*J. exp. Biol.* **140**, 257–271 (1988)
*Printed in Great Britain* © *The Company of Biologists Limited 1988*



# *IN VIVO* CARDIAC PRESSURE AND HEART RATE, AND HEART MASS, OF *BUSYCON CANALICULATUM* (L.)

BY H. D. JONES*

*Department of Zoology, University of Rhode Island, Kingston, RI 02881, USA*

*Accepted 26 May 1988*

## Summary

*In vivo* cardiac pressure and heart rate, and relative heart mass, have been measured in *Busycon canaliculatum*. Base pressure was about 1·3 $cmH_2O$ above ambient and effectively the same in the ventricle, atrium, pericardium and veins. Ventricular systolic pressures were very variable. Highest pressure was over 80 $cmH_2O$, but sustained levels were lower, up to about 40 $cmH_2O$. Heart rate was also very variable, 5–15 beats $min^{-1}$. Heart mass was about 0·19 % of body mass. Cardiac refilling, control and power output are discussed.

## Introduction

Several factors act collectively to control the gastropod heart in the intact animal (Jones, 1983; Smith, 1985*a*, 1987; Hill, 1987). Smith (1985*b*) and Smith & Hill (1986, 1987) have shown how the isolated, perfused heart of *Busycon canaliculatum* responds to different preload (= venous) and afterload (= aortic) pressures, and to various cardioregulatory substances. Heart output was independent of afterload pressure. The experimental preload and afterload pressures were set to levels necessarily inferred from measurements from other gastropods since there are no *in vivo* measurements of cardiac pressure or rate from this or indeed any other neogastropod.

Relative heart mass is potentially useful in comparing cardiac performance (Schmidt-Nielsen, 1984) and in predicting circulatory pressure (Jones, 1983). The ventricle mass of *Busycon* is about 0·17 % of the body mass (less the shell) (Smith, 1985*b*). This is a high proportion of body mass, and systolic pressure in *B. canaliculatum* might be correspondingly high.

The work reported here was primarily undertaken to determine *in vivo* cardiac pressure and heart rate in *B. canaliculatum*. Relative heart mass was also determined on a wider range of animal mass than used by Smith (1985*b*) and further unpublished data of P. J. S. Smith are also included.

*Present address: Department of Environmental Biology, University of Manchester, Manchester, M13 9PL, UK.

Key words: heart, heart mass, heart rate, cardiac pressures, cardiac power, *Busycon*.

## Materials and methods

Specimens of *Busycon canaliculatum* (L.) were obtained from the Marine Biological Laboratory, Woods Hole, MA, and stored in running sea water at ambient temperature at the Narragansett Bay campus of the University of Rhode Island (URI). Ambient seawater temperature varied from about 15°C in September to about 4°C in December, 1986, between which dates the work was carried out. During this period of the year the animals are quiescent, but at other times they are more active and it is possible that results may differ.

Experiments were carried out initially at 20–25°C in the Department of Zoology, Kingston campus, URI. Ultimately, experiments were carried out in the Marine Aquarium Building of the Graduate School of Oceanography, Narragansett Bay campus, URI where, 1 week before use, experimental animals were transferred to tanks containing running sea water at about 15°C and experiments were carried out at about that temperature. It is assumed that this transferral to constant temperature masked any differences due to the varying ambient temperature.

Before use, each animal was made to retract fully into its shell by means of repeated tactile stimulation, weighed and the shell length determined as follows. The shell was placed with the aperture down on a flat surface with the spire against a vertical bar. Shell length was taken as the distance from the bar to the tip of the siphon tube. After use the shell was removed and the masses of the body and heart (some as whole heart, others as ventricle and atrium separately) were determined.

To obtain access to the heart a triangular piece of shell was removed from the body whorl mid-ventrally, adjacent to the confluence of the lip of the shell with the body whorl. A diamond-edged saw in a small hand-held drill was used to make three cuts in the shell and the piece prised out. After careful washing to remove debris, animals were ready for use. Some specimens were used immediately, others after several days. Some survived for several weeks and were used on more than one occasion.

Pressures were recorded using Statham P23BB transducers connected to a Grass Polygraph model 79C *via* low-level d.c. preamplifiers. A system of reservoirs, tubes and taps allowed the transducer domes and catheters to be flushed with degassed (by vacuum) distilled water and sea water as appropriate. Catheters were 25-gauge hypodermic needles held in manipulators. Experimental animals were clamped upside-down in a container filled with sea water to a level just sufficient to cover the animal. The needles were then positioned over the heart and manipulated into the recording site. Zero pressure levels were determined before and after each recording by recording with the needle under the surface of the water in the container. Calibration, in $cmH_2O$ ($1\,cmH_2O = 98{\cdot}4\,Pa$), was carried out by raising or lowering the transducers in 1-cm steps relative to the experimental container, the catheter remaining in the container.

Heart rate was determined from the pressure recordings. In some specimens direct counts were made by visual observation of the heart.

*Symbols and units*

$M_b$, body mass (wet mass) of the retracted animal less the shell (g);
$M_h$, mass (wet mass) of the heart including the atrium (g);
$M_v$, mass (wet mass) of the ventricle (g);
$L_s$, shell length (cm).

Pressures are expressed in $cmH_2O$ throughout. Some pressures are expressed as absolute values above ambient (zero), others as pulse pressure amplitudes above or below base pressure.

## Results

*Pressure recordings*

Pressures were recorded successfully from the efferent branchial vein, the atrium, the ventricle and the pericardial cavity. Attempts were made to record from the anterior aorta, but the position of this in the body made it impossible to penetrate without considerable further surgery. In several specimens recordings were made from two sites simultaneously. In a few cases recordings were made consecutively, for example after recording from the pericardial cavity the cannula could be advanced into the ventricle.

Most recordings showed a clear, consistent and readily determinable base level between contractions of the ventricle since there was usually a well-defined diastolic pause. The base level could undergo transient fluctuations due to body contractions. The base level ranged from 0 to 5 $cmH_2O$ above ambient (Table 1), but was most often about 1 $cmH_2O$ with higher values present during body activity. The values of base pressure are clearly similar in all sites and can be assumed to be effectively identical.

Ventricular pressure pulses varied enormously from barely detectable pulses of less than 1 $cmH_2O$ above base level to values in excess of 50 $cmH_2O$ (Table 1). The amplitude varied considerably among individuals and was rarely consistent in an individual (Fig. 1A,B). There was no obvious reason to account for the variation. Activity of the snail in the form of an extended and active head and foot affected base level, but high systolic pressures seemed unrelated to any obvious activity. The highest value recorded was a single pulse of at least 80 $cmH_2O$ (Fig. 1D). The results produce a mean value of ventricular systolic pressure of 12·74 $cmH_2O$, but because of the large range this should be regarded with caution. It is difficult to give even 'typical' values, though in quiescent individuals the ventricular systolic pressure was usually less than 20 $cmH_2O$. In active animals the pressure usually exceeded 20 $cmH_2O$.

Generally the ventricular pulse showed a rapid and smooth rise in pressure to peak, followed by a drop to base level. However, there were occasions when the pressure pulse took a different form. Double pulses occurred in some specimens interspersed wtih normal pulses (Fig. 2A,B). The double pulses themselves varied a little in form. The amplitude of the first pulse could be larger than the second or *vice versa*. On other occasions the pulse took the form of a stepped rise in pressure

Table 1. *A summary of the pressures from the various recording sites*

| Site | Base | Pulse | *N* | *n* |
|---|---|---|---|---|
| Ventricle | 1·23 ± 0·14 (0–3) | 12·74 ± 1·52 (1 to >80) | 28 | 21 |
| Atrium | 1·31 ± 0·14 (0·5–2) | −0·92 ± 0·25 (−0·1 to −4) | 12 | 11 |
| (at atrial systole) | | 0·46 ± 0·07 (0·25 to 0·6) | 4 | 4 |
| Efferent branchial vein | 1·22 ± 0·25 (0·7–2) | −0·73 ± 0·22 (−0·25 to −1·8) | 6 | 6 |
| Pericardial cavity | 1·37 ± 0·25 (0·25–5) | −1·43 ± 0·28 (−0·25 to −4) | 19 | 18 |
| Overall base | 1·29 ± 0·25 (0–5) | | | |

Base pressure (diastolic) is expressed in $cmH_2O$ above zero, mean ± S.E. (range).

Pulse pressures are expressed in $cmH_2O$ above or below base pressure, *not* zero, mean ± S.E. (range).

In most recordings the atrium showed only a pressure drop synchronous with ventricular systole but in four recordings small positive pulses were evident in the atrium, the results of atrial contractions.

*N*, number of recordings; *n*, number of animals.

(Fig. 1C), the contraction taking place in either two or three stages. Again, these were interspersed with normal pulses.

Amplification of ventricular pressure records (Fig. 3) allowed comparison to be made of the amplitude of ventricular systolic pressure with the preceding diastolic level. There appears to be no correlation between the two, diastolic level not influencing the subsequent contraction.

The wall of the atrium is extremely thin and flexible and was thus difficult to penetrate. Recordings were variable in form. In all cases as the ventricle contracted there was a coincident drop in atrial pressure (Fig. 2B).

One puzzling feature of most of the atrial recordings is that there appeared to be no positive pulse (above base level) due to contraction of the atrium (Fig. 4A,B). In these cases it would appear that the ventricle alone was contracting. When the recording exhibited a clear atrial contraction, the pulses were up to $0{\cdot}6\,cmH_2O$ above base level (Table 1; Fig. 2A). The timing of the apparent atrial contraction varied. The atrial systolic contraction shown in Fig. 2A occurs at various intervals just before or after the ventricular contraction. Later, the same specimen (Fig. 2B) exhibited atrial contraction immediately before ventricular contraction.

The efferent branchial vein supplies blood directly to the atrium and pressure recordings from this site are very similar to atrial recordings (Fig. 4A). Typical pulse values in the efferent branchial vein were $-0{\cdot}5$ to $-1\,cmH_2O$ below base level (Table 1), with the reduction in pressure being coincident with, and

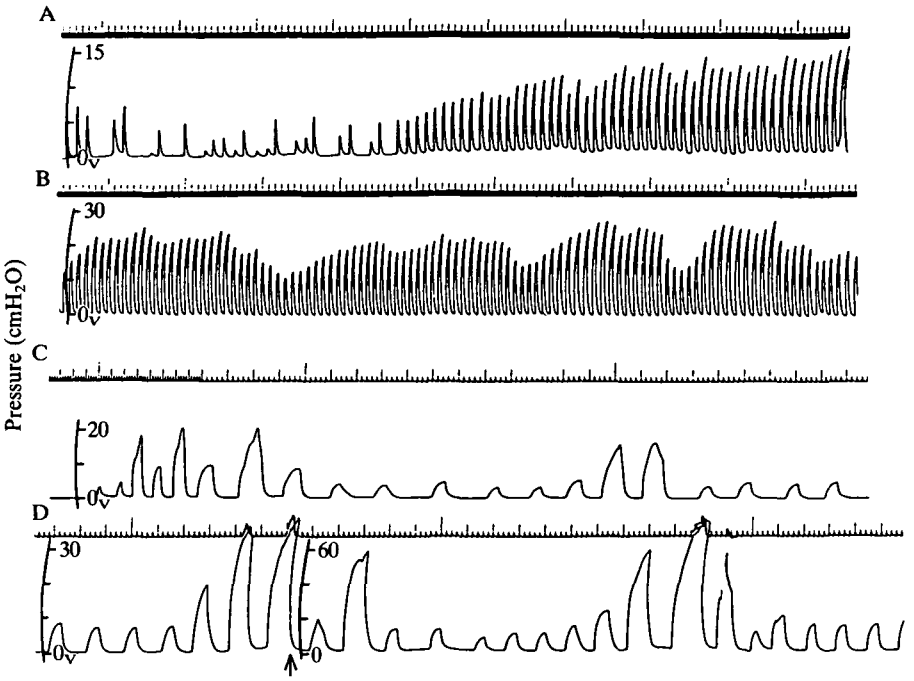


Fig. 1. Pressure recordings from the ventricle. (A) Initial irregular contractions (start of record is 15 min after penetration by the cannula), followed by regular contractions to about 15 $cmH_2O$ at 8·5 beats $min^{-1}$. (B) 8 min after record A, note different scale. Regular contractions of 10–25 $cmH_2O$ at about 10 beats $min^{-1}$. (C) Initial penetration by the cannula and subsequent record. D follows on from C. The largest contraction recorded is shown towards the end, estimated as at least 80 $cmH_2O$. Note the change of amplification at the arrow in D. Pressure in these and all other recordings is calibrated in $cmH_2O$ above or below ambient pressure (0) in the experimental container. The recording site of each record is indicated adjacent to zero by the following abbreviations: A, atrium; Ebv, efferent branchial vein; Pc, pericardium; V, ventricle. Time marks are at 1, 5 and 60 s on this and all other recordings.

proportional to, the amplitude of ventricular contraction. No positive pulses were recorded in any of the recordings from the efferent branchial vein, indicating that the atrium was inactive in these specimens. The pulse in the efferent branchial vein was slightly less than in the atrium (Fig. 4A).

Pericardial pressure recordings were relatively simple to obtain, though the pericardial wall is quite muscular and can contract very considerably. Pericardial pulses took the form of negative pulses (below base level) coincident with ventricular systole (Fig. 5). The amplitude of the pericardial pulse again seemed to depend on ventricular amplitude. The amplitude of the pericardial pulse was slightly greater than the corresponding pulse in either the atrium (Fig. 4B) or the efferent branchial vein (Fig. 4C).

Pressure changes in the various sites of measurement are summarized in Fig. 6.

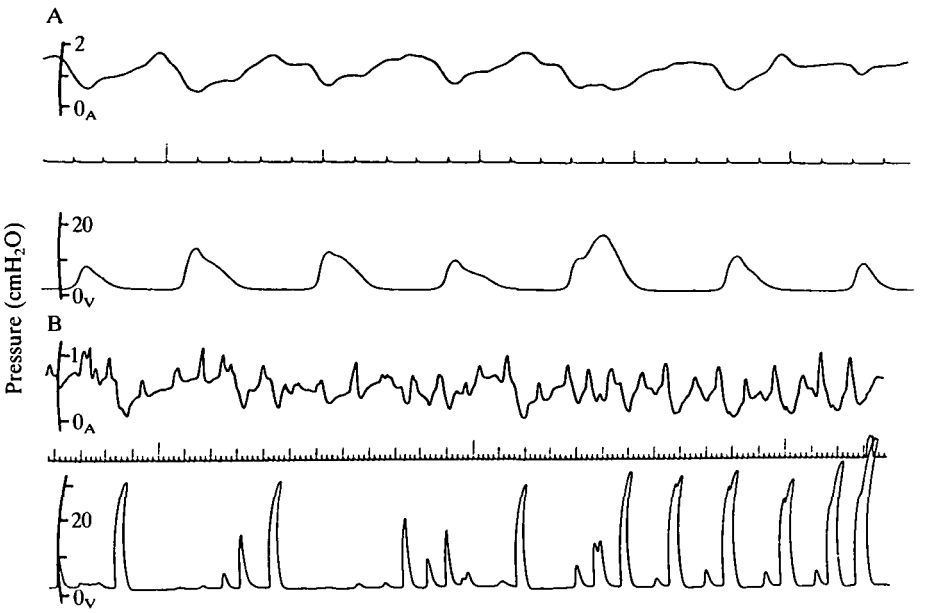


Fig. 2. (A) Simultaneous recording from the atrium (upper trace) and ventricle (lower trace) at fast chart speed. (B) As A but at slower chart speed. Note that in B the atrium produces pulses above base level (taken as about $+0{\cdot}5\,cmH_2O$) and that these immediately precede ventricular contraction, the latter evident as a sharp drop in pressure.

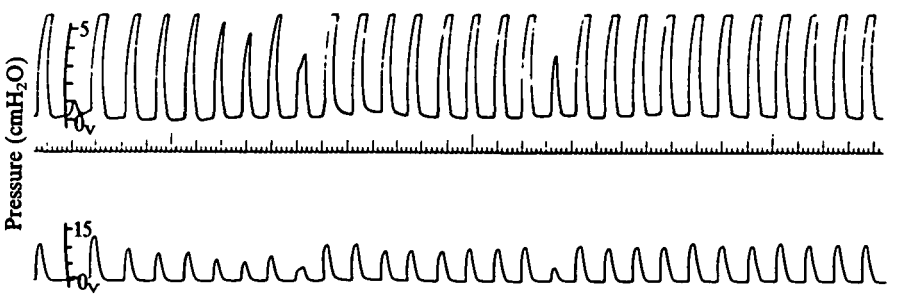


Fig. 3. Ventricular pressure recording (lower trace) with amplified recording (upper trace) to compare systolic amplitude with diastolic level. There appears to be no correlation of systolic level with the preceding diastolic level.

*Heart rate*

Heart rates were quite variable within an individual over time, and between individuals (Fig. 7). Some preparations beat in bursts (Fig. 4C) in which case the rate of contractions during the bursts was determined by measuring beat interval. Occasionally a preparation would stop beating for several minutes. By direct observation of the heart of unused specimens (but with the shell piece removed) it

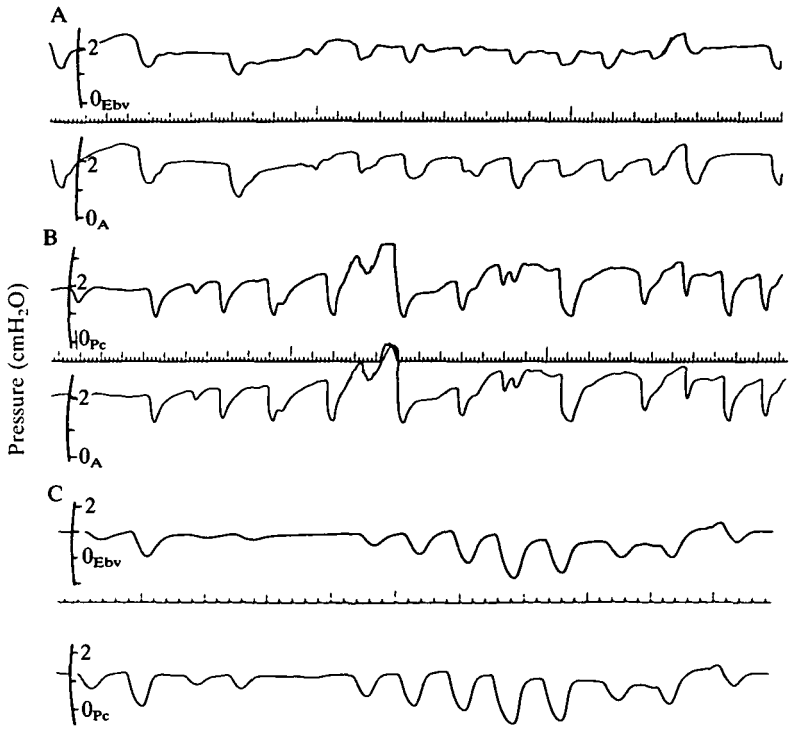


Fig. 4. (A) Simultaneous recording of pressure in the efferent branchial vein (upper trace) and atrium (lower trace). (B) Simultaneous recording of pericardial (upper trace) and atrial (lower trace) pressures in the same specimen. There is a 3 min break between A and B whilst the cannula recording the upper trace was moved from efferent branchial vein to pericardium. Note the relative amplitudes of the pulses in each site. Pericardial amplitude is slightly greater than atrial amplitude which, in turn, is greater than amplitude in the efferent branchial vein. (C) Simultaneous recording of pressure in the efferent branchial vein (upper trace) and pericardium (lower trace). Again note larger amplitude in the pericardium than in the efferent branchial vein.

was clear that the heart may be quiescent for quite long periods. To what extent this happens in unoperated specimens remains to be seen.

Though a mean heart rate could be calculated for any individual and for the species (at the two ranges of temperature) it would be relatively valueless because the rate varied from zero to 1–2 beats $min^{-1}$ to a maximum of 15 beats $min^{-1}$. The rate was usually between 5 and 15 beats $min^{-1}$, a similar overall range to that of the *in vitro* preparations of Smith (1985*b*) and Smith & Hill (1986, 1987). There is little apparent correlation with body size at either range of temperature. The cube of shell length has been used to give a measure of animal mass.

### *Heart and body measurements*

Fig. 8A shows how $M_h$ varied with $M_b$ for 42 specimens. Also plotted is $M_v$ against $M_b$ for 14 of the 42 specimens. The regression lines are clearly different

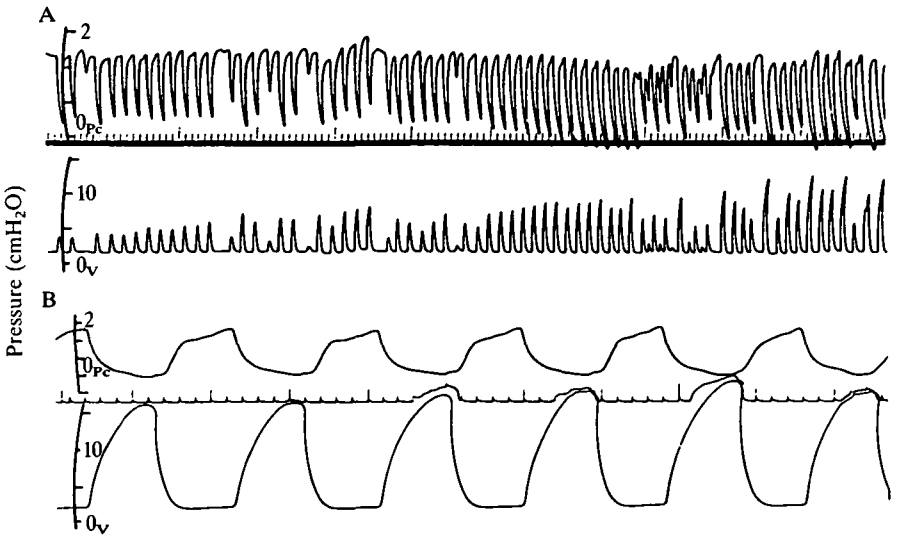


Fig. 5. (A) Simultaneous recordings of pericardial (upper trace) and ventricular (lower trace) pressures. (B) As A but at a faster chart speed.

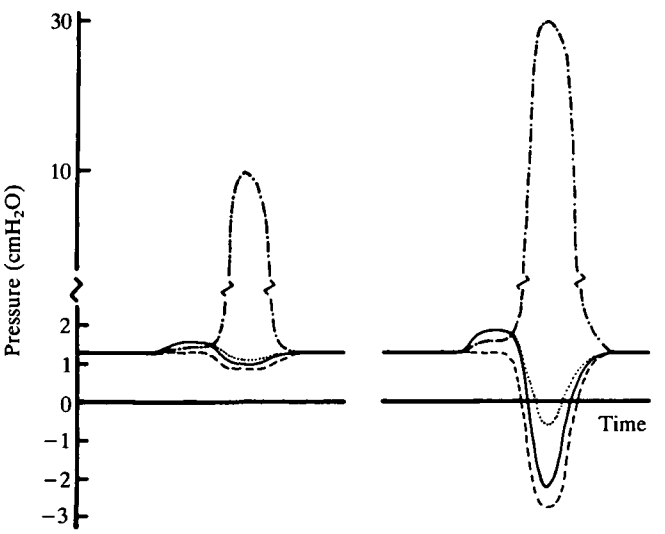


Fig. 6. Diagram summarizing the pressure changes in the heart and efferent branchial vein of *Busycon canaliculatum* at two arbitrarily selected levels of ventricular systolic pressure. Pressures in $cmH_2O$, note the change of scale in the pressure axis. During diastolic pauses pressure in all parts is at base level, about 1·3 $cmH_2O$. The time axis is uncalibrated, but at a heart rate of say 10 beats $min^{-1}$, each cycle of contraction would take 6 s. ····· pressure in the efferent branchial vein; —·— pressure in the ventricle; – – – pressure in the pericardial cavity; —— pressure in the atrium.

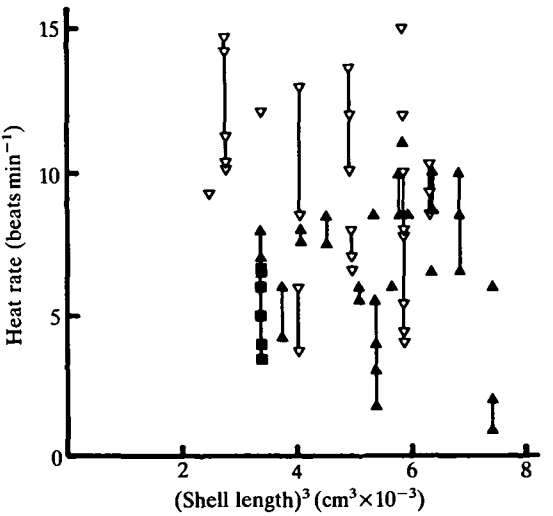


Fig. 7. Heat rate (in beats min$^{-1}$) plotted against body size (as cube of shell length). Vertical lines join different data from one specimen at different times. (▽) Experiments at 20–25°C; (▲) experiments at about 15°C; (■) experiments at a range of lower temperatures, down to 8°C.

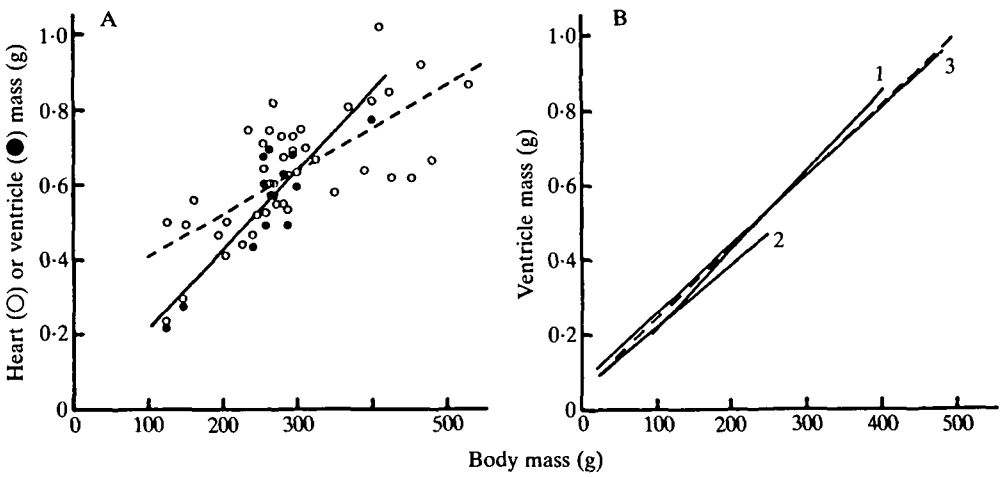


Fig. 8. (A) Heart mass ($M_h$, ○) and ventricle mass ($M_v$, ●) plotted against body mass ($M_b$). For $M_v$, $y = 0{\cdot}0021x + 0{\cdot}0026$, $r = 0{\cdot}86$, $N = 14$. For $M_h$, $y = 0{\cdot}0012x + 0{\cdot}29$, $r = 0{\cdot}70$, $N = 42$. (B) Regression lines of ventricle mass ($M_v$) plotted against body mass ($M_b$). Line 1 is taken from Fig. 1A; line 2 is taken from Smith (1985*b*), $y = 0{\cdot}0016x + 0{\cdot}053$, $r = 0{\cdot}92$, $N = 19$; line 3 is taken from further unpublished data from P. J. S. Smith, $y = 0{\cdot}0018x + 0{\cdot}065$, $r = 0{\cdot}82$, $N = 51$. Combined data (dashed line): $y = 0{\cdot}0019x + 0{\cdot}054$, $r = 0{\cdot}88$, $N = 84$.

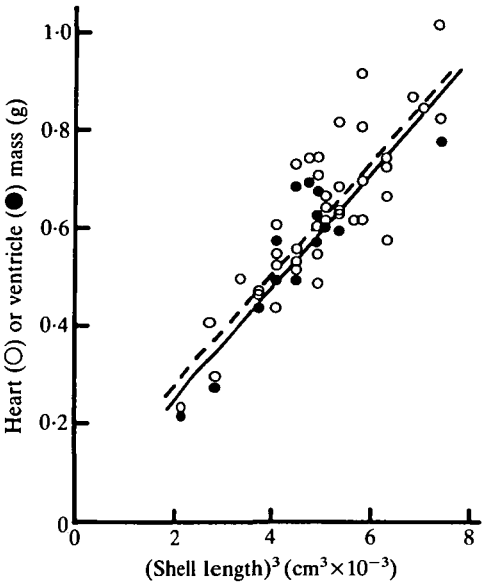


Fig. 9. Heart mass ($M_h$, ○) and ventricle mass ($M_v$, ●) plotted against size as the cube of shell length (in cm). For $M_v$, $y = 0{\cdot}0001x + 0{\cdot}03$, $r = 0{\cdot}87$, $N = 14$; for $M_h$, $y = 0{\cdot}0001x + 0{\cdot}06$, $r = 0{\cdot}84$, $N = 42$.

with the former not passing reasonably close to the origin, and with less, though still significant, correlation. For reasons to be discussed, the data for $M_b$ of the 42 specimens is considered suspect. $M_v$ was 93 % of $M_h$.

Three sets of measurements of $M_v$ and $M_b$ (Fig. 8B) are not significantly different ($P = 3{\cdot}84$ %) and the combined regression line is also shown. The value of the slope of each line gives $M_v$ as a percentage of $M_b$. The combined data show that the average $M_v$ was 0·19 % of $M_b$.

When $M_h$ and $M_v$ are plotted against $L_s^3$ for the same specimens (Fig. 9), the regression lines are closely parallel with intercepts close to zero and with similar correlations.

## Discussion

Base pressure is effectively the same at each site of measurement, and is the result of general body turgor produced by the tension of the body wall against the totality of its enclosed fluids. Base pressure tended to be higher when animals were more active, but this alone would not be expected to affect the hydrodynamic conditions around the heart since all parts of the circulatory system are similarly affected.

The relatively large size of the ventricle of *Busycon* and the presence of myoglobin in the muscle fibres of the ventricle (Ellington, 1985) both suggest that the ventricle is quite powerful and capable of producing large systolic pressures.

The recordings show that pressures of over 50 $cmH_2O$ may occur, the maximum recorded pressure being a single contraction of at least 80 $cmH_2O$. However, it is not clear at what levels the heart of *Busycon* can maintain systolic pressure for long periods. Pressures of over 50 $cmH_2O$ were only sustained for a few contractions at a time, sometimes single beats. During the periods of regular contractions recorded pressures were lower, variable among individuals and the amplitude fluctuated within a sequence. Sustained values of up to about 40 $cmH_2O$ were recorded, but more usual values were 15–30 $cmH_2O$.

Smith (1985*b*) and Smith & Hill (1986, 1987) recorded output pressure of *in vitro* preparations. Pulse amplitudes were usually a few $cmH_2O$, but treatment with 5-hydroxytryptamine resulted in amplitudes of up to about 16 $cmH_2O$ (Smith & Hill, 1986). In the *in vitro* preparations, output pressure level was preset. However, output resistance was not controlled and was probably quite low since the heart was pumping into an open reservoir. *In vivo* systolic pressures are the result of cardiac contractions working against the peripheral resistance and it would be valuable to carry out *in vitro* experiments which imposed a varied output resistance, by using tubes of differing diameter or length, as well as a varied output pressure.

The highest values of ventricular systolic pressure in *Busycon* are larger than those recorded from most other gastropods. The highest systolic ventricular pressures recorded from gastropods are from terrestrial slugs, typically of 74·5 $cmH_2O$, exceptionally of 90 $cmH_2O$, with diastolic pressures of about 52 $cmH_2O$ (Duval, 1983). The ventricle is thus raising pressure by about 23 $cmH_2O$. In *Helix* spp. ventricular systolic pressures are consistently sustained at levels of about 25 $cmH_2O$ with diastolic levels of about 6 $cmH_2O$ (Jones, 1971). Thus in *Helix* the ventricle is consistently raising pressure by about 19 $cmH_2O$. The diastolic level in *Busycon* is low, 1·3 $cmH_2O$, so that the ventricle itself is raising pressure virtually to the full systolic level. Though systolic pressures in *Busycon* are very variable, the pressure rise produced is the largest yet recorded from any gastropod.

Cephalopods show sustained levels of systolic pressure varying from about 30 to about 70 $cmH_2O$ (Bourne, 1987; Wells & Smith, 1987) though diastolic levels in some may be quite high. Thus the ventricle of *Busycon* can produce pressures for a few contractions that are at least as great as sustained pressures in some cephalopods. Sustained values in *Busycon* may approach those in cephalopods, but are usually lower. For comparative purposes it would be interesting to know what maximum pressure can be produced by the ventricle of different animals in isometric contraction, with the cardiac output closed.

It is now well established that in animals with an enclosed fluid-filled pericardial cavity the atrium is hydrostatically coupled to the ventricle, and at each ventricular contraction a volume of venous blood equivalent to ventricular stroke volume is drawn into the atrium. Thus, in such animals the ventricle not only empties the heart on every stroke, it also refills the atrium and this 'constant-volume' mechanism has been confirmed for gastropods (Jones, 1983) and fish (Satchell,

1971). Smith (1985*b*) and Smith & Hill (1986, 1987) used *in vitro* preparations of the atrium and ventricle with no artificial pericardium so that the atrium was not hydrostatically coupled to the ventricle. They also applied a constant (but adjustable) preload (venous) pressure and under these circumstances the heart was being filled by a means other than ventricular contraction, and could be considered as being driven by an external power source, the applied pressure head. This is unlikely to occur *in vivo* except when such movements as gross body deformation takes place and blood sinuses are thus forced to change their contained volume and cause an increased venous return of blood. To represent *in vivo* conditions more closely, *in vitro* experiments would be better carried out using an artificial pericardium (Civil & Thompson, 1972; Sommerville, 1973).

The high systolic pressures recorded here can be assumed to indicate high stroke volume as they also produced pericardial pulses of large amplitude. High systolic pressure could be the result of high aortic resistance, in turn the result of constriction of more distal vessels. But in this case there would be a small stroke volume and the resulting pericardial pulse would be small. High ventricular pressures with simultaneous large pericardial amplitude could also be the result of restriction in venous flow and consequent limitation on atrial expansion. Although this cannot be ruled out, the efferent branchial vein was always inflated which suggests that venous flow was unrestricted and that there was a volume of blood readily available for each atrial expansion. There is also a nephridial vein supplying the atrium which could not be readily observed.

Smith (1985*b*) and Smith & Hill (1986, 1987) found that cardiac output varied with preload (= venous) pressure which was set at various levels (0·5–10 $cmH_2O$). Smith (1985*b*) concluded that expected *in vivo* atrial pressures would be somewhere between 2 and 6 $cmH_2O$. It is only during ventricular systole that the atrium is filled and the effective refilling gradient is a transient one equivalent to the difference between pericardial pressure at systole and pressure in the efferent branchial vein and other veins. Mean pericardial pulse pressure was 1·43 $cmH_2O$ below base, maximally 4 $cmH_2O$ below base. Though the pressure simultaneously drops below base level in the atrium and the veins, 4 $cmH_2O$ must be considered as the maximum atrial filling gradient, and it is only a transient gradient, not a constant one. If input pressure did affect the amplitude of contraction, the pressure recordings would be expected to show amplitude variation as a result of diastolic pressure variation. This was not the case even in recordings amplified to show diastolic pressures in more detail (Fig. 2).

It was not possible to measure *in vivo* aortic pressures during this investigation, but in any circulatory system, aortic pressure is the result of ventricular output acting against peripheral resistance. During prolonged diastolic pauses at a low heart rate, as in *Busycon*, aortic pressure might fall back to, or very close to, base pressure.

Mean aortic pressure, if known, could be used to calculate power output (Schmidt-Nielson, 1984). Systolic pressure values are available and can also be used for the calculation (Prosser, 1973) but will obviously result in a higher figure.

Ideally, *in vivo* stroke volumes should be used, but again they are not available. However, Smith (1985*b*) found that for hearts of between 0·2 and 0·35 g, stroke volumes varied between 0·1 and 2·4 ml $g^{-1}$ at various preload pressures, and that a doubling of heart mass correlates with more than a doubling of stroke volume. Thus stroke volumes for the size of heart used here (mean mass 0·62 g) might be between 0·2 and 2·4 ml $g^{-1}$ heart tissue, giving stroke volumes in the range 0·125–1·48 ml. If heart rate is assumed to be 10 beats $min^{-1}$ (mid-range), the power for ventricular pressure of 10 $cmH_2O$ and stroke volume of 0·125 ml, is $30\times10^{-6}$ W $g^{-1}$. With ventricular pressure of 30 $cmH_2O$ and stroke volume of 1·48 ml, the power would be $1{\cdot}9\times10^{-3}$ W $g^{-1}$. These values would be reduced in direct proportion to aortic:systolic pressure ratio if aortic pressure was used in the calculation. These, of course, are not precise values but estimates based on reasonably realistic assumptions of stroke volume and heart rate.

This range of *in vivo* cardiac power output compares with the *in vitro* values of Smith (1985*b*), who found the highest power output to be about $70\times10^{-6}$ W $g^{-1}$, with an afterload pressure of 10 $cmH_2O$ and a preload pressure of between 4 and 6 $cmH_2O$. Treatment of the *in vitro Busycon* heart with 5-hydroxytryptamine (up to $10^{-7}$ mol $l^{-1}$) caused heart rate to increase by about 20 % compared with control values, but stroke volume showed no clear response (Smith & Hill, 1986). Power output was not calculated but would also be increased by 20 %, to about $84\times10^{-6}$ W $g^{-1}$. The low *in vivo* value is about half this maximum *in vitro* value, but the highest *in vivo* value is well in excess of this. It should also be borne in mind that systolic pressures may be even higher than the 30 $cmH_2O$ used to calculate the high figure. This suggests that *in vivo* figures, if confirmed, may well be more than *in vitro* values. Similarly in *Octopus*, Wells & Smith (1987) comment that the calculated *in vitro* cardiac power output, $0{\cdot}14\times10^{-3}$ W $g^{-1}$, 'is far too low in comparison with the *in vivo* performance', $1{\cdot}2\times10^{-3}$ W $g^{-1}$.

Systolic pressure varied considerably between successive contractions during these recordings, and even large single contractions occurred. Cardioregulation *in vivo* is dependent on several factors and none will act in isolation (Jones, 1983; Hill, 1987; Smith, 1987). Circulating cardioactive substances may continuously modulate tone and amplitude. Cardiac innervation will additionally modulate amplitude and perhaps rate (Kuwasawa & Hill, 1973; Kuwasawa *et al.* 1975; Koch *et al.* 1984; Furukawa & Kobayashi, 1987). Immediate and short-term changes in amplitude, as seen here, and pulses which rise in two or three stages or show two peaks (Figs 1C and 3B), must be the result of a neural influence rather than any other factor. Neural influences would seem to be the dominant factor controlling heart beat in *Busycon*, at least in the short term.

During these experiments it became clear that the wall of the pericardium is quite contractile. When a cannula penetrated the wall it would often contract very considerably and the contained pericardial volume would thus be reduced, including the volume of the heart. End-diastolic volume is of importance in affecting cardiac output in molluscs (Koester *et al.* 1974; Smith & Hill, 1986, 1987) and it seems possible that control of pericardial musculature may be an important

factor in cardioregulation, in this species at least, since base pressures and particularly end-diastolic volume of the heart could be controlled in this manner.

The results of body and heart measurements illustrate the difficulty of accurately and consistently establishing $M_b$ for these animals. Fig. 8A particularly, shows poor, though still significant, correlation between $M_h$ and $M_b$, yet when the same $M_h$ and $M_v$ results are plotted against $L_s^3$ (Fig. 10), the correlation is closer and $M_h$ varies more consistently with $M_v$. This variation in $M_b$ is due to the absorption of water into the vascular system (Mangum, 1979). Shell length at least does not vary as a result of this factor and seems to be a more consistent and better indication of size than body mass for this (and similar) species, though obviously of less value for interspecific comparisons owing to shape differences.

The combined data (Fig. 8B) suggest that the ventricle is about 0·19 % of the body mass in *B. canaliculatum*. Different sets of data give values varying from 0·17 % to 0·21 %. The ventricle mass is about 0·145 % of the whole animal mass including the shell.

There is little comparable data for other gastropods with regard to $M_v$. In *Lymnaea stagnalis* the ventricle is 0·10 % of the body mass, and in *Helix aspersa* the ventricle is 0·22 % of the body mass (dry masses, Jones, 1975). The ventricle mass of cephalopods varies from 0·071 % of body mass in *Architeuthis* to 0·16 % of body mass in *Octopus dofleini*, *Eledone* (Wells & Smith, 1987) and *Loligo pealei* (Bourne, 1982). These figures do not include the accessory hearts of cephalopods (see Wells & Smith, 1987).

This work was carried out whilst the author was a Visiting Professor in the Department of Zoology, University of Rhode Island. I am grateful to all members of the Zoology Department, but particularly to Professor R. B. Hill for arranging my visit and for reading the manuscript. Specimens were purchased with funds from NSF grant no. PCM 8309809 to RBH. Additional financial support was received in the form of a University of Manchester travel grant. I would also like to thank Bob Munkelwitz and Angelique Blowe for their enthusiastic assistance, and the referees for their constructive comments.

## References

BOURNE, G. B. (1982). Blood pressure in the squid, *Loligo pealei*. *Comp. Biochem. Physiol.* **72A**, 23–27.

BOURNE, G. B. (1987). Hemodynamics in squid. *Experientia* **43**, 500–502.

CIVIL, G. W. & THOMPSON, T. E. (1972). Experiments with the isolated heart of the gastropod *Helix pomatia* in an artificial pericardium. *J. exp. Biol.* **56**, 239–247.

DUVAL, A. (1983). Heartbeat and blood pressure in terrestrial slugs. *Can. J. Zool.* **61**, 987–992.

ELLINGTON, W. R. (1985). Cardiac energy metabolism in relation to work demand and habitat in bivalve and gastropod molluscs. In *Circulation, Respiration and Metabolism* (ed. R. Gilles), pp. 356–366. Berlin: Springer-Verlag.

FURUKAWA, Y. & KOBAYASHI, M. (1987). Neural control of heart beat in the African giant snail, *Achatina fulica* Ferussac. I. Identification of the heart regulatory neurones. *J. exp. Biol.* **129**, 279–293.

HILL, R. B. (1987). Conclusion: Comparative physiology of cardiovascular control. *Experientia* **43**, 994–997.

JONES, H. D. (1971). Circulatory pressures in *Helix pomatia* L. *Comp. Biochem. Physiol.* **39**A, 289–295.

JONES, H. D. (1975). Locomotion. In *Pulmonates*, vol. 1 (ed. V. Fretter & J. Peake), pp. 1–32. London, New York: Academic Press.

JONES, H. D. (1983). Circulatory systems of gastropods and bivalves. In *The Mollusca*, vol. 5, *Physiology*, part 2 (ed. A. S. M. Saleuddin & K. M. Wilbur), pp. 189–238. New York, London: Academic Press.

KOCH, U. T., KOESTER, J. & WEISS, K. R. (1984). Neuronal mediation of cardiovascular effects of food arousal in *Aplysia*. *J. Neurophysiol.* **51**, 126–135.

KOESTER, J., MAYERI, E., LIEBESWAR, G. & KANDEL, E. R. (1974). Neural control of the circulation in *Aplysia*. II. Interneurons. *J. Neurophysiol.* **37**, 476–496.

KUWASAWA, K. & HILL, R. B. (1973). Regulation of ventricular rhythmicity in the hearts of prosobranch gastropods. In *Neurobiology of Invertebrates*, Tihany, 1971 (ed. J. Salanki), pp. 143–165. Budapest: Akademiai Kiado.

KUWASAWA, K., NEAL, H. & HILL, R. B. (1975). Afferent pathways in the innervation of the ventricle of a prosobranch gastropod *Busycon canaliculatum* L. *J. comp. Physiol.* **96**, 73–83.

MANGUM, C. P. (1979). A note on blood and water mixing in large marine gastropods. *Comp. Biochem. Physiol.* **63**A, 389–391.

PROSSER, C. L. (1973). *Comparative Animal Physiology*, 3rd edn. Philadelphia: W. B. Saunders.

SATCHELL, G. H. (1971). *Circulation in Fishes*. Cambridge: Cambridge University Press.

SCHMIDT-NIELSEN, K. (1984). *Scaling: Why is Animal Size so Important*? Cambridge: Cambridge University Press.

SMITH, P. J. S. (1985*a*). Molluscan circulation: Haemodynamics and the heart. In *Circulation, Respiration, and Metabolism* (ed. R. Gilles), pp. 344–355. Berlin: Springer-Verlag.

SMITH, P. J. S. (1985*b*). Cardiac performance in response to loading pressures in *Busycon canaliculatum* (Gastropoda) and *Mercenaria mercenaria* (Bivalvia). *J. exp. Biol.* **119**, 301–320.

SMITH, P. J. S. (1987). Cardiac output in the Mollusca: Scope and regulation. *Experientia* **43**, 956–965.

SMITH, P. J. S. & HILL, R. B. (1986). Cardiac performance in response to loading pressures and perfusion with 5-hydroxytryptamine in the isolated heart of *Busycon canaliculatum* (Gastropoda, Prosobranchia). *J. exp. Biol.* **123**, 243–253.

SMITH, P. J. S. & HILL, R. B. (1987). Modulation of output from an isolated gastropod heart: effects of acetylcholine and FMRFamide. *J. exp. Biol.* **127**, 105–120.

SOMMERVILLE, B. A. (1973). The circulatory physiology of *Helix pomatia*. II. The isolated heart. *J. exp. Biol.* **59**, 283–289.

WELLS, M. J. & SMITH, P. J. S. (1987). The performance of the octopus circulatory system: A triumph of engineering over design. *Experientia* **43**, 487–499.