CIRCULATION TIME, BLOOD RESERVES AND EXTRACELLULAR SPACE IN A CEPHALOPOD

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Octopus vulgaris Cuvier has a fully enclosed blood system that includes large blood sinuses behind the eyes and around the gut. Experiments on the response of the hearts in exercise and hypoxic stress (Wells, 1979; Wells & Wells, 1983) and the response of the system to drugs or extracts from neurosecretory systems that discharge into the bloodstream (Wells & Mangold, 1980; Wells, 1983) have revealed a number of situations in which it would be useful to know the mean circulation time, the minimal number of heartbeats needed for blood to return to the hearts, and/or the extent to which the sinuses constitute a reserve of blood which can be mobilized in times of stress.

We installed stainless steel T-pieces into the aortas of four free-ranging male octopuses of 890 to 1250 g. Attached to the free arm of the T, a large cannula allowed us to record the aortic pulse, as in Wells (1979). Through the wall of the large cannula, down the arm of the T and into the lumen of the aorta passed two finer cannulae that allowed (a) the removal of blood samples from the aorta and (b) the injection of ^{14}C-inulin. The sampling cannula just entered the through-flow part of the T. The injection cannula turned downstream in the T, so that injection was made about half a centimetre downstream of the sampling point. The deadspace of the sample cannula was 0·25 ml and that of the injection line less than 0·05 ml.

Animals were operated upon under 2·5 % ethanol anaesthesia. Aortic pressure was monitored during the recovery period and throughout each experiment. No experiment was begun until the animal was sitting quietly in its tank with the heartbeat frequency, mean aortic pressure and pulse amplitude remaining steady at a value typical of resting animals in this size range (about 35 cmH$_2$O in systole and 20 cmH$_2$O in diastole with a frequency of about 0·8 Hz).

Each experiment began with the injection of 100 μl of ^{14}C-inulin (New England Nuclear; inulin ^{14}C-carboxylic acid, 2·1 mCi g^{-1}) following expulsion of 50 μl of sea water in the deadspace of the tube. According to the manufacturer's specification this dose ought to have contained 2 μCi. We checked 10 μl aliquots and found the total count of a 100 μl dose to be 4·58×10^6 d.p.m. Injection took 3 s.

Blood samples (0·4 or 0·5 ml) were then drawn at intervals after injection into a succession of 1 ml syringes. In each case the blood in the deadspace of the sample cannula was first discarded; exceptions were made in the first 15 samples from C91,
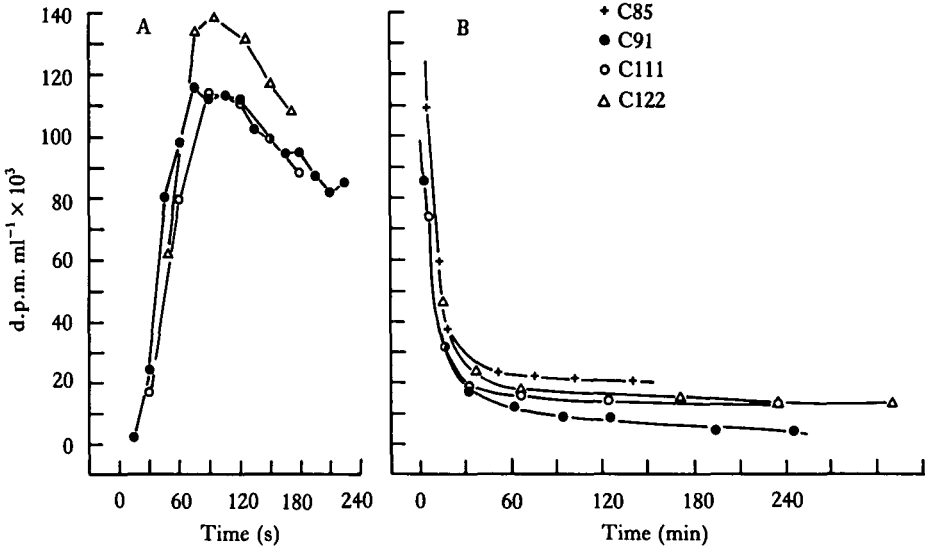
Fig. 1. Radioactive counts in blood samples taken from the dorsal aortas of octopuses at intervals after injection of $2 \mu Ci$ of ^{14}C-inulin in four separate experiments. (A) shows the counts from three of the animals where samples were removed at frequent intervals over the first 3–4 min. (B) shows the fall in counts over a period of hours.

which were withdrawn as rapidly as possible, at 15-s intervals. Blood samples were diluted with 2 ml H_2O and 15 ml 'Instagel' before being counted in an Intertechnique-60 spectrometer. Corrections were made for quenching and the results are plotted in d.p.m. ml^{-1} in Fig. 1.

After injection, radioactivity in the samples of returning blood rose to a maximum between 60 and 120 s. A substantial amount of radioactivity was recorded after 30 s, within 20 or 30 heartbeats of injection (Fig. 1). The absence of a sharp peak in the returning radioactivity is a reflection of the alternative pathways by which the blood may return within the first 120 s. The shortest, though not necessarily the quickest, route would be *via* the brain and the orbital sinuses, which discharge directly into the anterior vena cava. The longest could be out and back from the tips of the second pair of arms.

There is evidence that the radioactivity is still by no means evenly distributed in the blood by the end of the peak. The expected blood volume in our experimental animals was about 4·9 % of the body weight. This figure is derived from four experiments (O'Dor & Wells, 1973, and two more recent repeats) in which labelled haemocyanin was transferred from one octopus to another and allowed to distribute over periods of hours or days; haemocyanin does not penetrate into the extracellular space and it has a half-life of several weeks (R. K. O'Dor & M. J. Wells, unpublished; the actual values obtained were 3·8, 4·4, 4·8 and 6·4 % of the body weight). In the present experiments dividing the $4·58 \times 10^6$ d.p.m. doses injected (minus radioactivity already withdrawn in samples and deadspaces) by the peak radioactivity, averaging $1·19 \times 10^5$ d.p.m. ml^{-1}, gives distribution volumes of 3·3, 3·5, 3·7 and 3·9 % of the body weight (Table 1). This ignores any radioactivity that might have penetrated into the extracellular space, and any that might have been excreted during the first 2 min. Any such losses would reduce the estimates to less than the present average of 3·6 %. Thus during the first

Table 1. *Summary of circulatory parameters in four octopuses*

Animal	Weight (g)	Circulating blood volume* (% body wt)	Extracellular space including blood (% body wt)	Blood removed in course of experiment up to 60 min (ml)	Systolic and diastolic blood pressures (cmH$_2$O) At start	After 60 min
C85	1250	3·3	15·6	3·3	43–25	43–28
C91	1060	3·7	27·2	8·2	31–24	38–25
C111	970	3·9	25·2	9·0	29–20	39–20
C122	890	3·5	24·5	6·0	36–26	32–22

* These figures are derived by dividing the counts injected (minus counts removed) by the counts per ml in samples taken about 90 s after injection (see Fig. 1). The average, 3·6 %, is considerably lower than the 4·9 % obtained by alternative means (see text) following a much longer mixing time. The implication is that a substantial proportion of the blood volume has not become fully mixed with the blood returning within the first 2 min.

2 min the inulin was fully mixed with less than 75 % of the total blood volume. Presumably the more slowly equilibrating blood lies within the large blood sinuses around the gut, and might constitute a reserve that can be drawn upon in times of stress, exercise or blood loss. It is worth noting that blood pressures and pulses did not fall in these experiments, despite the removal of substantial quantities of blood (see Table 1).

As well as yielding figures for the circulation time and demonstrating unequal equilibration times for various blood pools, the results of the injection experiments can be used to estimate the total extracellular space, including blood. After the initial peak within 2 min of injection, the radioactivity recovered in blood samples falls rapidly to reach a relatively steady state at about 60 min. After this radioactivity declines almost linearly. If one assumes that the radio-inulin is now distributed evenly throughout the extracellular space it is possible to estimate extracellular space from the total radioactivity remaining divided by the blood radioactivity per ml at 60 min. The total radioactivity remaining will, of course, be less than at the start of each experiment. One must allow for the radioactivity removed in samples and deadspace discards and that excreted in the period up to 60 min. To estimate excretion we have assumed that the rate of loss is proportional to the concentration in the blood using the rate of loss during the 'steady state' 60–120 min period as a baseline.

The resulting estimates of inulin space are given in Table 1. They average 23 % of the body weight (26 % if one excludes the estimate from C85).

The figures for extracellular (inulin) space and blood volume (from the labelled-haemocyanin data) given here for *Octopus vulgaris* are plainly comparable with those (28 ± 7·3 % and 5·8 ± 1 %) reported by Martin, Harrison, Huston & Stewart (1958) for *Octopus dofleini*, which is an order of magnitude heavier in weight.

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