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BLOOD ACID–BASE BALANCE IN THE LUGWORM *ARENICOLA MARINA* VENTILATING IN HYPO- OR HYPEROXIC SEA WATER

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Summary

The time course of variation in blood acid–base balance was examined in lugworms, *Arenicola marina* (L.), experimentally acclimated for up to 72 h in hypoxic (P_{O_2} = 80 mmHg) (1 mmHg = 133·3 Pa), normoxic (P_{O_2} = 160 mmHg) or hyperoxic (P_{O_2} = 500 mmHg) sea water. In hyperoxic animals, a blood acidosis is entirely compensated 12 h after the beginning of the acclimation. In hypoxic animals, a blood alkalosis develops very quickly, persists and increases, reaching a maximum 72 h after the beginning of the acclimation. In both cases, variation in blood acid–base balance is mainly of respiratory origin. These data are consistent with previous results showing that the lugworm hypoventilates in hyperoxic sea water and hyperventilates in hypoxic sea water.

Introduction

When ventilatory activity and respiratory gas exchange of the lugworm *Arenicola marina* are analysed in animals acclimated for 3 h at different ambient P_{O_2} values (from 20 to 700 mmHg), in sea water of constant pH and P_{CO_2}, ventilation is oxygen-dependent: the lugworm hyperventilates in moderate hypoxia and hypoventilates in hyperoxia. Moreover, in comparison with the normoxic responses, hyperoxia led to a hypercapnia (and acidosis) and moderate hypoxia to a hypocapnia (and alkalosis) in the expired water (Toulmond & Tchernigovtzeff, 1984). These variations of the expired water acid–base balance (ABB) were assumed to reflect blood ABB variations similar to those demonstrated in other water breathers. For instance, in most of the fishes and decapod crustaceans that have been studied, decreased or increased ventilation induced by external hyper- or hypoxia is correlated with a respiratory blood acidosis or alkalosis which is more or less rapidly and completely compensated (for a detailed review, see Truchot, 1987).

In annelids, the effects of experimental exposure to hypo- or hyperoxic sea

Key words: lugworm, *Arenicola marina*, ventilation, hyperoxia, hypoxia, blood acid–base balance.

water on blood ABB have not yet been systematically analysed. In the present study, the time course of blood ABB variation was examined in lugworms acclimated for up to 72 h to ambient P_{O_2} values of 80, 160 or 500 mmHg, in slightly hypercapnic sea water (P_{CO_2} approx. 0·35 mmHg).

Materials and methods

Experiments were carried out in Roscoff, Nord-Finistère, France, in May and June 1987.

Animals

Medium-sized lugworms, wet mass 15–20 g, were collected on the nearby Penpoull beach, brought back to the laboratory and kept unfed overnight in local running sea water (temperature 14–16°C), by which time their gut was free of sand.

Experimental protocol

Blood ABB variations in response to changes in ambient oxygen level were studied in lugworms acclimated for 1, 3, 5, 8, 12, 18, 24, 48 or 72 h at P_{CO_2} of about 0·35 mmHg and P_{O_2} = 80 or 500 mmHg, or acclimated for 3, 12, 24, 48 or 72 h at P_{O_2} = 160 mmHg. For each acclimation time at a given P_{O_2}, one series of eight animals was analysed, except at 3 h where nine series of eight animals were studied. More 3-h acclimated animals were studied to evaluate more precisely the ABB parameters at the acclimation time used in our previous studies (Toulmond & Tchernigovtzeff, 1984; Conti & Toulmond, 1986).

Twelve hours after collection, each lugworm was placed, unrestrained, in an artificial burrow, a straight glass tube 30 cm long, i.d. 1 cm, and immersed horizontally in a 35-l holding tank supplied with flowing natural sea water. To keep the lugworm from escaping, the ends of the tube were plugged with rubber stoppers with a 4 mm i.d. hole, through which ventilated sea water circulated easily. The lugworm was allowed to acclimate to its artificial burrow for 12 h.

At time zero of an acclimation experiment, eight lugworms in their glass tubes were transferred to the experimental tank containing 100 l of confined natural sea water (chlorinity approx. 19·5 ‰, titration alkalinity approx. 2·42 mequiv l^{-1}), thermostatted at 15°C and continuously mixed by an external pump and by the bubbling of the gas phases used to equilibrate the sea water at fixed values of the respiratory parameters: pH approx. 8·1, P_{CO_2} approx. 0·35 mmHg and P_{O_2} approx. 80, 160 or 500 mmHg. Parameter constancy was monitored with home-made pH/P_{CO_2}- and P_{O_2}-stats (Dejours & Armand, 1980; Dejours, 1988). The titration alkalinity of the 100-l of sea water had not changed significantly after 72 h, the longest acclimation time.

At the end of a given acclimation time, each animal was rapidly removed from its tube, 150 µl of prebranchial blood was anaerobically withdrawn from the ventral vessel and the eight blood samples were immediately and anaerobically

pooled and mixed in a 3-ml glass syringe kept in melting ice. The whole blood sampling process took about 5 min for each group of eight animals.

Measurements and calculations

Blood pH ($pH\bar{v}$) was immediately measured and the percentage oxygen saturation of the respiratory pigment, $S\bar{v}_{O_2}$, determined using a modification of Tucker's technique (1967). Both values fairly represent *in vivo* conditions (Toulmond, 1973). Since the blood pH depends strongly on the respiratory pigment oxygenation state (see Discussion), the prebranchial blood carbon dioxide partial pressure, $P\bar{v}_{CO_2}$, was evaluated using a 'double Astrup method'. Briefly, four samples of the pooled blood were equilibrated *in vitro* for 30 min against pure nitrogen ($S_{O_2} = 0\%$) or pure oxygen ($S_{O_2} = 100\%$), at two P_{CO_2} values bracketing the *in vivo* estimate. $P\bar{v}_{CO_2}$ was calculated by linear interpolation, using $S\bar{v}_{O_2}$, $pH\bar{v}$ and pH values measured on the four samples.

All measurements on sea water and blood were made at 15 °C with thermostatted P_{O_2} (Radiometer E5046) and pH (Radiometer G299A and GK2301/C) electrodes, respectively coupled to a Strathkelvin 781b oxygen meter, a Radiometer PHM73 pH/blood gas monitor or a Radiometer PHM74 pH meter. Equilibration of the blood samples against the different gas mixtures prepared from pure gases with Wösthoff pumps was obtained in a Radiometer BMS2 MK2 blood microsystem apparatus.

The sea water titration alkalinity was determined by a modification of the method of Strickland & Parsons (1965). The coefficients of oxygen solubility in the acidified 6 g l^{-1} ferricyanide solution, used in Tucker's technique, and in the blood were taken as 1·97 and 1·61 μmol l^{-1} $mmHg^{-1}$ at 15 °C, respectively. Bicarbonate concentrations in the prebranchial blood samples, $[HCO_3^-]\bar{v}$, were calculated using corresponding values of $pH\bar{v}$ and $P\bar{v}_{CO_2}$, a coefficient of carbon dioxide solubility in the blood of 0·05 mmol l^{-1} $mmHg^{-1}$ and operational pK_1' values derived from the relationship: $pK_1' = 6{\cdot}896 - 0{\cdot}118pH\bar{v}$ (Fig. 1).

Mean values are given ± 1 S.D. Differences between means were evaluated using the Student's *t*-test with $P = 0{\cdot}05$ as the fiducial limit of significance.

Results

In lugworms ventilating normoxic ($P_{O_2} = 160$ mmHg) and slightly hypercapnic sea water (P_{CO_2} approx. 0·35 mmHg) for 3 h, the mean prebranchial blood ABB parameters were as follows: $pH\bar{v} = 7{\cdot}383 \pm 0{\cdot}021$, $P\bar{v}_{CO_2} = 1{\cdot}49 \pm 0{\cdot}71$ mmHg and $[HCO_3^-]\bar{v} = 1{\cdot}68 \pm 0{\cdot}78$ mequiv l^{-1}. Since similar values were observed in animals acclimated for 12, 24, 48 and 72 h in the same ambient respiratory conditions, these means have been taken as control values corresponding to time zero of the acclimation process in the hyperoxic and hypoxic conditions (point *C* in Figs 2 and 3).

In animals ventilating in hyperoxic ($P_{O_2} = 500$ mmHg) sea water in the same slightly hypercapnic conditions, the time course of ABB changes was clearly

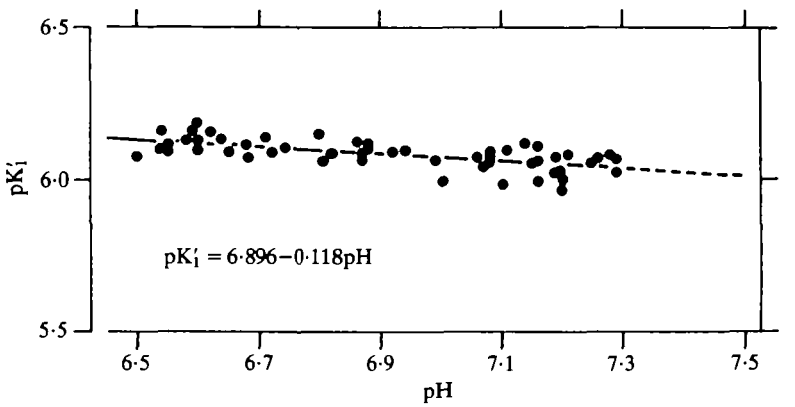

Fig. 1. Variation in pK_1', first apparent dissociation constant of carbonic acid in lugworm blood at 15 °C, expressed as a function of blood pH. pH values were measured on blood samples equilibrated against gas phases of known P_{CO_2}. On the same samples, the total CO_2 concentrations, C_{CO_2}, were determined with a Natelson microgasometer. pK_1' values were calculated as: $pK_1' = pH - \log(C_{CO_2} - \alpha_{CO_2} \times P_{CO_2}) + \log(\alpha_{CO_2} \times P_{CO_2})$, using $\alpha_{CO_2} = 0{\cdot}05\ mmol\,l^{-1}\,mmHg^{-1}$. Parameters of the regression line were calculated by the method of least squares on the pK_1' values corresponding to pH values less than 7·3 ($P_{CO_2} > 3$ mmHg) ($r = -0{\cdot}641$ for $N = 53$). When pH is greater than 7·3 ($P_{CO_2} < 3$ mmHg), microgasometric method inaccuracies and the likely unequal formation of carbamino compounds lead to a large scattering of the corresponding pK_1' values, which were not used in the calculations.

biphasic (Fig. 2). The first phase, ending after 3 h of acclimation, was characterized by a significant fall of $pH\bar{v}$ from the control value down to $7{\cdot}288 \pm 0{\cdot}023$, and a significant rise of $P\bar{v}_{CO_2}$ and $[HCO_3^-]\bar{v}$ from their control values to $3{\cdot}37 \pm 0{\cdot}53$ mmHg and $3{\cdot}00 \pm 0{\cdot}38$ mequiv l^{-1}, respectively. During the second phase, from 3 to about 12 h of acclimation, $pH\bar{v}$ increased, returning to the normoxic control value, and varied around this control value as acclimation was extended to 18, 24, 48 or 72 h. $P\bar{v}_{CO_2}$ and $[HCO_3^-]\bar{v}$ rose after between 3 and 12 h of acclimation to values of approximately 6–7 mmHg and 6·5–8·5 mequiv l^{-1}, respectively, where they remained until 72 h of acclimation.

Variations of the ABB parameters were much less marked during the acclimation of lugworms to moderate hypoxia ($P_{O_2} = 80$ mmHg; Fig. 3). In animals acclimated for 3 h, $pH\bar{v} = 7{\cdot}423 \pm 0{\cdot}017$, a value slightly but significantly higher than the control in normoxia. Conversely, $P\bar{v}_{CO_2}$ and $[HCO_3^-]\bar{v}$ decreased significantly to $0{\cdot}43 \pm 0{\cdot}19$ mmHg and $0{\cdot}54 \pm 0{\cdot}20$ mequiv l^{-1}, respectively. This new blood ABB was obtained within 1 h and persisted for at least 5 h. With longer acclimation, up to 72 h, all three ABB parameters showed a general tendency to drift towards slightly higher values.

For a given P_{O_2} condition, the values of $S\bar{v}_{O_2}$, the percentage oxygen saturation of the respiratory pigment in prebranchial blood, were similar at all acclimation times. Compared using the Kruskal–Wallis nonparametric test (Siegel, 1956), the $S\bar{v}_{O_2}$ values in animals acclimated for 3 h were significantly different in hypoxia

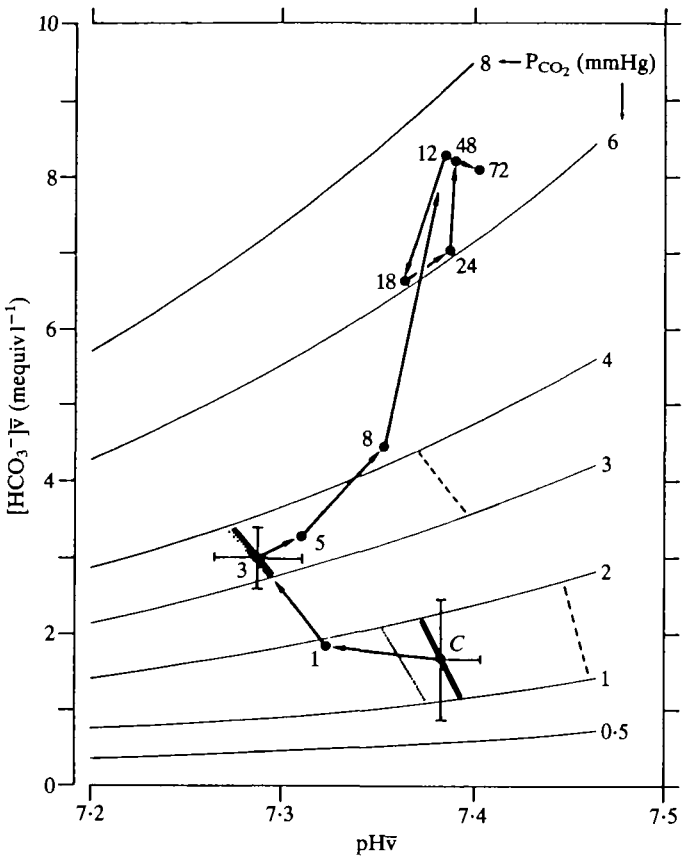

Fig. 2. Time course of variations in blood acid–base balance in normoxic lugworms *Arenicola marina* acclimated to hyperoxic water (P_{O_2} = 500 mmHg; P_{CO_2} = 0·35 mmHg) at 15 °C. *C*, control values in animals ventilating in normoxic water (P_{CO_2} = 0·35 mmHg) in the experimental conditions. Numbers indicate the duration of the hyperoxic acclimation period in hours. Heavy lines, blood buffer lines corresponding to *in vivo* $S\bar{v}_{O_2}$ values. For control and 3-h acclimated animals, dashed buffer lines correspond to *in vitro* equilibrations of blood samples with pure nitrogen (S_{O_2} = 0 %) and dotted lines to equilibration with pure oxygen (S_{O_2} = 100 %). Vertical and horizontal bars correspond to ±1 S.D. [*N* = 9 for control and 3-h values: other values from a single pooled sample (see Materials and methods)].

(mean value = 67 %), normoxia (mean value = 83 %) and hyperoxia (mean value = 96 %). In addition, the overall O_2-binding site concentration in the prebranchial blood, $C^{max}_{HbO_2}$, was significantly higher in hyperoxia than in normoxia and hypoxia (Table 1).

In Figs 2 and 3, an *in vitro* buffer line corresponding to blood protein buffering has been drawn through the points describing the *in vivo* values of the ABB parameters in control (*C*) and 3-h acclimated animals. In 3-h acclimated animals, the absolute value of the slope of these buffer lines, β, was always high, but was

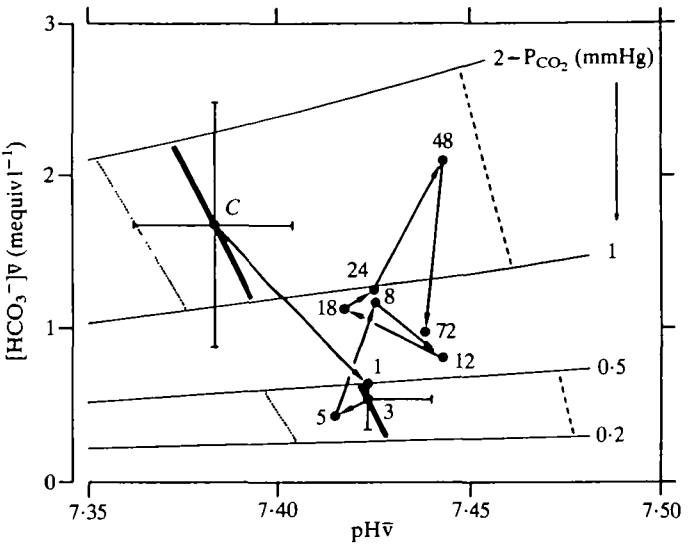

Fig. 3. Time course of variations in blood acid–base balance in normoxic lugworms *Arenicola marina* acclimated to hypoxic water (P_{O_2} = 80 mmHg; P_{CO_2} = 0·35 mmHg) at 15 °C. *C*, control values in animals ventilating normoxic water (P_{CO_2} = 0·35 mmHg) in the experimental conditions. Symbols and conventions as in Fig. 2.

Table 1. *Influence of pH and S_{O_2}, percentage oxygen saturation of the respiratory pigment, on the buffering capacity β of the prebranchial blood of lugworms acclimated for 3 h at three values of P_{O_2}*

P_{O_2} (mmHg)	pH$\bar{v}$	$P\bar{v}_{CO_2}$ (mmHg)	$S\bar{v}_{O_2}$ (%)	$C^{max}_{HbO_2}$ (mmol l^{-1})	β (mequiv l^{-1} pH unit^{-1} per mmol O_2-binding sites) S_{O_2} = 0 %	$S_{O_2} = S\bar{v}_{O_2}$	S_{O_2} = 100 %
80	7·423	0·43	67	5·54	−18·1	−9·8	−7·4
	0·017	*0·19*		*0·37*			
160	7·383	1·49	83	5·63	−16·7	−8·7	−7·5
	0·021	*0·71*		*0·18*			
500	7·288	3·37	96	6·10	−5·3	−4·8	−4·8
	0·023	*0·53*		*0·20*			

pH$\bar{v}$ and $S\bar{v}_{O_2}$, *in vivo* values of blood pH and S_{O_2}, $C^{max}_{HbO_2}$, blood concentration of O_2-binding sites.

The values of β for S_{O_2} = 0 and 100 % were obtained *in vitro* (see text).

Standard deviation in italics (N = 9).

According to the Kruskal–Wallis nonparametric test, the three $S\bar{v}_{O_2}$ values differ significantly from each other.

significantly lower in hyperoxia, $\beta = -29 \pm 3$ mequiv l^{-1} pH unit^{-1}, than in normoxia, $\beta = -49 \pm 10$ mequiv l^{-1} pH unit^{-1}, and hypoxia, $\beta = -54 \pm 8$ mequiv l^{-1} pH unit^{-1}. For control and 3-h acclimated animals, the interrupted buffer lines were obtained by *in vitro* equilibrations of the blood against pure nitrogen ($S_{O_2} = 0\%$, dashed lines) or pure oxygen ($S_{O_2} = 100\%$, dotted lines). When all available β values are considered, it is clear that the absolute value of β, expressed in mequiv l^{-1} pH unit^{-1} per mmol of O_2-binding sites, is strongly pH- and S_{O_2}-dependent; β values are higher, the higher the pH and the lower the S_{O_2} (Table 1, for 3-h acclimated worms).

Discussion

Evaluation of acid–base balance parameters in the lugworm's blood

The lugworm's blood contained in a closed circulatory system can be considered as a practically pure solution of an extracellular haemoglobin dissolved in sea water. The pigment concentration ranges from 100 to 160 g l^{-1}, corresponding to a 4·5–6·5 mmol l^{-1} concentration of O_2-binding sites (Toulmond, 1979).

The study of the parameters describing the *in vivo* ABB of this apparently simple blood is complicated by some special features of the haemoglobin, characterized by an important Bohr effect and by pH- and O_2-dependent buffering properties (see Toulmond, 1977). The consequences of these properties on the blood buffer curves are amplified by the high blood O_2-binding site concentration and can be summarized as follows. (1) For a given O_2-binding site concentration, at physiological pH and P_{CO_2} values, the position of the blood buffer curve on a Davenport diagram is strongly dependent on the oxygenation state of the haemoglobin. (2) For a given oxygenation state, the curve's slope β is extremely pH-dependent and the curve can be considered as linear only if very small pH intervals are considered. (3) At high pH and low S_{O_2} values, the buffering capacity of the blood is so high that rather large variations of P_{CO_2} induce only very small changes of blood pH (see Figs 2, 3; Table 1). As a result, it is clear that the extrapolation of *in vivo* P_{CO_2} values implies very accurate blood pH measurements and the knowledge of both *in vivo* pH and S_{O_2} values: hence the 'double Astrup method' described above. Since this method requires a greater number of linear interpolations to determine P_{CO_2} than the normal Astrup method, its precision is highest when $pH\bar{v}$ is lowest (because the absolute value of β is lowest) and $S\bar{v}_{O_2}$ is nearest to zero or 100 % (because the errors due to linear interpolations are then smallest).

Effects of hyperoxia on the lugworm's blood acid–base balance

Hyperoxia ($P_{O_2} = 500$ mmHg) induces important variations of prebranchial blood ABB in the lugworm, leading first to a significant blood acidosis which peaks after 3 h and which is fully compensated after 12 h of acclimation. Examination of Fig. 2 shows that the acidosis which develops during the first hour of acclimation has at least two components: (1) a small respiratory element, with $P\bar{v}_{CO_2}$ increasing

from 1·5 to 1·9 mmHg, probably as a consequence of the nearly instantaneous lowering of the ventilation rate in lugworms placed in hyperoxia (Toulmond & Tchernigovtzeff, 1984; Dejours & Toulmond, 1988); (2) a 'pseudo-metabolic' element, which originates in the more complete O_2 saturation of the haemoglobin, the mean $S\bar{v}_{O_2}$ value increasing from 83 to 96%. However, summation of these cannot explain the total buffer line shift from the control to its new position after 1 h of acclimation. Obviously a significant part of the total acidosis has a strictly metabolic origin which cannot yet be explained, the hypothesis of an anaerobic production of acidic byproducts being quite unlikely in hyperoxia. Matters are simpler between 1 and 3 h of acclimation: the acidosis increase during that interval appears to be of a strictly respiratory origin, $P\bar{v}_{CO_2}$ increasing from 1·9 to 3·4 mmHg at a constant $S\bar{v}_{O_2}$ value. This result fits well with previous observations of increased P_{CO_2} in the expired water and of lowered ventilation, total CO_2 excretion and respiratory ratio in lugworms acclimated for 3 h in similar respiratory conditions (Toulmond & Tchernigovtzeff, 1984; Conti & Toulmond, 1986).

After 3 h, a progressive compensation occurs, $pH\bar{v}$ increasing from 7·29 to 7·38, a value very similar to that of the control. $P\bar{v}_{CO_2}$ and $[HCO_3^-]\bar{v}$ increase progressively from 3·4 to 7·2 mmHg and from 3 to 8·3 mequiv l^{-1}, respectively. This occurs at a constant value of $S\bar{v}_{O_2}$, the haemoglobin being practically saturated with oxygen. At 12 h, one can consider that the compensation is complete, since the pH values remain practically constant and very similar to the control value when the acclimation time is extended to 18, 24, 48 or 72 h. This metabolic compensation allows the bicarbonate concentration to be more than three times the normoxic control value, with P_{CO_2} at about 7 mmHg. Such nearly perfect compensation has been observed in only a few cases: in *Carcinus maenas* (Truchot, 1975), the dogfish *Scyliorhinus canicula* (Truchot *et al.* 1980), the trout *Salmo gairdneri* (Wood & Jackson, 1980; Hõbe *et al.* 1984), the rock crab *Cancer irroratus* (Wheatly, 1987) and the large spotted dogfish *Scyliorhinus stellaris* (Heisler *et al.* 1988). The mechanisms of this compensation have never been clearly established but modifications in ionic exchanges and particularly in base excretion and/or production must be involved (see Truchot, 1987). For the lugworm, very few data are available except the observation that in 3-h acclimated animals the net transfer of base from the animal to the ambient water is about two times lower in hyperoxia than in normoxia, indicating that the compensation process has already begun (Toulmond & Tchernigovtzeff, 1984).

Effects of hypoxia on the lugworm's blood acid–base balance

Variation in the prebranchial blood ABB is not as striking in hypoxia (P_{O_2} = 80 mmHg). A hypocapnic alkalosis occurs within the first hour of acclimation in hypoxia, persists for at least 5 h and is clearly of ventilatory origin since $P\bar{v}_{CO_2}$ decreases from 1·49 to 0·43 mmHg after 3 h. Hypoxia-induced ventilatory increases, leading to increased CO_2 excretion and a respiratory ratio higher than one, have been observed in lugworms acclimated for 3 h in similar conditions (Toulmond & Tchernigovtzeff, 1984). This respiratory alkalosis is slightly rein-

forced by a pseudo-metabolic component due to the small but significant decrease, from 83 to 67%, of the percentage haemoglobin oxygen saturation (Fig. 3; Table 1).

In animals acclimated for more than 5 h in hypoxia, the general trend is towards an aggravation of the alkalosis despite slightly increasing $P\bar{v}_{CO_2}$ values. A pseudo-metabolic component cannot be put forward as an explanation in this case, since $S\bar{v}_{O_2}$ remains practically unchanged after the first hour of acclimation. In other words, in prolonged hypoxia, the lugworm does not compensate for the respiratory alkalosis which, secondarily, tends to become metabolic through unknown mechanisms. A similar unexplained hypocapnic and metabolic alkalosis has been observed in the shore crab *Carcinus maenas* (Truchot, 1975; Burnett & Johansen, 1980), the lobster *Homarus vulgaris* (McMahon *et al.* 1978) and the crayfish *Astacus leptodactylus* (Sinha & Dejours, 1980). Truchot's proposition (1975) that the resulting increase of blood pH may be important in enhancing the haemocyanin oxygen-affinity in hypoxic *Carcinus via* a normal Bohr effect was found to be pertinent in the case of *Homarus vulgaris* (McMahon *et al.* 1978). In the lugworm, where a strong Bohr effect does exist, the magnitude of the $S\bar{v}_{O_2}$ decrease, from 83 to 67% in animals acclimated for 3 h in hypoxia, is perhaps limited by the concomitant blood alkalosis. Our results, however, do not show any significant increase of $S\bar{v}_{O_2}$, whatever the length of the acclimation time.

In conclusion, in *Arenicola marina* as in most water breathers that have been studied, a change in the water oxygenation rapidly induces a blood acidosis in hyperoxia and a blood alkalosis in hypoxia. Generally, both these acid–base balance variations have a respiratory origin, being explainable in terms of changes in the balance between CO_2 production and excretion rates. Despite some debate (see Truchot, 1987), it is generally considered that, as the first aim of the ventilatory flow is to provide the organism with sufficient oxygen, hypoxic water induces hyperventilation which, in turn, provokes a blood alkalosis by enhancing the CO_2 excretion. Conversely, breathing hyperoxic water leads to hypoventilation favouring CO_2 retention, and hence blood acidosis. Our findings support this general interpretation, with some restrictions: in the lugworm, variation in acid–base balance is not of a strictly respiratory origin since, both in hyperoxia and in hypoxia, a small part of the response is of pseudo-metabolic origin and comes from readjustments of the respiratory pigment oxygen saturation. In addtion, in hyperoxia, the acidosis seems also to have a true, as yet unexplained, metabolic component.

The lugworm's compensations for these two blood acid–base disturbances are not equally successful. In animals acclimated to hyperoxic sea water, the acidosis is entirely compensated after 12 h through metabolic processes leading to a net increase of the blood bicarbonate concentration. Conversely, in animals acclimated to hypoxic sea water, the blood alkalosis is never compensated and is still increasing after 72 h of acclimation. The same discrepancy has been observed in the intertidal green crab *Carcinus maenas* (Truchot, 1975) and has never been clearly explained. In the case of the lugworm, also an intertidal dweller, a blood

respiratory and metabolic acidosis develops at every low tide, when the animal's metabolism becomes anaerobic, and it is quite clear that the lugworm is well prepared and equipped, both biochemically and physiologically, to overcome a recurrent natural blood acidosis (see Toulmond, 1987). The aggravation of the blood alkalosis observed during a long-term moderate hypoxia may correspond to an unphysiological response to quite unusual respiratory conditions and blood acid–base status.

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