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On the Anaerobic Metabolism of Three Species of *Nereis* (Annelida)

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ABSTRACT: Endproducts of anaerobic metabolism were examined in *Nereis diversicolor, N. virens* and *N. pelagica*. These nereids tend to be exposed to different degrees of hypoxia in their natural habitats. Accordingly, they exhibit different degrees of tolerance to anaerobiosis. The present study reveals: (1) In contrast to all previously examined facultative anaerobe annelids all three nereids produce high amounts of D-lactate. (2) *N. diversicolor* and *N. virens* (to a lesser degree) are able to switch, during longlasting anaerobiosis, to the energetically more convenient degradation of glycogen to succinate and volatile fatty acids. (3) In *N. pelagica*, however, production of volatile fatty acids is only of minor importance. (4) In all three nereids, alanine is accumulated during the beginning of anaerobiosis; but in *N. virens* alanine production continues during longlasting anaerobiosis, although at a lower rate. Variations in responses to anaerobic conditions are also reflected by the 'energy charge', which decreases within 36 h from 0.88 to 0.66 in *N. pelagica*, whereas in *N. virens* and *N. diversicolor* it decreases within 72 h from 0.90 or 0.88, respectively, to 0.70. The metabolic pathway of energy production during functional anaerobiosis, caused by extensive muscular work (electrical stimulation), was examined. All three species accumulate D-lactate in considerable amounts; however, succinate or volatile fatty acids were not synthesized.

INTRODUCTION

Several members of the genus *Nereis* live in remarkably different habitats. *Nereis diversicolor* inhabits the upper region of the tidal zone. Frequently it is found in places covered by water only for one or two hours during high tide. *N. virens* mainly dwells on sublittoral bottoms, but is also found near the low tide level exposed to emergence for 1 or 2 h. *N. pelagica* lives in the sublittoral phytal. As demonstrated by Theede et al. (1973), these species differ in their ability to survive oxygen deficiency, a fact which may have been expected on the basis of the different habitats occupied. *N. diversicolor* is highly resistant to anoxia, while *N. pelagica* succumbs after a relatively short time; the anaerobic capacity of *N. virens* is intermediate. Theede et al. did not investigate the cause of this striking difference in the tolerance to anoxic conditions. Since differences in metabolic properties appeared to be responsible, I have studied quality and quantity of the metabolic endproducts accumulated during experimental anaerobiosis. Furthermore ATP, ADP and AMP concentrations before and after exposure to anaerobic conditions were measured in order to elucidate the underlying energy dynamics.

MATERIAL AND METHODS

Material

Nereis diversicolor and *N. virens* were dug out from tidal flats of the German North Sea coast (*N. diversicolor* at Carolinensiel/Ostfriesland, and *N. virens* at List/Sylt) and kept in artificial sea water (34 ‰ S) at 6 °–8 °C without food for periods of 6 to 14 d before being used in experiments. *N. pelagica* were dredged from the phytal of the Kiel Bay (Baltic Sea). They were kept at 8 °C in artificial sea water (15 ‰ S) without food for 4 d. Previous experiments had shown that starvation does not influence the mode of anaerobic metabolism.

Exposure to Anaerobic Conditions

All experiments were carried out at 12 °C in artificial sea water (*Nereis diversicolor* and *N. virens*: 34 ‰ S, and *N. pelagica*: 15 ‰ S). Five or more test individuals were kept in 800 ml medium in 1-l flasks which could be closed by stopcocks. The test water was flushed with N_2 1 h prior and 1 h after inserting the nereids. Thereafter the stopcocks were closed.

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Electrical Stimulation

Three individuals of *Nereis virens* and 5 individuals of *N. pelagica* or *N. diversicolor* were stimulated up to 20 min by 0.5 s pulses of alternating current (8 V) with a frequency of 12 min^{-1}.

Preparations of Extracts

At the end of an experiment the worms were rapidly blotted on paper tissue, dropped into liquid nitrogen and ground to powder in a porcelain mortar cooled by liquid nitrogen. The powder, still frozen, was then added to three volumes of ice cold 3N perchloric acid, stirred and homogenized in an 'Ultra Turrax' homogenizer. After centrifugation the pellet was extracted once more. Both extracts were combined, neutralized and centrifuged for 30 min at 45 000 · g. The incubation water was frozen for later analysis only when all worms had survived the incubation period.

Quantitative Analysis

D-lactate, succinate, alanine, glutamate, aspartate, ATP, ADP and AMP were estimated by standard enzymatic methods (Bergmeyer, 1974). Volatile fatty acids were measured after steam distillation by gas-liquid chromatography according to Kluytmans et al. (1975).

RESULTS

Nereis diversicolor and *N. virens* survived anaerobic periods of 72 h without casualties. In experiments with *N. pelagica* 40 % of the test animals were found dead after 36 h of anaerobiosis.

There is uniformity in D-lactate being quantitatively the most important endproduct in all three species, but the rates at which it is formed strongly depend on the duration of anaerobiosis. Furthermore, in all three species succinate, acetate and propionate were produced, although in different quantities and varying proportions (Fig. 1).

Rather large quantities of propionate were formed by *Nereis diversicolor*, much less by *N. virens* and only very little by *N. pelagica*. Most of the propionate was excreted into the medium. After 24 h the concentrations in the worms were 10–15 μmol g^{-1} dry weight in *N. diversicolor*, 8–12 μmol g^{-1} dry weight in *N. virens* and 5 μmol g^{-1} dry weight in *N. pelagica*. These concentrations remained constant over longer exposure periods. Accumulation of succinate was also highest in *N. diversicolor*, very low in *N. pelagica*, with *N. virens* being intermediate. No succinate appeared in the medium. In the three species acetate was produced in rather small quantities and partly excreted into the medium. The concentrations in the worms were nearly the same as reported for propionate.

In order to find out whether amino acids contribute to anaerobic metabolism in the species studied, the concentrations of aspartate, alanine and glutamate were also estimated. The data obtained are shown in Table 1.

In all three species alanine is an endproduct of short-term anaerobiosis. In *Nereis virens* also a rather small increase in alanine concentration took place during prolonged anaerobic exposure. No change in glutamate concentration occurred in *N. pelagica* and *N. diversicolor*, whereas a small increase after 24 h was apparent in *N. virens*.

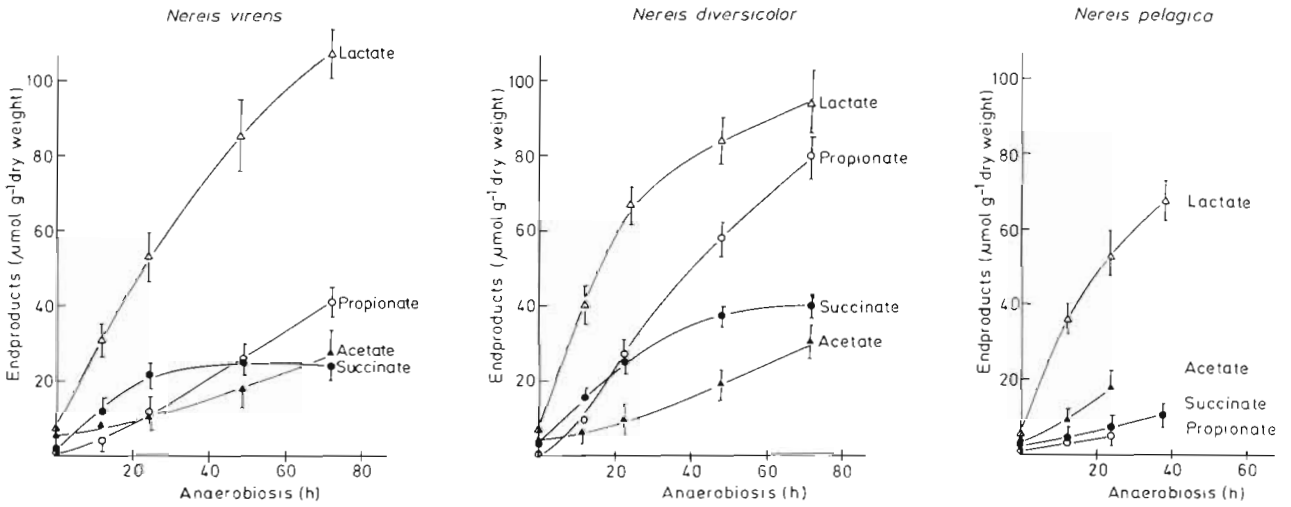

Fig. 1. Concentrations of D-lactate, succinate, propionate and acetate in the nereids specified as a function of duration of exposure to anaerobic conditions. Mean values ± standard deviation (N = 4)

Table 1. Concentrations of glucogenic amino acids after various periods of anaerobiosis. All values expressed as μmol g^{-1} dry weight. Mean values ± S.D. (N = 4)

Species	Time of anaerobic incubation	Aspartate	Alanine	Glutamate
Nereis diversicolor	0	18.9 ± 2.5	70.0 ± 6.7	39.1 ± 3.2
	12	3.5 ± 0.3	83.2 ± 7.3	43.7 ± 3.4
	24	3.9 ± 0.3	88.4 ± 5.1	38.3 ± 3.9
	48	4.3 ± 0.9	84.7 ± 6.8	41.6 ± 2.7
	72	6.6 ± 0.7	92.4 ± 8.9	40.3 ± 4.1
Nereis virens	0	16.8 ± 0.4	83.1 ± 8.2	25.5 ± 1.6
	12	6.0 ± 1.1	101.6 ± 10.1	24.1 ± 3.5
	24	2.8 ± 0.8	116.5 ± 14.4	26.2 ± 1.4
	48	1.5 ± 0.5	128.6 ± 16.7	33.2 ± 3.9
	72	1.9 ± 0.4	134.8 ± 9.8	36.2 ± 2.8
Nereis pelagica	0	7.7 ± 0.6	35.4 ± 2.8	23.9 ± 1.3
	12	3.2 ± 0.4	46.3 ± 3.3	25.5 ± 0.9
	24	1.8 ± 0.6	50.8 ± 5.3	24.8 ± 1.4
	36	2.2 ± 0.4	55.9 ± 3.2	26.5 ± 4.0

In order to assess the relative importance of the different endproducts in relation to anaerobic exposure, the proportions of the sum of the concentrations of all metabolic endproducts were calculated. As is evident from Table 2, the proportions differ greatly in the three species; in addition they depend on the duration of anaerobiosis.

In *Nereis pelagica* D-lactate accounts for more than 50 % of the endproducts accumulated, and alanine for about 28 %. Succinate, propionate and acetate, prevailing endproducts in most facultative anaerobes, together make up a share of less than 25 % in this species.

In *Nereis diversicolor* D-lactate was also the main endproduct during the first 24 h, but during prolonged anaerobiosis its proportion decreased to 25 %. During the first 24 h the amount of alanine was 15 % of the total. Succinate, propionate and acetate increased from 37.5 % after 12 h to more than 70 % between 48 and 72 h of anaerobiosis.

Concerning the accumulation of D-lactate, *Nereis virens* is intermediate between *N. diversicolor* and *N. pelagica*. The share of D-lactate was found to be close to 40 % during different periods of exposure. At prolonged anaerobiosis, between 48 and 72 h, the percentage of volatile fatty acids accumulated increased to nearly 40 %. Although the concentration of alanine increased rather slowly during anaerobic exposure its percentage of the total accumulation of the metabolites decreased.

Table 3 shows the concentrations of adenosine phosphates estimated in the worms after different periods of anaerobiosis. It is evident that in all Nereidae studied ATP decreased from its initial level as anaerobic exposure was extended. However, there are striking differences between the species. In *Nereis pelagica* ATP

Table 2. Percentages of the endproducts listed

Species	Time (h)	D-Lactate	Succinate	Propionate	Acetate	Alanine	Glutamate
Nereis diversicolor	0–12	46.1	15.4	11.9	8.4	18.2	–
	12–24	42.2	15.6	29.7	4.7	7.8	–
	24–48	25.3	19.4	43.2	11.9	–	–
	48–72	20.4	–	49.0	30.6	–	–
Nereis virens	0–12	44.1	11.9	8.5	5.1	30.5	–
	12–24	37.3	18.6	11.9	6.8	25.4	
	24–48	40.5	7.6	20.2	8.8	15.7	7.6
	48–72	42.6	–	24.1	16.7	11.1	5.6
Nereis pelagica	0–12	60.0	4.4	4.4	6.7	24.4	–
	12–24	53.1	9.3	6.3	18.8	12.5	

Table 3. Concentrations of adenosine phosphates (μmol g^{-1} dry weight) and the calculated 'energy charge' after various periods of anaerobiosis

Species	Time (h)	ATP	ADP	AMP	ATP + ADP + AMP	Energy charge
Nereis diversicolor	0	9.73	2.28	0.29	12.30	0.88
	12	8.68	3.08	0.74	12.50	0.82
	24	7.43	3.22	0.99	11.64	0.78
	48	6.08	3.68	1.28	11.04	0.72
	72	5.71	3.84	1.32	10.87	0.70
Nereis virens	0	10.16	1.82	0.27	12.25	0.90
	12	8.68	2.48	0.57	11.73	0.85
	24	8.04	3.39	0.84	12.27	0.79
	48	6.88	3.68	1.26	11.82	0.74
	72	5.82	3.73	1.37	10.92	0.70
Nereis pelagica	0	11.26	2.79	0.34	14.39	0.88
	12	9.68	3.42	0.88	13.98	0.81
	24	7.44	3.87	1.25	12.56	0.75
	36	5.71	4.28	1.97	11.96	0.66

dropped to nearly 50 % of the normal value within 36 h, whereas in *N. virens* and *N. diversicolor* the decrease of ATP was much slower: after 72 h the ATP content was nearly 60 % of the initial level in both species. The concentrations of ADP and AMP obviously increased in all worms during anaerobiosis, but in *N. diversicolor* and *N. virens* maximum concentrations were reached within 48 h; simultaneously a decrease of the total content of adenosine nucleotides was observed. The quotient (ATP) + ½ (ADP)/(ATP) + (ADP) + (AMP) was calculated from the concentrations of adenosine nucleotides. Termed 'energy charge' of the adenosine system, this quotient was introduced in 1967 by Atkinson and Walton. It can vary between 0 and 1.0 and is an excellent parameter for calculating the energy content of a system. The basal metabolism of an animal cell is characterized by an energy charge between 0.88 and 0.92. A decrease of the energy charge is assumed to indicate excessive stress. In *N. pelagica* the energy charge dropped from 0.88 to 0.66 during 36 h of anaerobiosis. In *N. virens* and *N. diversicolor* there was a much slower decrease; a value of 0.70 was attained after 72 h of anaerobiosis.

In addition to the measurements described, the water content of the worms was determined during anaerobiosis. All three species were shown to take up water, although in different proportions. In *Nereis virens* and *N. diversicolor* the water content only increased from 79 to 82 % of the fresh weight within 72 h; in *N. pelagica* the water uptake was substantially higher, increasing from 80 to 87 % of the fresh weight within 36 h.

As lactate is preferentially formed during extensive muscular activity, and Nereidae are very motile annelids, all three species were subjected to electrical stimulation in order to evoke maximum muscle work.

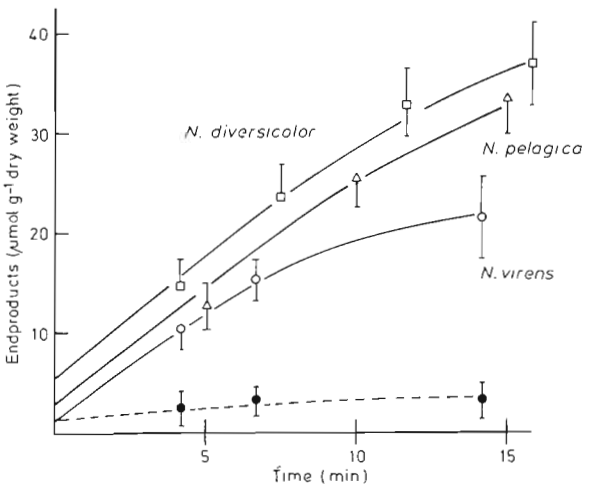

Fig. 2. Formation of D-lactate and succinate in the nereids specified following electrical stimulation. Solid lines: D-lactate; dotted line: succinate. The succinate concentration shown, is representative of all three species

In Figure 2 the results of these experiments are shown. After electrical stimulation D-lactate was accumulated in considerable concentrations, but no succinate was produced under these conditions by all three species.

During their individual development many Nereidae are most active at the beginning of sexual maturation, when they switch from living at the bottom and in caves to continuous swimming close to the water surface until reproduction and following death. Heteronereid individuals of *Nereis virens* were examined for accumulation of typical endproducts of anaerobic metabolism after strong and continuous swimming for 30 min. Contrary to sexually immature individuals, however, neither succinate nor lactate could be detected at this stage.

DISCUSSION

In all animals glycogen is the main fuel for anaerobic energy production. In invertebrates two metabolic pathways for anaerobic glycogen breakdown are known: (1) The Embden-Meyerhof pathway leading to the accumulation of lactate or, in some cases in a modified form, to the formation of octopine or ethanol. (2) A pathway in which phosphoenolpyruvate is carboxylated to oxaloacetate giving rise to malate and succinate and finally to volatile fatty acids as propionate and acetate.

In the Nereidae studied both alternatives of anaerobic energy production occur together. As these analyses show, the ratio of lactate to volatile fatty acids formed differs anaerobically from species to species. It is conspicuous that the capacity to form volatile fatty acids parallels the ability to survive periods of anaerobiosis. *Nereis pelagica*, with its relatively low tolerance to anaerobic conditions, produces succinate and volatile fatty acids only to a minor extent and acetate production exceeds the synthesis of propionate. *N. diversicolor*, however, which produces predominantly lactate during an early phase of anaerobiosis, apparently switches to volatile fatty acid formation during prolonged anoxia. It is still unknown how this change is effected. Although the production of D-lactate decreases drastically, its formation still continue to some degree during prolonged anaerobiosis. Possibly, the continuous formation of lactate at a decreased rate is due to some tissues which are unable to switch to the production of volatile fatty acids or at least have difficulties in doing so.

Nereis virens occupies an intermediate position between *N. pelagica* and *N. diversicolor*. Volatile fatty acids increased absolutely and relatively during prolonged anaerobiosis. However, the absolute increase is distinctly lower than in *N. diversicolor* and the increase of the percentage of the metabolites is due to the fact that during prolonged anoxia alanine only rises very slowly and succinate does not increase at all. Lactate accumulation in relation to the formation of the other metabolic endproducts remains constant over 72 h of anaerobiosis in *N. virens*, contrary to *N. diversicolor*.

Nereidae also accumulate alanine in addition to lactate, succinate and volatile fatty acids. As in other facultative anaerobes (Schöttler and Schroff, 1976; de Zwaan, 1977; Felbeck, 1979) alanine accumulation in *Nereis diversicolor* only takes place shortly after the onset of anaerobiosis. On the other hand, *N. virens* apparently accumulates alanine as long as the anaerobic state lasts. The significance of alanine production for anaerobic energy production is still unknown. In other annelids the C-skeleton of this amino acid was shown to be derived from glycogen (Zebe, 1975; Schöttler and Schroff, 1976); however the origin of the amino group remains unexplained. Theoretically it seems possible that aspartate, which is metabolized during the initial phase of anaerobiosis, may be the main donor of this group. However, most of the aspartate is metabolized during the first hour of anaerobiosis and at this time the increase of alanine concentration does not parallel the decrease of aspartate; it was found to be distinctly lower.

While in *Nereis pelagica* and in *N. diversicolor* the concentrations of glutamate remained constant during anaerobiosis some increase was found in *N. virens* during prolonged anoxia, as has been reported previously from *Tubifex tubifex* (Schöttler and Schroff, 1976). The origin of this accumulated glutamate is still unknown.

The results presented here indicate that the degree of resistance to anaerobic conditions is coupled with the capacity to produce volatile fatty acids from carbohydrates. The death of *Nereis pelagica* after rather short periods of anoxia is probably caused by insufficient rates of ATP formation. This seems obvious from rapid decrease of the energy charge. However, in *Arenicola marina* body-wall musculature, similar values (0.65) were measured after 24 h of anaerobiosis without lethal effects to this species (Surholt, 1977). Therefore, still other causes – in addition to falling energy charge – must be responsible for the low anaerobic resistance of *N. pelagica*. Since *N. pelagica* takes up substantially more water during anaerobiosis than the other species studied, osmotic imbalance might be an additional cause of the low anaerobic resistance of this species. This presumption is supported by the observation of Theede et al. (1973) that *N. pelagica* is rather sensitive to changing osmotic conditions.

The question still remains, why the capability of lactate formation is so highly developed in Nereidae. Unlike other facultative anaerobic annelids, Nereidae are quite mobile. They are able to swim and creep quickly by twisting their body and using their parapods as levers. This twisting is mainly performed by the longitudinal musculature, in which mitochondria are quite rare (Lindner and Fischer, 1964; Defretin and Wissocq, 1969). Probably, the capacity for aerobic energy production of the muscles cannot meet the energy demand during exercise. Therefore these muscles have to rely on lactate formation or, in other words, the muscles of Nereidae may have been developed to meet functional anaerobiosis to varying degrees in addition to environmental anoxia.

In mature heteronereid individuals, however, the situation is completely different. Their parapods have greatly increased in size, and function as oar blades,

enabling the worms to swim quickly and for a long time almost without interruption. The impossibility to demonstrate the accumulation of anaerobic end-products in heteronereid forms of *Nereis virens* agrees well with the results of electron microscopic investigations, showing the muscle cell of heteronereis of *N. irrorata* and of *Perinereis cultrifera* being well equipped with mitochondria (Lindner and Fischer, 1964; Defretin and Wissocq, 1969). Thus the worms are adapted excellently to long-lasting muscular activity, which is probably the basis of successful reproduction in the free water close to the surface.

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LITERATURE CITED

Atkinson, D. E. and Walton, G. M. (1967). Adenosine triphosphate conservation in metabolic regulation. *J. biol. Chem., 242,* 3239–3241.

Bergmeyer, H. U. (1974). *Methoden der enzymatischen Analyse,* 4th. ed. Verlag Chemie, Weinheim.

Defretin, R. and Wissocq, J. C. (1969). La musculature longitudinale dorsale et ventrale des *Nereis irrorata Malmgren* (Annelides Polychetes) atoques et epitoques. *C. r hebd. Séanc. Acad. Sci. Paris, 249,* 2117–2120.

Felbeck, H. (1979). Die Rolle der Aminosäuren im Stoffwechsel von *Arenicola marina:* Bedeutung für Osmoregulation und Anaerobiose. Ph. D. thesis, University of Münster, FRG.

Kluytmans, J. H. F. M., Veenhof, P. R. and de Zwaan, A. (1975). Anaerobic production of volatile fatty acids in the sea mussel *Mytilus edulis L. J. comp. Physiol., 104,* 71–78.

Lindner, E. and Fischer, A. (1964). Zur Feinstruktur nereider und heteronereider Muskulatur von *Platynereis dumerilii. Naturwissenschaften, 17,* 410.

Schöttler, U. (1978). Investigations of the anaerobic metabolism of the polychaete worm *Nereis diversicolor* M. *J. comp. Physiol., 125,* 185–189.

Schöttler, U. and Schroff, G. (1976). Untersuchungen zum anaeroben Glykogenabbau bei *Tubifex tubifex* M. *J. comp. Physiol., 108,* 243–254.

Surholt, B. (1977). The influence of oxygen deficiency and electrical stimulation on the concentration of ATP, ADP, AMP and phosphotaurocyamine in the body-wall musculature of *Arenicola marina. Hoppe-Seyler's Z. physiol. Chem., 358,* 1455–1461.

Theede, H., Schaudinn, J. and Safe, F. (1973). Ecophysiological studies on four *Nereis* species of the Kiel bay. *Oikos, 15* (Suppl.), 246–252.

Zebe, E. (1975). In vivo-Untersuchungen über den Glucose-Abbau bei *Arenicola marina* (Annelida, Polychaeta). *J. comp. Physiol., 101,* 135–145.

Zwaan, A. de (1977). Anaerobic energy metabolism in bivalve molluscs. *Oceanogr. mar. Biol. A. Rev., 15,* 103–187.

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