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BLOOD GAS TRANSPORT IN THE CEPHALOPOD, *SEPIA OFFICINALIS*

BY KJELL JOHANSEN, OLE BRIX AND GUNNAR LYKKEBOE
Department of Zoophysiology, University of Aarhus, DK-8000 Aarhus C., Denmark

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SUMMARY

Blood gas transport was studied in unrestrained free-swimming cuttlefish, *Sepia officinalis*, following cannulations of an efferent branchial (arterial) vessel and the vena cava cephalica with indwelling catheters.

In well-aerated water the arterial p_{O_2} averaged about 100·00 mmHg and was fully saturated with O_2. Mixed venous p_{O_2} varied between 17 and 40 mmHg, typically corresponding to blood O_2 utilizations of 80% or higher. Some blood samples showed venous pH to exceed arterial, a tendency becoming more distinct during exposure to hypoxic water. The resulting higher O_2 affinity of venous compared to arterial blood discourages O_2 unloading in the tissues, while promoting efficient O_2 loading in the gills. A high n-value of *Sepia* blood ($n = 4{\cdot}7$) is important for maintaining a large arteriovenous O_2 content difference and a high utilization of circulating O_2.

INTRODUCTION

The high aerobic metabolism of most cephalopods exceeds that for other aquatic invertebrates of similar size and also surpasses that of many fast-swimming fishes (Altman & Ditmer, 1971).

Since cephalopod blood has a low O_2 capacity, typically not exceeding 5 vol%, the high tissue O_2 requirements must be satisfied by high perfusion rates (cardiac outputs) and by maximum utilization of the blood-borne, circulating O_2 pool. The utilization of O_2 transported by the blood and the physiological significance of blood respiratory properties studied *in vitro* can never be fully assessed unless information about *in vivo* circulating levels of blood gases and pH are available from arterial and venous blood. Such information is extremely rare for cephalopods, due to the obvious technical difficulties associated with implanting chronically indwelling intravascular catheters in these animals. Studying the active squid, *Loligo*, Redfield & Goodkind (1929) measured near-maximal arteriovenous O_2 content differences by direct blood sampling from pinned-down, cut open and artificially ventilated specimens. Based on these measurements blood O_2 utilization in *Loligo* exceeded 90%. The authors implied that values of blood pH (p_{CO_2}) measured concurrently established that nearly 40% of the large O_2 turnover to the tissues depended on the uniquely high Bohr

shifts in *Loligo*. The Bohr factor ϕ ($\Delta \log P_{50}/\Delta pH$) was reported to be $-1{\cdot}8$ for *Loligo* (Redfield, Coolidge & Hurd, 1926).

Studies of the large *Octopus dofleini* at rest, based on blood samplings from chronically cannulated animals, showed O_2 utilizations averaging 72% with a maximum of 85% (Johansen & Lenfant, 1966). *O. dofleini* also has a large Bohr factor ($\phi = -0{\cdot}80$) (Lenfant & Johansen, 1965).

Blood O_2 transport in the phylogenetically primitive *Nautilus pompilius* showed a much lower value for O_2 utilization of 35%. *Nautilus* is sluggish and has a small Bohr factor ($\phi = -0{\cdot}20$) (Johansen, Redmond & Bourne, 1978).

The study to be reported concerns an evaluation of blood gas transport in the common North-Atlantic cuttlefish, *Sepia officinalis*.

MATERIALS, METHODS AND SURGICAL PROCEDURES

Seven specimens of *Sepia officinalis*, ranging in weight from 1500 to 1600 g, were used in the study. The animals were obtained by trawling in the English Channel near Plymouth. Before experiments, the animals had been kept for several days in well aerated running sea water at 17 °C. They were fed live crabs at intervals, but food was withheld for at least 24 h prior to anaesthesia.

Anaesthesia was induced by slow addition of ethyl alcohol to an aerated seawater bath to a final concentration of about 3% in the sea water. Muscle relaxation occurred after about 30 min exposure to the alcohol–water mixture. The surgical procedures lasted about 20 min. The large vena cava cephalica, cannulated for mixed venous blood, is accessible slightly posterior to the anus when the mantle musculature is relaxed. A PE 60 polythene catheter was passed downstream through a hole made in the vessel wall with a cutting-edge surgical suture needle. The catheter was advanced 6–8 cm downstream and tied to the vessel wall by a pursestring ligature.

The cannulation presented no significant obstruction to venous flow since the vena cava cephalica is a large vessel of 8–10 mm diameter. Arterial blood was obtained by cannulation of the efferent branchial artery accessible at the lateral aspect of the gill when the mantle was relaxed. After tying a holding thread around the tip of the gill, a hole was cut in the efferent branchial vessel about 10–15 mm from the holding thread.

Polyethylene catheters (PE 50) were used except in one specimen where the highly contractile gill tissue precluded insertion of a catheter larger than PE 10. The catheters had a standard length of 40 cm and each blood sampling procedure lasted 20–30 s. The catheters were advanced 3–5 cm upstream from the point of insertion. A ligature was tied around the vessel immediately proximal to the point of cannulation.

This cannulating procedure occluded the distal portion of one of the two gills. The reduction in the overall gas exchange area from this occlusion can, however, only be small since the 40–60 cm long gills taper sharply off at the distal tips where the cannulations were made. Following cannulations the animals were transferred to well-aerated sea water and normally recovered within 10–20 min. No blood sampling or other experimentation was done until the following day, when the animals were assumed to have fully recovered from the cannulating procedures.

During experiments, arterial and venous blood were drawn simultaneously. The samples were kept in stoppered syringes in iced water until analysed for p_{O_2}, p_{CO_2} and pH using Radiometer equipment. Total blood CO_2 content was analysed on a modified Van Slyke apparatus (Brix, 1981). During hypoxia experiments, water p_{O_2} was brought down to, and kept at, the desired level by nitrogen bubbling. Information on blood respiratory properties used in calculations was taken from Brix, Lykkeboe & Johansen (1981) and unpublished information. O_2 capacity was calculated as half of the blood copper content (Ghiretti, 1966) measured on a Perkin Elmer 503 atomic absorption spectrophotometer. All experiments were performed at 17–19 °C.

Table 1. In vivo *values for oxygen tension and pH of arterial and venous blood in normoxic (top panel) and hypoxic water*

Also shown are derived values for utilization (*Ut*) and perfusion requirement $\dot{Q}/\dot{V}_{O_2}$, the latter calculated as $((a-\bar{v})O_2)^{-1} \times 100$.)

Animal no.	P_{I,O_2} (mmHg)	P_{a,O_2} (mmHg)	$P_{\bar{v},O_2}$ (mmHg)	pH_a	pH_v	O_2 capacity (vol.%)	S_{a,O_2} (%)	$S_{\bar{v},O_2}$ (%)	*Ut* (%)	$(a-\bar{v})O_2$ (vol.%)	$\dot{Q}/\dot{V}_{O_2}$
2	135	85·0	27·8	7·43	7·40	3·61	84·6	2·0	97·6	3·16	31·6
2	150	113·5	26·0	7·64	7·57	3·32	99·9	22·1	77·9	2·85	35·1
2	150	100·0	25·1	7·64	7·67	3·00	99·8	57·2	42·7	1·51	66·2
3	147	63·0	29·9	7·49	7·45	3·79	81·9	6·4	92·2	2·96	33·8
4	143	108·0	18·0	7·55	7·66	3·23	99·4	19·3	80·6	2·87	34·8
5	153	100·5	17·0	7·69	7·73	3·70	99·9	37·8	62·2	2·56	39·1
5	153	125·4	17·0	7·65	7·73	3·49	99·9	37·8	62·2	2·51	39·8
7	133	115·0	40·0	7·55	7·58	3·23	99·5	71·8	27·8	1·12	89·3
4	50	37·5	7·5	7·63	7·70	3·23	81·6	0·8	99·0	2·70	37·0
4	67	24·0	14·5	7·70	7·68	3·07	64·7	10·8	83·3	1·68	59·5
6	55	29·5	19·0	7·67	7·72	3·14	74·1	46·4	37·4	0·90	111·1
6	30	16·0	14·0	7·83	7·82	3·02	71·9	53·4	35·5	0·57	175·4
7	51	33·3	20·0	7·61	7·70	3·67	64·2	43·7	31·9	0·79	126·6

RESULTS

Table 1 gives values for arterial and venous p_{O_2} and pH from steady-state conditions in normoxic (133 mmHg < p_{O_2} < 153 mmHg) and hypoxic water (30 < p_{O_2} < 80 mmHg). In normoxic water the arterial p_{O_2} was very high, exceeding 100 mmHg for 4 out of 5 animals. In two animals arterial p_{O_2} was higher than 110 mmHg, in one case 125 mmHg, when ambient water p_{O_2} was 135 mmHg. This implies near equilibrium between arterial blood and inspired water with respect to O_2 tension. Arterial blood was nearly fully saturated with O_2 (Table 1). Mixed venous p_{O_2} in normoxic animals ranged between 17 and 40 mmHg. Table 1 shows the venous O_2 saturations to vary considerably, because of variable blood pH and the very large Bohr factor. The utilization or turnover of O_2 in arterial blood to the tissue also varied and could be as high as 99%. The blood O_2 capacity averaged 3·1 vol%. In 4 animals, total blood CO_2 content was measured in arterial and venous samples at normoxic conditions. The increases in blood CO_2 content (in mM), comparing arterial and venous blood for the 4 specimens, were from 2·7 to 4·5, from 3·3 to 5·0, from 3·0 to 4·6, and from 4·7 to 5·4.

During hypoxic exposure the arterial O_2 tension fell markedly (Table 1, Fig. 2), while the venous tension fell initially, but as the hypoxia progressed it realigned with

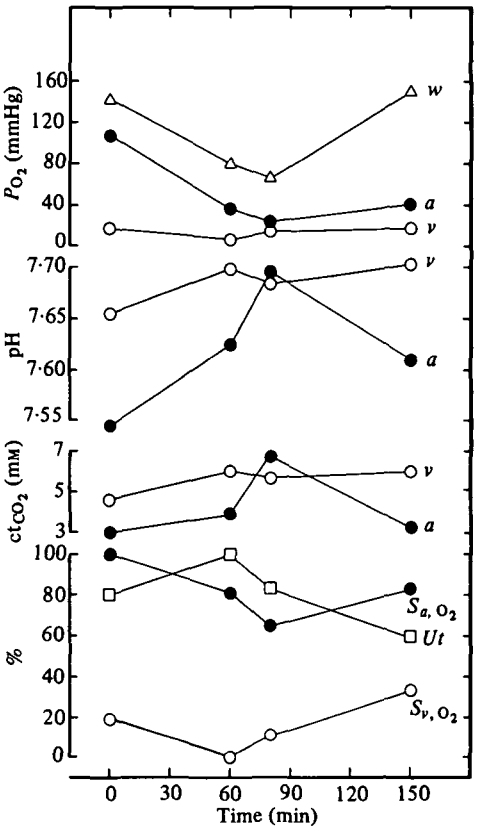

Fig. 1. Time course of blood gases, pH and percentage utilization of blood O_2 in *Sepia officinalis* associated with exposure to hypoxic water. *w*, Water; *a*, arterial; *v*, venous; *S*, % O_2 saturation; *Ut*, utilization. The data are from one animal.

pre-hypoxic values. Blood pH rose markedly during hypoxia, also correlated with a state of hyperventilation. In some animals under hypoxic conditions, pH of venous blood was higher than that of arterial blood, although this finding was not consistent in all sample sets (Table 1). Hypoxia also caused the total CO_2 content of arterial blood to rise (Fig. 1). The O_2 saturation fell in both arterial and venous blood, but the relative decline of the two, and thus of the O_2 utilization, varied between animals and experiments.

Fig. 1 (lower panel) also shows that O_2 utilization from circulating blood initially rises during hypoxic exposure (at $P_I \simeq 80$ mmHg), but declines at more severe O_2 deficiency in the water.

Fig. 2 shows how the arterial and venous O_2 tensions relate to ambient p_{O_2} during hypoxic exposure. The decline in arterial O_2 tension with reduced ambient p_{O_2} was associated with a reduced gradient from arterial blood to inspired water. This trend was correlated with a marked hyperventilation. The venous O_2 tensions stayed rather unchanged, after an initial drop in moderately hypoxic water. In combination with a marked alkalosis, this implies an increase in venous O_2 saturation and hence a reduced utilization of circulating O_2 during hypoxia (Fig. 2, Table 1).

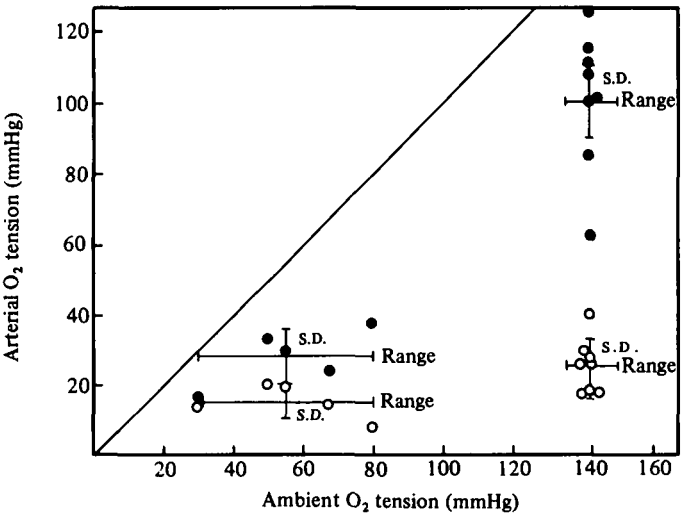

Fig. 2. Arterial and venous p_{O_2} in relation to ambient p_{O_2} for *Sepia officinalis* at 17 °C. ●, arterial; ○, venous.

Fig. 3 shows the relationship between blood O_2 affinity (P_{50}), HcO_2 saturation and blood p_{O_2}, based on O_2-binding curves determined for *Sepia in vitro* (Brix *et al.* 1981). The relationship between pH and O_2 affinity is expressed by the equation: $\log P_{50} = -1{\cdot}6\,\mathrm{pH} + 13{\cdot}644$ at a temperature of 17 °C. The empirically determined n-value for the O_2 binding curves was 4·7. The broken lines show calculated p_{O_2} isopleths based on an n-value of 2·5. The pairs of arterial and venous sample sets plotted on to the nomogram (larger data points) express the *in vivo* blood gas levels from unrestrained *Sepia* during normoxia (data set I) and during progressive hypoxia (set II, p_{O_2} 80 mmHg; set III, p_{O_2} 67 mmHg; and set IV, p_{O_2} 30 mmHg). The smaller data points connected by arrows to those experimentally determined show what effect a reduction in n-value would have on arterial and venous O_2 saturations. It is clear that, for some data sets, the venous values are aligned with or displaced to the left of the arterial, implying that venous blood had a higher O_2 affinity, *i.e.* lower P_{50} values than arterial. This result reflects the fact that circulating blood pH in some cases was higher in venous compared to arterial blood (Table 1). At a water p_{O_2} of 67 and 30 mmHg, arterial and venous blood pH, and hence the corresponding O_2 affinity, were nearly similar.

Fig. 3 also reveals that a reduction in n-value from the very high level present in *Sepia* blood, results in a marked reduction in arterial saturation and a concurrent increase in the venous saturation, causing a drastic fall in the arteriovenous O_2 content difference and hence in utilization of the circulating blood O_2.

DISCUSSION

The combination of high tissue O_2 requirement and low blood O_2 carrying capacity such as is the case for cephalopods (Johansen *et al.* 1981; Ghiretti, 1966), place special demands on efficiency in utilizing the oxygen transport potential of the blood. This

P_{I,O_2} (mmHg)	135–150		80		67		30	
n-value	2·5	4·7	2·5	4·7	2·5	4·7	2·5	4·7
P_{a,O_2} (mmHg)	108·0	108·0	37·5	37·5	24·0	24·0	16·0	16·0
P_{v,O_2} (mmHg)	18·0	18·0	7·5	7·5	14·5	14·5	14·0	14·0
S_{a,O_2} (%)	93·7	99·4	68·9	81·6	58·0	64·7	62·2	71·9
S_{v,O_2} (%)	31·9	19·3	7·0	0·8	24·6	10·8	51·8	53·4
Ut (%)	65·9	80·6	89·8	99·0	57·0	83·3	16·7	35·5
$(a-v)O_2$ (vol. %)	2·3	2·9	2·1	2·7	1·0	1·7	0·3	0·6
Q/V_{O_2}	43·9	34·8	47·8	37·0	97·1	59·5	326·6	175·4

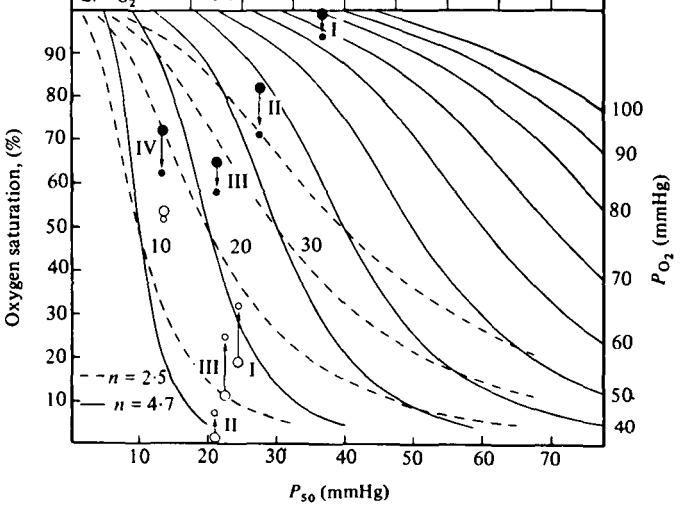

Fig. 3. The table in the top panel gives the numerical changes in arteriovenous O_2 content difference, O_2 utilization and the perfusion requirement ($\dot{Q}/\dot{V}_{O_2}$) in relation to ambient water p_{O_2} for *Sepia officinalis*. The nomogram (bottom panel) is constructed from the O_2 affinity values, Bohr shift, and *n*-value published by Brix *et al.* (1981), for the same species. The larger data symbols in the nomogram are based on *in vivo* blood sampling from animal 4 at a water p_{O_2} of 140 mmHg (symbols I), 80 mmHg (symbols II), 67 mmHg (symbols III) and animal 6 at 30 mmHg water p_{O_2} (symbols IV). ●, Arterial; ○, venous blood. The smaller symbols express the *in vivo* data points transformed to a nomogram based on an *n*-value of 2·5.

in turn will depend on the respiratory properties of blood and the ventilatory and circulatory pumps, as well as the diffusion barriers in the gills and tissues. A high blood O_2 utilization expressed by a near-maximal difference in O_2 content between arterial and venous blood has long been advocated as a unique trait of cephalopod blood and alleged to depend on an exceptionally high pH sensitivity (Bohr effect) of the binding of O_2 to most cephalopod haemocyanins. Recently, however, Lykkeboe, Brix & Johansen (1980), Brix *et al.* (1981) and Lykkeboe & Johansen (1982) have pointed out that a Bohr factor ($\Delta \log P_{50}/\Delta pH$) numerically exceeding 1·0, which is typical of many cephalopods, brings about an entirely different interaction between blood acid–base status and oxygenation of the haemocyanin than when the Bohr factor is numerically less than 1·0. The important point emphasized in these recent studies is that the maximal yield of protons from aerobic metabolism will numerically match that of oxygen used in metabolism on a molar basis, if the gas exchange ratio, $R_E = 1{\cdot}0$. It should be remembered that the binding of O_2 to haemocyanin is tanta-

mount to the release of the same mol fraction protons, or, conversely, during O_2 unloading one mol protons will be bound to the O_2 carrier when one mol O_2 is unloaded. This relationship formally expressed in the linkage equation (Wyman, 1964) has the important consequence that if the pH sensitivity of the blood numerically exceeds 1·0, there will not be an excess of free protons produced in aerobic metabolism to shift the O_2-binding equilibrium of haemocyanin towards that of unbound O_2, i.e. O_2 unloading in the tissues will consequently not be promoted by the aerobic metabolism. Rather, as predicted by Lykkeboe *et al.* (1980), the pH of venous blood may exceed that of arterial blood, handicapping O_2 unloading rather than promoting it as has been traditionally advocated (Redfield & Goodkind, 1929).

The present study on *Sepia* is one of very few allowing an evaluation of the importance of the uniquely high Bohr shifts in cephalopods for blood gas transport *in vivo* based on blood samples drawn from unrestrained specimens.

The high arterial O_2 tensions in *Sepia* (Table 1, Fig. 1) at normoxic ambient conditions, suggest a highly efficient gas exchange in the gills. An arterial p_{O_2} of 100 mmHg implies that an O_2 extraction from the ventilatory current of 28·5% or higher will suffice to produce a negative p_{O_2} gradient between expired water and arterial blood, characteristic of a counter-current exchange process. Hazelhoff (1939) reported a range for O_2 extraction between 50% and 80% in cephalopods, while the large *Octopus dofleini* showed an average extraction of 26·8% (Johansen & Lenfant, 1966).

Arterial blood for *Sepia* in well-aerated water will therefore typically be fully saturated with O_2 (Table 1). The more variable venous p_{O_2} corresponds to venous O_2 saturations that are about 20% or lower, implying O_2 utilizations 80% or higher. At conditions of increased O_2 requirement during swimming, or at reduced water p_{O_2}, the cephalopod solution to increased or maintained O_2 delivery (hypoxic water) can thus only modestly depend on an increased unloading of O_2, but must instead depend on maximizing the arterial O_2 saturation and an increase in cardiac output.

Our data testify that in *Sepia* venous pH may exceed arterial pH, in both normoxic and hypoxic water (Table 1). This *in vivo* situation accords with the predictions of Lykkeboe *et al.* (1980), explainable as a result of the interaction between proton binding and the state of oxygenation of haemocyanins with very high Bohr shifts. As is apparent from Fig. 3, this tendency gives venous blood a higher O_2 affinity than arterial blood, a fact which will actually impede O_2 unloading from haemocyanin.

In addition, the O_2 affinity of all circulating blood will increase during hypoxia as a result of a general alkalosis, a finding recently also reported for blood of *Octopus vulgaris* (Houlihan *et al.* 1982). This tendency will further reduce O_2 unloading, while at the same time favouring O_2 loading. The possibility that anaerobic production of protons could aid O_2 unloading, for example during exercise, is small due to the relatively undissociated state of octopine, which is the principal product of anaerobic metabolism in cephalopods (Zammit, 1978).

The very high O_2 utilization of *Sepia* blood in normoxic and moderately hypoxic water ($P_{I,O_2} \cong 80$ mmHg) is clearly related to the highly sigmoid shape (high n-value) of the O_2Hc binding curves (Fig. 3). Calculated data points show that arterial saturations would decline and the venous saturations markedly rise, if the blood had a

smaller n-value. The high n-value can hence be regarded as a counterbalance for the apparent adverse influence of the blood acid–base status on O_2 unloading. Fig. 3 (top panel) also gives the numerical changes in arteriovenous O_2 content difference, O_2 utilization and the perfusion requirement ($\dot{Q}/\dot{V}_{O_2}$). The latter is seen to increase dramatically as the blood loses importance in O_2 transport.

Accompanying the alkalosis in *Sepia* during exposure to hypoxia was a notable increase in total CO_2 content of the blood (Fig. 1). The increase may possibly be related to the breakdown of arginine phosphate that occurs during hypoxia (Storey & Storey, 1979), since hydrolysis of this compound leads to a build-up of bicarbonate (Burton, 1978). The phenomenon will act to amplify the tendency towards an alkalotic status during hypoxia and thus further substantiate the O_2 loading strategy of compensatory O_2 transport.

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