THE EFFECT OF ENVIRONMENTAL OXYGEN CONCENTRATION ON THE RATE OF OXYGEN CONSUMPTION OF THE POLYCHAETE *STHENELAIS BOA* (JOHNSTON)

by

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Résumé

La consommation d'oxygène $\dot{V}_{02}$ de *Sthelenais boa* a été mesurée en fonction de la pression partielle d'oxygène dans le milieu extérieur, P_{02} (E). Dans chaque expérience, les valeurs de $\dot{V}_{02}$ ont été calculées à partir de la courbe, enregistrée en continu, des variations de P_{02} (E) dans une enceinte close contenant un seul individu dont le métabolisme aérobie réduit progressivement la valeur de P_{02} (E). Les variations de la pression partielle d'oxygène dans le milieu intérieur P_{02} (I), ont été mesurées en fonction de P_{02} (E) grâce à une microélectrode à oxygène implantée dans la cavité coelomique.

Chez les 19 individus étudiés, la consommation d'oxygène est directement proportionnelle à la pression partielle de ce gaz pour des valeurs de P_{02} (E) comprises entre 0 et 159 torr (« oxyconformité »). Pour la P_{02} (E) *ca* 159 torr, les valeurs de V_{02} sont supérieures à celles trouvées chez d'autres espèces de poids comparable (compris entre 0,99 et 3,31 g) et la valeur moyenne de P_{02} (I) est de 33 torr. Cette valeur diminue avec P_{02} (E) et elle est nulle pour P_{02} (E) *ca* 6 torr. Aucune dette d'oxygène n'a pu être mise en évidence.

Nous considérons que 1' « oxyconformité » observée est le résultat des mécanismes de diffusion fournissant l'oxygène aux tissus en l'absence d'un système circulatoire efficace dépourvu, en particulier, de pigment respiratoire. Le pigment de type myoglobine entourant le système nerveux est susceptible de fournir à cette structure un meilleur approvisionnement en oxygène. Cette « oxyconformité », mise en évidence chez l'individu au repos, exige que l'énergie supplémentaire dépensée en période d'activité soit fournie par un métabolisme de type anaérobic.

Introduction

Most polychaetes, even those of quite small body size, have hemoglobin-type pigments in solution in the blood or contained in corpuscles suspended in the coelmic fluid or both. In contrast, members of the family Aphroditidae (2) have no such pigments in the circulatory system, which is poorly developed, or in the coelomic

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(2) We have followed the nomenclature of Cabioch, *et al.* (1967) throughout this paper.

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fluid; an intracellular pigment (myoglobin?) occurs surrounding the central nervous system and the proximal portions of the segmentai nerves (Bloch-Raphaël, 1939). Nonetheless, several members of this family reach large body sizes and are at least moderately active. We report here studies on the rate of oxygen consumption of *Sthenelais boa,* which is comparable in body size to the polychaetes *Nephthys* sp. and *Audouinia* sp. which have circulatory systems containing hemoglobin and with which it shares a common habitat (Fauvel, 1923).

Materials and Methods

Specimens of S. *boa* supplied by the collecting staff of the Station biologique de Roscoff were kept in aquaria of running sea-water and used within four days of collection.

We measured oxygen consumption of individual worms by continuous monitoring of the oxygen concentration in a closed respiration chamber (Fig. 1) with a galvanic cell oxygen electrode (1) (Mancy *et al.* 1962) of our own fabrication, its output being fed into a 10-inch Linear Instruments chart recorder in parallel with a Beckman 10-turn helipot adjusted to give nearly full-scale chart readings with air-satured sea-water. The chamber was immersed in a bath of flowing sea-water which maintained the temperature constant to within ± 0.25°C during the course of a determination.

Before each determination, we calibrated the electrode by recording its output in sea-water made oxygen-free with sodium sulfite and in sea-water saturated with air. These initial and final calibrations never differed by more than five percent. The electrode output being a linear function of the chemical activity of oxygen in the sea-water, knowing the barometric pressure and relative humidity and assuming 20.9 percent oxygen in air, we were able to calculate for any value of the electrode output (1) the partial pressure of oxygen in equilibrium with the sea-water in the respiration chamber and, knowing the chamber volume, (2) the total oxygen content of this sea-water. We verified the accuracy of these calculated values by occasional Winkler determinations (method of Carritt and Carpenter, 1966).

Recording of the output of the electrode in the respiration chamber continued until the chart trace approached asymptoty with the time axis (Fig. 2A). We then removed the worm from the chamber, rolled it on filter paper to remove adherent water, weighed it rapidly

(1) Galvanic oxygen electrodes and polarographic oxygen electrodes both measure the chemical activity of oxygen in solutions. This chemical activity is the fundamental thermodynamic quantity governing the behaviour of oxygen in physical processes such as diffusion and in chemical reactions of metabolism; it is therefore the physiologically important quantity. It is customarily expressed as the partial pressure of oxygen (P_{02}) in a gas phase in equilibrium with the solution. The concentration of oxygen (amount per unit volume of solution) is proportional to the partial pressure of oxygen in equilibrium with the solution and therefore to the chemical potential as measured by the electrode. Knowing the proportionality constant, the oxygen concentration can be calculated from the electrode output. We have calculated the necessary proportionality constants from our electrode calibrations and the data supplied in Carpenter (1966).

and returned it to an aquarium of running sea-water. In all cases, the worms resumed normal activity and survived for at least two days.

From each chart trace, we obtained the X and Y coordinates (as minutes and total oxygen content in microliters of the sea-water in the respiration chamber) for points approximately five millimeters apart over the whole length of the curve using a Ladd Graphical Digitizer. We entered these values in a Hewlett-Packard Model 9320A

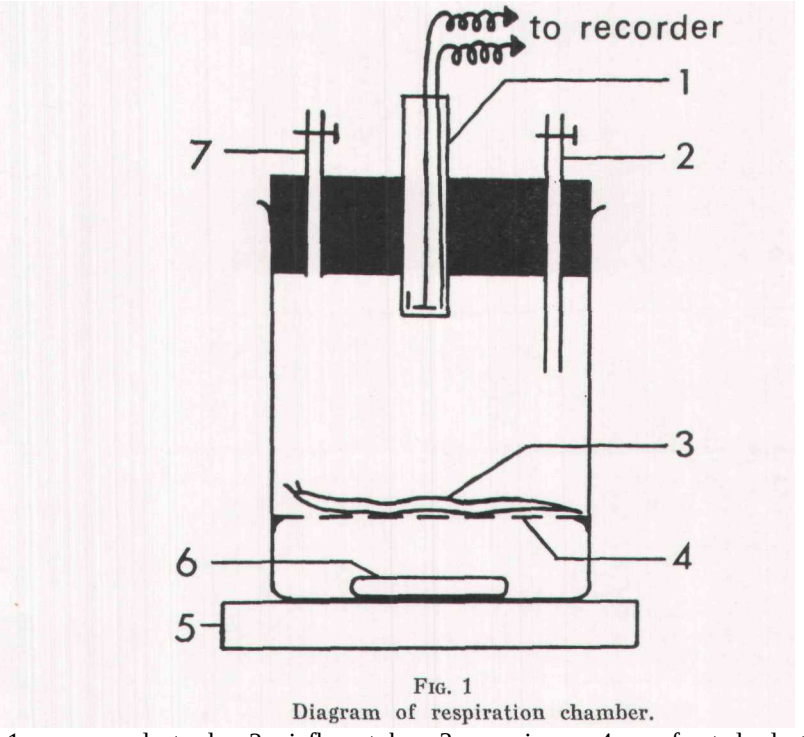

FIG. 1
Diagram of respiration chamber.
1: oxygen electrode; 2: inflow tube; 3: specimen; 4: perforated plastic disc; 5: submersible magnetic stirrer; 6: stirring bar; 7: outflow tube.

Calculator-Plotter programmed to calculate partial pressure of oxygen **(P_{02})** in torr of the mid-point of successive pairs of **X, Y** values and the rate of oxygen consumption **(V_{02})** in µl.min-1 from $\Delta Y/\Delta X$, to fit the expressions $V_{02s} = a + b\ (P_{02})$ and $V_{02} = a' + b'\ (P_{02}) + c\ (P_{02})^2$ to these date and to plot the individual points and the fitted curves.

We measured internal partial pressures of oxygen with a naked **micro-0_2** electrode (Clark-type) mounted in **18G** hypodermic tubing (Transidyne, Ann Arbor, Michigan) and inserted into the mid-region of the body of a worm pinned in a plastic tray filled with sea-water in which **we** varied the partial pressure of oxygen by equilibration with gas mixtures of different known oxygen contents or by mixing appropriate volumes of air-saturated sea-water and of sea-water deoxygenated by bubbling with nitrogen. **We** recorded the output **of** the electrode at each partial pressure of oxygen until it reached

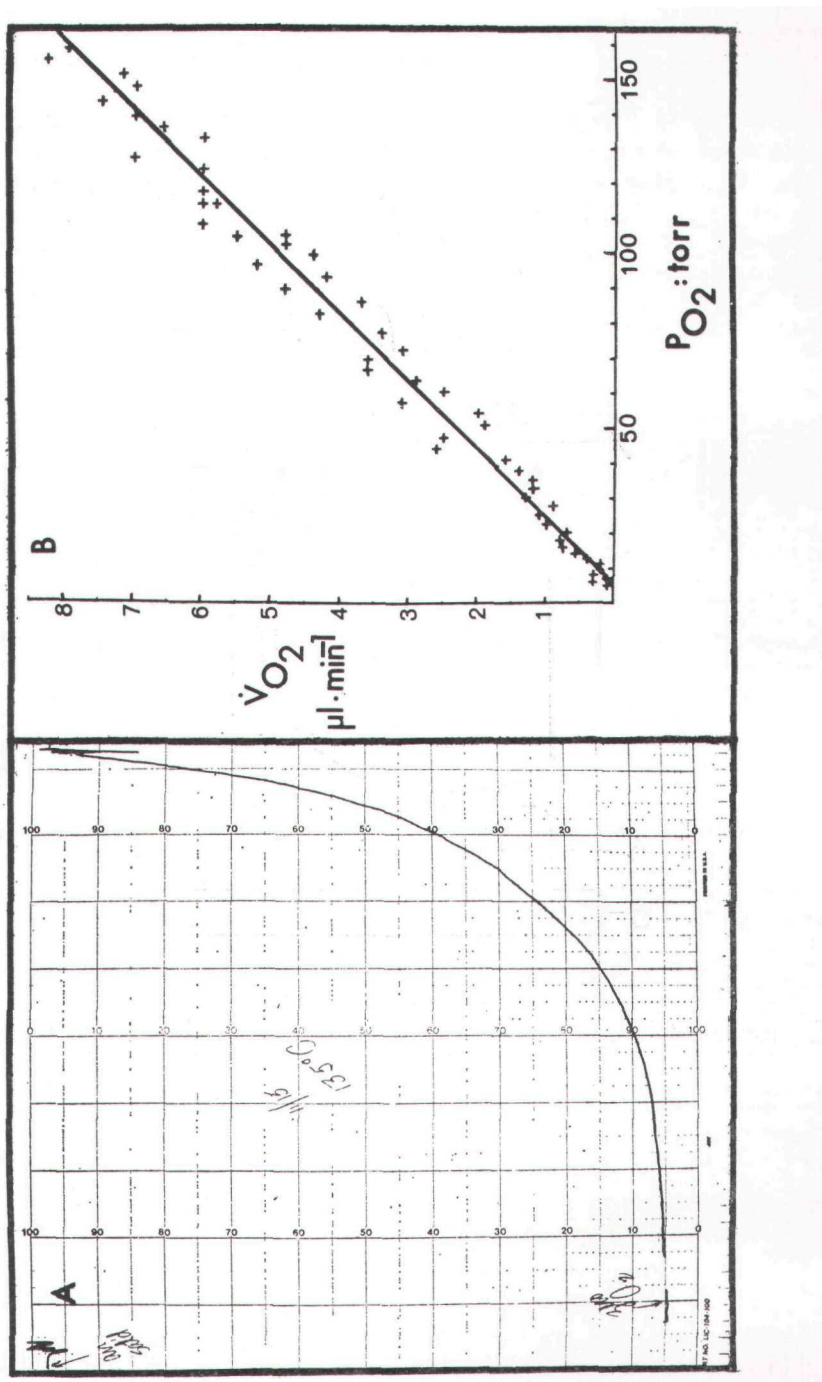

FIG. 2

A: unretouched chart record of determination on specimen No. 14; B: regression of $_{O2}$ V_{O2} on P_{O2} plotted as described in text.

The regression lines are based on 108 sets of values of V_{O2} and P_{O2} but for clarity only every other set of values is plotted; on the scale of this figure the linear and quadratic regression lines are indistinguishable.

a constant value and converted this value to internal P_{O2} (in torr) using a calibration of the electrode made in the same way as with the galvanic cell electrode. Although we attempted always to insert the electrode to a depth which would place the sensing tip in the coelomic space, there was some uncertainty about its exact placement.

Results

Fig. 2 shows, for a randomly selected determination, the original chart record and the regressions of V_{O2} on P_{O2} For each determination, we tested the hypothesis that the regression is linear by the method of Snedecor and Cochran (1967); in no case was the hypothesis rejected at the 5 percent level so we conclude that the relationship between rate of oxygen consumption and environmental oxygen concentration is linear in all the individuals studied. To test the possibility that this linearity might result from a high rate of oxygen consumption at higher P_{O2}'s (which necessarily correspond to the earlier portion of each determination) resulting from repayment of an oxygen debt incurred by the vigorous activity triggered by transfer to the respiration chamber, in five additional determinations we allowed, to rest for an hour in the chamber while air-saturated water flowed through it; in each case, the record then obtained showed the same linear dependence of V_{O2} on P_{O2} at high P_{O2}'s.

We also satisfied ourselves that prolonged exposure to the low oxygen concentrations toward the end of a determination and to the restricted volume of sea-water in the chamber had no permanent effect on V_{O2} by (1) comparing the results of determinations on the same worm as described in Materials and Methods and following abrupt decrease in oxygen concentration at the beginning of a determination by replacement of part of the sea-water in the closed respiration chamber with sea-water deoxygenated with nitrogen and (2) by replacing the oxygen depleted sea-water at the end of a determination with air-saturated sea-water through the inlet tube and immediately repeating the determination. Differences in slopes and intercepts of the regression lines were not significant at the 5 percent level.

Table 1 summarizes the results of all determination. Because of the time required for equilibration of the electrode after inserting it in the chamber, the chart records on which the data in Table 1 are based do not provide direct estimates of V_{O2} in air-saturated water. We have calculated these values, assuming that air-saturated water contains a concentration of oxygen in equilibrium with a P_{O2} of 159 torr; the results of these calculations are shown in Table 2. A calculation of the regression of log (V_{O2}) on log (body weight) showed so poor a correlation (R^2=0.1) that the weight-specific rates in column B have been calculated simply by dividing each value in column A by the weight of the worm. We consider that the poor correlation between log (V_{O2}) and log (body weight) is the consequence of the small range of weights in our material, good correlation usually

requiring weights ranging over at least an order of magnitude. During these determinations, the worms were quiescent except for brief periods of activity and slight movements of the elytrae which we interpret as ventilatory movements; consequently the values of V_{O2} given in this table represent resting rates.

The calculated maximum V_{O2}'s under natural conditions appear to be higher than the values reported for most other annelids except species considerably smaller than our specimens of S. *boa*. (For representative data see Dales, 1961a, b; Hoffman and Mangum, 1970; Mangum, 1963, 1972; Mangum and Sassaman, 1969; May, 1972; Toulmond, 1973). The control experiments described above, which failed to show any evidence of an oxygen debt resulting from either vigorous muscular activity or from partially anoxic conditions rule out an explanation based on repayment of an oxygen debt. We have no explanation to offer for this higher metabolic rate.

We have also used the regression constants of Table 1 to estimate the environmental oxygen concentrations at which oxygen consumption ceases; the results are shown in column C of Table 2.

Fig. 3 shows the results of determinations of internal P_{O2}. Attempts to determine internal P_{O2}'s at very low environmental oxygen concentrations resulted in electrode readings indicating internal concentrations substantially higher than the known external concentrations. Such internal concentrations are physically impossible in these worms which have no oxygen stores that could be unloading oxygen to the tissues under these conditions. We have not included these determinations in Fig. 3 and have no explanation for them. HOW-

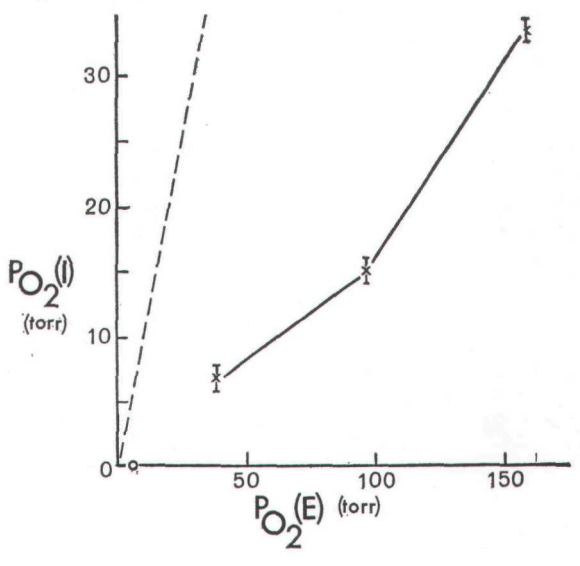

FIG. 3
Relationship between environmental partial pressure of oxygen, P_{O2} (E), and internal partial pressure of oxygen, P_{O2} (I).
The dashed line is the oxygen isobar; o is the predicted point for P_{O2} (I) = 0 from table 2; vertical lines indicate standard errors of means.

ever, even if they indicate failure of the electrodes to respond linearly to oxygen concentration, they do not vitiate the conclusion, from measurements at higher environmental concentrations of oxygen, that the internal concentration is substantially lower than the environ-

TABLE 1

Regression of rate of Oxygen consumption, V_{O2} on environmental partial pressure of Oxygen, P_{O2} (E).

$V_{O2} = a + bP_{O2}$ (E)

Specimen No	Temperature °C	Body weight (grams)	a(x 10^2) (µl. $min.^{-1}$)	b(x 10^2) (µ1. min^{-1} $torr^{-1}$)	
1	14.0	1.35	—12.6	3.8	0.888
2	14.0	1.16	—32.2	7.1	0.947
3	13.5	1.76	—20.8	3.8	0.833
4	13.0	1.40	—10.2	3.6	0.881
5	14.0	0.99	—10.3	3.1	0.880
6	13.0	3.31	—43.0	4.1	0.970
7	13.5	2.03	—23.2	3.9	0.932
8	14.0	1.92	—44.5	4.5	0.891
9	13.5	1.18	—21.2	5.4	0.898
10	13.0	1.40	—52.9	4.8	0.867
11	13.0	1.26	—15.0	1.4	0.813
12	12.5	2.15	—33.5	4.8	0.865
13	13.0	1.66	—15.1	4.4	0.862
14	13.5	1.34	—30.1	5.2	0.913
15	13.0	1.37	—34.0	4.3	0.865
16	13.0	2.56	—25.6	2.9	0.876
17	14.0	1.01	—36.2	4.3	0.804
18	14.5	1.83	—21.3	5.1	0.939
19	13.0	1.56	—37.4	3.4	0.855

TABLE 2

Calculated values of V_{O2} in air-saturated sea-water [P_{O2} (E) = 159 torr] and of P_{O2} (E) at which V_{O2} becomes zero.

Specimen No	V_{O2} at P_{O2} (E) = 159 torr		P_{O2} (E) (V_{O2} = 0)
	(A) µ1. min^{-1}	(B) µ1. $min^{-1}.g^{-1}$	(C) torr
1	5.92	4.38	3.3
2	10.97	9.45	4.5
3	5.83	3.31	5.5
4	5.62	4.02	2.8
5	4.83	4.87	3.3
6	6.09	1.84	1.1
7	5.97	2.94	5.8
8	6.71	3.4ï)	9.9
9	8.37	7.10	3.9
10	7.10	5.07	11.0
11	2.08	1.65	10.7
12	7.30	3.39	7.0
13	6.85	4.12	3.4
14	7.97	5.95	5.8
15	6.50	4.74	7.9
16	4.36	1.70	8.8
17	6.48	6.41	8.4
18	7.90	4.31	4.2
19	5.03	3.23	11.0
mean	*6.46*	*4.34*	*6.2*

mental concentration since the direction of this putative electrode error would tend to reduce the apparent difference between internal and environmental concentrations.

Discussion

Mangum and van Winkle (1973) proposed that the relationship between rate of oxygen consumption and environmental concentration of oxygen can best be analysed and stated quantitatively by fitting experimental data to a quadratic expression, $Y = a + b(P_{O2}) + c(P_{O2})^2$ where Y is the relative weight-specific rate of oxygen consumption expressed as a fraction of the weight-specific rate in air-saturated water (1). If c is not significantly different from zero, strict oxyconformity is demonstrated; values of c significantly different from zero denote oxyregulation. This method of analysis provides an objective criterion for deciding whether oxyregulation or oxyconformity occurs and also allows a quantitative comparison of the degree of oxyregulation among individuals or species in which it occurs. Of the six species of annelids for which Mangum and van Winkle give data, all individuals of *Hyalinoecia tubicola* and of *Nereis succinea* were oxyconformers whereas in the other four species, some individuals showed oxyregulation. All of the individuals of S. *boa* which we studied were strict oxyconformers. Hoffman and Mangum (1970) reported that *Glycera dibranchiata* is also a conformer, as is *Abarenicola pacifica* (May, 1972); *Arenicola marina* and *A. cristata* are moderately good oxyregulators (Toulmond, 1975; Mangum, 1976). Mangum *et al.* (1975) have reported that when the gills of *Amphitrite ornata* and of *Euplobranchus sanguineus* are ligated, these oxyregulators become conformers; *Lysilla alba,* sympatric with *E. sanguineus* but lacking hemoglobin, is a conformer under normal conditions. During our studies of S. *boa,* we were also able to make determinations on six specimens of *Cirratulus cirratus,* which has vascular hemoglobin, an to two specimens of *Harmothoe imbricata* and one small specimen of *Aphrodite aculeata.* These two species of aphroditids lack hemoglobin. All the specimens of *C. cirratus* showed oxyregulation (range of c: —0.05 to —0.08 x 10^{-3}); all the aphroditids were oxyconformers. No generalizations correlating oxyregulation or conformity with body size, habitat or life habits are possible among polychaetes but oxyregulation seems not to occur in species lacking hemoglobin.

In any oxyregulator, at environmental partial pressures of oxygen above the critical pressure, the measured rate of oxygen consumption reflects the rate of operation of the terminal oxidase systems. These rates in turn reflect the amounts and properties of the systems themselves or the rate at which they are supplied with anaerobically-

(1) The use of Y or "normalized" weight-specific rate of oxygen consumption allows the ready comparison of degrees of oxyregulation in different individuals or species but is not essential to the mathematical argument on which the distinction between oxyregulation and oxyconformity is based. Because our experimental procedure did not yield values of V_{O2} in air-saturated water, we have not normalized our data when testing for oxyconformity.

produced substrates. In contrast, when V_{02} is proportional to environmental oxygen concentration, whether in oxyregulators below their critical pressures or in oxyconformers at all pressures, the terminal oxidase systems must be operating at less than capacity; to an increasing extent as environmental oxygen concentration decreases, energy requirements must be supported by anaerobic processes.

It seems clear that in oxyregulators, oxygen-transport pigments, respiratory surfaces and circulatory systems all serve to maintain, in conjunction with direct diffusion of oxygen, internal partial pressures of oxygen sufficient to saturate the terminal oxidase systems. The relative importance of the oxygen-transport pigments and of physical solution of oxygen in the blood can be assessed by comparison of the effects of inactivation of the pigment and of gill ligation on V_o over a range of environmental partial pressures of oxygen. Since *S. boa* has neither a well-developed circulatory system nor any oxygen-transport pigment other than the myoglobin surrounding the central nervous system, its strict oxyconformity is to be expected.

Internal (= coelomic fluid) P_{02}'s in annelids generally are well below environmental P_{02}'s (Mangum, 1976; Mangum *et al.*, 1975), the extent of the difference increasing under natural conditions when gametes are present in the coelom and experimentally when oxygen transport by hemoglobin is blocked or oxygen exchange through the respiratory surface is prevented. The reproductive season for *S. boa* in the Roscoff area is early summer. All of our determinations were made late in October and in November so none of our specimens contained gametes; under our experimental conditions there was no interference with the normal functioning of the respiratory exchange surface. Low internal P_{02}'s would also be expected in an annelid in which the body wall consists of massive layers of muscle but the body wall of *S. boa* is not so constructed. Bloch-Raphaël (1939) points out that « au lieu des quatre masses de fibres musculaires qu'on rencontre chez la plupart des Polychètes, il n'y a que des fibres musculaires en paquets peu volumineux suspendus dans le coelome ». We have estimated relative masses of epithelial, muscular and nervous tissues from the areas occupied by these tissues in cross-sections prepared at various distances along the length of the bodies of our specimens and in her Fig. 12. Approximately 55 percent of the total volume of the body consists of coelom and intestinal lumen, which should have low rates of oxygen consumption; the remainder is about equally divided between muscle and epithelium. Since there are no massive muscle layers which would hinder the diffusion of oxygen into the coelomic fluid, we conclude that the low internal P_{02}'s show that the respiratory surface is relatively inefficient in oxygen exchange.

The brain and ventral nerve cord contain conspicuous amounts of a hemoglobin-like pigment which appears to be entirely intracellular and is probably a myoglobin. The brain is separated from the environment by a thick epithelial layer and the ventral nerve cord lies at a distance from the respiratory surface and close to major bundles of muscle fibers. We suggest that the myoglobin-like pigment increases that diffusional flux of oxygen into the central

nervous system in a fashion analogous to its function in vertebrate red muscle fibers (Murray, 1974) with the result that this nervous tissue receives an adequate supply of oxygen even when the P_{02} of its oxygen sources is low. We tried to test this function of this myoglobin by exposing worms in the respiration chamber to carbon monoxide but could not detect any effect on V_o, although the pigment took on the characteristic appearance of the carbon monoxide form and the worms upon removal from the chamber seemed more sluggish than usual. This failure may result from the inability of our technique to detect reductions in oxygen consumption in structures which we estimate to constitute less than 2 percent of the total tissue mass. A more critical test will be the comparison of oxygen consumption rates by isolated central nervous system in the presence and absence of carbon monoxide at various oxygen concentrations.

Our results show that S. *boa* is a strict oxyconformer over the whole range of environmental oxygen concentrations to which it is exposed in nature, that vigorous muscular activity in air-saturated sea-water neither increases V_{02} nor results in an oxygen debt. Although we cannot say whether any of the energy expenditure of resting specimens in air-saturated sea-water is supported by anaerobic processes, it is obvious that as environmental P_{02} declines an increasing percentage of total energy expenditure must be so supported, as must all of the additional energy expenditure during activity at all environmental P_{02}'s. The end-products of these anaerobic processes must be lost from the body. This ability to rely on anaerobic processes to support its energy requirements is not unusual among invertebrates where the capacity for facultative anaerobiosis is well-developed (see Hochachka, 1973 for a review). We have made no studies of the nature of the anaerobic aspects of intermediary metabolism in this worm. Such studies should be undertaken to increase our understanding of the metabolic adaptations of invertebrates and of the bioenergetics of this active, predatory worm.

Acknowledgments

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Summary

We have calculated the oxygen consumption, V_{02}, of *Sthenelais boa* from continuous recordings of the partial pressure of oxygen in a sealed respiration chamber containing a single individual and have measured the partial pressure of oxygen in the coelom, P_{02} (I), at several environmental partial pressures of oxygen, P_{02} (E), with a micro-0_2 electrode inserted into the coelomic cavity. All of the 19 individuals studied were strict oxyconformers at least up to P_{02} (E) of 159 torr; at that partial pressure, V_{02}'s were higher than values reported for other species of annelids of comparable size (0.99 to 3.31 g body weight). Mean P_{02} (I) was 33 torr at P_{02} (E) of 159 torr, decreasing to zero at a predicted mean P_{02} (E) of 6.2 torr. We found no evidence of the incurring of oxygen debts.

The strict oxyconformity observed indicates that oxygen activity in the tissues is insufficient to saturate the terminal oxidases over the whole range of environmental partial pressures oxygen encountered under natural conditions. We propose that this insufficiency results from the combination of a respiratory surface which is inefficient in oxygen exchange and a circulatory system which is poorly developed and lacks hemoglobin to assist in the transport of oxygen. The myoglobin-like pigment surrounding the central nervous system may provide this structure with an improved supply of oxygen. The strict oxyconformity which prevails in the resting individual requires that the extra energy expended during activity be supplied by anaerobic metabolic processes.

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