J. exp. Biol. **122**, 345–353 (1986)
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BLOOD FLOW IN ACUTE HYPOXIA IN A CEPHALOPOD

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Accepted 22 November 1985

SUMMARY

This paper reports a method of measuring blood flow in the dorsal aorta of *Octopus vulgaris* using fixed magnet flow meters. Blood flow, like blood pressure, falls progressively during acute hypoxia. Flow increases greatly as soon as the ambient P_{O_2} rises, with values for a given external P_{O_2} exceeding those found during the preceding development of hypoxia. On average, about 70% of the total systemic blood flow runs down the aorta to the head and arms; the rest goes to the gonad, to the mantle, to the coronary circulation and to the guts. This pattern remains unchanged as the ambient P_{O_2} falls.

INTRODUCTION

Cephalopods regulate their oxygen uptake over a wide range of ambient oxygen tensions (Borer & Lane, 1971; Johansen, Brix & Lykkeboe, 1982; Maginnis & Wells, 1969). Their response to acute hypoxia (here meaning a progressive fall in ambient oxygen tension over a period of an hour or two) is unusual; it includes a marked increase in the blood pigment affinity, associated with a rise in pH as the oxygen tension falls (Houlihan, Innes, Wells & Wells, 1982; Johansen, Brix & Lykkeboe, 1982). The increased oxygen affinity allows the blood to remain saturated after passage through the gills despite the fall in ambient P_{O_2}, so that the animals do not need to pump more blood as the external P_{O_2} falls, provided that they can somehow compensate for the decline in the P_{O_2} gradient between blood and tissues.

Studies of the changes in blood pressure in the dorsal aorta and afferent branchial vessels of *Octopus* during acute hypoxia show that the hearts slow down, pulse amplitude decreases, and the mean blood pressure falls (Wells & Wells, 1983). This suggests that, far from increasing the blood flow, blood flow actually declines in these circumstances. But the matter cannot be resolved from pressure measurements alone. A fall in peripheral resistance would allow the flow to be sustained or even increased, despite the fall in blood pressure.

One purpose of the present series of experiments was to examine flow directly. A second was to examine changes in the stroke volume output from the hearts, since this is the parameter that the animal is most likely to manipulate to increase blood flow: frequency, we already know, remains steady or actually declines in these

Key words: blood flow, hypoxia, cephalopod.

circumstances (Wells & Wells, 1983). The third purpose, using total blood flow calculated on the Fick principle and comparing this with the measured aortic blood flow, was to assess whether oxygen lack was accompanied by any gross change in the proportion of the blood going to the head and arms, as might be expected if, for instance, the animal were to favour the brain at the expense of the guts, or increase cutaneous respiration during acute hypoxia.

METHODS

The experiments were made with *Octopus vulgaris* at the Laboratoire Arago, Banyuls, France, during 1982–1984. The eight animals concerned weighed from 1117 to 2959 g. FW-series permanent magnet transducers (*in vivo* metric systems, Healdsburg, California, IVM) with internal diameters of 3 and 4 mm were used to record blood flow in the dorsal aorta, operations being carried out as described for the installation of T-pieces leading to pressure transducers in Wells (1979). In one experiment, a pressure transducer (Radiospares 303-343) was added, in series with the flow probe. The animals, with wires emerging from the middle of the back of the abdomen, were free to move around their aquaria, and appeared to be undisturbed by the surgery. A recovery period of at least 2 h was allowed after operation and before any experiment, experience having shown that this is sufficient for the heart beat rate and the pressure pulse to return to normal and for oxygen consumption to return to normal following the apparent repayment of the limited oxygen debt that *Octopus* is able to sustain (Wells, 1979; Wells & Wells, 1984).

Fixed magnet transducers give a voltage output which varies linearly with the rate of flow, so that it is in principle possible to derive the flow directly from the recorded pulses. At very low flow rates, however, the voltage output ($0{\cdot}5$–$1{\cdot}0\,\mu V\,ml^{-1}\,min^{-1}$) requires considerable amplification and we found that both our own amplification–recording system (a one-off design from the electronics workshop of the Zoology Laboratory, Cambridge) and that used by IVM to calibrate their probes tended to show a small overshoot and a recovery lasting for several seconds when flow was abruptly reduced to zero. Our *Octopus* hearts beat at $0{\cdot}5$–$0{\cdot}8$ Hz. To overcome the overshoot and recovery problem and to guard against possible non-linearity arising from the system's reaction time to pulsating flow, we calibrated our apparatus by producing a mimic series of pulse envelopes, corresponding in form and voltage to the original records. Each mimic was made by placing the flow probe downstream from a fluid reservoir and immediately upstream of a tap rotating at the appropriate heartbeat frequency. The height of the reservoir and the shape of holes cut in the tap regulated, respectively, the flow rate and the flow pattern, so that it was possible to mimic the pulse envelopes recorded from the animal with some accuracy (Fig. 1). The reservoir contained sea water; cephalopod blood contains few haemocytes – the pigment is in solution – and the salinity is close to that of sea water. Small differences in salinity make no difference to the output of fixed magnet meters.

Fig. 1 shows the first mimic series that we made. Sixty-two mimics were prepared in this way. Pulse envelope area × frequency showed a good correlation with

measured flow ($r = 0{\cdot}92$ for the smaller and 0·93 for the larger of the two probes used) and flow rates were thereafter read off on a basis of pulse envelope area.

Animals were held in closed respirometers (with a small central hole in the lid for wires) as described in Wells, O'Dor, Mangold & Wells (1983). Oxygen levels were measured using EIL 15A or EIL 7130 probes.

In *Octopus vulgaris*, blood leaves the systemic heart through two arteries. The smaller of the two runs posteriorly to the gonad. The larger, the dorsal aorta, runs anteriorly and at once gives off a pair of small vessels, which provide the coronary circulation for the systemic and branchial hearts. A second pair of branches supplies the mantle musculature and a group of vessels downstream of this run to the gut with branches to the stomach, caecum and digestive gland. These branches are all quite small (the largest less than 0·5 mm in diameter) compared with the main trunk (up to 4 mm diameter in an octopus of 2 kg) which continues forward to the head and arms. The flow probe was always inserted downstream of the branches to the guts. This was a place where the probe could be inserted with minimal disturbance to the surrounding tissues. It also had the convenience of coming at a point where the arterial supply divides into two distinct halves. Branches upstream of the probe supply the abdomen. Branches downstream (apart from 2–3 very tiny vessels to the crop) run to the head and arms, connected to the abdomen by a narrow neck. There is little scope for anastomosis between the two halves of the system. Isgrove (1909) has published figures showing the arterial circulation in *Eledone*, and the circulation in *Octopus* differs from this only in minor details.

RESULTS

Fig. 1 shows how the blood flow pulse in the aorta changed in a typical animal, which reduced the P_{O_2} of the oxygen in its respirometer from 145 to 55 mmHg in the course of a little over 60 min. The figure also shows how the measured flow changed in a mimic series.

Fig. 2 summarizes simultaneous records of the ambient oxygen tensions, the exhalant oxygen tension (sampled through a cannula attached to the vertical muscular septum just behind the funnel in the exhalant stream, as in Wells & Wells, 1982) and the flow along the aorta in an octopus during a progressive fall in oxygen tension and in a subsequent recovery period. The same figure summarizes values for the stroke volume passing the probe in the aorta, and calculated values for the total blood flow and stroke volume for the period during which the respirometer was closed. Total blood flow was calculated from the measured oxygen consumption and from an estimate of the likely oxygen delivery from the blood, derived from previous experiments on the effects of acute hypoxia carried out under the same conditions as the present series on blood flow. Houlihan *et al.* (1982) cannulated the afferent branchial vessels and dorsal aortae from a series of octopuses to obtain blood samples at various stages during the development of acute hypoxia. They found that the arterial oxygen content averaged $3{\cdot}3 \pm 0{\cdot}23$ vol % and the venous return averaged $0{\cdot}52 \pm 0{\cdot}09$ vol %, a difference of 2·8 vol % in normoxia at an ambient P_{O_2} of

143 mmHg. As the oxygen content of the respirometer was reduced progressively over 60–90 min, the amount of oxygen delivered fell progressively, to 2·6 vol % at 94 mmHg and 2·3 vol % at 49 mmHg. These figures have been used as the basis for calculating total blood flows, after deducting 10 % from the total oxygen consumption to allow for cutaneous respiration (Wells & Wells, 1983, measured cutaneous respiration as 13 % of the total, under conditions where all the likely errors would have inflated the value found; the proportion did not change in acute hypoxia).

Table 1 lists similar measurements and calculated values for eight further experiments, during the period of declining P_{O_2}.

It is clear that flow along the aorta declines as the ambient P_{O_2} falls. The change is not marked over the range 140 to 95 mmHg (90 % to 60 % saturated); indeed some individuals (Y1 and Y14 in Table 1) actually increased the measured flow in this

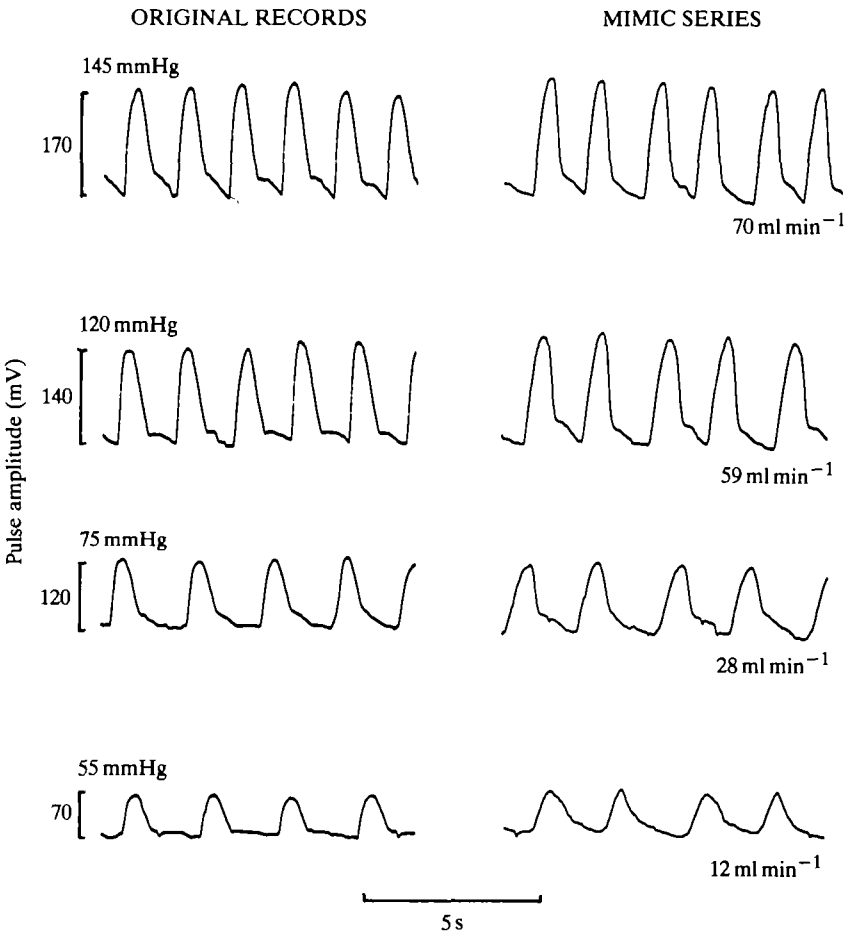

Fig. 1. Blood flow in the aorta of octopus W38 (weight 1459 g) at 21 °C. On the left are flow pulses recorded from the animal as it reduced the oxygen tension in a closed respirometer. Pulses on the right are a mimic series, made by passing sea water through the same flow probe; this series shows the actual flow volume passed per minute.

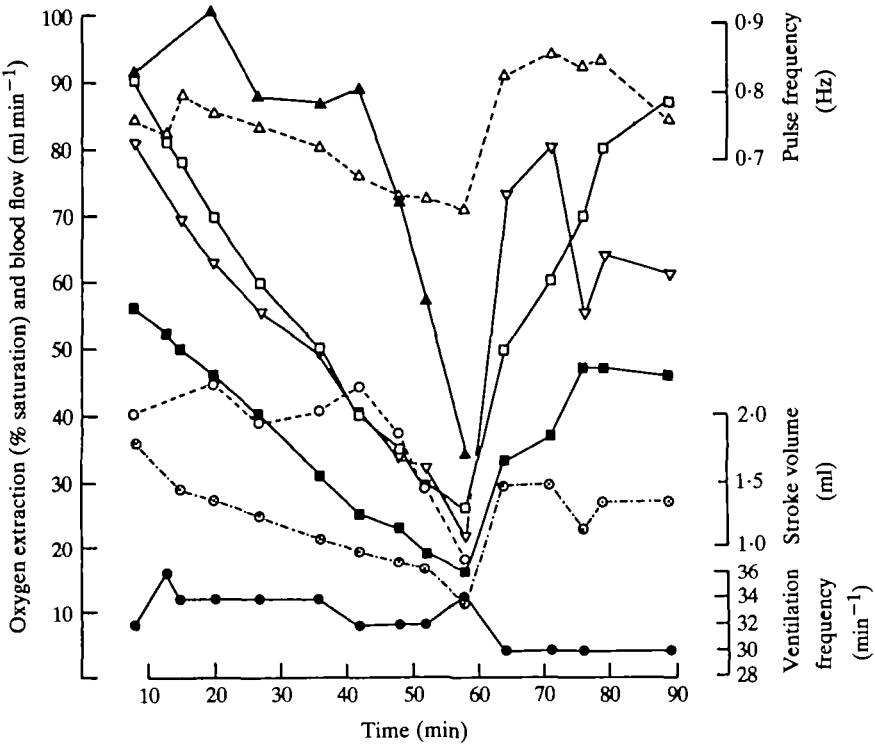

Fig. 2. Blood flow in the aorta and oxygen extraction in octopus Y14 (weight 1687 g) at 23 °C. The plots show oxygen saturation of the tank water (□) and in the exhalant stream (■), the heartbeat frequency (△), the blood flow down the aorta (▽), the total blood flow (Fick) (▲) and the calculated stroke volume of the systemic heart on a basis of the aortic flow (◉) and from the total blood flow (○) and the ventilation frequency (●). This experiment followed that summarized in Table 1, after an interval during which the animal was anaesthetized and a cannula attached to the median pallial adductor to sample the exhalant stream. The experiment shown here began only 35 min after recovery from the anaesthetic, and the high initial blood flow (nearly twice that shown in the first experiment, in Table 1) is probably a reflection of this.

period. But flow has greatly declined by the time a P_{O_2} of 65 mmHg is reached. This is in the range where the capacity to regulate collapses (Wells & Wells, 1983). The important point is that flow measurements, like pressure measurements already reported (Wells, 1979; Wells & Wells, 1983) decline as the external P_{O_2} falls. The matter was confirmed in the only instance in the present series where flow and pressure were recorded from the same individual (X26, Table 2). *Octopus* does not compensate for the fall in external P_{O_2} by dropping peripheral resistance and increasing blood flow.

Flow increases considerably as soon as more oxygenated water is provided. For a given P_{O_2}, it is generally greater in a rising ambient P_{O_2} than in a falling ambient P_{O_2} (Tables 2, 3; Fig. 2). This was to be expected. *Octopus* can sustain a limited oxygen debt (in the region of 22 ml kg^{-1}; Wells & Wells, 1984) and evidently accumulates a debt in acute hypoxia which is repaid as soon as additional oxygen becomes available.

Table 1. *Oxygen consumption and blood flow in* Octopus

No.	Wt (g) and °C	P_{O_2} (mmHg)	O_2 consumption (ml min^{-1})	Total flow (Fick) (ml min^{-1})	Pulse frequency (Hz)	Stroke volume (ml)	Measured flow (ml min^{-1})	% down aorta
W38	1459	125	1·88	60·6	0·60	1·68	58·0	96
	21°C	95	1·29	44·7	0·53	1·41	42·5	95
		55	0·50	19·6	0·45	0·73	12·0	61
X12	2387	140	4·47	182·0	0·83	3·65	88·5	47
	24°C	95	3·74	171·0	0·80	3·56	87·0	51
		65	2·96	139·0	0·71	3·26	69·5	50
X26	1745	140	2·24	72·0	0·64	1·86	58·5	81
	24°C	95	2·14	74·2	0·62	1·99	46·5	75
		65	1·29	48·3	0·57	1·41	44·0	91
X47	1018	140	1·68	54·1	0·65	1·39	33·5	62
	23°C	95	1·57	54·5	0·65	1·40	31·0	57
		65	1·23	48·4	0·60	1·35	23·0	48
X51	1983	140	2·13	68·5	0·78	1·46	53·0	77
	23°C	95	1·68	58·3	0·77	1·26	44·0	75
		65	1·34	50·6	0·71	1·19	35·0	69
Y1	2959	140	3·94	126·6	0·78	2·71	72·0	57
	22°C	95	3·27	113·2	0·71	2·66	76·0	67
		65	1·76	68·9	0·64	1·80	59·0	86
Y14	1687	140	1·42	45·8	0·63	1·21	30·8	67
	22°C	95	1·76	61·0	0·65	1·56	36·8	60
		65	0·76	29·6	0·59	0·89	22·0	74
Y29	1210	140	1·88	60·3	0·65	1·55	48·5	80
	20°C	95	1·35	46·6	0·52	1·49	33·0	71
		65	0·99	38·9	0·48	1·35	32·5	84

The difference in flow in developing hypoxia and in recovery tells us that the circulatory response is to changes in the blood or tissues rather than a response to the external P_{O_2}.

The stroke volume output of the systemic heart can be estimated by dividing the flow (total, by Fick, or measured at the aorta) by the heartbeat frequency. In either case, it falls as the external P_{O_2} declines, again not markedly in the P_{O_2} range 140 to 95 mmHg, but often considerably thereafter (Table 1).

DISCUSSION

Criticism of the method

In calibrating the flow probe, it has always been assumed that the lowest voltage output found in a series of pulses represents zero flow. Zero flow is a reasonable assumption to make because actual observations of the blood flow in the aorta (small, transparent individuals, and the exposed aortae of larger animals) always show flow to be pulsed, with no detectable movement at some stage in the cycle. Any errors arising on this account will tend to underestimate slightly the flow along the aorta. It was not possible to establish a zero by occlusion because of the problems outlined under Methods.

Values derived from Fick calculations are relatively unreliable because we used average values for arterial and venous blood oxygen contents taken from an earlier study which also showed some variability from one individual to the next. It would, of course, have been better in theory to measure blood oxygen in the experimental animals rather than adopt values from a previous series of experiments. The practical objection to attempting to do this was that it would have required extra cannulae and some loss of blood from animals that we wanted to stress as little as possible. Our octopuses were free to move around their respirometers, adopt whatever positions they found comfortable and, more important, there were no cannulae to obstruct free movement of the gills. Efficient oxygen extraction depends critically upon the way in which these soft structures lie against neighbouring tissues in the mantle cavity (Wells & Wells, 1982). We already knew that regulation of oxygen uptake was liable

Table 2. *Measured flow, and pressure pulses, in developing hypoxia and in recovery (animal X26)*

	P_{O_2}	Flow ($ml\,min^{-1}\,kg^{-1}$)	Pressure pulse (systolic–diastolic in mmHg)
Falling	130	33	65
	115	30	65
	100	26	55
	85	25	32
	70	25	32
Rising	85	33	53
	100	34	—
	115	40	70
	130	39	70

Table 3. *Flow along the aorta (in ml min^{-1} kg^{-1}) when the tank water was at a P_{O_2} of 80 mmHg*

No.	Weight (g)	T (°C)	In developing hypoxia	In recovery
W38	1459	21	19	25
X12	2387	24	30	37
X26	1745	24	25	32
Y1	2959	23	22	21
Y14	1687	22	30	43
Y14*	1687	22	31	46

* Second experiment, shown in Fig. 2.

Specific metabolic rate decreases as weight$^{0.67}$ and rises with a Q_{10} of 1·77 (Wells, O'Dor, Mangold & Wells, 1983).

to be imperfect if the gills were encumbered with cannulae, presumably because the gills can then no longer form a tight seal dividing the mantle into pre- and post-branchial cavities (Wells & Wells, 1983; Wells & Smith, 1985). It seemed preferable to us to aim for animals where we could reasonably assume that the circulatory and ventilatory systems were performing normally (so that the measured flow, at least, would be reliable), since this was the prime purpose of the experiments, and accept that the adoption of oxygen delivery data from previous experiments would inevitably reduce the accuracy of the total flow estimates, since the possible detection of gross changes in blood distribution was a secondary objective.

Discussion of the results

The results are consistent, despite the possibly dubious procedure adopted to calculate total flow. Total estimated flow was always greater than the measured aortic flow to the head and arms. Both declined as the external P_{O_2} fell, with little change in heartbeat frequency and stroke volume over the first part of the range, from a P_{O_2} of 140 down to 95 mmHg, followed by greater and more variable falls between 95 and 65 mmHg. Stroke volume changes were greater than frequency changes. The proportion of the total blood flow passing through the flow probe in the dorsal aorta showed no systematic change as the ambient oxygen was depleted (Table 1).

The main finding is that flow remains steady or declines, whatever way one measures it, as the external P_{O_2} falls. Flow and pressure pulses tell the same story. The animal does *not* drop its peripheral resistance as the P_{O_2} falls, increasing flow while holding pressure steady.

The increase in pH that takes place as the ambient P_{O_2} falls ensures that the arterial blood remains saturated, or nearly so, down to P_{O_2} values well below the 75 mmHg required in normoxia. The venous return is always very deficient in oxygen (0·5 vol% even in fully saturated sea water) and becomes even more so (to 0·35 vol%) in extreme hypoxia (Houlihan *et al.* 1982). The net effect is that the system delivers almost as much (2·3 vol% cf. 2·8 vol%) oxygen from the blood at each circuit at an ambient P_{O_2} of 50 mmHg as at 150 mmHg, but at a much lower oxygen tension. Since the blood flow does not increase, this implies a considerable reduction

in the length of the mean diffusion path from blood to tissues, or a considerably increased circulation time. The flaccid appearance of octopuses subjected to severe hypoxia suggests engorgement of the tissues with blood. Where is the extra fluid coming from? The large sinuses around the guts are the only obvious source of extra blood, and the suggestion has been made elsewhere that these represent a relatively slowly-circulating reserve (O'Dor & Wells, 1984).

The proportion of the total blood flow going to the head and arms appears to remain unchanged as hypoxia develops. At this crude level there is evidently no change in the pattern of blood distribution. At a more detailed level (is the blood rerouted so that more goes to the brain or the coronary circulation?) we have no evidence. But a very large number of neurones in the brain are dedicated to the control of blood vessel musculature; Young (1963) has estimated that the vasomotor lobes alone include more than $1{\cdot}3 \times 10^6$ nerve cells. These neurones must be concerned with adjusting peripheral resistance when the animal moves. Muscles in a hydrostatic skeleton are extended only under pressure and the animal must close down relatively low-resistance pathways to the guts or the arm musculature will be starved of blood just when it needs it as the animal moves about. With the degree of fine control that is potentially available, it would be remarkable if the animal did not also adjust its blood flow to favoured organs when oxygen is scarce.

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