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PROTON-EQUIVALENT ION TRANSFER IN *SIPUNCULUS NUDUS* AS A FUNCTION OF AMBIENT OXYGEN TENSION: RELATIONSHIPS WITH ENERGY METABOLISM

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Accepted 1 November 1990

Summary

Proton-equivalent ion transfer processes between animals and ambient water were determined under normoxic control conditions during anaerobiosis and the subsequent recovery period in the marine worm *Sipunculus nudus* L. During anaerobiosis and recovery, transepithelial H^+-equivalent ion transfer was generally correlated with changes in extracellular pH, with some disparities in 'spring' animals. The typical initial alkalosis induced by phosphagen cleavage during early anaerobiosis was reflected by a loss of basic equivalents. The acidosis, which developed later, reflecting production of acidic metabolic intermediates, resulted in a relatively small net extrusion of protons into the water. The coelomic acidosis during recovery was greatly exaggerated by the release of protons during phosphagen repletion and by the considerable elevation of P_{CO_2} after normoxia had been reattained. The acidosis stimulated the net release of H^+ to the water at a rate several times higher than that during anaerobiosis. The efficient transfer of protons from the body fluids to the environmental water during recovery facilitated normalization of coelomic pH, long before protons dissociated from the large amounts of organic acids produced as anaerobic intermediates could be removed from the body fluids by metabolism.

Although the transfer of net H^+ equivalents to the water coincided with coelomic acidosis, the rates of transfer during different periods of the experiment were primarily correlated with overall metabolic rate. Low net proton transfer rates associated with anaerobiosis were not sufficient to maintain acid–base parameters typical for normoxia, whereas re-establishment of aerobic conditions facilitated a greatly increased transepithelial H^+ transfer rate. These data suggest that the transfer capacity of the energy-consuming translocation mechanism may

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status in intracellular and extracellular body fluid compartments and the environmental water.

Materials and methods

Experimental animals

Specimens of *Sipunculus nudus* L. were dug up close to the low water line of intertidal flats at Locquemeau, Brittany, France, in March and September (referred to as 'spring' or 'autumn' animals, respectively). Large animals (26–74 g) were selected for experimentation and kept in tanks with a 10–20 cm deep layer of sand from the original habitat. The holding tank was supplied with filtered and recirculated artificial sea water at 10–15 °C. Net release of base equivalents by the spring animals during long-term holding caused an increase of water bicarbonate levels from 2.3 to 3 mmol l^{-1}, whereas the [HCO_3^-] for autumn animals was adjusted by titration with HCl to a constant level of about 2.3 mmol l^{-1}.

Experimental apparatus

The experiments were conducted in a closed seawater recirculation system (Fig. 1), with dimensions chosen according to the size of the animal to mimic a natural burrow (Pörtner, 1982). The system contained between 300 and 470 ml of artificial sea water and was thermostatted to 15±0.05 °C. The initial seawater bicarbonate level was adjusted to 2.3 mmol l^{-1}, but was allowed to rise up to 3 mmol l^{-1} during the experiment as a result of the release of bicarbonate equivalent ions by the animal. Matching sizes of animals and chambers prevented the animals from turning around and allowed us to use a minimal water volume. The chamber was connected to a gas exchange column *via* a 2.5 mm mesh grid. To prevent evaporative loss of water the gases feeding the gas exchange column were thermostatted to 15 °C and humidified. Circulation of the water was provided at a rate of 100 ml min^{-1} by a roller pump (type MP-GE, Ismatec, Zürich, Switzerland). The water flow was directed at the animal from the anterior, and flowed along the body surface (Pörtner, 1982). As well as mimicking the water flow in the natural habitat, this flow direction also kept the animals from exploring and clogging the lateral water inlet of the chamber. The animal tube and aeration column were darkened to minimize any stimulus for muscular activity.

Water from the aeration column was continuously sampled by means of a second roller pump at a rate of 6.6 ml min^{-1} (Ismatec IP 4, Zürich), feeding a system for detecting changes in bicarbonate levels before being returned to the animal system (Heisler, 1978, 1984, 1989). During passage through this 'Δ-HCO_3^-' system the water was thermostatted to 30±0.05 °C and equilibrated in a series of three glass columns with fritted bottoms with humidified gas at constant P_{CO_2} (1 % CO_2, delivered by a gas-mixing pump: M 303/a-F, Wösthoff, Bochum, FRG). pH was measured by means of a glass electrode and a double electrolyte bridge Ag/AgCl reference with sleeve diaphragm connected to a high-impedance isolation amplifier (model 87, Knick, Berlin, FRG). The recording system was

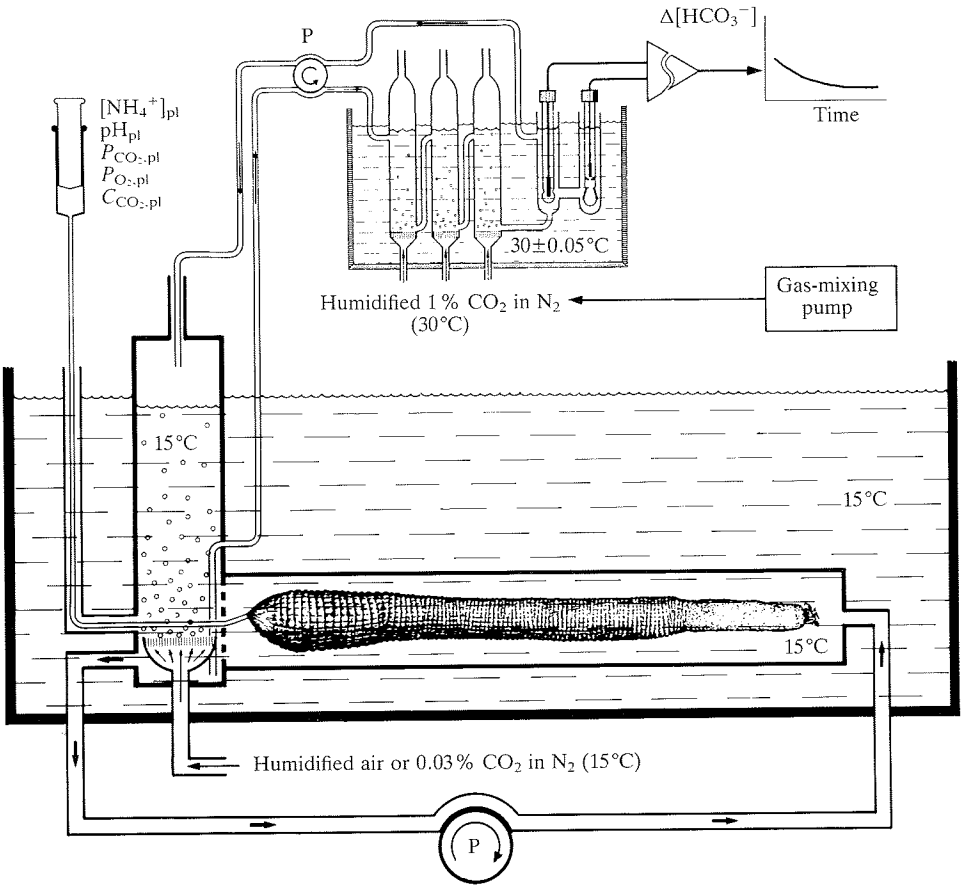

Fig. 1. Diagram of the experimental apparatus utilized for the analysis of H^+-equivalent ion fluxes in *Sipunculus nudus*. The water is recirculated through the aeration column, the animal chamber and the system for the determination of changes in bicarbonate concentration. Coelomic fluid samples were collected from an indwelling catheter. For further details see text. P, recirculation pump.

completely floating in electronic terms with the environmental water and was grounded *via* a platinum electrode close to the electrodes. After amplification and conditioning, the signal was recorded on a chart recorder and, after A/D conversion, fed into a microcomputer system for on-line analysis (PSI 98, Kontron).

This type of apparatus and water conditioning (30°C) provided a sufficiently fast electrode response and a low drift of the electrode chain (<0.001 pH units per 24 h; see Heisler, 1989). The equilibration gas was chosen to give a pH close to 7, a range where non-bicarbonate buffering is negligible in sea water (Heisler, 1986*a*, 1989). At constant P_{CO_2} and temperature, changes in pH result exclusively from changes in water bicarbonate concentration (Heisler, 1986*a*, 1989). The accuracy of the respective calculations (Heisler, 1986*a*) was checked using a calibration

procedure including the addition of known amounts of HCl and $NaHCO_3$ to the system and by analysis of total CO_2 in water sampled from the Δ-HCO_3^- system.

Experimental procedure

Prior to experimentation the animals were catheterized by introducing a 2–3 cm length of PE 60 or PE 90 tubing (75 cm) into the body cavity after puncturing the posterior end of the body. The tubing was secured with cyanoacrylate glue (type 7432, Bostik, Oberursel, FRG.). After placing the animal in the chamber as described above (Fig. 1), the cannula was fed out of the system through the grid and the aeration column. After 24 h of acclimation at P_{O_2} values between 13.3 and 16 kPa (100–120 mmHg) and P_{CO_2} between 0.053 and 0.093 kPa (0.4–0.7 mmHg), the control rate of H^+-equivalent ion release was determined for another 24 h. Hypoxic conditions (P_{O_2}<0.4 kPa=<3 mmHg) were introduced within 15–30 min by bubbling the water with normocapnic nitrogen during passage through the gas exchange column. 0.03 % CO_2 in pure N_2 was provided by gas-mixing pumps. After 24 h of hypoxic incubation, normoxic conditions (see above) were restored by aerating the water. This led to an increase of water P_{O_2} to above 12 kPa (90 mmHg) within 5 min. Recovery from anaerobiosis was followed for 48 h after the end of hypoxic incubation.

During the whole period of aerobiosis, anaerobiosis and subsequent recovery, coelomic fluid was sampled anaerobically *via* the indwelling catheter at the intervals indicated in the figures. System water was exchanged with fresh sea water at intervals of 24 h. At these times, 50–100 ml samples were taken for the analysis of volatile fatty acids until it became evident that they did not accumulate in the recirculated water because of the vigorous gas flow in the gas exchange and equilibration columns. Water samples taken at shorter intervals for measurement of water ammonium concentration were replaced with fresh sea water to maintain water levels in the aeration column. Thus, the release of protonated end products did not contribute to net H^+-equivalent ion transfer to the water. The dilution of the environmental water with fresh sea water was taken into account during evaluation.

Analyses

Coelomic fluid samples (about 700 μl from a total volume of 13.5–18 ml) were analyzed for extracellular pH, P_{CO_2} and P_{O_2} using a thermostatted microelectrode assembly (15±0.1 °C, 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. Total CO_2 in coelomic plasma was determined after centrifugation and analysis of supernatant plasma samples. The CO_2 contents of plasma and water samples were analyzed by means of a Capnicon III apparatus (Cameron Instruments, Port Aransas, TX, USA), calibrated with $NaHCO_3$ standards. The resulting apparent bicarbonate values were compared with those obtained by calculation based on the Henderson–Hasselbalch equation using values for CO_2 solubility and pK_1'''

derived from the polynomials of Heisler (1984, 1986*a*). (Note: the last line term of the α-formula in Heisler, 1984, is misprinted and should read '+'.) In order not to deplete the animals of coelomic fluid, the samples used for blood gas analyses (180–250 μl) were re-infused into the animal.

Water bicarbonate levels were monitored as described above. Ammonium concentrations in water and plasma samples were determined enzymatically using standard tests (Bergmeyer, 1974). Acetate and propionate levels in water samples were analyzed by high performance liquid chromatography, as previously reported (Pörtner *et al.* 1984*c*).

Calculations

The apparent bicarbonate concentrations of plasma and water directly determined from measurement of total CO_2 were checked by application of the Henderson–Hasselbalch equation on the basis of measured pH and P_{CO_2}, and values for CO_2 solubility (α_{CO_2}) and pK_1''' derived from the polynomials of Heisler (1986*a*, 1984; see note above). The net amount of H^+-equivalent ions transferred between animal and water was determined from the changes in water bicarbonate and ammonium levels according to equation 1 (Heisler, 1986*a*):

$$H^+_{\rightarrow w} = \frac{(\Delta NH_4^+{}_w - \Delta HCO_3^-{}_w)V_w}{V_A} (\text{mmol kg}^{-1}\text{ body mass}), \quad (1)$$

where V_A is body mass (kg) of the animal and V_w is the volume of ambient water (l). This approach is in accordance with the proton balance of protein and amino acid catabolism (Pörtner, 1989).

Values are presented throughout as mean±S.E. Significance of differences ($P<0.05$) was evaluated by application of Student's *t*-test.

Results

The first series of experiments with autumn animals (collected in September, see above), performed approximately 2 months after collection, was focused on steady-state acid–base parameters and the control rate of H^+-equivalent ion exchange during long-term incubation. The acid–base status remained essentially unaffected during 1 week of incubation in aerated sea water. Coelomic pH (plasma pH, pH_{pl}) varied insignificantly between 7.87±0.10 after 24 h and 7.92±0.07 after 162 h; plasma bicarbonate (7.4±0.3 to 8.7±0.7 mmol l^{-1}) and plasma P_{CO_2} (0.28±0.04 to 0.29±0.03 kPa, 2.1±0.3 to 2.2±0.2 mmHg) did not change significantly (Fig. 2A). The apparent bicarbonate concentrations derived from measurements of total CO_2 and those calculated according to Heisler (1984, 1986*a*; see above) were essentially identical (Table 1), confirming the validity of the respective equations for the body fluids of this invertebrate. Average coelomic P_{O_2} varied insignificantly between 3.7 and 4.8 kPa (28 and 36 mmHg) (Fig. 2B), and coelomic ammonium levels increased slightly, but also insignificantly (from 0.16 to 0.28 mmol l^{-1}; Fig. 2B), during these control experiments.

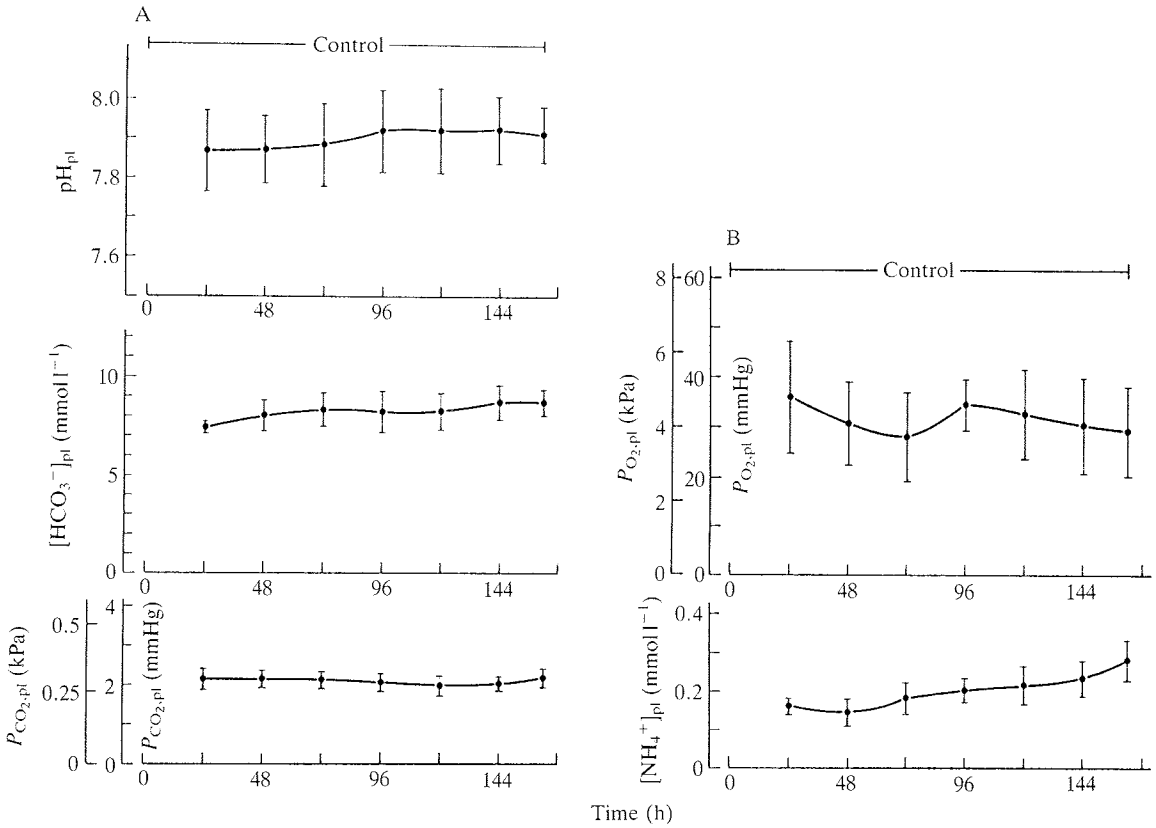

Fig. 2. (A) Acid–base variables (pH, $[HCO_3^-]$, P_{CO_2}) and (B) P_{O_2} and ammonium levels in coelomic fluid of *Sipunculus nudus* during long-term acclimation to the experimental apparatus. Homeostasis in the experimental animals is expressed by essentially constant values over 7 days (autumn animals, mean ±S.E., N=3; see text). 1 kPa=0.133 mmHg.

Bicarbonate and ammonium levels in the ambient water increased steadily in this series of experiments ($\Delta NH_4^+{}_w$=0.041 mmol kg^{-1} h^{-1}; $\Delta HCO_3^-{}_w$= 0.080 mmol h^{-1} kg^{-1} body mass). The resulting average net H^+-equivalent ion transfer from animals to water was accordingly −0.039 mmol h^{-1} kg^{-1} (Fig. 3).

Experiments on proton movements during anaerobiosis were performed about 4 (autumn animals) or 5–6 months (spring animals) after animal collection. Since volatile fatty acids did not accumulate in the ambient water, determination of net proton transfer did not include the release of protonated metabolic end products. Upon exposure to anoxia, both autumn and spring animals exhibited an extracellular alkalosis, with pH rising 0.17 units above the control value of 7.81±0.01 in autumn animals and by 0.13 units above the control value of 7.99±0.07 in spring animals (Figs 4A, 6). No acidosis occurred until 12 h after exposure of the animals to anoxia; after 24 h the depression of pH was much more pronounced in autumn animals (ΔpH=−0.21) than in spring animals

Table 1. *Comparison of bicarbonate concentrations determined from total CO_2 measurements in coelomic plasma with values calculated from measured pH and P_{CO_2} by application of the Henderson–Hasselbalch equation, based on constants for pK_1''' and α_{CO_2} determined from the polynomials of Heisler (1984*, 1986b)*

Condition	Bicarbonate concentration (mmol l^{-1} coelomic plasma)		
	Measured	Calculated	Difference
Normoxia	8.45±0.85	8.15±1.02	0.30
Anoxia (24 h)	4.90±0.67	4.84±0.76	0.06
Recovery (12 h)	5.24±0.42	5.20±0.54	0.04
Recovery (48 h)	7.82±0.42	7.86±0.60	−0.04

* Note: the last line term of the α-formula in Heisler (1984) is misprinted and should read '+'.
Values are mean±S.E., N=6.

(ΔpH=−0.10). These changes in pH were linked to a drop in plasma apparent bicarbonate levels (from 5.2±0.3 to 2.6±0.2 mmol l^{-1}, autumn animals; 9.2±0.8 to 4.9±0.7 mmol l^{-1}, spring animals) and to a fall in coelomic P_{CO_2}, which reached its minimum after 8–16 h of anaerobiosis [from 0.25±0.01 to 0.15±0.01 kPa (1.9±0.1 to 1.1±0.1 mmHg) in autumn animals and from 0.30±0.03 to 0.2±0.03 kPa (2.3±0.2 to 1.5±0.2 mmHg) in spring animals after 8 h of anaerobiosis].

Upon return to normoxia (recovery), P_{CO_2} increased significantly above control levels. pH was further reduced by 0.23 units in spring animals and by 0.26 units in autumn animals. Minimal values were attained after recovery times of 3 h in autumn animals and 6 h in spring animals. Peak values of P_{CO_2} were attained after 6–12 h, with P_{CO_2} rising by 0.25 kPa (1.9 mmHg) in autumn and 0.17 kPa (1.3 mmHg) in spring animals. In autumn animals, P_{CO_2} remained above control values even after 72 h of recovery, so that plasma bicarbonate levels stayed slightly above control values even though pH was restored to its original level within 24 h. In spring animals, P_{CO_2} slowly started to decline after 12 h of recovery and pH_{pl} and plasma bicarbonate concentration reattained control values after 48 h of recovery (Figs 4A, 6).

P_{O_2} values in the coelomic fluid of autumn animals (Fig. 4B) followed the changes in water P_{O_2}. Upon initiation of environmental anoxia, P_{O_2} fell from 6.5±0.44 kPa (48.7±3.3 mmHg) to zero, but was elevated to only 2.9 kPa (22 mmHg) upon return to normoxia. Plasma ammonium levels (0.23± 0.02 mmol l^{-1}) were significantly reduced during the initial 8 h of anaerobiosis (to 0.13±0.01 mmol l^{-1}), but were later restored to control levels. Upon return to normoxia, the ammonium concentration was significantly reduced (to 0.18±0.02 mmol l^{-1}; 12 h) and then slightly elevated above control values (0.38±0.1 mmol l^{-1}) after 48 h of recovery.

During the control period before anoxic exposure the ammonium and bicarbon-

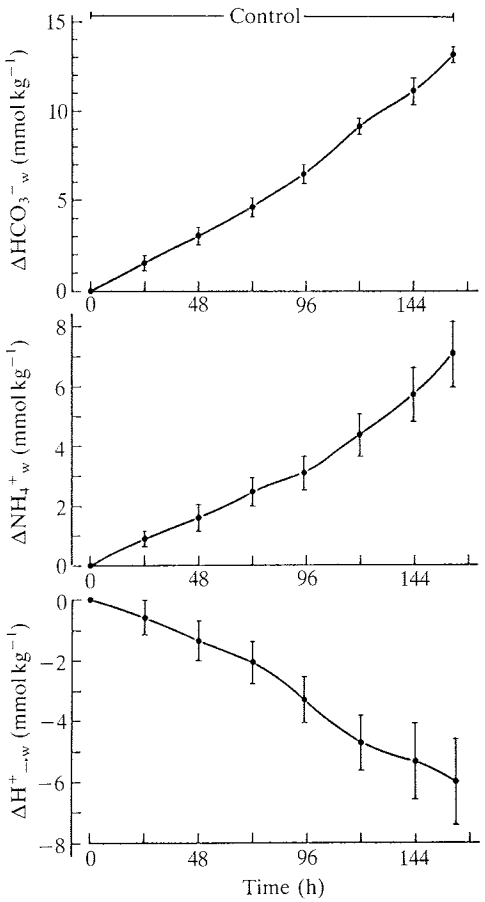

Fig. 3. Changes in bicarbonate and ammonium levels in the ambient water and net H^+ equivalent transfer ($H^+_{\rightarrow w}$) from animal to water. Under control conditions, a net uptake of H^+ equivalents compensates for the base load imposed by metabolism or gained from external sources (autumn animals, mean±S.E., N=3, see text and Fig. 2).

ate concentrations of the ambient water rose steadily (by 0.079 and 0.080 mmol kg^{-1} h^{-1}, respectively), but the rate of H^+-equivalent ion transfer was much lower (−0.001 mmol kg^{-1} h^{-1}) than that measured during the control experiments (Fig. 3), possibly because of uptake of $CaCO_3$ from the sand (see also Discussion). During anaerobiosis, the rates of both bicarbonate and ammonium accumulation were reduced compared with the control period. These changes were significant and reflected an increased net base release during the first 16 h of anaerobiosis, before the process was reversed, indicating a net proton release during the last 8 h of anaerobiosis in autumn animals (Fig. 5).

In spring animals (approximately 5–6 months after collection) the rate of net H^+-equivalent ion transfer under control conditions was much higher (−0.032 mmol kg^{-1} h^{-1}) than in autumn animals. In spring animals the rate of net

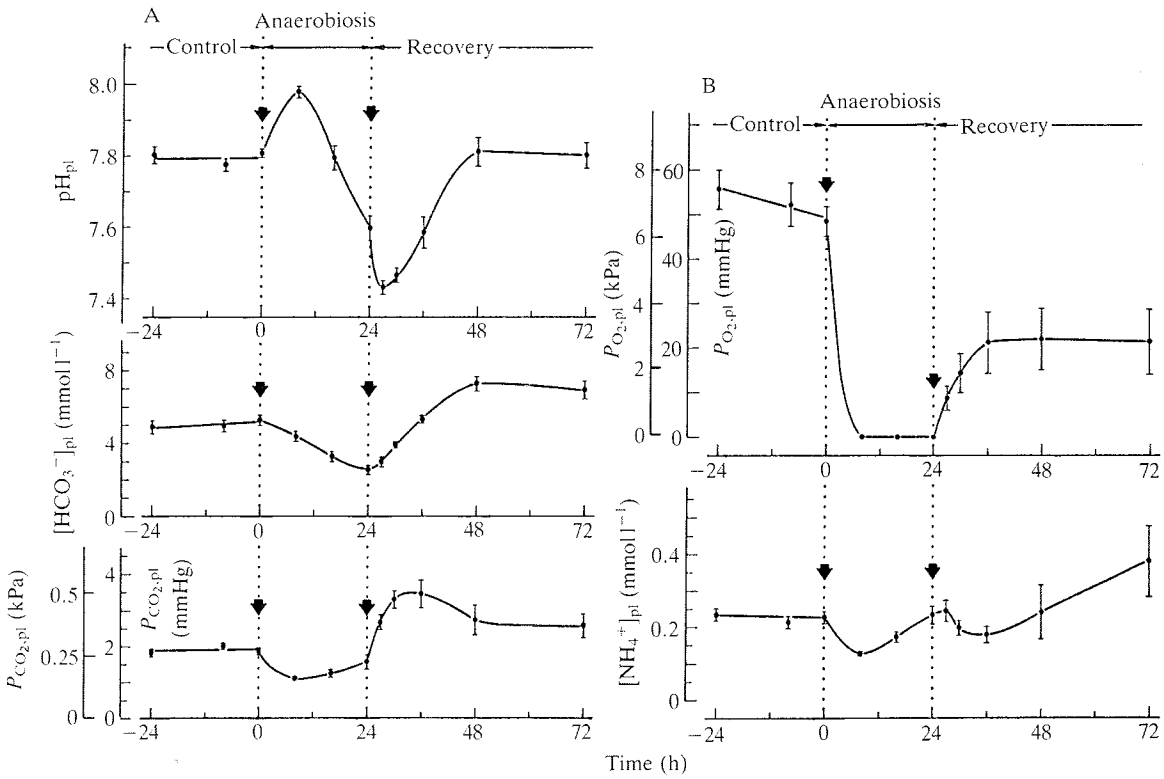

Fig. 4. (A) Acid–base variables and (B) P_{O_2} and ammonium levels in the coelomic fluid of *Sipunculus nudus* under control conditions, during 24 h of hypoxia and during the subsequent recovery (autumn animals, mean±S.E., N=8). The arrows mark the beginning and end of the period of anaerobiosis.

bicarbonate release was also higher (0.105 mmol kg^{-1} h^{-1}), whereas the rate of ammonium release was slightly lower (0.073 mmol kg^{-1} h^{-1}). In spring animals the net base release reversed to a net release of protons immediately upon exposure to anoxia, with an additional rate increase during the last 8 h of anaerobiosis (Fig. 7).

In both spring and autumn animals, the rate of H^+-equivalent ion transfer to the ambient water was greatly stimulated during the first hours of recovery (maximal rates: +0.320 mmol kg^{-1} h^{-1}, autumn animals; +0.087 mmol kg^{-1} h^{-1}, spring animals). This rise was primarily due to a considerable reduction in the rate of release of bicarbonate into the water, whereas the rate of ammonium release returned to control values in autumn animals (0.076 mmol kg^{-1} h^{-1}) or slightly reduced values in spring animals (0.057 mmol kg^{-1} h^{-1}). The control rate of bicarbonate release was reattained between 48 and 72 h of recovery in autumn animals (Fig. 5), whereas in spring animals this rate was still below the control rate at 72 h (Fig. 7). Accordingly, the net H^+ transfer in autumn animals was close to the control value after 48 h of recovery, whereas this variable did not reattain control values in spring animals for the entire recovery period (Fig. 7).

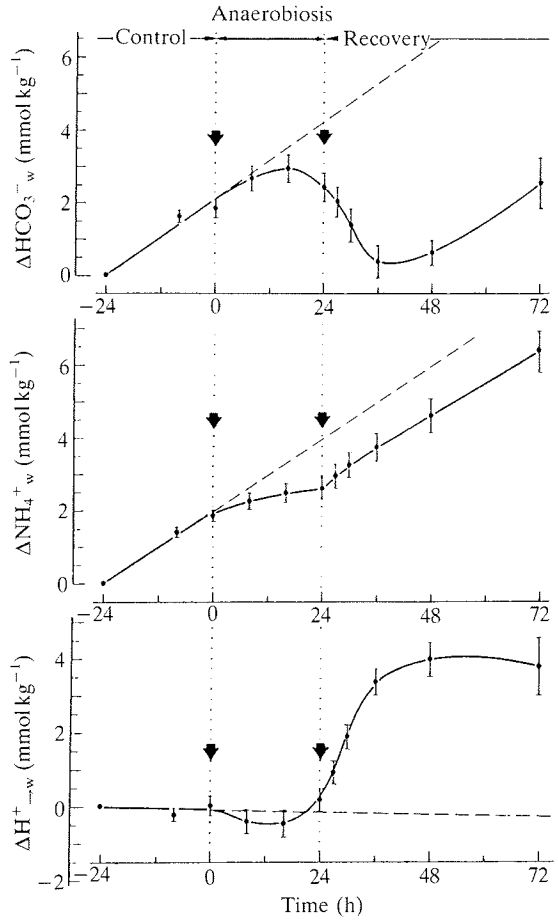

Fig. 5. H^+-equivalent ion transfer between animals and ambient water, calculated from changes in ammonium and bicarbonate levels of the water (autumn animals). Note the smaller control H^+ influx compared with Fig. 3 (see text). The control rates are extrapolated (dashed line) for the entire experimental period (mean±S.E., N=8).

Discussion

Control conditions

Determination of transepithelial H^+-equivalent ion transfer requires that the experimental animals are kept in a system with relatively high water flow and minimal volume. These somewhat unphysiological conditions applied during our experiments, however, did not significantly affect the status of the animals. Analysis of blood gases and acid–base parameters for 1 week under control conditions clearly demonstrated the ability of *Sipunculus* to maintain a steady state for prolonged experimental periods under these conditions. The rate of net H^+ transfer between animals and ambient water was also rather constant and may be taken as an indication of steady-state conditions. Net transfer of protons in

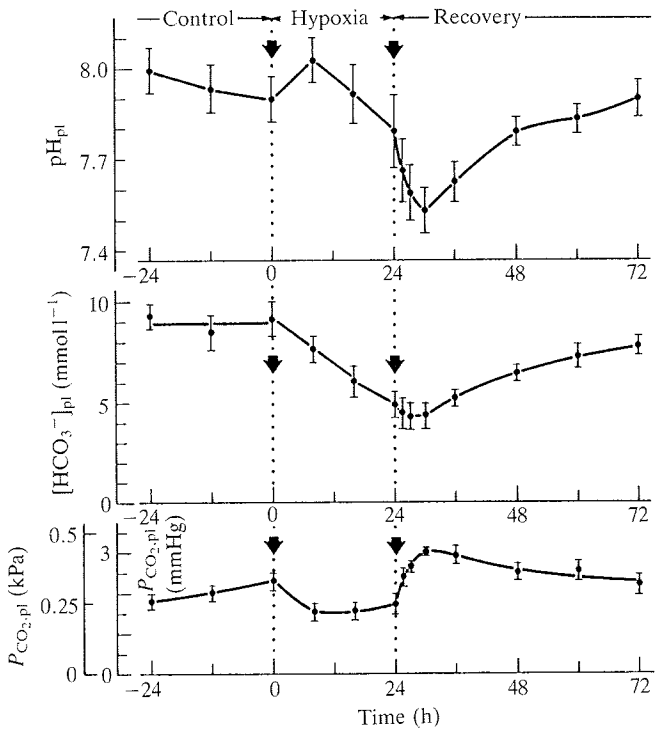

Fig. 6. Acid–base variables in the coelomic fluid of *Sipunculus nudus* under control conditions, during 24 h of hypoxia and during subsequent recovery (spring animals, mean±S.E., N=6).

Sipunculus, however, is from the animal to the water and accordingly opposite in direction to that in fish. In fish, the net proton release is due mainly to aerobic degradation of sulphur- and phosphate-containing protein and nucleic acids (Heisler, 1986*b*). The reverse fluxes observed in *Sipunculus* may be linked to differences in energy substrates utilized. Metabolic processes leading to proton consumption at aerobic steady state include degradation of carboxylic acids (for a recent review of the relationships between metabolism and acid–base regulation see Pörtner, 1989). Organic acids originating from the diatom content of sand ingested by the animals may be of some importance in this respect. Protein or amino acid catabolism, however, is evidently also a major route of metabolism, as indicated by ammonium accumulation in the ambient water. Autumn animals kept for 2 months before experimentation exhibited lower rates of ammonium production than animals from the same pool pre-acclimated in the laboratory for 4 months. The oxygen consumption/nitrogen excretion (O/N) ratio changed considerably from 27.8 after 2 months to 14.4 after 4 months. A ratio of 15.6 was measured for spring animals after 5–6 months of acclimation (based on oxygen consumption measurements by Pörtner *et al.* 1985). In squid, which depend largely on protein catabolism for energy production, the O/N ratio was reported to be

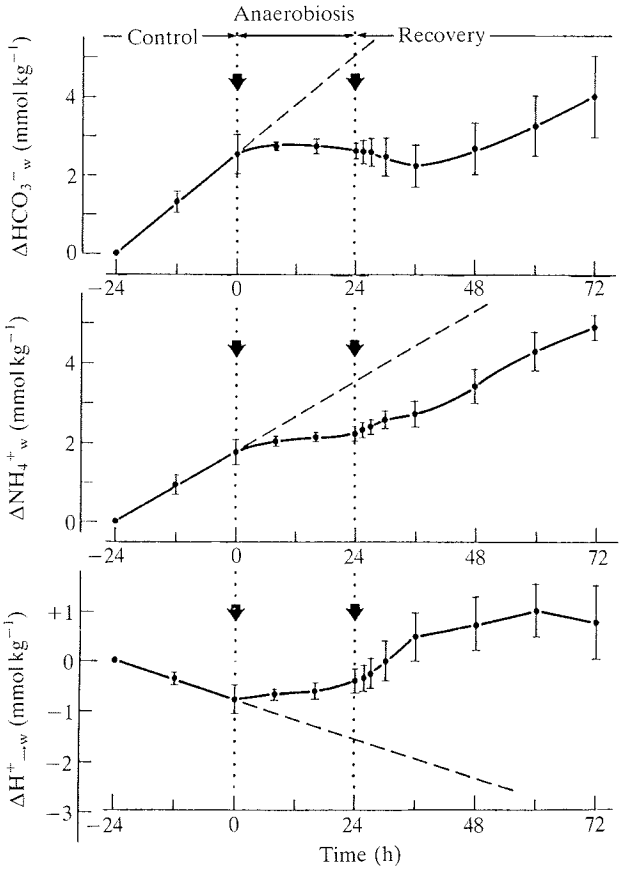

Fig. 7. H^+-equivalent ion transfer between animals and ambient water, calculated from changes in water ammonium and bicarbonate levels (spring animals). Note the difference between autumn and spring animals with respect to the net base release during the initial alkalosis of early anaerobiosis and the net rate of proton exchange during recovery (mean±S.E., N=6).

14.9 (Hoeger *et al.* 1987). This suggests that after 4 months the (autumn) animals had switched to protein catabolism for a larger fraction of energy production because of depletion of potentially basic nutrients from the sand.

Another process possibly involved in promoting a net base release by the animals may be mobilization of carbonate deposits in the sand. A lowering of pH of the gut contents during passage through the digestive tract from the naturally rather alkaline sea water to values close to coelomic pH may well support resorption of basic equivalents originating from carbonate mobilization. These, in turn, have to be eliminated from the animal in soluble form. This source of basic equivalents will be depleted after the animals have used the same sand for two or more feeding cycles (especially in those tanks where water bicarbonate levels were kept low by the addition of acid), leading to an apparently more acidotic overall

output of metabolism (as seen in autumn animals acclimated for 4 rather than for 2 months) (cf. Figs 3 and 5).

Extracellular acid–base status: anaerobiosis and recovery

Exposure to an anoxic environment and accordingly anaerobiosis led to an initial alkalosis in both spring and autumn animals. This alkalization is mainly attributable to the reduced production of CO_2 during anaerobiosis and the resulting lower coelomic P_{CO_2}, whereas the non-respiratory component is likely to be attributable to hydrolysis of phospho-L-arginine (Pörtner *et al.* 1984*b*). The production of acidic anaerobic metabolic end products leads to normalization and finally to a considerable acidification of coelomic plasma pH after 16–24 h of anoxia. This acidosis is further aggravated upon return to normoxia and aerobiosis, partly as a result of the pronounced elevation of P_{CO_2} during the first 12 h of recovery indicative of the repayment of an oxygen debt (Pörtner *et al.* 1986*b*). This interpretation is supported by the observation that, owing to the elevated metabolic rate, coelomic P_{O_2} values remain low for the entire recovery period (Fig. 4B). The non-respiratory component of the acidosis is related to the resynthesis of phospho-L-arginine (see below). Coelomic bicarbonate levels are restored and even elevated above normal in the course of the recovery period. The elevation during late recovery is related to the increased P_{CO_2} and serves to restore plasma pH.

The changes in plasma ammonium levels are probably governed to some extent by the changes in P_{NH_3} of the environmental water, which are closely correlated with the procedure of flushing the system with fresh sea water. Flushing was performed at the beginning of the anoxic and the recovery periods, and is clearly accompanied by reductions in plasma $[NH_4^+]$ levels (Fig. 4B). The plasma ammonium level returned to normal at the end of the anoxic period, but rose considerably above control values during the second half of the recovery period. This is probably due to the considerable elevation of metabolic rate associated with repayment of the oxygen debt. Two other factors should be mentioned which may also be involved in the initial reduction of plasma ammonium levels upon return to normoxia: a severe intracellular acidosis developing in parallel with the extracellular acidosis will cause considerable trapping of ammonium in the tissues, and amino acid stores depleted during anaerobiosis may be replenished. There is, however, little direct evidence for such speculation.

H^+-equivalent ion transfer: anaerobiosis

In autumn animals, the alkalosis during the first 16 h of anoxia resulted in a very slight enhancement of the normoxic rate of base release (negative net H^+ flux). A reversal of the net H^+ flux occurred only at the end of the anoxic period. The offset of the cumulative ammonium release curve during recovery from the extrapolated normoxic control curve (Fig. 5) – due to an almost complete halt in the elimination of ammonium – was essentially matched by a similar reduction in net bicarbonate release. Since plasma ammonium levels were not enhanced during

anoxia and tissue storage of ammonium is naturally limited, the rate of elimination of ammonium clearly reflects a reduction in metabolic ammonium production. This reduction indicates a switch to glycogen as the main substrate for anaerobic metabolism (see Grieshaber *et al.* 1988, for the pathways involved). The observed transepithelial transfer of H^+ equivalents during anaerobiosis is closely linked to changes in plasma pH, a base release during alkalosis being reversed to a net proton release during the period of progressive metabolic acidosis. This finding supports the conclusion that acid–base regulation is not completely terminated during anaerobiosis, but that ion exchange rates are probably reduced (see below).

The relationship between coelomic pH and net transepithelial ion transfer observed in autumn animals is not duplicated in spring animals. A net extrusion of protons takes place during the early stages of anoxia (Fig. 7). This phenomenon could be explained if the initial alkalosis were reduced owing to a decrease in the rate of phospho-L-arginine depletion compared to that in autumn animals. Another possible explanation depends on the anatomical arrangement of muscle tissues in *S. nudus*. Intracellular muscle compartments represent a large fraction of the fluid space interfacing with the ambient water. Intracellular muscle pH, rather than coelomic fluid pH, may accordingly be the controlling factor for transepithelial ion transfer processes, especially if disequilibria between coelomic fluid and intracellular fluid pH occur. Further study is required to clarify whether the vertebrate model of bulk extracellular fluid as a 'second defence' line for intracellular compartments is applicable to the fluid compartment system in *S. nudus*.

Another explanation for the discrepancy between coelomic fluid pH (alkalotic) and the apparent net extrusion of protons from the body fluids may be sought in the difficulty of comparing extrapolated normoxic control fluxes with fluxes obtained using different substrates under completely different metabolic states. During anaerobiosis, metabolism is shifted towards the breakdown of carbohydrates, which during aerobiosis would produce no acid–base relevant effect. Thus, the normoxic control rate should not be taken into consideration during anaerobiosis, and the amount of protons released to the water would have to be determined as the difference between bicarbonate and ammonium release rates alone. On this basis, the average net acid–base relevant H^+ release during anaerobiosis would be similar in spring ($0.44\,mmol\,kg^{-1}\,24\,h^{-1}$, Fig. 7) and in autumn ($0.31\,mmol\,kg^{-1}\,24\,h^{-1}$, Fig. 5) animals. These low rates would be independent of metabolic rate and in good accordance with the limited extent of acid–base disturbances during anoxia in both groups of animals.

H^+-equivalent ion exchange: recovery

Significant amounts of H^+ were net transferred only during recovery, when more than $4\,mmol\,kg^{-1}$ body mass was eliminated from the autumn animals (Fig. 5, see below). This was based exclusively on a net reduction of the water bicarbonate level. Immediately upon return to normoxia, ammonium release was

restored to control rates, indicating that ammonium metabolism was not involved in repayment of the oxygen debt. Rather, the enhanced metabolism was probably based on the breakdown of some of the accumulated intermediary products.

During recovery, the net release of protons to the water is accelerated, coinciding with the development of an acidosis in the extracellular space. The non-respiratory fraction of the additional extracellular acidosis is quantitatively explained by resynthesis of phospho-L-arginine (Meyerhof and Lohmann, 1928), depleted during early anaerobiosis. This resynthesis of phosphagen, restoring the high-energy stores, results in a delayed release of metabolic protons transiently associated with inorganic phosphate during anaerobiosis (Pörtner *et al.* 1986*a*,*b*). Previous experiments have indicated (Pörtner *et al.* 1986*b*) that intracellular pH remains constant during the initial phases of recovery, which may mean that the acid–base relevant transepithelial ion transfer is mainly governed by the extracellular acid–base status. At this stage, however, intracellular pH (pHi) is still well below control values (Pörtner *et al.* 1986*b*), and may contribute to eliciting high rates of net H^+ release into the water. These observations clearly demonstrate that transmembrane transfer of protons is a valuable (though relatively inefficient) mechanism during anaerobiosis, but that transepithelial H^+ translocation, in particular during early recovery, is the prime mechanism for acid–base regulation in *S. nudus*.

The large increase in the rate of transepithelial elimination of protons after the re-establishment of normoxia coincides with an increase in the coelomic acidosis, induced by increased metabolic CO_2 production and release of metabolic protons from resynthesis of phosphagen (Meyerhof and Lohmann, 1928). The rate increase, however, is larger than would be expected from the extent of the additional acidosis. The deflection of pH roughly doubles, whereas the H^+ extrusion rate rises by a much larger factor to 320 μmol kg^{-1} h^{-1}, a rate even higher than that observed during environmental hypercapnia with a larger pH deflection (110 μmol kg^{-1} h^{-1}; H. O. Pörtner and N. Heisler, unpublished results). This suggests that, under these conditions, either the intracellular muscle compartments experience a more severe acidosis than the extracellular fluid or other factors, such as the reavailability of aerobic energy, are involved in facilitating an extremely fast transfer.

Even after 72 h of normoxic recovery the curves of cumulative net H^+ transfer (Figs 5 and 7) did not approach the extrapolation of the control curve, but were displaced in parallel by about 4 mmol kg^{-1} H^+. This pattern is different from that found in fishes after extensive activity associated with severe lactacidosis (Heisler, 1984, 1986*b*). Part of this offset may be attributable to the different metabolic substrates used under aerobic and anaerobic conditions (see above). The magnitude of the displacement, however, is too large to be explained on this basis alone. A more probable interpretation is that the anoxia-induced production of organic acids has not yet been reversed by aerobic metabolism. Previous experiments have indicated that anoxia-induced acetate accumulation is not totally reversed during 24 h of recovery (Pörtner *et al.* 1986*a*). Quantitatively more

Table 2. *Comparison of net proton transfer to the ambient water and quantity of residual end products in the body fluids of control animals and during 24 h of post-anaerobic recovery in* Sipunculus nudus *(autumn animals, amounts and changes presented as mmol kg^{-1} body mass)*

	Strom-bine	Octo-pine	Succi-nate	Propio-nate	Acetate	Sum	H^+ transfer
Control	1.7	0.4	0.05	0.03	0.26	2.4	
Recovery (24 h)	5.2	1.7	0.34	0.11	0.49	7.8	
ΔH^+						5.4	4.0

The calculated amount of excess protons released from metabolism is based on data on metabolite concentrations (dissociation equivalents of strombine, octopine, succinate, propionate and acetate) and fluid volumes of musculature and coelomic plasma given by Pörtner *et al.* (1986*a*) and Pörtner (1987*b*). See text.

important is that strombine levels remain high more or less constant during 24 h of recovery. This is due to the characteristics of the respective opine dehydrogenase, which rapidly catalyzes the formation of this intermediate (Grieshaber and Kreutzer, 1986). Succinate and propionate were also found at relatively high concentrations, even after 24 h of recovery (Pörtner *et al.* 1986*a*). The amount of protons released from by these intermediates is actually very similar to the offset (4 mmol kg^{-1}) of the net H^+ transfer curves (Figs 5 and 7) from the extrapolation of the control rate of H^+ release (Table 2). The extent to which these intermediates remain at elevated levels in the body fluids or the environmental water, even after 48 h of recovery, is unknown and a more detailed evaluation is impossible until further data are available.

Acid–base regulation and metabolic rate

The pronounced differences found between the regulatory pattern in autumn and spring animals are probably attributable to differences in anaerobic and post-anaerobic metabolic rate (see also Pörtner, 1987*b*). The initial alkalosis is much greater in autumn animals, indicating a faster degradation of phospho-L-arginine than in spring animals. Differences in metabolic rates are also indicated by the rates of ammonium accumulation during recovery and from the observation that larger quantities of anaerobic end products are metabolized in autumn, as compared to spring, animals (Pörtner *et al.* 1986*a*). The higher metabolic rate of autumn animals is evidently linked to higher rates of ionic exchange, resulting in significantly earlier recovery.

Generally, re-establishment of aerobic (and overall much faster) metabolism after anaerobiosis is also correlated with a considerable elevation in the rate of H^+ transfer. This high rate of H^+ extrusion is required to facilitate repletion of phosphagen by establishing the appropriate homeostatic conditions. Resynthesis of phospho-L-arginine, however, results in release of large quantities of protons, which in turn have to be eliminated in order not to disturb the recovery process.

The energy demand of the active translocation mechanism could thus explain part of the observed increase in the metabolic turnover.

This positive correlation between ionic transfer and metabolic rate will not provide any limitation for ionic transfer as long as sufficient energy can be provided without exceeding a certain ratio of gained energy/simultaneously produced protons. If the amount of protons produced by a metabolic process exceeds the amount that can be translocated, then the resulting acidosis cannot be corrected and further metabolism may even be inhibited. This may be the case during anaerobiosis, when the energy yield from substrates is small compared to that in aerobic conditions.

We conclude that, during long-term anaerobiosis, the rate and efficiency of ionic pH regulation in *Sipunculus nudus* are considerably diminished. pH regulation is primarily performed through a minimization of proton production from metabolism. The contribution of transmembrane acid–base relevant ion transfer is small and not sufficient to improve the homeostatic conditions of intracellular body compartments significantly. Transepithelial ion transfer processes are greatly reduced during anaerobiosis, and are closely correlated with the reduction of energy turnover reflected by a decrease in metabolic rate. The observed restriction to closed-system organismal pH regulation during anaerobiosis may accordingly be related to the reduction of energy consumption by epithelial net H^+ transfer.

The tremendous increase in transepithelial ion transfer after return to normoxia facilitates the establishment of the homeostatic conditions required for the restoration of high-energy stores and sufficient energy production, coupled with a lower H^+ yield than during anaerobiosis. The observed increase in metabolic rate forms the basis for the energy-consuming transfer mechanisms for acid–base relevant ions, which are capable of restoring the acid–base status long before the original stress factors, the protons dissociating from anaerobic intermediates, have been removed from the body fluids by further oxidation.

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