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OXYGEN CONSUMPTION AND MODE OF ENERGY PRODUCTION IN THE INTERTIDAL WORM *SIPUNCULUS NUDUS* L.: DEFINITION AND CHARACTERIZATION OF THE CRITICAL P_{O_2} FOR AN OXYCONFORMER

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Abstract. Oxygen consumption, anaerobic metabolism, and oxygen supply of inner tissues were analysed in *Sipunculus nudus* at different oxygen tensions. Oxygen consumption, energy expenditure, and the P_{O_2} in the coelomic fluid decreased linearly with declining ambient P_{O_2}. Below a certain range of P_{O_2}, which was a function of the size of the animals, the rate of oxygen consumption deviated progressively from the linear $P_{O_2}/\dot{M}_{O_2}$ function. In the same range of ambient P_{O_2} the coelomic P_{O_2} levelled off. Anaerobic glycolysis, phosphagen degradation, and the succinate–propionate pathway became apparent with concentration changes of anaerobic metabolites first occurring in inner tissues.

In extension of the conventional definition (Prosser, 1973; Dejours, 1981) the term critical P_{O_2} (P_c) is applied to the oxyconforming *Sipunculus nudus*. The P_c is redefined as the steady-state P_{O_2} below which environmental oxygen availability becomes insufficient for complete aerobic metabolism (as indicated by the onset of anaerobic energy production). It is discussed to be closely linked to the oxygen supply of inner tissues.

This redefined critical P_{O_2} is shifted to higher partial pressures with increasing size of the animals because of the diffusion distance related decrease in coelomic P_{O_2}. Accordingly, with decline of ambient P_{O_2}, oxygen starts to be released from haemerythrin at higher ambient P_{O_2} values in larger animals. The pigment, which is likely to function as an oxygen store, defers anaerobiosis and, thereby, supports compensation of a higher P_c in large individuals by means of an increased haematocrit.

The P_c is discussed as crucial factor for survival of individual animals in intertidal oxygen-depleted environments.

Aerobic metabolism | Haemerythrin | Oxygen stores
Critical oxygen tension | Oxyconformity | Size

In comparative physiology two types of oxygen consuming organisms can be distinguished: animals that keep their oxygen consumption independent of the ambient

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oxygen tension (at least down to a certain level) and which are referred to as 'oxygen regulators', and animals that reduce their oxygen uptake in a linear function with falling oxygen partial pressure and which are referred to as 'oxygen conformers' (Prosser, 1973; Dejours, 1981). The rate of oxygen uptake of a regulating animal becomes dependent on environmental oxygen only, when the ambient oxygen tension falls below a certain threshold called the critical oxygen tension (P_c). Below the P_c the regulator is incompletely supplied with oxygen and, therefore, may gradually turn to anaerobiosis.

Clear distinction between oxyconformers and oxyregulators has been questioned for invertebrates several times (Mangum and van Winkle, 1973; Herreid, 1980). Intermediate and variable responses were not only found for different species (Mangum and van Winkle, 1973), but also in individuals of the same species during different physiological states (*e.g. Mytilus edulis*, Bayne, 1971; *Crepidicula fornicata*, Newell *et al.*, 1978). These discrepancies lead to the conclusion that the critical P_{O_2} as defined for oxyregulators may be inapplicable to these animals, since a decline in oxygen consumption rate does not necessarily indicate incomplete oxygen supply. There must, though in a low range, clearly be a P_{O_2} below which the organism is forced by its energy demands to perform anaerobic metabolism. This has been suggested for instance for *Littorina irrorata* by Pamatmat (1978).

Oxygen consumption is only directly correlated to the metabolic rate or the turnover of energy during complete supply of all animal tissues with oxygen. Answering the question whether oxyconformity or oxyregulation reflect conformity or regulation of energy expenditure, therefore, requires the analysis of both aerobic and anaerobic metabolism in declining oxygen tension.

The present study was designed to investigate the relation between aerobic and anaerobic metabolism as a function of ambient oxygen tension in *Sipunculus nudus*, a marine invertebrate which has already been classified as oxyconformer by Henze (1910) and more recently by Jones (1972). During severe anoxia (N_2-application) the energy metabolism of *Sipunculus nudus* comprises cytosolic and mitochondrial mechanisms of ATP generation. The transphosporylation of the phosphagen phospho-L-arginine and the accumulation of strombine and octopine as end products of anaerobic glycolysis are observed early, whereas long term anaerobiosis results in glycogen degradation along the succinate–propionate pathway (Pörtner *et al.*, 1984; for reviews of anaerobic metabolism see de Zwaan, 1977; Zebe *et al.*, 1980; Grieshaber, 1982; Livingstone, 1982).

Materials and methods

Animals. Specimens of *Sipunculus nudus* were dug out of intertidal flats of Brittany, France. Large animals (30–35 g) were found near Loquémeau and small specimens (7–12 g) were collected near Morgat. The animals were kept for several weeks in tanks which contained a bottom layer of sand (10–20 cm) and which were circulated with artificial sea water of 10–15 °C.

Experimental procedure. The animals were adapted to the experimental conditions for at least 24 h in darkened, aerated, artificial sea water which was thermostatted to 15 ± 0.1 °C.

Determination of oxygen uptake. The oxygen consumption of *Sipunculus nudus* was measured in sealed, darkened respirometers filled with an accurately known volume of sea water. Large animals were studied separately, whereas the oxygen uptake of small animals was analysed from groups of 3–5 animals. The rate of oxygen depletion was monitored continuously with Radiometer Model E 5047 polarographic oxygen electrodes for usually 12–20 h.

Incubation at different P_{O_2} values. Prior to acclimatization the animals were catheterized by introducing a 75 cm length of PE 60 tubing into the body cavity by puncture of the posterior body pole. The tubing was secured by application of cyanoacrylate glue (No. 7432, Bostik GmbH, Oberursel, F.R.G.). P_{O_2} in coelomic fluid samples was determined exclusively in samples withdrawn via indwelling catheters with thermostatted oxygen electrodes (Radiometer E 5047) which were calibrated with humidified gas mixtures of adequate oxygen partial pressures. All gas mixtures used throughout the experiments were prepared from pure nitrogen and oxygen by means of gas mixing pumps (Type M 303/a-F, Wösthoff, Bochum, F.R.G.).

Coelomic P_{O_2} values at different ambient oxygen tensions were investigated by repeated sampling of coelomic fluid from large and small animals lying in darkened tanks which contained 14 L of artificial sea water.

In a second set of experiments anaerobic metabolites and coelomic P_{O_2} values were analysed in groups of 2 and 3 small animals incubated for 24 h at various ambient oxygen tensions in darkened bottles containing 500 ml of sea water each. In addition a constant P_{O_2} of 7.5 torr was selected for incubation periods of 0.5, 1, 3, 6, 12, and 24 h after the equilibration of the water which was achieved after about 6 min.

Preparation of perchloric acid extracts. After measuring coelomic P_{O_2} the animals were blotted dry and dissected as quickly as possible. The body wall musculature and the introvert reactors were freeze-clamped separately (Wollenberger *et al.*, 1960). The frozen tissue of the body wall was extracted according to Beis and Newsholme (1975) and that of the retractor muscles according to Pette and Reichmann (1982). Coelomic fluid was treated as described previously (Pörtner *et al.*, 1984).

Measurements of metabolites. The enzymatic analysis of the concentrations of L-aspartate and malate followed standard procedures described in Bergmeyer (1974). The content of succinate was measured according to Michal *et al.* (1976). Concentrations of phospho-L-arginine, L-arginine, and octopine were assayed according to Grieshaber *et al.* (1978) and those of strombine, alanopine, acetate and propionate were estimated by high-pressure liquid chromatography (Siegmund and Grieshaber, 1983; Pörtner *et al.*, 1984).

Determination of haemerythrin oxygen equilibria

(a) In vitro studies. The oxygen binding characteristics of the haemerythrin of *Sipunculus nudus* were studied by tonometry of coelomic fluid (Instrumentation Laboratory, Model 273, Padorno Dugano, Italy). Pooled samples of coelomic fluid obtained from 5 aerobic animals were equilibrated for 30 min with humidified gas mixtures of constant P_{CO_2} of 1.5 torr and varying P_{O_2} (between 0 and 140 torr). Samples of 50 μl were analysed for oxygen content from the increase of P_{O_2} after release of bound oxygen in 0.5 ml of ferricyanide (Florkin, 1933; Tucker, 1967) using a 'Tucker chamber' (Tucker, 1967) modified according to Bridges *et al.* (1979). Haematocrit was measured by centrifugation of coelomic fluid (Compur Minicentrifuge M 1100, Compur-Electronic GmbH, Munich, F.R.G.). This method is suitable to determine the haematocrit, since haemerythrocytes are the first of the coelomic cells to sediment during centrifugation (*cf.* Mangum and Kondon, 1975).

(b) In vivo studies. Three large animals were kept in artificial sea water at different oxygen tensions. Coelomic fluid was sampled repeatedly and anaerobically via indwelling catheters from individual animals. P_{O_2}, oxygen content and haematocrit of the coelomic fluid samples were determined as described above. Since the haematocrit was quite variable in different animals, the oxygen content was recalculated for an average haematocrit of 7%.

The oxygen dissociation curves followed a merely hyperbolic function (*cf.* fig. 2). Accordingly the oxygen combining capacity was obtained by utilization of Lineweaver–Burk plots (Lineweaver and Burk, 1934) both for *in vivo* and *in vitro* oxygen dissociation curves. The characteristic parameters oxygen tension at half saturation (P_{50}) and Hill coefficient (n) were evaluated from Hill plots.

Results

Aerobic metabolism at different oxygen tensions. At P_{O_2} = 150 torr large animals (35.3 ± 1.2 g, n = 3) consumed 0.69 ± 0.07 μmol $O_2 \cdot g^{-1} \cdot h^{-1}$. With increasing hypoxia at P_{O_2} values of 50 to 70 torr the oxygen consumption fell linearly (r = 0.992) and attained 0.41 ± 0.08 μmol $O_2 \cdot g^{-1} \cdot h^{-1}$ or 60% of the initial consumption at a P_{O_2} of 50 torr. At lower P_{O_2} values the decline in oxygen uptake was more pronounced deviating from the linear function progressively towards zero (fig. 1).

A similar pattern could be observed for the oxygen uptake of small animals (8.9 ± 2.0 g, n = 11) although environmental P_{O_2} and oxygen consumption were not quite as linearly correlated as described for large animals (r = 0.977). The relationship started to deviate from the linear function at P_{O_2} values around 30 torr which was about 20 torr lower than for large animals. At normoxia (P_{O_2} = 150 torr) small animals consumed 0.84 μmol $O_2 \cdot g^{-1} \cdot h^{-1}$. The oxygen uptake rate fell to about 40% of the initial value (0.33 μmol $O_2 \cdot g^{-1} \cdot h^{-1}$) at P_{O_2} = 30 torr (fig. 1).

Steady-state coelomic P_{O_2}, which was reached after 3–4 h of equilibration, was linearly correlated to the ambient oxygen tension as well (fig. 1). It decreased from

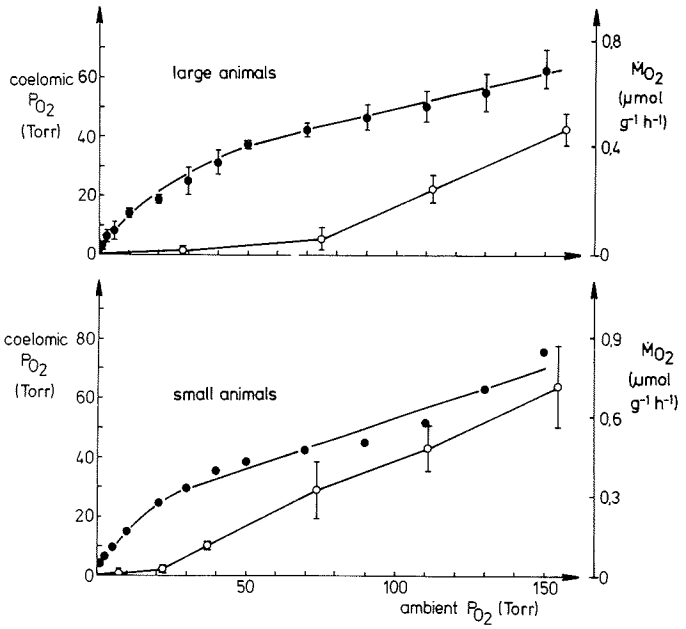

Fig. 1. Oxygen consumption (● ——— ●) under declining oxygen tension in large ($\bar{x} \pm$ SD, n = 3) and in small animals. Coelomic P_{O_2} values (○ ——— ○) in both large and small individuals ($\bar{x} \pm$ SD, n = 4) reflect the changes of the oxygen uptake rate (T = 15 °C).

42.0 ± 5.5 torr in large (34.0 ± 4.1 g, n = 4) and from 64.0 ± 12.2 torr in small animals (8.4 ± 1.5 g, n = 4) to 5.5 ± 3.7 torr or 2.3 ± 0.9 torr, respectively. The latter values were attained at environmental oxygen tensions of 75 torr in large and of 22 torr in small animals. At lower ambient oxygen partial pressures the decline of the coelomic P_{O_2} levelled off.

Contribution of haemerythrin to the oxygen supply. The oxygen equilibria of haemerythrin from *Sipunculus nudus* have already been studied several times before (*e.g.* Florkin, 1933; Boeri and Ghiretti-Magaldi, 1957; Bates *et al.*, 1968). The present study was intended to evaluate how haemerythrin is involved in the oxygen supply of inner tissues.

No difference could be found between the oxygen dissociation characteristics of haemerythrin *in vitro* and *in vivo* (fig. 2). Both dissociation curves are hyperbolic. Hill plots of the *in vivo* and *in vitro* data are linear with slopes of approximately n = 1 (n = 0.977 *in vitro*, n = 1.013 ± 0.032 *in vivo*). Also P_{50} values were not found to be significantly different between *in vitro* (P_{50} = 3.2 torr) and *in vivo* conditions (P_{50} = 2.7 ± 0.8 torr). At P_{O_2} = 20 torr 85–90% of haemerythrin was saturated with oxygen and saturation reached 95% at P_{O_2} = 50 torr.

The oxygen capacity $C^{max}_{HmrO_2}$ (Hmr: Haemerythrin) as evaluated from Lineweaver–Burk plots was 0.95 mmol · L^{-1} *in vitro* (haematocrit = 9%) and

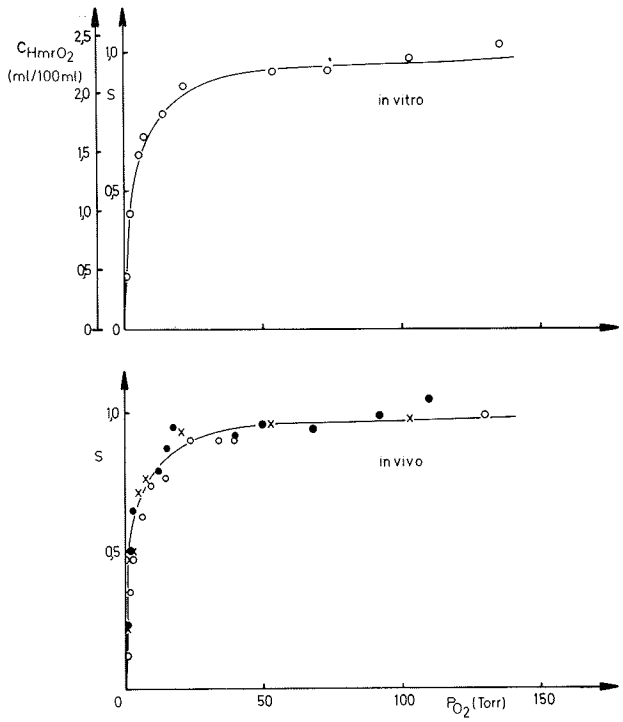

Fig. 2. Oxygen binding characteristics (C_{HmrO_2}: volume of haemerythrin-bound oxygen; S: fractional saturation) of haemerythrin from *Sipunculus nudus* at 15 °C *in vitro* (O ——— O, tonometry of coelomic fluid pooled from 5 animals) and *in vivo* (O, ●, ×, measurement of P_{O_2} and oxygen content in coelomic fluid samples withdrawn via catheter from living animals; each symbol represents one individual animal).

0.89 ± 0.08 mmol · L^{-1} *in vivo* (haematocrit = 7%) resulting in an average specific combining capacity of 0.12 mmol · L^{-1} haematocrit-$\%^{-1}$.

Large animals (31.3 ± 8.1 g, n = 28) generally had a higher haematocrit ($7.8 \pm 2.3\%$) than small animals (10.3 ± 2.6 g, n = 11; haematocrit = $4.1 \pm 2.0\%$). In *Sipunculus nudus* the oxygen combining capacity, therefore, appears to be correlated with the size of the respective specimens.

Anaerobic metabolism at different oxygen tensions. The transitions described for the dependence of $\dot{M}_{O_2}$ and coelomic P_{O_2} on the ambient oxygen tension may indicate that the animals and in particular inner tissues become anaerobic even in the presence of ambient oxygen. Therefore inner tissues (introvert retractor muscles) and outer tissues (body wall musculature) of *Sipunculus nudus* were analysed in order to delineate the utilization of anaerobic metabolism at various ambient oxygen tensions.

Succinate content was increased in retractors and body wall of small specimens (9.1 ± 1.4 g) only at low oxygen tensions (fig. 3). At 24 torr a significant increase occurred in the retractors whereas in the body wall succinate concentration did not rise

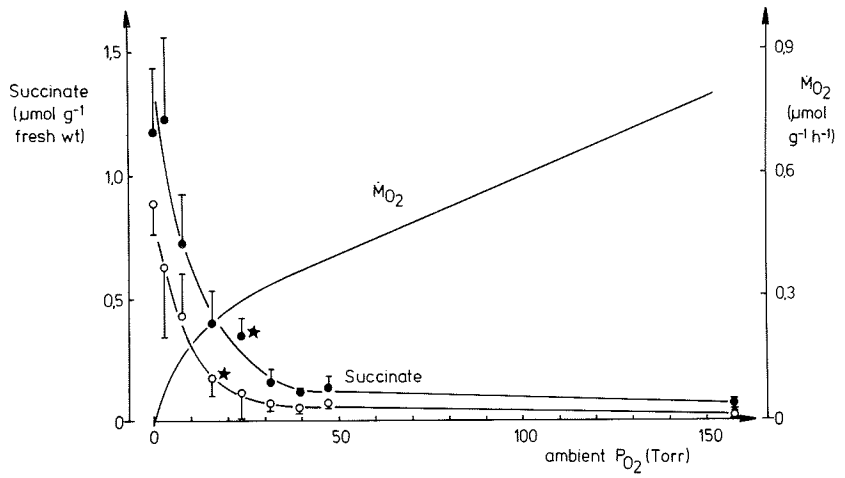

Fig. 3. Succinate concentrations in introvert retractor muscles (● ——— ●) and in the body wall musculature (○ ——— ○) measured after 24 h of exposure to different ambient oxygen tensions ($\bar{x} \pm$ SD, n = 5, T = 15 °C) and compared to the oxygen consumption curve (see fig. 1). A star indicates the onset of significant deviation from normoxic control values in declining oxygen tension ($P < 0.05$).

before the external P_{O_2} fell below 24 torr. In introvert retractors the start of succinate accumulation coincided with the onset of the pronounced decline of oxygen consumption. The contents of aspartate as an early substrate of anaerobiosis and of acetate and propionate as end products of anaerobic mitochondrial metabolism changed in accordance with the increase in succinate content (tables 1 and 2). Malate only slightly varied in concentration. Succinate, propionate and acetate were not only accumulated in the tissues but were also released into the coelomic fluid. Generally the observed concentration changes of anaerobic metabolites were gradually augmented at lower P_{O_2} values.

Apart from mitochondrial mechanisms of ATP synthesis, the cytosolic energy production by anaerobic glycolysis also increased when the external oxygen tension declined. Among the possible end products of anaerobic glycolysis in *Sipunculus nudus* (Pörtner *et al.*, 1984) the synthesis of strombine exceeded octopine formation two- or threefold. The concentration changes of alanopine were negligible (table 3). At low values of ambient P_{O_2} the amount of opines formed was higher in the body wall than in the introvert retractors (fig. 4). Anaerobic glycolysis started, as deduced from the first accumulation of end products, at P_{O_2} = 32 torr in the retractors and at P_{O_2} = 24 torr in the body wall.

As reported earlier the adenylates are not affected by environmental hypoxia (Pörtner *et al.*, 1984). Phospho-L-arginine, however, started to be transphosphorylated in the same range of P_{O_2} elaborated for the participation of other anaerobic processes. The ratio of the phospho-L-arginine concentration over the sum of phospho-L-arginine and L-arginine contents decreased progressively below P_{O_2} = 32 torr (table 3).

In order to evaluate if anaerobic glycolysis and succinate–propionate formation in

TABLE 1

Concentrations of L-aspartate, malate, acetate and propionate (in $\mu mol \cdot g^{-1}$ fresh wt.) in the body wall musculature after 24 h of exposure to different ambient oxygen tensions ($\bar{x} \pm SD$, n = 5, T = 15 °C).

P_{O_2} (torr)	L-Aspartate	Malate	Acetate	Propionate
158	1.0 ± 0.12	0.10 ± 0.05	0.61 ± 0.14	0.21 ± 0.07
47	0.81 ± 0.25	0.07 ± 0.03	0.76 ± 0.31	0.08 ± 0.03
40	0.91 ± 0.48	0.10 ± 0.04	0.57 ± 0.25	0.14 ± 0.04
32	0.98 ± 0.14	0.09 ± 0.02	0.82 ± 0.13	0.08 ± 0.04
24	0.78 ± 0.21	0.11 ± 0.05	0.66 ± 0.14	0.14 ± 0.06
16	0.74 ± 0.21	0.12 ± 0.02	0.65 ± 0.10	0.19 ± 0.12
8	0.62 ± 0.09*	0.18 ± 0.04*	0.84 ± 0.22	0.54 ± 0.30
3	0.42 ± 0.20	0.14 ± 0.05	0.85 ± 0.21	0.88 ± 0.14*
≈0	0.40 ± 0.23	0.12 ± 0.04	0.76 ± 0.26	0.89 ± 0.34

* Onset of significant deviation from normoxic control values in declining oxygen tension ($P < 0.05$).

TABLE 2

Concentrations of succinate, propionate and acetate in the coelomic fluid (in $mmol \cdot L^{-1}$ plasma) after 24 h of exposure to different ambient oxygen tensions ($\bar{x} \pm SD$, n = 5, T = 15 °C).

P_{O_2} (torr)	Succinate	Propionate	Acetate
158	0.01 ± 0.01	0.05 ± 0.03	0.17 ± 0.06
47	0.01 ± 0.01	0.06 ± 0.05	0.18 ± 0.02
40	0.01 ± 0.01	0.08 ± 0.06	0.18 ± 0.02
32	0.01 ± 0.01	0.08 ± 0.05	0.22 ± 0.04
24	0.01 ± 0.01	0.07 ± 0.02	0.22 ± 0.05
16	0.01 ± 0.01	0.12 ± 0.03*	0.33 ± 0.06*
8	0.04 ± 0.03	0.42 ± 0.11	0.48 ± 0.18
3	0.09 ± 0.02*	0.90 ± 0.24	0.81 ± 0.58
≈0	0.26 ± 0.19	0.76 ± 0.37	0.64 ± 0.13

* Onset of significant deviation from normoxic control values in declining oxygen tension ($P < 0.05$).

the body wall exhibit different delay periods after the onset of hypoxic exposure, incubation at a P_{O_2} of 7.5 torr was performed for different time periods. Succinate content was elevated already after 6 h whereas opines were found to accumulate after 12 h. Aspartate metabolisation started 3–6 h after the onset of hypoxic exposure and became significant after 12 h. During 24 h of hypoxia aspartate degradation and succinate formation seemed to level off whereas opine formation proceeded continuously (fig. 5).

Discussion

Oxygen supply and aerobic metabolic rate. The oxygen consumption of *Sipunculus nudus* conforms to the ambient oxygen tension as already pointed out by Henze (1910). This

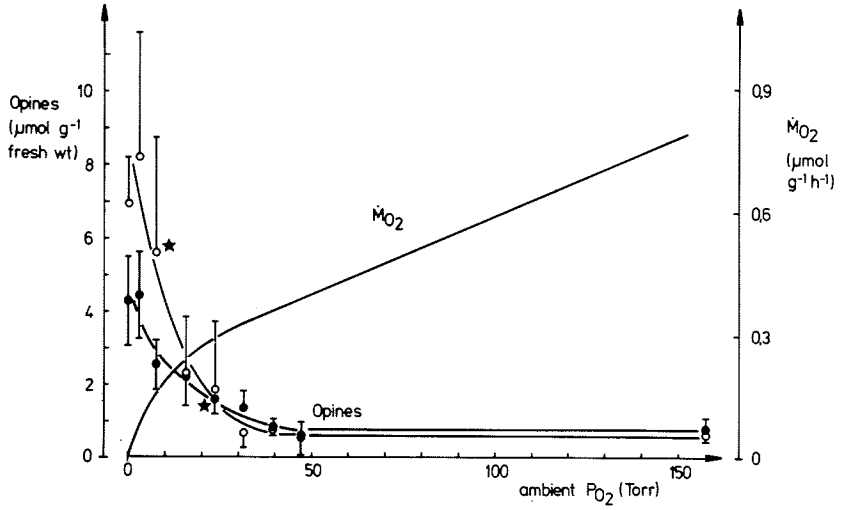

Fig. 4. Sum of opine concentrations (alanopine, strombine, octopine) in introvert retractor muscles (● ——— ●) and in the body wall musculature (○ ——— ○) measured after 24 h of exposure to different ambient oxygen tensions ($\bar{x} \pm$ SD, n = 5, T = 15 °C) and compared to the oxygen consumption curve (see fig. 1). For concentrations of alanopine, strombine and octopine, see table 3. A star indicates the onset of significant deviation from normoxic control values in declining oxygen tension ($P < 0.05$).

TABLE 3

Concentrations of octopine, strombine, alanopine, phospho-L-arginine and L-arginine (in μmol · g^{-1} fresh wt.) and ratio of phospho-L-arginine concentration (PLA) over L-arginine (L-Arg) plus phospho-L-arginine (PLA) contents in the body wall musculature after 24 h of exposure to different ambient oxygen tensions ($\bar{x} \pm$ SD, n = 5, T = 15 °C).

P_{O_2} (torr)	Octopine	Strombine	Alanopine	Phospho-L-arginine	L-Arginine	$\frac{[PLA]}{[PLA] + [L\text{-}Arg]}$
158	0.06 ± 0.10	0.54 ± 0.09	< 0.1	41.0 ± 8.6	5.8 ± 1.7	0.88 ± 0.03
47	0.07 ± 0.05	0.46 ± 0.45	< 0.1	40.0 ± 5.6	5.1 ± 0.5	0.88 ± 0.02
40	0.08 ± 0.06	0.71 ± 0.12	< 0.1	39.2 ± 5.7	5.7 ± 1.0	0.87 ± 0.01
32	0.07 ± 0.07	0.58 ± 0.30	< 0.1	44.6 ± 5.9	5.8 ± 0.6	0.88 ± 0.02
24	0.38 ± 0.56	1.45 ± 1.37	< 0.1	41.3 ± 9.5	9.6 ± 5.0	0.80 ± 0.12
16	0.42 ± 0.31	1.86 ± 1.35*	< 0.1	44.8 ± 4.1	8.6 ± 2.6	0.84 ± 0.04
8	1.46 ± 1.15*	3.97 ± 1.98	0.18 ± 0.05	32.2 ± 8.5	15.1 ± 5.9*	0.67 ± 0.14*
3	1.87 ± 0.89	6.09 ± 2.42	0.22 ± 0.09	27.2 ± 7.8	24.7 ± 11.3	0.53 ± 0.17
≈0	1.92 ± 0.86	4.86 ± 0.80	0.18 ± 0.02	22.4 ± 11.4*	24.3 ± 6.3	0.46 ± 0.21

* Onset of significant deviation from normoxic control values in declining oxygen tension ($P < 0.05$).

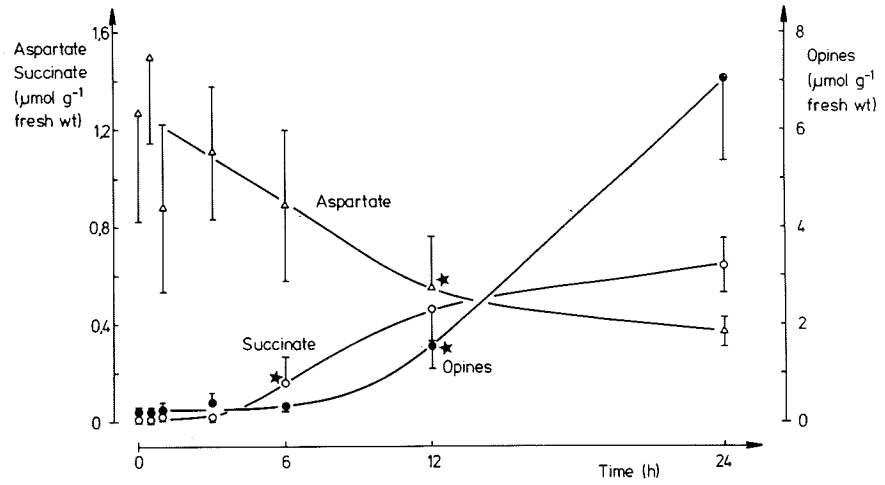

Fig. 5. Succinate (○———○), aspartate (Δ———Δ) and sum of opine concentrations (●———●) (alanopine, strombine, octopine) in the body wall musculature after different periods of hypoxic exposure at P_{O_2} = 7.5 torr ($\bar{x} \pm$ SD, n = 5, T = 15 °C). Please note the different scale for concentrations of succinate, aspartate, and opines. A star indicates the onset of significant deviation from normoxic control values in declining oxygen tension ($P < 0.05$).

is valid also for other sipunculid worms (*Dendrostomum cymodoceae* and *Phascolopsis gouldi*; *cf.* Edmonds, 1957, and Mangum and van Winkle, 1973). Anaerobic metabolism is not involved in energy production in the linear range of the oxygen consumption curve, indicating that real oxyconformity of metabolism occurs instead of partial energy production by anaerobic metabolism which was discussed by Famme *et al.* (1981) and Hammen (1983) for marine bivalves. Lower metabolic rates may be explained by the reduction of activity and by changes in anabolism, since uncoupling of the respiratory chain and concomitant heat loss is disadvantageous and thus has to be considered unlikely.

For an animal which like *Sipunculus nudus* has to adapt to regular changes in oxygen supply, the gradual reduction of energy requirements may well be a strategy to tolerate low ambient oxygen tensions, thereby saving residual oxygen and anaerobic sources of energy. From another point of view oxyconformity is presumably required because of the morphological organization of the animal. The body wall musculature of *Sipunculus nudus* is involved in all body movements including ventilation. Therefore, it may very well need an enhanced oxygen supply. It also represents the animal's respiratory organ (Pörtner, Heisler and Grieshaber, unpubl.) as indicated by longitudinal coelomic channels in this tissue which facilitate the entrance of oxygen into the main coelomic cavity (*cf.* Baltzer, 1931). In declining ambient oxygen tension a continuously high oxygen consumption of the body wall would endanger the oxygen supply of inner tissues. To avoid anaerobiosis at moderately hypoxic conditions, the reduction of energy expenditure appears indispensable. Accordingly the linear decrease of the coelomic oxygen tension with falling environmental P_{O_2} is accompanied by a linear decrease of

the difference between internal and external P_{O_2} which may at least partially be caused by a decreasing oxygen consumption of the body wall musculature.

Animals lying in sea water with little or no activity, however, are not as dependent on ventilatory activity as when dwelling in sand. In the lugworm *Arenicola marina*, the degree of regulation appears to be dependent on the environment. Complete regulation was observed by Krüger (1958) above a P_{O_2} of 22 torr when the animals were irrigating an artificial burrow, but oxyconformity was described to prevail over a wide range of P_{O_2} when they were directly exposed to sea water (Toulmond, 1975; Shumway, 1979). Accordingly further experiments are required to analyse the regulatory pattern in burrowed *Sipunculus nudus*.

Involvement of anaerobic metabolism. Above a certain range of low P_{O_2} values in the ambient water (30–70 torr, depending on the size of the animals) the oxygen uptake rate falls linearly with water P_{O_2}. Below this size-dependent threshold the oxygen consumption of *Sipunculus nudus* is progressively reduced towards zero (fig. 1). A similar pattern is observed for the difference between ambient and coelomic P_{O_2} values (*cf.* fig. 1) indicating enhanced oxygen transfer through the body wall as a result of its reduced oxygen uptake and declining oxygen consumption from the coelomic fluid by the tissues. These transitions, however, do not represent a corresponding decline of total metabolic rate since evidently part of the energy is started to be delivered by anaerobic processes as suggested by concentration changes of anaerobic metabolites (Pörtner *et al.*, cited in Grieshaber, 1982).

The mitochondrial formation of succinate starts at a higher ambient P_{O_2} in the inner tissues (introvert retractors) than in the body wall. Moreover, the retractors accumulate a larger amount of succinate at all P_{O_2} values applied, a fact which is consistent with the assumption mentioned above, that the body wall is the first tissue to consume the available oxygen. The body wall is likely supplied with oxygen predominantly from the sea water rather than from the coelomic fluid. This assumption is supported by the observation that at an ambient P_{O_2} of 24 torr the coelomic fluid is largely depleted of oxygen ($P_{O_2} < 1$ torr) whereas the body wall musculature of small individuals of *Sipunculus nudus* produces energy still mainly via aerobic pathways. Correspondingly the concentrations of Krebs cycle enzymes are higher in the external layer of the body wall (Pörtner, 1982) indicating a higher content of mitochondria in this part of the tissue. In contrast, the introvert retractors relying on oxygen from coelomic fluid start to accumulate succinate when the coelomic P_{O_2} falls below 5 torr, a value attained at an ambient oxygen tension of about 35 torr (fig. 6).

Concentration changes of aspartate, acetate, and propionate demonstrate that all processes characteristic for anaerobic mitochondrial energy production during environmental hypoxia in *Sipunculus nudus* (Pörtner *et al.*, 1984) are started simultaneously even when the animals become only slightly anaerobic. Below $P_{O_2} = 16$ torr the amount of succinate and propionate accumulated in the body wall even surpasses the amount of metabolized aspartate which is known to be the initial substrate of anaerobic metabolism (Schöttler, 1980). Therefore malate as an intermediate of succinate and propionate

formation, which by itself is only slightly accumulated (table 1, *cf.* Pörtner *et al.*, 1984), has to be delivered by carboxylation of either phosphoenolpyruvate or pyruvate. Consequently a carboxylation reaction known to occur during long-term anaerobiosis (Schöttler, 1980) becomes involved as well, although oxygen is still available to the animals. Simultaneous contribution of aerobic and anaerobic processes to energy production similar to that demonstrated here for *Sipunculus nudus* has been suggested for *Mytilus edulis* (Livingstone and Bayne, 1977; Livingstone, 1978), *Mytilus californianus* (Bayne *et al.*, 1976), *Arenicola marina* (Schöttler *et al.*, 1983) and *Nephthys hombergii* (Schöttler, 1982).

Cytosolic energy production by anaerobic glycolysis and by the transphosphorylation of phospho-L-arginine also supplies ATP during progressive hypoxia (fig. 4, table 3). Like the formation of succinate glycolysis seems to start at somewhat higher ambient P_{O_2} values in the introvert retractor muscles than in the body wall. At low ambient oxygen tensions the glycolytic rate is, however, higher in the body wall. This tissue is involved in the ventilation of the animal, so that a high metabolic rate is found as expression of the high energy demand. Strombine formation is two to three times higher than octopine production under the experimental conditions applied (table 3). For still unknown reasons, strombine dehydrogenase and not the highly active octopine dehydrogenase preferably terminates anaerobic glycolysis during environmental hypoxia in *Sipunculus nudus* (Pörtner *et al.*, 1984).

Anaerobic glycolysis started in both the body wall as well as the introvert retractors at higher P_{O_2} values than mitochondrial anaerobic ATP formation, though the elevation of opine concentrations was not yet significant (*cf.* figs. 3 and 4). Therefore a P_{O_2} of 7.5 torr was chosen for a detailed investigation of the time course of anaerobic changes. This P_{O_2} assured that the animal and especially the body wall was still partially aerobic (*cf.* figs. 3 and 4). In this experiment anaerobic glycolysis, however, could not be confirmed to start earlier than succinate formation, in contrast, succinate production and aspartate degradation seemed to be involved somewhat earlier than glycolysis (fig. 5). From this experiment and from comparison of aspartate and opine concentrations displayed in tables 1 and 3 and fig. 4 it is obvious, however, that opine formation starts before the aspartate pool is depleted, an observation which is at variance with the prediction of Fields (1983). Anaerobic glycolysis, which is also utilized during exhausting activity without important participation of anaerobic mitochondrial metabolism (Pörtner *et al.*, 1984), probably becomes involved when the energy requirements surpass the mitochondrial ATP production. This very likely does not occur under the applied experimental conditions in quiescent animals as long as oxygen is still sufficiently available to maintain aerobic mitochondrial metabolism.

It can be concluded that all reported anaerobic processes start below a certain P_{O_2} more or less simultaneously in resting *Sipunculus nudus*. With declining oxygen tension anaerobic metabolism becomes progressively involved in energy production.

Definition of the P_c. Changes of oxygen consumption, steady-state coelomic P_{O_2} and anaerobic metabolite contents in declining oxygen tension correspond below a certain

P_{O_2}. Quasi steady-state conditions may, therefore, be assumed for the measurements of oxygen uptake during long-term oxygen depletion in a closed respirometer. Oxygen bound to haemerythrin very likely insignificantly affected the shape of the oxygen consumption curve.

The correlation between aerobic metabolism (oxygen uptake) of the total animal, anaerobic energy production (represented by succinate formation) in inner tissues (introvert retractors), and oxygen supply of inner tissues is summarized in fig. 6, depicting both 'physiological' and 'biochemical' characteristics of our definition of the critical oxygen partial pressure (P_c) in *Sipunculus nudus*: Below the P_c

(a) oxygen consumption is progressively and non-linearly reduced, whereas the $P_{O_2}/\dot{M}_{O_2}$ function is linear above the P_c;
(b) changes occur in the relation between P_{O_2} in the coelomic fluid and P_{O_2} in the ambient water;
(c) anaerobic energy production commences, starting in tissues supplied with oxygen via coelomic fluid ('inner tissues').

Accordingly the critical P_{O_2} is defined as the steady-state P_{O_2} below which anaerobic metabolism becomes involved in energy production as indicated by correlated changes of 'physiological' and 'biochemical' parameters. This characterization of the P_c fits only partially into the conventional definition used in comparative physiology (Prosser, 1973; Dejours, 1981). Consideration of anaerobic metabolism may lead, however, to extend the applicability of the term P_c which should not be restricted to characterize the cessation of oxygen uptake regulation. The critical oxygen tension should generally indicate oxygen demand in excess of environmental oxygen delivery which is unequivocally connected with the change towards anaerobic energy production.

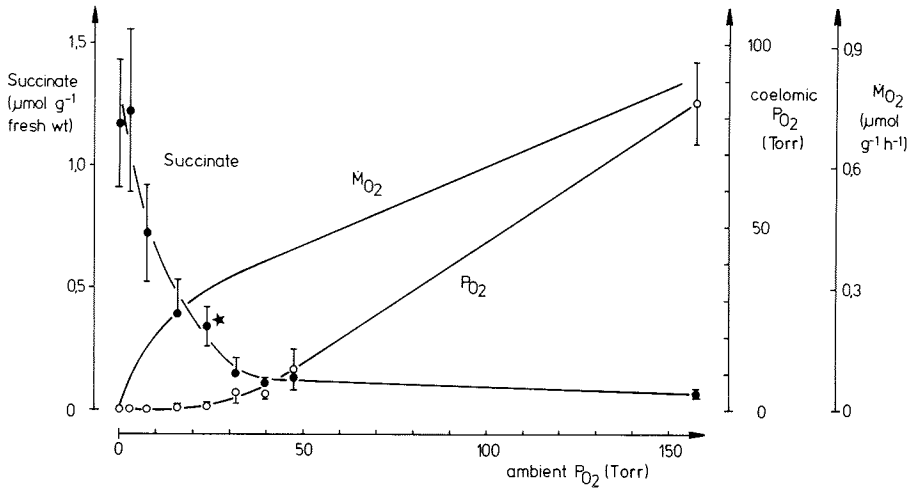

Fig. 6. Comparison of succinate concentrations in the introvert retractor muscles (●———●, see fig. 3) with coelomic P_{O_2} values (○———○, measured in the same animals) and oxygen consumption data (see fig. 1) at different ambient oxygen tensions for summary of 'physiological' and 'biochemical' characteristics of the critical P_{O_2}.

Oxygen, however, may not only be supplied from the environment but also, during transition phases to hypoxia, from internal oxygen stores. In the present study this could be observed under moderate hypoxia (P_{O_2} = 7.5 torr) when the onset of anaerobic metabolism in the body wall was delayed 3–6 h (fig. 5). The oxygen bound to haemerythrin was consumed during this time period. Depletion of internal oxygen stores may affect the evaluation of the P_c in animals not being in steady state. Therefore, our definition of the P_c is at variance with the definition of Toulmond (1975) who, presumably because of the utilization of oxygen bound to respiratory pigments, assumed changes in the rate of oxygen consumption and in the mode of energy production to occur at different ambient oxygen tensions in *Arenicola marina*. We propose that the term critical P_{O_2} should only be applied to animals which totally rely on ambient oxygen, since internal stores provide oxygen only during restricted time periods.

In steady state, analysis of anaerobic metabolism helps to elucidate the background of changes in oxygen uptake rates especially in the invertebrates whose oxygen consumption curves exhibit a large diversity (Mangum and van Winkle, 1973). For the future it may be worthwhile to investigate whether the prediction of 'physiological' and 'biochemical' transition phases being always as closely related as described here for *Sipunculus nudus* holds true for other oxyconformers and also for oxyregulating animals (*cf.* Pamatmat, 1978). When investigating the P_c in quiescent animals, it has to be considered, however, that long-term acclimation of the oxygen uptake rate to hypoxia may occur (*cf.* Bayne and Livingstone, 1977) which is equivalent to a shift of the steady state in metabolism and presumably also implies a shift of the P_c to lower oxygen tensions. Under the experimental conditions applied this could not be observed in *Sipunculus nudus*.

Interrelations: P_c, body weight, haematocrit and habitat. The coincidence of 'biochemical' and 'physiological' processes at the P_c has been elaborated during this study for small specimens of *Sipunculus nudus*, but is likely valid also for large individuals. According to fig. 1 the critical P_{O_2} increases in *Sipunculus nudus* with increasing size of the animals. This is clearly correlated to the comparatively low coelomic P_{O_2} values in large specimens. Oxygen is taken up by the animal only via the external surface. Since the surface to weight ratio decreases, the diffusion distance through the oxygen consuming body wall musculature becomes longer with increasing body weight. The amount of oxygen passing through the body wall, per unit weight, is thereby diminished. Accordingly the oxygen supply to inner tissues becomes worse.

Large individuals compensate for this disadvantage by higher haematocrit, the respective values being twice as large in animals of about 30 g compared to specimens of about 10 g. Haemerythrin starts to release oxygen below a coelomic fluid P_{O_2} of 20 torr. CO_2 or pH have been reported not to influence the binding of oxygen to the pigment of *Sipunculus nudus* (Florkin, 1933; Kubo, 1953; Bates *et al.*, 1968). The ambient P_{O_2} below which oxygen may be released in the living animal is lower in small (P_{O_2} = 55 torr) than in large individuals (P_{O_2} = 105 torr). Since haemerythrin is utilized as an oxygen store in *Sipunculus nudus* when environmental or functional hypoxia occurs

(Pörtner, Heisler and Grieshaber, unpubl.), the lower coelomic P_{O_2} values of large animals indicate that they possibly have to rely on the stored oxygen more often than small ones. Presuming an oxygen consumption at the P_c (near P_{O_2} = 50 torr in large and near P_{O_2} = 30 torr in small individuals) the stored oxygen would support a merely aerobic metabolism for 71 min in large and for 46 min in small animals, thereby extending the resistance of the animals to hypoxic exposure. In the present study the storage function of haemerythrin in *Sipunculus nudus* is substantiated by the delay of anaerobic energy production under moderate hypoxia (fig. 5).

In the natural habitat of *Sipunculus nudus* the body weight of the animals influences the selection of the dwelling-place. Large animals (of about 30 g) only live in sand with larger grains, *i.e.* with larger interstices compared to the sand from the natural habitat of small ones (of about 10 g). Since the preliminary burrow of *Sipunculus nudus* possesses no opening for extrusion of the respiratory water the animals have to press it into the sand. Due to the wide interstices large individuals are able to pump more water into their burrow. In addition, their ventilatory activity requires less muscular work (Pörtner, Heisler and Grieshaber, unpubl.). The ability of providing themselves with more respiratory water also helps large animals to compensate for the higher critical P_{O_2}. Correspondingly this value very likely indicates the limit for the ability to survive in an environment poor in oxygen.

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