RECOVERY FROM ANAEROBIOSIS IN THE INTERTIDAL WORM *SIPUNCULUS NUDUS*

II. GAS EXCHANGE AND CHANGES IN THE INTRA- AND EXTRACELLULAR ACID-BASE STATUS

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SUMMARY

Intra- and extracellular acid–base status and changes of coelomic P_{O_2} were investigated during recovery following 24 h of anaerobiosis in *Sipunculus nudus* L.. Metabolism, gas exchange and acid–base status were compared in animals collected during March and October.

Anaerobiosis led to an uncompensated metabolic acidosis, the degree of the acidosis depending on the metabolic rate of the animals. During initial recovery in March animals, the acidosis was transiently aggravated in the extracellular, but not in the intracellular, compartment, indicating an efficient regulation of intracellular pH as soon as oxygen was available in the coelomic fluid. The extracellular acidosis was predominantly of non-respiratory origin.

The non-respiratory part of the acidosis is attributed to the repletion of the phospho-L-arginine pool. The proton yield calculated from phosphagen resynthesis was highly correlated in time and in quantity to the observed negative base excess in the extracellular compartment. In October animals, strombine accumulation may have contributed to the acidosis that develops during recovery. The amount of succinate, propionate, and acetate in the coelomic plasma had already decreased when the acidosis developed. This discrepancy supports the conclusion that protons move between the body compartments independent of the distribution of anionic metabolites.

The respiratory part of the acidosis is attributed to the repayment of an oxygen debt. The increase of P_{CO_2} is higher in October than in March animals, probably because of differences in metabolic rate.

The time course of acid–base disturbances and their compensation is discussed in relation to the time course of metabolic events during recovery and to the priorities of the different processes observed.

INTRODUCTION

Restoration of aerobic homeostasis after exposure to hypoxia in marine invertebrates not only includes the disposal of accumulated end-products and the

replenishment of high energy phosphates (Ellington, 1983; Pörtner, Vogeler & Grieshaber, 1986), but also requires the readjustment of physiological parameters like P_{O_2} and pH in body fluids and tissues. Anaerobic degradation of glycogen, for instance, always leads to an accumulation of protons, the amount of which may exceed the amount of protons absorbed by phosphagen hydrolysis (Pörtner, Heisler & Grieshaber, 1984*b*). In *Sipunculus nudus*, long-term experimental hypoxia was found to lead to a decrease of intra- and extracellular pH *in vivo* (Pörtner, Grieshaber & Heisler, 1984*a*), which must be reversed during postanaerobic recovery.

In this paper, gas exchange and changes in the intra- and extracellular acid–base status during recovery in *Sipunculus nudus* are described. Since the restoration of aerobic homeostasis in the energy metabolism has been investigated in the same animals (Pörtner *et al.* 1986), we can discover whether or not a correlation exists between recovery processes in energy metabolism and acid–base events. From theoretical considerations, oxidation of accumulated end-products, for instance, would lead to proton consumption, but resynthesis of the phosphagen should lead to an acidification of the cell (cf. Pörtner *et al.* 1984*b*). The latter might be important, especially in *Sipunculus nudus*, since cleavage of a high amount of phosphagen during anaerobiosis has been reported for the body wall musculature of this species (Pörtner *et al.* 1984*c*).

MATERIALS AND METHODS

Specimens of *Sipunculus nudus* were collected in the intertidal zone near Morgat (small animals of 7–12 g) and Locquemeau (large animals 25–35 g), Brittany, France, and kept in Düsseldorf for several weeks before the experiments were started. Cannulation of the animals, acclimation to the experimental conditions, and exposure to hypoxia and postanaerobic normoxia are described in detail in the preceding paper (Pörtner *et al.* 1986).

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

Coelomic fluid was withdrawn anaerobically *via* the indwelling catheter. Samples were analysed for pH_e, P_{CO_2} and P_{O_2} using a thermostatted (15 ± 0.1°C) micro-electrode 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 (Type M 303/a-F, Wösthoff, Bochum, FRG).

At the end of each experiment, the (March) animals were dissected quickly in the dorso-ventral plane. The left or right half of the musculature of each animal was utilized for pH_i analysis in the body wall (cut into four pieces, introvert excluded) and in the introvert retractors (two retractors in one sample). The remaining half was utilized for the analysis of metabolites (Pörtner *et al.* 1986). Intracellular pH 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 *et al.* 1984*a*).

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 was adopted from Pörtner *et al.* (1984*a*). Constants for the coelomic plasma were calculated according to Heisler (1984). Bicarbonate concentrations evaluated using these constants were identical with values yielded from total CO_2 measurements in coelomic plasma samples (H. O. Pörtner & N. Heisler, unpublished data). Changes in the acid–base status were analysed quantitatively according to Pörtner *et al.* (1984*a*) using the following equations (non-resp. = non-respiratory; resp. = respiratory; β_{NB} = non-bicarbonate buffer value, adopted from Pörtner *et al.* 1984*a*):

$$\Delta H^+_{\text{non-resp.}} = -\,|\,\beta_{NB}\,| \times \Delta pH - \Delta HCO_3^- \ (\text{mmol}\,l^{-1}),$$

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

The significance of changes in the acid–base status of March animals was tested by the two-sample or paired-sample *t*-test.

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

In March animals, 24 h of anaerobiosis led to a non-significant decrease of extracellular but a significant drop of intracellular pH by 0·07 and 0·18 pH units, respectively, starting from $pH_e = 8\cdot21 \pm 0\cdot05$ and $pH_i = 7\cdot37 \pm 0\cdot02$ under normoxia. These pH reductions were accompanied by a decrease of intra- and extracellular bicarbonate concentrations and of coelomic P_{CO_2} (Fig. 1). Furthermore, the oxygen stores of the animals were depleted during anaerobiosis, the P_{O_2} being zero after 24 h (Fig. 2).

During postanaerobic recovery, more prominent pH changes occurred in the coelomic fluid (Fig. 1). Instead of returning to control values, pH_e continued to fall and reached a value of $7\cdot85 \pm 0\cdot30$ after 3 h of recovery. Such a decrease of pH was not observed in the intracellular compartment. pH_i remained constant during the first 3 h of recovery, but then started to rise. Both pH_e and pH_i approached control values within 24 h of recovery.

During initial recovery, the coelomic P_{CO_2} rose above control values, reaching a maximum after 3 h. After 1 h, the P_{O_2} had increased to above 40 Torr (Figs 1, 2). The bicarbonate content remained low in the coelomic fluid during the first hours of recovery, whereas it was elevated in the tissue. After 24 h of recovery, intra- and extracellular bicarbonate contents and P_{CO_2} had more or less reached control levels (Fig. 1).

Extracellular pH values and bicarbonate concentrations during long-term recovery were highly variable in small animals, which had to be killed for the determination of intracellular pH and tissue metabolite concentrations. Observed differences were sometimes not significant (cf. Fig. 1). Therefore, in a more sensitive design, the influence of postanaerobic recovery on the extracellular acid–base status was monitored individually in three large specimens of *Sipunculus nudus* by repeated

sampling of coelomic fluid during the normoxic, hypoxic and posthypoxic incubation periods. This procedure is impossible in small specimens because of the limited volume of coelomic fluid. In every animal the pattern of results was essentially the
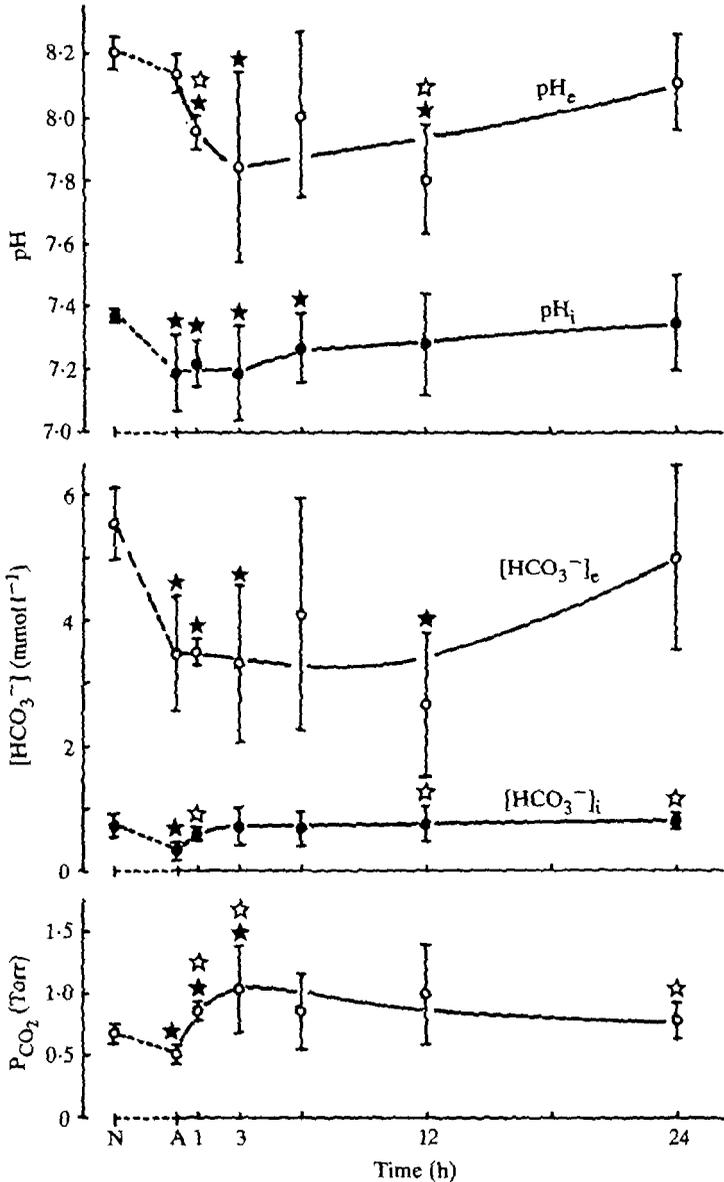

Fig. 1. Extracellular (coelomic plasma) and intracellular (muscle cell) pH, bicarbonate concentrations and P_{CO_2} during recovery following 24 h of experimental anaerobiosis in small specimens of *Sipunculus nudus* collected in March (N, normoxia; A, anaerobiosis; $\bar{x} \pm$ s.D., $N = 5$; March animals). Closed stars (★) indicate significant differences (two-sample t-test) from control values. Open stars (☆) represent significance of deviation from values obtained after 24 h of anaerobiosis ($P < 0.05$).
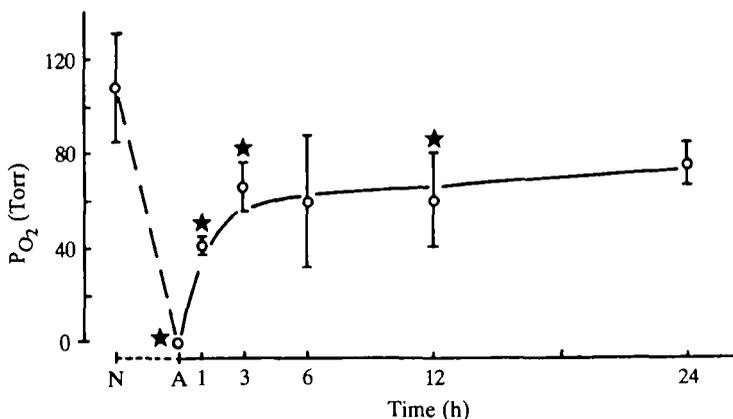
Fig. 2. Coelomic fluid P_{O_2} during recovery following 24 h of experimental anaerobiosis (N, A, ★, see Fig. 1; $\bar{x} \pm$ s.d., $N = 5$; March animals).

same as described for small animals (Fig. 3). The mean pH_e decreased significantly by 0·14 pH units during anaerobiosis, starting from 7·97 ± 0·04, and fell significantly by an additional 0·29 pH units during the first 3 h of recovery. After 6 h, pH_e began to rise and reached normoxic control values after 24 h of recovery. Extracellular bicarbonate concentration and P_{CO_2} dropped during anaerobiosis. P_{CO_2} rose during the first 3 h of recovery, whereas the bicarbonate content decreased slightly. From 6 to 24 h of recovery, P_{CO_2} and bicarbonate concentrations approached control levels.

Presentation of pH, P_{CO_2} and bicarbonate concentration in a pH–bicarbonate diagram allows evaluation as to whether the observed changes in the acid–base status are caused by respiratory or non-respiratory processes (Figs 4, 5). Buffer values of coelomic fluid and muscle tissue were adopted from Pörtner *et al.* (1984*a*). During 24 h of anaerobiosis, a non-respiratory acidosis developed in both intra- and extracellular compartments. In the intracellular space, pH started to increase after 3 h of recovery, when the P_{CO_2} had reached a maximum. The acidosis was compensated progressively, mainly by base equivalents of non-respiratory origin during 24 h (Fig. 4). In the coelomic fluid, the non-respiratory acidosis observed during 24 h of anaerobiosis was aggravated by protons of respiratory and mainly non-respiratory origins during 3–6 h of recovery (Fig. 5). Subsequently, non-respiratory processes led to an increase of pH as already pointed out for the intracellular space.

The changes in the intra- and extracellular acid–base status were analysed quantitatively (Fig. 6). Respiratory processes could be demonstrated to be of minor importance for the observed changes, exhibiting a slightly positive base excess during anaerobiosis and a negative base excess during initial recovery which turned into a positive base excess during long-term recovery. Plotting of the non-respiratory changes demonstrated the severe proton load in the extracellular compartment, especially during initial recovery, which was progressively reduced after 6 h. The amount of protons expected from organic acids present in the coelomic plasma (measured in plasma samples of the same animals) changed independently of the amount of non-respiratory H^+ ions (Fig. 6, dissociation of 1 mol H^+ mol^{-1} of acetate

and propionate and $2\,\text{mol}\,H^{+}\,\text{mol}^{-1}$ of succinate is assumed). The concentration of organic acid anions had already decreased when the amount of non-respiratory protons rose during initial recovery.

The amounts of non-respiratory protons in the intra- and extracellular compartments were calculated from the mean values of pH and bicarbonate content in small March animals (Fig. 7). During anaerobiosis most of the protons were trapped intracellularly. During recovery, an increase of the amount of non-respiratory protons was observed only in the extracellular space.
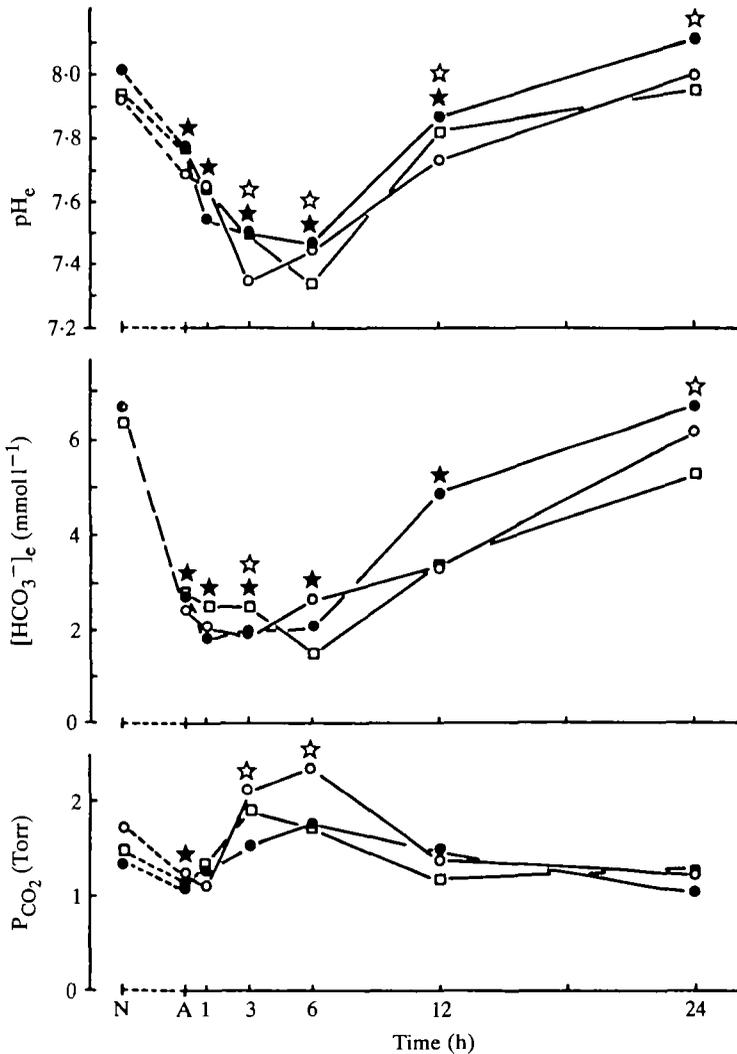

Fig. 3. Coelomic plasma pH (pH_e), bicarbonate concentration and P_{CO_2} during recovery following 24 h of anaerobiosis monitored in three large *Sipunculus nudus* (● 43·6 g, □ 37·7 g, ○ 35·8 g) in order to demonstrate that the pattern of changes in the acid–base status is similar in every animal (N, A, ★, ☆, see Fig. 1; paired-sample *t*-test).

Comparison of March and October animals

In animals collected during October, metabolite concentrations (see Pörtner *et al.* 1986) and extracellular acid–base status were investigated (Table 1). pH_e decreased by 0·45 pH units during anaerobiosis, starting from 7·97 under normoxia, and fell by
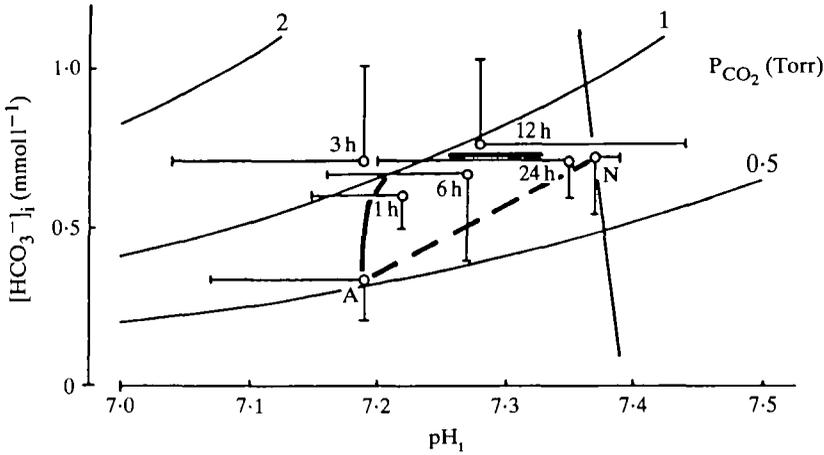

Fig. 4. Changes in the intracellular acid–base status of the musculature during recovery following 24 h of experimental anaerobiosis, presented in a pH–bicarbonate diagram. The slope of the buffer line was adopted from Pörtner, Grieshaber & Heisler (1984*a*). N, A, see Fig. 1.
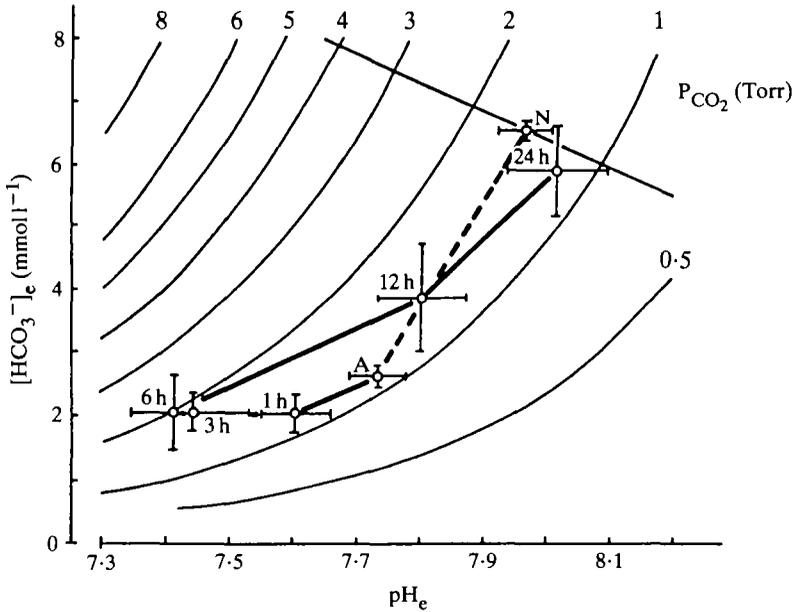

Fig. 5. Changes in the coelomic fluid acid–base status during recovery following 24 h of experimental anaerobiosis, presented in a pH–bicarbonate diagram. The slope of the buffer line was adopted from Pörtner, Grieshaber & Heisler (1984*a*). N, A, see Fig. 1.
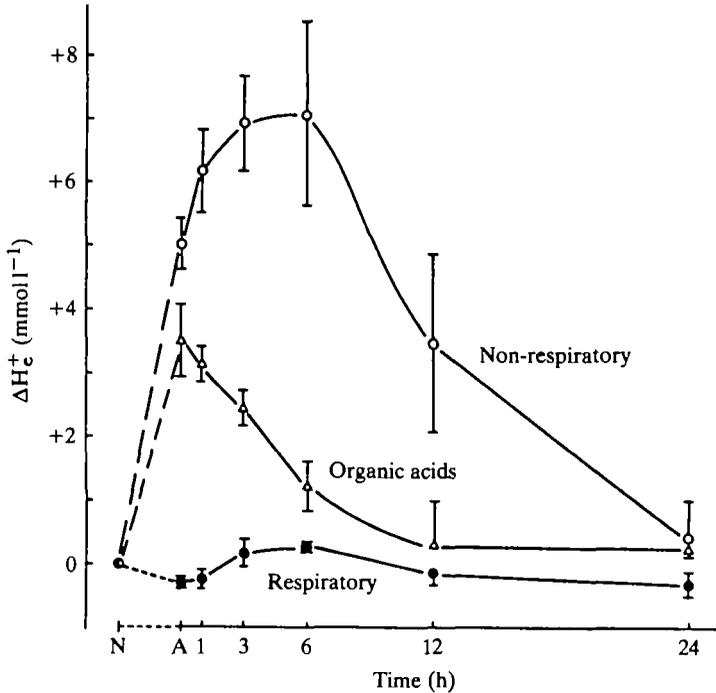
Fig. 6. Respiratory and non-respiratory changes in the extracellular acid–base status of large specimens of *Sipunculus nudus* during recovery following 24 h of anaerobiosis compared to the amount of protons calculated from the stoichiometric dissociation of accumulated organic acids (succinic, propionic and acetic acid). The negative base excess observed during the first 3–6 h of recovery is not reflected by concomitant changes in the concentration of organic acid anions. N, A, see Fig. 1.

an additional 0·39 pH units to 7·03 during the first 3 h of recovery. The bicarbonate concentration fell from 4·4 to 0·7 mmol l^{-1} and did not start to rise until after 3 h of recovery. P_{CO_2} reached a maximum of 1·7 Torr after 6 h. After 24 h of recovery, pH, P_{CO_2} and bicarbonate concentration had approached normoxic values.

DISCUSSION

Comparison of March and October animals not only reveals differences in concentrations of anaerobic metabolites (Pörtner *et al.* 1986) but also differences in changes in the acid–base status during 24 h of anaerobiosis. In October animals, the higher levels of anaerobic end-products, predominantly of octopine and strombine, represent a higher amount of protons generated by anaerobic metabolism (Pörtner, 1982; Pörtner *et al.* 1984*b*). This obviously led to a drastic fall of extracellular pH during anaerobiosis. Since changes in extra- and intracellular pH correlate during experimental hypoxic exposure (cf. Pörtner *et al.* 1984*a*), a severe intracellular acidosis may be assumed to occur as well. These conclusions clearly demonstrate that the extent of the acidosis observed during environmental hypoxia depends on the amount of accumulated metabolites and, therefore, is correlated to the rate of

anaerobic metabolism. Comparison of March and October data shows that an increase of the metabolic rate mainly means an increase of cytosolic glycolysis. This implies that the ratio of proton generation to ATP formation in metabolism is changed from a lower value towards unity. Apart from high expenditure of stored substrates, the ratio of $1 H^+$ per ATP formed is another disadvantageous feature of the Embden–Meyerhof pathway as compared to the succinate–propionate pathway (acetate and propionate formation from aspartate or glycogen, succinate and alanine formation from aspartate; cf. Pörtner *et al.* 1984*b*).

As already pointed out by Pörtner *et al.* (1984*a*), respiratory processes only negligibly changed the picture of variations in the acid–base status which were caused by the metabolic impact during experimental hypoxia, the more so since normocapnic conditions were maintained during the present study. This is also true for the recovery period. Respiratory protons transiently accumulated during the first 6 h of recovery and slightly aggravated the extracellular non-respiratory acidosis (Fig. 6). The increase of P_{CO_2} above control values is very likely to be caused by an increase of oxygen consumption, indicating the repayment of an oxygen debt during initial recovery. Inhibition of gas exchange can be excluded as an improbable reason for this finding, because the animals were probably ventilating and the concentration of CO_2 was kept constant in the ambient water throughout the experiments. Possibly the increase in $\dot{V}_{O_2}$ is also linked to an augmented ventilatory activity. Probably due to ventilation, the P_{O_2} in the coelomic fluid quickly rose during initial recovery. Haemerythrin, which was found to be saturated with oxygen at 20 Torr (Pörtner, Heisler & Grieshaber, 1985), can be expected to have been completely loaded after 1 h of recovery in March and after 3 h in October animals. The increase of P_{CO_2} was

Table 1. *Coelomic plasma pH (pH$_e$), bicarbonate concentration (mmol l^{-1}), P_{CO_2} (Torr) and P_{O_2} (Torr) during recovery following 24 h of anaerobiosis in animals collected during October*

|	pH$_e$	[HCO$_3^-$]$_e$	P_{CO_2}	P_{O_2}
Normoxia	7·97	4·42	1·02	42·6
	(7·80, 8·13)	(3·27, 5·57)	(0·72, 1·32)	(27·7, 57·5)
Anaerobiosis	7·42	0·71	0·73	0
	(7·32, 7·51)	(0·52, 0·90)	(0·39, 1·07)	
Recovery				
1 h	7·22	0·52	0·78	13·8
	(7·18, 7·26)	(0·43, 0·61)	(0·72, 0·84)	(6·5, 21·1)
3 h	7·03	0·51	1·31	37·9
	(6·97, 7·10)	(0·47, 0·55)	(1·11, 1·51)	(28·3, 47·5)
6 h	7·12	0·97	1·73	32·0
	(6·96, 7·28)	(0·60, 1·34)	(1·69, 1·77)	(19·1, 45·0)
12 h	7·62	1·67	0·91	44·8
	(7·53, 7·72)	(1·17, 2·17)	(0·84, 0·98)	(28·6, 61·0)
24 h	7·87	2·66	0·81	36·0
	(7·78, 7·95)	(2·38, 2·94)	(0·71, 0·91)	(31·0, 41·0)

Mean values and values from individual animals are shown, $N = 2$.

higher after 6 h of recovery in October than in March animals, an observation that corresponds to the higher metabolic rate in October animals as derived from the concentrations of anaerobic metabolites (Pörtner et al. 1986).

The correlation between changes in the intra- and extracellular acid–base status during hypoxic exposure was not as clear as in the study of Pörtner et al. (1984a), who reported pH_i and pH_e to be similarly affected by hypoxia. In the present study, the mean intracellular pH dropped by 0·18 pH units, whereas extracellular pH fell insignificantly by only 0·07 pH units in the same animals. A closer examination reveals that, in the study of Pörtner et al. (1984a), pH_i and pH_e had both fallen during 24 h of anaerobiosis by 0·3–0·4 pH units as compared to control values, i.e. pH reduction was higher than in the March animals of the present study, presumably because of different metabolic rates. The discrepancy between pH_e and pH_i reductions in small March animals may be attributed to the kinetics of proton distribution. Non-respiratory protons are consumed or generated in the intracellular compartment, and there may be a delay in proton exchange with the extracellular fluid, which is more clearly seen in animals with low metabolic rates and minor pH changes.

During recovery, in contrast to the situation in anaerobiosis, there was no correlation between changes of pH_e and pH_i. During anaerobiosis most of the protons had been trapped in the intracellular space. With the onset of recovery under normoxia, however, additional non-respiratory protons appeared in the extracellular space. Since proton absorption or release by metabolism occurs in the intracellular compartment, the protons, which during 3–6 h of recovery were found in the coelomic plasma, have probably been released from the musculature into the extracellular space. Metabolic changes in the coelomic cells are minor compared with the amounts of protons observed (Pörtner, 1982). Consequently, the extracellular pH dropped by more than it had fallen during anaerobiosis. Proton release, however, was not accompanied by an accumulation of organic acid anions in the coelomic plasma. On the contrary, anions were removed from the coelomic plasma right from the beginning of recovery (Fig. 6). In accordance with the observations of Pörtner et al. (1984a), this finding leads to the conclusion that there is a discrepancy between the kinetics of proton and anion distribution. In addition, it becomes clear from the present study that intracellular pH is regulated at the expense of the extracellular acid–base status when oxygen is sufficiently available to the tissues.

The origin of proton accumulation during initial recovery becomes evident from the comparison of acid–base and metabolic events. According to the ratio of phospho-L-arginine to L-arginine plus phospho-L-arginine concentrations (Fig. 7), the major part of the phosphagen is resynthesized during the first 3 h of recovery, so that proton accumulation in the coelomic plasma and phospho-L-arginine resynthesis are highly correlated in time and also in kinetics (cf. Fig. 7). pH_i is kept constant near a value of 7·20. For this pH value, cleavage or resynthesis of any phosphagen may be written as follows (cf. Pörtner et al. 1984b):

$$R - PO_3^{2-} + H_2O + 0·289 H^+ \rightarrow R - H + 0·289 P_i^- + 0·711 P_i^{2-}.$$
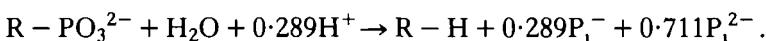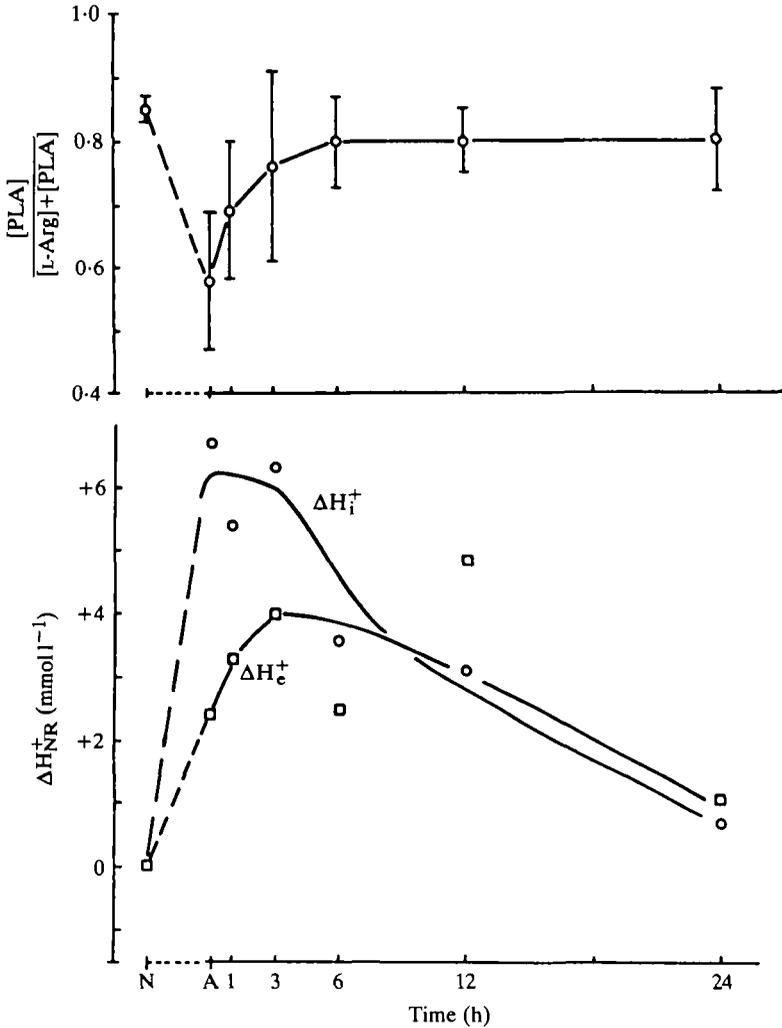
Fig. 7. Changes of the ratio of phospho-L-arginine to L-arginine plus phospho-L-arginine contents in the musculature during recovery following 24 h of anaerobiosis (data from Pörtner, Vogeler & Grieshaber, 1986) compared with non-respiratory (NR) changes in the acid–base status of the same animals. During the first 3 h of recovery surplus protons accumulate in the extracellular (squares) but not in the intracellular (circles) compartment. The repletion of the phospho-L-arginine pool and the acidosis developing during the first 3 h of recovery display similar time courses (cf. Fig. 6). N, A, see Fig. 1.

Resynthesis of phospho-L-arginine is clearly accompanied by proton generation as can be derived from the general physico-chemical properties of the phosphagens (Meyerhof & Lohmann, 1928; Lipmann & Meyerhof, 1930). Obviously, protons which have been absorbed by cleavage of the phosphagen during periods of organic acid accumulation in hypoxia or exercise are released during the subsequent recovery period. This underlines the H^+ buffer function of the phosphagens during periods of anaerobiosis. Postanaerobic restoration of the aerobic energy status may allow an

easier disposal of the extra proton load. Proton release by phosphagen resynthesis, however, has rarely been described in the literature since it was done by Lipman & Meyerhof (1930). In human muscle, for example, a transient intracellular acidosis was observed during recovery from exercise by high time resolution $^{31}P_{NMR}$ (Taylor et al. 1983). In Sipunculus nudus, the metabolic rate is much lower, especially after periods of environmental hypoxia. In March animals, efficient regulation of pH_i, therefore, led to an immediate release of these protons to the extracellular space.

In order to evaluate whether there was a quantitative correlation between proton release by phosphagen resynthesis and proton accumulation in the plasma, phospho-L-arginine, L-arginine and ΔH_e^+ values are given in Table 2. Since the sum of the concentrations of all L-arginine derivatives was rather variable (cf. Table 1, Pörtner et al. 1986), but is assumed to be kept constant during anaerobiosis (cf. Pörtner et al. 1984c), the concentrations have been recalculated from the mean value of this sum for all animals investigated. The phosphagen resynthesized during 3 h of recovery amounts to $7·4 \mu mol g^{-1}$ wet weight, which is equivalent to a proton yield of $2·1 \mu mol g^{-1}$ wet weight. The musculature, which may be assumed to be the only tissue exhibiting a high turnover of phosphagen, constitutes about 80 % of the tissues, whereas 52 % of the animal is coelomic fluid (Pörtner, 1982). The total extracellular fluid represents 62 % of the animal (calculated from fractional values of water content and extracellular space, Table 2), and of this portion approximately 15 % is coelomic cells (H. O. Pörtner, unpublished data). In the coelomic cells, $1·34 \pm 0·28 \mu mol$ phospho-L-arginine g^{-1} fresh weight has been found during aerobiosis, which is totally depleted during 24 h of anaerobiosis (Pörtner, 1982) and which during resynthesis will only slightly influence the proton load of the plasma

Table 2. Concentrations ($\mu mol g^{-1}$ fresh weight) of phospho-L-arginine and L-arginine in the musculature (mean values from five March animals, see text) and the proton yield of phospho-L-arginine resynthesis during 3 h of recovery compared to the negative base excess observed in the extracellular acid–base status

	Phospho-L-arginine	L-Arginine	ΔH^+ Phospho-L-arginine	ΔH^+ AB status
Normoxia	33·5	5·9		
Anaerobiosis	22·2	16·4		
Recovery			+1·5	+1·6
1 h	27·1	12·1		
3 h	29·6	9·4		
6 h	31·3	7·9		
12 h	31·7	7·9		
24 h	31·8	7·4		

The amount of $1·5 mmol H^+ l^{-1}$ coelomic plasma originating from the repletion of the phosphagen pool is close to the value of $1·6 mmol H^+ l^{-1}$ coelomic plasma derived from the events in the acid–base (AB) status.

The fractional values of water content and extracellular space in the musculature (80 % of the tissues) were $0·798 \pm 0·016$ and $0·257 \pm 0·059$, respectively.

(0·06 mmol $H^+ l^{-1}$, assuming $pH_i = 7·2$). An amount of 1·5 mmol $H^+ l^{-1}$ plasma, however, may result from the resynthesis of phospho-L-arginine in the musculature of March animals, which is close to the amount of H^+ evaluated from the observed changes in the acid–base status (1·6 mmol $H^+ l^{-1}$ plasma). This balance is feasible only for a period of 3 h recovery, since afterwards ionic exchange with the ambient sea water significantly contributes to changing the amount of non-respiratory protons present in the extracellular compartment (H. O. Pörtner & N. Heisler, unpublished observations). In addition, during the first hours of recovery, con-centration changes of other metabolites had only a minor influence on the acid–base status in March animals. A decrease of succinate and propionate contents, for instance, was accompanied by an accumulation of malate and aspartate and, there-fore, did not lead to a marked reduction of the amount of carboxyl groups present (cf. Pörtner *et al.* 1984*b*).

In October animals, strombine appeared to increase by 5 μmol g^{-1} wet weight, which during the first hour of recovery may have aggravated the acidosis provoked by replenishment of the phosphagen. Therefore, pH_e dropped further than in March animals and a transient decrease of pH_i cannot be excluded because of the higher metabolic rate observed.

A period of 24 h of recovery led to the restoration of values of pH_i, pH_e, P_{CO_2} and bicarbonate content typical for normoxia. The analysis of metabolism demonstrated, however, that strombine, alanine and, to a minor extent, acetate concentrations remained elevated in March animals. Formation of these metabolites from glycogen yields a stoichiometric amount of protons (Pörtner, 1982; Pörtner *et al.* 1984*b*: for the net proton balance of alanine formation, it is irrelevant whether the ammonium group originates from aspartate or from free ammonia, since the pattern of dissociation is the same in both cases). Therefore the organism obviously disposes of surplus non-respiratory protons which are not reabsorbed by gluconeogenesis or by oxidation to CO_2. Correspondingly, pH regulation during recovery could be demonstrated to occur also *via* ionic exchange with the ambient water (H. O. Pörtner & N. Heisler, unpublished observations).

In summary, restoration of aerobic homeostasis after a period of anaerobiosis in *Sipunculus nudus* proceeds with a rapid replenishment of the phosphagen and, thereby, restoration of the energy status. The priority of the resynthesis of arginine phosphate is such that even an additional acid–base disturbance is accepted for the extracellular fluid. Intracellular pH is regulated efficiently as soon as oxygen is available to the tissues. The plasma is the medium utilized as a sink for protons or base equivalents during ionic regulation of intracellular pH (cf. Pörtner *et al.* 1984*a*). During periods of high metabolic rate or energy consumption, which are likely to depend on the season (Pörtner *et al.* 1986), anaerobic glycolysis becomes involved during recovery and even aggravates the acidosis. Aerobic metabolism is then not sufficient to restore the energy status rapidly enough, although repayment of an oxygen debt is observed during the same period. During long-term recovery, restoration of the aerobic acid–base status is accomplished even if some anaerobic metabolites remain: a high concentration of these obviously has little effect on

homeostasis in the sense that their disposal is of lower priority in the preparations for the next period of anaerobiosis.

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