

OXYGEN CONSUMPTION OF THE ISOLATED HEART OF *OCTOPUS*: EFFECTS OF POWER OUTPUT AND HYPOXIA

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SUMMARY

A technique is described which allowed the measurement of the oxygen consumption of the isolated heart of *Octopus vulgaris*. Contraction of the heart resulted in an aortic output and a flow through the heart muscle into coronary veins (the coronary output). The flow and oxygen content of the aortic output and the coronary output were measured with variable input pressures and constant output back pressure (volume loaded), variable output back pressure and constant aortic output (pressure loaded), and during hypoxia. Volume loading of the heart resulted in an increase in aortic output, power output and total oxygen consumption. Pressure loading increased power output and total oxygen consumption of the heart. Exposure to hypoxia decreased the aortic output, power output and total cardiac oxygen consumption. In the response of the heart to reduced work, brought about either by a reduced input pressure or by hypoxic perfusate, the power output was linearly related to the total oxygen consumption of the heart.

The oxygen extracted from the coronary output accounted for 80–100 % of the total oxygen consumption of the heart. Coronary output amounted to 30 % of the total cardiac output at maximum power output. In volume-loaded hearts the volume of the coronary output increased as aortic output increased; in pressure-loaded hearts coronary output increased as power output increased, but aortic output remained constant. In hypoxia, the coronary output increased as the aortic output fell. At a perfusate P_{O_2} of around 50 Torr (1 Torr = 133 Pa), the aortic output ceased although the heart continued to beat and the coronary output continued, accounting for all of the oxygen consumption of the heart. The coronary output flow *in vitro* therefore has the capacity to be varied independently of the aortic output flow to maintain the oxygen supply to the perfused cardiac muscle.

INTRODUCTION

The systemic heart of *Octopus vulgaris* consists of a ventricle and two auricles and is capable of generating pressures above 100 cmH₂O (1 cmH₂O = 98.1 Pa) in the dorsal aorta (Wells, 1979). The cephalopod heart appears to be a highly aerobic issue. Ghiretti-Magaldi, Guiditta & Ghiretti (1958) found that the metabolic rate of

Key words: *Octopus*, heart, oxygen consumption, coronary efflux.

octopod cardiac muscle was higher than that of any other octopod tissue measured. The systemic hearts of squids have been shown to contain mitochondria capable of high rates of oxidation (Ballantyne, Hochachka & Mommsen, 1981; Mommsen & Hochachka, 1981) and slices of the hearts have respiratory rates exceeding those of rat heart (Barron, Sights & Wilder, 1953). Unlike fish hearts, the systemic heart of *Octopus* receives oxygenated blood directly from the gills and therefore would seem to be well sited in terms of oxygen supply.

However, there have been some reports which indicate that the heart muscle receives a separate coronary supply. Smith & Boyle (1983) describe a coronary arterial supply to the heart of the octopod *Eledone* and Smith (1985) mentions that the thick octopod ventricle has well-developed ventricular arteries. Wells & Wells (1986) state that the aorta, after leaving the heart, gives off a pair of small vessels that provide the coronary circulation for the systemic and branchial hearts. In contrast, Foti, Trara Genoino & Agnisola (1985) report that with each contraction of the heart a proportion of the total cardiac output passes through the walls of the heart, and is collected in coronary veins which lead back to the vena cava and the gills. They also provide evidence for a linkage between the coronary efflux and the cardiac output in the heart *in vitro*. In the absence of any previous measurements of the oxygen consumption of the working ventricle of *Octopus* the present study aims to measure the oxygen consumption of the isolated heart, differentiating between the oxygen consumed from the aortic output and from the coronary output.

The regulation of cardiac output by the isolated heart of *Octopus* has been shown to be primarily effected by changes in stroke volume, with perfusion pressures having little effect on heart rate (Smith, 1981; Foti *et al.* 1985). Smith (1981), using input pressures between 10 and 40 cmH₂O with the *in vitro* heart of *Eledone cirrhosa*, found large increases in aortic output, while Foti *et al.* (1985) found maximum cardiac output values with an input pressure of 20 cmH₂O in the *in vitro* heart of *Octopus*. In the present study variations in input pressure have been used to drive the work output of the heart by increasing aortic output. The aim was to increase the aortic output by at least two-fold, simulating the 2.5-fold increase in cardiac output which occurs during exercise (Houlihan *et al.* 1986). The hearts have also been made to work against variable output pressures within the range 25–50 cmH₂O (*in vivo* aortic pressures range from 15 to 40 cmH₂O at rest; Wells, 1983), while maintaining constant aortic output.

Octopus can continue to extract oxygen from sea water at a constant rate as the ambient P_{O₂} falls to values of around 70 Torr (Maginnis & Wells, 1969). Measurements *in vivo* showed that the blood P_{O₂} falls in parallel with the ambient P_{O₂} but that changes in blood pH ensure that the haemocyanin remains saturated (Houlihan, Innes, Wells & Wells, 1982; Smith, Duthie, Wells & Houlihan, 1985). In acute hypoxia, heart rate falls and the rate of blood flow may be reduced (Wells, 1983). In view of the oxygen demand of the heart, we decided to investigate the heart's response to hypoxic perfusates and the regulatory mechanisms permitting continued cardiac function in hypoxia.

MATERIALS AND METHODS

Animals

The experiments were carried out at the Stazione Zoologica, Naples, Italy. The animals, of both sexes, were maintained in a circulating seawater aquarium (20°C) and used within 7 days of capture. Seventy-one animals were used for the study. For the investigation of the effects of body mass on heart mass, cardiac output and myocardial oxygen consumption, a size range of 0.19–1.50 kg ($N = 39$) was used. In the investigation of the effects of volume loading, pressure loading and hypoxia, a size range of 0.450–0.980 kg was used (0.661 ± 0.042 kg, mean \pm S.E., $N = 32$).

Isolated heart preparation

In *Octopus vulgaris*, blood enters the heart through two auricles and leaves the ventricle through three arteries: the major aorta and the abdominal and gonadal arteries. Over the surface of the heart there is a network of coronary veins which run to the vena cava. The arrangement of the coronary flow was determined by cannulating *in situ* the efferent branchial vessel leading to an auricle and perfusing the heart with Mercox (Japan Vilene Company Ltd) or with methylene blue in sea water.

The isolated systemic heart was prepared as described by Foti *et al.* (1985). Both the auricles and the dorsal aorta were cannulated and the abdominal and gonadal aortae ligatured at their bases. The heart was then installed in the perfusion apparatus which was similar to that described by Foti *et al.* (1985), but was modified as a respirometer for the aortic and coronary circuits (Fig. 1). Approximately 10 min elapsed between killing the animal and placing the heart in the chamber.

The two auricles received perfusion fluid (sea water + 0.05 % glucose maintained at 20°C, pH 8.0) at the same controlled input pressures and its P_{O_2} was measured throughout the experiment. Contraction of the heart resulted in a proportion (the aortic output) of the input perfusion fluid passing out of the cannulated aorta and the sealed chamber: we measured the flow and P_{O_2} of this output. With each contraction of the heart, fluid also passed through the walls of the heart into the cut coronary veins and out into the chamber (the coronary output). The chamber was initially filled with aerated saline and the output flow and P_{O_2} from the coronary output was measured throughout the experiment. The chamber had a volume of 56 ml; the fluid in it was stirred continuously (Fig. 1) and the turnover time (volume of chamber/coronary output) was between 8 and 10 min. The temperature in the chamber was 20°C. Because of the chamber arrangement, the heart was bathed in the coronary output and there was the possibility of oxygen consumption occurring over the surface of the heart. Experiments were therefore carried out on hearts with no input pressure, and the chamber was initially filled with aerated saline and sealed. Repeated sampling and measurement of the P_{O_2} of the chamber fluid gave an estimate of the surface oxygen consumption of the heart and the microbial oxygen consumption in the saline. Values obtained were less than 3 % of the total oxygen consumption of the heart and were not included in the calculations. Because of the coronary flow it

is not possible to measure surface oxygen consumption in working hearts. We have assumed that the P_{O_2} gradient between the chamber fluid and the heart is maximal, even in the resting heart, and that surface oxygen consumption would therefore not increase.

The heights of the input and output tubes were measured with a ruler to within 1 mm with reference to the levels of the input cannulae and the aortic output, respectively. The coronary output tube was always fixed to the same level as the input tubes to the heart, i.e. the difference between input and output on the coronary flow was equal to the input pressure to the heart. Input and output pressures and coronary output pressures were also measured through saline-filled sidearms.

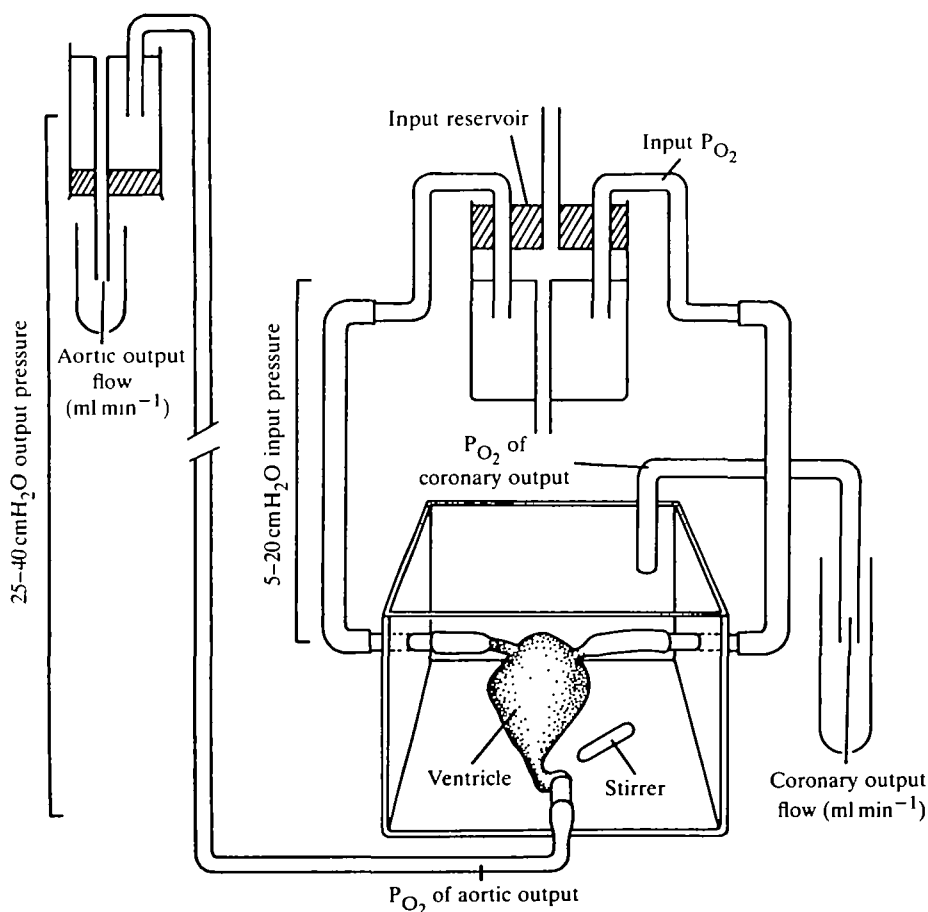


Fig. 1. Diagram of the apparatus used. The two auricles received perfusion fluid with an input pressure controlled by the height of the input reservoir. The aortic output was collected from the cannulated aorta and the output pressure was controlled by the height of the output reservoir. The coronary output was collected from the outer chamber which was fitted with a magnetic stirrer. Samples of perfusate, aortic output and coronary output were collected for measurement of P_{O_2} .

Protocols

Following the cannulation procedures and placing the heart in the chamber, it was supplied with aerated saline at an input pressure of 20 cmH₂O and the aortic output pressure was set at 40 cmH₂O (Δ pressure = 40–20 cmH₂O). These values were chosen as the input value gave the maximum cardiac output in the *in vitro* heart (Foti *et al.* 1985) and the output was similar to *in vivo* aortic values (see Introduction). In these conditions, aortic output was $16.5 \pm 1.0 \text{ ml g}^{-1} \text{ min}^{-1}$. Over the course of 10 min, samples of cardiac output and coronary outputs were collected for measurements of flow rates and P_{O_2} . If these variables had not stabilized after 10 min, the heart was discarded. Experiments were run for no more than 40 min after setting up the heart; returning input and output pressures to control values up to this time resulted in the heart being able to return to initial values.

Volume loading

These experiments ($N = 12$) examined the effects of changing power output of the heart while changing O₂ delivery to the heart. Varying input pressure, while keeping the Δ pressure at a constant value of 20 cmH₂O, resulted in variable aortic output; the P_{O_2} of the input perfusate remained at around 150 Torr. The input pressure was reduced in steps of 5 cmH₂O to decrease aortic output from the maximum value, and perfusate P_{O_2} , aortic and coronary output flows and P_{O_2} were measured over 10 min. By this time, repeated measurements gave the same values and sufficient time had elapsed for the coronary efflux to have stabilized relative to the chamber volume. It has previously been demonstrated that the range 5–20 cmH₂O input pressure gave the greatest range of cardiac outputs (Foti *et al.* 1985).

Pressure loading

These experiments ($N = 5$) examined the effects of changing the power output of the heart, while maintaining O₂ delivery (maximum aortic output and P_{O_2}) constant. The input and output pressures were arranged to give a Δ pressure of 30 cmH₂O (20 cmH₂O input, 50 cmH₂O output pressure). After a 10-min stabilization period and the measurement of variables, the Δ pressure was changed to 25 cmH₂O while keeping aortic output constant. Measurement of variables was followed by continued step changes of 5 cmH₂O in Δ pressure at constant aortic output. Foti *et al.* (1985) describe the relevant input and output pressure necessary to achieve constant aortic output.

Progressive hypoxia

These experiments ($N = 11$) initially examined the effects of a reduction in the perfusate P_{O_2} with constant input and output pressures of 20 and 40 cmH₂O, respectively. In addition to the control (air-saturated) perfusate, four other P_{O_2} levels were examined: 120, 100, 80 and 50 Torr. The P_{O_2} of the perfusate was constant and was controlled by the mixing of N₂ and air streams. After 10 min with air-saturated saline, the P_{O_2} was reduced to the first level of hypoxia, and after 8 min of

continuously recording cardiac variables the P_{O_2} was reduced again. It was found that after 8 min at any particular P_{O_2} the flow rates and P_{O_2} values had stabilized. In a further series of experiments with five animals, after reducing the P_{O_2} to 70 Torr for 15 min after normoxia, the Δ pressure was reduced and cardiac variables were monitored.

Measurements and calculations

Throughout the experiments, the aortic output and coronary output were collected over 1 min, weighed, values were corrected for temperature and salinity and expressed as a volume measurement. Mean values for input and output pressures were obtained from the directly measured heights of the input and output reservoirs and from pressure measurements made with a Statham P23 pressure transducer coupled to a chart recorder (Gould, Cleveland). Pressures were referenced to the level of saline in the bath. Pressure drops across the input and output cannulae were measured at variable flows and corrections applied where necessary. Separate samples were taken anaerobically by syringe of the input and output flows for the measurement of P_{O_2} . Heart rate was measured by counting contractions over 1 min.

Samples of input perfusate, aortic output and coronary output were withdrawn anaerobically in the intervals between the measurements of fluid flow, and P_{O_2} was measured with Radiometer oxygen sensors mounted in thermostatted cuvettes and connected to oxygen meters (Strathkelvin Instruments). The sensors were calibrated with a sodium sulphite/borax solution for zero P_{O_2} and using air of calculated P_{O_2} . Normally, the heart received aerated perfusion medium, but in a series of experiments on the effects of hypoxia, N_2 and air were bubbled into the input saline at controlled rates to give the required perfusate P_{O_2} .

Utilization of oxygen during the passage of the perfusion fluid through the heart through either the aortic output or the coronary output flows was calculated as:

$$\% \text{ utilization} = \frac{P_{IO_2} - P_{EO_2}}{P_{IO_2}} \times 100,$$

where P_{EO_2} was either that for the aortic output or for the coronary output.

All pressures are expressed in cmH_2O . Aortic output ($\text{ml g}^{-1} \text{ min}^{-1}$) = heart rate \times stroke volume. Power output of the heart from the aortic output (\dot{V}_b) was calculated as (mW g^{-1}) = (mean output pressure – mean input pressure) $\times \dot{V}_b \times (980/60) \times 10^{-4}$ (Farrell, Wood, Hart & Driedzic, 1985). The power output from the coronary flow (mW g^{-1}) was calculated as the product of the aortic pressure and the coronary flow (V_c); coronary flow power output = aortic pressure $\times \dot{V}_c \times (980/60) \times 10^{-4}$. Total power output of the heart was calculated from the sum of the aortic and coronary power outputs. Total oxygen uptake of the heart ($\mu\text{l O}_2 \text{ g}^{-1} \text{ min}^{-1}$) = $[\dot{V}_b \times P_{O_2} \times a] + [\dot{V}_c \times \Delta P_{O_2} \times a]$, where a is the solubility coefficient of oxygen determined from its solubility in aerated sea water plus glucose by the method of Strickland & Parsons (1972). Mechanical efficiency of the heart (%) =

(100) [power (mW) \times 0.0498] [\dot{V}_{O_2} (μ l O_2 min⁻¹)]. The ventricle was weighed at the end of each experiment, and all the results are expressed per gram ventricle mass.

The results were compared using Student's *t*-test and, in the case of the scaling investigation, analysis of covariance was used. The 5 % level of confidence has been employed throughout.

RESULTS

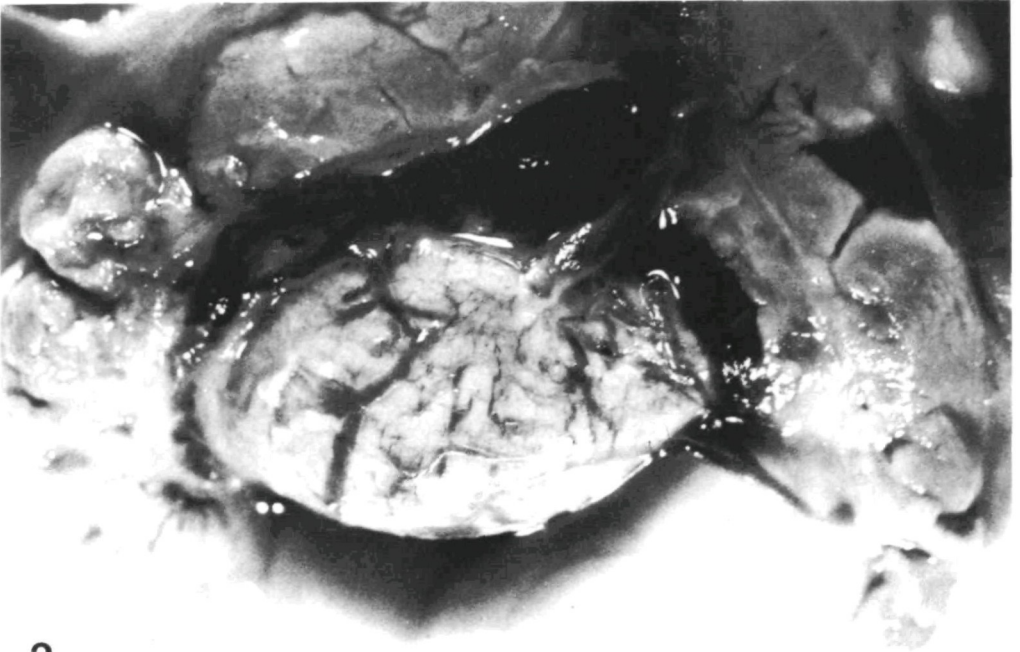
As described by Foti *et al.* (1985), contraction of the isolated ventricle was accompanied by the loss of some fluid from the cut coronary veins. Observations of isolated contracting hearts clearly revealed the streams of coronary output from the cut coronary vessels on the anterior lateral edges of the heart. Injection of methylene blue into a cannulated efferent branchial vessel *in situ* revealed that contraction of the undamaged heart is accompanied by the passage of dye through the walls of the ventricle, and its collection in coronary veins which empty into the lateral vena cava. Inspection of the *in situ* Mercox-injected (Fig. 2) heart revealed a network of fine capillaries over the surface of the ventricle which were connected to the coronary veins and led to the vena cava and hence to the gills. In the isolated heart, there was no leakage of fluid out of the heart except through the cut coronary veins; contraction of the ventricle resulted in flow of perfusion fluid through the cannulated aorta and a coronary output. The latter only occurred during ventricular contraction.

The *in vitro* heart preparation generated its own rhythm. Generally the hearts did not perform well after 50 min in the apparatus; after this there was a notable decline in cardiac output, particularly at the higher input pressures. After several trials, a protocol was developed for the volume-loading experiments, as shown in Fig. 3. In initial experiments, the hearts were started with an input pressure of 5 cmH₂O, but under these conditions steady-state conditions were not reached and the heart performance steadily declined.

Volume loading

A decrease in input pressure with constant Δ pressure produced a decrease in the maximum aortic output and coronary output (Fig. 3). The coronary output amounted to 41.5 ± 3.6 % of the aortic output (30 % of the total of cardiac output) over the range of pressures used. Aortic output was linearly related to coronary output where coronary output = $0.37 \times$ aortic output + 0.98 ($r = 0.83$, $df = 24$, $P < 0.001$) (Fig. 4). The P_{O_2} of the aortic output was only 10 Torr less than the perfusion P_{O_2} (145.8 ± 0.6 and 156 ± 0.1 Torr, respectively), and this and the percentage utilization (mean 6.7 ± 0.5 %) did not change significantly with aortic output (Fig. 3).

The tissue supplied *via* the network of coronary vessels was clearly the major site of oxygen consumption, with the coronary output accounting for 80 ± 0.5 % of the total oxygen consumption of the heart over the range of input pressures used. Both aortic output and coronary output were linearly related to the total oxygen



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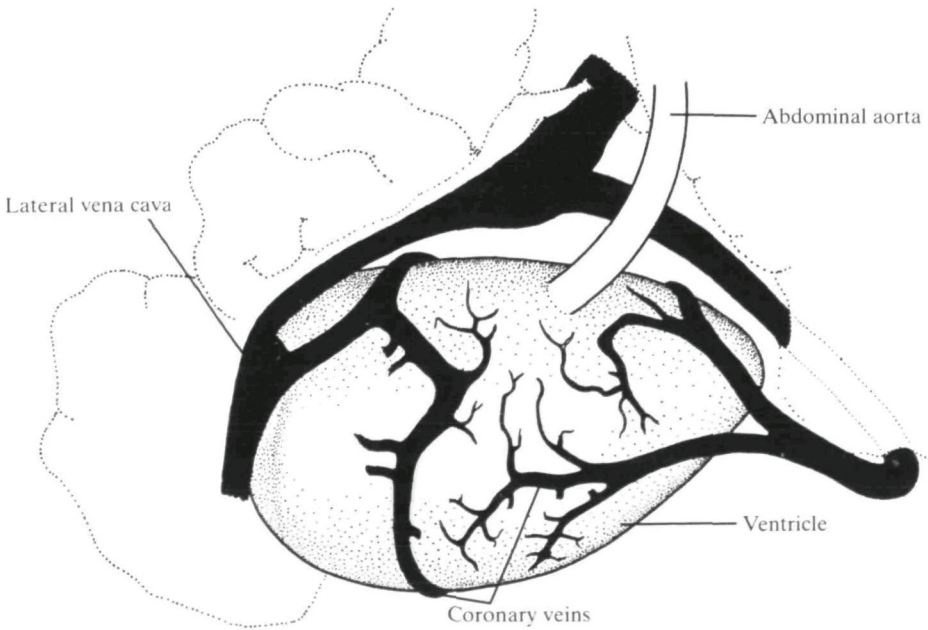


Fig. 2. Ventricle of *Octopus vulgaris* injected *in situ* through the efferent branchial vessel with Mercor resin. The resin entered the ventricle through one auricle (not visible) and left *via* the arteries and through the coronary veins. The resin passed through the walls of the ventricle to be collected in fine capillaries uniting to form the coronary veins.

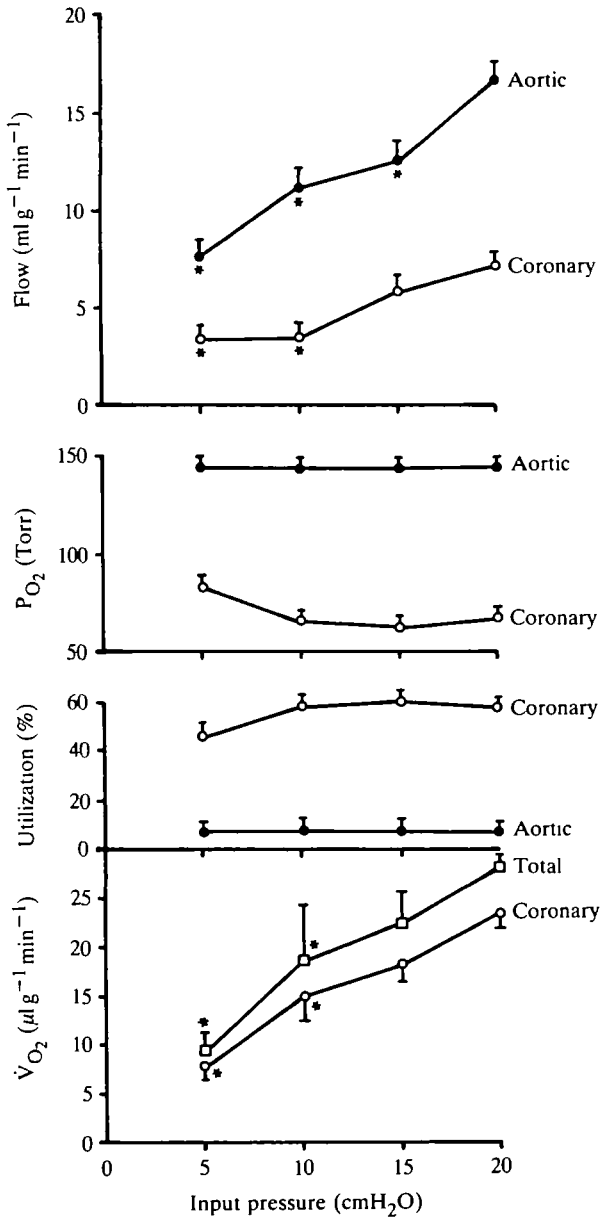


Fig. 3. Mean (\pm S.E.) aortic output and coronary output flow rates, P_{O_2} , % utilization, total oxygen consumption (aortic output and coronary output) and coronary output oxygen consumption from 12 perfused hearts (mean mass of animals 765.0 ± 69.1 g, mean mass of hearts 0.83 ± 0.07 g). Input pressure was started at 20 cmH₂O and reduced every 10 min by 5 cmH₂O, aortic output pressure was always 20 cmH₂O above input pressure. * denotes a statistically significant difference from the 20 cmH₂O input pressure results.

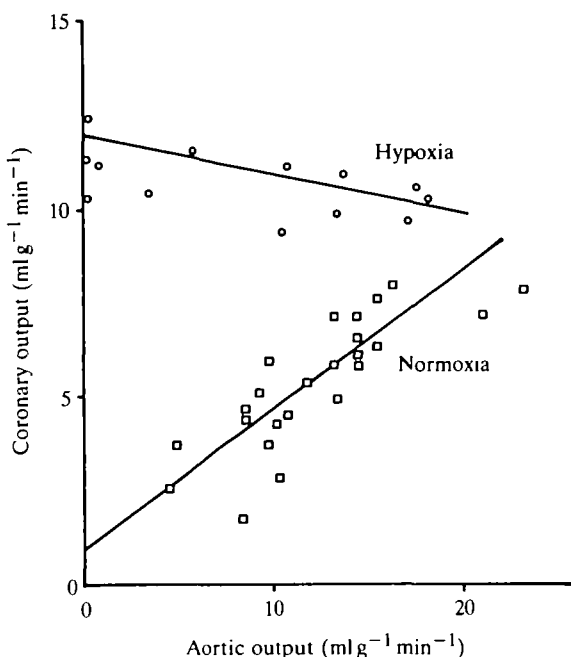


Fig. 4. Relationships between aortic output and coronary output in normoxic and hypoxic hearts. The hypoxia results are taken from input P_{O_2} values below 100 Torr. Each point is the result from an individual determination. For values of linear regressions, see text.

consumption of the heart. Fig. 5 shows aortic output and the total oxygen consumption of the heart, where total oxygen consumption = $1.49 \times$ aortic output + 1.25 ($r = 0.81$, $df = 22$, $P < 0.001$). The total oxygen consumption showed a 2.8-fold increase with a 2.2-fold increase in aortic output.

The total power output of the heart was almost equally divided between the aortic flow and overcoming the resistance of the coronary vessels. Total oxygen consumption was linearly related to total power output (Fig. 6A), where total oxygen consumption = $28.46 \times$ power - 0.87 ($r = 0.89$, $df = 25$, $P < 0.01$). Mechanical efficiency of the heart was $12.4 \pm 0.7\%$ at the maximum and $12.4 \pm 1.9\%$ at the minimum aortic outputs, an insignificant difference.

There was no significant change in heart rate with increase in input pressure; mean values were 31.0 ± 1.13 and 30.0 ± 0.7 beats min^{-1} at 20 and 5 cmH_2O , respectively; the increases in aortic output were due to increases in stroke volume. In three hearts, the coronary output pressure was increased by 5 cmH_2O while the hearts were receiving 20 cmH_2O input pressure and 40 cmH_2O output pressure. There was no significant change in the coronary output flow rate.

Thus, when total power output was increased by increasing aortic output and coronary output, there was an increase in total oxygen consumption due to a increase in coronary volume flow. There was a negligible change in the oxygen

removal from the perfusate and a direct linear linkage between aortic output and coronary output.

Pressure loading

Changing Δ pressure from 30 to 5 cmH₂O without significantly changing aortic output resulted in a fall in coronary output (Fig. 7). The utilization of oxygen in the aortic and coronary outputs had mean values of $6.5 \pm 0.4\%$ and $56 \pm 2.3\%$, respectively, not significantly different from values obtained in the volume-loading experiments. The total oxygen consumption of the heart declined as Δ pressure fell, but in this case total oxygen consumption correlated with coronary output and not aortic output (Fig. 7). Total oxygen consumption varied by 2.3-fold with a 3.5-fold increase in coronary output. The coronary output accounted for 80% of the total oxygen consumption, as with the volume-loaded hearts.

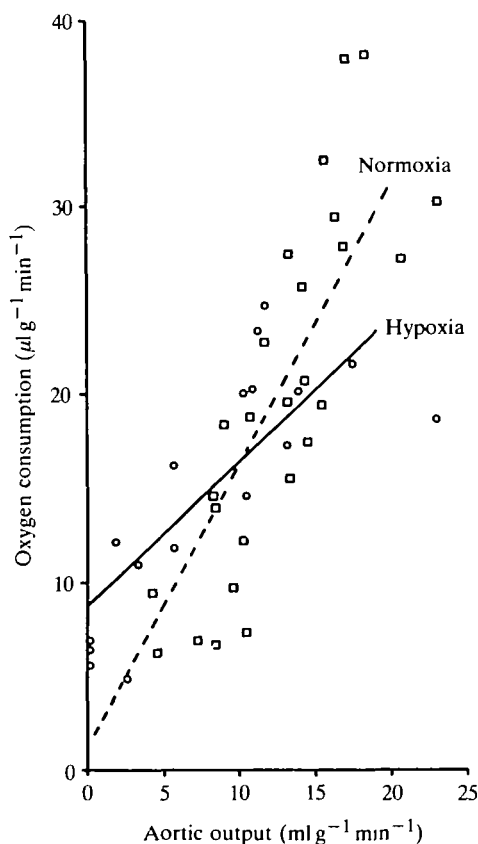


Fig. 5. The relationships between aortic output and total myocardial oxygen consumption (aortic output and coronary efflux) for hearts in normoxic (□) and hypoxic conditions (—, ○). The normoxic results are from volume-loaded hearts while the hypoxic results are from 20 cmH₂O input, 40 cmH₂O output pressures. For values of linear regressions, see text.

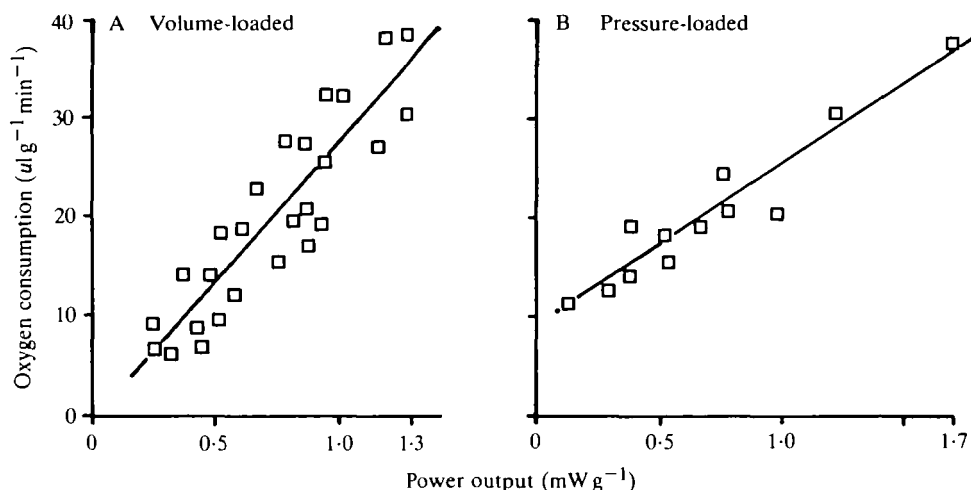


Fig. 6. The relationships between myocardial power output (from aortic and coronary outputs) and total myocardial oxygen consumption (aortic output and coronary output) for volume-loaded (A) and pressure-loaded (B) hearts. For values of linear regressions, see text.

Total oxygen consumption was linearly related to total power output (Fig. 6B), where total oxygen consumption = $16.34 \times \text{power} + 9.29$ ($r = 0.95$, $df = 10$, $P < 0.01$). This regression equation is significantly different in slope from that obtained with the volume-loaded hearts ($P < 0.01$) but not significantly different in elevation. Mechanical efficiency increased with pressure loading; across the range of power outputs efficiency improved from 2.7% to 13.7%.

Heart rate did not change significantly with pressure loading. Thus, when power output is increased with a constant O_2 delivery (aortic output and P_{O_2} constant) an increase in \dot{V}_{O_2} is achieved through improved coronary perfusion with little change in O_2 removal from the perfusate.

Progressive hypoxia

In the series of experiments on hypoxia the mean values for the cardiac variables of the normoxic hearts were not significantly different from those obtained in the previous experiments at input and output pressures of 20 and 40 cmH₂O, respectively. There was a significant decline in aortic output with hypoxia with a cessation of aortic output (but not heart rate) at 50 Torr (Fig. 8). Coronary output rose during hypoxia whereas aortic output fell. Plotting the aortic output and coronary output data for hypoxia below a perfusate P_{O_2} of 100 Torr gives a linear relationship where coronary output = $-0.10 \times \text{aortic output} + 11.99$ ($r = 0.51$, $df = 11$, $P < 0.05$) (Fig. 4). This line is significantly different from that obtained in the volume-loading experiments where coronary output fell as aortic output fell (Fig. 4).

Utilization of oxygen in the aortic output was the same as in the volume-loaded experiments and did not change significantly during hypoxia. The oxygen utilization in the coronary output decreased significantly ($P < 0.01$) at the lowest perfusate P_{O_2} .

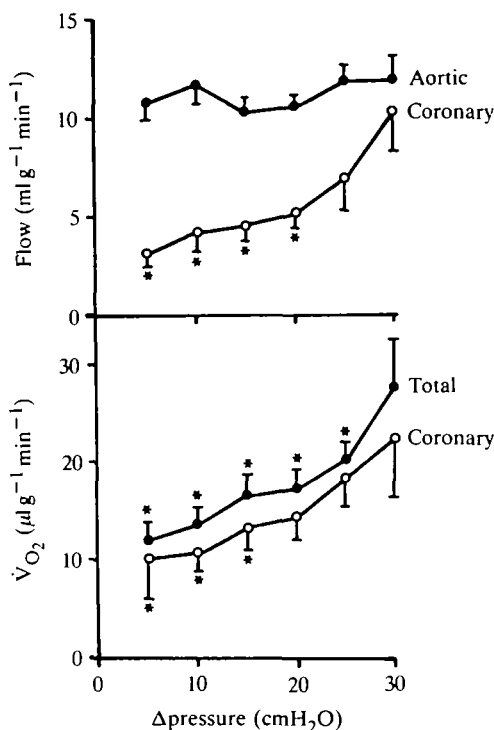


Fig. 7. Mean (\pm S.E.) aortic output and coronary output flow rates, total oxygen consumption (aortic output and coronary output) and coronary output oxygen consumption from five perfused hearts (mean mass of animals 624.55 ± 42.61 g). Input and output pressures were varied to keep the aortic output constant while varying the Δ pressure (output - input pressure). * denotes a statistically significant difference from the Δ pressure = 30 cmH₂O pressure results.

Total oxygen consumption declined linearly as aortic output fell (Fig. 5), where total oxygen consumption = $0.77 \times$ aortic output + 8.59 ($r = 0.81$, $df = 15$, $P < 0.01$). This regression equation is not significantly different from that obtained from the volume-loading experiments, but in this case the fall in aortic output is accompanied by an increase in coronary output. At 50 Torr, all the oxygen consumption was due to the flow through the coronary output. Combining all the data on total cardiac oxygen consumption and aortic output (i.e. from the volume-loading and hypoxia experiments, Fig. 5) gave the relationship: total oxygen consumption = $1.08 \times$ aortic output + 6.35 ($r = 0.79$, $df = 39$, $P < 0.001$).

Total power output and total oxygen consumption were linearly related, where total oxygen consumption = $24.5 \times$ power output - 8.42 ($r = 0.86$, $df = 13$, $P < 0.01$). The elevation of this line is significantly lower than that for the volume-loaded normoxic hearts ($P < 0.001$) (Fig. 6A) and for any given power output in hypoxia, oxygen consumption was approximately 50% less. Total power output in hypoxia is mainly due to the coronary output and we may be overestimating the pressure fall across the coronary vessels when the aortic pressure is used in these calculations.

Because of these low \dot{V}_{O_2} values, mechanical efficiency appears to be high in hypoxia (over 30%).

Heart rate was 32.36 ± 1.77 in normoxia and 29.80 ± 4.05 beats min^{-1} at 50 Torr, an insignificant decrease. Thus, the hypoxia-induced reduction in aortic output was brought about by a decrease in stroke volume until, at 50 Torr, there was no aortic output but only a coronary output.

Fig. 9 shows the mean results from five hearts in which after a progressive reduction in perfusate P_{O_2} to 70 Torr there was an almost complete cessation of the

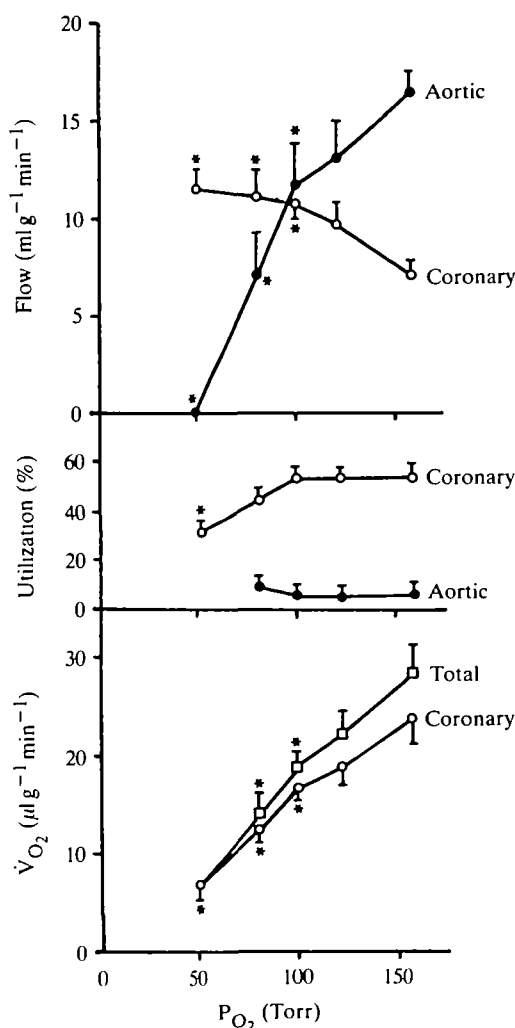


Fig. 8. The mean (\pm S.E.) effects of progressive hypoxia on aortic output, coronary output, percentage utilization, total oxygen consumption (aortic output and coronary output) and coronary output oxygen consumption of 11 perfused hearts (mean mass of animals 624.6 ± 42.0 g, mean mass of hearts 0.71 ± 0.04 g). Input pressure was $20 \text{ cmH}_2\text{O}$, aortic output pressure was $40 \text{ cmH}_2\text{O}$. * denotes a statistically significant difference from the normoxic control values.

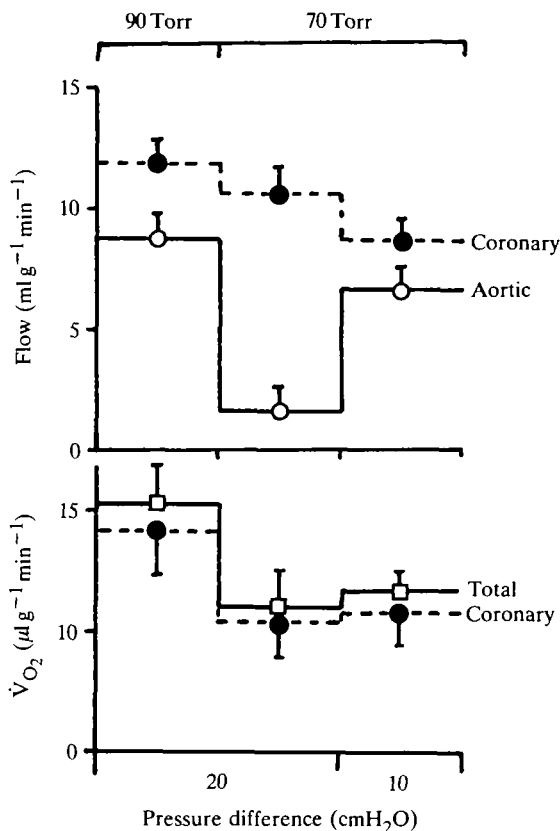


Fig. 9. The mean (\pm S.E.) effects of hypoxia and change in back pressure on aortic output, coronary output, total oxygen consumption (aortic output and coronary output) and coronary output oxygen consumption of five animals (mean mass of animals 595.0 ± 29.5 g, mean mass of hearts 0.65 ± 0.05 g). After lowering the input P_O₂ from 90 to 70 Torr, the aortic output pressure was lowered from 40 to 30 cmH₂O (input pressure was constant at 20 cmH₂O).

aortic output. The output pressure was then reduced from 40 to 30 cmH₂O (constant 20 cmH₂O input pressure). Aortic output immediately increased with an insignificant increase in total oxygen consumption. Calculation of the total power output showed an insignificant change. However, with the reduction in output pressure the power output to the aortic flow increased and that to the coronary flow decreased. Thus the work done by the heart had been transferred from pressure work to volume work.

Scaling of cardiac variables

Allometric equations describing the relationships between systemic heart mass, *in vitro* aortic output, *in vitro* coronary output and *in vitro* total myocardial oxygen consumption are given in Table 1. In each case the *in vitro* exponents are significantly less than one.

Total oxygen consumption of resting *Octopus cyanea* has been found to be proportional to mass^{0.833} (Maginnis & Wells, 1969). Testing the exponent for the total myocardial oxygen consumption against 0.833 revealed no significant difference.

DISCUSSION

Limitations of the in vitro heart preparation

The *in vitro* heart in the present experiments performed in a similar way to previous reports for *Octopus vulgaris* and *Eledone cirrhosa* hearts in terms of the responsiveness of the aortic output to input pressures (Smith, 1981; Foti *et al.* 1985). Heart rate has been found to be affected by input pressures when these ranged up to 40 cmH₂O (Smith, 1981; Foti *et al.* 1985); this was not observed in the present experiments, where a smaller range of input pressures was used. The calculated range of power output is above that for the *in vitro* heart of *Eledone cirrhosa* working at 10°C (Smith, 1985).

As in previous experiments with isolated octopod hearts, the heart continued to beat regularly for several hours after isolation (Smith, 1981; Foti *et al.* 1985). However, input pressures of at least 5 cmH₂O are needed to ensure regular contractions [as also found by Smith (1981) for *Eledone*], and in the present experiments the ventricle displayed maximum contractility at an input pressure of 20 cmH₂O. Because the heart showed a marked decline in performance after 40 min, it was necessary to start all experiments with the highest levels of performance. Clearly there are a number of problems with interpreting the performance of the *in vitro* heart in terms of the likely conditions *in vivo*.

First, the cardiac output *in vitro* is below that *in vivo*. *In vivo*, the cardiac output calculated from pre- and postbranchial blood gas measurements and allowing for cutaneous oxygen consumption is approximately 44 ml kg⁻¹ min⁻¹ for resting

Table 1. *The effects of body mass on heart mass, in vitro aortic output, in vitro coronary output and in vitro total myocardial oxygen consumption in relation to whole body mass (x in g) for Octopus vulgaris using the allometric equation $y = ax^b$*

Parameter	a	b	r	N
Heart mass (g)	0.0015	0.942	0.798	39
Aortic output (ml min ⁻¹)	0.060	0.817**	0.927	12
Coronary output (ml min ⁻¹)	0.171	0.542**	0.825	18
Total oxygen consumption (μl min ⁻¹)	0.113	0.763*	0.885	12

The *in vitro* values are taken from an input pressure of 20 cmH₂O and an output pressure of 40 cmH₂O.

Values of b = 1 in the regressions against mass indicate that the parameter is a constant fraction of mass, whereas values significantly different from one (indicated by an asterisk) indicate decreasing fractions of the body mass.

The number of animals (N) and the correlation coefficient (r) for each regression are given. In each case the correlation coefficient is significant.

*P < 0.01; **P < 0.001.

animals (Houlihan *et al.* 1986). As a 1-kg *Octopus* has a heart mass of approximately 1 g, this gives a cardiac output of $44 \text{ ml g}^{-1} \text{ min}^{-1}$ and a stroke volume of 0.6 ml beat^{-1} (Houlihan *et al.* 1986; Wells, 1979). *In vitro*, the minimum heart output, combining aortic output and coronary output, was $11.0 \text{ ml g}^{-1} \text{ min}^{-1}$ for a 765-g animal (Fig. 3) with a stroke volume of 0.4 ml beat^{-1} . The similarities in stroke volume between the *in vivo* and *in vitro* hearts are deceptive as the *in vitro* heart is only beating at 69 % of the *in vivo* rate (*in vivo* values from Wells, 1979). The total maximum cardiac output values for the *in vitro* heart ($24 \text{ ml g}^{-1} \text{ min}^{-1}$, stroke volume 0.8 ml beat^{-1}) are approximately 55 % of the resting *in vivo* values. However, it has been calculated that the cardiac output *in vivo* shows a 2.5-fold increase when the animals become active, mainly due to an increase in stroke volume (Wells, 1983; Houlihan *et al.* 1986). Thus, the maximum total cardiac output values of the *in vitro* heart as reported here and by Foti *et al.* (1985) are approximately 22 % of the likely maximum values *in vivo*. The *in vitro* heart could also show a 2.2-fold increase in total cardiac output but only from the lowered baseline values.

Second, the input pressures used here (5–20 cmH₂O) and in other *in vitro* experiments (Smith, 1981; 5–40 cmH₂O) are much higher than the likely pressures in the efferent branchial vessels returning blood to the heart (0.5–3.0 cmH₂O, Smith, 1985). One exception are the *in vivo* input pressures recorded for *Octopus dofleini* (10–20 cmH₂O; Johansen & Martin, 1962). Output pressures (25–40 cmH₂O) compare with aortic pressures of 35 cmH₂O (systole) and 15 cmH₂O (diastole) reported to be typical of *Octopus vulgaris* at rest (Wells, 1983). Following exercise, the pressures may rise to between two- and three-fold above those recorded at rest (Wells, 1983).

Third, we have no data on the pressures in the coronary veins *in vivo* or the likely extent of the coronary flow. Wells & Wells (1986) have reported that 70 % of the total systemic flow passes down the aorta to the head and arms leaving the rest for the gonad, mantle, gut and coronary circulation. Therefore, it seems likely that the coronary flows reported here are higher than those *in vivo*, possibly due to the necessity for cutting the coronary veins, the absence of any venous back pressure and the lower oxygen content of the perfusion fluid compared with blood; the perfusate only delivers 0.22 vol% with a 50-Torr fall in P_{O_2} whereas the blood under similar conditions would deliver 2.8 vol%. Nevertheless, the experiments using increased coronary output pressures indicated that coronary flow was rather insensitive to back pressure *in vitro*.

Fourth, the relatively poor performance of the *in vitro* heart in terms of total cardiac output is also evident in its susceptibility to changes in P_{O_2} . *In vivo* the heart receives arterial blood with a P_{O_2} of 78 Torr (Houlihan *et al.* 1982) but *in vitro* aortic output was reduced when the perfusate P_{O_2} fell below 120 Torr (Fig. 8). Also, *in vivo* the P_{O_2} of the arterial blood may fall below 30 Torr during environmental hypoxia (Houlihan *et al.* 1982; Smith *et al.* 1985), but *in vitro* at a perfusate P_{O_2} of 10 Torr the heart beat generated no aortic output and only a coronary output. It is likely that the reduced oxygen content of the perfusate compared with that of blood is

responsible for the poor performance in hypoxia. It is possible that the heart has some anaerobic capacity (Pritchard, Huston & Martin, 1963; Driedzic, 1985), but we have no information on anaerobic metabolism *in vitro*. Also, *in vivo* the heart will be subject to a range of neurotransmitters and circulating cardioactive substances (Wells, 1983) which will presumably be absent *in vitro*.

Aortic output and coronary output in the in vitro heart

This is the first report of the myocardial oxygen consumption of an intact cephalopod heart. Based on the present observations, maximum myocardial oxygen consumption is $15 \mu\text{l O}_2 \text{ min}^{-1}$ for a 600-g *Octopus* with a 0.6-g ventricle. This represents approximately 3% of the total oxygen consumption of the resting animal for a tissue that occupies 0.1% of the total body mass. Clearly, as cardiac output and cardiac oxygen consumption scale in proportion to total oxygen consumption (Table 1) comparisons must be made for defined sizes of animal. Allowing for these scaling relationships, the rates of oxygen consumption, power output and mechanical efficiency of the *in vitro* *Octopus* heart are comparable with values for the isolated teleost heart (Driedzic, Scott & Farrell, 1983), but are slightly lower than the values from an *in situ* teleost heart preparation (Farrell *et al.* 1985). In both the teleost and *Octopus* heart, oxygen consumption is linearly correlated with power output, and in both groups pressure work and volume work have the same metabolic costs.

With a cardiac output of $44 \text{ ml kg}^{-1} \text{ min}^{-1}$ and a blood oxygen content of 3 vol% (Houlihan *et al.* 1982) the oxygen consumption of the heart would make a 1% difference to the oxygen content of the arterial blood as it passed through the heart. As this is not likely to be significant, it seems probable that the value of the coronary circulation is in overcoming the problem of oxygen diffusion through the thick ventricular muscle.

The coronary circulation of the *Octopus* heart accomplishes the important task of perfusing the organ which generates the driving pressure for the systemic circulation. The *Octopus* arrangement of the coronary perfusion provides oxygenated blood from the gills which flows directly from the ventricle through the walls of the heart. This is in contrast to the arrangement in fish, where the inner spongy myocardium derives oxygen from the venous blood pumped through the ventricular chamber, and the outer compact layer of the ventricle, where it is present (Santer & Greer Walker, 1980), receives a coronary supply from arterial blood. Blood flow through the coronary vessels of *Octopus* must be determined by the driving pressure and the resistance of the coronary vascular bed. The driving pressure across the bed is considered to be the difference between the intraventricular pressure and the downstream pressure of the coronary veins. *In vitro* it appears that the coronary flow is rather insensitive to the output back pressures. The coronary resistance will depend upon the number, size and calibre of the coronary vessels.

Interestingly, the coronary perfusion only seemed to occur during systole; For *et al.* (1985) have estimated that the diastolic efflux was only 3% of the systolic value.

In the mammalian heart there is a systolic obstruction of ventricular coronary blood flow and only 16–25 % of the stroke flow enters the coronary vasculature in systole. As diastole becomes abbreviated during exercise, the proportion of the flow occurring during systole increases to 32–50 % (Barnard, Duncan, Livesay & Buckberg, 1977; Sanders, White, Peterson & Bloor, 1978). In mammals and fish, the arterial blood pressure is the main driving force for coronary perfusion and coronary resistance is the main regulator of cardiac blood flow. However, in *Octopus* it appears that the critical opening pressure for the coronary vessels is only reached during systole.

The *in vitro* heart of *Octopus* demonstrated remarkable abilities in the control of the coronary circulation. The coronary output was found to vary in three different ways with respect to aortic output in the present experiments, and any explanation of how the aortic output is varying must also take into account the coronary output. The aortic output and coronary output may be modelled as two sets of resistances in parallel. First, volume-loaded hearts are filled to varying amounts by the end diastolic pressure under the influence of input pressure. The increased input pressure increases systolic pressure as aortic output is increased. Thus coronary output can increase with both output resistances remaining constant, while there is constant oxygen utilization and increased oxygen delivery to the cardiac muscle. Thus, the degree of contraction of the cardiac muscle under the influence of volume loading appears to supply the cardiac muscle automatically with the appropriate level of coronary perfusion.

During pressure loading at constant aortic output, increased systolic pressure produces increased coronary flow (Fig. 7), with the resistance of the coronary circuit presumably remaining constant. Thus, the increased power output of the heart due to pressure loading results in increased coronary flow, and with constant oxygen utilization, increased oxygen supply to the heart. Overall, the power output/oxygen consumption relationship is very similar in the volume-loaded and pressure-loaded hearts.

During hypoxia with constant input and output pressures, aortic output, power output and, presumably, systolic pressure declined, but we must postulate a fall in coronary output resistance to account for the increased coronary perfusion. Coronary output remained almost constant as aortic output fell below a perfusion P_{O_2} of 100 Torr, implying that the coronary vessels were almost maximally open at these P_{O_2} values. This ensured the best possible oxygen supply to the hypoxic cardiac muscle.

How coronary output is regulated *in vitro* is not clear. In mammals, hypoxia is the most potent stimulator of a reduction in coronary resistance and it is likely that the metabolite adenosine is responsible for this (Berne & Rubio, 1969). As we have demonstrated that the coronary output is increased during hypoxia, presumably through dilation of the coronary vessels, it is possible that adenosine formed from the γ -nucleotidase found in *Octopus* heart (Sciurba, Agnisola, Foti & Trara Genoino, 1985) is also involved in the cardiac muscle's response to hypoxia.

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REFERENCES

- BALLANTYNE, J. S., HOCHACHKA, P. W. & MOMMSEN, T. P. (1981). Studies on the metabolism of the migratory squid, *Loligo opalescens*: enzymes of tissues and heart mitochondria. *Mar. biol. Letters* **2**, 75–85.
- BARNARD, R. J., DUNCAN, H. W., LIVESAY, J. J. & BUCKBERG, G. D. (1977). Coronary vasodilator reserve and flow distribution during near-maximal exercise in dogs. *J. appl. Physiol.* **43**, 988–992.
- BARRON, E. S. G., SIGHTS, W. P. & WILDER, V. (1953). The carbohydrate metabolism of heart slices. *Arch. exp. Path. Pharmacol.* **219**, 338–348.
- BERNE, R. M. & RUBIO, R. (1969). Acute coronary occlusion: Early changes that induce coronary dilation and the development of collateral circulation. *Am. J. Cardiol.* **24**, 776–781.
- DRIEDZIC, W. R. (1985). Contractile performance of cephalopod hearts under anoxic conditions. *J. exp. Biol.* **117**, 471–474.
- DRIEDZIC, W. R., SCOTT, D. L. & FARRELL, A. P. (1983). Aerobic and anaerobic contributions to energy metabolism in perfused isolated sea raven (*Hemiteuthis americana*) hearts. *Can. J. Zool.* **61**, 1880–1883.
- FARRELL, A. P., WOOD, S., HART, T. & DRIEDZIC, W. R. (1985). Myocardial oxygen consumption in the sea raven, *Hemiteuthis americana*: the effects of volume loading, pressure loading and progressive hypoxia. *J. exp. Biol.* **117**, 237–250.
- FOTI, L., TRARA GENOINO, I. & AGNISOLA, C. (1985). *In vitro* cardiac performance in *Octopus vulgaris* (Lam.). *Comp. Biochem. Physiol.* **82C**, 483–488.
- GHIRETTI-MAGALDI, A., GUIDITTA, A. & GHIRETTI, F. (1958). Pathways of terminal respiration in marine invertebrates. *J. cell. comp. Physiol.* **52**, 389–429.
- HOULIHAN, D. F., DUTHIE, G., SMITH, P. J., WELLS, M. J. & WELLS, J. (1986). Ventilation and circulation during exercise in *Octopus vulgaris*. *J. comp. Physiol.* **156**, 683–689.
- HOULIHAN, D. F., INNES, A. J., WELLS, M. J. & WELLS, J. (1982). Oxygen consumption and blood gases of *Octopus vulgaris* in hypoxic conditions. *J. comp. Physiol.* **148**, 35–40.
- JOHANSEN, K. & MARTIN, A. W. (1962). Circulation in the cephalopod *Octopus dofleini*. *Comp. Biochem. Physiol.* **5**, 161–176.
- MAGINNIS, L. A. & WELLS, M. J. (1969). The oxygen consumption of *Octopus cyanea*. *J. exp. Biol.* **51**, 607–613.
- MOMMSEN, T. P. & HOCHACHKA, P. W. (1981). Respiratory and enzymatic properties of squid heart mitochondria. *Eur. J. Biochem.* **120**, 345–350.
- PRITCHARD, A. W., HUSTON, M. J. & MARTIN, A. W. (1963). Effects of *in vitro* anoxia on metabolism on octopus heart tissue. *Proc. Soc. exp. Biol. Med.* **112**, 27–29.
- SANDERS, M., WHITE, F. C., PEDERSON, T. M. & BLOOR, C. M. (1978). Characteristics of coronary blood flow and transmural distribution in miniature pigs. *Am. J. Physiol.* **235**, H601–H609.
- SANTER, R. M. & GREER WALKER, M. (1980). Morphological studies on the ventricle of teleost and elasmobranch hearts. *J. Zool., Lond.* **190**, 259–272.
- SCIURBA, M., AGNISOLA, C., FOTI, L. & TRARA GENOINO, I. (1985). Evidence for 5'-nucleotidase activity in the heart of *Octopus vulgaris* Lam. *Comp. Biochem. Physiol.* **80B**, 67–72.
- SMITH, P. J. S. (1981). The role of venous pressure in regulation of output from the heart of the octopus, *Eledone cirrhosa* (Lam.). *J. exp. Biol.* **93**, 243–255.
- SMITH, P. J. S. (1985). Molluscan circulation: Haemodynamics and the heart. In *Circulation, Respiration and Metabolism* (ed. R. Gilles), pp. 344–355. Berlin: Springer-Verlag.
- SMITH, P. J. S. & BOYLE, P. R. (1983). The cardiac innervation of *Eledone cirrhosa* (Lamarck) (Mollusca: Cephalopoda). *Phil. Trans. R. Soc. Ser. B* **300**, 493–511.

- SMITH, P. J. S., DUTHIE, G. G., WELLS, M. J. & HOULIHAN, D. F. (1985). Continuous recording of arterial blood P_{O_2} in *Octopus vulgaris* during progressive hypoxia and movement. *J. exp. Biol.* **117**, 475–479.
- STRICKLAND, J. D. H. & PARSONS, T. R. (1972). *A Practical Handbook of Seawater Analysis*. Bull. Fish. Res. Bd Can. **167**, 310pp.
- WELLS, M. J. (1979). The heartbeat of *Octopus vulgaris*. *J. exp. Biol.* **78**, 87–104.
- WELLS, M. J. (1983). Circulation in cephalopods. In *The Mollusca*, vol. 5, part 2 (ed. A. S. M. Saleuddin & K. M. Wilbur), pp. 239–290. New York: Academic Press.
- WELLS, M. J. & WELLS, J. (1986). Blood flow in acute hypoxia in a cephalopod. *J. exp. Biol.* **122**, 345–353.