HAEMODYNAMICS IN TRESUS NUTTALLII AND CERTAIN OTHER BIVALVES

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INTRODUCTION

Molluscs, having a number of relatively large species, offer an opportunity to study general circulation dynamics in an open type of circulatory system. Certain aspects of their cardiac function, such as filtration through the heart wall, are found only in molluscan hearts. Other features, such as the myogenic nature of the heart and the pressure dynamics, are of more general interest. Few investigators, however, have looked at bivalves in terms of their internal pressures, filling mechanisms, the presence and structure of valves, or the function of the aortic bulb. None has done so with modern electronic equipment.

In their comprehensive review of molluscan hearts Krijgsman & Divaris (1955) suggest that ventricular filling requires functional auricles and that auricular filling requires an intact pericardium. They infer that in clams with an immovable pericardium, ventricular contraction creates a negative pressure which in turn causes the expansion and filling of the auricle. Then the auricular contraction, according to their hypothesis, is required for ventricular filling. We used isolated ventricles to test this hypothesis.

The aortic bulb—a hollow, contractile organ attached to the posterior aorta just inside the pericardium—is found in some bivalves, but its role has not been satisfactorily explained. We anticipated that measurements of its pumping activity would correlate with some other measurable function and thereby provide clues to its functional significance.

A few secondary questions also attracted our attention. Does the heart provide pressure for the extension of the foot or siphons? Is there increased venous return during sudden bodily contractions? What valves, if any, control the direction of blood flow? Such problems have been widely examined in many vertebrates, but scarcely considered for bivalves.

MATERIALS AND METHODS

The bivalves used were collected in the vicinity of Victoria, B.C., Canada, and kept in cooled, aerated sea water until used. Most experiments were performed at approximately 20°C. This temperature did not affect blood pressure or heart adversely. We used a hand-held, electric grinding tool with a mandrel saw to remove shell from over the heart. In some experiments with Tresus nuttallii, we found little or no difference in heart activity of clams removed entirely from the shell as compared with those having only a portion of the shell removed. Blood loss was not noticed from clams properly
prepared in either way. However, we removed as little shell as possible for most experiments.

The pressure recording system consisted of a Sanborn model 321 two-channel recorder, two 267 Sanborn transducers, and plastic tubing connecting the transducers to the clam. With 0.011 in. bore polyethylene tubing (Clay-Adams, PE10) 25 cm. long the system gave an overall frequency response of about 0.3 cyc./sec., while with 0.023 in. bore tubing (Clay-Adams, PE50) the frequency response was about 30 cyc./sec. While simultaneous records from one heart using the two sizes of tubing gave apparently identical records, the larger tubing was used whenever possible. Total sensitivity was such that 1 mm. Hg pressure gave either 0.5 or 1.25 cm. deflexion of the writing stylus.

Temperature and pH were not rigidly controlled except for preliminary experiments in which it seemed that there were only minor changes in heart rate and pressure over fairly wide limits. Because of the inconvenience of maintaining low temperatures and the fact that hearts often continued beating for 3-5 hr. at room temperature in an apparently normal fashion, experiments were performed at room temperature (18-20°C) even though such temperatures are probably outside the normal environmental extremes encountered by these bivalves.

RESULTS

Morphology

A knowledge of the structure of the auricular and ventricular walls is important for the understanding of the heart’s ability to produce pressure and to filter blood. The occurrence and position of valves controls direction of flow. The following description applies only to *Tresus nuttalli* and *T. capax* in which we observed heart structure under ×7-20 magnification *in vivo*.

The ventricle of *Tresus* is somewhat valentine-shaped—the posterior end being the pointed one and the two auricles connecting laterally to the ventricle at its broadest point. The auricles are so thin that their broad connexions with the veins from the ctenidia are readily visible through the auricular walls. The wall of the ventricle, on the other hand, is comparatively thick and opaque, obscuring the intestine inside in most places. By inflating the ventricle with air, however, one sees that both chambers have similar walls, composed of a meshwork of muscle fibres lined internally with an extremely thin membrane. The spaces between the muscle fibres of the ventricle are not noticeable until the ventricle is drastically stretched. The wall of the aortic bulb, which at first looks like the unstretched ventricle, in uniformly thick with no membranous ‘windows’ between muscle strands.

Perhaps because valves are not conspicuous unless observed in a beating heart, we found no previous reports on the occurrence of valves in the hearts of bivalves. There appear to be no valves guarding the broad opening between ctenidial veins and auricles. Where the auricles join the ventricle, however, the wall of the ventricle forms a broad, smooth-edged collar which folds inward when the ventricle contracts and prevents backflow into the auricles. The posterior aorta between the ventricle and the aortic bulb is constricted around the intestine so that blood flow occurs only when a wave of relaxation preceding ventricular contraction opens the constriction. We
believe that a second valve exists in the posterior aorta distal to the aortic bulb, but the evidence for its presence rests not on direct observation but solely on the pumping characteristics of the isolated ventricle.

The anterior aorta is also valved, but in a different manner. The contraction of the ventricle continues into the anterior aorta, depressing a muscular, V-shaped area over which blood flows. After watching dye and small bubbles pass this valve we concluded that the flow does not continue during the entire period for which the valve is open, and therefore that the valve action is controlled by the contractile process of the ventricle and not by the pressure gradient.

Recording 'normal' blood pressures

We devoted considerable effort toward determining the effects on heart haemodynamics of the various operative techniques, particularly the effects of opening the pericardium. By inserting a cannula through the pericardium into the ventricle and recording pressures during the removal of the pericardium, we were convinced that no changes in pressure or rate occurred. Even when Tresus was completely removed from its shell the pressure records still appeared 'normal'. However, making similar measurements in the auricles without removing the pericardium was impossible because there was no obvious pressure pulse to signal when the pressure transducer cannula was inside the auricle. The pericardium was too opaque to position the
cannula visually. Further, the fragile nature of the walls of the auricle predisposed it to tearing and leaking around the cannula. The only instance when we recorded an auricular pressure was during increased venous return (discussed later). *Crassostrea gigas* is an exception to this rule since we normally recorded auricular pressures (Fig. 1). Some very small pulses were commonly recorded from auricles, but these pulses correlated with ventricular contraction rather than auricular contraction.

Although several investigators hypothesized a pericardial pressure we found none which was measurable with transducers. Upon opening the pericardium, however the ventricle 'ballooned' out of the incision as though it had been released from compression, even though the pressure transducer measured no pericardial pressure. Waiting for an hour between shell removal and opening the pericardium eliminated the 'ballooning', probably because the clam had relaxed and reduced its venous return and blood pressure.

Table 1 shows the values obtained for ventricular pressures in several local bivalves. (See also Fig. 5). These values, together with the figures of the pressure records obtained suggest several generalizations. Ventricular systolic pressures are not correlated with the size of the bivalves. Diastolic pressure is zero except when there is increased venous return. Heart rate is usually 6–10 beats/min., rarely above 15 beats/min. except in *Crassostrea* where heart rate is 20–30 beats/min. Diastole usually lasts longer than systole. Although there is considerable irregularity in the height of the ventricular pressure pulse, the records show a tendency to alternation between larger and smaller pulses (Fig. 2, upper) or may show a rising and falling series of pulses (Fig. 1; Fig. 2, lower). These regular variations disappear after blood loss or ventricle isolation, suggesting that stronger pressure pulses might trigger an inhibitory system. We first hypothesized that the stretching of the aortic bulb by a strong pressure pulse produced

<table>
<thead>
<tr>
<th>Table 1. Ventricular systolic pressures in selected bivalves</th>
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<tr>
<td>Mean Pressure (mm. Hg) Range (mm. Hg)</td>
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<tr>
<td><strong>Mytilidae</strong></td>
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<tr>
<td><em>Mytilus californianus</em> 1.9 0.4–3.0</td>
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<tr>
<td><strong>Pectinidae</strong></td>
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<tr>
<td><em>Himnites giganteus</em> (multirugosus) 0.2 0.1–0.4</td>
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<tr>
<td><em>Pecten sp.</em> 0.5 0.4–0.7</td>
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<tr>
<td><strong>Ostreidae</strong></td>
</tr>
<tr>
<td><em>Crassostrea gigas</em> 1.4 0.8–2.0</td>
</tr>
<tr>
<td><strong>Tellinidae</strong></td>
</tr>
<tr>
<td><em>Macoma nasuta</em> 0.8 0.2–2.9</td>
</tr>
<tr>
<td><strong>Mactridae</strong></td>
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<tr>
<td><em>Tresus</em> (Schizothaerus) <em>nuttalli</em> 1.3 0.4–3.5</td>
</tr>
<tr>
<td><em>Tresus</em> (Schizothaerus) <em>capex</em> 1.3 0.4–3.5</td>
</tr>
<tr>
<td><strong>Cardiidae</strong></td>
</tr>
<tr>
<td><em>Clinocardium nuttalli</em> 1.7 0.4–2.6</td>
</tr>
<tr>
<td><strong>Veneridae</strong></td>
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<tr>
<td><em>Protothaca staminea</em> 1.3 1.1–2.8</td>
</tr>
<tr>
<td><em>Saxidomus gigantea</em> 1.5 1.0–2.5</td>
</tr>
<tr>
<td><em>Compsomyax subdiaphana</em> 1.5 1.2–2.0</td>
</tr>
<tr>
<td><strong>Myidae</strong></td>
</tr>
<tr>
<td><em>Mya arenaria</em> 0.6 0.3–1.1</td>
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Inhibition of the ventricle, but discarded the idea when we found that pinching any tissue near the heart gave the same effect.

The aortic bulb also contracts, but less frequently than the ventricle and not in synchrony with any particular phase of ventricular contraction. An exception was the isolated heart in which the contraction of the aortic bulb consistently followed that of the ventricle. The pressure in the aortic bulb approximately equalled that in the ventricle, but was maintained over longer periods of time (Fig. 2).

![Graphs showing haemodynamics in Tresus capax and Tresus nuttallii](image)

Fig. 2. Examples of the aortic bulb contractions, showing lack of synchrony with the ventricle. Note that the aortic bulb contractions are related to the ventricular beat in the isolated heart.

**Normal and experimental changes in heart output**

Constrictions of blood-filled organs such as siphons or foot could be expected to cause an inrush of peripheral blood and thereby increase heart rate and pressure. Such appears to be true only in some instances. We watched siphons and foot extend and contract in several bivalves (Fig. 4, upper) without any corresponding change in heart rate or pressure. Pinching the extended siphons of *Tresus* caused contraction of the siphons, but changed the heart rate only if sea water had been previously injected into the blood sinuses. Conversely, continued tapping on the shell of *Mytilus californianus* caused closure of the shell, a brief inhibition of the heartbeat, and greatly increased aortic pressure for a longer time (Fig. 3, lower). Although the ventricular pressure record appears normal in this record, it is difficult to see how there could have been any net output during the period of increased aortic pressure.

In two cases we saw contractions such as might occur under normal environmental
Mytilus californianus

Fig. 3. Examples of normal and excessive aortic pressures. Note that the ventricular response to contraction of the adductor muscle is similar in both cases on the lower record, but that the aortic pressure did not change in the first case.

Macoma nasuta

Fig. 4. Examples in which body movements did affect (lower record) and did not affect (upper record) blood pressure or heart rate.
Conditions and which did affect heart output. In Fig. 3 the notation 'spontaneous contraction' marks the typical ejection of pseudofaeces in *Mytilus*. Correlated with the ejection is an increase in ventricular pressure, both systolic and diastolic, for a few beats. In *Pecten* sp. (Fig. 4, lower) a sudden increase in heart rate occurred in 'anticipation' of a swimming-type contraction. When digging *Tresus* in shallow water we occasionally observed a forceful jet of blood being expelled from the anterior aorta as the foot and siphons contracted rapidly. We interpreted this as a 'safety valve' mechanism operating in the presence of increased aortic pressure which prevented overfilling of the heart. A second contraction within a few hours did not produce the jet of blood again, presumably because the lost volume of blood had not yet been replaced. After the animal had been left undisturbed in an aquarium for a week or so the phenomenon usually appeared again.

We experimentally produced increased venous return in two ways. Sea water injected into the blood sinuses of the siphon produced no effect on heart rate or pressure until we pinched the siphon and made it contract. Then the auricles and ventricle became dilated, although blood pressure did not rise in proportion to the increased heart volume. The heart did not return to its normal volume within the 1–2 hr. limit of these experiments. Secondly, sea water injected directly into the auricle produced a transitory rise in auricular pressure and rate so that the auricle was not synchronized with the ventricle. This was somewhat startling to us, but did not seem to compromise the pumping ability of the ventricle. Injection into one auricle had no effect on the other auricle.

Stopping blood flow by ligaturing vessels gave the expected results. Both ventricular pressure and volume rose when the two aortas were tied off, but did not change with one aorta open; ventricular pressure fell when both auricles were ligated, but not with only one blocked. Retrograde flow apparently did not occur, even when the heart became greatly dilated.

**Isolated ventricle**

The most meaningful test we could devise to measure the ability of the ventricle to fill itself in the absence of either pericardium or auricles was to isolate the ventricle with part of the posterior aorta. Briefly, the isolation procedure was as follows.

A large *Tresus* was removed from its shell and the exposed pericardium was removed. The auricles were severed, the anterior aorta tied off as far anteriorly as possible, and both aortas severed where they passed through the pericardium. After moving the heart into a dish of sea water a polyethylene tube (PE 50, Clay-Adams) was put into the posterior aorta. Pressure recordings were obtained by inserting a cannula through the wall of the ventricle and of the aortic bulb. The pressure head against which the heart pumped was regulated by raising and lowering the free end of the aortic cannula and the output was collected into a 0.1 ml. pipette for measurement.

The pumping of the isolated heart resembled that of the intact heart. Intraventricular pressure records were not noticeably different from records of other *Tresus* hearts *in vivo* (Fig. 2, lower right). The manner in which the ventricle expanded and contracted appeared 'normal'. There was no back-flow in the aortic cannula even though its free end was as much as 2.0 cm. above the heart, except when the cannula was inserted 100 far into the posterior aorta toward the ventricle.
The primary difference in vitro was that contractions of the aortic bulb followed each ventricular contraction. The fluid pumped by the aortic bulb flowed into the ventricle and not out of the aortic cannula.

The output from isolated hearts varied inversely with the height of the cannula outflow above the heart. In one heart, at an outflow height of 1.0 cm., the stroke volume was 0.010 ml., but at 2.0 cm. the output was only half that. The greatest stroke volume recorded for any isolated ventricle was 0.015 ml. at 2.0 cm. height, but the average for this heart was only 0.010 ml./beat.

**DISCUSSION**

The pressures we recorded in the ventricles of the various bivalves are about the same as that reported for *Anodonta*: 3 mm. Hg (Picken, 1937; Potts, 1954). This 'pump' does not normally inflate the foot or siphon as they extend and is not generally affected by bodily movement, although some intriguing correlations between heart rate and body movements were recorded.
Since the isolated heart pumps sea water in the absence of auricles or pericardium, these are eliminated as being necessary for ventricular filling. However, we cannot say to what degree the ventricular filling in the isolated heart resembles that in the intact heart.

Several molluscan hearts have been identified as sites of blood filtration (Picken, 1937; Potts, 1954; Harrison, 1962). Although the intraventricular pressures in bivalves seem very low compared to those in mammalian kidneys, the thin lining of the heart chambers makes low-pressure filtration not unlikely. And since the auricle rarely produces much pressure it seems probable that most of the filtration takes place through the ventricle wall. The increased activity of the auricle during increased venous return suggests that the auricle could function as an auxiliary filtration site for reducing excessive blood volume. We plan further experimentation on the filtration phenomena.

The function of the aortic bulb is still unclear. It seems improbable that it is either glandular or a site of filtration. It might also serve as a Windkessel, but if so it only functions intermittently. It might also serve to keep the ventricle beating by refilling it when aortic pressures exceed heart pressures, but such high aortic pressures have been observed only in Mytilus californianus which has no aortic bulb. Injections into the aortic bulb to test the effect of inflating it have all failed because of leakage around the puncture site.

We spent considerable time trying to get consistent results from changes in temperature and from aerating or oxygenating the water baths containing the experimental clams. While heart rate did increase with increased temperature, the changes were rarely repeatable in any one clam. Aeration or oxygenation seemed to have little effect, although low oxygen or high carbon-dioxide content of the water could have been expected to depress heart rate (Schlieper, 1955). Our final conclusion was that when the experiments were performed at room temperature the heart performance was not conspicuously different from that seen at environmental temperatures, but that any detailed study of temperature effect would require an extensive series of separate experiments.

A possible explanation for not finding any auricular pressure except under special circumstances is that the auricular contraction occurred in perfect synchrony with ventricular expansion. Under such circumstances the net pressure gradient between the two chambers could have been too small to detect with pressure transducers. That the ventricule could exert the negative pressure required by such a hypothesis is demonstrated by the filling of the isolated ventricle.

**SUMMARY**

1. Blood pressures were measured in the hearts and aortas of several bivalves.
2. Systolic pressures in the ventricles were commonly 0.5–2.0 mm. Hg, while diastolic pressure was zero.
3. The aortic bulb produced pressures similar to those produced by the ventricle, but beat less frequently and not in synchrony with the ventricle, except in the isolated heart.
4. Pericardial and auricular pressures were normally undetectable, except that
small pressure pulses were recorded in the auricle during experimentally produced increases in venous return.

5. Aortic pressure sometimes exceeded ventricular pressure, although unusually strong contractions of the body muscles raised aortic pressures far above that in the ventricle. The heart does not provide pressure for extension of siphons or foot.

6. Sea water pumped by isolated Tresus ventricles demonstrated that neither pericardium nor auricles are needed for ventricular filling, as had been hypothesized earlier.

7. The morphology of the heart walls supports the concept of bivalve hearts as being a site of filtration, with the ventricle thought to play a larger role than the auricles.

8. Valves in the heart and aortas which control direction of blood flow are described.

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REFERENCES


