J. exp. Biol. ***175****, 211–218 (1993)*
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FLUID UPTAKE AND THE MAINTENANCE OF BLOOD VOLUME IN *OCTOPUS*

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Accepted 8 October 1992

Summary

The replacement of fluid following withdrawal of up to 40% of the blood from *Octopus vulgaris* can be tracked over a period of days by measuring the dilution of haemocyanin, which is not simultaneously replaced. Haemocyanin concentration was measured from the copper content or the oxygen-carrying capacity of further small blood samples. Fluid lost was replaced within 1–2h, provided that the digestive gland ducts were left intact. If these were ligated, the haemocyanin concentration remained the same as before withdrawal of the initial large blood sample and the animals died within a few hours. Evidence presented elsewhere has indicated that the site of the fluid uptake is the digestive gland appendages. Urine production would be continued or increased during the restoration of blood volume. When urine volume is added to the volume of fluid replaced, it appears that this fluid transport system must be capable of moving at least its own volume of fluid from the gut into the blood every 5min. An immediate consequence of blood withdrawal is a fall in blood pressure and pulse amplitude, followed within minutes by a transient rise to high blood pressures, apparently as a result of an increase in peripheral resistance as circulation to the arms is restricted, conserving the blood for vital central organs. Following these transient swings, the diastolic blood pressure returns to normal values despite blood loss; pulse amplitude returns as blood volume is replaced. Duct-ligated animals continue to show a reduced pulse.

Introduction

Octopuses, in common with other coleoid cephalopods, have an excretory system based on ultrafiltration, with pressure provided by the branchial hearts (Harrison and Martin, 1965). In *Octopus vulgaris*, urine production varies, depending on the time of day, from 9.5% to 14.0% (mean, overall, 11.1±3.3, N=15; throughout this paper rates stated are means ± S.D.) of the body mass per day in fasted animals (Wells and Wells, 1990).

These figures match the rate of mass loss (11.6±3.0%, N=26) found in animals that have had operations preventing water from reaching the digestive gland appendages (Wells and Wells, 1989). Water uptake, it appears, is entirely *via* the gut, with no net input through the rest of the body surface.

Key words: *Octopus vulgaris*, fluid uptake, blood volume, blood pressure.

In this report the effect of suddenly withdrawing up to 40% of the total circulating blood volume is considered, the hypothesis being that this would force the animal to replace the lost fluid as rapidly as possible. It was reasoned that this would give information about the maximum rate of uptake, allowing us to confirm that all the fluid enters through the digestive gland ducts (deduced from the effect of ligating these) and to show how the animal manages to keep a high-pressure circulatory system functional in the event of this sort of emergency.

Materials and methods

The work was carried out at the Laboratoire Arago, Banyuls, in 1986 and 1991. *Octopus vulgaris* Cuvier, ranging in size from 290 to 1040g, were caught in trawls or by SCUBA diving and were kept for a few days before experimentation to ensure that they were undamaged. Most were fed, then starved for at least 24h before use in experiments.

Surgery was carried out under 2.5% ethanol anaesthesia. In most instances a stainless-steel T-piece was installed in the dorsal aorta, as described in Wells (1979). A cannula from the T-piece allowed blood samples to be withdrawn (deadspace being removed and returned on each occasion) and pressure records to be made using an Elcomatic EM 750 transducer. In four experiments, blood was taken instead through a cannula inserted through one of the branchial hearts into an afferent branchial vessel, as described by Wells and Wells (1983). Animals were held for an hour or more after the operation before the beginning of the withdrawal of the first blood sample. In three animals the digestive gland ducts were ligated, as described by Wells and Wells (1989), at the time that the T-piece was installed.

The fall in the concentration of haemocyanin was used to follow the uptake of fluid replacing lost blood. It will be shown below that there appears to be no reserve of haemocyanin that the animal can call upon, because the blood haemocyanin concentration, once reduced by fluid replacement, remains stable over a period of days.

Haemocyanin concentration was measured in two ways: from the copper content of the blood and from the oxygen-carrying capacity of the blood. In the former case samples were diluted with $0.1 mol\,l^{-1}$ HNO_3 to release the copper, before being transported to Cambridge and analysed using a Unicam SP 90A atomic absorption spectrometer, calibrated against copper nitrate standards (BDH Spectrosol). Samples taken to measure the copper content of the digestive and branchial glands were treated similarly. Oxygen capacity was assessed by aerating each fresh blood sample in a tonometer and then measuring the oxygen content in a Tucker cell, based on a Kent EIL 7130 oxygen meter. Cyanide was used instead of ferricyanide as recommended for haemocyanin by Bridges *et al.* (1979), and calculations were carried out as shown in that paper. Haemocyanin concentration is always expressed in terms of oxygen capacity, calculated where necessary from copper content on the basis that the copper atoms in haemocyanin combine 1:1 with oxygen (Ghiretti, 1966).

Predictions of the effect of blood removal on haemocyanin concentration were made on the basis that the blood volume in *Octopus vulgaris* is 4.85% of the body weight, as reported by O'Dor and Wells (1984). The actual values obtained in their experiments, in

which blood volume was measured from the dilution of radioactive haemocyanin, were 3.8, 4.4, 4.8 and 6.4%. Martin *et al.* (1958), using dye dilution to estimate blood volume in the much larger *O. dofleini,* found a value of 5.8±1%. If, as these figures suggest, the blood volume in octopuses is somewhat variable, the figures given for the predicted degree of dilution should all the fluid be replaced in any individual can only be approximate.

Results

The effect of removing blood on the haemocyanin concentration

Table 1 summarises the results obtained from 13 animals, five using copper content as an indicator of blood pigment concentration and eight using the oxygen-carrying capacity of the haemocyanin present.

Table 1A summarises the results obtained following the removal of a large blood sample (up to 37% of the total estimated volume) from the circulation. After an interval averaging 95min, a second much smaller sample (approximately 2%) was taken. If all of the fluid and none of the haemocyanin were replaced during the interval between the first and second samples, the blood pigment concentration in the second sample could be predicted by multiplying the original concentration by the percentage of the blood volume left after the blood withdrawal. This was done for each of the animals. Column 5 in Table 1 shows the average concentrations predicted, in terms of the oxygen-carrying capacities of the bloods. Column 6, similarly, shows the averages of the measured concentrations. If the predictions were correct, the ratio of the predicted to the measured values should be close to unity. For intact animals, the predictions did indeed come close to the concentrations actually measured (Table 1A). Column 8 in Table 1A shows the ratio of concentrations measured in the second sample to the concentrations measured in the first. This mean dilution corresponds closely to the dilution expected if all of the fluid and none of the haemocyanin were replaced.

The situation was plainly different in the three animals with ligated digestive gland ducts. For these animals the ratio of the predicted to the measured concentrations was 0.8 (the actual concentration was well in excess of that predicted) while the ratio of the concentrations found in the first and second samples was close to unity (no dilution had occurred; Table 1A).

In Table 1B, the haemocyanin concentrations measured in subsequent small blood samples, taken during the next 4 days, are compared with concentrations that were predicted on the basis that all of the fluid but none of the haemocyanin was replaced. In these cases the predictions were made on the basis that haemocyanin was removed at the starting concentrations in the first large samples only. If fluid were being replaced between each sample, the expected concentration would drop in each successive sample. For simplicity, and because the total volume of the second and subsequent samples was always small compared with the initial volume withdrawn, we have estimated the haemocyanin lost in these samples from the concentration measured at the end of the first (approximately 90min) period. The total volume removed listed in column 4 of Table 1B is the volume removed in the first sample plus the volume removed in subsequent samples

Table 1. *The oxygen-carrying capacity of the haemocyanin in blood samples taken from octopuses before, and 1–2h after, removal of a large volume (up to 37%) of the blood*

	Initial (vols%)	Interval (min)	Total blood removed (%)	Predicted (vols%)	Measured (vols%)	Predicted/ measured	Measured/ initial	Dilution (%)
A. *Octopus* haemocyanin oxygen-carrying capacity								
Animals with intact ducts								
Tucker (*N*=5)	4.2±0.7	98±21	37±5	2.6±0.6	3.0±1.0	0.9±0.2	0.7±0.2	33
Copper (*N*=4)	4.2±0.9	92±12	29±4	3.0±0.6	3.0±0.6	1.0±0.2	0.7±0.1	29
Copper (*N*=1)	4.3	255	31	3.0	2.9	1.0	0.68	32
Animals with ligated ducts								
Tucker (*N*=3)	3.3±0.7	84±5	25±2	2.5±0.5	3.2±0.8	0.8±0.1	1.0±0.1	2
B. *Octopus* haemocyanin oxygen-carrying capacity over 4 days following the initial sample (Intervals in h)								
2–8h (*N*=7)	4.1±0.9	5.6±1.9	38±5	2.5±0.7	2.6±1.0	1.0±0.3	0.6±0.2	38
16–24h (*N*=5)	4.2±0.7	20±3	38±4	2.6±0.7	2.5±0.7	1.0±0.1	0.6±0.1	40
2–4 days (*N*=5)	4.3±0.8	57±26	37±7	2.7±0.7	2.6±0.8	1.1±0.1	0.6±0.1	41
Animals with ligated ducts								
3–5h (*N*=3)	3.5±0.8	3.6±0.6	28±3	2.5±0.5	3.3±0.9	0.8±0.1	0.9±0.1	7

Blood oxygen capacities were predicted on the basis that the total blood volume is 4.85% of the body mass and that all of the fluid and none of the haemocyanin is replaced between samplings.

Values are mean ± S.D.

Oxygen-carrying capacities were measured from oxygen released from blood after treatment with cyanide in a Tucker cell or from the copper content of blood measured in a spectrophotometer (see text). In Table 1B results from the two techniques have been pooled and predicted haemocyanin concentrations have been calculated on the basis that the small samples removed all contained haemocyanin at the concentration found at the end of the first interval.

multiplied by the ratio of the concentration measured at the end to that found at the beginning of the first large blood withdrawal.

Once again, with the exception of samples taken from animals with ligated digestive gland ducts, the ratios of predicted to measured concentrations were close to unity, and the dilutions implied by the ratio of the measured to the original concentrations were close to those predicted.

Animals with ligated digestive ducts do not survive for more than 48h even when no blood is removed (Wells and Wells, 1989). The added stress of a massive blood withdrawal meant that the condition of these animals declined rapidly after about 6h, at which point they were killed.

The results from animals held for more than 24h after the initial large-volume blood removal indicate that the haemocyanin concentration remains stable following the initial rapid fluid replacement. Since in every case the haemocyanin concentration was very much less than the concentration before blood withdrawal, these results imply that there is no reserve of haemocyanin for the animal to draw upon should it lose blood, and that it cannot rapidly synthesise more of the pigment to restore the oxygen-carrying capacity of the blood.

Table 2. *Changes in* Octopus *heart function following removal of a large quantity of blood*

	Before blood withdrawal	30min	60min	Next day
Control animals (*N*=5)				
P_d	1.8±0.3	1.7±0.7	1.8±0.3	1.9±0.6
P_p	1.4±0.3	0.6±0.2	1.1±0.3	1.5±0.4
f_H	0.8±0.2	0.6±0.2	0.8±0.2	1.0±0.1
Animals with the digestive gland ducts ligated (*N*=3)				
P_d	2.0±0.9	1.8±1.4	1.9±0.8	–
P_p	1.0±0.2	0.7±0.3	0.6±0.2	–
f_H	0.7±0.2	0.8 (*N*=1)	0.5±0.2 (*N*=2)	–

	Maximum	Minimum
Maximum and minimum values found within 5min of bleeding in animals with the ducts ligated		
P_d	3.5±1.3	1.2±1.2
P_p	0.6±0.5	0±0

Each entry shows mean± S.D.
P_d, diastolic pressure; P_p, pulse pressure (both kPa); f_H, heartbeat frequency (Hz).
Times are periods elapsed since the end of blood removal.

Blood pressure changes following blood loss

Table 2 summarises the changes in aortic diastolic and pulse pressures and in heartbeat frequency in those of the animals in Table 1 in which haemocyanin concentration was assessed from Tucker cell measurements of oxygen-carrying capacity. A relatively small number of animals was concerned and there was a lot of scatter in the data. Nevertheless, some features stand out. By 30min after the end of blood withdrawal, diastolic pressures (P_d) had returned to values within a few per cent of those found in the same animals at the start of the experiments. The aortic pressure pulse (P_p), in contrast, had decreased; 30min after bleeding the five control animals showed less than half the pulse pressure recorded before blood removal; the three octopuses with ligated ducts, which had somewhat smaller volumes of blood withdrawn (see Table 1), showed a 30% reduction. Thereafter the pulse increased progressively in the controls; within 60min it had returned to its original value. The duct-ligated animals in contrast showed a progressive decline; pulse was reduced by 40% after 1h. These animals did not survive until the next day. Heartbeat frequency showed no consistent changes.

In the case of the three duct-ligated animals only, a continuous record of the blood pressure was kept from the completion of the first blood withdrawal. By the end of blood withdrawal the aortic pressure pulse had ceased in all three instances; pressure had fallen by 40% (minimum values at the foot of Table 2). During the next few minutes a return of the aortic pulse was accompanied by massive transient rises in diastolic pressure, which now exceeded the pressure found before bleeding by 75% (maximum values).

Copper in the digestive and branchial glands

There are massive quantities of copper sequestered in the digestive gland. 24 animals were examined. These averaged 1839±772mg of copper per kilogram wet mass, about 10 times as much per gram as is present in the blood. The branchial glands, which are believed to be the site of haemocyanin synthesis (Messenger *et al.* 1974), though perhaps not the site at which the copper is finally added to the pigment (Schipp and Hevert, 1978) contained, gram for gram, considerably less copper (60.4±30.8mgkg^{-1}, N=9) than the blood.

The mass of the digestive gland appendages

The digestive gland appendages of five animals were dissected out and weighed. They formed between 0.17 and 0.07% (average 0.12%) of the body mass. It should be noted that the wet mass of the appendages would have included fluid in the ducts and blood, so that the actual mass of the cells responsible for fluid transport would be rather less than 0.6g in a 500g animal.

Discussion

The results described above show that the volume of the blood is kept constant in *Octopus*. Fluid lost is replaced within an hour or two. Replacement is prevented by ligation of the digestive gland ducts. There is no corresponding replacement of haemocyanin, at least over a period of 2–4 days (Table 1).

The rate of fluid uptake in animals with intact digestive gland ducts must have been considerable. These octopuses had to replace the fluid lost when blood was withdrawn at the same time as replacing fluid lost in the urine. We did not measure urine production in these experiments. In the normal course of events, a urine volume amounting to 11.1±3.3% of the body mass passes out of the animal daily (Wells and Wells, 1990). The driving force producing the ultrafiltrate forming the basis of the urine is the branchial heart blood pressure. Schipp and Hevert (1981) showed that in *Octopus vulgaris* the branchial heart systolic pressure, peaking at 0.4–0.5kPa at rest, is sufficient to overcome the colloid osmotic pressure (largely due to haemocyanin in solution) of 0.3kPa. In the present experiments, fluid replacement of 30–40% of the blood volume would be expected to reduce the colloid osmotic pressure to 0.2kPa. Apart from falls during and immediately after periods of blood removal, systemic heart diastolic pressures were maintained. Systolic pressures (diastolic plus pulse in Table 2) fell by 30%. It is assumed that the branchial hearts behaved in the same manner. Experiments carried out under a variety of conditions (progressive acute hypoxia, exercise, effects of drugs; Wells, 1983; Wells and Mangold, 1980; Wells and Smith, 1987; Wells and Wells, 1983) show that systolic and diastolic pressures in the systemic and branchial hearts rise and fall together. A 30% fall in systolic pressure in the branchial hearts would reduce the urine filtration rate while the 30–40% drop in colloid osmotic pressure would increase it. The two changes would have occurred simultaneously during the time that fluid was being replaced following blood loss, suggesting that urine was likely to have been produced at a rate close to the normal pre-bleeding rate during this period. The animals must therefore

have taken up a quantity of fluid sufficient to form a normal volume of urine in addition to the fluid needed to replace the blood removed. In the 95min that a 500 g *Octopus* takes to restore 29% of its blood volume, it will have produced 3.7ml of urine as well as adding 7.0ml of fluid to the remaining blood; an uptake rate of 6.8ml h^{-1}. It should further be noted that any reduction of the colloid osmotic pressure will produce a tendency for fluid to move out of the blood into the tissues, until a new equilibrium is established. All this fluid, it appears, must come in through the digestive gland appendages.

The digestive gland appendages weigh about 0.6g in a 500g animal. This wet mass would include fluid in both the ducts and blood. To account for the rates of dilution observed, this tissue must transport at least its own volume of water from the lumen of the digestive gland ducts into the bloodstream every 5min. Even if one makes the unlikely assumption that no urine is produced during the period of blood fluid replacement, the cells of the appendages would be passing their own volume of fluid from the digestive gland ducts to the blood every 8min. Such high rates are not unknown in other systems: in what is claimed to be the fastest fluid-transmission system known, the Malpighian tubule system of the blood-sucking insect *Rhodnius prolixus*, the tissue can secrete its own volume of fluid in 15s (Maddrell, 1991), but this rate is maintained for minutes rather than hours.

The maintenance of diastolic blood pressure following blood withdrawal implies an increase in peripheral resistance. The sudden rise and fall observed immediately after blood removal in animals where a continuous record was kept shows that the system can overshoot if there is a sudden massive diminution of blood flow. It is perhaps a response designed to restrict the remaining blood to the central nervous system and other vital organs at the expense of the arms. The main reason for believing this is that animals dying from fluid loss following duct ligation often lose the use of the arm tips, which become white and flaccid, at a stage when there are no obvious changes to the head and abdomen (Wells and Wells, 1989). Young (1963) has pointed out that some 1.3×10^6 neurones in the brain appear to be devoted to vasomotor control, and one function of these could be to rearrange blood flow to maintain the circulation to vital organs in the event of blood loss. The location of the sense organs which detect blood pressure or flow is not known.

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