J. exp. Biol. (1975), 63, 647–660
With 7 figures
Printed in Great Britain

BLOOD OXYGEN TRANSPORT AND METABOLISM OF THE CONFINED LUGWORM *ARENICOLA MARINA* (L.)

BY ANDRÉ TOULMOND*

Laboratoire de Zoologie, Université Pierre-et-Marie-Curie, Paris; Station Biologique de Roscoff; Laboratoire de Physiologie Respiratoire, C.N.R.S., Strasbourg

(*Received* 24 *April* 1975)

SUMMARY

Oxygen consumption ($\dot{M}_{O_2}$), haemoglobin oxygen saturation level ($S\bar{v}_{O_2}$) and pH (pH$\bar{v}$) in prebranchial blood were measured in lugworms experimentally confined in sea water at 15 °C. Total blood flow through the gills ($\dot{V}b$) was estimated. For sea water oxygen partial pressure (Pw_{O_2}) between 120 and 150 Torr, $\dot{M}_{O_2}$, $S\bar{v}_{O_2}$ and $\dot{V}b$ were high and nearly constant. For $Pw_{O_2} < 120$ Torr, $\dot{V}b$ fell quickly, $\dot{M}_{O_2}$ progressively dropped, and metabolism remained aerobic at the expense of the prebranchial blood oxygen store. For $Pw_{O_2} < 50$ Torr, $\dot{V}b$ and $S\bar{v}_{O_2}$ values were extremely low, and the low pH$\bar{v}$ and the modified buffer power of the surrounding sea water showed that anaerobic metabolism was occurring. Changes in respiratory gas exchanges and metabolism during the tidal cycle are deduced from the comparison of these results with data obtained in the field.

INTRODUCTION

For many intertidal animals, such as the burrowing polychaete annelid *Arenicola marina* (L.), oxygen availability is reduced during low tide (Wells, 1945; Krüger, 1964*a*). How the lugworm overcomes this critical period is still obscure. The different hypotheses on the subject (Jones, 1955; Krüger, 1960, 1969; Wells, 1966) have in common the proposition, based on an analysis of the physico-chemical properties of the lugworm blood haemoglobin, that since this respiratory pigment exhibits a strong oxygen affinity ($P_{50} = 2$ Torr; pH $= 7{\cdot}5$; $t = 15$ °C; Toulmond, 1970), it must theoretically allow *Arenicola* to fix and transport oxygen at the low partial pressures (Jones, 1955; Rullier, 1959; Amoureux, 1963) occurring in the L-shaped burrow during low tide. This proposition is inconsistent with recent work (Toulmond, 1973) showing that the lugworm develops a respiratory and metabolic blood acidosis during low tide. The acidosis strongly suggests that both anaerobic metabolism and reduction of respiratory gas exchanges occur during this period.

There are no data on variations of the lugworm's oxygen consumption during a tidal cycle: Van Dam's (1938) and Krüger's (1964*b*) measurements were obtained in conditions corresponding to high tide. Such variations must be maximal during the transitory period immediately following the confinement of the lugworm to its burrow by

* Mailing address: Laboratoire de Zoologie, Université Pierre-et-Marie-Curie, 4 place Jussieu, 75230 Paris Cedex 05, France.

the receding tide. The subject of the present work is a study of this transitory period. Measurements, which were unfeasible in the field, were made on lugworms experimentally confined in a large volume of sea water.

MATERIAL

Lugworms were collected at the exit of the 'Vieux Port' of Roscoff (Nord-Finistère, France) in August, September and October, brought back to the laboratory and kept unfed in running sea water (15–17 °C) for 48 h before utilization in experiments. Sampling and conservation of blood have been described previously (Toulmond, 1973).

METHODS AND EXPERIMENTAL PROCEDURES

All measurements were made at 15 °C.

(1) pH values of prebranchial blood ($pH\bar{v}$) and sea water (pHw) samples were measured with a thermostatted Radiometer capillary pH-microelectrode (G297/G2) coupled to a Radiometer pH-meter (Model pH 27 with Gas Monitor for sea water; model PHM 72 for blood). The electrode was calibrated at pH = 6·900 and pH = 7·445 with Radiometer buffers. These buffer solutions have an ionic strength approximately 20 times lower than that of sea water and of *Arenicola* blood. Therefore, values of $pH\bar{v}$ and pHw obtained after standardizing of the pH-microelectrode with these buffers cannot be considered as 'true' (NBS) pH values. Nevertheless comparison between sets of measurements obtained in the same standard conditions remains valid. Readings were made 2 min after the application of the buffer or of the solution of unknown pH on to the electrode. In these conditions, measurements made on the same sample did not differ by more than 0·005 pH unit.

(2) Oxygen partial pressure in seawater samples (Pw_{O_2}) was measured using a thermostatted Radiometer oxygen microelectrode (E 5046/0) calibrated with an oxygen-free solution and with sea water equilibrated at adequate P_{O_2}.

(3) Carbon dioxide partial pressure in seawater samples (Pw_{CO_2}) was measured with a thermostatted Radiometer CO_2 microelectrode (E 5036/0), coupled to the model pH 27 with Gas Monitor Radiometer pH-meter. The calibration curve of the electrode was established in the following way: seawater samples were equilibrated at 15 °C against gas phases of known P_{CO_2} (prepared from pure gases with Wösthoff gas-mixing pumps) and injected on to the CO_2-electrode. Readings were made on the expanded pH-scale, 15 min after the first injection, and the values obtained were plotted on a log Pw_{CO_2} *vs* arbitrary units diagram. Further readings of pH were then reported on to the free-hand curve, giving corresponding Pw_{CO_2}.

(4) Percentage saturation of prebranchial blood pigment ($S\bar{v}_{O_2}$) was determined using a method described elsewhere (Toulmond, 1973).

Three types of experiments were carried out:

(1) A lugworm was confined in a 250 ml glass syringe together with a known volume of sea water equilibrated against air at 15 °C. The syringe, closed by a three-way stopcock and packed in a thin aluminium sheet, was placed in a thermostatted water-bath. 3-ml water-samples were then withdrawn at regular time intervals for Pw_{O_2} (and in some cases, see Table 1, pHw and Pw_{CO_2}) measurements, until Pw_{O_2} fell below

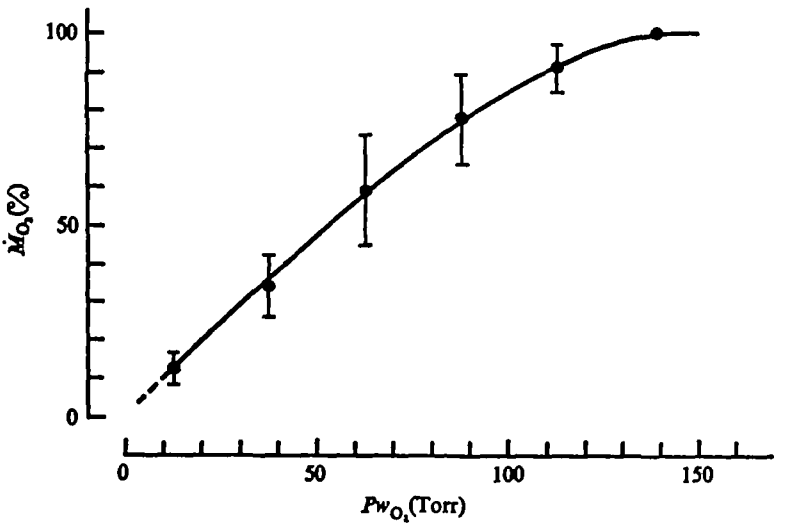

Fig. 1. Mean values of oxygen consumption ($\dot{M}_{O_2}$) as a function of partial pressure of oxygen in sea water (Pw_{O_2}) in confined *Arenicola* at 15 °C (10 experiments on 10 different animals, see Table 1). Vertical bar: SEM × t (Student) for N = 10 and P = 0·05.

Table 1. *Variations of oxygen consumption ($\dot{M}_{O_2}$) in experimentally confined lugworms at* 15 °C

Animal no.	W_f g	Total duration h	$\dot{M}_{O_2}$, in percentage of $\dot{M}_{O_2}$ measured for $125 < Pw_{O_2} < 150$ Torr: $100 < Pw_{O_2} < 125$	$75 < Pw_{O_2} < 100$	$50 < Pw_{O_2} < 75$	$25 < Pw_{O_2} < 50$	$Pw_{O_2} < 25$
	9·9	6·5	77	61	50	28	14
2	9·8	6·9	91	84	71	47	22
3	10·3	7·9	91	80	54	47	16
4	9·9	8·3	96	87	84	47	15
5	10·0	8·3	88	80	68	39	17
6*	8·2	6·9	98	60	32	17	7
7*	8·8	4·6	104	107	75	20	9
8*	8·8	6·3	80	57	31	19	6
9	7·2	2·7	89	74	51	32	12
10	7·2	4·4	92	91	77	39	11
Mean			91	78	59	34	13

* pHw and Pw_{CO_2} were measured in these animals.

Torr. Mean oxygen consumption ($\dot{M}_{O_2}$, μmol.h^{-1}) between two consecutive withdrawals was calculated using $\alpha w_{O_2} = 163 \cdot 10^{-5}$ μmol.ml^{-1}.Torr^{-1} (the solubility coefficient for oxygen at 15 °C in sea water of chlorinity, Cl = 19·5‰; Harvey, 1963) and plotted on a $\dot{M}_{O_2}$ *vs* Pw_{O_2} diagram. For comparisons between different experiments, the Pw_{O_2} scale was then divided in six standard intervals (15–25 Torr; 25–50 Torr, . . .; 125–150 Torr), mean $\dot{M}_{O_2}$ values were recalculated for these intervals and expressed, for each experiment, in percentage of $\dot{M}_{O_2}$ value for $125 < Pw_{O_2} < 150$ Torr. Ten experiments were carried out on ten different lugworms (Fig. 1, Table 1).

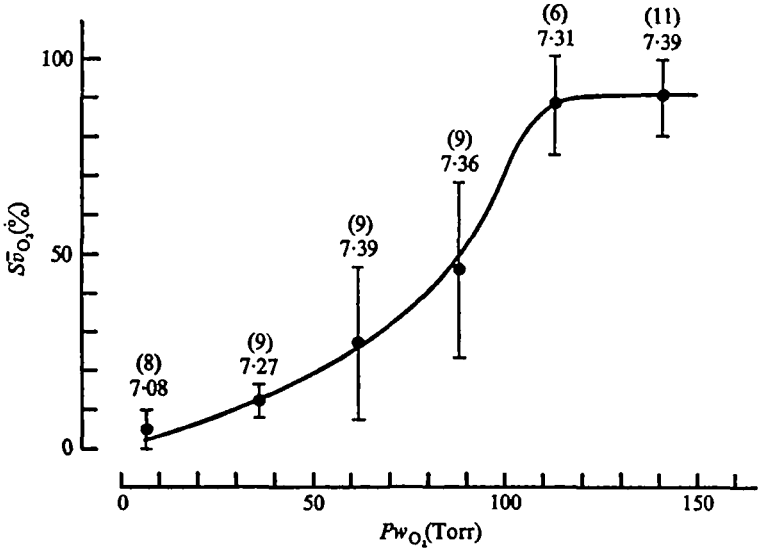

Fig. 2. Mean values of oxygen saturation of haemoglobin in prebranchial blood ($S\bar{v}_{O_2}$) as a function of Pw_{O_2} in confined *Arenicola* at 15 °C. Pw_{O_2} scale arbitrarily divided into 25-Torr intervals as in Fig. 1 and Table 1. Each point represents mean of $S\bar{v}_{O_2}$ and Pw_{O_2} values measured in each of these intervals. Above each point, number of measurements N (in brackets) and mean pH of prebranchial blood (pH$\bar{v}$). Vertical bar: SEM × t (Student) for N and $P = 0{\cdot}05$.

(2) In order to have corresponding data on percentage saturation of prebranchial blood pigment ($S\bar{v}_{O_2}$) for some of the values of Pw_{O_2} recorded in the above experiments, lugworms weighing approximately 10 g (fresh weight, W_f), were confined separately in 250 ml ground-glass stoppered bottles. These bottles were filled with air-saturated sea water at 15 °C and placed in a waterbath thermostatted at the same temperature. Each lugworm was allowed to respire for a certain period. Its bottle was then opened and Pw_{O_2} measured. $S\bar{v}_{O_2}$ and pH$\bar{v}$ were determined on prebranchial blood sampled in the lugworm ventral vessel (Ashworth, 1904). Data were plotted on a $S\bar{v}_{O_2}$ *vs* Pw_{O_2} diagram (Fig. 2).

(3) For further calculations, the relation between $\dot{M}_{O_2}$ and body weight at 15 °C had to be known. Taking into account the results of experiment (1), oxygen uptake rate was measured for $100 < Pw_{O_2} < 150$ Torr. As in experiment (2), lugworms were confined separately for various times in closed bottles. Bottles in which oxygen tension at the end of the confinement was found to be lower than 100 Torr were discarded. Values of $\dot{M}_{O_2}$ were computed as above and plotted against W_d (dry weight) on a log–log scale (Fig. 3).

LIST OF SYMBOLS

αb_{O_2}, αw_{O_2}	coefficient of oxygen solubility in blood and in sea water, μmol.ml^{-1}.Torr^{-1}
αw_{CO_2}	coefficient of carbon dioxide solubility in sea water
Ca_{O_2}, $C\bar{v}_{O_2}$	oxygen concentration in post- and prebranchial blood, μmol.ml^{-1}

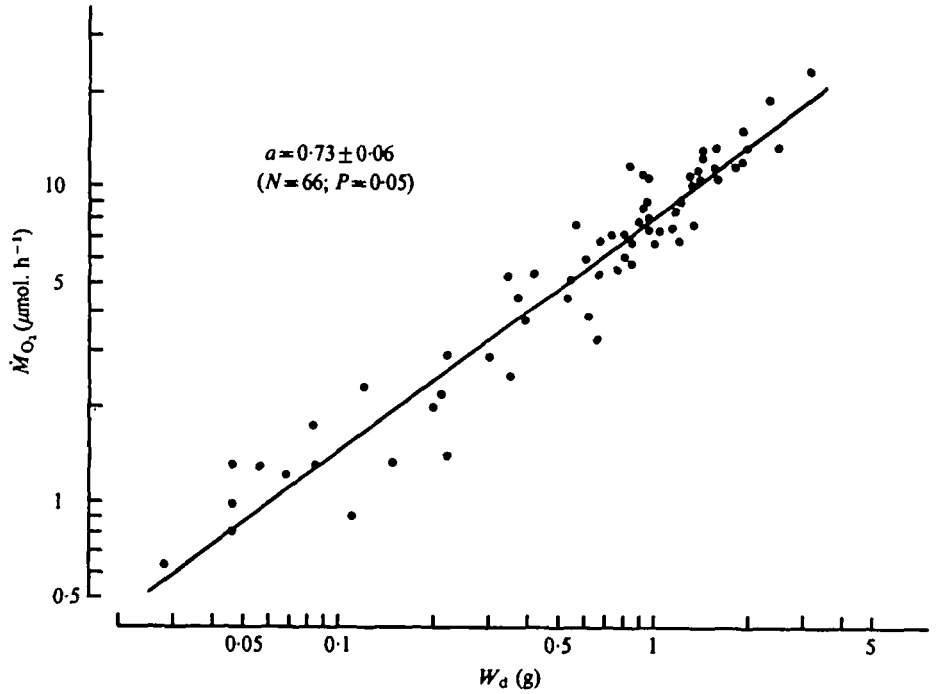

Fig. 3. Oxygen consumption ($\dot{M}_{O_2}$) as a function of dry body weight (W_d) in confined *Arenicola* at 15 °C, $Pw_{O_2} > 100$ Torr. Confidence interval on the regression line slope a: SEM $\times\, t$ (Student) for $N = 66$ and $P = 0{\cdot}05$.

$C^{max}_{HbO_2}$	concentration of combined oxygen in blood, when the haemoglobin is fully saturated, μmol.ml^{-1}
$\dot{M}_{O_2}$	oxygen consumption, μmol.h^{-1} (1 mole O_2 = 22393 ml)
Pa_{O_2}, $P\bar{v}_{O_2}$, Pw_{O_2}	oxygen partial pressure in post- and prebranchial blood and in sea water, Torr (= 1 mmHg)
Pw_{CO_2}	carbon dioxide partial pressure in sea water
pH$\bar{v}$, pHw	pH of prebranchial blood and of sea water
Sa_{O_2}, $S\bar{v}_{O_2}$	percentage saturation of post- and prebranchial blood haemoglobin.
Vb	total blood volume of *Arenicola*, ml
Vt	total volume of *Arenicola*, ml
$\dot{V}b$	total blood flow through the 26 gills of *Arenicola*, ml.h^{-1}
W_d	dry weight, g
W_f	wet weight, g

RESULTS

If oxygen uptake rate ($\dot{M}_{O_2}$) is expressed in μmol.h^{-1} and dry weight (W_d) in g, calculation of the parameters of the regression line $\log \dot{M}_{O_2} = f(\log W_d)$, gives (Fig. 3):

$$\log \dot{M}_{O_2} = 0{\cdot}90 + 0{\cdot}73 \log W_d$$

or

$$\dot{M}_{O_2} = 8{\cdot}0\,(W_d^{\,0{\cdot}73})$$

The data yield two other useful relationships: between dry weight (W_d) and wet weight (W_f)

$$W_d = 0{\cdot}145\ W_f\,(\mathrm{SEM} \times t\,(\mathrm{Student}) = 0{\cdot}012;\ N = 159;\ P = 0{\cdot}05),$$

between W_f and the total body volume (Vt)

$$W_f = 1{\cdot}074\ Vt\ (\mathrm{SEM} \times t\,(\mathrm{Student}) = 0{\cdot}020;\ N = 37;\ P = 0{\cdot}05).$$

Fig. 1 shows that $\dot{M}_{O_2}$ is Pw_{O_2}-dependent for $0 < Pw_{O_2} < 150$ Torr. Nevertheless this curve is not exactly that of a typical oxygen conformer and clearly suggests that oxygen uptake may be regulated at the higher values of Pw_{O_2}.

Fig. 2 shows that the percentage saturation of prebranchial blood ($S\bar{v}_{O_2}$) remains constant (*ca.* 91 %) for $Pw_{O_2} > 120$ Torr. Below this value, it rapidly decreases: when $Pw_{O_2} = 90$ Torr, $S\bar{v}_{O_2} = 50\%$; when $Pw_{O_2} = 15$ Torr, $S\bar{v}_{O_2} = 5\%$. When $Pw_{O_2} < 50$ Torr, the blood clearly becomes acidotic.

Results for type-experiment (Table 1, no. 6, and Fig. 4) show that, as Pw_{O_2} decreases from 148 to 7·3 Torr, pHw falls from 8·0 to 7·1, whereas Pw_{CO_2} increases from 0·3 to 5 Torr. Most of the Pw_{CO_2} rise occurs when $Pw_{O_2} < 50$ Torr. If pHw and Pw_{CO_2} values of Fig. 4 are substituted into the Henderson–Hasselbalch equation, one can draw the buffer line corresponding to the sea water in which the worm is confined (Fig. 5, curve *A*). This curve shows that the sea-water buffer power is saturated for Pw_{CO_2} *ca.* 2 Torr ($\Delta[HCO_3^-]/\Delta pHw = 0$). Saturation of running seawater buffer power occurs only when $Pw_{CO_2} > 3$ Torr (Fig. 5, curve *B*). This, and the divergence of curves *A* and *B* when Pw_{O_2} is about 50 Torr, must be correlated with the blood acidosis reported above. These findings strongly suggest that when $Pw_{O_2} < 50$ Torr, metabolism is anaerobic and acid is being liberated into the medium.

Estimation of the total blood flow through the gills during confinement ($\dot{V}b$)

In accordance with Ashworth's morphological and anatomical data (1904) we shall assume that *Arenicola*'s respiratory gas exchanges are wholly branchial. In these conditions Fick's first principle may be used:

$$\dot{V}b = \dot{M}_{O_2}/(Ca_{O_2} - C\bar{v}_{O_2})$$

where Ca_{O_2} and $C\bar{v}_{O_2}$ = oxygen concentration in post- and prebranchial blood (μmol .ml^{-1}). If then, the simultaneous variations of $\dot{M}_{O_2}$, Ca_{O_2} and $C\bar{v}_{O_2}$ as functions of Pw_{O_2} are known, the variations of $\dot{V}b$ during a confinement can also be known.

Let us consider a lugworm of $W_f = 10$ g, $W_d = 1{\cdot}45$ g and $\dot{M}_{O_2} = 10{\cdot}4$ μmol.h^{-1} (= *ca.* 240 μl.h^{-1}) (Fig. 3, $t = 15$ °C and $Pw_{O_2} = 150$ Torr). Mean variations of $\dot{M}_{O_2}$, in absolute values, are obtained from data of Fig. 1. Computation of Ca_{O_2} and $C\bar{v}$

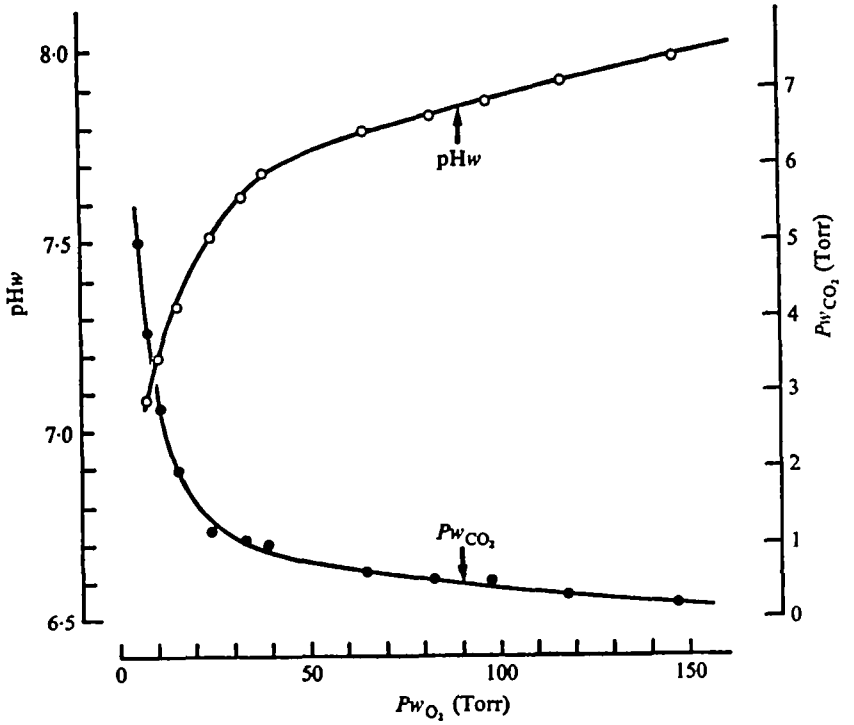

Fig. 4. pH (pH*w*) and P_{CO_2} (Pw_{CO_2}) in sea water as a function of Pw_{O_2} during the confinement of *Arenicola* at 15 °C (Table 1, no. 6).

variations involves several hypotheses and the utilization of previous results. Values of Ca_{O_2} and $C\bar{v}_{O_2}$ are given by the relations:

$$Ca_{O_2} = (C^{max}_{HbO_2} . Sa_{O_2}) + \alpha b_{O_2} . Pa_{O_2}$$

and

$$C\bar{v}_{O_2} = (C^{max}_{HbO_2} . S\bar{v}_{O_2}) + \alpha b_{O_2} . P\bar{v}_{O_2}$$

where $C^{max}_{HbO_2}$ = concentration of combined oxygen in blood, when haemoglobin is fully saturated, μmol.ml^{-1}, Sa_{O_2} = percentage saturation of postbranchial blood haemoglobin and αb_{O_2} = coefficient of oxygen solubility in blood at 15 °C ($161 \cdot 10^{-5}$ μmol.ml^{-1}.Torr^{-1}, Toulmond, unpublished).

$C^{max}_{HbO_2}$ is higher during high tide than during low tide (Toulmond, 1973). It is therefore assumed that, during lugworm confinement, $C^{max}_{HbO_2}$ falls linearly from 5·70 μmol.ml^{-1} (Pw_{O_2} = 150 Torr) to 4·95 μmol.ml^{-1} (Pw_{O_2} = 20 Torr). Variations of $C\bar{v}_{O_2}$ can be directly derived from $S\bar{v}_{O_2}$ variations (Fig. 2). Since $S\bar{v}_{O_2}$ values are always less than 100%, corresponding values of $P\bar{v}_{O_2}$ are very small (< 10 Torr) and the term $\alpha b_{O_2} . P\bar{v}_{O_2}$ may be neglected. Lugworm postbranchial blood being inaccessible, variations of Ca_{O_2} were calculated taking into account that postbranchial blood haemoglobin is oxygen-saturated when Pa_{O_2} > 10 Torr (Toulmond, 1970), and that a difference of 10 Torr remains between Pa_{O_2} and Pw_{O_2} (Jones, 1955). The greatest source of error upon the calculated values of $\dot{V}b$ certainly arises from this last hypothesis.

Full results of the calculations appear in Table 2 and Fig. 6. The $\dot{V}b$ *vs* Pw_{O_2} curve shows three parts: for 120 < Pw_{O_2} < 150 Torr, $\dot{V}b$ values approach 14 ml.h^{-1}; then,

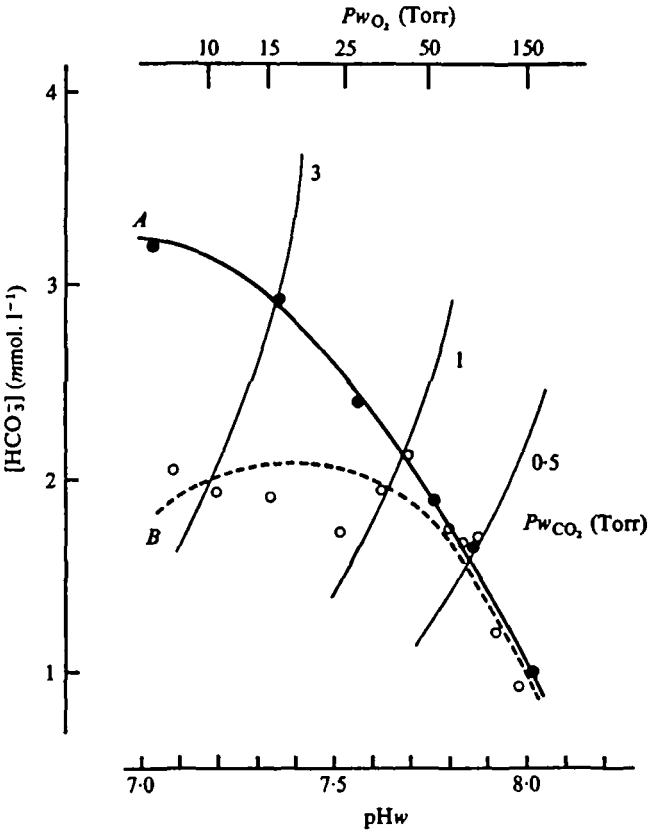

Fig. 5. Bicarbonate concentration [HCO_3^-] as a function of pHw, Pw_{CO_2} and Pw_{O_2}. Curve A: [HCO_3^-] of sea water containing confined *Arenicola* at 15 °C. Curve B: [HCO_3^-] of sea water equilibrated against gas phases of known P_{CO_2} at 15 °C. Coefficient of carbon dioxide solubility in sea water (αw_{CO_2}) = 0·051 μmol.ml^{-1}.Torr^{-1} and pK_1' = 6·06 for sea water of chlorinity Cl = 19·5‰ and t = 15 °C (from Harvey, 1963).

as Pw_{O_2} falls from 120 to 90 Torr, $\dot{V}b$ abruptly decreases from 14 to 3 ml.h^{-1}. In the final part of the curve, $\dot{V}b$ decreases more slowly until it reaches 0·45 ml.h^{-1} when Pw_{O_2} = 20 Torr (*ca.* 3% of $\dot{V}b$ value for Pw_{O_2} = 150 Torr).

DISCUSSION

Variations of blood oxygen transport during experimental confinement

From the data of Table 2, it is possible to calculate the percentage of consumed oxygen transferred from the gills to the tissues in combined form. This percentage is always higher than 68% (Pw_{O_2} = 150 Torr) and increases when Pw_{O_2} decreases. In the range of Pw_{O_2} explored, most of the consumed oxygen is thus transported by the haemoglobin. This confirms Krüger's hypothesis (1960, 1969) and Toulmond's preliminary calculations (1973), and refutes the opinion of Jones (1955, 1972) and of Wells (1966) that lugworm haemoglobin plays a role in oxygen transport only when external oxygen tensions are low. In fact, at low Pw_{O_2}, the absolute quantities of oxygen transferred are very small (Fig. 1), because of the very low blood flow ($\dot{V}b$) across th

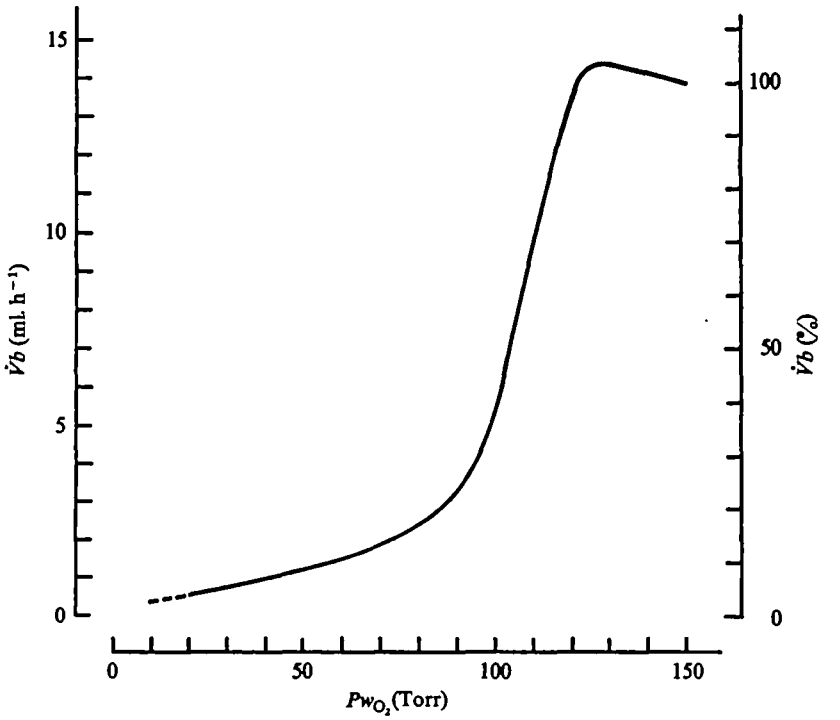

Fig. 6. Computed estimate of the total blood flow ($\dot{V}b$) through the branchial system, as a function of Pw_{O_2}, in confined *Arenicola* at 15 °C. Data from Table 2.

gills. If we consider that during their transfer from water to cells, oxygen molecules meet a series of resistances, such by-passing of the gills by the blood constitutes the main of these resistances, inducing in confined lugworm a type *E*, and more probably type *G*, hypoxia as defined by Hughes (1973).

Causes of $\dot{V}b$ *variations during experimental confinement*

The blood flow through the gills ($\dot{V}b$) varies enormously during confinement. A lugworm of 10 g wet weight contains about 0·54 ml of blood (Toulmond, 1971*a*). From the data of Table 2, it can be shown that an equivalent blood volume takes 140 s to cross the gills when $120 < Pw_{O_2} < 150$ Torr, and more than an hour when $Pw_{O_2} = 20$ Torr. What are the reasons for such a difference?

The lugworm pumps water through its burrow by means of peristaltic waves of the body wall five times a minute in aerated seawater at 15 °C (Seymour, 1972). Each gill shrinks and swells successively at the passage of these waves, injecting oxygenated blood into its efferent vessel, then sucking venous blood from the afferent one (Milne-Edwards, 1838). Blood flow through the branchial system is therefore directly dependent on the general ventilatory activity of the lugworm. Now, Wells (1949) showed that this activity was rapidly reduced when the animal was confined to a small volume of sea water, i.e., when it was submitted to conditions of decreasing Pw_{O_2} and increasing Pw_{CO_2}. This confirms Van Dam's (1938) observations that such a reduction occurs only in both hypoxic and hypercapnic conditions. These factors seem then to be the ultimate causes of $\dot{V}b$ variations in the confined lugworm.

Table 2. *Data and hypothesis used for computation of total blood flow through the branchial system in experimentally confined lugworms at* 15 °C ($\dot{V}b = f(Pw_{O_2})$ *curve of Fig.* 6)

Percentage saturation of postbranchial haemoglobin (Sa_{O_2}) and oxygen partial pressure in prebranchial blood ($P\bar{v}_{O_2}$) values are considered as constants ($Sa_{O_2} = 100\%$ and $P\bar{v}_{O_2} < 10$ Torr, see text).

Pw_{O_2} Torr	$\dot{M}_{O_2}$ μmol.h^{-1}	$C^{max}_{HbO_2}$ μmol.ml^{-1}	Pa_{O_2} Torr	$\alpha b_{O_2}.Pa_{O_2}$ μmol.ml^{-1}	Ca_{O_2} μmol.ml^{-1}	$S\bar{v}_{O_2}$ (%)	$C\bar{v}_{O_2}$ μmol.ml^{-1}	$\dot{V}b$ ml.h^{-1}	T*
150	10·4	5·70	140	0·24	5·94	91	5·19	13·87	68
140	10·4	5·65	130	0·23	5·88	91	5·14	14·05	69
130	10·2	5·59	120	0·21	5·80	91	5·09	14·37	70
120	9·9	5·53	110	0·19	5·72	90	4·98	13·38	74
110	9·4	5·47	100	0·18	5·65	86	4·70	9·89	81
100	8·9	5·41	90	0·16	5·57	72	3·90	5·33	90
90	8·3	5·35	80	0·14	5·49	52	2·84	3·13	95
80	7·6	5·30	70	0·13	5·43	41	2·17	2·33	96
70	6·8	5·24	60	0·11	5·35	32	1·68	1·85	97
60	5·9	5·18	50	0·10	5·28	25	1·30	1·48	98
50	5·1	5·12	40	0·08	5·20	19	0·97	1·21	98
40	4·1	5·07	30	0·06	5·13	13	0·66	0·92	99
30	3·1	5·01	20	0·05	5·06	9	0·45	0·67	99
20	2·1	4·95	10	0·03	4·98	6	0·30	0·45	99

* T = Percentage of consumed oxygen that is transported in combined form.

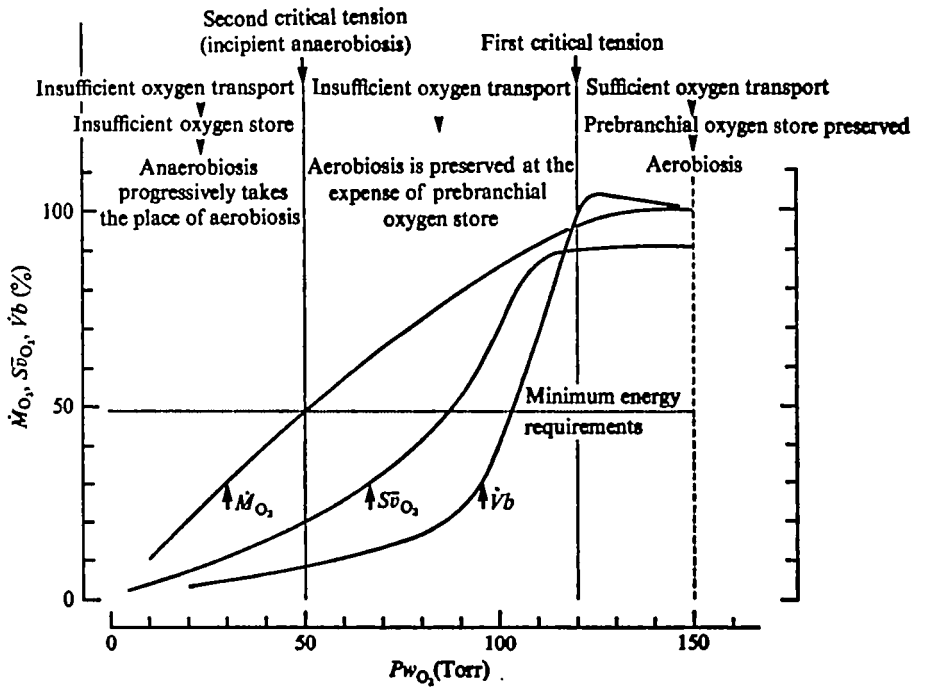

Fig. 7. Summary of data on blood oxygen transport and metabolism in *Arenicola* experimentally confined at 15 °C.

Variations of the confined lugworm's metabolism

The experimental confinement of a lugworm in a large amount of sea water (120–240 ml) can be divided into three successive phases (Fig. 7).

Phase 1. As Pw_{O_2} decreases from 150 to 120 Torr, the aerobic metabolism of the lugworm is maximal and nearly independent of Pw_{O_2}. Blood flow through the gills is sufficient to meet the metabolic oxygen needs and to maintain the percentage saturation of the prebranchial haemoglobin ($S\bar{v}_{O_2}$) and the prebranchial blood oxygen store (*ca.* $[C^{max}_{HbO_2}.S\bar{v}_{O_2}] \times$ total volume of prebranchial blood) at a maximum and constant level. Two-thirds of the consumed oxygen comes from oxyhaemoglobin.

Phase 2. When Pw_{O_2} decreases from 120 to 50 Torr, the ambient medium becomes hypoxic and hypercapnic. The lugworm's general activity probably drops, and this entails a progressive reduction of (*a*) the metabolic demand for oxygen, $\dot{M}_{O_2}$; (*b*) the total blood flow through the branchial system; (*c*) the oxygen transfer from sea water to tissues. Therefore, metabolism, which remains aerobic during this second phase, depends more and more on the prebranchial oxygen store, and $S\bar{v}_{O_2}$ decreases. At the end of phase 2, the fraction of the consumed oxygen transported in dissolved form becomes negligible.

Phase 3. As Pw_{O_2} falls from 50 to 20 Torr, the lugworm becomes more and more quiescent. Blood flow across the gills and oxygen transfer are extremely reduced. The increasing blood acidosis and modified acid-base equilibrium of the confined sea water strongly suggest that an anaerobic metabolism progressively takes the place of the declining aerobic metabolic processes and that acid by-products are excreted by the lugworm. In this phase, all the consumed oxygen comes from oxyhaemoglobin.

These different phases are well defined by two critical values of Pw_{O_2}. The first, Pw_{O_2} *ca.* 120 Torr, corresponds to the incipient decrease of $S\bar{v}_{O_2}$ (i.e. incipient utilization of the prebranchial oxygen store). The second, Pw_{O_2} *ca.* 50 Torr, corresponds to the incipient changes in the acid-base equilibrium of the sea water to which the lugworm is confined.

Variations of blood oxygen transport and metabolism during the tidal cycle

What are the connections between the phenomena occurring during an experimental confinement and during the tidal cycle? During high tide, the lugworm ventilatory activity is practically uninterrupted (Krüger, 1964*b*). The main respiratory variables are held almost constant: $C^{max}_{HbO_2}$ *ca.* 5·7 μmol.ml^{-1}; 88% < $S\bar{v}_{O_2}$ < 97%; 4·8 < $P\bar{v}_{O_2}$ < 6·8 Torr; 7·41 < pH$\bar{v}$ < 7·46; 0·3 < $P\bar{v}_{CO_2}$ < 0·5 Torr (Toulmond, 1973). During phase 1 of the experimental confinement, $S\bar{v}_{O_2}$ is nearly constant (*ca.* 91%), whereas total blood flow through the branchial system and oxygen consumption are at their maxima. It therefore seems possible to identify phase 1 with the immersion period corresponding to high tide. In both cases, the respiratory gas exchanges of the lugworm are high. They permit a maximum, roughly constant, aerobic metabolism and the maintenance of the prebranchial blood oxygen store at its highest level.

During low tide, the lugworm's spontaneous activity is suppressed, except for occasional 'testing movements' and defecation (Wells, 1949*a*, *b*). The body is tightly pressed against the burrow walls. Blood circulation and pressure are probably extremely reduced, as in *Carcinus maenas* (Flindt, 1971) and in several intertidal bivalves (*Mytilus edulis*, Schlieper, 1955, Helm & Trueman, 1967; *Cardium edule* and *Mya*, Trueman, 1967); although this has not been demonstrated in *Arenicola*, it can be deduced from several observations, particularly the $C^{max}_{HbO_2}$ fall from 5·7 μmol.ml^{-1} to 4·95 μmol.ml^{-1} (see Toulmond, 1973). In addition, at low tide, blood respiratory variables alter as a function of the duration of emersion. During a 4 h emersion, $S\bar{v}_{O_2}$ decreases from 13 to 7%, and $P\bar{v}_{O_2}$ from 1·2 to 0·6 Torr, whereas the pH of prebranchial blood (pH$\bar{v}$) falls from 7·48 to 7·35 as a result of the increasing $P\bar{v}_{CO_2}$ (from 0·8 to 2·4 Torr) and the accumulation of acid metabolites (Toulmond, 1973). In the same fashion, during phase 3 of the experimental confinement, blood flow across the branchial system is nearly thirty times lower than during phase 1, $S\bar{v}_{O_2}$ decreases from 19 to 6% and pH$\bar{v}$ falls from *ca.* 7·33 to 7·08. Changes in the acid-base equilibrium of the ambient medium suggest that acid metabolites are released by the lugworm. Thus, it seems possible to identify phase 3 of the experimental confinement with the emersion period during low tide. In both cases, the respiratory gas exchanges of the lugworm are extremely low. The prebranchial blood oxygen store is nearly exhausted and the deoxygenated haemoglobin acts essentially as a buffer (Toulmond, 1971*b*, 1973). The lugworm reduces its activity and the oxygen demand of the tissues is minimized. Despite this, the very low aerobic metabolism must be complemented by anaerobic processes. The energy production of both aerobic and anaerobic processes is then sufficient to meet the minimum metabolic needs of the organism, i.e. the minimum energy supply necessary to preserve cellular structure.

Therefore, in agreement with Shepard (1955) and Beadle (1961), phase 1 and high tide are periods of 'respiratory independence' whereas phase 3 and low tide are periods of 'resistance'.

Phase 2 of the experimental confinement constitutes a true transitory period and probably occurs in two different circumstances of the tide cycle: (*a*) in the first hour immediately following the emersion of the burrow and during which $S\bar{v}_{O_2}$, for example, abruptly falls from *ca.* 88–97% to 13%; (*b*) during the ventilatory rest periods, natural in the cyclical pattern of lugworm activity, during which the animal lies quiescent, feeds or burrows (Wells, 1949*a*, *b*, 1966).

It thus seems that, during low tide, the lugworm adopts an attitude of passive resistance. It would be now interesting to examine two questions: firstly, what are the true stimuli which provoke the beginning and the end of this resistance behaviour, secondly how are they perceived?

Special thanks are due to the Staff of the Station Biologique, Roscoff, for the facilities put at my disposal.

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