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ANAEROBIC METABOLISM AND CHANGES IN ACID–BASE STATUS: QUANTITATIVE INTERRELATIONSHIPS AND pH REGULATION IN THE MARINE WORM *SIPUNCULUS NUDUS*

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SUMMARY

The quantitative influence of anaerobic metabolism on acid–base status and on acid–base regulation is investigated in *Sipunculus nudus* L. Proton generation by metabolism is calculated from theoretical predictions. The quantitative comparison of metabolic protons with non-respiratory protons found in the acid–base status is performed assuming a simplified model of the total animal. Taking the proton-equivalent ion exchange between animals and ambient water into account, changes in the anaerobic acid–base status can be explained exclusively by proton generation in metabolism. It is concluded that the classical concept of acid–base physiology is adequate and that the consideration of strong ions is not required for a quantitative treatment of the acid–base status.

The hypothesis that a quantitative correlation exists between metabolic and acid–base events is tested by comparing changes in acid–base status and in metabolism in animals exhibiting different metabolic rates. For this purpose, a method is developed for the calculation of intracellular pH from metabolite concentrations and extracellular acid–base parameters. Proton exchange between intra- and extracellular compartments, which is found to depend upon the total amount of accumulated non-respiratory protons, demonstrates that pH_i is regulated even during anaerobiosis. The defended pH_i value, however, is lower during anaerobiosis than during subsequent recovery.

INTRODUCTION

The preceding paper (Pörtner, 1987*b*) led to several conclusions about how anaerobic metabolism in good animal anaerobes (in the sense that they can survive long periods of hypoxia) may contribute to pH regulation. Metabolism, however,

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may not be the only process influencing acid–base homeostasis during anaerobiosis. The rates of ionic exchange could change, thereby influencing the distribution of ions relevant to acid–base status. Variations in strong ion concentrations, for example, are supposed to be crucial for changes in acid–base parameters, the latter being seen as the dependent variables (Stewart, 1983). Classical acid–base concepts focus on the exchange of H^+ or bicarbonate for strong ions to explain acid–base regulation. The present paper is intended to analyse whether the yield of protons expected from anaerobic metabolic pathways (see Pörtner, 1982, 1987*b*; Pörtner, Heisler & Grieshaber, 1984*b*) is quantitatively sufficient to explain observed changes in acid–base status. For a quantitative comparison, the number of protons or base equivalents that caused the observed changes in pH must be evaluated. For this purpose, the tools of classical acid–base physiology have been utilized in this study (Siggaard-Andersen, 1974; Davenport, 1974; Heisler, 1986). Therefore, the present analysis also provides a useful test of whether the consideration of P_{CO_2}, pH, bicarbonate concentrations, non-bicarbonate buffer values and their interrelationships is a suitable quantitative approach for studying changes in acid–base status.

The marine sipunculid worm *Sipunculus nudus* L. proved to be very useful for the intended quantitative analysis, because it is morphologically simple and is able to sustain long periods of anaerobiosis. The morphological complexity of the vertebrates may explain why such a quantitative study has never been performed *in vivo*. Consideration of the major body compartments is required as well as consideration of net movements of protons or bicarbonate between animals and extracorporal medium (urine, water). In a first approach, Pörtner, Heisler & Grieshaber (1984*c*) compared acid–base and metabolic changes investigated in separate experiments. Since the strong influence of the season on metabolic rates and acid–base disturbances have become evident (see Pörtner, Vogeler & Grieshaber, 1986*a*,*b*) and since the theoretical basis for the analysis has been widened (Pörtner, 1987*b*), the present study was designed to investigate all those parameters that are important for the analysis in each individual animal.

For the analysis, tissue pH was monitored using the weak acid DMO (dimethyloxazolidinedione, Waddell & Butler, 1959). To allow for equilibrium distribution of DMO between tissues and body fluids (see Roos & Boron, 1981), long-term anaerobiosis and not muscular activity was selected for the evaluation of the quantitative correlation between metabolic and acid–base disturbances.

MATERIALS AND METHODS

Animals

Small specimens (7–12 g) of *Sipunculus nudus* were dug up near the low-water line of intertidal flats at Morgat, Brittany, France in March, 1984. The animals were kept for several weeks in tanks containing a bottom layer of sand (10–20 cm) from the original habitat. The aquarium was circulated with artificial sea water at 10–15 °C.

Experimental procedure

Prior to the experiments the animals were catheterized by introducing PE 60 tubing (total length approximately 75 cm) into the body cavity by puncturing the posterior end of the body and sealing it with cyanoacrylate glue (no. 7432, Bostik GmbH, Oberursel, FRG; Histidine blue, Braun Melsungen, FRG). Groups of five cannulated animals were placed in darkened bottles containing 0·5 l of aerated artificial sea water (15 °C), but no sand. Exclusion of light minimized the muscular activity of the animals. After 24 h of acclimation at normoxia, hypoxic conditions ($P_{O_2} < 3$ Torr, 1 Torr = 133·3 Pa) were introduced by continuous bubbling of the water with normocapnic nitrogen (0·03 % CO_2 in pure N_2) provided by gas mixing pumps (Type M 303/a-F, Wösthoff, Bochum, FRG). Hypoxic incubation was continued for 24 h. Animals were sampled after the acclimation period and after 24 h of anaerobiosis. Coelomic fluid was withdrawn anaerobically *via* the indwelling catheter, and the worms were dissected quickly in the dorsoventral plane.

Analysis of metabolites

The right or left half of the musculature (body wall and two of the introvert retractor muscles, introvert excluded) of each animal was freeze-clamped (Wollenberger, Ristau & Schoffa, 1960), and the remaining half was utilized for analysis of pH_i. The frozen tissue was extracted according to the method of Beis & Newsholme (1975). Coelomic plasma, which was obtained after centrifugation of coelomic fluid (for 1 min at 1000 ***g***), was extracted according to the method of Pörtner *et al.* (1984*d*).

The concentrations of L-alanine, D-alanine, malate, aspartate, ammonia, glutamine, glutamate, pyruvate and α-ketoglutarate were measured enzymatically using standard procedures (Bergmeyer, 1974). Succinate was analysed enzymatically according to the method of Michal, Beutler, Lang & Guenter (1976). Determinations of phospho-L-arginine, L-arginine and octopine followed the enzymatic test described by Grieshaber, Kronig & Koormann (1978). The concentrations of strombine, alanopine, acetate and propionate were measured by high-pressure liquid chromatography (Siegmund & Grieshaber, 1983; Pörtner *et al.* 1984*d*).

To correct for individual variations in the sum of metabolites containing L-arginine, the sum of the concentrations of phospho-L-arginine, L-arginine and octopine was calculated for all investigated animals (Table 1). This sum is assumed to remain constant during anaerobiosis (Pörtner *et al.* 1984*d*). For phospho-L-arginine and L-arginine, concentrations were recalculated according to the individual ratio of the phospho-L-arginine content to the sum of phospho-L-arginine and L-arginine concentrations, and according to the mean value of the sum of all L-arginine-containing metabolites minus the individual octopine concentration (see Pörtner *et al.* 1986*b*). The final values are shown in Table 5. Owing to the high number of investigated animals, the mean values in Table 1 are very close to those presented in Table 5. Therefore, this analysis confirms that L-arginine is not metabolized during anaerobiosis (Pörtner *et al.* 1984*d*).

Table 1. *Concentrations (μmol g^{-1} fresh mass, $\bar{x} \pm$ S.E.) of phospho-L-arginine (PLA), L-arginine (L-Arg), octopine, the sum of these values (Σ) and the ratio of the phospho-L-arginine content over L-arginine plus phospho-L-arginine concentrations in the musculature of* Sipunculus nudus *before and after 24 h of anaerobiosis*

Time (h)	PLA	L-Arg	Octopine	Σ	$\frac{[PLA]}{[PLA] + [L\text{-}Arg]}$
0 ($N = 10$)	44·8 ± 2·0	9·8 ± 0·5	0·07 ± 0·01	54·6 ± 2·0	0·82 ± 0·01
24 ($N = 10$)	31·5 ± 2·2	22·3 ± 1·8	1·17 ± 0·15	54·9 ± 2·3	0·58 ± 0·03

For recalculation of phospho-L-arginine and L-arginine concentrations (Table 5) the mean value of the sum for all animals (54·8 ± 1·5, $\bar{x} \pm$ S.E., $N = 20$) and individual values of the ratio and the octopine concentrations were used.

Determination of pH, P_{CO_2} and P_{O_2}

Samples of coelomic fluid were analysed for pH_e, P_{CO_2} and P_{O_2} using a thermostatted (15 ± 0·1 °C) microelectrode assembly (BMS 3, Radiometer, Copenhagen). The electrodes were calibrated with precision phosphate buffers (Radiometer, Copenhagen) or humidified gas mixtures of N_2, CO_2 and O_2 provided by gas-mixing pumps. Intracellular pH in the left or right half of the musculature of each animal (body wall musculature cut into four pieces, introvert excluded, two introvert retractors in one additional sample) was determined by application of the DMO-distribution method (Waddell & Butler, 1959). Details of the procedure have been described previously (Heisler, Weitz & Weitz, 1976; Pörtner, Grieshaber & Heisler, 1984*a*). The fractional values of extracellular space (Q) and water content (F_{H_2O}) of the tissues are given in Table 3.

Calculations and modelling

Bicarbonate concentrations in the intra- and extracellular fluids were calculated from measured pH and P_{CO_2} values by application of the Henderson–Hasselbalch equation. The pK_1''' value for the intracellular fluid of the musculature was adopted from Pörtner *et al.* 1984*a*. For the coelomic fluid, constants were calculated according to Heisler (1984). Calculations based on these constants yielded bicarbonate values identical with those derived from total CO_2 measurements in coelomic plasma samples (H. O. Pörtner & N. Heisler, unpublished data).

The amounts of protons or bicarbonate, which are thought to cause the observed non-respiratory* changes in the acid–base status, were calculated from pH changes and changes of bicarbonate concentration by using equation 1 (non-resp., non-respiratory; β_{NB}, non-bicarbonate buffer value; Bic, bicarbonate; adopted from Pörtner *et al.* 1984*a*).

* For the sake of clarity, the term non-respiratory is utilized to address changes in acid–base parameters or those proton quantities that have been evaluated as being caused by either ionic exchange or metabolism. The term metabolic is used only for protons derived from metabolic pathways.

Table 2. *Mass fractions (g) of tissues and body fluid in* Sipunculus nudus

Body mass	$7{\cdot}91 \pm 0{\cdot}37$ ($N = 44$)
Coelomic fluid	$0{\cdot}52 \pm 0{\cdot}006$ ($N = 19$)
Musculature	$0{\cdot}38 \pm 0{\cdot}006$ ($N = 23$)
Residue	$0{\cdot}14 \pm 0{\cdot}011$ ($N = 19$)

$\bar{x} \pm$ S.E. N, number of animals; taken from Pörtner, 1982.

$$\Delta H^{+}_{\text{non-resp.}} = -|\beta_{NB}| \times \Delta pH - \Delta Bic\,(\text{mmol}\,l^{-1}). \quad (1)$$

These calculations were performed for the intracellular compartment of the musculature and for the coelomic plasma. For modelling, the changes in the plasma were assumed to be uniform for all of the extracellular space in the animal. Mass fractions of tissues and coelomic fluid were taken from Pörtner (1982, see Table 2), and a mean coelomic fluid cell content of 15 % was used (H.-O. Pörtner, unpublished data). For the sake of simplicity, the analysis was restricted to the major compartments of the animal, assuming that the musculature (80 % of the tissues) quantitatively determines metabolite contents, concentration changes and acid–base changes in the extracellular fluid of the whole animal. Table 3 shows how the fractional values of the extracellular compartment and the intracellular compartment were calculated (based on Table 2 and the fractional values for water content, F_{H_2O}, and extracellular space of the musculature, Q). Together the compartments represent 85 % of the total animal. The fluid summed for both compartments is called body water.

The amounts of protons representing the non-respiratory changes in the acid–base status can be summed for both compartments according to equation 2 (see Table 3; bw, body water; ew, extracellular water; cw, cell water).

$$\Delta H^{+}_{bw} = (F_{ew} \times \Delta H^{+}_{ew} + F_{cw} \times \Delta H^{+}_{cw})/(F_{ew} + F_{cw})\ (\text{mmol}\,l^{-1}\,\text{body water}). \quad (2)$$

The amounts of protons consumed or produced by metabolism were calculated on the basis of Table 1 and fig. 6 of Pörtner (1987*b*), including the removal or

Table 3. *Model of body compartments in* Sipunculus nudus *based on Table 2 and fractional values of extracellular space (Q) and water content (F_{H_2O}) in the musculature (see text)*

Compartment 1: extracellular water
$(0{\cdot}52-0{\cdot}08) + (0{\cdot}28\times0{\cdot}48) = 0{\cdot}58 = F_{ew}$
coelomic plasma + extracellular tissue water

Compartment 2: cell water of the musculature
$(0{\cdot}38\times0{\cdot}79) - (0{\cdot}28\times0{\cdot}38) = 0{\cdot}19 = F_{cw}$
muscle water − extracellular muscle water

Compartments 1+2: body water (77 % of the organism) in 85 % of the animal

Q: $0{\cdot}283 \pm 0{\cdot}005$ ($N = 20$)
F_{H_2O}: $0{\cdot}788 \pm 0{\cdot}001$ ($N = 20$)

production of carboxyl groups, amino groups and ammonium as well as the metabolism of high-energy phosphates. Since alanine accumulation exceeded aspartate and glutamine degradation (Table 5), reductive amination or protein catabolism had to be taken into account in considering the origin of the amino group in alanine. Proton consumption by phospho-L-arginine hydrolysis was calculated for the cell pH reached after 24 h of anaerobiosis. This procedure covers the proton consumption during hydrolysis of the phosphagen and the increase in the non-bicarbonate buffer value by phosphate accumulation (for the proton balance of metabolism see Pörtner, 1982, 1987*b*; Pörtner *et al.* 1984*b*). Calculations referring to the body water of the animal had to be modified for the evaluation of the proton yield of metabolism, $\Delta H^{+}_{M,bw}$, since perchloric acid extraction of the body wall musculature comprises cell water and extracellular muscle water (equation 3, pl., plasma; musc., musculature; M, metabolic; for fractional values see Tables 2, 3 and text).

$$\Delta H^{+}_{M,bw} = (F_{pl.} + Q \times 0{\cdot}098)\Delta H^{+}_{M,ew} + F_{musc.} \times \Delta H^{+}_{M,musc.}/(F_{ew} + F_{cw}) \quad (\text{mmol}\,l^{-1}\,\text{body water}). \quad (3)$$

(The value of 0·098 represents the fraction of the remaining 20 % of the tissues in addition to the musculature. The term $F_{pl.} + Q \times 0{\cdot}098$ originates from the consideration that the plasma fraction $F_{pl.}$ plus the fraction of extracellular water in the residual tissues yields the fraction of extracellular water not covered by perchloric acid extraction of the musculature. The term $F_{musc.} \times \Delta H^{+}_{M,musc.}$ was originally $F_{musc.} \times F_{H_2O} \times \Delta H^{+}_{M,musc}/F_{H_2O}$ for the calculation of the fractional amount of protons in the fraction of total muscle water.)

For literature data taken from Pörtner *et al.* (1986*a*,*b*), tissue pH was calculated from the changes in metabolite concentrations and the extracellular acid–base disturbances. The discrepancy between the amount of non-respiratory protons in the extracellular acid–base status and the metabolic proton yield in the whole animal was evaluated using the model described above. Metabolic protons ($\Delta H^{+}_{met.}$) were calculated for the extracellular water (i.e. as if they were all released from the intracellular to the extracellular space). The difference was taken to be a measure of the non-respiratory proton load of the tissues ($\Delta H^{+}_{i,met.} = \Delta H^{+}_{i,non\text{-}resp.}$).

For the evaluation of pH_i changes during anaerobiosis, the control value of pH_i (pH_0) was taken as the value found in the animals of the present study. The overall pH change is reflected by the bicarbonate change during respiratory processes, which is reduced or increased by non-respiratory events. The term ΔBic in equation 4 (Table 4, derived from equation 1) therefore had to be readjusted. In the pH–bicarbonate diagram (Fig. 1), the respiratory pH changes were determined graphically by following the buffer line (equation 5). In the first approach, the metabolic pH change was calculated using equation 6 (see equation 1). Adding the resulting difference in equation 7 to pH_1 originating from equation 5 yields the pH value resulting from metabolic and respiratory changes, to a first approximation. The term ΔH^{+}_{i} in equation 4 has to be corrected for the term ΔBic (equation 8). The change in bicarbonate concentration results from the different positions of pH_0 and

pH_2 in the pH–bicarbonate diagram (calculated by using the Henderson–Hasselbalch equation). By iterative utilization of equations 9–12, the terms ΔBic_i and ΔpH are brought close to the values which finally yield the correct value of $\Delta H^+_{met.}$ or $\Delta H^+_{non\text{-}resp.}$ in equations 4 and 1, respectively. For the final estimate of pH_i, the

Table 4. *Iterative approximation of intracellular pH values evaluated from extracellular acid–base disturbances and metabolic proton production on the basis of equation 1 and the graphic presentation of the Henderson–Hasselbalch equation in the pH–bicarbonate diagram (see Fig. 1 and text)*

$\Delta pH_i = (\Delta H^+_{met.} + \Delta Bic)/-\lvert\beta_{NB}\rvert = \Delta H_i^+/-\lvert\beta_{NB}\rvert.$	(4)
$pH_0 \rightarrow \Delta pH_{resp.}$; $pH_0 + \Delta pH_{resp.} = pH_1.$	(5)
$\Delta H^+_{i,met.}/-\lvert\beta_{NB}\rvert = \Delta pH_1.$	(6)
$pH_1 + \Delta pH_1 = pH_2.$	(7)
$\Delta H^+_{i,met.} + \Delta Bic_{i,1(pH2-0)} = \Delta H_i^{+\prime}.$	(8)
$\Delta H_i^{+\prime}/-\lvert\beta_{NB}\rvert = \Delta pH_2.$	(9)
$pH_0 + \Delta pH_2 = pH_3.$	(10)
$\Delta H^+_{i,met.} + \Delta Bic_{i,2(pH3-0)} = \Delta H_i^{+\prime\prime}.$	(11)
$\rightarrow$ equation 9 $\rightarrow$ equation 10 $\rightarrow pH_4$ etc.	(12)

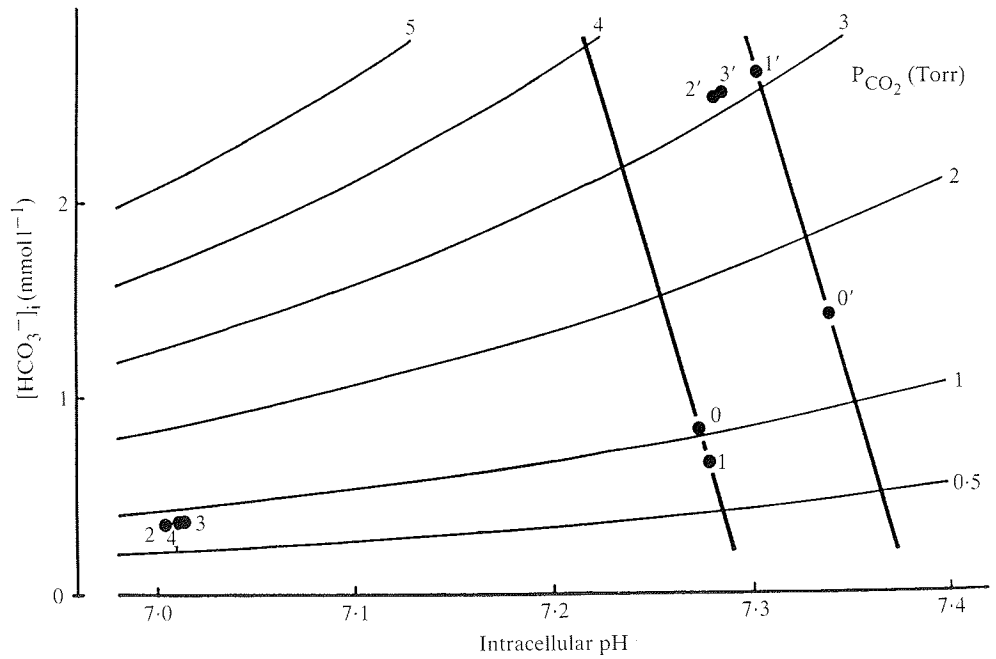

Fig. 1. Presentation of the method of pH evaluation (Table 4) in a pH–bicarbonate diagram. During iterative approximation, changes in the bicarbonate concentration and in pH are evaluated taking respiratory acid–base disturbances into account (see text). The procedure is demonstrated for two examples: (a) points 0–4 reflect mainly non-respiratory changes in pH (for the results see Table 9), whereas (b) points 0′–3′ show mainly respiratory influences on pH (during digging activity, results presented by Pörtner, 1987*a*, based on data by H.-O. Pörtner, M. K. Grieshaber & N. Heisler, unpublished).

amount of protons produced in metabolism had to be recalculated, since the total amount of protons bound during phospho-L-arginine hydrolysis depends on the final intracellular pH. (Changes in the non-bicarbonate buffer value of the tissues, resulting from the accumulation of inorganic phosphate, are taken into account by this procedure.)

This significance of changes was evaluated using Student's t-test.

RESULTS

Table 5 demonstrates the effect of 24 h of anaerobiosis on the concentrations of several metabolites in the body wall musculature. The proton balance is equal to the changes in the amounts of carboxyl groups, amino groups, ammonia and phosphate, the latter being calculated from changes in the concentrations of high-energy phosphates.

Proton production during anaerobiosis results from anaerobic glycolysis which includes octopine, strombine and alanine formation. Malate, succinate and propionate accumulation in the tissues during anaerobiosis also represent an increase in the amount of carboxyl groups, whereas the degradation of aspartate as well as the

Table 5. *Concentrations of metabolites ($\mu mol\,g^{-1}$ fresh mass, $\bar{x} \pm$ S.E.) in the musculature of* Sipunculus nudus *before and after 24 h of anaerobiosis*

	Aerobic ($N = 10$)	Anaerobic (24 h) ($N = 10$)	ΔC	$\Delta H^+_{met.}$
Octopine	0·07 ± 0·01	1·17 ± 0·15*	+1·10	+1·10
Strombine	1·05 ± 0·24	5·18 ± 0·34*	+4·13	+4·13
Alanopine	0·10 ± 0·02	0·09 ± 0·01	—	—
Pyruvate	<0·02	<0·02	—	—
Glutamate	1·04 ± 0·03	1·05 ± 0·10	—	—
Glutamine	0·36 ± 0·08	0·25 ± 0·05	−0·11	−0·22
α-Ketoglutarate	<0·02	<0·02	—	—
Aspartate	1·04 ± 0·06	0·52 ± 0·03*	−0·52	−1·04
Alanine	1·40 ± 0·15	3·03 ± 0·25*	+1·63	+1·63
Malate	0·04 ± 0·01	0·13 ± 0·01*	+0·09	+0·18
Succinate	0·06 ± 0·01	0·90 ± 0·09*	+0·84	+1·68
Propionate	0·10 ± 0·02	0·47 ± 0·03*	+0·37	+0·37
Acetate	0·30 ± 0·02	0·27 ± 0·02	—	—
NH_3	2·63 ± 0·27	3·91 ± 0·20*	+1·28	−1·28
$-NH_2$			+0·89	−0·89
Phospho-L-arginine	44·8 ± 0·6	31·3 ± 1·7*	−13·5	−3·95
L-arginine	10·0 ± 0·6	22·2 ± 1·7*	+12·2	—
ATP	3·36 ± 0·16	3·21 ± 0·14	—	—
ADP	0·44 ± 0·02	0·47 ± 0·02	—	—
				+1·71

The proton balance of anaerobic metabolism ($\Delta H^+_{met.}$) was evaluated from concentration changes (ΔC) according to changes in the amounts of carboxyl groups, amino groups, ammonia and high-energy phosphates (Pörtner, 1987*b*).

An asterisk indicates a significant change ($P < 0·05$).

Table 6. *Changes in metabolite concentrations (ΔC) and proton equivalents ($\Delta H^+_{met.}$) during 24 h of anaerobiosis in the coelomic fluid (mmol l^{-1} plasma, $\bar{x} \pm$ S.E., significance indicated by asterisks, $P < 0{\cdot}05$, see Table 5 and text)*

	Aerobic ($N = 10$)	Anaerobic (24 h) ($N = 10$)	ΔC	$\Delta H^+_{met.}$
Succinate	0·01 ± 0·01	0·30 ± 0·02*	+0·29	+0·58
Propionate	0·01 ± 0·01	0·74 ± 0·07*	+0·73	+0·73
Acetate	0·07 ± 0·01	0·36 ± 0·03*	+0·29	+0·29
NH_3	0·15 ± 0·02	0·20 ± 0·02	+0·05	−0·05
				1·55

Table 7. *Acid–base parameters in the musculature and the coelomic plasma before and after 24 h of anaerobiosis ($\bar{x} \pm$ S.E., asterisks indicate a significant change, $P < 0{\cdot}05$)*

	Aerobic ($N = 10$)	Anaerobic ($N = 10$)	$\Delta H^+_{non\text{-}resp.}$ (mmol l^{-1})
P_{CO_2} (Torr)	0·88 ± 0·03	0·50 ± 0·02*	
pH_i	7·27 ± 0·01	7·19 ± 0·04*	+3·22 (cell water)
$[HCO_3^-]_i$ (mmol l^{-1})	0·68 ± 0·03	0·34 ± 0·03*	
pH_e	8·09 ± 0·02	8·20 ± 0·02*	+0·65 (coelomic plasma)
$[HCO_3^-]_c$ (mmol l^{-1})	5·50 ± 0·29	4·86 ± 0·43*	
			body water:
		$\Delta H^+_{non\text{-}resp.}$:	+1·39
		$\Delta H^+_{met.}$:	+1·79
		Discrepancy:	+0·40 (22 %)

The amounts of non-respiratory protons were calculated based on equations 1 and 2. The amount of metabolic protons in the body water (equation 3) exceeds the non-respiratory protons by 0·4 mmol l^{-1} body water or 22 %.

cleavage of phospho-L-arginine cause proton consumption. Proton consumption is also linked to the accumulation of ammonium ions or amino groups. Changes in alanopine, pyruvate, glutamate, α-ketoglutarate, acetate, ATP and ADP concentrations are very small and insignificant under the experimental conditions applied and considered to be irrelevant for the acid–base status. The changes in metabolite concentrations of the musculature represent a proton load in the tissue of 1·71 μmol g^{-1} fresh mass.

Succinate and propionate also accumulate in the coelomic plasma (Table 6), and, in contrast to its behaviour in the tissues, acetate levels also increase. The ammonia concentration increases only slightly, leading to an overall proton load of 1·55 mmol l^{-1} plasma.

Changes in the intra- and extracellular acid–base status are presented in Table 7. Intra- and extracellular bicarbonate concentrations fall, as do the P_{CO_2} and

intracellular pH. Extracellular pH, however, increases slightly. The overall proton load for the intra- and extracellular compartment is 1·39 mmol l^{-1} body water for the whole animal.

Table 7 also compares the amount of protons representing the changes in the acid–base status and the quantity of protons expected from metabolism. Metabolic protons exceed by 22 % the non-respiratory protons that were actually found in acid–base status.

DISCUSSION

Methodology and model

Long-term hypoxia was selected in the present study for the quantitative comparison of metabolic and acid–base events. In order to make an exact evaluation of protons from measured acid–base parameters, it was assumed that the following preconditions held. First, equilibrium distribution of DMO is achieved during long-term anaerobiosis due to low rates of pH changes (see Pörtner *et al.* 1984*a*). This is not necessarily so during muscular activity, when pH changes or changes in the pH gradient between intra- and extracellular compartments may occur too rapidly for DMO to follow its equilibrium distribution (Roos & Boron, 1981). Second, the P_{CO_2} of the body fluids is accepted as being the same as that of the tissues. This appears to be very likely under control conditions and more so during anaerobiosis, because of the reduction in metabolic rate and CO_2 production (Pörtner, 1982; see Piiper, 1986, for the estimation of P_{CO_2} values in muscle tissues). Third, a stoichiometric increase in the concentration of free inorganic phosphate was assumed for the calculations of proton quantities consumed during phospho-L-arginine hydrolysis. This has been confirmed during ^{31}P-NMR studies in many tissues and also for long-term incubations using isolated muscle preparations from marine invertebrates (Barrow, Jamieson & Norton, 1980; Graham & Ellington, 1985).

The reliability of the calculations of non-respiratory and metabolic proton quantities, therefore, is accepted for the present study. Unfortunately, the extent of acid–base changes during anaerobiosis was small (see the study of Pörtner *et al.* 1986*b* on recovery from anaerobiosis, which was performed using animals collected during the same period). The number of animals investigated for each sampling time (10), however, should ensure that the described changes represent true tendencies. The extent of acidosis was found to be slightly smaller in animals investigated during the present study than in animals utilized in the study of Pörtner *et al.* (1986*a*,*b*). This could be caused by the accumulation of ammonium in the muscle tissue, which indicates proton consumption. Recovery was investigated using an experimental chamber containing 16 l of sea water, whereas the number of animals per unit volume and the resultant ammonia accumulation in the water was higher in the present study which used 0·5 l water in the incubation bottles.

The simplified quantitative model is based on the morphology of *Sipunculus nudus* (see Tables 2, 3). The model takes 85 % of the total animal into account and neglects tissues such as nephridia, nervous tissues, gut and coelomic cells. The

musculature of *Sipunculus nudus*, however, not only comprises 80 % of the tissues (Pörtner, 1982), but also may be the most metabolically active, since it is involved in feeding, ventilation and digging.

For the calculation of both non-respiratory and metabolic protons, acid–base and metabolic parameters of the extracellular space of all tissues were assumed to depend on the influence of the musculature rather than that of any other tissue. The coelomic plasma, as part of the extracellular water, would be the substance to mirror any major changes in the other tissues. If the same or more intensive acid–base and metabolic changes in tissues different from the musculature were to occur, an underestimation of the described discrepancy between metabolic and non-respiratory protons in the animal would result. During higher rates of metabolism, the amount of protons leaving the tissues would be in excess of the amount of released organic acid anions (see Pörtner *et al.* 1984*a*). However, the opposite is the case, indicating that the extracellular space is only influenced by the other tissues in a minor way. Smaller changes in these tissues would have no significant influence on the calculated discrepancy, since they represent only a small fraction by mass of the animal and since small changes would mean negligible exchange with the extracellular water.

Only a big excess in the release of metabolites over the release of protons from the residual tissues into the extracellular water would lead to an overestimation of the actual excess of expected metabolic protons. Such a discrepancy in metabolite production without simultaneous acid–base disturbances, however, is considered to be most unlikely. An overestimation of the excess of protons expected from metabolism could also originate from the fact that there may have been no exchange between coelomic plasma and the extracellular water of the residual tissues at all, an assumption which can also be considered as being most unlikely, since inulin is successfully used as the marker for the extracellular space of the tissues after injection into the coelomic plasma.

Quantitative interrelationships and acid–base regulation

If the calculated excess of metabolic protons over non-respiratory protons actually exists, one might question the fate of the respective non-respiratory protons. In separate experiments, the proton-equivalent ion transfer between animals and ambient water was investigated (H.-O. Pörtner & N. Heisler, unpublished results). A net proton release to the ambient water was found after 24 h of anaerobiosis, even in animals which exhibited only minor deflections in the extracellular pH (see Table 8; Pörtner, 1987*a*). The total amount of protons released by ionic exchange, therefore, could be quite independent of the anaerobic metabolic rate of the animal. The observed quantity, which does not reflect the release of volatile acids (acetic and propionic acid) by passive diffusion, equals between 0·3 and 0·5 mmol l^{-1} body water and covers the discrepancy between metabolic and non-respiratory protons quite well (Table 8). Obviously, a good quantitative correlation can be evaluated for the proton balance of metabolism and the changes in the intra- and extracellular acid–base status.

Table 8. *Proton release into the ambient water ($\Delta H^{+}_{\rightarrow w}$) during 24 h of aerobiosis and during subsequent anaerobiosis (24 h, H.-O. Pörtner & N. Heisler, unpublished data)*

Animals collected in	pH_e aerobic	pH_e anaerobic	$\Delta H^{+}_{\rightarrow w}$ aerobic (mmol kg^{-1} body mass)	$\Delta H^{+}_{\rightarrow w}$ anaerobic (mmol kg^{-1} body mass)	net $\Delta H^{+}_{\rightarrow w}$ (mmol l^{-1} body water)
Spring 1984 ($N = 6$)	7·90 ± 0·07	7·79 ± 0·12	−0·77	−0·41 ± 0·23	0·49
Autumn 1985 ($N = 8$)	7·81 ± 0·01	7·60 ± 0·03	−0·07	0·19 ± 0·31	0·34

0–24 h of anaerobiosis (mmol l^{-1} body water)	
$\Delta H^{+}_{met.}$:	+1·79
$\Delta H^{+}_{non\text{-}resp.}$:	+1·39
Discrepancy:	+0·40 (22 %)
net $\Delta H^{+}_{\rightarrow w}$:	+0·3 to +0·5

The net anaerobic proton release is assumed to be represented by the difference between aerobic and anaerobic values. This amount seems to be quite independent of the extent of acid–base disturbances (shown for the extracellular compartment) and covers the observed discrepancy between proton accumulation in the acid–base status ($\Delta H^{+}_{non\text{-}resp.}$) and proton generation by metabolism ($\Delta H^{+}_{met.}$) ($\bar{x} \pm$ S.E., N, number of investigated animals).

It is interesting to note that after 24 h of anaerobiosis the amount of organic acid anions in the plasma of the animals of the present study exceeded the amount of non-respiratory protons. This is quite opposite to the quantitative interrelationships found in earlier investigations (Pörtner *et al.* 1984*a*). More pronounced extracellular pH changes had been found in those animals, indicating a higher metabolic rate. With the greater extent of proton accumulation, intra- and extracellular pH changes were observed to change within the same order of magnitude during anaerobiosis. When, however, the acid–base status was only minimally affected by anaerobiosis, it was calculated that the release of protons from the intra- to the extracellular space would be delayed (Pörtner *et al.* 1986*b*). The movement of organic acid anions, however, is independent of the movement of protons (Pörtner *et al.* 1984*a*,1986*b*). This may explain why, in the present study, the change in organic acid anion concentrations (representing 1·5 mmol $H^+ l^{-1}$) exceeded the amount of non-respiratory protons (0·7 mmol l^{-1}) in the extracellular space.

It could be argued that the amount of organic acid anions, which was higher in the coelomic plasma than the amount of non-respiratory protons, is evidence that the acids leave the tissues in dissociated form. In the musculature, 2·3 mmol $H^+ l^{-1}$ cell water (corrected for the extracellular metabolites) originate from metabolism, whereas 3·2 mmol $H^+ l^{-1}$ cell water are found in the acid–base status. This discrepancy, however, is more likely to support the assumption of a downward shift of pH_i during anaerobiosis due to ionic exchange (see below; Graham & Ellington, 1985). The discrepancy found in the extracellular space could also originate from the ion transfer between animals and water and not necessarily from ionic movements of metabolites.

In earlier investigations, acetate and propionate have been found to leave the animals (Pörtner *et al.* 1984*d*). The quantitative correlation between anaerobic metabolism and acid–base events strongly suggests that these metabolites are released together with the associated protons in a 1:1 relationship. The easiest explanation for this would be to assume passive diffusion, since the volatile fatty acids are certainly the most diffusible among the end-products. Experimental evidence for passive diffusion, however, has not been published.

Changes in the acid–base status during anaerobiosis were small in the present study. Further evidence should therefore be provided in order to support the conclusion that quantitative correlations between anaerobic metabolic and acid–base events exist. Higher metabolic rates and higher rates of proton accumulation were observed in animals collected during October than in the animals collected in March (Pörtner *et al.* 1986*a*,*b* and present study). Intracellular pH, however, has not been determined in these animals. Therefore, on the basis of the evidence presented above for a quantitative correlation between anaerobic metabolism and changes in the acid–base status, the intracellular pH change was calculated from changes in metabolite concentrations and extracellular acid–base parameters, all measured in the same October animals. Calculations were performed using mean values reported by Pörtner *et al.* (1986*a*,*b*), considering the net proton release into the ambient water. Table 9 compares these values with the mean values from the present study. Only those metabolites are shown which, in October animals, represent the enhanced metabolic rate as discussed by Pörtner *et al.* (1986*a*). The extracellular acid–base parameters exhibit a clear correlation with the extent of metabolic proton formation (Pörtner *et al.* 1986*b*). It was calculated that the intracellular pH decreased by 0·26 pH units. This is less than the fall in extracellular pH but close to the change in pH_i found in previous experiments (Pörtner *et al.* 1984*a*; Table 10).

In this context it is interesting to compare the extent of intracellular pH regulation in these animals. During anaerobiosis, it is mainly proton-equivalent ion transfer between intra- and extracellular compartments which contributes to pH_i regulation, since proton release into the water is minor, especially when high rates of anaerobic metabolism occur (H.-O. Pörtner & N. Heisler, unpublished results). Table 9 demonstrates that the extent of proton transfer depends upon the amount of accumulated non-respiratory protons, 35 % being transferred during minor acid–base disturbances in March animals, whereas more than 60 % is released in October animals. Table 10 presents a comparison of the degree of proton transfer with the total amount of non-respiratory protons (from data reported up to now for *Sipunculus nudus*) together with intra- and extracellular pH values before and after 24 h of anaerobiosis. There is a tendency for intracellular pH to be regulated above a value of about 7·0 with extracellular pH being increasingly affected by hypoxia. The assumption that ionic pH_i regulation takes place during anaerobiosis is confirmed by the fact that the excess of non-respiratory over metabolic protons in the intracellular space (see above) turns into an excess of metabolic over non-respiratory protons at high metabolic rates (19·9 over 9·6 or 12·5 mmol $H^+ l^{-1}$ cell water, in October animals; see Table 10). During recovery from anaerobiosis, however, pH_i is more

Table 9. *Comparison of acid–base and metabolic changes during 24 h of anaerobiosis in animals collected during March (present study) and October (Pörtner, Vogeler & Grieshaber, 1986*a,b*)*

		pH_e	$[HCO_3^-]_e$ (mmol l^{-1})	P_{CO_2} (Torr)	$\Delta H^+_{e,nr}$ (mmol l^{-1})	$\Delta H^+_{i,nr}$ (mmol l^{-1})	pH_i	Strombine (musculature) (μmol g^{-1} fresh mass)	Octopine (musculature) (μmol g^{-1} fresh mass)	Acetate (μmol g^{-1} fresh mass)	Acetate (plasma) (mmol l^{-1})
March	Control	8·09	5·50	0·88			7·27	1·05	0·07	0·30	0·07
	Anaerobic	8·20	4·86	0·50	+0·65 (35 %)	3·22	7·19	5·18	1·17	0·27	0·36
October	Control	7·97	4·42	1·02			7·27	4·5	1·0	0·36	0·27
	Anaerobic	7·42	0·71	0·73	+6·24 (63 %)	9·61	7·01	12·8	6·7	0·81	1·25

The increase in metabolic rate and proton accumulation in October animals leads to a more pronounced intracellular and extracellular acidosis. 63 % of the non-respiratory (nr) protons are released into the extracellular water in October animals, whereas in March animals only 35 % are found to leave the muscle tissue (see Table 10). Underlined values are (based on) calculated values of pH_i.

Table 10. *Comparison of anaerobic proton accumulation in the musculature and extracellular water of* Sipunculus nudus *exhibiting different degrees of changes in the acid–base status (data from Pörtner, Grieshaber & Heisler, 1984*a*; Pörtner, Vogeler & Grieshaber, 1986*b*, present study)*

pH_e control	pH_e 24 h	pH_i control	pH_i 24 h	$\Delta\beta_{NB}$ (mmol pH^{-1} l^{-1} cw)	ΔH^+_{nr} (mmol l^{-1} bw)	$\Delta H^+_{e,nr}$ (mmol l^{-1} ew)	$\Delta H^+_{i,nr}$ (mmol l^{-1} cw)	% $\Delta H^+_{e,nr}$	Source
8·09	8·20	7·27	7·19	9·8	+1·39 (+1·61)	+0·65	+3·22 (+4·0)	35 (30)	Present study
8·09	8·14	7·37	7·19	9·8	+3·54 (+4·04)	+2·20	+6·72 (+8·5)	47 (41)	Pörtner, Vogeler & Grieshaber, 1986*b*
8·09	7·71	7·32	7·02	11·1	+6·87 (+7·78)	+5·06	+10·92 (+14·2)	55 (49)	Pörtner, Grieshaber & Heisler, 1984*a*
7·97	7·42	7·27	7·01	11·2	+7·37 (+8·19)	+6·24	+9·61 (+12·5)	63 (57)	Pörtner *et al.* 1986*b*, present study

The respiratory contribution to changes in the acid–base status is only minor under the experimental conditions applied (cf. Fig. 1, example a).

Values in brackets include proton binding assuming an increase in non-bicarbonate buffering during hydrolysis of 11·6 μmol phospho-L-arginine g^{-1} fresh mass. (This value represents the mean value from all considered experiments. nr, non-respiratory; bw, body water; ew, extracellular water; cw, cell water; β_{NB}, non-bicarbonate buffer value.)

efficiently regulated and metabolic protons are rapidly transferred to the extracellular space and to the ambient water (Pörtner *et al.* 1986*b*; H.-O. Pörtner & N. Heisler, unpublished results). Obviously, ionic regulation of pH is stimulated by oxygen availability, whereas during anaerobiosis the efficiency of pH_i regulation may be reduced, allowing pH_i to fall to some extent. The minimum value of pH defended by ionic regulation seems to be lower during anaerobiosis (7·0) than during aerobiosis (7·3, both values are valid for 15 °C), and thereby probably contributes to metabolic regulation (see Pörtner *et al.* 1984*a*).

According to the data presented, a quantitative correlation can be derived for the whole animal between proton production by metabolism, changes in the acid–base status and proton release into the ambient water. Recently, Graham & Ellington (1985) qualitatively compared pH changes and changes in metabolite concentrations during treatment of isolated mollusc hearts with anoxia and anoxia plus aminooxyacetate. For anoxia, the authors revealed a discrepancy between net proton consumption by metabolism (they neglected, however, proton production by alanine formation) and the observed acidosis. Proton consumption during anaerobiosis is due to aspartate degradation and hydrolysis of phospho-L-arginine (see Pörtner, 1982, 1987*b*; Pörtner *et al.* 1984*b*). Application of aminooxyacetate blocks the degradation of aspartate and the accumulation of alanine and succinate and is indicative of a reduction in proton consumption. Graham & Ellington, however, could not find a significant increase in the acidosis which would be expected during aminooxyacetate application. Contrary to their conclusion, the mean fall in pH_i in their experiments increased from a value between 0·1 and 0·12 towards 0·2, which means an almost 100 % increase in the amount of protons causing these pH changes: despite the high variability in their results, this could be related quantitatively to the block of aspartate degradation. For a substantial and quantitative analysis, it is necessary, however, to take proton and metabolite movements between body compartments and between animals and ambient water into account.

The quantitative correlation found between anaerobic metabolism and changes in the acid–base status indicates that classical acid–base physiology can provide useful tools to study these interrelationships. The evaluation of proton quantities and proton movements from pH, P_{CO_2}, bicarbonate concentration and non-bicarbonate buffer values provides accurate results and does not require consideration of strong ions as proposed by Stewart (1983). From a biochemical point of view it is essential to state that the observed changes in the acid–base status are described by the known end-products; in other words, there are no other organic acids which can be expected to be formed as end-products in anaerobic *Sipunculus nudus* L. Research may now focus on pH regulation in these animals, for example in relation to metabolic regulation.

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