosus (14 °C)	182	7.78	2.0	Claiborne and Evans (1988); see text
Conger conger (17 °C)	182	7.84	2.0	Toews et al. (1982)
Platichthys flesus	165	7.79	2.3	Nonnotte and Truchot (1990)
Scophthalmus maximus	163	7.84	2.3	Gaumet et al. (1994)
Oncorhynchus mykiss	146	7.87	2.6	See text
Salmo trutta	148	7.85	1.5	Butler and Day (1993); Gordon (1959)
Salmo salar (12 °C)	176	7.94	2.2	Maxime et al. (1990)
Squalus acanthias (17.5 °C)	240	7.87	2.1	Claiborne and Evans (1992); Robin et al. (1964)
Scyliorhinus canicula	284	7.84	0.55	Truchot et al. (1980); Tierney et al. (1998)
Scyliorhinus stellaris, juvenile	284	7.82	1.1	Heisler et al. (1976); see text
Scyliorhinus stellaris, adult	284	7.88	1.5	Heisler et al. (1976); see text
Eptatretus cirrhatus (16 °C)	544	7.92	0.7	Wells et al. (1986); Urist and Van de Putte (1967)

Where there are two references on a line, the first is for pHa and Pa_{CO_2} and the second is for [Na^+].
Temperatures other than 15 °C are as indicated.
1 mmHg=0.133 kPa.

both is taken as that for *S. canicula*. The *Amia calva* of Table 1 rarely breathed air at 24.5 °C (Randall et al., 1981).

Analysis of interspecific correlations

Fig. 1 confirms the existence of the disputed negative correlation between pHa and [Na^+] (plotted as log[Na^+]) in tetrapods at approximately 25 °C. Two air-breathing fish (*Protopterus aethiopicus* and *Electrophorus electricus*), both with high Pa_{CO_2}, can be seen as conforming to the same relationship, but for most of the other fish pHa is higher for a given log[Na^+]. For water-breathing fish, Fig. 1 shows no correlation between pHa and log[Na^+] at approximately 25 °C; as shown below, this relates to the large variation in Pa_{CO_2}. Data for approximately 15 °C (Table 2) confirm the negative correlations between pHa and log[Na^+] in teleosts (r=–0.61) as well as in the ectothermic tetrapods (r=–0.86). However, these data are not presented here as in Fig. 1 because the separate correlations are better unified by taking Pa_{CO_2} into account.

The next stage in the analysis concerns A in equation 1. *Eptatretus cirrhatus* (Wells et al., 1986) is of special interest for its high [Na^+] and low Pa_{CO_2} (Table 2). The data for this species yield unusually high values of A (1.36) and of D′pH (0.52, with interstitial P_{O_2} taken as 7 mmHg). With data for *E. cirrhatus* included with those for the 22 species tabulated by Burton (2001), the regression of A on logPa_{CO_2} (r=–0.54) is given by the equation:

$$A = 1.24 - 0.13 \log Pa_{CO_2} . \quad (2)$$

This relationship is assumed in the subsequent data analysis. That it is inexact is unimportant because A has much less influence on D″pH than does m/Pa_{CO_2}.

With A thus calculated, the parameters n and m were obtained by trial and error so as to minimize the standard deviation of pHa+log([Na^+]+n)–D″pH at each temperature. This process suggested that m should be 2.3 mmHg at 25 °C and 1.2 mmHg at 15 °C, with n at the two temperatures being respectively 94 and 70 mmol l^{-1}. Since an intermediate n of 80 mmol l^{-1} raises the standard deviation only beyond the third

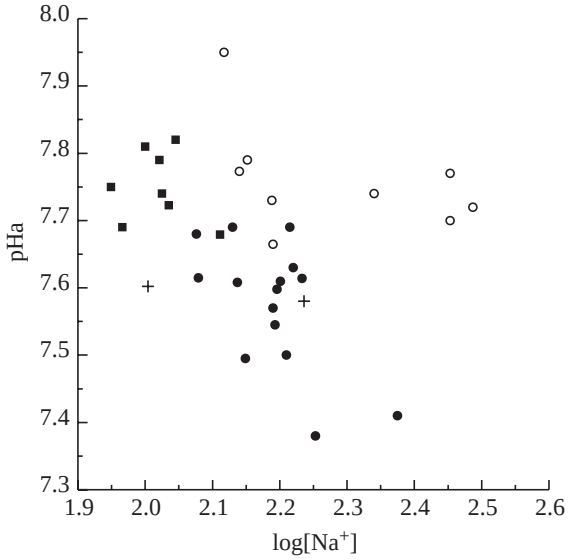

Fig. 1. Arterial pH (pHa) at 25 °C *versus* log[Na^+] for reptiles (●), amphibians (■), water-breathing fish including *Amia calva* (○) and air-breathing fish (+).

decimal place, D″pH is hereafter as calculated using that single value of *n*, together with the two values of *m*. The expression pHa+log([Na^+]+80)–D″pH then averages 9.89±0.08 at 25 °C and 10.00±0.08 at 15 °C, a difference of –0.01 °C^{-1}. As already noted, the relevant surface pH is probably lower than these means by nearly 4 units.

The expression pHa–D″pH may be seen as an estimate of mean interstitial pH. Fig. 2 shows this plotted against log[Na^+]. The curves conform to the results of the previous paragraph. At 25 °C, *r*=–0.65 in the tetrapods and *r*=–0.72 in the fish. At 15 °C, the corresponding values are *r*=–0.84 and *r*=–0.74, respectively, with these correlations discernible in both the reptiles alone (*r*=–0.70) and the teleosts alone (*r*=–0.65). Thus, the overall correlations seem not to result merely from the merging of disparate data sets.

Fig. 3 shows pHa+log([Na^+]+80) plotted against D″pH, the line through the points being of gradient +1. Since D″pH is an inverse function of Pa_{CO_2}, Fig. 3 shows that pHa, adjusted for variations in [Na^+], is negatively correlated with Pa_{CO_2}. For all fish, *r*=0.82 at 25 °C and *r*=0.72 at 15 °C. For the tetrapods at 15 °C, *r*=0.60.

Figs 2 and 3 show that pHa depends separately on both [Na^+] and D″pH. In Fig. 4, these two variables are combined as log([Na^+]+80)–D″pH. The negative correlation between pHa and that expression is evident within the reptiles (*r*=–0.61 at 25 °C; *r*=–0.77 at 15 °C) as well as in the tetrapods collectively (*r*=–0.75 at 25 °C; *r*=–0.87 at 15 °C). At 25 °C, *r*=–0.84 for the teleosts and –0.64 for all the fish. (*Eptatretus cirrhatus* is a notable outlier at 15 °C.) For all data taken together, *r*=–0.74 at 25 °C and *r*=–0.65 at 15 °C.

The analysis has been in terms of equations 1 and 2, but the correlations amongst pHa, Pa_{CO_2} and [Na^+] may also be expressed using functions other than D″pH and log([Na^+]+*n*). It would be surprising if multiple regression analysis, coupled

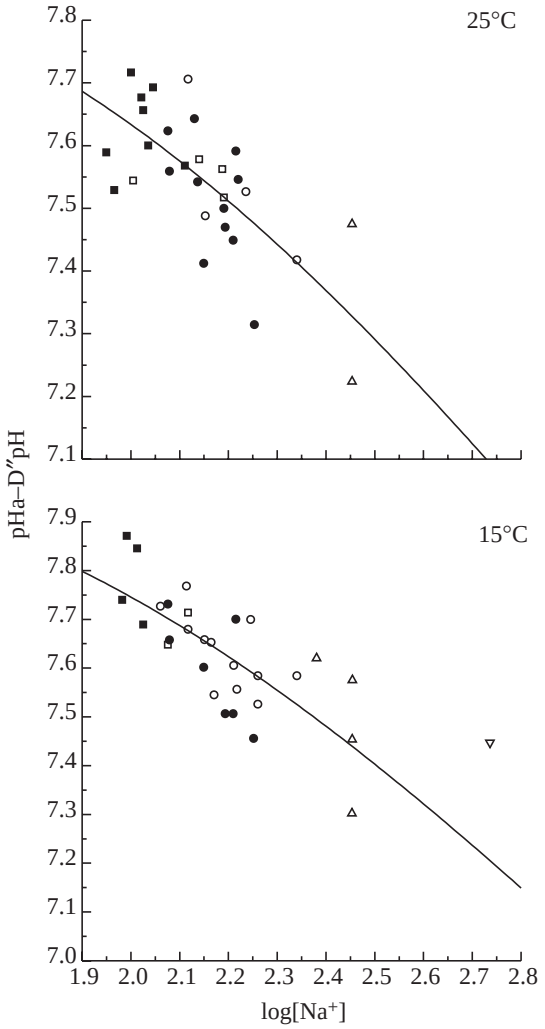

Fig. 2. (pHa–D″pH) (see Materials and methods) *versus* log[Na^+] at 25 and 15 °C for reptiles (●), amphibians (■), teleosts (○), elasmobranchs (△), *Eptatretus cirrhatus* (▽) and other fish (□). The curves are such that pHa+log([Na^+]+80)–D″pH equals 9.89 at 25 °C and 10.00 at 15 °C.

with a free choice of such functions, did not yield enhanced correlations. One expression correlating better with pHa than does log([Na^+]+80)–D″pH is log[Na^+]+*k*logPa_{CO_2}, where *k* is 0.51 at 25 °C and 0.58 at 15 °C. The correlation coefficients are –0.77 at 25 °C and –0.76 at 15 °C (compare the values of –0.74 and –0.65 in the previous paragraph). The expression *k*logPa_{CO_2} has no obvious functional meaning here.

The analysis of all these correlations is simplified by the fact that there is little correlation between Pa_{CO_2} and [Na^+] at either temperature, except that Pa_{CO_2} is low and [Na^+] high in elasmobranchs and *Eptatretus cirrhatus*.

Discussion

Functional interpretation

The correlations accord with the hypothesis that the pH at particular cell surface sites is normally more nearly similar in

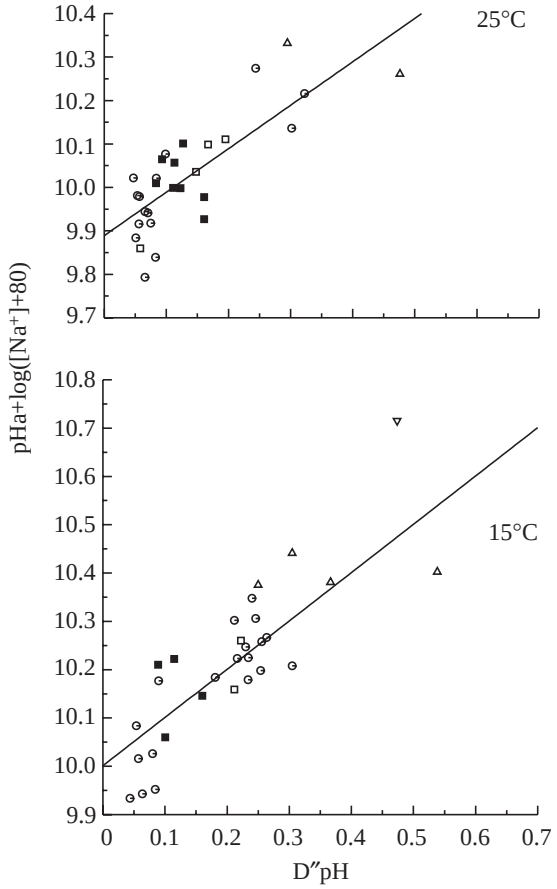

Fig. 3. pHa+log([Na^+]+80) and D″pH (see Materials and methods) at 25 and 15 °C: symbols are as in Fig. 2. The lines, of gradient +1, conform to the relationships given in Fig. 2.

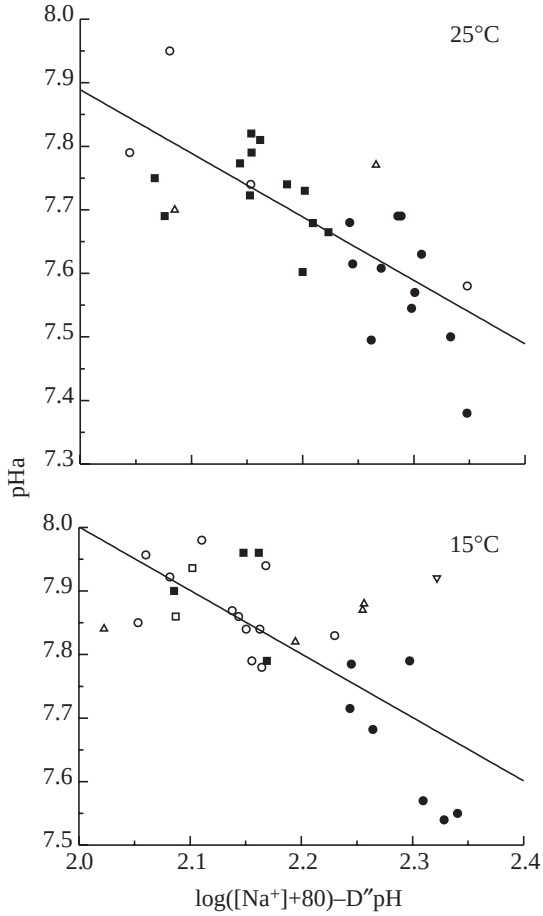

Fig. 4. Arterial pH (pHa) and log([Na^+]+80)–D″pH (see Materials and methods) at 25 and 15 °C: symbols are as in Fig. 2. The lines, of gradient –1, conform to the relationships given in Fig. 2.

different species (at a given temperature) than is pHa. It is a noteworthy test of the hypothesis both that the two estimates of *m*, i.e. 2.3 mmHg and 1.2 mmHg, correspond to the likely values for *KRG* of 1–3 mmHg given in the Introduction and that the data points in Fig. 3 are compatible with a straight line of gradient 1.0. Within their limitations, the data therefore accord also with the model of Burton (2001). That model was originally formulated in terms of whole-body mean interstitial pH, since most venous data on gas tensions are for mixed venous blood. In this paper, venous gas tensions are relevant only to *A*, which is a minor determinant of D″pH. It is therefore permissible to interpret *R* and *G* in terms of particular tissues, e.g. those deemed most likely to require good extracellular pH homeostasis.

Although the scatter of points in Figs 2–4 must be due largely to inadequacies in the data, it is also required by the theoretical model. Thus, there are other variables in equation 1, notably *G*, that, for lack of sufficient data, have not been considered here.

If the cell-surface sites bear a constant density of fixed negative charges, then surface pH would be raised by the screening effect of cations in the bulk interstitial fluid. If Na^+ were the only cation present other than H^+, the effect would be proportional to log[Na^+] (see Introduction). In fact, K^+, Ca^{2+} and Mg^{2+} would contribute to screening too, to an extent that depends on the concentrations of their free ions and on the fixed charge density at the cell surface (Burton, 1973a,b). That interspecific variations in [Ca^{2+}] and [Mg^{2+}] do not obscure the supposed effect of [Na^+] could result from a sufficiently low fixed charge density together with the known interspecific correlation between [Ca^{2+}] and [Na^+] in vertebrate plasma (Burton, 1973a). However, screening by [Ca^{2+}] and [Mg^{2+}] might explain why *n* in the expression log([Na^+]+*n*) is not zero, but approximately 80 mmol l^{-1}. Only an approximate treatment is possible here, since the geometry of the supposed structures is unknown and full data on free [K^+], [Ca^{2+}] and [Mg^{2+}] in plasma are lacking. However, we may apply diffuse double-layer (Gouy–Chapman) theory to other data. Burton (1973a) gives an approximate form of the Grahame equation relating the surface potential in volts, Ψ, to the molar concentrations of the four cations and to σ, the density of fixed electronic charges per nm^2 on the cell surface. In terms appropriate to the present context, it may be re-written as:

$$\exp(F\Psi/\boldsymbol{R}T) = Q/S, \qquad (3)$$

where F is the Faraday constant and $\boldsymbol{R}$ is now the gas constant, and where:

$$Q = [Na^+] + [K^+] + \sqrt{([Na^+] + [K^+])^2 + 2S([Ca^{2+}] + [Mg^{2+}])}, \quad (4)$$

and

$$S = 4[Na^+] + 4[K^+] + 6[Ca^{2+}] + 6[Mg^{2+}] + 2(2.72\sigma)^2. \quad (5)$$

Table 1 of Burton (1973a) gives data for 10 species in which mean plasma $[Na^+]$ is 0.087–0.544 mol l^{-1}, and for these it is possible to find a value of σ that makes ($[Na^+]$+0.08) (in mol l^{-1}) a good approximation on average for $Q/2$. The data used in these calculations are for diffusible $[Ca^{2+}]$ or, where possible, free $[Ca^{2+}]$, with concentrations of free Mg^{2+} taken as 70% of the total concentrations (this being the mean percentage for Ca^{2+}). The required value of σ is approximately 0.8 electronic charges nm^{-2}. This is plausible (Burton, 1973a). For the 10 species in question, the corresponding surface potentials are –56 to –86 mV. With estimates of log$S$ being 1.00–1.08, with log(2/[1000 mmol mol^{-1}]) being 2.70 and with the mean of pHa+log($[Na^+]$+80)–D″pH being 9.89 at 25 °C and 10.00 at 15 °C (see above), the Boltzmann equation (Burton, 1973b) implies surface pH values near 6.2.

Is there a common determinant of optimum pHa?

It now seems at least plausible that there exists a common determinant of optimum pHa in ectothermic vertebrates, namely a cell-surface structure with a conserved requirement for surface pH. Minor variations in the optimum cannot be ruled out, but there is no evidence in the present data for consistent differences amongst reptiles, amphibians and fish. Regarding mammals, our own pHa is typical of reptiles at the same temperature that share our plasma $[Na^+]$ of approximately 145 mmol l^{-1} (Burton, 1994). The human Pa_{CO_2} of 40 mmHg is higher than that typical of reptiles at 37 °C, namely approximately 29 mmHg as calculated from a regression equation for reptiles given by Ultsch and Jackson (1996). Accordingly, D″pH would be approximately 0.02 higher in reptiles than in man. This is small enough not to belie our own conformity to reptilian data.

According to this conserved determinant hypothesis, optimal (and normal) pHa has evolved and diversified in such a way as to maintain constant the pH-dependent functional properties of key molecular structures (probably proteins or protein complexes). These are sited on cell surfaces bathed by an interstitial fluid that is more acid than arterial plasma – and especially so in species with low Pa_{CO_2}. The pH-dependent functional properties are also affected by temperature and by the screening action of other cations in the extracellular fluid. The plasma $[Na^+]$ typical of each species seems to be determined independently; it is generally close to the concentration needed to minimize the work of Na^+ transport while remaining compatible, so it seems, with an optimum range of cell $[K^+]$ (Burton, 1973b). Because DpH may be temperature-dependent, the hypothesis does not require ΔpHa/ΔT to be the same in all species.

The cells bearing the relevant molecular structures are probably not the same as those acting as pH sensors in extracellular pH homeostasis. The latter would presumably evolve in such a way as to regulate pH appropriately, but both their evolution and their moment-to-moment efficacy would be aided if they showed similar sensitivities to temperature and salt concentration. How far the interspecific pHa/$[Na^+]$ relationship is maintained in individuals when $[Na^+]$ varies has yet to be properly explored (Burton, 1996); there may be an advantage to this, but no logical necessity.

Are there alternative interpretations?

The pH must generally be lower in interstitial fluid than in arterial blood, and lower still in those diffuse double layers that are associated with excesses of fixed negative charge. Nevertheless, it is important to seek alternative explanations for the correlations explored here. Thus, the apparent antagonism between H^+ and Na^+ might involve, not screening, but a straightforward competition for binding to specific protein sites. If only those two ionic species were involved, the proportion of protonated sites might be expected to be proportional to the ratio of $[H^+]$ to $[Na^+]$ in the interstitial fluid; Δ(pHa–D″pH)/Δlog$[Na^+]$ should then be –1.0. Since n is not zero, it would seem necessary to postulate binding of one or more other ionic species. A quite different possibility is that Cl^-, not Na^+, is relevant. Since Cl^- is the main anion balancing the charge on Na^+ in plasma, the concentrations of the two ions in plasma are strongly correlated within the vertebrates.

It should also be questioned whether attention is correctly focused on P_{CO_2}, rather than on $[HCO_3^-]$ or $[CO_3^{2-}]$ (or even on ion pairs involving one or other of these). Since $[HCO_3^-]$ and $[CO_3^{2-}]$, at a given temperature and salt concentration, may be calculated from pH and P_{CO_2} (with appropriate equilibrium constants and CO_2 solubility coefficient), correlations must exist amongst other sets of these variables than the trio investigated (e.g. $[Na^+]$ and $[HCO_3^-]$ with either pHa or Pa_{CO_2}). Whether a more direct functional basis exists for any of these is an open question.

Future research

Since the validity of some data sets in Tables 1 and 2 may be questioned (especially when $[Na^+]$ and acid–base variables are from separate sources), one may hope that more and better data will be obtained, especially for species in which the variables have extreme values. Ideally, all variables relevant to the model should be measured, including R and both arterial and venous gas tensions. This could prove revealing in relation to the changes in maturing *Scyliorhinus stellaris* (Heisler et al., 1976). The possibility of acid–base disequilibrium and variations in D_{O_2}/D_{CO_2} should be considered (Burton, 2001). Since all animals should ideally be studied unstressed and under conditions corresponding as far as possible to their typical habitats and preferred temperature ranges, a unified survey of many species would be ambitious. More realistically, one may urge that plasma $[Na^+]$ and P_{O_2} be more routinely measured in acid–base studies.

Equation 1 was originally formulated in terms of mixed venous blood and whole-body mean D′pH. However, if extracellular pH homeostasis is assumed to be more important to particular tissues, it may be preferable to calculate D′pH just for these.

One component of the overall conserved determinant hypothesis may be tested more directly than through correlational studies. This is the predicted greater DpH in species with low $P_{a_{CO_2}}$, i.e. in water-breathers. Most studies so far have been on mammals (Burton, 2001). The most decisive insight could emerge, however, through the identification of a conserved cell-surface structure with appropriate local charge density and appropriate sensitivites to pH and temperature.

References

Bhattacharya, S. and Lecomte, J. T. J. (1997). Temperature dependence of histidine ionization constants in myoglobin. *Biophys. J.* **73**, 3241–3256.

Bickler, P. E. (1981). Effects of temperature on acid–base balance and ventilation in desert iguanas. *J. Appl. Physiol.* **51**, 452–460.

Bickler, P. E. (1984). Effects of temperature on acid and base excretion in a lizard, *Dipsosaurus dorsalis*. *J. Comp. Physiol. B* **154**, 97–104.

Bickler, P. E. (1986). Day–night variations in blood and intracellular pH in a lizard, *Dipsosaurus dorsalis*. *J. Comp. Physiol. B* **156**, 853–857.

Boutilier, R. G., Glass, M. L. and Heisler, N. (1987). Blood gases and extracellular/intracellular acid–base status as a function of temperature in the anuran amphibians *Xenopus laevis* and *Bufo marinus*. *J. Exp. Biol.* **130**, 13–25.

Burleson, M. L., Shipman, B. N. and Smatresk, N. J. (1998). Ventilation and acid–base recovery following exhausting activity in an air-breathing fish. *J. Exp. Biol.* **201**, 1359–1368.

Burton, R. F. (1969). Buffers in the blood of the snail, *Helix pomatia* L. *Comp. Biochem. Physiol.* **29**, 919–930.

Burton, R. F. (1973a). The balance of cations in the plasma of vertebrates and its significance in relation to the properties of cell membranes. *Comp. Biochem. Physiol.* **44A**, 781–792.

Burton, R. F. (1973b). The significance of ionic concentrations in the internal media of animals. *Biol. Rev.* **48**, 195–231.

Burton, R. F. (1986). Ionic regulation in fish: the influence of acclimation temperature on plasma composition and apparent set points. *Comp. Biochem. Physiol.* **85A**, 23–28.

Burton, R. F. (1994). The dependence of normal plasma pH on sodium concentration in amphibians, reptiles and man. *Comp. Biochem. Physiol.* **108A**, 1–5.

Burton, R. F. (1996). The dependence of normal arterial blood pH on sodium concentration in teleost fish. *Comp. Biochem. Physiol* **114A**, 111–116.

Burton, R. F. (2001). Differences in pH between interstitial fluid and arterial blood in water-breathing and air-breathing vertebrates. *Physiol. Biochem. Zool.* **74**, 607–615.

Bushnell, P. G., Lutz, P. L., Steffensen, J. F., Oikari, A. and Gruber, S. H. (1982). Increases in arterial blood oxygen during exercise in the lemon shark (*Negaprion brevirostris*). *J. Comp. Physiol.* **147**, 41–47.

Butler, P. J. and Day, N. (1993). The relationship between intracellular pH and swimming performance of brown trout exposed to neutral and sublethal pH. *J. Exp. Biol.* **176**, 271–284.

Cameron, J. N. (1978). Regulation of blood pH in teleost fish. *Respir. Physiol.* **33**, 129–144.

Cameron, J. N. (1980). Body fluid pools, kidney function and acid–base regulation in the freshwater catfish *Ictalurus punctatus*. *J. Exp. Biol.* **86**, 171–185.

Cameron, J. N. and Kormanik, G. A. (1982). Intracellular and extracellular acid–base status as a function of temperature in the freshwater channel catfish, *Ictalurus punctatus*. *J. Exp. Biol.* **99**, 127–142.

Claiborne, J. B. and Evans, D. H. (1988). Ammonia and acid–base balance during high ammonia exposure in a marine teleost (*Myoxocephalus octodecimspinosus*). *J. Exp. Biol.* **140**, 89–105.

Claiborne, J. B. and Evans, D. H. (1992). Acid–base balance and ion transfers in the spiny dogfish (*Squalus acanthias*) during hypercapnia: a role for ammonia excretion. *J. Exp. Zool.* **261**, 9–17.

Covington, A. K., Bates, R. G. and Durst, R. A. (1985). Definition of pH scales, standard reference values, measurement of pH and related terminology (Recommendations 1984). *Pure Appl. Chem.* **57**, 531–542.

Crawford, E. C. and Gatz, R. N. (1974). Carbon dioxide tension and pH of the blood of the lizard *Sauromalus obesus* at different temperatures. *Comp. Biochem. Physiol.* **47A**, 529–534.

Davies, D. G., Thomas, J. L. and Smith, E. N. (1982). Effect of body temperature on ventilatory control in the alligator. *J. Appl. Physiol.* **52**, 114–118.

Dean, J. B. and Gratz, R. K. (1983). The effect of body temperature and CO_2 breathing on ventilation and acid–base status in the northern water snake *Nerodia sipedon*. *Physiol. Zool.* **56**, 290–301.

DeLaney, R. G., Lahiri, S., Hamilton, R. and Fishman, A. P. (1977). Acid–base balance and plasma composition in the aestivating lungfish (*Protopterus*). *Am. J. Physiol.* **232**, R10–R17.

Garey, W. F. and Rahn, H. (1970). Normal arterial gas tensions and pH and the breathing frequency of the electric eel. *Respir. Physiol.* **9**, 141–150.

Gaumet, F., Boeuf, G., Truchot, J.-P. and Nonnotte, G. (1994). Effects of environmental water salinity on blood acid–base status in juvenile turbot (*Scophthalmus maximus* L.). *Comp. Biochem. Physiol.* **109A**, 985–994.

Glass, M. L., Boutilier, R. G. and Heisler, N. (1985). Effects of body temperature on respiration, blood gases and acid–base status in the turtle *Chrysemys picta bellii*. *J. Exp. Biol.* **114**, 37–51.

Gonzalez, R. J., Milligan, L., Pagnotta, A. and McDonald, D. G. (2001). Effect of air breathing on acid–base and ion regulation after exhaustive exercise and during low pH exposure in the bowfin, *Amia calva*. *Physiol. Biochem. Zool.* **74**, 502–509.

Gordon, M. S. (1959). Ionic regulation in the brown trout (*Salmo trutta* L.). *J. Exp. Biol.* **36**, 227–252.

Gordon, M. S. (1962). Osmotic regulation in the green toad (*Bufo viridis*). *J. Exp. Biol.* **39**, 261–270.

Heisler, N. (1986). Comparative aspects of acid–base regulation. In *Acid–Base Regulation in Animals* (ed. N. Heisler), pp. 397–450. Amsterdam: Elsevier.

Heisler, N., Forcht, G., Ultsch, G. R. and Anderson, J. F. (1982). Acid–base regulation in response to environmental hypercapnia in two aquatic salamanders, *Siren lacertina* and *Amphiuma means*. *Respir. Physiol.* **49**, 141–158.

Heisler, N., Weitz, H. and Weitz, A. M. (1976). Extracellular and intracellular pH with changes of temperature in the dogfish *Scyliorhinus stellaris*. *Respir. Physiol.* **26**, 249–263.

Hicks, G. H. and Stiffler, D. F. (1984). Patterns of acid–base regulation in urodele amphibians in response to variations in temperature. *Comp. Biochem. Physiol.* **77A**, 693–697.

Howell, B. J., Baumgardner, F. W., Bondi, K. and Rahn, H. (1970). Acid–base balance in cold-blooded vertebrates as a function of body temperature. *Am. J. Physiol.* **218**, 600–606.

Ishimatsu, A., Hicks, J. W. and Heisler, N. (1988). Analysis of intracardiac shunting in the lizard, *Varanus niloticus*: a new model based on blood oxygen levels and microsphere distribution. *Respir. Physiol.* **71**, 83–100.

Jackson, D. C., Palmer, S. E. and Meadow, W. L. (1974). The effects of temperature and carbon dioxide breathing on ventilation and acid–base status of turtles. *Respir. Physiol.* **20**, 131–146.

Jensen, F. B. and Weber, R. E. (1987). Internal hypoxia–hypercapnia in tench exposed to aluminium in acid water: effects on blood gas transport, acid–base status and electrolyte composition in arterial blood. *J. Exp. Biol.* **127**, 427–442.

Kao, Y.-H., Fitch, C. A., Bhattacharya, S., Sarkisian, C. J., Lecomte, J. T. J. and Garcia-Moreno, B. (2000). Salt effects on ionization equilibria of histidines in myoglobin. *Biophys. J.* **79**, 1637–1654.

Katz, U. (1980). Acid–base status in the toad *Bufo viridis in vivo*. *Respir. Physiol.* **41**, 105–115.

Kraus, D. R. and Jackson, D. C. (1980). Temperature effects on ventilation and acid–base balance of the green turtle. *Am. J. Physiol.* **239**, R254–R258.

Lutcavage, M. E. and Lutz, P. L. (1991). Voluntary diving metabolism and ventilation in the loggerhead sea turtle. *J. Exp. Mar. Biol. Ecol.* **147**, 287–296.

Mangum, C. P., Haswell, M. S., Johansen, K. and Towle, D. W. (1978). Inorganic ions and pH in the body fluids of Amazon animals. *Can. J. Zool.* **56**, 907–916.

Maxime, V., Peyraud-Waitzenegger, M., Claireaux, G. and Peyraud, C. (1990). Effects of rapid transfer from sea water to fresh water on respiratory

variables, blood acid–base status and O_2 affinity of haemoglobin in Atlantic salmon (*Salmo salar* L.). *J. Comp. Physiol. B* **160**, 31–39.

Minnich, J. E. (1979). Reptiles. In *Comparative Physiology of Osmoregulation in Animals*, vol. 1 (ed. G. M. O. Maloiy), pp. 391–641. London: Academic Press.

Mitchell, G. S. and Gleeson, T. T. (1985). Acid–base balance during lactic acid infusion in the lizard *Varanus salvator*. *Respir. Physiol*. **60**, 253–266.

Moalli, R., Meyers, R. S., Ultsch, G. R. and Jackson, D. C. (1981). Acid–base balance and temperature in a predominantly skin-breathing salamander, *Cryptobranchus alleganiensis*. *Respir. Physiol.* **43**, 1–11.

Nonnotte, G., Maxime, V., Truchot, J. P., Williot, P. and Peyraud, C. (1993). Respiratory responses to progressive ambient hypoxia in the sturgeon, *Acipenser baeri*. *Respir. Physiol*. **91**, 71–82.

Nonnotte, G. and Truchot, J.-P. (1990). Time course of extracellular acid–base adjustments under hypo- or hyperosmotic conditions in the euryhaline fish *Platichthys flesus*. *J. Fish Biol.* **36**, 181–190.

Oikari, A. (1975). Hydromineral balance in some brackish-water teleosts after thermal acclimation, particularly at temperatures near zero. *Ann. Zool. Fenn.* **12**, 215–229.

Oppelt, W. W., Adamson, R. H., Zubrod, C. G. and Rall, D. P. (1966). Further observations on the physiology and pharmacology of elasmobranch ventricular fluid. *Comp. Biochem. Physiol.* **17**, 857–866.

Rahn, H. (1966). Aquatic gas exchange: theory. *Respir. Physiol*. **1**, 1–12.

Rahn, H. (1967). Gas transport from the external environment to the cell. In *Development of the Lung*, Ciba Foundation Symposium (ed. A. V. S. de Reuck and R. Porter), pp. 3–23. London: Churchill.

Randall, D. J., Cameron, J. N., Daxboeck, C. and Smatresk, N. (1981). Aspects of bimodal gas exchange in the bowfin, *Amia calva* L. (Actinopterygii: Amiiformes). *Respir. Physiol.* **43**, 339–348.

Reeves, R. B. (1972). An imidazole alphastat hypothesis for vertebrate acid–base regulation: tissue carbon dioxide content and body temperature in bullfrogs. *Respir. Physiol.* **14**, 219–236.

Robin, E. D. (1962). Relationship between temperature and plasma pH and carbon dioxide tension in the turtle. *Nature* **195**, 249–251.

Robin, E. D., Murdaugh, H. V. and Weiss, E. (1964). Acid–base, fluid and electrolyte metabolism in the elasmobranch. I. Ionic composition of erythrocytes, muscle and brain. *J. Cell. Comp. Physiol*. **64**, 409–418.

Seymour, R. S. and Webster, M. E. D. (1975). Gas transport and blood acid–base balance in diving sea snakes. *J. Exp. Zool*. **191**, 169–182.

Smatresk, N. J. and Cameron, J. N. (1982). Respiration and acid–base physiology of the spotted gar, a bimodal breather. II. Responses to temperature change and hypercapnia. *J. Exp. Biol.* **96**, 281–293.

Stiffler, D. F. (1991). Partitioning of acid–base regulation between renal and extrarenal sites in the adult, terrestrial stage of the salamander *Ambystoma tigrinum* during respiratory acidosis. *J. Exp. Biol.* **157**, 47–62.

Stiffler, D. F., Tufts, B. L. and Toews, D. P. (1983). Acid–base and ionic balance in *Ambystoma tigrinum* and *Necturus maculosus* during hypercapnia. *Am. J. Physiol.* **245**, R689–R694.

Stinner, J. N. (1982). Ventilation, gas exchange and blood gases in the snake, *Pituophis melanoleucus*. *Respir. Physiol.* **47**, 279–298.

Stinner, J. N. and Hartzler, L. K. (2000). Effect of temperature on pH and electrolyte concentration in air-breathing ectotherms. *J. Exp. Biol*. **203**, 2065–2074.

Stinner, J. N., Hartzler, L. K., Grguric, M. R. and Newton, D. L. (1998). A protein titration hypothesis for the temperature-dependence of tissue CO_2 content in reptiles and amphibians. *J. Exp. Biol.* **201**, 415–424.

Sulya, L. L., Box, B. E. and Gunter, G. (1960). Distribution of some blood constituents in fishes from the Gulf of Mexico. *Am. J. Physiol*. **199**, 1177–1180.

Takeda, T. (1990). Ventilation, cardiac output and blood respiratory parameters in the carp, *Cyprinus carpio*, during hyperoxia. *Respir. Physiol.* **81**, 227–240.

Tierney, M. L., Takei, Y. and Hazon, N. (1998). A radioimmunoassay for the determination of Angiotensin II in elasmobranch fish. *Gen. Comp. Endocrinol*. **111**, 299–305.

Toews, D. P., Holeton, G. F. and Heisler, N. (1982). Regulation of the acid–base status during environmental hypercapnia in the marine teleost fish *Conger conger*. *J. Exp. Biol*. **107**, 9–20.

Truchot, J.-P., Toulmond, A. and Dejours, P. (1980). Blood acid–base balance as a function of water oxygenation: a study at two different ambient CO_2 levels in the dogfish, *Scyliorhinus canicula*. *Respir. Physiol.* **41**, 13–28.

Ultsch, G. R. and Jackson, D. C. (1996). pH and temperature in ectothermic vertebrates. *Bull. Alabama Mus. Nat. Hist*. **18**, 1–41.

Ultsch, G. R., Ott, M. E. and Heisler, N. (1981). Acid–base and electrolyte status in carp (*Cyprinus carpio*) exposed to low environmental pH. *J. Exp. Biol.* **93**, 65–80.

Urist, M. R., Uyeno, S., King, E., Okada, M. and Applegate, S. (1972). Calcium and phosphorus in the skeleton and blood of lungfish *Lepidosiren paradoxa*, with comments on humoral factors in calcium homeostasis in the Osteichthyes. *Comp. Biochem. Physiol*. **42A**, 393–408.

Urist, M. R. and Van de Putte, K. A. (1967). Comparative biochemistry of the blood of fishes. Identification of fishes by the chemical composition of serum. In *Sharks, Skates and Rays* (ed. P. W. Gilbert, R. F. Mathewson and D. P. Rall), pp. 271–285. Baltimore: Johns Hopkins Press.

Wells, R. M. G., Forster, M. E., Davison, W., Taylor, H. H., Davie, P. S. and Satchell, G. H. (1986). Blood oxygen transport in the free-swimming hagfish, *Eptatretus cirrhatus*. *J. Exp. Biol.* **123**, 43–53.

Wilkes, P. R. H., Walker, R. L., McDonald, D. G. and Wood, C. M. (1981). Respiratory, ventilatory, acid–base and ionoregulatory physiology of the white sucker *Catostomus commersoni*: the influence of hyperoxia. *J. Exp. Biol.* **91**, 239–254.

Wood, S. C., Johansen, K., Glass, M. L. and Hoyt, R. W. (1981). Acid–base regulation during heating and cooling in the lizard, *Varanus exanthematicus*. *J. Appl. Physiol*. **50**, 779–783.

Wood, S. C. and Moberly, W. R. (1970). The influence of temperature on the respiratory properties of iguana blood. *Respir. Physiol.* **10**, 20–29.