THE MECHANISM
OF BLOOD CIRCULATION IN *ANODONTA ANATINA* (L.)
(BIVALVIA, UNIONIDAE)

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INTRODUCTION

Early experimental studies of blood pressures in molluscs were confined almost exclusively to the heart with little consideration of the dynamics of blood flow in the peripheral circulation (Scheer, 1948). As a consequence, knowledge of the mechanism of blood circulation at this time was derived principally from functional interpretations of the morphology. More recently, modern electronic pressure-recording techniques have been used in a series of investigations into the mechanism of burrowing in various bivalves (Trueman, Brand & Davis, 1966; Trueman, 1968a) and other molluscs (Trueman, 1968b, c). The recording of high pressures in the haemocoele during movement in these investigations has substantiated the opinion, previously expressed by Chapman (1958), that the forces generated by the muscles of the body wall are far greater than those derived from the heart. As a result there is now some evidence for the suggestion that body movements are largely responsible for circulating the blood, and that the heart may only serve to maintain a sluggish circulation when the animal is at rest and the body musculature inactive (Trueman, 1966; Chapman, 1967). At the same time, however, the work on burrowing has also shown how the efficient functioning of the foot is dependent upon the retention of blood within the pedal sinus (Trueman, 1968a), and Keber's valve, situated at the exit of the pedal sinus in some bivalves (Willem & Minne, 1898; Pelseneer, 1906; Schwanecke, 1913) is obviously positioned to fulfil this function. This would suggest that the circulation of blood through the foot is actually restricted during burrowing, yet it is at this time that the bivalve is most active.

Previous work on heart pressures in bivalves includes determinations of ventricular pressure in *Anodonta cygnea* (Willem & Minne, 1898; Picken, 1937; Chadwick, 1962), and Smith & Davis (1965) have recorded ventricular pressures in 12 species of marine bivalves. There have been some comparable investigations of circulatory pressures in cephalopods (Johansen & Martin, 1962; Smith, 1962), and of the work on gastropods the relevant investigations are those of Picken (1937) on *Limnaea peregra* and *L. stagnalis*, Chadwick (1962) on *Helix* spp. and Jones (1968, 1970, 1971) on *Patella vulgata* and *Helix pomatia*. Electronic pressure transducers were used in most of these investigations but the method of preparation and size of the cannula usually resulted in considerable damage to the animal. Only Trueman (1966), working on *Mya*, has recorded ventricular and pericardial pressures in any bivalve using techniques which reduce the disturbance to the animal.

The purpose of this work was to study some of the problems of the bivalve circulatory mechanism. By using techniques which cause a minimum of damage and leave the animal free enough to be able to burrow actively it was intended particularly to determine whether body movements play an important part in blood circulation and to investigate the mechanism of venous return and diastolic refilling of the heart. *Anodonta anatina* (L.) was chosen for this study because it is a comparatively large and active species, readily available and easy to keep in the laboratory.

MATERIALS AND METHODS

Anodonta was collected from gravel pits at Catwick, E. Yorks., and kept until required in tanks containing gravel substrate and water collected from the natural habitat. The main stock was kept in large tanks in a cooled aquarium room at approximately 7 °C and these were used to maintain a smaller number of experimental animals which were kept at normal room temperature in the laboratory where all experiments were performed.

Hydrostatic pressures throughout the haemocoele were recorded by means of E. and M. Linear Core and Statham P23 BB fluid pressure transducers coupled to a multichannel pen recorder (E. and M. Instrument Co. Ltd.; Physiograph). The cannulae were short no. 20 hypodermic needles connected to this pressure transducers by a 30 cm length of 3 mm bore rubber pressure tubing. To record pressures from the heart and pericardial cavity a small hole was bored through the shell dorsal to the heart and the animal was set in molten black dissecting wax to the side of a dish and covered with water. The cannula was then lowered through this hole in the shell using a Palmer adjustable screw clamp. With the animal rigidly fixed in this manner it was possible to change the position of the needle and make consecutive recordings from the heart and pericardial cavity. Recordings of auricular pressure were more difficult to obtain and required a more lateral entry. When a good recording was obtained, the needle could be rigidly fixed to the shell and the hole sealed with wax. The cannula was then released from the clamp and the animal freed from its mounting on the side of the dish. It was then possible to record heart or pericardial pressures from freely burrowing animals. *Anodonta* with cannulae fixed into the heart by this method could be kept alive and in good condition for several weeks before the needle became blocked by a ball of amoebocytes and recording was no longer possible. To record pressures from the pedal haemocoele during burrowing it was necessary to use a specially adapted flexible cannula constructed from the tip of a hypodermic needle, cut off and bent over to form a barb, attached to a 15 cm length of very fine flexible plastic tubing of the type used for insulating electrical wires. The barbed cannula was held in forceps and plunged into the extended foot; this caused immediate retraction, but after some time the shell opened and burrowing usually commenced. The fine plastic tubing did not appear to impede the normal movements of burrowing and although the cannula frequently became blocked by the musculature, or often slipped out, many recordings were made through complete digging cycles. In this paper all pressures are expressed as centimetres of water using the tank surface as the zero reference level.

Electrocardiograms (ECG) were recorded on the pen recorder using an E. and M

'condenser-coupled Hi-Gain pre-amplifier. Electrodes were constructed from a 1 cm length of fine chlorided silver wire, insulated with Silicoset (I.C.I. Ltd.) except at the tip and soldered on to a long length of light flexible plastic-covered wire. The best results were usually obtained when both electrodes were inserted through very small holes in the shell over the heart, fixed in place with wax and insulated with Silicoset. In some recordings heart activity was monitored from the same electrodes by recording changes in impedance across the heart by means of an impedance pneumograph (Helm & Trueman, 1967). An impedance pneumograph was also used to record shell gape from electrodes attached to the shell (Hoggarth & Trueman, 1967).

<div align="center">MORPHOLOGY</div>

In order to understand the haemodynamics of *Anodonta* it was necessary to determine the course of the circulatory system and the position and structure of valves.

The diagrams showing the arterial and venous systems (Fig. 1 a, b) were constructed from dissections of several animals following the injection of rubber latex solution
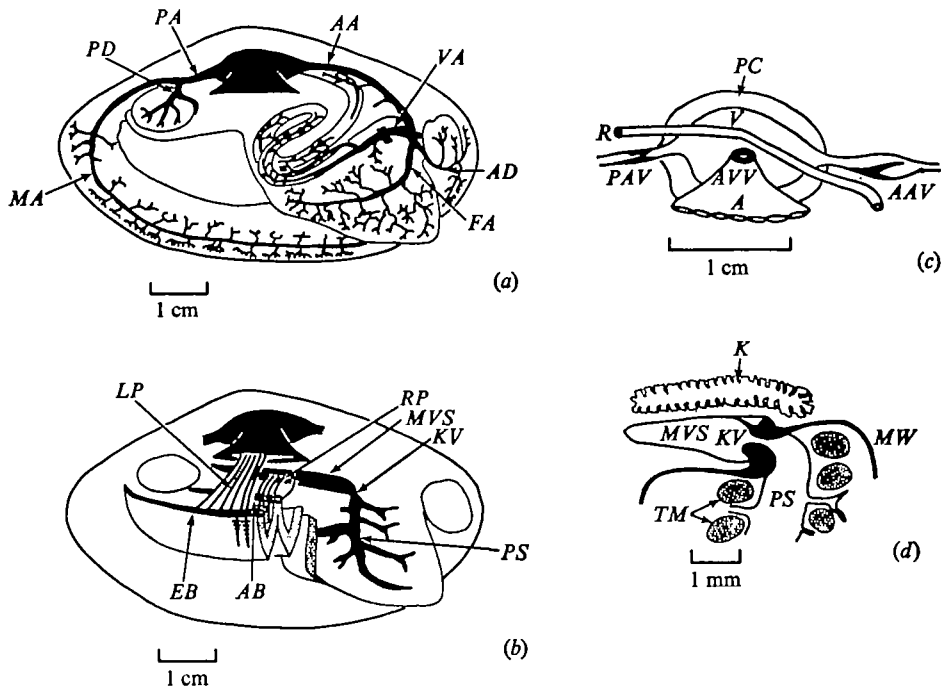

Fig. 1. Diagrams showing the course of the blood circulatory system of *Anodonta anatina*, and the position and structure of circulatory valves. All viewed from the right. (a) Stereogram showing the arterial system with the right ctenidium and mantle lobe removed. (b) Stereogram showing the venous system with the right mantle lobe and part of the right ctenidium removed. (c) Generalized diagram showing the structure of the heart and the position of circulatory valves. (d) Generalized sagittal section through Keber's valve. A, Auricle; AA, anterior aorta; AB, afferent branchial vessel; AD, anterior adductor artery; AAV, anterior aortic valve; AVV, auriculo-ventricular valve; EB, efferent branchial vessel; FA, pedal artery; K, kidney; KV, Keber's valve; LP, lateral plexus; MA, mantle artery; MW, muscular wall of foot; MVS, median ventral sinus; PA, posterior aorta; PC, pericardial cavity; PD, posterior adductor artery; PS, pedal sinus; PAV, posterior aortic valve; R, rectum; RP, renal plexus; TM, transverse muscles of foot; V, ventricle; VA, visceral artery.

(Revertex Ltd; Revultex) through the heart. Blood is pumped from the ventricle into an anterior and a posterior aorta. The anterior aorta gives off, in turn, branches to the intestine, foot, labial palps (not figured), anterior adductor muscle and mantle lobes. The visceral artery branches greatly to form a plexus of vessels around the intestine and also gives off a few small vessels which pass into the pedal musculature. The pedal artery supplies blood to all regions of the foot through a number of small branch arteries. Each branch divides many times to form a large number of very small vessels which open out into the haemocoelic spaces between the muscle fibres. The posterior aorta supplies blood to the posterior adductor muscle, then divides and continues around each mantle margin as the mantle artery and is continuous with the mantle artery given off by the anterior aorta. On the venous side of the circulation (Fig. 1b) the spaces between the muscles in the foot form a large pedal sinus. Blood from all parts of the foot is gathered into this sinus and released through Keber's valve (*KV*) into the median sinus. From here most of the blood passes laterally in a plexus of fine vessels around the kidney and is collected into the afferent branchial vessels which run longitudinally along the bases of the ctenidia. After passing through the ctenidia the blood is collected up into the longitudinal efferent branchial vessels and returned to the auricles through a lateral plexus. It is possible that some blood returns directly to the auricles from the median ventral sinus without passing through the renal plexus and ctenidia. Blood from the sinuses in the mantle is collected up laterally and also passes direct to the auricles. The course of the circulatory system of *Anodonta anatina* is very similar to that previously described for *A. cygnea* (Howes, 1885) and *A. cellensis* (Schwanecke, 1913).

The structure and position of valves in the region of the heart is shown diagrammatically in Fig. 1(c). Within the heart the auriculo-ventricular openings are surrounded by muscular lips which prevent blood being forced back into the auricles during ventricular systole. The anterior aortic valve is a thin crescent-shaped flap extending anteriorly from the muscular thickening of the wall of the blood vessel; this is a typical one-way valve, for the reflux of blood towards the heart raises the flap and occludes the aorta. Anterior to the valve the aorta is distended to form a small aortic bulb which acts as a reservoir for blood forced back towards the heart. The posterior aortic valve has a more simple structure and consists of a well-developed muscular ridge which forms a sphincter around the vessel. Keber's valve, situated between the pedal sinus and the median ventral sinus (Fig. 1b), and a valve in the siphonal artery of some siphonate bivalves (Pelseneer, 1906), are the only other circulatory valves which have been found in any bivalve. In *Anodonta anatina*, Keber's valve is a small but powerful muscular ring made up of three small blocks of muscle which fit together to form a sphincter and is shown in longitudinal section in Fig. 1(d). A small slip of muscle arises from one of the muscle blocks and is inserted on the roof of the median ventral sinus. Keber's valve has only been previously described in a few species, including *Cardium edule* (Johnstone, 1899), *Lyonsia norvegica* (as 'l'orifice bojano-pédieux' by Pelseneer, 1911), *Anodonta cygnea* (Willem & Minne, 1898) and *A. cellensis* (Schwanecke, 1913).

EXPERIMENTAL RESULTS

(a) *Pressures in the heart and pericardial cavity*

It was impossible to observe the exact position of the pressure cannulae when introduced through small holes in the shell, so pressure was correlated with simultaneous recordings of the ECG. Pressure recordings from different chambers of the heart were thus identified. The ECG typically consists of a relatively fast component
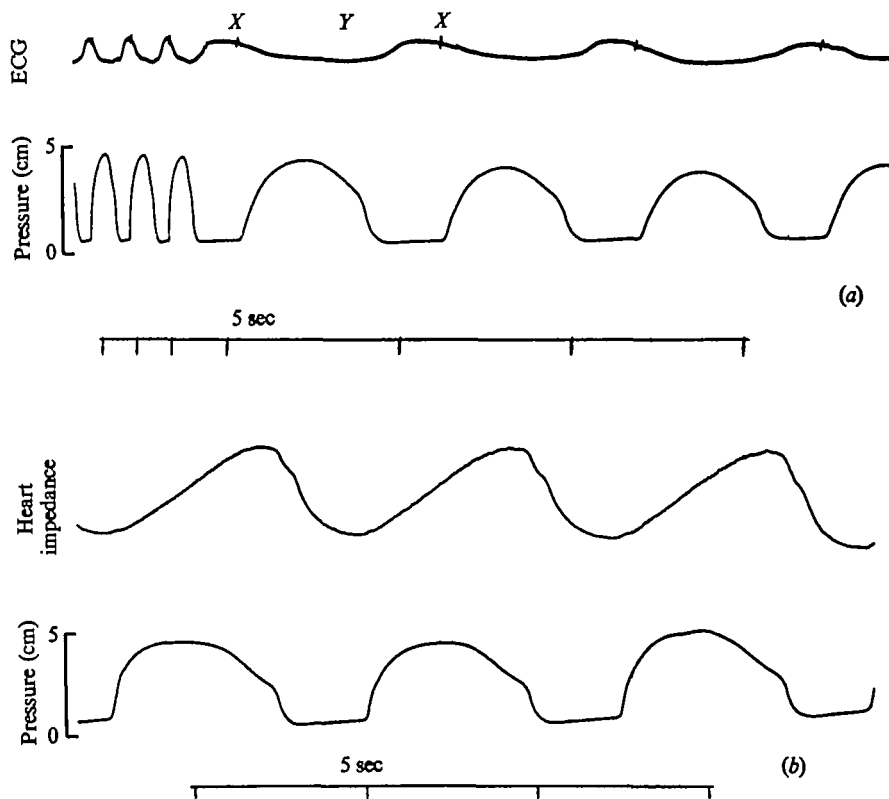

Fig. 2. Simultaneous recordings of ventricular pressure of *Anodonta anatina* with (*a*) the electrocardiogram (ECG, upper trace) and (*b*) an impedance recording of the heart beat (heart impedance, upper trace), recorded from electrodes inserted through one shell valve into the anterior and posterior ends of the pericardium. The calibration of pressure in this and subsequent figures is in centimetres of water. *X*, The fast component of the ECG which marks the beginning of ventricular systole; *Y*, the slow waves of the ECG.

(Fig. 2*a* at *X*), followed by a varying number of slow waves (*Y*). In animals with exposed hearts the fast component was observed to coincide with the beginning of ventricular systole and probably represents the initial depolarization of the heart muscle prior to contraction. This fast deflexion therefore provides a good reference point on the ECG, but unfortunately it was not always well displayed on all recordings, possibly because of differences in the placing of the electrodes. The slow waves of the ECG (*Y*) do not show the same regularity of form and cannot be related to any observable part of the heart-beat cycle. When electrodes implanted through the shell failed to give a

satisfactory ECG, connexion to the impedance pneumograph allowed heart activity to be monitored (Fig. 2b), but correlations with ventricular pressure recordings, the ECG and visual observations, showed that heart impedance recordings are more difficult to interpret reliably in terms of the phase of the heart-beat cycle.
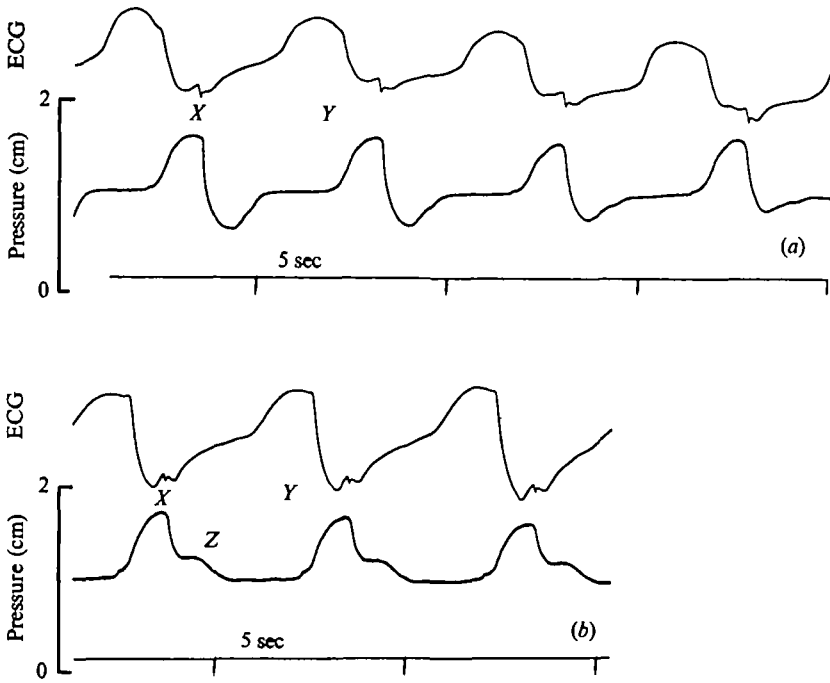

Fig. 3. Simultaneous recordings of auricular pressure of *Anodonta anatina* (lower trace) and the ECG (upper trace), showing the variation in wave-form recorded. In (a) the pressure in the auricle falls rapidly during ventricular systole (X), then rises again and shows a peak during ventricular diastole (Y). In (b) the peak of pressure during diastole is similar to that in (a), but the rapid fall in pressure at systole is interrupted by a smaller secondary peak of pressure (Z). Variations in the amplitude of the secondary peak (Z) produced wave-forms intermediate between these two conditions.

It was soon apparent that pressure recordings from the ventricle, auricles and pericardial cavity had a characteristic amplitude and wave-form and were easily distinguishable. The systolic pressure in the ventricle is usually between 2 and 4 cm in the resting animal (Fig. 2a, b) but may rise to 8 or, exceptionally, even to 10 cm during burrowing. The pressure pulse has a characteristic 'square' wave-form: the pressure rises rapidly with the contraction of the ventricle to the maximum level and then falls, slowly at first, but then more rapidly to the diastolic level. The change in rate of pressure decline produces a well-marked inflexion on the pressure pulse. During diastole there are no recordable pressure waves in the ventricle, but in most recordings a level of pressure of at least 0·5 cm is maintained. This corresponds to a similar resting pressure recorded in the pedal haemocoele and pericardial cavity (Figs. 4, 7, 8) and is a measure of the tone of the muscles of the body and subject to some variation. The pressure recorded in the ventricle during diastole may not be entirely a true resting pressure for it does rise by about 0·1 cm during the time the

ventricle is filling (Figs. 2, 4a). This indicates that the pressure produced by the auricles to fill the ventricle is low, and direct recordings of auricular pressure confirm that this is so (Fig. 3).

Auricular pressure recordings were difficult to obtain since the auricles are very thin-walled and collapse easily. Those that were obtained showed some variation in
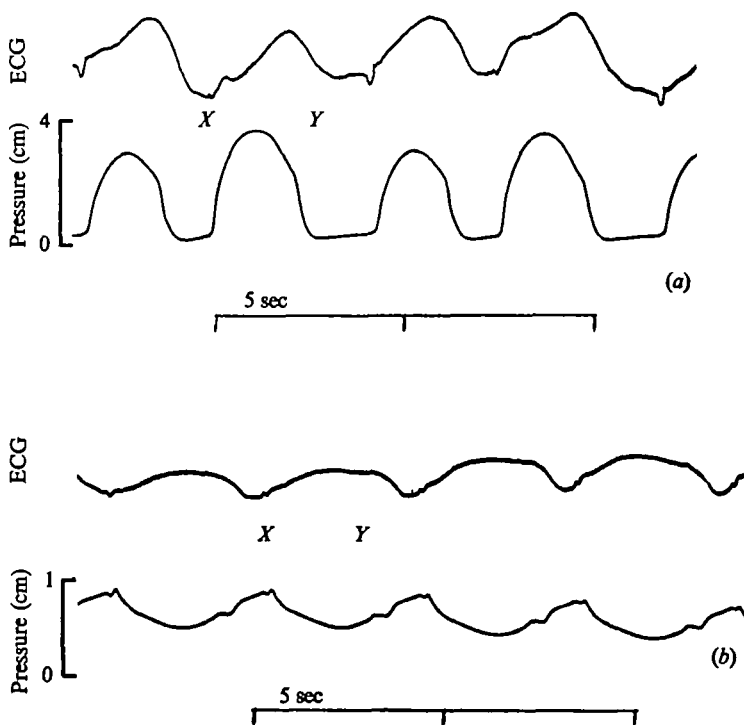

Fig. 4. Consecutive recordings of pressure from the ventricle (a) and pericardial cavity (b) of *Anodonta anatina*, recorded simultaneously with the ECG. The recording cannula was first lowered into the ventricle through a hole bored dorsally in the shell (a), then raised slightly into the pericardial cavity (b). X, Ventricular systole; Y, ventricular diastole.

wave-form but were readily distinguishable from pericardial or ventricular pressure recordings. The pressure in the auricles falls rapidly during ventricular systole (Fig. 3 at X), then rises again and produces a small peak during ventricular diastole (Y). In some recordings the rapid fall in pressure at systole was interrupted by a smaller secondary peak of pressure (Fig. 3b at Z) and variations in the amplitude of this secondary peak produced intermediate wave-forms between these two conditions.

A fall in pressure during ventricular systole also occurs in the pericardial cavity but this decline is not as rapid, nor usually as large, as in the auricles. Fig. 4 shows consecutive recordings from the ventricle and pericardial cavity of the same animal. Whereas the pressure in the ventricle increased by about 3·5 cm during systole (X), the pressure in the pericardial cavity at this time fell by about 0·5 cm and rose again during diastole (Y). The pressure pulses in the pericardial cavity are produced principally by the beating of the ventricle, for the pericardial wall, although slightly elastic,

is not contractile; the amplitude of the pericardial pressure pulses must therefore depend upon the amplitude or force of contraction of the ventricle.

The relationship of pressure changes in the ventricle, auricles and pericardial cavity during the heart-beat cycle is summarized in Fig. 5. This shows the precise alternation between the pressure pulse in the auricle and that in the ventricle, with the ventricular pulse occupying two-thirds of the duration of the heart-beat cycle. The levels of the pressure curves are typical of resting animals and indicate the probable gradients of pressure between the chambers of the heart during the heart-beat cycle.
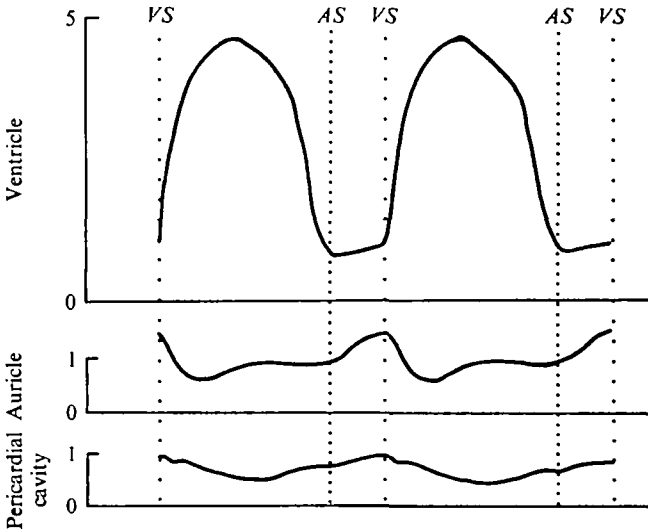

Fig. 5. Diagram summarizing typical pressure changes in the ventricle, auricles and pericardial cavity during two cycles of heart beat. *VS*, Start of ventricular systole (auricular diastole); *AS*, start of auricular systole (ventricular diastole).

However, since these gradients are very small, they could only be determined precisely by simultaneous recordings from the same animal and this was not successfully achieved. It also proved impossible to obtain satisfactory simultaneous recordings of pressure from the lateral plexus of fine vessels entering the auricles, so it was not possible to estimate the gradient of pressure between the venous system and the heart during the heart-beat cycle. However, auricular and pericardial cavity pressures were always low, with a similar base-line, and the rapid fall in pressure in these chambers during ventricular systole must exert a suction-like effect which would tend to draw blood into the auricles from the veins outside the pericardium. After the initial rapid fall the auricular pressure starts to rise quickly, and some recordings show a small secondary peak of pressure as blood enters the auricles (Fig. 3*b* at *Z*), which could indicate a small venous return pressure, but the occurrence of this peak could not be related to any observable movement of the animal. In contrast, pericardial pressure falls more slowly at ventricular systole and continues to fall until the ventricle starts to relax. Typical pressure changes in the ventricle, auricles and pericardial cavity during ventricular diastole and ventricular systole are summarized diagrammatically in Fig. 6.

It is of interest to compare the pressure curves of the *Anodonta* heart (Fig. 5),

with the classical work on the pressure curves of the mammalian heart figured in text-books of physiology (e.g. Bell, Davidson & Scarborough, 1968). The similarity in the form of the ventricular pressure curve is remarkable, and pericardial pressure curves of *Anodonta*, which are generated principally by the beating of the ventricle, show a marked resemblance to recordings of mammalian ventricular volume. This suggests a fundamental similarity in the mechanism of operation of the ventricle, and it is therefore tempting to interpret the pressure recordings of *Anodonta* by analogy with the more completely understood system of the mammal. This would suggest, for example, that the inflexion on the curve of ventricular pressure decline after systole marks the closing of the aortic valves. Auricular pressure curves show greater differences between the two groups, but this is not surprising in view of the different mechanisms responsible for filling the auricles.
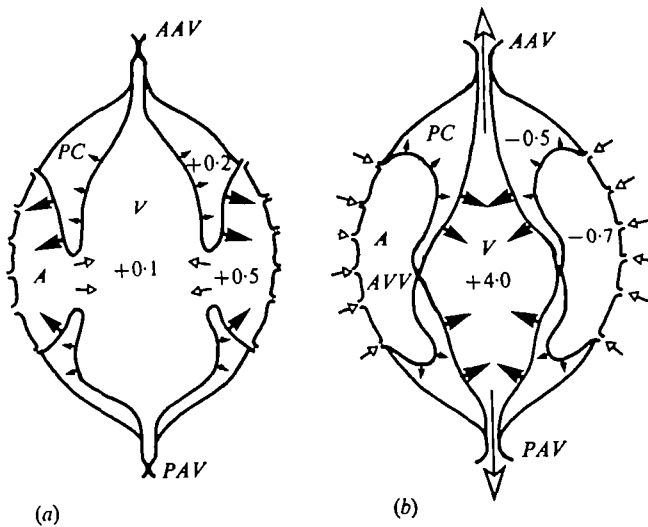

Fig. 6. Generalized diagrammatic longitudinal horizontal sections through the heart, showing movements and typical pressure changes in the chambers of the heart and pericardial cavity during (*a*) ventricular diastole and (*b*) ventricular systole. For clarity the rectum has been omitted. Large solid arrows represent the contracting chamber, small solid arrows represent the relaxing chamber and open arrows represent the flow of blood. *A*, Auricle; *AAV*, anterior aortic valve; *AVV*, auriculo-ventricular valve; *PC*, pericardial cavity; *PAV*, posterior aortic valve; *V*, ventricle.

(b) *Pressures in the pedal haemocoele, heart and pericardial cavity during body movements*

The delicate nature of the tissues, and the difficulty of accurately positioning cannulae implanted through the shell, make it extremely difficult to study blood pressures in the sinuses and vessels of the peripheral circulation. Only the foot is both easily accessible and sufficiently muscular to retain a cannula, and in this work it was only possible to obtain satisfactory recordings of peripheral blood pressure from the pedal haemocoele. By correlating the changes of pressure in the foot during body movements with those in the pericardial cavity and heart it was possible to estimate gradients of pressure which could result in the movement of blood in and out of the pedal haemocoele.

In the normal behaviour of *Anodonta* the large and powerful adductor and peda.
muscles are responsible for spontaneous adductions of the shell and postural move-
ments of the foot respectively, and during burrowing these two muscle systems are
closely co-ordinated in the movements of a digging cycle, which is essentially similar
to that previously described for some common marine bivalves by Trueman *et al.*
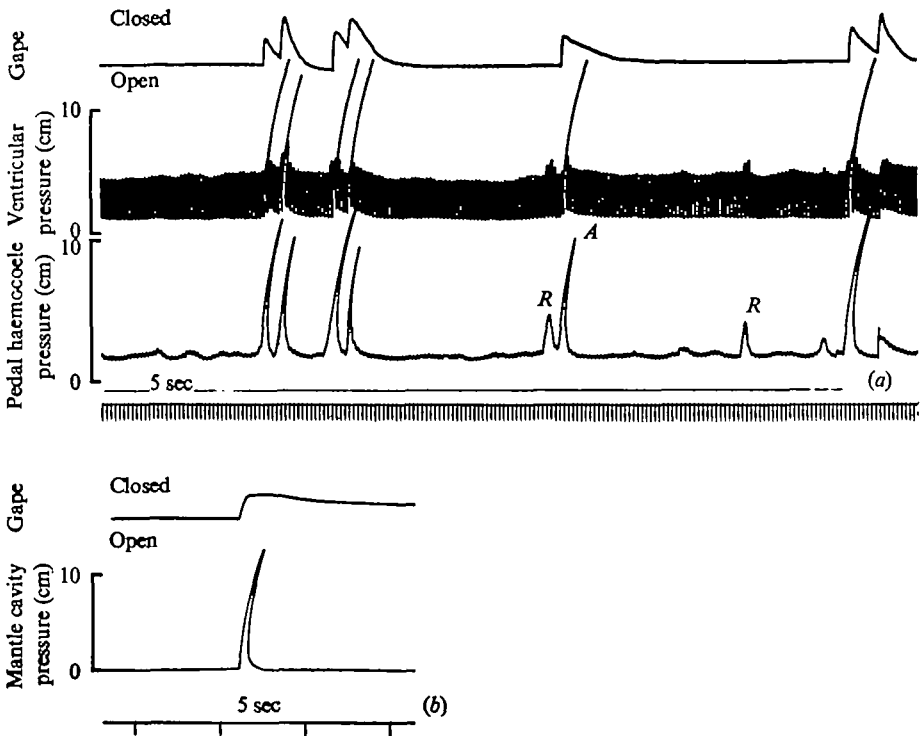

Fig. 7. (*a*) Simultaneous recording of ventricular pressure, pedal haemocoele pressure and
shell movement (gape, upper trace), showing major peaks of pressure throughout the haemo-
coele at spontaneous phasic adduction (*A*), but not during small withdrawal movements of the
foot (*R*). (*b*) Simultaneous recording of mantle cavity pressure and shell movement (gape,
upper trace) at spontaneous phasic adduction.

(1966). In all bivalves the digging cycle basically involves the extension and probing
of the foot down into the substrate, followed by adduction of the shell and retraction
of the foot drawing the shell down towards the anchored tip of the foot. Recordings
of pressure changes in the ventricle, pericardial cavity and pedal haemocoele show that
major peaks of pressure are produced throughout the haemocoele during spontaneous
phasic adductions of the shell (Figs. 7, 8) and during the adduction stage of the
digging cycle (Figs. 9, 10). Such peaks of pressure have been previously demonstrated
in many species and are responsible for pedal dilation and anchorage during burrowing
(e.g. Trueman *et al.* 1966; Trueman, 1968*a*). These pressure peaks are large; but
delicate organs like the ctenidia, kidney and heart are not subjected to their full effect,
for the simultaneous peak of pressure of nearly equal amplitude in the mantle cavity
(Fig. 7*b*) means that large differential pressures are not generated between any parts
of the animal contained within the shell.

There are, however, considerable differences in the duration of these pressure peaks in the pedal haemocoele, the pericardial cavity or heart, and the mantle cavity. The rapid rise in pressure in the heart, pericardial cavity and mantle cavity at spontaneous phasic adduction corresponds closely with the closure of the shell (Figs. 7*b*, 8). The pressure in the pedal haemocoele, however, starts to increase some 5 or 6 sec before adduction (Fig. 8*b*), and this must result from tension in the pedal muscles, for there
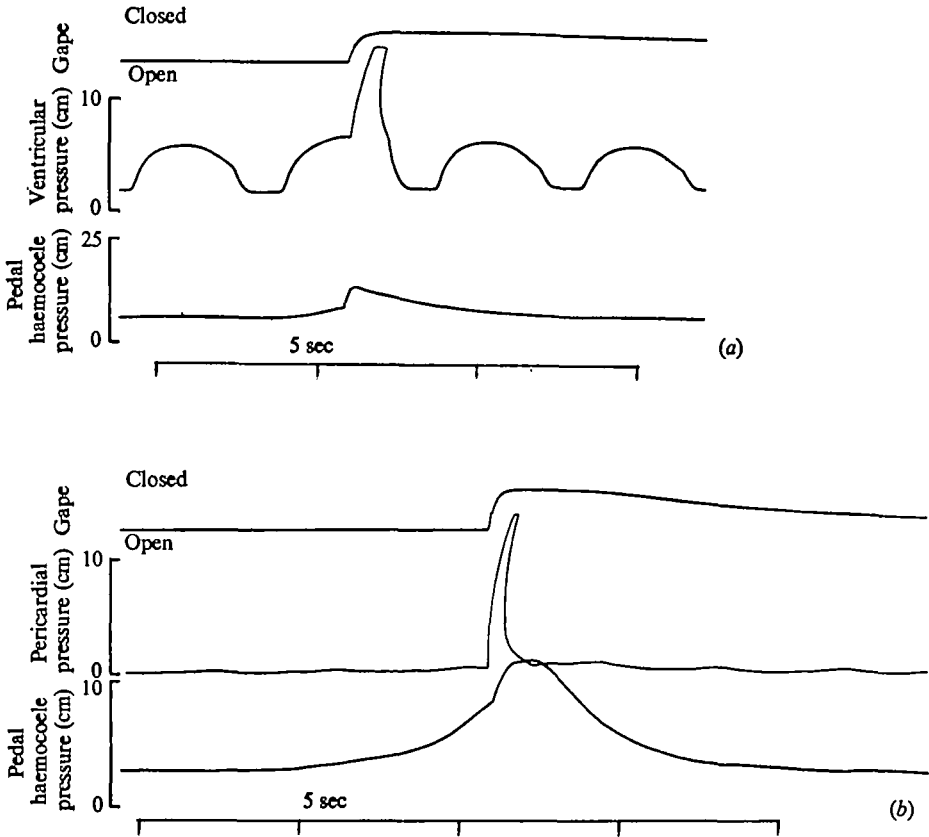

Fig. 8. Pressures recorded in the heart, pericardial cavity and pedal haemocoele of *Anodonta anatina* at spontaneous phasic shell adduction. (*a*) Simultaneous recording of ventricular pressure, pedal haemocoele pressure and shell movement (gape, upper trace) shows that major peak of pressure at adduction is of short duration in the ventricle and does not affect the rhythm of the heart beat. (*b*) Simultaneous recording of pericardial cavity pressure, pedal haemocoele pressure and shell movement (gape, upper trace). Both recordings show that pressure in the pedal haemocoele starts to increase several seconds before adduction and then falls more slowly than in the ventricle or pericardial cavity.

is no corresponding movement of the shell. After adduction the pressure falls more slowly in the pedal haemocoele than elsewhere, taking some 5–10 sec longer to return to the normal resting level. At each phasic adduction there is therefore a period of some 10–15 sec when a gradient of pressure of up to 10 cm exists between the pedal haemocoele and the heart or mantle cavity. A pressure gradient is also present during the adduction and retraction movements of the digging cycle for the multiple high-

pressure peak generated in the pedal haemocoele and heart at this time is of consider·ably longer duration than the pressure peak in the mantle cavity. Pressure recordings during burrowing also show a progressive increase in pressure in the pedal haemocoele during the extension and probing stage of the digging cycle (Fig. 9). This increase in the level of pressure is not present in recording from the heart or pericardial cavity (Fig. 10), so it is evident that a gradient of pressure between the two situations exists for some time before the adduction stage of the digging cycle (probably for at least one minute in most of the digging cycles in Fig. 9). When the level of pressure in the pedal haemocoele is greater than elsewhere, blood would be forced from the pedal haemocoele if Keber's valve was open.
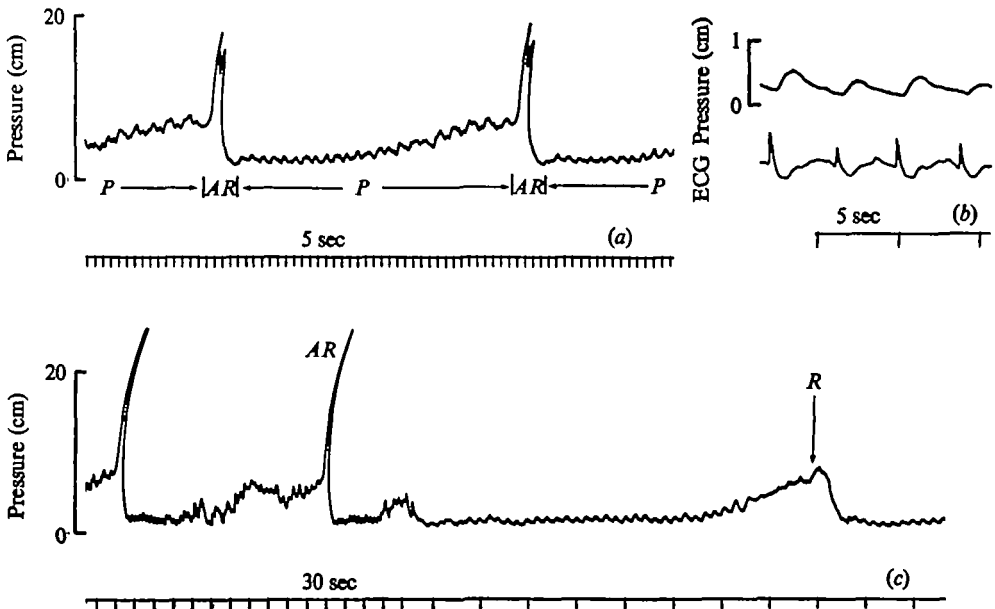

Fig. 9. Pressures recorded in the pedal haemocoele of *Anodonta anatina* during free burrowing. (a) Pressures recorded throughout the digging cycle, with the animal about one-quarter buried. The level of pressure rises slowly during the stage of pedal extension (*P*), with major peaks occurring only during the adduction and retraction movements (*AR*). (b) Simultaneous recording showing the correspondence of small peaks of pressure in the pedal haemocoele (upper trace) to the ECG (lower trace). (c) One normal digging cycle showing the major peak of pressure during the adduction and retraction movements (*AR*), followed by the withdrawal of the foot back into the shell (*R*).

Ventricular pressure recordings, however, show no evidence of an increased venous return to the heart following movements of the shell, or of the foot. The major peaks of pressure at adduction have very little effect on the rhythms or rate of beat of the ventricle (Fig. 8a), for they affect the pressure throughout the bivalve, and the small increase in diastolic pressure observed after adduction represents an increase in the resting pressure throughout the animal, there being a comparable increase in the pedal haemocoele (Fig. 7a). Retractions of the foot, which occur without movement of the shell (Fig. 7a at *R*), produce a small increase in pressure in the pedal haemocoele but do not increase the diastolic pressure in the ventricle. Similarly, during burrowing, there is no indication of an increased diastolic pressure in the heart. In fact, the

diastolic pressure characteristically falls during pedal extension (Fig. 10*b*) and, since pedal haemocoele pressure at this time is rising, this fall would seem to indicate that Keber's valve is restricting the flow of blood from the pedal haemocoele. During the adduction and retraction movements which follow it is of great functional importance that Keber's valve remains closed, both to protect the ctenidia from a surge of blood occasioned by the rapid fall in mantle cavity pressure, and to retain blood in the foot in order to maintain the anchorage necessary during pedal retraction (Trueman, 1968*a*).
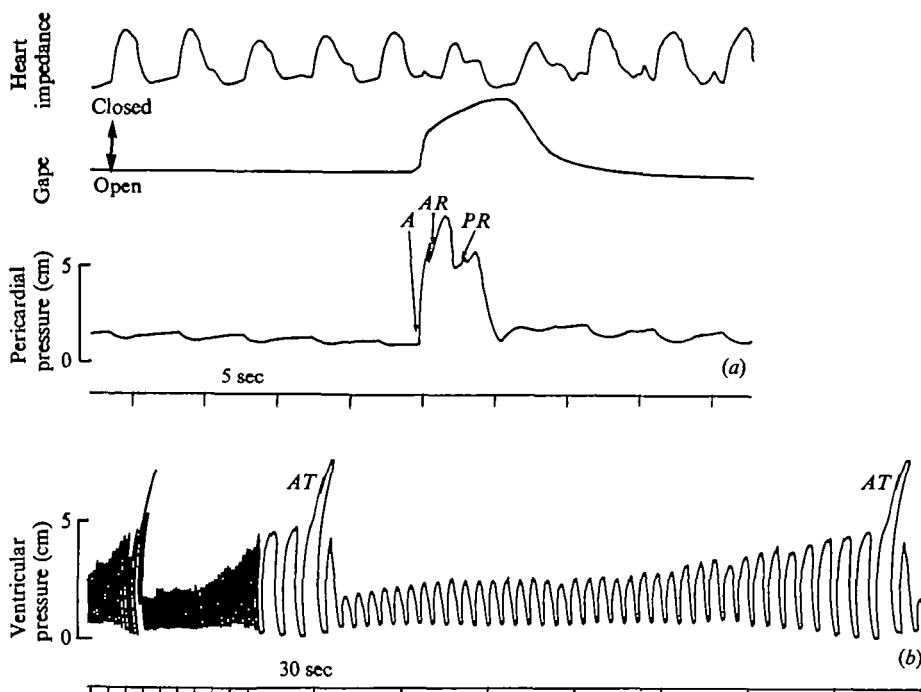

Fig. 10. Pressures recorded in the pericardial cavity and ventricle of *Anodonta anatina* during free burrowing. (*a*) Simultaneous recording of pericardial cavity pressure (lower trace), shell gape (middle trace) and an impedance recording of heart beat (heart impedance, upper trace) from electrodes inserted through one shell valve into the anterior and posterior ends of the pericardium, showing the form of the multiple pressure wave during the adduction and retraction movements of the digging cycle. *A*, Adduction; *AR*, anterior retractor muscles contract; *PR*, posterior retractor muscles contract. (*b*) Ventricular pressure recorded during two complete digging cycles, showing the progressive increase in systolic pressure and fall in diastolic pressure during the probing stage of the digging cycle, prior to the adduction and retraction movements (*AT*).

Recordings of pressure in the pedal haemocoele (Figs. 7, 9) all show systolic pressure pulses superimposed upon the fluctuating level of pressure produced by the musculature. These systolic pressure waves are evident at all stages of the digging cycle, except during the high-pressure peaks at adduction and retraction when they would, in any case, be difficult to detect. During the pedal extension and probing stage of the digging cycle the level of pressure in the pedal haemocoele rises from 2–4 cm to between 6 and 10 cm (Fig. 9), but there is a corresponding increase in ventricular systolic pressures (Fig. 10*b*). A similar increase in systolic pressure accompanies the

increase in pedal haemocoele pressure during small withdrawal movements of the foot (Fig. 7). While the presence of systolic pressure pulses in the foot does not prove that blood is actually entering the pedal haemocoele, the amplitude of the increased ventricular systolic pressures, and the high amplitude of the pressure pulses in the pedal haemocoele in some recordings, suggest that systolic pressures are high enough to force blood into the foot at all times during the extension and probing stage of the digging cycle.

DISCUSSION

The recordings of pressure in the pedal haemocoele, heart and pericardial cavity of freely burrowing *Anodonta* confirm the conclusions drawn from previous work carried out on *Margaritifera* and *Ensis* under conditions which restricted free movement (Trueman, 1967, 1968a). Relatively low pressures are produced in the pedal haemocoele during extension and probing movements of the foot, and large peaks of pressure are only generated at the adduction and retraction movements of the digging cycle and at spontaneous phasic adduction. Since these peaks of pressure are generated equally throughout all parts of the animal enclosed within the shell, including the mantle cavity, they do not create large gradients of pressure in the haemocoele and can have little effect upon the movement of blood, except within the extended foot where they may cause dilation or extension.

The gradual build-up of pressure in the pedal haemocoele during the extension and probing movements of the foot increases the arterial resistance and this evokes a more powerful heart beat, so that ventricular systolic pressures increase from the normal resting level of between 2 and 4 cm to as high as 8 or even 10 cm. These ventricular pressures are considerably higher than those previously reported for other bivalves, possibly because they were recorded under more natural conditions. Systolic pressures of 2 cm (Chadwick, 1962), 3 cm (Willem & Minne, 1898) and 5 cm (Picken, 1937) have been determined for *Anodonta cygnea*, and Smith & Davis (1965) list the mean systolic pressure of 12 species of marine bivalve, which range from 0·25 cm in *Hinnites giganteus* to 2·47 cm in *Mytilus californianus*, with most species producing less than 2 cm. Trueman (1966) found the systolic pressure of *Mya* was normally about 1·5 cm and the maximum recorded value was 2·5 cm. In other molluscs systolic pressures have been reported to be about 3 cm in the resting limpet (Jones, 1970), 24 cm in resting *Helix* (Jones, 1971) and as high as 60 cm in the large cephalopod *Octopus dofleini* (Johansen & Martin, 1962). Both Hill & Welsh (1966) and Jones (1970) comment on the obvious general correlation between heart pressures and the level of activity in different groups of molluscs. The present study has shown that *Anodonta* has a considerable ability to adapt its heart pressures according to the requirements of its own level of activity, and the increased ventricular pressure during burrowing is probably sufficient to supply blood to the foot throughout most of the digging cycle. Since it is most unlikely that blood ever enters the foot through Keber's valve, arterial pressure generated by the heart is the only mechanism responsible for the movement of blood into the pedal haemocoele, although some adjustment in the distribution of blood in the foot occurs during movement. Local increases in hydrostatic pressure in other sinuses and vessels are unlikely to exceed those produced in the foot during burrowing, so systolic pressure is normally sufficient to

supply blood to all parts of the arterial system. While this must also be generally applicable to other bivalves, one notable exception must be the siphonal vessels of species with very long siphons. In *Mya*, for example, it is doubtful if the heart can effectively maintain a circulation of blood through the extended siphons for they may extend for considerably more than 10 cm above the shell (Trueman, 1966).

The mechanism responsible for the return of blood to the heart from the pedal or any other sinus is not easy to determine, for it is difficult to distinguish between general increases in hydrostatic pressure and increases in venous pressure. Sensitive pressure-recording methods, preferably using differential pressure techniques, are necessary to determine accurately gradients of pressure; in the absence of such studies in the Mollusca there has been much controversy regarding the mechanism of venous return and, in particular, the mechanism of refilling the heart. A number of previous workers have proposed a volume-compensating mechanism, where blood is drawn into the auricles to compensate for that pumped from the ventricle during systole (Ramsay, 1952; Krijgsman & Divaris, 1955). The successful operation of this mechanism depends upon the pericardial cavity maintaining a more-or-less constant volume throughout the heart-beat cycle, and the auricles and ventricle maintaining a regular alternation of beat.

Chadwick (1962) argued against such a mechanism in the gastropod *Helix* on the grounds that, in this genus, the pericardial wall is not rigid but moves with the heart beat, the total volume of the auricles and ventricle can vary considerably, and there is not always a precise alternation of beat between the chambers. She also considered that the large reno-pericardial opening of many molluscs would interfere with any volume-compensating mechanism. These objections have been recently refuted by Jones (1970, 1971), who obtained some confirmatory evidence of a volume-compensating mechanism when he recorded a reduction in pressure in the pericardial cavity and auricle during ventricular systole in *Patella* and in *Helix*, and he suggested that outflow through the reno-pericardial opening may actually be essential to maintain the pressure in the pericardial cavity at a lower level than in the auricle or ventricle.

However, while this recent evidence supports the operation of a so-called 'constant volume' mechanism in the Gastropoda under resting conditions, both Chadwick (1962) and Jullien & Ripplinger (1953) have recorded large venous return pressures which would serve to fill the heart and are likely to be more important during activity. In the Bivalvia the evidence strongly favours an important role for the volume-compensating mechanism and large venous return pressures have not been reported. The pericardial wall is well supported by the shell in this class, and the precise alternation of beat between the auricles and the ventricle has often been noted (Koch, 1917; Smith & Davis, 1965).

In the present study on *Anodonta* the precise alternation of beat was confirmed, and although the small gradients of pressure within the heart during the heart-beat cycle could not be accurately determined, a marked fall in pressure, to a level which was probably below the general body pressure, was demonstrated in the pericardium and the auricle during systole, and this would tend to draw blood into the auricles from the veins outside the pericardial cavity. A similar reduction in pericardial pressure has also been noted in *Mya* (Trueman, 1966). In addition, it was found that heart action of *Anodonta* was affected by cutting the pericardial wall. Whereas systole

usually occupied about two-thirds of the duration of the heart-beat cycle, after cutting the pericardium the duration of diastole increased so that it took longer than systole. Compared with recordings of *Helix* (Chadwick, 1962), the heart rhythm of *Anodonta anatina* shows little variation with body movements. The heart of *Anodonta* is therefore less dependent upon a high venous pressure for maintaining the normal rhythm, and the volume-compensating mechanism is usually sufficient to refill the heart and maintain circulation.

The same mechanism also assists the refilling of the ventricle. Since there are no valves between the auricles and the venous system, some blood may be forced back into the veins when the auricles contract, but this backflow is likely to be small for, with the aortic valves closed at this time, the volume-compensating mechanism will act to resist the movement of blood from within the pericardium and favour the transfer of blood from auricles to ventricle. In addition, the close proximity in the venous system of the lateral plexus and ctenidia means that a high resistance to the backflow of blood soon builds up.

Accessory hearts have been described in the mantle of *Crassostrea gigas* and *C. virginica* (Hopkins, 1934, 1936; Eble, 1958), and these serve to receive blood from the renal vessels and pump it on to the ctenidia and other parts of the mantle. Other pulsatile organs with a similar function may be discovered in other bivalves but are obviously not of widespread occurrence. Local increases in pressure resulting from movements of the body will, of course, cause the movement of blood in some parts of the peripheral circulation and undoubtedly aid the venous circulation of blood. In *Anodonta* a pressure gradient which could result in the movement of blood out of the pedal haemocoele is present throughout most of the digging cycle and, more briefly, at spontaneous phasic adduction. During burrowing there are strong functional reasons for retaining blood within the pedal haemocoele (Trueman, 1968*a*) and the reduction in diastolic pressure in the ventricle and pericardial cavity towards the end of the digging cycle, recorded in the present investigation, suggests that the closure of Keber's valve at this time is restricting the amount of blood returning to the heart. At spontaneous phasic adduction there is no similar evidence to suggest that Keber's valve is closed (although the duration of this event may not be long enough to affect diastolic pressure), or any functional reason why it should be. The gradient of pressure is set up several seconds before adduction of the shell valves, and this could only be caused by an increase in tension in the pedal muscles which has not been previously noticed to occur at this time in any species. It seems possible that, in addition to ejecting faeces and pseudo-faeces and replenishing the water in the mantle cavity, phasic adductions of the shell, co-ordinated with contractions of the pedal muscles, may also periodically pump blood from the pedal sinus on through the venous system. It has been suggested that phasic adductions of the shell valves are also responsible for pumping the fluid contents of the stomach into the digestive diverticula (Morton, 1969), but this possibility requires further investigation since the stomach and digestive diverticula are in close proximity and may not experience a pressure gradient. Recordings of heart and pericardial pressures show no indication of an increased diastolic pressure following movements of the shell, or of the foot, but this is not surprising for in *Anodonta* most of the blood leaving the pedal haemocoele passes through the renal plexus, the ctenidia and the lateral plexus before returning to the heart,

and these vessels would themselves present a high resistance to blood flow. The form of the circulatory system of *Anodonta* therefore effectively isolates the heart from the changes in pressure which occur in the pedal haemocoele, and although body movements may assist the movement of blood through parts of the peripheral circulation, including the movement of blood from the pedal haemocoele, they do not contribute directly to a high venous return pressure. In more active molluscs the effect of body movements on circulation is likely to be greater. The heart is relatively reduced in size and unlikely to play an important part in circulating blood in the highly active *Ensis* (Trueman, 1967), and entirely absent from the scaphopod *Dentalium*, where rhythmic locomotory and feeding movements are thought to maintain the circulation (Morton & Yonge, 1964).

The circulatory system of *Anodonta* is relatively simple, for the posterior aorta only supplies blood to the posterior adductor muscle and the mantle. The backflow of blood through the posterior aorta during contractions of the adductor muscle or mantle is unlikely to be large and, since the posterior aortic valve is morphologically suitable for stopping blood flow in either direction, it could also serve to channel all available blood into the anterior aorta during burrowing and therefore increase the blood supply to the foot. In the siphonate bivalves the form of the circulatory system is more complex, with an additional and important blood system through the siphons. Bivalves of the families Veneridae, Petricolidae, Tridacnidae and Mactridae often possess a highly developed posterior aortic bulb contained within the pericardial cavity (Pelseneer, 1906). Blood is presumably forced back into this bulb when the siphons retract, and this must seriously affect the volume-compensating mechanism and prevent or reduce the movement of blood through the heart. It would therefore be of great interest to make a comparative investigation into the effects of body movements on blood circulation in these bivalves.

SUMMARY

1. The structure of the blood circulatory system of *Anodonta anatina* is described and the haemodynamics have been investigated by recording pressures in the ventricle, auricle, pericardial cavity and pedal haemocoele at rest and during burrowing.

2. Ventricular systolic pressure is usually 2–4 cm in the resting animal; during burrowing it increases to between 6 and 10 cm and this is sufficient to maintain the blood supply to the foot for most of the digging cycle.

3. Auricular and pericardial cavity pressures fall rapidly (by about 1·0 cm) during ventricular systole, confirming the operation of a volume-compensating mechanism for refilling the heart.

4. High peaks of pressure at spontaneous phasic adduction and during the adduction and retraction movements of the digging cycle are generated equally throughout all parts of the animal enclosed within the shell and do not create large gradients of pressure in the haemocoele; the longer duration of these pressure peaks in the pedal haemocoele produces small transient gradients of pressure which could result in the movement of blood out of the pedal haemocoele.

5. At spontaneous phasic adduction contraction of the pedal muscles may assist

the flow of blood from the pedal haemocoele. There is some evidence that Keber's valve limits blood flow from the pedal haemocoele during active burrowing.

6. Although body movements may assist the movement of blood through parts of the peripheral circulation, they do not generate a high venous return pressure. The form of the circulatory system effectively isolates the heart from pressure changes in the pedal haemocoele.

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