

tine, which resulted in significant depression of the levels of the microtubule-forming protein tubulin, did not affect electrically-evoked mechanical responses in the nerve. Since other contractile proteins (e.g., actin, myosin)<sup>14</sup> are known to be present in neural and perineural cells their possible role in neural mechanical activity deserves further exploration.

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## Oxygen equilibrium of *Priapulus hemerythrin*<sup>1</sup>

R.E. Weber and R. Fänge

*Institute of Biology, Odense University, DK-5230 Odense M (Denmark)<sup>2</sup>, and Department of Zoophysiology, University of Gothenburg, Gothenburg (Sweden), 23 July 1979*

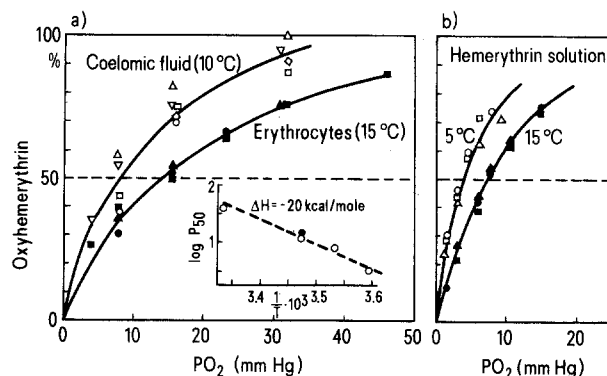
**Summary:** The hemerythrin-containing coelomic fluid of *Priapulus caudatus* shows a relatively low O<sub>2</sub> affinity (half-saturation O<sub>2</sub> tension P<sub>50</sub>=8 mm at 10 °C) and a low O<sub>2</sub> capacity (near 1 vol.%). O<sub>2</sub> affinity is independent of pH but shows a large temperature sensitivity. A major role as a continuous O<sub>2</sub> transporter seems to be excluded.

Hemerythrin is the least known of the classes of respiratory oxygen-binding proteins found in animals. It shows restricted phylogenetic distribution, occurring in an annelid (*Magelona*), most sipunculids, the brachiopod genera *Lingula* and *Glottidia*, and the priapulids *Priapulus* and *Hali-cryptus*<sup>3</sup>. In contrast to the situation in the former 3 genera, virtually no information is available on the pigment in the priapulids. This paper reports the oxygenation properties of hemerythrin from *Priapulus caudatus*, a large burrowing worm encountered in cold, O<sub>2</sub>-rich northern seas<sup>4,5</sup>. It possesses a conspicuous, branched anal appendage, whose lumen communicates, via an opening surrounded with circular muscles, with a voluminous coelomic fluid containing hemerythrin-laden erythrocytes; it contracts rhythmically when O<sub>2</sub> availability returns after anoxia<sup>6</sup>.

Specimens of *Priapulus caudatus* weighing 3–8 grams were trawled from grey muds at a depth of about 45 m near Kristineberg Marine Biology Laboratory, Fiskebäckskil, Sweden<sup>7</sup>. Coelomic fluid samples were equilibrated with gas mixtures of different O<sub>2</sub> tension (delivered by Wösthoff gas mixing pumps) and the O<sub>2</sub> content of each sample was then measured following a method<sup>8</sup> modified after Tucker<sup>9</sup>, where bound O<sub>2</sub> liberated by K<sub>3</sub>Fe(CN)<sub>6</sub> is recorded using a Radiometer O<sub>2</sub> electrode. Pigment-bound O<sub>2</sub> was obtained after correction for dissolved O<sub>2</sub> (calculated assuming that O<sub>2</sub> solubility in coelomic fluid is the same as in the ambient 35‰ sea water). O<sub>2</sub> saturation at different O<sub>2</sub> tensions was derived expressing the corresponding O<sub>2</sub> content as a percentage of O<sub>2</sub> carrying capacity (defined as O<sub>2</sub> content at atmospheric O<sub>2</sub> tension). O<sub>2</sub> equilibria of erythrocytes washed in sea water and of hemerythrin solutions were measured spectrophotometrically using a modified diffusion chamber technique<sup>10,11</sup>.

The coelomic fluid of *Priapulus* showed low values of hematocrit (4–12%) and hemerythrin O<sub>2</sub> carrying capacity (0.4–1.4 vol.%), both parameters increasing with body weight. Oxygenation studies of the coelomic fluid reveal a

low O<sub>2</sub> affinity compared to that of most burrowing worms (P<sub>50</sub> approximates 8 mm at 10 °C), essentially hyperbolic O<sub>2</sub> equilibrium curves (Hill's coefficient,  $n \sim 1.2$ , reflecting the absence of marked cooperativity between the O<sub>2</sub> binding sites), and the absence of a significant Bohr effect ( $\Delta \log P_{50} / \Delta \text{pH}$ ) (figure, a). O<sub>2</sub> affinity, however, decreases drastically with temperature increase (figure, a, inset), reflecting an overall heat of oxygenation (calculated as  $2.30R \cdot \Delta \log P_{50} / (\Delta 1/T)$ )<sup>12</sup> of about –20 kcal/mole, i.e. almost twice the value generally found with hemoglobins and hemocyanins<sup>12,13</sup>. At the same temperature the washed erythrocytes show an identical O<sub>2</sub> affinity, excluding the possibility of



Oxygen equilibrium of *Priapulus* hemerythrin and its temperature and pH dependence. a) Erythrocytic hemerythrin. Open symbols, whole coelomic fluid; closed symbols, washed erythrocytes suspended in sea water. pH values (varied by admixture of CO<sub>2</sub> to equilibration gases): □, 8.10; ○, 8.05; △, 7.45; ◇, 7.30; ▽, 7.04; ■, 7.62; ●, 7.35; ▲, 7.22. Inset: temperature dependence of oxygen affinity. b) Hemerythrin dissolved in 0.1 M tris buffer of varying pH. pH values: ○, 8.20; △, 7.33; □, 7.05; ●, 8.13; ▲, 7.67; ■, 7.14.

plasmatic influences on  $O_2$  affinity. In solution the hemerythrin  $O_2$  affinity is, however, distinctly higher ( $P_{50}$  near 7.5 mm at 15 °C, see figure, b). A similar influence of lysis has been observed in hemerythrin from the sipunculids *Dendrostomum* and *Phascolosoma*<sup>14,15</sup>, suggesting that erythrocytic factors may depress the  $O_2$  affinity of hemerythrins as do allosteric phosphate cofactors with hemoglobin in vertebrates.

In the absence of a Bohr effect and cooperative  $O_2$  binding, *Priapulus* hemerythrin differs from that of brachiopods (*Lingula*)<sup>16</sup>, but resembles sipunculid hemerythrins, which generally, however, have higher  $O_2$  affinities<sup>17,18</sup>. The relatively low  $O_2$  affinity, however, resembles that found in the annelid *Magelona* ( $P_{50} = 13$  at 15 °C)<sup>19</sup>.

The  $O_2$  affinities suggest that *Priapulus* hemerythrin can only load  $O_2$  when the ambient  $O_2$  tension is high, temperature is low, and/or the uptake of  $O_2$  is efficient, limiting the fall in  $O_2$  tension across the respiratory surfaces. However, measurements of coelomic  $O_2$  tensions of live animals exposed to water with atmospheric  $O_2$  tensions for periods exceeding 1.5 h, demonstrate the occurrence of steep  $O_2$  tension gradients across the body walls of *Priapulus*<sup>7</sup>. The present findings thus correlate neatly with the observed restriction of *Priapulus* in nature to waters of low temperature and high  $O_2$  tension<sup>4,5</sup>.

Our data suggest that *Priapulus* hemerythrin cannot play an important role as a continuous transporter of  $O_2$  from the respiratory surfaces to the metabolizing tissues. The pigment is extravascular and moreover lacks a sigmoid, pH dependent  $O_2$  equilibrium curve – factors which enhance  $O_2$  unloading in acid tissues by vascular pigments. It is, however, probable that the pigment will play a role as a short-term store of  $O_2$ , tiding over infrequent bouts of activity associated with burrow ventilation, anal appendage

contractions and coelomic fluid mixing<sup>6,7</sup>.  $O_2$  uptake rates of 15–20 ml · kg<sup>-1</sup> · h<sup>-1</sup> at 10 °C suggest that the measured pigment-bound  $O_2$  could sustain the animal's respiration for about 25 min in well aerated water<sup>7</sup>.

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## Baroreceptor reflex sensitivity after acute blood volume expansion in anesthetized dogs<sup>1</sup>

J.P. Dujardin

Department of Physiology, The Ohio State University, 1645 Neil Avenue, Columbus (Ohio 43210, USA), 5 June 1979

**Summary.** Changes in heart rate resulting from mechanically induced changes in arterial blood pressure were used as a measure of baroreflex sensitivity. This sensitivity was shown to be decreased after volume expansion.

In conscious dogs, rapid blood volume expansion causes an increase in mean arterial pressure, pulse pressure and heart rate<sup>2</sup>. The heart rate response is known as the Bainbridge reflex<sup>3</sup>. Since baroreceptor reflex characteristics would predict a decreased heart rate in response to an increased arterial pressure, a modification of these reflex characteristics after volume expansion has been postulated. To determine the sensitivity of the baroreflex, Vatner et al.<sup>2</sup> measured the relationship between the cardiac pulse interval and the systolic arterial blood pressure following an i.v. injection of methoxamine in conscious dogs. The increase in cardiac pulse interval for a given increase in arterial pressure was reduced after volume expansion. The authors concluded that the baroreflex sensitivity was decreased. The same conclusion was reached by Sinnott et al.<sup>4</sup> in a study on anesthetized rabbits. These authors found that heart rate responses to left aortic nerve stimulation were reduced after volume loading.

It was the purpose of this research to use mechanically induced blood pressure changes to determine the sensitivity of the baroreceptor reflex before and after blood volume expansion.

**Methods.** Experiments were performed on 6 mongrel dogs weighing from 18 to 25 kg. The dogs were premedicated with morphine sulfate (2 mg/kg) and anesthetized with sodium pentobarbital (20 mg/kg). Central venous pressure was monitored using a Statham (P23AC) manometer connected to the thoracic vena cava at the level of the right atrium by way of a cardiac catheter. Blood volume was determined in all animals at the onset of the experiment, using Evans Blue dye (T 1824). Aortic pressure was measured with a Pieper catheter-tip pressure gauge<sup>5</sup>. This gauge

SD of the normalized cardiac pulse intervals before and after volume expansion

Experiment	Control	Volume expansion
1	0.334	0.134
2	0.363	0.260
3	0.114	0.021
4	0.332	0.150
5	0.227	0.210
6	0.089	0.048