RECOVERY FROM ANAEROBIOSIS IN THE INTERTIDAL WORM *SIPUNCULUS NUDUS*

I. RESTORATION OF AEROBIC, STEADY-STATE ENERGY METABOLISM

BY H. O. PÖRTNER, S. VOGELER AND M. K. GRIESHABER

Lehrstuhl für Stoffwechselphysiologie, Institut für Zoologie, Universität Düsseldorf, D-4000 Düsseldorf 1, FRG

SUMMARY

Recovery from 24 h of anaerobiosis was investigated in *Sipunculus nudus* L. by monitoring changes in the concentrations of anaerobic metabolites in the musculature and in the coelomic plasma. The metabolic events in animals collected during March and October were compared.

Anaerobiosis led to an increase of succinate, propionate and acetate in the muscle tissues and the coelomic plasma. Strombine, octopine and alanine (predominantly L- rather than D-alanine) accumulated in the musculature, whereas aspartate and phospho-L-arginine levels decreased. A higher metabolic rate was observed during anaerobiosis in October than in March animals, as indicated by the higher amounts of strombine, octopine and acetate formed. An increase in metabolic rate appears to entail an increase of flux through the Embden–Meyerhof pathway which favours the accumulation of direct derivatives of pyruvate.

During recovery, regeneration of phospho-L-arginine occurred during the first 3 h, whereas disposal of succinate, octopine and propionate was observed during the entire 24-h period of recovery. Strombine, alanine and, to a lesser extent, acetate contents remained elevated. The concentration of D-alanine approached that of L-alanine during recovery, indicating the activity of alanine racemase. Malate levels increased transiently, possibly linked to the repletion of the aspartate pool.

In October animals, strombine seemed to accumulate transiently during initial recovery, indicating that the energy demand was not met by aerobic metabolism alone. In contrast to the situation in March animals, however, anaerobic glycolysis during recovery obviously becomes important only when the metabolic rate during anaerobiosis was high enough.

INTRODUCTION

Anaerobiosis and postanaerobic recovery are alternately experienced by animals which are periodically exposed to environmental hypoxia. When fluctuations of ambient P_{O_2} occur frequently, not only the ability to tolerate hypoxia but also the

rapid return to aerobic homeostasis may become essential for survival. Tolerance of hypoxia is greatest in animals which are able to reduce drastically their energy requirements during anaerobiosis and which possess efficient mechanisms of energy production. This has been demonstrated in marine intertidal invertebrates, which are able to endure anaerobiosis for days; firstly due to the reduction of energy expenditure, as in *Mytilus edulis* (de Zwaan & Wijsman, 1976; Shick, de Zwaan & de Bont, 1983), and secondly due to efficient pathways regenerating ATP. Modes of energy production during environmental hypoxia in marine invertebrates include phosphagen degradation as well as the exploitation of the succinate–propionate pathway and of anaerobic glycolysis with lactate and/or opine formation (for reviews see Grieshaber, 1982; Livingstone, 1982).

Restoration of the aerobic steady-state after exposure to hypoxia requires disposal of accumulated end-products and replenishment of high energy phosphates, which has been demonstrated for *Arenicola marina* and *Mytilus edulis* as typical inhabitants of the intertidal zone (Pörtner, Surholt & Grieshaber, 1979; Zurburg, de Bont & de Zwaan, 1982; for a review see Ellington, 1983). Disposal of anaerobic metabolites, however, does not always start immediately after the onset of postanaerobic exposure to normoxia. Anaerobic glycolysis, as evidenced by strombine accumulation, has been shown to contribute to ATP production during initial recovery in *Mytilus edulis* (de Zwaan *et al.* 1983). In fact de Zwaan *et al.* (1983) have suggested that the main functional role of strombine accumulation in *Mytilus edulis* is to meet the elevated energy demands during postanaerobic recovery.

The present study was designed to investigate postanaerobic recovery after 24 h of environmental hypoxia in *Sipunculus nudus*, which is a cosmopolitan species widely distributed in marine sandy sediments (Hérubel, 1907). In this habitat, the animals may undergo irregular periods of hypoxia during digging and feeding in the substratum without building an elaborate, permanent burrow (Hérubel, 1907; Hyman, 1957). When living in the intertidal zone the worm can be regularly exposed to periods of hypoxia, which are of maximum duration at spring tides. Hypoxia may also be induced by wave action when the animals are buried by the sand.

Sipunculus nudus survives environmental hypoxia by generating ATP from the utilization of phospho-L-arginine and the formation of succinate, propionate and acetate from aspartate and glycogen. Of the opines, strombine is accumulated mainly during the early phase of environmental hypoxia. The involvement of alanopine is negligible, whereas octopine may become important during exercise (Pörtner *et al.* 1984).

In this paper, attention is focused on the restoration of aerobic homeostasis in energy metabolism. The time course of changes in metabolite concentrations was monitored during recovery following 24 h of anaerobiosis and the degree of metabolic changes compared in animals collected in spring and autumn. Thus a possible anaerobic contribution to ATP production during recovery could be investigated in animals exhibiting different metabolic rates. A companion paper (Pörtner, Vogeler & Grieshaber, 1986) deals with gas exchange and acid–base regulation during the same experiments.

MATERIALS AND METHODS

Animals

Small specimens (7–12 g) of *Sipunculus nudus* were dug out near the low-water line of intertidal flats at Morgat, Brittany, France, in October 1982 and March 1984. The animals were kept for several weeks in tanks which contained a bottom layer of sand (10–20 cm) from the worms' original habitat and which were circulated with artificial sea water at 10–15 °C.

Experimental procedure

Prior to the experiments the animals were catheterized by introducing a PE 60 tubing (total length approximately 75 cm) into the body cavity by puncture of the posterior end of the body. The tubing was secured by the application of cyano-acrylate glue (no. 7432, Bostik GmbH, Oberursel, FRG; Histidin blue, Braun Melsungen, FRG). The cannulated animals were placed in darkened tanks containing 14 l of aerated artificial sea water but no sand, thermostatted to 15 °C. Exclusion of light helped to minimize the muscular activity of the animals. After 24 h of acclimatization at normoxia, hypoxic conditions ($P_{O_2} < 3$ Torr) were induced 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. Subsequently, normoxic conditions were restored by vigorous aeration of the water, which led to an increase of water P_{O_2} above 100 Torr within 15–30 min. After the acclimatization period, 24 h of anaerobiosis and various periods of postanaerobic recovery, metabolite concentrations were determined in the musculature and pH (pH_e), P_{CO_2}, P_{O_2} and metabolite concentrations were measured in coelomic fluid samples. In addition, intracellular pH was measured in the musculature of small animals collected in March.

For the procedure and results of pH, P_{CO_2} and P_{O_2} determinations see Pörtner *et al.* (1986).

Analysis of metabolites

At the end of each experiment, coelomic fluid was withdrawn anaerobically *via* the indwelling catheter. The March animals were dissected quickly in the dorso-ventral plane. The left or right half of the musculature (body wall musculature and two of four introvert retractor muscles, introvert excluded) of each animal was freeze-clamped (Wollenberger, Ristau & Schoffa, 1960), whereas the remaining half was utilized for pH_i analysis (Pörtner *et al.* 1986). From October animals the whole body wall musculature (introvert excluded) was employed for the analysis of metabolites. The frozen tissue was extracted according to Beis & Newsholme (1975). Coelomic plasma, which was obtained after centrifugation of coelomic fluid (for 1 min at 1000 *g*), was extracted according to Pörtner *et al.* (1984).

The concentrations of L-alanine, D-alanine, malate and aspartate were estimated enzymatically using standard procedures (Bergmeyer, 1974). Succinate was analysed enzymatically according to Michal, Beutler, Lang & Guenter (1976). Enzymatic

determinations of phospho-L-arginine, L-arginine and octopine followed the procedure of Grieshaber, Kronig & Koormann (1978). The concentrations of strombine, alanopine, acetate and propionate were estimated by high pressure liquid chromatography (Siegmund & Grieshaber, 1983; Pörtner *et al.* 1984). The significance of differences was tested for concentration changes in the March animals by the application of Student's *t*-test.

RESULTS

Anaerobiosis over 24 h resulted in a net production of succinate, propionate and acetate as indicated by significant changes in the levels of these compounds in the musculature and/or in the coelomic plasma (Figs 1, 2). Aspartate was depleted in the muscle tissues. The accumulation of malate was not significant. It was predominantly L- rather than D-alanine that increased in concentration (Fig. 3). Phospho-L-arginine was transphosphorylated (Table 1), resulting in a significant decrease in the ratio of phospho-L-arginine to L-arginine plus phospho-L-arginine (Fig. 4). The fact that the decrease in the phospho-L-arginine level during anaerobiosis (Table 1) is insignificant is interpreted to be due to the high scatter of the sum of L-arginine derivatives. Strombine accumulated as the main end-product of anaerobic glycolysis, the final level being four to five times as high as the content of octopine (Fig. 4). Alanopine levels remained below $0.2\,\mu$mol g^{-1} fresh weight. D- and L-lactate are known not to be formed during anaerobiosis in *Sipunculus nudus* (Pörtner *et al.* 1984). No change was found in the levels of the adenylates and, consequently, in the energy charge.

During recovery from anaerobiosis, replenishment of the phospho-L-arginine pool and disposal of most of the anaerobic end-products were observed. Within 3–6 h of recovery the phospho-L-arginine pool had been regenerated (Fig. 4; Table 1), and octopine and succinate had returned to near control levels in the muscle (Figs 1, 2, 4). Strombine, however, was not metabolized during 24 h of recovery.

The succinate content approached control values by 6 h of recovery in the tissues and by 12–24 h in the coelomic plasma (Figs 1, 2). The volatile fatty acid propionate was removed from the coelomic plasma by 6–12 h of recovery. In the muscle tissues, propionate approached control levels within 24 h. The level of acetate changed only slightly in the musculature; observed differences were not always significant. Acetate was significantly accumulated, however, in the plasma. A decrease in plasma acetate concentration was not observed until after 12 h of recovery (Figs 1, 2). Aspartate levels remained low during 1 h of recovery but then reached control levels within 6 h (Fig. 3). The sum of D- and L-alanine levels remained elevated during 24 h of recovery. D-alanine was found to increase during recovery, whereas L-alanine decreased, approaching D-alanine levels after 24 h. Malate concentration rose transiently during 1 h of recovery, reaching a maximum value of $0.28 \pm 0.14\,\mu$mol g^{-1} fresh weight (Fig. 3).

Comparison of March and October animals

In another experiment, metabolite levels and extracellular acid–base status (see Pörtner *et al.* 1986) were investigated at the same temperature of 15 °C in small animals collected during October. For comparison with the above data, which were obtained from small animals collected in March, the levels of various metabolites in the body wall musculature and the coelomic plasma of October animals are compiled in Table 2. Strombine was found to accumulate from 4·4 to 12·8 μmol g^{-1} fresh weight and reached 17·9 μmol g^{-1} fresh weight after 1 h of recovery. The accumulation during recovery, however, can only be described as a trend, because of the high scatter of the two replicates. During the 24 h of recovery the strombine level did not fall below control values. The octopine content also changed during
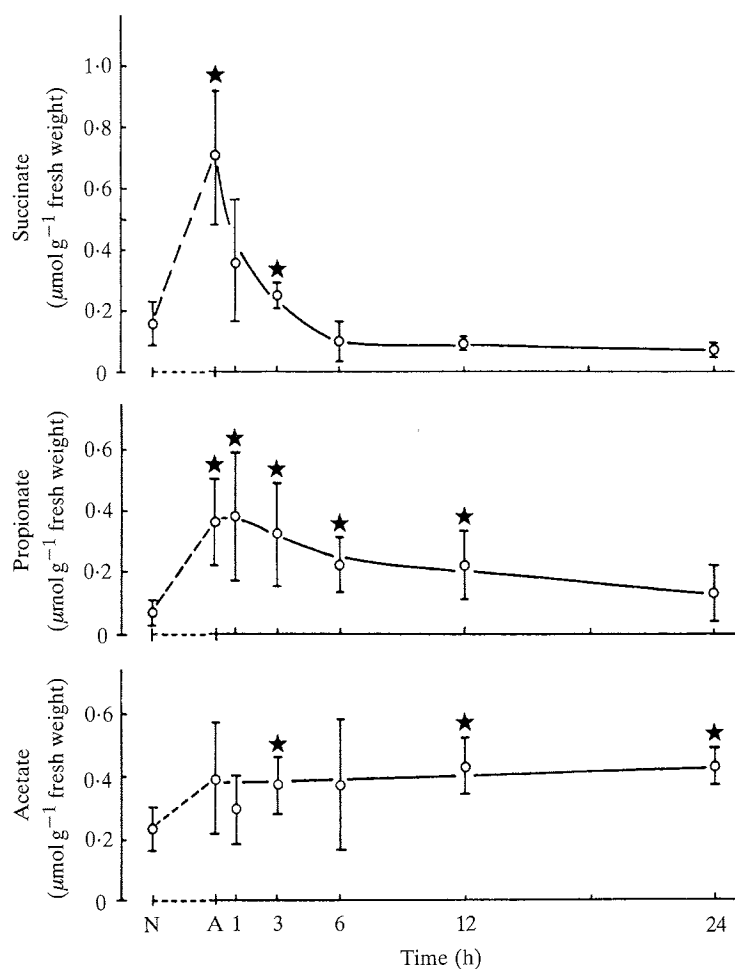

Fig. 1. Levels of succinate, propionate and acetate in the muscle tissues of specimens of *Sipunculus nudus* (collected in March) during recovery after 24 h of experimental anaerobiosis. N, normoxia; A, anaerobiosis; $\bar{x} \pm$ S.D., $N = 5$; March animals). A star ($\bigstar$) indicates significance of deviation from normoxic control values ($P < 0.05$).
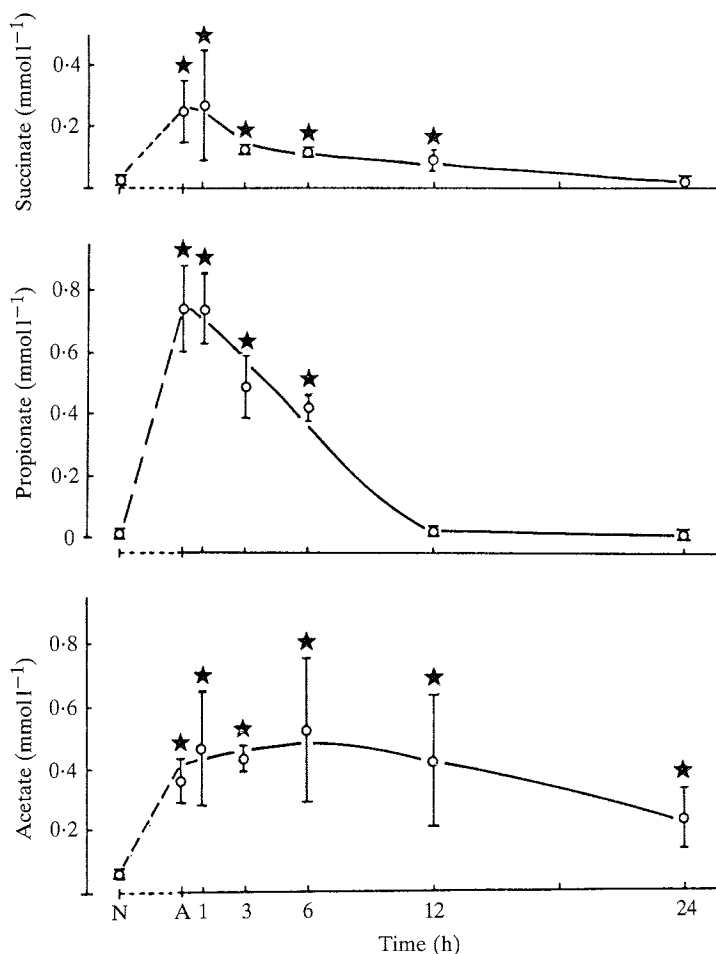
Fig. 2. Concentrations of succinate, propionate and acetate determined in the coelomic plasma during recovery following 24 h of experimental anaerobiosis (N, A, ★, see Fig. 1; $\bar{x} \pm$ s.D., $N = 5$; March animals).

Table 1. *Levels ($\mu mol g^{-1}$ fresh weight) of phospho-L-arginine and L-arginine and the sum of L-arginine derivatives (phospho-L-arginine, L-arginine, octopine) in the musculature during recovery following 24 h of anaerobiosis ($\bar{x} \pm$ s.D., $N = 5$, March animals)*

	Phospho-L-arginine	L-Arginine	L-Arginine derivatives
Normoxia	$33 \cdot 0 \pm 12 \cdot 8$	$5 \cdot 8 \pm 1 \cdot 9$	$38 \cdot 8 \pm 14 \cdot 6$
Anaerobiosis	$27 \cdot 2 \pm 5 \cdot 3$	$20 \cdot 1 \pm 5 \cdot 9^*$	$48 \cdot 2 \pm 4 \cdot 9$
Recovery			
1 h	$25 \cdot 8 \pm 5 \cdot 1$	$11 \cdot 6 \pm 3 \cdot 9^*$	$37 \cdot 6 \pm 3 \cdot 2$
3 h	$33 \cdot 1 \pm 16 \cdot 9$	$10 \cdot 5 \pm 8 \cdot 5$	$43 \cdot 9 \pm 18 \cdot 3$
6 h	$25 \cdot 2 \pm 8 \cdot 2$	$6 \cdot 4 \pm 3 \cdot 3$	$31 \cdot 6 \pm 10 \cdot 6$
12 h	$31 \cdot 9 \pm 6 \cdot 1$	$7 \cdot 9 \pm 1 \cdot 4$	$39 \cdot 5 \pm 6 \cdot 0$
24 h	$29 \cdot 4 \pm 10 \cdot 7$	$6 \cdot 9 \pm 1 \cdot 8$	$36 \cdot 4 \pm 10 \cdot 2$

* Indicates significance of deviation from normoxic control value ($P < 0 \cdot 05$).

anaerobiosis from 1·0 to 6·7 μmol g^{-1} fresh weight and fell only slightly during the subsequent recovery period. During anaerobiosis, succinate, propionate and acetate reached levels of 0·6, 0·6 and 0·8 μmol g^{-1} fresh weight, respectively, in the musculature, and concentrations of 0·5, 1·0 and 1·3 mmol l^{-1} in the coelomic plasma. The pattern of concentration changes during subsequent recovery was essentially the same as described above.

DISCUSSION

In contrast to species like *Arenicola marina* and *Mytilus edulis, Sipunculus nudus* may not be classified as being exclusively specialized to live in the intertidal zone. These sipunculids are widely distributed in marine sandy sediments at various
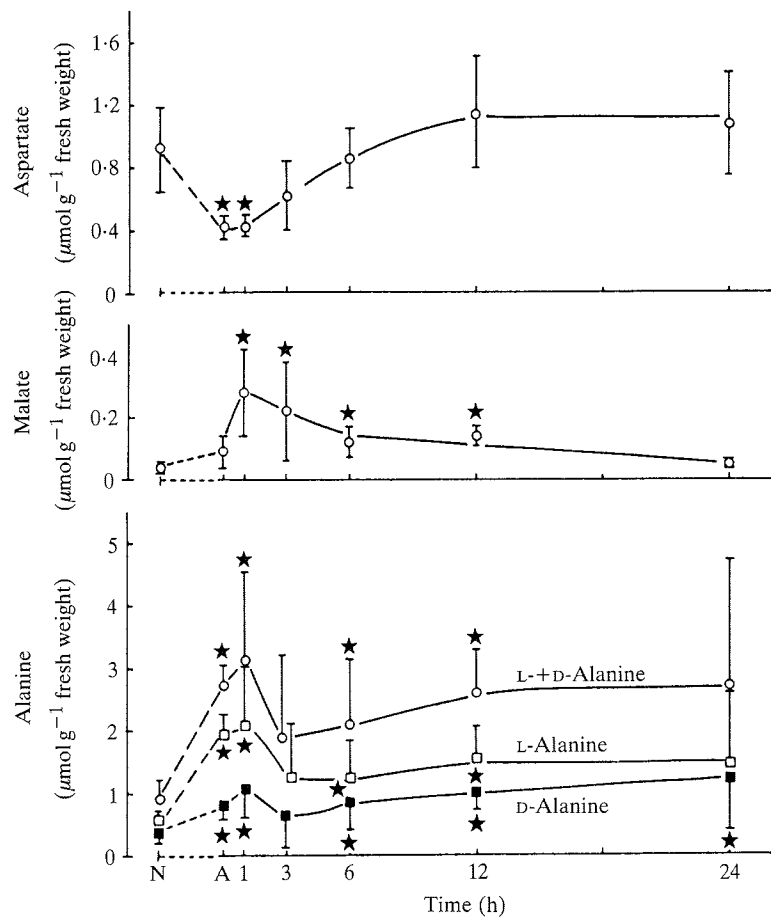

Fig. 3. Levels of aspartate, malate and alanine extracted from the muscle tissue after 24 h of anaerobiosis and different periods of recovery (N, A, ★, see Fig. 1; x̄ ± s.d., $N = 5$; March animals).
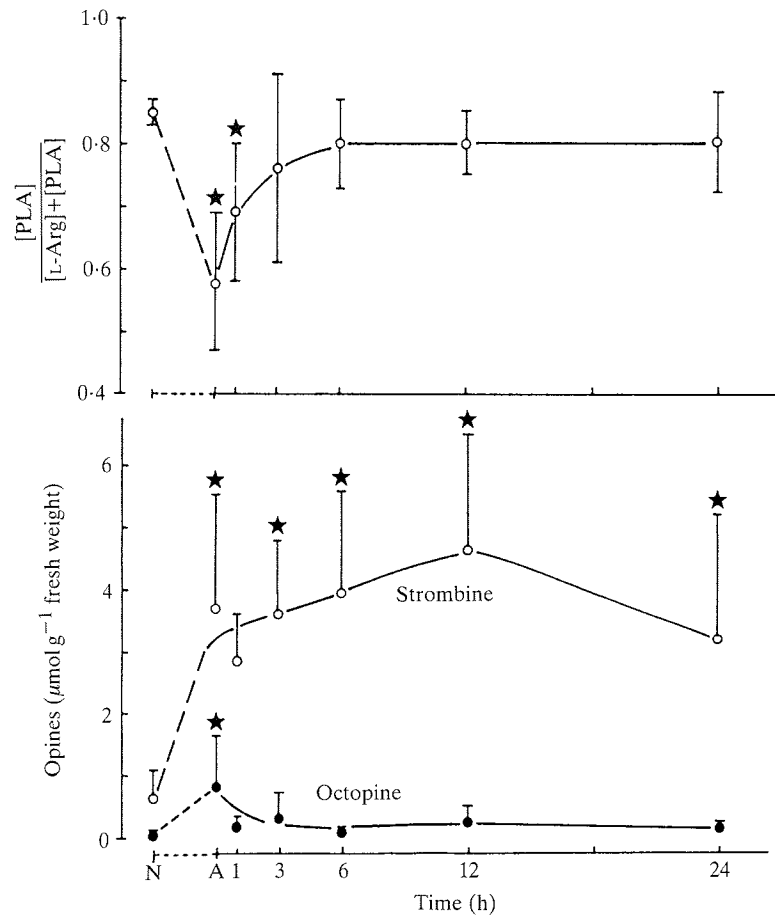
Fig. 4. Levels of strombine and octopine, and ratio of phospho-L-arginine (PLA) to phospho-L-arginine plus L-arginine (L-Arg) contents in the muscle tissue during recovery following 24 h of anaerobiosis (N, A, ★, see Fig. 1; $\bar{x} \pm$ S.D., $N = 5$; March animals).

depths. They must cope with hypoxia during spontaneous burrowing, feeding and migration, which all may lead to anaerobiosis. This preadaptation also enables *Sipunculus nudus* to colonize the lower margin of the intertidal zone, where periods of environmental hypoxia are short (2–6 h). A period of 24 h of hypoxia was selected for the present study so that the metabolism during recovery in *Sipunculus nudus* could be compared with that investigated after 24–48 h of anaerobiosis in the intertidal invertebrates *Arenicola marina* (Pörtner *et al.* 1979) and *Mytilus edulis* (de Zwaan *et al.* 1983).

It is evident that *Sipunculus nudus*, according to its mode of life, requires longer to regain an aerobic state of metabolism than species which are more specialized for survival in the intertidal zone. The replenishment of the phospho-L-arginine pool, which is of priority for the function of the musculature, required 3–6 h of recovery. A faster resynthesis of phosphagen was observed in *Arenicola marina* (84 % within 1 h after 24 h of anaerobiosis, Pörtner *et al.* 1979) and *Cardium edule* (30 min to 1 h

Table 2. *Levels (µmol g⁻¹ fresh weight) of succinate, propionate, acetate, strombine, octopine and the ratio of phospho-L-arginine (PLA) to L-arginine (L-Arg) plus phospho-L-arginine contents in the musculature (M) and the coelomic plasma (CP) during recovery after 24 h of anaerobiosis*

| | Succinate | | Propionate | | Acetate | | Strombine | Octopine | $\dfrac{[PLA]}{[L\text{-}Arg] + [PLA]}$ |
	M	CP	M	CP	M	CP	M	M	M
Normoxia	0·05 (0·02, 0·08)	0·01 (0·01, 0·01)	<0·02	<0·05	0·36 (0·33, 0·39)	0·27 (0·14, 0·40)	4·5 (4·1, 5·0)	1·0 (0·8, 1·2)	0·82 (0·79, 0·85)
Anaerobiosis	0·58 (0·51, 0·65)	0·46 (0·34, 0·58)	0·62 (0·41, 0·83)	0·99 (0·96, 1·02)	0·81 (0·76, 0·86)	1·25 (0·81, 1·69)	12·8 (9·9, 15·7)	6·7 (4·0, 9·5)	0·66 (0·63, 0·69)
Recovery 1 h	0·39 (0·38, 0·40)	0·24 (0·18, 0·30)	0·55 (0·35, 0·75)	0·88 (0·82, 0·94)	0·72 (0·70, 0·74)	0·72 (0·65, 0·79)	17·9 (15·5, 20·2)	7·5 (5·0, 10·0)	0·71 (0·68, 0·74)
3 h	0·36 (0·20, 0·52)	0·33 (0·30, 0·36)	0·52 (0·40, 0·64)	0·55 (0·52, 0·58)	0·99 (0·80, 1·18)	0·71 (0·60, 0·82)	15·1 (12·8, 17·3)	6·8 (5·9, 7·7)	0·69 (0·63, 0·75)
6 h	0·14 (0·10, 0·18)	0·23 (0·11, 0·35)	0·30 (0·27, 0·33)	0·27 (0·24, 0·30)	0·97 (0·82, 1·12)	0·60 (0·40, 0·80)	13·0 (11·5, 14·5)	5·4 (4·8, 6·0)	0·73 (0·72, 0·74)
12 h	0·15 (0·10, 0·20)	0·19 (0·02, 0·36)	0·23 (0·08, 0·38)	0·06 (0·05, 0·07)	0·73 (0·50, 0·96)	0·51 (0·24, 0·78)	11·0 (4·3, 17·7)	7·4 (4·5, 10·3)	0·85 (0·83, 0·87)
24 h	0·24 (0·22, 0·26)	0·17 (0·16, 0·18)	0·22 (0·12, 0·32)	<0·05	0·79 (0·77, 0·81)	0·41 (0·32, 0·50)	13·7 (13·4, 14·0)	4·4 (3·5, 5·3)	0·84 (0·83, 0·85)

Mean values and (in brackets) values from individual animals collected in October are shown, $N = 2$.

after 12 h of anaerobiosis, Gäde & Meinardus, 1981). That *Sipunculus nudus* is highly adapted to anaerobiosis is demonstrated, however, by the fact that under the experimental conditions applied, the adenylate concentrations and the energy charge were not affected by hypoxia (cf. Pörtner *et al.* 1984). This may be due to the high phosphagen content in the body wall which amounts to 33 μmol g^{-1} wet weight as compared to 9 μmol g^{-1} in the body wall of *Arenicola marina* (Pörtner *et al.* 1979), 4–10 μmol g^{-1} in the muscle tissue of *Mytilus edulis* (Zurburg *et al.* 1982), and 6–10 μmol g^{-1} wet weight in the foot muscle of *Cardium edule* (Gäde & Meinardus, 1981). This quantity represents a high 'buffering capacity' for maintenance of a high concentration of ATP (Wilson, Nishiki & Erecinska, 1981). Similar amounts of phosphagen have been observed in anaerobically performing musculature of species exhibiting bursts of activity (cf. Beis & Newsholme, 1975). This finding, and also the non-permanent character of the burrow, support the picture of a temporarily mobile species relying on anaerobic sources of energy. The quick initiation of the replenishment of the phosphagen may have been enabled by the immediate oxygen uptake of the animals during early recovery, the P_{O_2} in the coelomic fluid already being near 40 Torr after 1 h (Pörtner *et al.* 1986).

Of the substrates metabolized during anaerobiosis, only aspartate was analysed. The aspartate pool was replenished after 6–12 h of recovery, possibly by using succinate and propionate as precursors, malate being transiently elevated in concentration as an intermediate of aspartate synthesis. It is possible, however, that the elevation of the concentration of malate represents a high spin of the citric acid cycle during initial recovery. In the same experiments an increase of P_{CO_2} was observed, which is probably due to the repayment of an oxygen debt (Pörtner *et al.* 1986) and implies a transient increase of the aerobic metabolic rate including an enhanced turnover in the Krebs cycle.

The time course of disposal of each of the different end-products during recovery was different. In March animals, succinate concentrations fell quickly during 3–6 h of recovery in the muscle tissue and the coelomic fluid. Octopine, which was low in concentration, was also rapidly metabolized. Propionate clearance was more rapid in the coelomic plasma than in the muscle tissue, possibly because propionate was taken up from the plasma by the tissues. Acetate accumulated significantly only in the coelomic fluid of March animals, but its content was found to increase in muscle tissues and coelomic fluid of October animals. In general, higher amounts of acetate and especially of octopine and strombine were found in October specimens after 24 h of anaerobiosis. Acetate formation, therefore, seems to be important only in animals which, depending on the season, have high metabolic rates. Seasonal changes in acetate formation were also observed by Kluytmans, Zandee, Zurburg & Pieters (1980) for *Mytilus edulis*. Since the higher metabolic rate in October as compared to March animals obviously means an increased flux through the Embden-Meyerhof pathway rather than through the succinate–propionate pathway, acetate formation may require an increased delivery of glycolytic pyruvate (cf. Pörtner *et al.* 1984, fig. 1). During recovery, disposal of acetate was even more time-consuming than the reversal of propionate accumulation.

Alanine levels were low in control animals in the present study (sum of L- + D-alanine: $0.9 \pm 0.3 \, \mu\text{mol g}^{-1}$ fresh weight) compared with the values reported by Pörtner *et al.* (1984) ($6.9 \pm 3.3 \, \mu\text{mol g}^{-1}$ fresh weight). In contrast to the results of Pörtner *et al.* (1984), the amount of accumulated alanine exceeded the amount of degraded aspartate three to four times without being reduced by alanopine formation. For this reason, transamination (cf. Felbeck, 1980) cannot be the exclusive mechanism of alanine production during anaerobiosis in *Sipunculus nudus*. Reductive amination of pyruvate (cf. de Zwaan & van Marrewijk, 1973; Zurburg & de Zwaan, 1981), and thereby elimination of ammonia, is another mechanism to be considered. Since the amount of alanine formed during the present experiments ($1.8 \, \mu\text{mol g}^{-1}$ fresh weight, $P < 0.01$) exceeded the amount of alanine formed during the experiments of Pörtner *et al.* (1984) ($1 \, \mu\text{mol g}^{-1}$ fresh weight, insignificant change) the situation may well be as in *Mytilus edulis*, where an inverse correlation was found between alanine concentration and alanine accumulation depending on the season (Kluytmans *et al.* 1980).

In control animals, D-alanine was present in only slightly smaller amounts than L-alanine (Fig. 3). During anaerobiosis, it was mainly L-alanine that accumulated. Previous studies have observed higher equimolar concentrations of both L- and D-alanine throughout anaerobiosis (Pörtner, 1982). In *Arenicola marina*, slightly higher concentrations of L-alanine were found, with isomer ratios remaining unchanged during anaerobiosis (Schöttler, Wienhausen & Westermann, 1984). In the present study, L-alanine seemed to be converted to D-alanine during recovery (Fig. 3), probably by the action of alanine racemase, which, from previous studies (Pörtner, 1982) and from control values and values after 24 h of recovery, may be assumed to possess an equilibrium constant close to unity (as was demonstrated also for bivalve molluscs, Matsushima, Katayama, Yamada & Kado, 1984). A delayed conversion of L- to D-alanine during anaerobiosis could be caused by a low concentration or by a high K_m for L-alanine of this enzyme and would indicate that L-alanine is an intermediate of D-alanine formation during anaerobiosis.

Like alanine and unlike octopine in March animals, strombine concentration did not change significantly during 24 h of recovery. A delayed metabolism of strombine during recovery could be attributed to the apparent K_m for strombine ($13 \, \text{mmol l}^{-1}$) of strombine dehydrogenase from *Sipunculus nudus*, which is considerably higher than the K_m of octopine dehydrogenase for octopine ($0.6 \, \text{mmol l}^{-1}$; U. Kreutzer, personal communication). In *Mytilus edulis*, the apparent K_m of strombine dehydrogenase for strombine is $2.4 \, \text{mmol l}^{-1}$ (de Zwaan & Dando, 1984), which may explain why strombine is metabolized during 24 h of recovery in *Mytilus edulis* (after 24 h of anaerobiosis, de Zwaan *et al.* 1983), whereas it is not metabolized during this period in *Sipunculus nudus*. For two reasons, the oxidation of strombine after long periods of anaerobiosis (e.g. during spring tides) could be of lower priority for the animals. First, the osmolarity of the cell water is not affected by opine synthesis, since accumulated pyruvate by condensation stoichiometrically reduces the amount of amino acids present. Secondly, concentrations of glycine as a substrate of strombine dehydrogenase are not limiting for further strombine

formation ($180\,\mu\text{mol}\,\text{g}^{-1}$ wet weight, Pörtner et al. 1984). Octopine degradation may be more important than strombine oxidation, since arginine is involved in phospho-L-arginine synthesis.

In October animals, considerably higher amounts of both strombine and octopine were accumulated during anaerobiosis. This seasonal influence was remarkable despite a constant temperature of 15 °C maintained throughout all experiments. During environmental hypoxia, octopine probably only becomes important compared to strombine when the glycolytic rate is high enough. Consistent with this assumption, octopine formation also becomes involved when, during muscular activity, the animals increase their glycolytic rate to a far higher extent than during environmental hypoxia (Pörtner et al. 1984).

In October animals, strombine may have accumulated during initial recovery, thus indicating a high energy demand which could not be met by aerobic metabolism alone. The energy demand was highest during the first hours of recovery. This is also indicated by the increase of P_{CO_2} due to the repayment of an oxygen debt (Pörtner et al. 1986). An increase in the concentrations of glycolytic end-products during recovery from environmental anaerobiosis was also described by Eberlee, Storey & Storey (1983), Nicchita & Ellington (1983) and de Zwaan et al. (1983) for marine bivalves. It is also pronounced in active species during recovery from muscular exercise, e.g. in Chlamys opercularis (Grieshaber, 1978) or Crangon crangon (Onnen & Zebe, 1983). In Sipunculus nudus, the involvement of strombine in energy production during recovery seems to depend on the metabolic rate of the animals during different seasons. In the experiment with October animals, not only strombine but also octopine concentrations remained elevated during 24 h of recovery. Obviously, the period required for metabolic recovery is prolonged when the energy demand is high during anaerobiosis.

Generally, the period of metabolic recovery is longer in Sipunculus nudus than in, for example, Arenicola marina or Mytilus edulis, which are exposed to longer periods of low tide (Pörtner et al. 1979; Zurburg et al. 1982; Siegmund, Grieshaber, Reitze & Zebe, 1985). This difference in the duration of recovery may be linked to the fact that, in the intertidal zone, Sipunculus nudus is only found near the low-water line. By shortening the period of exposure to hypoxia, this species obviously avoids excessive metabolic changes, which, as demonstrated above, take longer to be reversed and may even increase the length of the recovery period above a critical limit.

REFERENCES

Beis, J. & Newsholme, E. A. (1975). The contents of adenine nucleotides, phosphagens and some glycolytic intermediates in resting muscles from vertebrates and invertebrates. Biochem. J. **152**, 23–32.

BERGMEYER, H. U. (1974). *Methoden der Enzymatischen Analyse*. 3. Aufl. Bd. II, pp. 1171–2353. Weinheim: Verlag Chemie.

DE ZWAAN, A., DE BONT, A. M. T., ZURBURG, W., BAYNE, B. L. & LIVINGSTONE, D. R. (1983). On the role of strombine formation in the energy metabolism of adductor muscle of a sessile bivalve. *J. comp. Physiol.* **149**B, 557–563.

DE ZWAAN, A. & DANDO, P. R. (1984). Phosphoenolpyruvate–pyruvate metabolism in bivalve molluscs. *Molec. Physiol.* **5**, 285–310.

DE ZWAAN, A. & VAN MARREWIJK, W. J. A. (1973). Anaerobic glucose degradation in the sea mussel *Mytilus edulis* L. *Comp. Biochem. Physiol.* **44**B, 429–439.

DE ZWAAN, A. & WIJSMAN, T. C. M. (1976). Anaerobic metabolism in Bivalvia (Mollucsa). *Comp. Biochem. Physiol.* **54**B, 313–324.

EBERLEE, J. C., STOREY, J. M. & STOREY, K. B. (1983). Anaerobiosis, recovery from anoxia, and the role of strombine and alanopine in the oyster *Crassostrea virginica*. *Can. J. Zool.* **61**, 2682–2687.

ELLINGTON, W. R. (1983). The recovery from anaerobic metabolism in invertebrates. *J. exp. Zool.* **228**, 431–444.

FELBECK, H. (1980). Investigations on the role of the amino acids in anaerobic metabolism of the lugworm *Arenicola marina* L. *J. comp. Physiol.* **137**B, 183–192.

GÄDE, G. & MEINARDUS, G. (1981). Anaerobic metabolism of the common cockle *Cardium edule*. V. Changes in the level of metabolites in the foot during aerobic recovery after anoxia. *Mar. Biol.* **65**, 113–116.

GRIESHABER, M. (1978). Breakdown and formation of high energy phosphates and octopine in the adductor muscle of the scallop, *Chlamys opercularis* (L.), during escape swimming and recovery. *J. comp. Physiol.* **126**B, 269–276.

GRIESHABER, M. K. (1982). Metabolic regulation of energy metabolism. In *Exogenous and Endogenous Influences on Metabolic and Neural Control* (ed. A. D. F. Addink & N. Spronk), pp. 225–242. Oxford, New York: Pergamon Press.

GRIESHABER, M., KRONIG, E. & KOORMANN, R. (1978). A photometric estimation of phospho-L-arginine, arginine and octopine using homogeneous octopine dehydrogenase isozyme 2 from the squid, *Loligo vulgaris* Lam. *Hoppe Seyler's Z. physiol. Chem.* **359**, 133–136.

HÉRUBEL, M. A. (1907). Recherches sur les sipunculides. *Mém. Soc. zool. Fr.* **20**, 107–418.

HYMAN, L. H. (1959). The protostomatous coelomates – phylum Sipunculida. In *The Invertebrates*, vol. 5 (ed. L. H. Hyman), pp. 610–696. New York: McGraw Hill Book Company.

KLUYTMANS, J. H., ZANDEE, D. I., ZURBURG, W. & PIETERS, H. (1980). The influence of seasonal changes on energy metabolism in *Mytilus edulis* (L.). III. Anaerobic energy metabolism. *Comp. Biochem. Physiol.* **67**B, 307–315.

LIVINGSTONE, D. R. (1982). Energy production in the muscle tissues of different kinds of molluscs. In *Exogenous and Endogenous Influences on Metabolic and Neural Control* (ed. A. D. F. Addink & N. Spronk), pp. 257–274. Oxford, New York: Pergamon Press.

MATSUSHIMA, O., KATAYAMA, H., YAMADA, K. & KADO, Y. (1984). Occurrence of free D-alanine and alanine racemase activity in bivalve molluscs with special reference to intracellular osmoregulation. *Mar. biol. Lett.* **5**, 217–225.

MICHAL, G., BEUTLER, H. O., LANG, G. & GUENTER, U. (1976). Enzymatic determination of succinic acid in foodstuffs. *Z. anal. Chem.* **279**, 137–138.

NICCHITA, C. V. & ELLINGTON, W. R. (1983). Energy metabolism during air exposure and recovery in the high intertidal bivalve mollucs *Geukensia demissa granosissima* and the subtidal bivalve mollusc *Modiolus squamosus*. *Biol. Bull. mar. biol. Lab., Woods Hole* **165**, 708–722.

ONNEN, T. & ZEBE, E. (1983). Energy metabolism in the tail muscles of the shrimp *Crangon crangon* during work and subsequent recovery. *Comp. Biochem. Physiol.* **74**A, 833–838.

PÖRTNER, H. O. (1982). Biochemische und physiologische Anpassungen an das Leben im marinen Sediment: Untersuchungen am Spritzwurm *Sipunculus nudus* L. Dissertation, Universität Düsseldorf.

PÖRTNER, H. O., KREUTZER, U., SIEGMUND, B., HEISLER, N. & GRIESHABER, M. K. (1984). Metabolic adaptation of the intertidal worm *Sipunculus nudus* to functional and environmental hypoxia. *Mar. Biol.* **79**, 237–247.

PÖRTNER, H. O., SURHOLT, B. & GRIESHABER, M. (1979). Recovery from anaerobiosis of the lugworm, *Arenicola marina* L.: changes of metabolite concentrations in the body wall musculature. *J. comp. Physiol.* **133**B, 227–233.

PÖRTNER, H. O., VOGELER, S. & GRIESHABER, M. K. (1986). Recovery from anaerobiosis of the intertidal worm *Sipunculus nudus* L. II. Gas exchange and changes in the intra- and extracellular acid–base status. *J. exp. Biol.* **122**, 51–64.

SCHÖTTLER, U., WIENHAUSEN, G. & WESTERMANN, J. (1984). Anaerobic metabolism in the lugworm *Arenicola marina* L.: the transition from aerobic to anaerobic metabolism. *Comp. Biochem. Physiol.* **79**B, 93–103.

SHICK, J. M., DE ZWAAN, A. & DE BONT, A. (1983). Anoxic metabolic rate in the mussel *Mytilus edulis* L. estimated by simultaneous direct calorimetry and biochemical analysis. *Physiol. Zool.* **56**, 56–64.

SIEGMUND, B. & GRIESHABER, M. K. (1983). Determination of meso-alanopine and D-strombine by high pressure liquid chromatography in extracts from marine invertebrates. *Hoppe Seyler's Z. Physiol. Chem.* **364**, 807–812.

SIEGMUND, B., GRIESHABER, M., REITZE, M. & ZEBE, E. (1985). Alanopine and strombine are end products of anaerobic glycolysis in the lugworm *Arenicola marina* L. (Annelida, Polychaeta). *Comp. Biochem. Physiol.* **82**B, 337–345.

WILSON, D. F., NISHIKI, K. & ERECINSKA, M. (1981). Energy metabolism in muscle and its regulation during individual contraction–relaxation cycles. *Trends biochem. Sci.* **6**, 16–19.

WOLLENBERGER, A., RISTAU, O. & SCHOFFA, G. (1960). Eine einfache Technik der extrem schnellen Abkühlung größerer Gewebestücke. *Pflügers Arch. ges. Physiol.* **270**, 399–412.

ZURBURG, W., DE BONT, A. M. T. & DE ZWAAN, A. (1982). Recovery from exposure to air and the occurrence of strombine in different organs of the sea mussel *Mytilus edulis* L. *Molec. Physiol.* **2**, 135–147.

ZURBURG, W. & DE ZWAAN, A. (1981). The role of amino acids in anaerobiosis and osmoregulation in bivalves. *J. exp. Zool.* **215**, 315–325.