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THE ROLE OF VENOUS PRESSURE IN REGULATION OF OUTPUT FROM THE HEART OF THE OCTOPUS, *ELEDONE CIRRHOSA* (LAM.)

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SUMMARY

1. The influence of perfusion pressure on stroke volume and heart rate was examined in the isolated ventricle. Input pressure, within the physiological range (10–20 cm of water), had a direct effect upon stroke volume and heart rate. Output back pressure had an inverse effect upon stroke volume and no effect upon heart rate.

2. Sites that could vary input pressure were investigated by selective denervation in the whole animal. The results indicated that the efferent branchial vessel and auricle may be involved, as well as the branchial hearts and lateral venae cavae.

3. It is proposed that the pressure of venous blood has a limited effect upon ventricular output *in vivo*.

INTRODUCTION

The pressure generated by the ventricle of cephalopods is higher than in other molluscs, and is similar to those found in the lower vertebrates (Packard, 1972). The pressure of the blood returning to the ventricle from the gill vascular bed is also higher in the octopods (Johansen & Martin, 1962) than those reported from other molluscs at equivalent sites (for example Jones, 1971; Bourne & Redmond, 1977).

The pressure of the blood returning to the ventricle is the result of the contractions of organs and vessels upstream of the ventricle, in particular the lateral venae cavae and the branchial hearts (Johansen & Martin, 1962). It is not clear whether the gills and auricles are also involved.

The molluscan ventricle generally shows a contraction rate dependent upon the pressure of blood in the auricle (reviewed by Hill & Welsh, 1966). The ventricle of *Octopus vulgaris* has been shown by Fredericq (1914) to have a contraction rate that is directly related to the input pressure in the isolated preparation, although study of free-swimming *Octopus dofleini* (Johansen & Martin, 1962) indicates that the pressures employed by Fredericq were higher than those present in the auricles. An influence of vascular haemodynamics upon the ventricular performance is indicated by the experiments of Wells (1980). The present study compares the effect of input pressure

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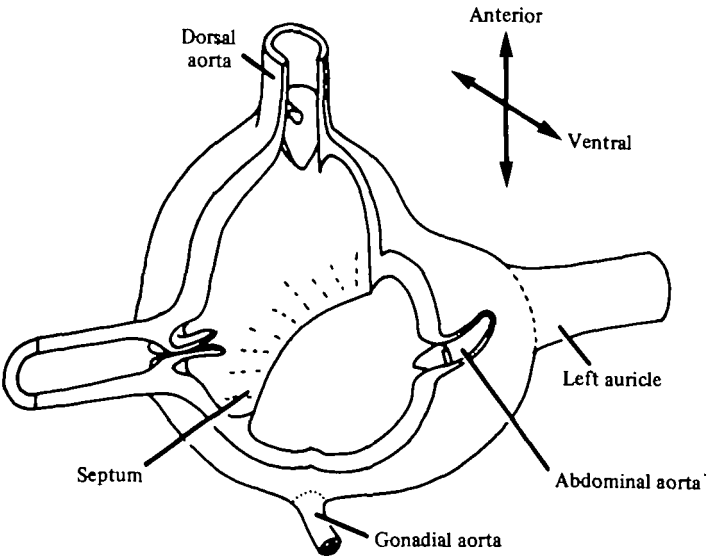

Fig. 1. Diagrammatic representation of the ventricle and associated vessels. A section of the ventral surface is cut away to show the partial septum and the valves at the junctions with the auricles and the aortae. The direction of blood flow is regulated by these valves.

and output back pressure upon the ventricular output, and examines the sites of generation of input pressure.

MATERIALS AND METHODS

For the recording from the isolated ventricle, specimens of the Lesser or Northern Octopus, *Eledone cirrhosa* (Lam.) were acquired locally from fishing grounds within 60 miles of Aberdeen and maintained in a recirculating aquarium at the Department of Zoology, University of Aberdeen. Ventricles were taken from healthy female animals weighing between 700 and 1000 g.

To isolate the ventricles, the animals were anaesthetized by 3% ethanol in sea water. The central nervous system was then surgically destroyed and the ventricle dissected out. The right auricle was cannulated with an L-shaped glass tube of internal diameter 5 mm. The left auricle was ligatured as close to the auriculo-ventricular junction as possible. Three aortae leave the ventricle (Fig. 1). The two minor aortae, the abdominal and gonadial, were ligatured at their base and the dorsal aorta cannulated. The cannula (Portex tubing, softness 45, 2 mm i.d.) was tied as close to the ventricular junction as possible without obstructing the action of the aortic valve. The ventricle was then installed in the perfusion apparatus (Fig. 2) which contained 20 l of sea water at 10 °C, circulated through an aeration column. Input pressure and output back pressure were controlled by the heights of reservoirs (Fig. 2). The perfusion pressures used in these experiments cover the physiological range (10–25 cm of water) set by *in vivo* work on *Octopus dofleini* (Johansen & Martin, 1962).

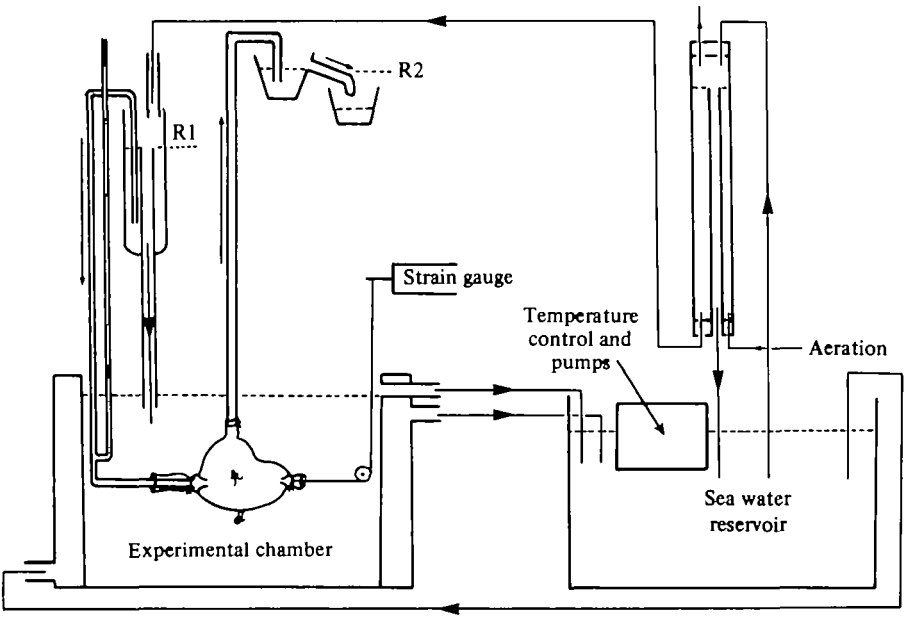

Fig. 2. Diagram of the experimental apparatus used to maintain and perfuse physiological preparations. The input pressure to the isolated ventricle was set by adjusting the height of reservoir R_1. The output back pressure was set by the height of the reservoir R_2. The arrows indicate the direction of flow. The component parts of this set-up are not drawn to scale.

Output from the preparation was collected, weighed, corrected for temperature and salinity and expressed as a volume measurement. By recording the number of ventricular contractions during the collection period a value for the mean stroke volume was estimated.

Heart rate was measured using either displacements of a CFP wire strain gauge, tied to the ligated auricle, or by monitoring the ventricular cardiogram via two trimel-coated stainless steel electrodes (0·006 in dia.) implanted in the myocardium. The cardiogram was amplified using a CFP 121 preamplifier. Permanent records were made on a Devices Pen Recorder.

For the recording of cardiograms from free-moving preparations, two species were used, *Octopus vulgaris* Cuvier and *Eledone cirrhosa* (Lam.). The work was carried out at the Department of Zoology, University of Aberdeen and at the Laboratoire Arago, Banyuls-sur-Mer, France. Animals were anaesthetized in 2% ethanol in sea water. The ventral septum was cut and the mantle inverted. The exposed cut edges of the median pallial adductor muscles were sewn together. Care was taken to ligature the septal artery. Two trimel-coated stainless steel electrodes, with the insulation stripped from the tips, were inserted through the renal sacs into the ventricular myocardium. This was facilitated by passing the electrodes through syringe needles and hooking their ends over the points. Withdrawal of the needles left the electrodes firmly implanted in the ventricular muscle. The electrode wire was then sewn through the dorsal surface of the mantle and connected to an Epil 111 A preamplifier. The interval between successive cardiograms was measured from permanent records, made on a

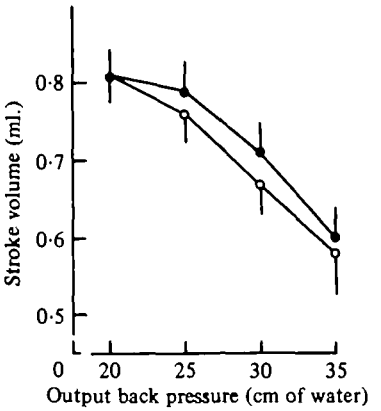

Fig. 3. The mean stroke volume response from four isolated ventricles in response to the variation of the output back pressure. The input pressure was held constant at a level of 20 cm of water. The stroke volume values for both descending values (●) and the subsequent ascending values (○) of the output back pressure are presented. Bars represent standard deviation. Stroke volume varies inversely with the value of the output back pressure and the difference between the input pressure and the output back pressure.

Washington 400 MD2R recorder fitted with a d.c. input coupler. Cardiogram intervals were measured at the same time relative to recovery from anaesthesia, in all control and experimental preparations.

RESULTS

The octopod ventricle showed only localized contractions, along the ventral midline between the auricles, when isolated from the body. If briefly touched the ventricle gave a single strong contraction. When perfused as described (Materials and Methods) and therefore stretched by the internal hydrostatic pressure, the ventricle contracted strongly and regularly for up to 12 h. Activity could continue for 36 h.

Effect of perfusion pressure on stroke volume

(*a*) In the first series of experiments the input pressure was held constant at 20 cm of water. The level of the output pressure was dropped in steps of 5 cm of water, from 35 to 20 cm, and then returned by 5 cm increments. Each run was completed within 40 min. Any alteration in the output back pressure caused an immediate and constant change in stroke volume. The ventricles studied all responded in the same manner, with the stroke volume being inversely related to the output back pressure and the difference between the input and output back pressure. Fig. 3 plots the mean stroke volume measurement for four preparations at the same pressure levels. Standard deviations shown in the figure indicate how stroke volume varied considerably between individuals. The size range of the experimental animals was too restricted to establish the relationship between size and stroke volume. The mean stroke volume measurements were lower upon the return of the output back pressure to the original level.

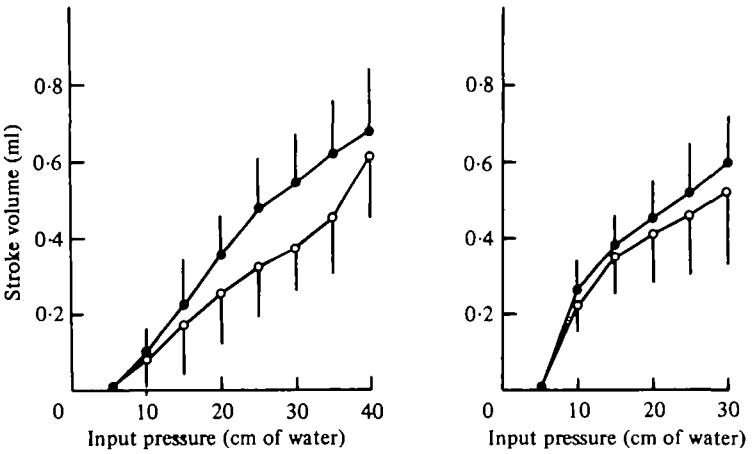

Fig. 4. The mean stroke volume response from four isolated ventricles in response to the variation of the input pressure, at a constant output back pressure of 40 cm of water. The stroke volume values for both descending values (●) and the subsequent ascending values (○) of the input pressures are given. Bars represent standard deviation. Stroke volume varies directly with the value of the input pressure and inversely with the difference between input and output back pressure.

Fig. 5. As in Fig. 4 but with the starting value of the input pressure and output back pressure being 30 cm of water. Mean values and standard deviations are from three preparations.

(*b*) In the second series of experiments the output back pressure was held constant at either 40 or 30 cm of water. The level of the input pressure was dropped in steps of 5 cm of water, from either 30 or 40 cm to 5 cm, and then returned to the original level by 5 cm increments. Each run was completed within 50 min. The input pressure was not taken below 5 cm of water as at these levels the ventricle frequently ceased contracting. Often it would then take an input pressure in excess of 5 cm of water to establish a regular rate of contraction.

With either 40 cm of water back pressure (Fig. 4) or 30 cm (Fig. 5), stroke volume changed directly with the value of the input pressure and inversely with the difference between the input and output back pressures. Mean values obtained with ascending input pressures were lower than the mean values measured at descending pressure levels.

(*c*) The above changes in stroke volume could have been caused either by the alteration of the absolute hydrostatic pressure at the end of diastole, or by the change in the difference between the input and the output back pressure. In two preparations (Fig. 6*a*, *b*) this was investigated by maintaining a constant difference between the input pressure and the output back pressure while the absolute hydrostatic pressures were varied. With pressures between 10 and 20 cm of water, stroke volume clearly depended upon perfusion pressure, both when output pressure was 5 cm of water higher than input pressure (○), and when at the same pressure (●). At higher perfusion pressures, stroke volume was not affected by the absolute level, and was presumably dependent on the difference between input and output back pressures.

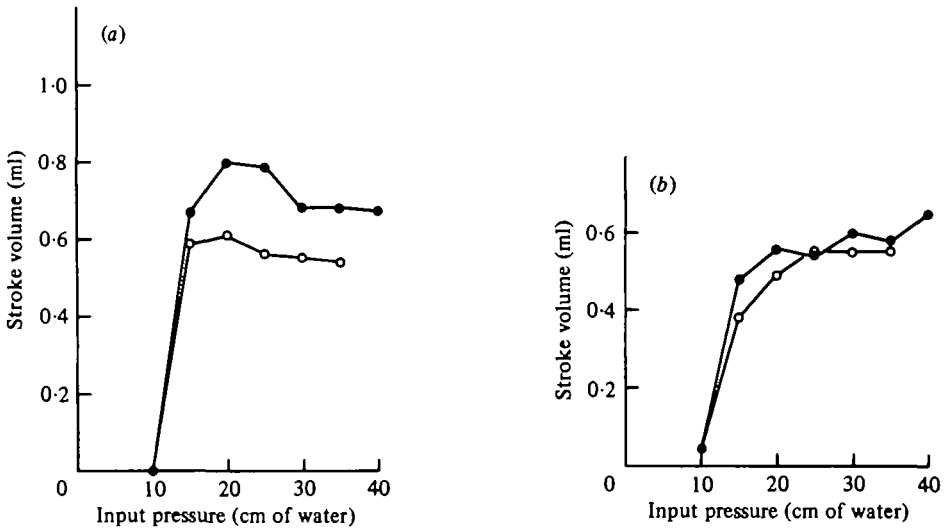

Fig. 6. Stroke volumes from two preparations (*a* and *b*) observed with descending input and output back pressure, with a constant difference between these pressures. Output back pressure was either 5 cm water higher than input pressure (○) or at the same pressure (●). In both *a* and *b* the stroke volume values show a rapid rise between the pressure levels of 10 and 15 cm of water, levelling off at higher values.

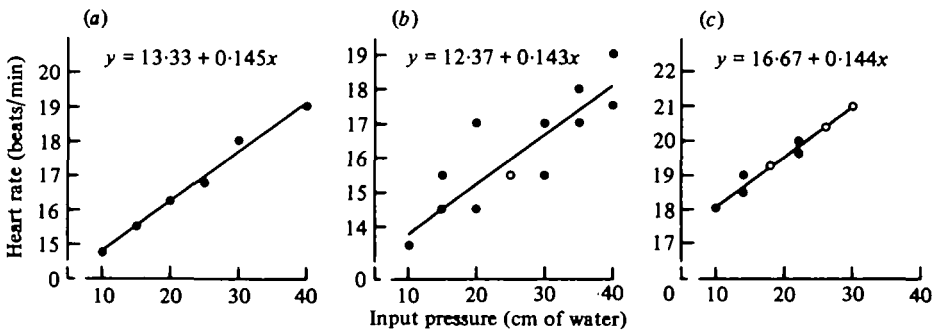

Fig. 7. Changes in the isolated ventricular heart rate in response to the alteration of the input pressure. In all three cases the output back pressure was constant (*a* and *b*, 40 cm, *c*, 30 cm of water). In all three preparations the heart rate varied directly with the level of the input pressure ($P < 0{\cdot}01$) and there was no significant difference in the rate of change between the preparations (see text). The open circles represent overlapping values.

Effect of perfusion pressure on heart rate

Input pressure had a direct, linear effect upon heart rate (Fig. 7*a–c*). Analysis of covariance showed no significant difference between the slopes of the relationships for the preparations tested (regression 7*a vs b*, $P < 0{\cdot}05$; 7*a vs c*, $P < 0{\cdot}01$; 7*b vs c*, $P < 0{\cdot}01$).

In three further preparations, heart rate showed no apparent relationship with changes in output back pressure (Fig. 8*a–c*). The results presented in Fig. 7 are therefore due solely to the alteration of the input pressure level.

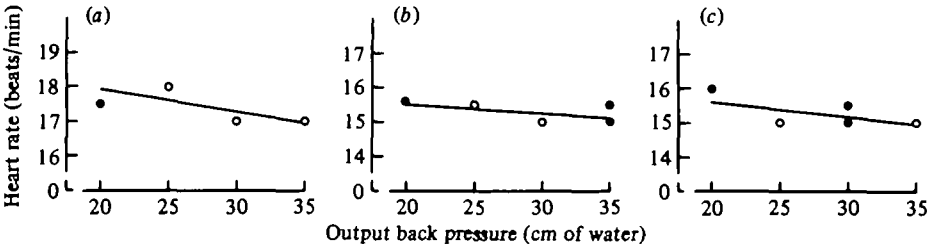

Fig. 8. The response of heart rate to the variation in the output back pressure at a constant input pressure level of 20 cm of water. In all three cases there was no significant relationship between heart rate and the level of the output back pressure. The open circles represent overlapping values.

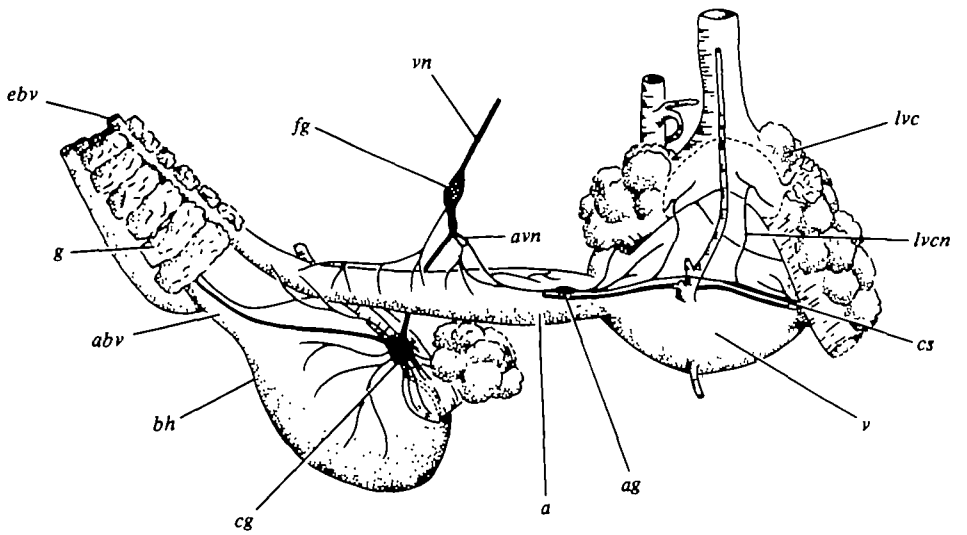

Fig. 9. The nerve supply to the hearts. A semi-diagrammatic illustration from a ventral dissection showing the generalized pattern of innervation for *Eledone cirrhosa* (Lam.). Only the right side is illustrated for, with the exception of the auricular ganglion, the innervation is bisymmetrical. Sections of the lateral venae cavae are omitted, as is the anterior tip of the gill. Innervation from the fusiform ganglion to the gonoduct, renal papillae and renal sac membrane are also omitted.

Legend: *a*, auricle; *abv*, afferent branchial vessel; *ag*, auricular ganglion; *avn*, auriculoventricular nerve; *bh*, branchial heart; *cg*, cardiac ganglion; *cs*, commissural strands; *ebv*, efferent branchial vessel; *fg*, fusiform ganglion; *g*, gill; *lvc*, lateral vena cava; *lvcn*, nerve to the lateral vena cava; *v*, ventricle; *vn*, visceral nerve.

Effect of nerve lesions on the ventricular inter-cardiogram interval

From the results of the previous experiments it is apparent that the pressure of blood filling the ventricle can affect the rate of contraction. The contribution of different organs and vessels to the generation of this input pressure was investigated by selective denervation. Ventricular performance was monitored by measurements of the inter-cardiogram interval, at least 1 h after recovery from anaesthesia.

The innervation to the main components of the circulatory system was found to be more complex (Fig. 9) than suggested by the published material (reviewed by Young,

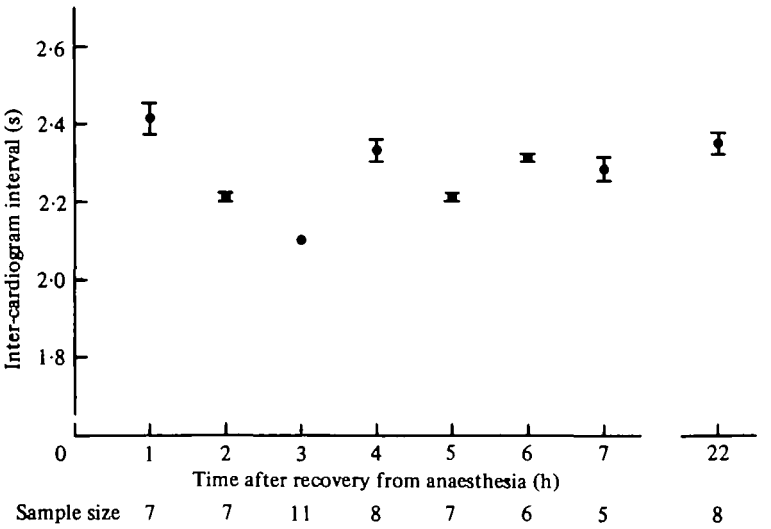

Fig. 10. *In vivo* ventricular activity from an intact free-moving *Eledone cirrhosa*. The mean inter-cardiogram interval and the standard deviation of each sample are plotted against the time of sampling after recovery from anaesthesia. Within each sampling period there is little variation in the interval between successive cardiograms.

1971). Note in particular that the lateral venae cavae receive nerve bundles from auricular and ventricular nerves at the level of the ventricle, that a small ganglion can be found in the auricle and that the efferent branchial vessel receives innervation from the ipsilateral cardiac ganglion. The cardiac ganglion also innervates the pallial vein, the valve region of the lateral vena cava and the branchial heart itself. The auricular ganglion was not located in every preparation examined and never on both ventricular nerves in the same animal.

In intact animals the ventricular contractile rate was very stable within a sampling period. In six preparations, the standard deviation about the mean was less than 0·1 s. An example of the intact condition is given in Fig. 10. The smaller sample sizes in this figure were taken from the same period as in the subsequent denervated preparations. The limited variation about the mean interval in the intact animals indicates that any extreme variation in heartbeat interval cannot therefore be attributed to the operative procedures required to implant the recording electrode. Nor could any variation be attributed to stress or incidental damage on sectioning of the peripheral nervous system, as cutting the visceral nerves ($n = 3$) or the ablation of the fusiform ganglia ($n = 3$) caused no apparent increase in the standard deviation of one sampling period, when compared to the intact condition. The standard deviation was less than 0·1 s for both operations.

Ablation of one or both cardiac ganglia ($n = 5$) produced a quite different response. In such cases there was a considerable deviation about the mean value of the inter-cardiogram interval for any one sampling period (Fig. 11). The effect of such an operation is however complicated by the number of sites innervated from the cardiac ganglion. For example, sectioning of the innervation from the cardiac ganglion to th

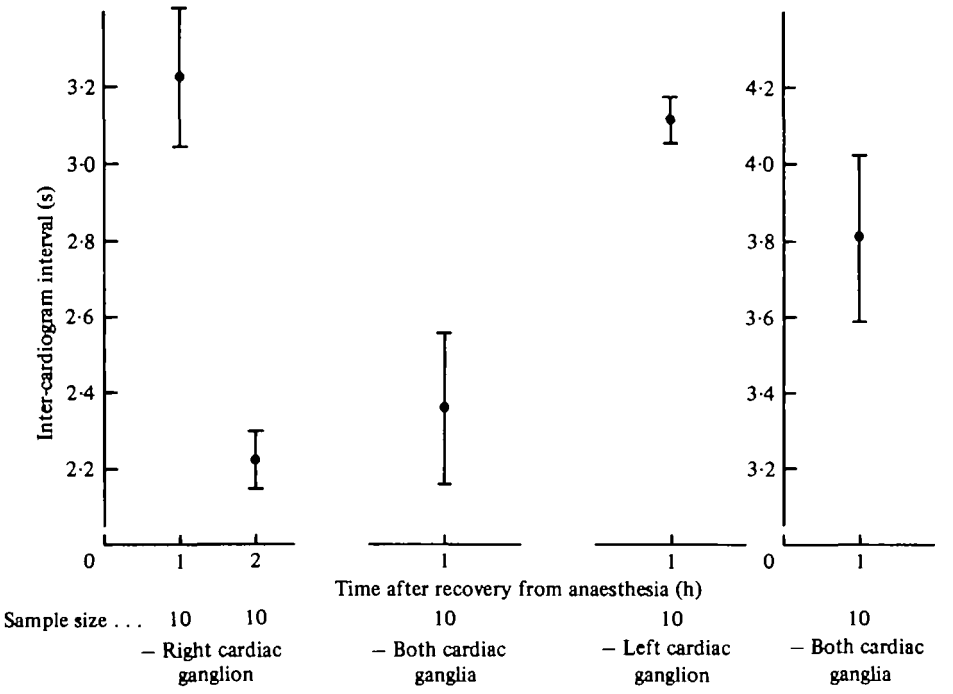

Fig. 11. As in Fig. 10, but for four preparations of *Eledone cirrhosa* with either one or both cardiac ganglia ablated. The deviation about the mean cardiogram interval for each sampling period is greater when compared to the intact condition.

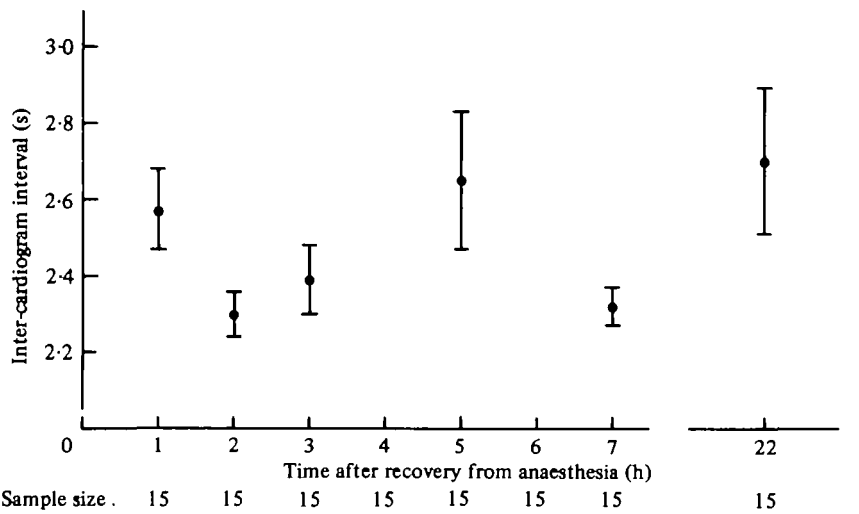

Fig. 12. As in Fig. 10 but with the nerve running between the cardiac ganglion and the efferent branchial vessel sectioned on one side only. As with the ablation of the cardiac ganglia this operation results in an increase in the deviation about the mean for the sampling period when compared to the intact condition.

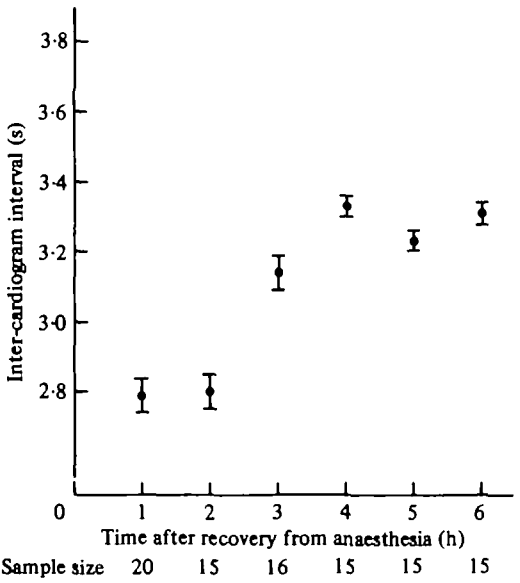

Fig. 13.

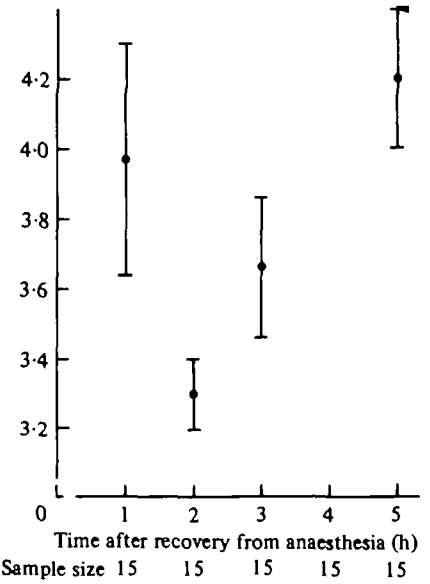

Fig. 14.

Fig. 13. *In vivo* control operation for the sectioning of the innervation to the lateral venae cavae. In this the inter-cardiogram intervals were measured from an *Eledone cirrhosa* after the renal sacs had been opened and the venae cavae mechanically disturbed. As in Fig. 10, the mean inter-cardiogram interval is presented for each sampling period along with the standard deviation for the sample. The standard deviations are slightly greater than was the case in the intact preparation.

Fig. 14. Variation in the inter-cardiogram intervals for four sampling periods after the sectioning of the innervation to the lateral venae cavae. This experiment was conducted on the same animal as the control operation presented in Fig. 13. After sectioning, the standard deviation about the mean inter-cardiogram interval for each sampling period is greatly increased compared to the control operation. (The acceleration shown after nerve sectioning in this preparation was not seen with the other two preparations upon which this experiment was performed.)

efferent branchial vessel also caused considerable fluctuation in the intervals sampled (Fig. 12).

Sectioning of the nerves to the lateral venae cavae also caused extreme variation in the inter-cardiogram interval ($n = 3$). To section these nerves it was necessary to open the renal sacs over the ventral surface of the ventricle. This operation involved the unavoidable loss of renal fluid and the possibility of anaesthesia entering the renal sacs. Both these problems were kept to a minimum, but a separate control operation was also run.

In the control operation the renal sacs were opened at the ventricular midline to allow access of a scalpel. The lateral venae cavae were then mechanically disturbed and the sac membranes sewn up. The standard deviation about the mean interval was slightly increased by this operation (Fig. 13) when compared to the intact condition. Subsequent sectioning of the nerves to the lateral venae cavae, as well as the required operative procedures, caused a considerable increase in the standard deviation about the mean (Fig. 14) when compared to the separate control experiment.

DISCUSSION

The isolated molluscan ventricle beats more effectively when stretched by internal pressure (reviewed by Hill & Welsh, 1966). The results of this study indicate that the normal rhythm and pumping action of the isolated octopod ventricle is dependent on an internal diastolic pressure more than 2–5 cm of water above ambient. This agrees with the measurements of Skramlik (1941), but is at odds with the view of Carlson (1906), who maintained that contractions would occur in the unstretched octopod ventricle.

The rate at which the molluscan heart will beat also appears to depend upon the internal pressure and therefore the degree to which the myocardium is stretched at the end of diastole. The lower the pressure the slower the heart rate. The present results show that the isolated octopod ventricle varies the contraction rate with input pressure in the range 5–40 cm of water. This range includes that to be expected in the intact animal (10–25 cm of water systolic; Johansen & Martin, 1962).

If the resting heart rate is governed primarily by the filling pressure *in vivo* as well as *in vitro*, then this would help to explain the rather steady ventricular contraction rates recorded in free-moving animals (Wells, 1979). *In vitro* alteration of the input pressure in the physiological range (10–25 cm of water; Johansen & Martin, 1962) caused only small changes in the contraction frequency.

In this study and that of Wells (1980), ablation of the fusiform ganglia had no effect upon the *in vivo* resting ventricular contraction rate or the systolic pressure. After such an operation it should be noted however that the ventricle may not be totally devoid of a neural influence because of the presence of the auricular ganglion (Fig. 9) and putative nerve cell bodies in the ventricular epicardium (Smith, 1979). Ablation of the cardiac ganglia and sectioning of the innervation to the lateral venae cavae disrupt contraction of the ventricle, as would be expected from the contractile role of the branchial hearts and lateral venae cavae (Johansen & Martin, 1962; Smith, 1979).

The observation that the sectioning of the small nerves to the efferent branchial vessel can disrupt ventricular contraction indicates that the efferent branchial vessel and auricle might be playing a more active role in the filling of the ventricle than has been proposed (Johansen & Martin, 1962).

The constant volume hypothesis of Krigsman & Divaris (1955) is advanced to explain the filling of the ventricle in molluscs with lower returning vascular pressures. Although Narain (1976) states that this could not work in an animal with a flaccid pericardium or equivalent, it has been shown that the hypothesis does apply to *Patella* where a non-rigid pericardium is present (Jones, 1970). The situation in the cephalopods however is complicated. First of all, the true pericardium is restricted to the area of the branchial heart appendages (Marthy, 1968) and second, there are other contractile components within the renal sacs (tor example the lateral venae cavae) and lastly, the main circulatory organs are contained within the contracting mantle sac. At this stage it cannot be ruled out that co-ordination of the contraction of these components might mutually aid the refilling of the ventricle. However, mantle and ventricular contraction rates are not linked in any immediately obvious [redacted]ationship (Wells, 1979; Smith, 1979).

A stability of heart rate does not preclude an ability to adjust volume output. From the present results it might be expected that, *in vivo*, small changes in the auricular pressure over the range of 10–20 cm of water would cause considerable change in stroke volume without noticeably affecting the short-term measurement of contraction rate. Filling pressures of 10–20 cm of water correspond to the efferent branchial vessel pressure range measured in *Octopus dofleini* (Johansen & Martin, 1962). Wells (1979) estimated an *in vivo* stroke volume of 0·49 ml from the ventricle of a 500 g *Octopus vulgaris*. *In vitro*, the ventricle of *Eledone cirrhosa* requires an input pressure of at least 10 cm of water to pump such a volume, even when the difference between the input pressure and the output back pressure is minimal. Above the input pressure level of 20 cm of water the stroke volume is governed by the difference between the input (auricular) pressure and the output (aortic diastolic) back pressure. The range of stroke volume changes would appear to be more limited than in the teleosts, where increases in the order of 10 times have been reported between resting and active values (reviewed by Jones & Randall, 1978). As the heart rate also shows a limited scope for increase it would seem possible that the octopods could incur a sizeable oxygen debt during prolonged activity.

The observation that the back pressure on the isolated ventricle affects stroke volume, but not heart rate, implies that *in vivo* the diastolic aortic pressures will make an important contribution to the regulation of volume output. In the experiments described, the value of the output back pressure was set independently of the input pressure and the consequent volume output. This would not be the case *in vivo*. A reduction in stroke volume at a relatively constant heart rate would reduce the pressure stored by the elastic component of the dorsal aorta (the octopod 'windkessel', Johansen & Martin, 1962). This would reduce the subsequent resistance to flow from the ventricle. The extensive innervation of the cephalopod blood vessels (Alexandrowicz, 1928; Barber & Graziadei, 1967) suggests the possibility of complex control reflexes on this parameter. Large areas of the central nervous system appear to be devoted to the regulation of the peripheral circulation (see Young, 1971). The more complex interactions affecting volume output cannot be investigated using an isolated preparation. To understand the role of these factors, studies must be undertaken on more complex physiological preparations or with the *in vivo* recording of the interactions between pressure, flow and stroke volume. For the moment, however, it can be concluded that the haemodynamics *in vivo* might be expected to perform an important role in the regulation of the volume output from the octopod ventricle, principally by the regulation of stroke volume.

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