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Fine structure, mechanism of heart function and haemodynamics in the prosobranch gastropod mollusc *Littorina littorea* (L.)

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Summary. The heart, main blood vessels, and associated structures of *Littorina littorea* were examined by scanning and transmission electron microscopy. The auricle is subdivided into two compartments, one receiving blood from the gill and opening to the nephridial gland vein, the other connecting with the latter anteriorly and the ventricle posteriorly.

Video recordings were made of the beating heart in vivo and revealed that the auricle expelled blood not only to the ventricle, but also the nephridial gland vein at systole and provided further evidence of tidal flow of blood in the vein. There is clear indication that the constant volume mechanism of auricular re-filling is not strictly true in *Littorina*.

Blood pressure in the heart and major vessels was measured using a servo-nulling micropressure system. The rate of formation of urine (derived by filtration of blood through the auricular wall) was measured using [^{51}Cr] EDTA as a blood marker.

Basal blood pressure was slightly above ambient (0.7 cm H_2O). Peak systolic pressure in the ventricle (3.8 cm H_2O) was synchronised with a subambient trough in pericardial pressure (−1.0 cm H_2O); these pressure pulses were out of phase with that of the auricle (2.3 cm H_2O) at systole. The observations are consistent in broad terms with a constant volume mechanism, but this does not take into account urine formation or filling of the nephridial gland vein.

A filtration pressure of 1.5 cm H_2O has been demonstrated across the auricular wall throughout the cardiac cycle. Colloidal back pressure appears to be negligible. The mean rate of urine formation is 0.26 µl g^{-1} min^{-1}.

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Introduction

It is generally accepted that the systemic heart in prosobranch gastropods, as in other molluscs, is responsible for unidirectional circulation of blood, though muscular contraction may affect blood flow locally in such regions as the pedal and cephalic haemocoels. The low venous return pressure, insufficient to refill the auricle at diastole, is thought to be coupled with a constant volume mechanism (Ramsay 1952; Krijgsman and Divaris 1955), in which reduction of pericardial fluid pressure at ventricular systole sucks blood into the expanding auricle, as shown in the archaeogastropods *Patella vulgata* L. (Jones 1970) and *Haliotis corrugata* Wood (Bourne and Redmond 1977). Strict adherence to the principle has been challenged, however, by Sommerville (1973), working on *Helix*. The possible effect on a constant volume mechanism of a site of urine formation in the heart has not been considered.

Blood pressure and circulatory pressure gradients in prosobranchs are lower than in terrestrial pulmonates, whilst blood volume and (unexpectedly) peripheral resistance are high (Jones 1983). Kamel (1979), cited by Jones, calculated that circulation time in *Littorina littorea* is about 19.8 min, compared with an estimated 4.28–6.38 min in *Helix pomatia*.

There are several aspects of the functioning of the blood system in prosobranchs which have yet to be adequately examined. Recent ultrastructural evidence (Andrews 1976, 1981) strongly supports the view that primary urine (=pericardial fluid)

is formed by filtration of blood through part of the auricular wall, though there is no conclusive experimental evidence that the auricle can generate an effective filtration pressure. Andrews (1979), in a study of the freshwater mesogastropod *Viviparus*, suggested how filtration may be achieved in a way largely compatible with the constant volume hypothesis, but this proposal is yet to be tested experimentally. The role of the renopericardial canal is believed to be important in maintaining dynamic equilibrium (Potts 1968; Jones 1971; Civil and Thompson 1972; Andrews 1979) but is poorly understood and needs further investigation.

A survey of the fine structure and function of the excretory system of archaeogastropods (Andrews 1985) has corroborated earlier evidence (Crofts 1929; Fretter 1964, 1966) of tidal, as distinct from unidirectional flow of blood in the vein connecting the left kidney with the left auricle in most archaeogastropods. This vessel is the homologue of the so-called efferent vein of the nephridial gland of the single (left) kidney of meso- and neogastropods (Fretter and Graham 1962), in which Andrews (unpublished observations) has also seen indications of tidal flow. The excretory dorsal wall of the kidney in these monotocardians has a renal portal system, through which most of the venous blood passes before reaching the mantle skirt and ctenidium. Oxygenated post-branchial blood from the efferent ctenidial vein may therefore be modified to a greater or lesser extent in the nephridial gland by travelling to and fro between auricle and gland along the single nephridial gland vein before entering the systemic circulation. The physiological implications discussed by Andrews (1985) in relation to diotocardians therefore merit consideration in relation to monotocardians.

In this study of *L. littorea*, these problems have been approached in three different ways: examination of the fine structure of the heart and associated structures by scanning and transmission electron microscopy and by injection of latex; observation and analysis of video recordings of the beating heart in vivo; and experimental investigation including measurement of hydrostatic pressures in the heart, pericardial cavity and various blood vessels. Measurements of blood pressure in the beating ventricle of *Littorina* by Kamel (1979) gave a mean pulse pressure of 3.3 cm H_2O ($\approx$323 Pa), but he used a passive transduction system, the technical limitations of which precluded recording from less robust sites. In the present study, the use of an active micropressure system has made possible recording from within both auricle and pericardial cavity for the first time.

Materials and methods

Snails for experimental work were collected from the shore at Rottingdean, Sussex. They were supplemented for some structural observations by specimens supplied by the University Marine Station, Millport, Cumbrae. Parasitised snails were rejected according to the method of Jones and Kamel (1984) and healthy animals were kept in re-circulating marine aquaria at 34.5% salinity.

Anatomical techniques. Snails were examined either without narcotisation or after immersion overnight in equal parts of sea water and 7.5% magnesium chloride.

Latex casts of the heart and main veins were made by injecting the efferent ctenidial and nephridial gland veins, subsequently hardened in ammonia. Injected specimens were examined with a stereomicroscope and the tissue was then digested in aqueous potassium hydroxide. Casts were critical-point dried and coated with gold-palladium for scanning electron microscopy. Some injected specimens were fixed in glutaraldehyde and dissected to trace the latex.

The volume of the nephridial gland vein and some of its branches was estimated in two snails, after obtaining their wet weights, by weighing hardened casts and calculating their original volume from the weight after hardening of a known volume of latex.

Other snails were dissected for scanning and transmission electron microscopy. Tissues were fixed in 2.5% glutaraldehyde in 0.1 *M* Sørensen's phosphate buffer, pH 7.2, containing 14% sucrose, followed by post-fixation in 1% osmium tetroxide in the same buffer. Specimens were dehydrated in ethanol, critical-point dried and sputter-coated with gold-palladium for scanning or embedded in Araldite, sectioned and stained in aqueous uranyl acetate and Reynolds' lead citrate for transmission electron microscopy.

Serial sections of snails fixed in Bouin's or Stieve's fixative were stained in Heidenhain's iron haematoxylin or Mallory's triple stain for optical microscopy. The Spirit blue method of Elder and Owen (1967) was used to corroborate ultrastructural identification of elastic fibres.

Video recordings were made of the beating heart in snails, from which the shell over part of the mantle cavity and body whorl was removed, leaving the columellar muscle still attached. The pericardium was intact and the heart was visible by transparency. The snail was covered by sea water and usually attached to the dish with its foot, remaining quiescent for long periods. A Zeiss SV8 stereomicroscope and Hitachi DK81 colour camera were used for filming, and a Mitsubishi video copy processor was used to analyse the recordings.

Experimental methods. Experiments were carried out at 12°–15 °C. Results are expressed as the mean ± 1 standard error (SEM); *N* is the number of experimental animals.

Fluid pressures. Blood and pericardial fluid (PCF) pressures were measured with pressure transducers (Elcomatic EM751), using either a passive or an active (micropressure) system, in which a steel needle (25G hypodermic; $\approx$0.5 mm o.d.) or a glass micropipette ($\approx$5 μm o.d.) respectively, acted as the pressure probe. Hydraulic couplings were made using 1.2 mm i.d. Portex tubing. The experimental animal was restrained on a bed of plasticine in an open chamber. The renopericardial complex was exposed by cutting a window in the overlying shell using a dental burr and the chamber was flooded with sea water. The pressure probe, mounted on a micromanipulator, was manoeuvred into position with the aid of a microscope.

The passive pressure system consisted of a needle probe

coupled to a transducer with degassed sea water, and was used to record PCF pressure. The needle tip was sealed into the pericardium with a small quantity of tissue cement (Histoacryl; Braun Melsungen A.G.). In paired recordings the active servo-nulling micropressure system (Riegel 1986a) was used to measure blood pressure in the heart and selected veins, as it was much less invasive of these delicate recording sites than the passive system and required no sealant. The micropipette probes were pulled from 1.2 mm o.d. capillary tubing, and had tips (initial diameter about 5 μm) bevelled with alumina abrasive-film (0.3 μm grain) using a record turntable with tone-arm modified as a micropipette holder. Bevelled micropipettes were filled with dilute saline (3 m*M* NaCl) having a higher electrical resistance than *Littorina* body fluid. The micropressure system operates to prevent displacement of the saline/body fluid interface at a probe tip by generating hydraulic counter-pressure via a servo-feedback system. The position of the interface is determined from the probe tip resistance, which is inversely proportional to the degree of fluid displacement from the tip. An alternating current is generated in the probe electrical circuit by a 465 Hz oscillator and probe resistance is measured using an ac bridge in conjunction with a negative capacitance amplifier. Pressure is generated in the hydraulic circuit by a pump (Vibrator 201; Ling Dynamic Systems), through a Neoprene diaphragm, to maintain resistance at a preset value (5–10 mΩ to ac) somewhat lower than maximum; this enables subambient pressure to be recorded. The generated pressure, equal to the fluid pressure near the probe tip, is measured by a transducer. Hydraulic fluid was degassed before use; liquid paraffin was interfaced with saline in the hydraulic circuit to afford protective electrical insulation for the pump. Micropipettes were filled with 3 m*M* NaCl in preference to the usual 1–3 *M* KCl because preliminary experiments indicated that, for our requirements, a high resistance pressure probe (within the capacitance balance limits of the system) afforded the greater measurement sensitivity.

Pressure transducer output was recorded on a Servogor 460-02 multi-pen chart recorder (Servoscript Ltd) and calibrated for each experiment by subjecting the pressure probe to standard heads of sea water in a test chamber. Results are expressed in terms of cm H_2O (1 cm H_2O = 98.1 Pa); zero pressure is that ambient at the level of the renopericardial complex. Pressure response was linear in the range studied (-3 to 10 cm H_2O) and there was no loss of amplitude at observed frequencies of pressure oscillation (<1.5 Hz). Performance characteristics of the micropressure system outside the quoted ranges of pressure and frequency were not investigated. Measurement accuracy was ± 0.05 cm H_2O for both systems.

Body fluids. Samples of blood and PCF (5–10 μl) were taken from the exposed renopericardial complex using glass micropipettes; blood was taken from the ventricle. Protein concentration was measured by the method of Lowry et al. (1951) using bovine serum albumin as a standard. Colloidal osmotic pressure (COP) was estimated using a membrane electro-osmometer (Riegel 1986a) fitted with a synthetic 10000 Da filter (Millipore PTGC); COP standards were prepared with Ficoll 70 (Pharmacia).

Blood volume and rate of urine formation. Blood volume (BV) was measured using [^{51}Cr] EDTA (Amersham International). Co-identity of marker distribution space with BV was assumed. The rate of [^{51}Cr] EDTA clearance from the blood was also measured, enabling urine filtration rate to be calculated.

The marker was injected into the pedal haemocoel in 10 μl phosphate buffer (pH 7.6) using a microsyringe. Animals were then placed in fresh, aerated medium for 1.5 h to recover from the injection, after which time the marker was fully distributed throughout the extracellular fluid (Jones and Kamel 1984). A rapid distribution of injected marker is to be expected in intact animals where the integrity of the circulatory system is maintained.

^{51}Cr-activity (counts min^{-1}; CPM) was determined using an LKB 1275 minigamma counter: counting efficiency was 33% throughout. Approximately 9 kBq of [^{51}Cr] EDTA were injected into a snail, and the rate of clearance was estimated from the rate of appearance of radioactivity in the medium. The recovered experimental animal was maintained in 25 ml sea water, from which 2 ml samples were withdrawn at intervals (for a period of up to 30 h) and their ^{51}Cr-activity determined, to be replaced immediately by 2 ml fresh medium. The quantity of [^{51}Cr] EDTA lost by an animal between successive samples of the medium was determined from sample counts after the (diluted) residual radioactivity had been subtracted. At the end of the experiment the [^{51}Cr] EDTA concentrations (CPM ml^{-1}) of the blood (Cb) and urine (Cu) were determined from 10 μl samples in 2 ml sea water. The animal was then removed from its shell, [^{51}Cr] EDTA was extracted from the crushed, dry flesh with 2 ml distilled water and the radioactivity of the extract measured; when added to the blood sample activity this gave the residual ^{51}Cr-activity of the animal (Qr; CPM). The initial animal radioactivity (Qi; CPM) was calculated as the sum of Qr and the cumulative ^{51}Cr losses over the experimental period; hence, animal radioactivity at each sampling time t (Qt; CPM) was derived. The rate constant for clearance, k(h^{-1}), was obtained as the slope of a plot of ln(Qt/Qi) against time (h). BV (ml g^{-1} flesh) was calculated as Qr/Cb D, where D is the flesh weight (g). BV measured 1.5 h and 30 h post [^{51}Cr] EDTA injection did not differ significantly from one another, indicating that EDTA penetration of cells was not significant over the experimental period adopted. The rate of clearance (Vcl; ml g^{-1} h^{-1}), which we assume reflects the rate of filtration of urine, was estimated from the relation $\mathrm{Vcl} = k$. BV and the rate of urine production (Vu; ml g^{-1} h^{-1}) was obtained as $\mathrm{Vu} = \mathrm{Vcl} \cdot \mathrm{Cb}/\mathrm{Cu}$.

Results

Fine structure of the heart and associated blood vessels

The blood system of *Littorina* (Fig. 1) is a single circulation with a systemic heart. The latter is connected to the nephridial gland of the kidney by a shunt along which post-branchial blood travels for modification before entering the systemic circulation. This arrangement is made possible by a complexity of organisation in the single (left) auricle impossible to resolve without the aid of a scanning electron microscope. Its structure is further complicated by the location in part of its wall of the site of formation of primary urine.

The auricle and main veins

The auricle (Figs. 2–4) is attached to the pericardium anteriorly and is subdivided by powerful longitudinal muscles into an S-shaped ventral com-

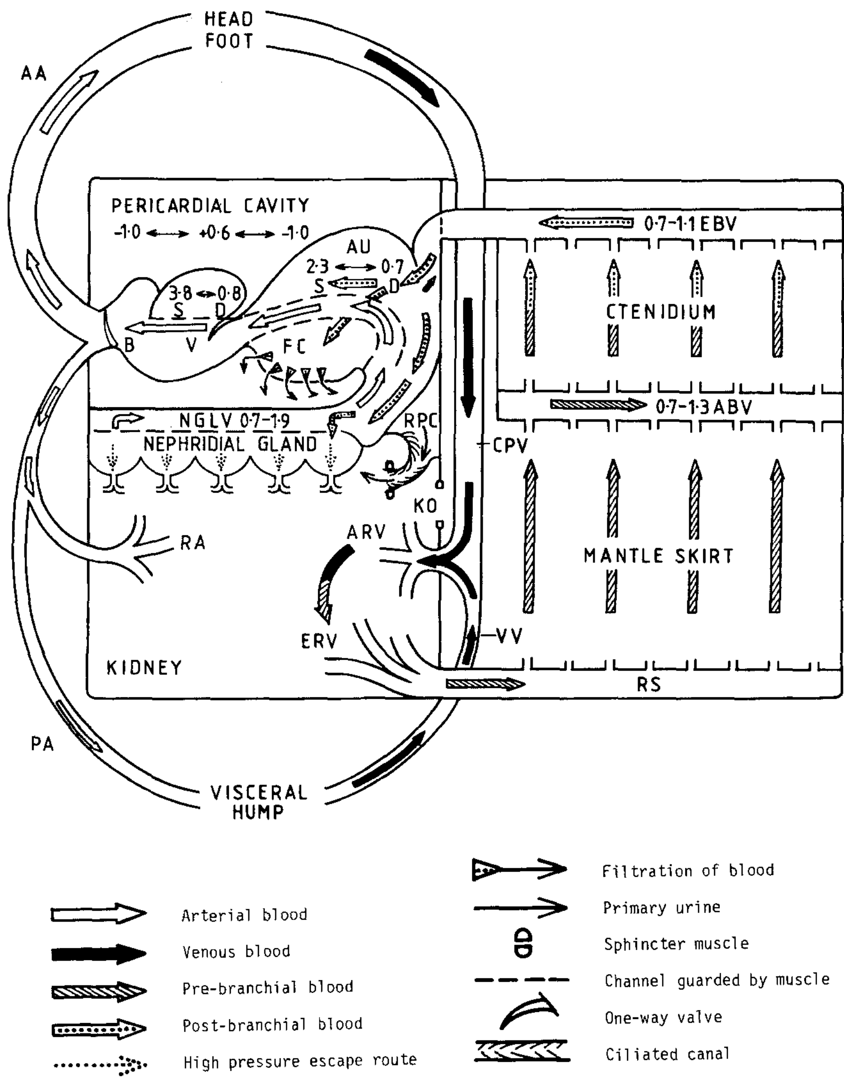


Fig. 1. Diagrammatic representation of the blood system of *Littorina littorea* and its haemodynamics. Fluid pressures are expressed as cm H_2O. The colloidal osmotic pressure (COP) gradient across the auricular wall is 0.2 cm H_2O, giving an effective filtration pressure of 1.5 cm H_2O throughout the cardiac cycle. *AA* Anterior aorta; *ABV* afferent ctenidial vein; *AU* auricle; *ARV* afferent renal vein; *B* bulbus aortae; *CPV* cephalopedal vein; *D* diastole; *EBV* efferent ctenidial vein; *ERV* efferent renal vein; *FC* filtration chamber; *KO* kidney opening; *NGLV* nephridial gland vein; *PA* posterior aorta; *RA* renal artery; *RPC* renopericardial canal; *RS* rectal sinus; *S* systole; *V* ventricle; *VV* visceral vein

partment bearing the filtration chamber on its median side, and a dorsal channel seen superficially as a ridge. The efferent ctenidial vein (ebv) opens into the ventral compartment, which is blind-ending posteriorly but connects with the nephridial gland vein (nglv) antero-dorsally. The dorsal channel is a through-route between the nephridial gland vein anteriorly and the auriculo-ventricular opening posteriorly. In a relaxed auricle the nephridial gland vein can be seen to open widely to the dorsal channel but at auricular systole the contracted longitudinal muscles occlude the anterior end of the channel, giving only the ventral chamber access to the vein (Fig. 2). The auriculo-ventricular valve opens, giving access from the dorsal channel to the ventricle. The opening of the nephridial gland vein to the auricle lacks a valve so that blood flow between it and one or other of the compartments in the auricle appears to be controlled entirely by the longitudinal muscles. The efferent ctenidial vein, by contrast, has an occlusor valve in the form of a transverse muscle (Fig. 2) in its outer wall anterior to its auricular opening. At this point the vein turns through 90°, and auricular muscles extend across it, their contraction creating a small bulge (Fig. 2) which seems to act as a baffle restriction reflux of blood into the vein. The auriculo-ventricular opening is guarded by a passive one-way valve (Figs. 2 and 3).

The nephridial gland vein is approximately 4 mm long and 0.5 mm wide in an adult snail with a tissue wet weight of about 1 g and it has two distinct groups of branches: superficial and deep. Eight superficial branches not only open to a reticulum of blood spaces in the gland but also to efferent renal sinuses of the dorsal wall. Twenty or more deeper branches connect only with the blood spaces of the nephridial gland. Anteriorly the main

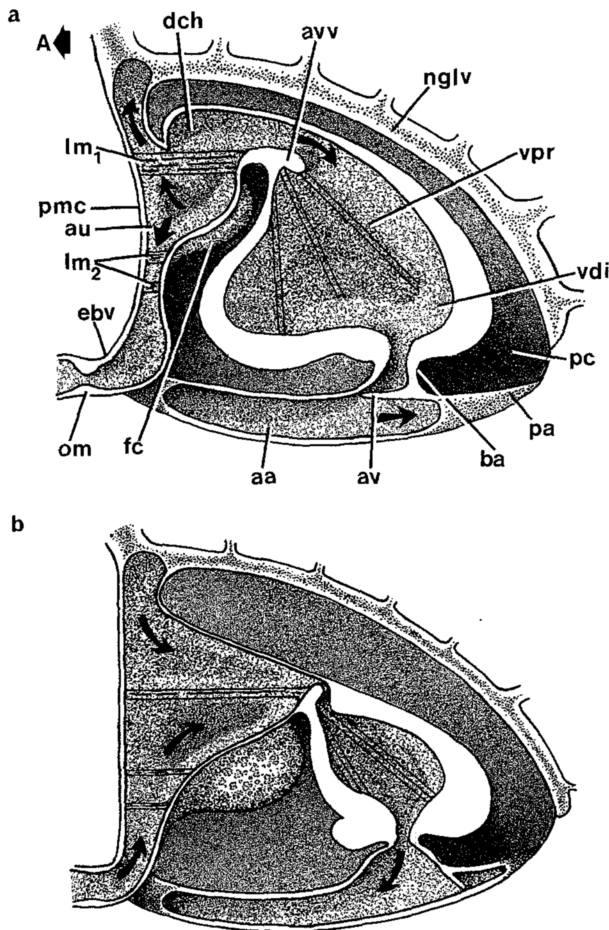


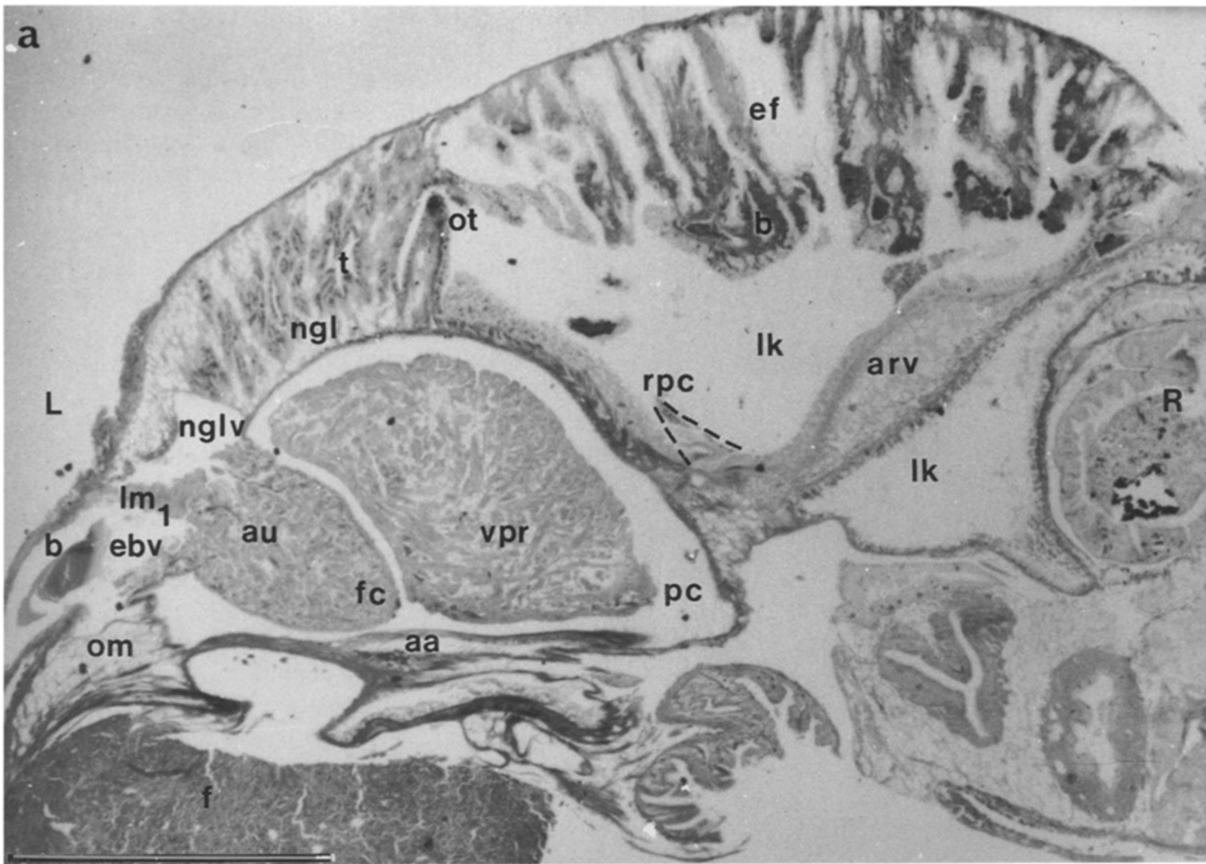
Fig. 2a, b. Diagrammatic representation of the heart of *Littorina* in sagittal section drawn from the left side. Cut surfaces are white. *Arrows* indicate direction of blood flow. Only a few trabecular muscles are shown. **a** Auricular systole; **b** ventricular systole. *A* Anterior; *aa* anterior aorta; *au* auricle; *av* aortic valve; *avv* auriculo-ventricular valve; *ba* bulbus aortae; *dch* dorsal channel of auricle: *ebv* efferent ctenidial vein; *fc* filtration chamber; lm_1 longitudinal muscles forming floor of dorsal channel; lm_2 longitudinal muscles controlling reflux to efferent ctenidial vein; *nglv* nephridial gland vein; *om* occlusor muscle; *pa* posterior aorta; *pc* pericardial cavity; *pmc* posterior wall of mantle cavity; *vdi* distal chamber of ventricle; *vpr* proximal chamber of ventricle

vein dives inwards and branches over the luminal surface of the gland. The main vein and its superficial branches are readily injected with latex, but only a small proportion of the blood spaces is filled. Even so, the volume of casts is of the order of 5 μl, double the value given by Kamel (1979) for ventricular stroke volume in unparasitised specimens of *Littorina*, indicating that it is a considerable reservoir of blood for systematic circulation.

The prominence of a row of stout pillar-like muscles, innervated by branches of the auricular nerve, in the nephridial gland vein contrasts markedly with the weak musculature of the efferent ctenidial vein, as does the shape of the vessel in cross-section, triangular as distinct from ovoid. The nephridial gland vein therefore appears to be capable of generating the higher hydrostatic pressure of the two and of accommodating greater changes in volume.

The junctions between the superficial branches of the vein and the renal plexus are demarcated by a change to weak musculature in the efferent renal sinuses and by a constriction at each junction coupled with crossed diagonal muscles, which appear to form a pressure valve. Observations suggest that the muscles relax at a particular pressure threshold, allowing any surge of blood to bypass the systemic circulation and enter the renal sinuses. The renal plexus drains into a capacious rectal sinus, at the distal end of which is a muscular papilla alongside the anus. Fretter (1982) has described an external vascular opening on the papilla in other littorinaceans which acts as an escape route for blood displaced from cephalic and pedal sinuses on sudden retraction of the snail into its shell. No permanent pore has been found in *L. littorea*, but there is clearly a zone of weakness in the papilla easily ruptured if pressure exceeds a threshold, so protecting the heart from damage and allowing escape of blood and reduction in volume when space is needed to accommodate the retracted head/foot in the body whorl.

The observations therefore challenge the generally accepted view that the nephridial gland vein serves as a second efferent route from the kidney (Fretter and Graham 1962) and other evidence also indicates that this is not so. Small volumes of either vital methylene blue or latex injected into the nephridial gland vein with little pressure reached the boundary between nephridial gland and dorsal wall, but no further. Higher pressure resulted in penetration of the dorsal wall. In sections of snails fixed without narcotisation, the dorsal wall is usually gorged with blood and the gland contains little, suggesting that blood does not escape from the renal sinuses to the gland. There therefore appears normally to be constriction of the vessels between the two parts of the kidney which stops blood flow between nephridial gland and dorsal wall. On retraction of a snail blood is forced out of the mantle skirt at high pressure and the vessels open widely, supporting the view that it provides an escape route and protects the delicate auricle from rupture. The indications are that pressure in the renal plexus does not reach the threshold to open the valves to the nephridial gland vein, so making it improbable that venous blood from the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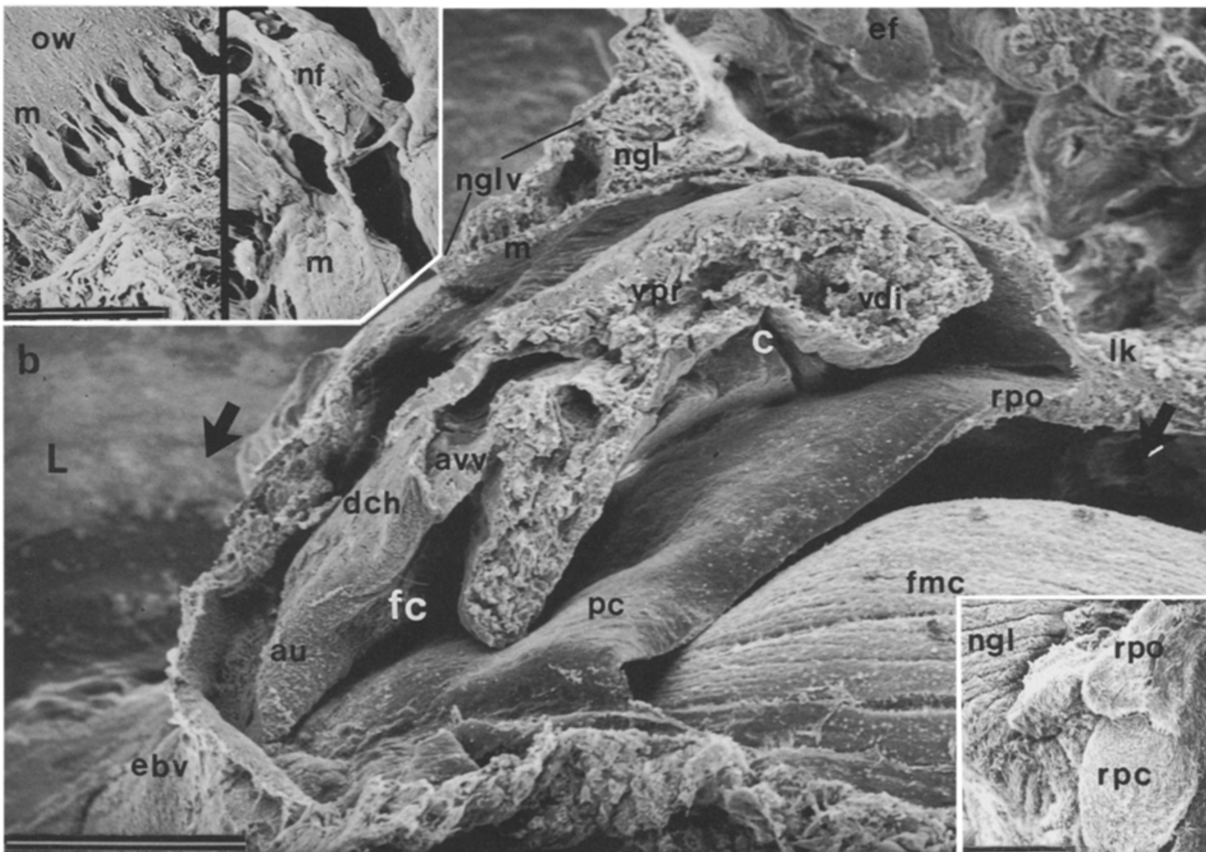
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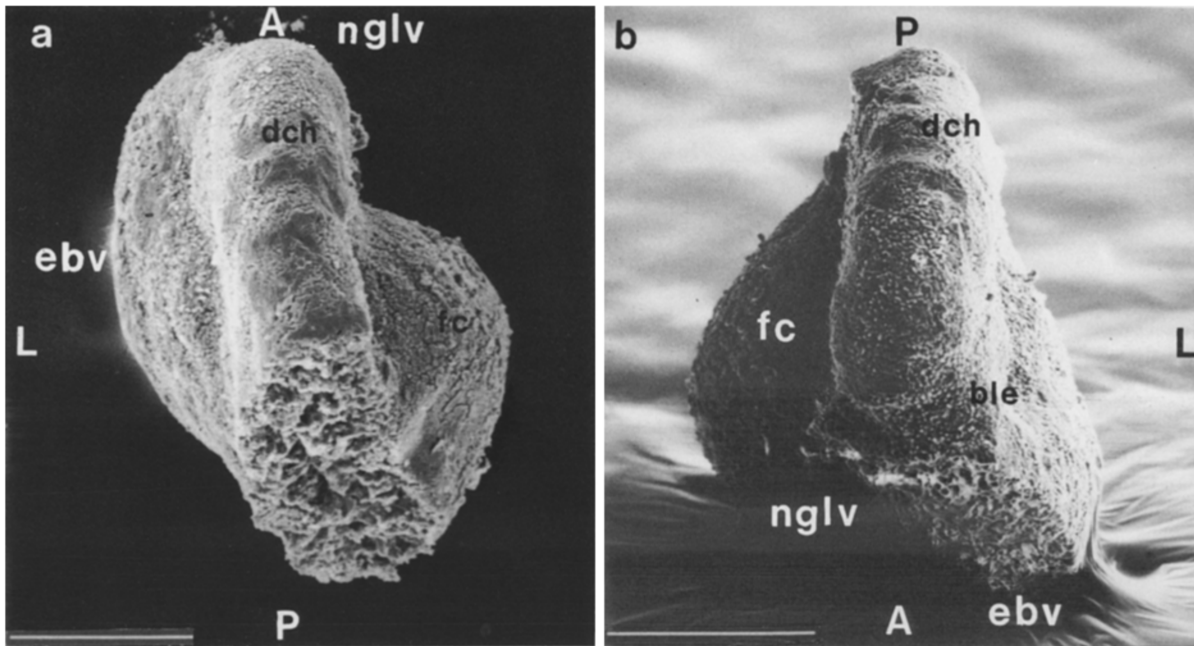

Fig. 4. **a** SEM of a contracted auricle of *Littorina*, postero-dorsal view ×125, *scale bar* = 200 µm. **b** Antero-dorsal view of the auricle showing the blind-ending dorsal channel; ×140, *scale bar* = 200 µm. *A* Anterior; *ble* blind end of dorsal channel; *dch* dorsal channel, *ebv* efferent ctenidial vein; *fc* filtration chamber; *L* left; *nglv* nephridial gland vein; *P* posterior

kidney is mixed with post-branchial blood in the auricle.

Further evidence to support this interpretation of blood flow between kidney and heart is the provision of an arterial supply from the posterior aorta to the dorsal wall, but the lack of one to the nephridial gland. It implies that the dorsal wall does not normally receive oxygenated blood from the auricle, but that the nephridial gland does.

The ventricle and main arteries

The ventricle (Figs. 2 and 3) lies postero-ventral to the auricle, to which its main axis is at an oblique angle. It is subdivided into a capacious thinner-walled proximal chamber and a thick-walled distal portion with a smaller lumen, suggesting that it is a two-stage pump, perhaps operating more smoothly and generating more power than a single pump, the more muscular distal region producing maximum systolic pressure. Powerful longitudinal muscles traverse the lumen of the proximal chamber and are inserted radially in the wall around the entrance to the distal chamber, so that their contractions would close off the proximal chamber and assist propulsion of blood into the bulbus aortae. The bulbus, demarcated from the ventricle by a change from striated cardiac to smooth muscle, bears a passive valve in its posterior wall at the bifurcation into the anterior and posterior aortae. The valve occludes the smaller posterior aorta on opening, allowing preferential flow of blood into the anterior aorta, as observed in other gastropods by Sommerville (1973).

The aorta (Fig. 2) is separated from the pericardium by a thick collagenous layer, minimising the

Fig. 3. **a** Transverse section of *Littorina* in the region of the heart and kidney, cut tangentially to the nephridial gland to show the opening of the veins into the auricle. Stained in Mallory's triple stain. ×42.5; *scale bar* = 1 mm. **b** SEM of the heart and left side of kidney cut transversely. ×63.5; *scale bar* = 0.5 mm. The ventricle has been cut longitudinally, the auricle is unopened. *Arrows* indicate plane of section in **a**. *Upper inset*: SEM of the nephridial gland vein cut open to show the stout muscles along its left side, ×105, *scale bar* = 200 µm, and detail of innervation, ×840. *Lower inset*: SEM of closed renopericardial canal from renal side. ×140, *scale bar* = 100 µm. *aa* Anterior aorta; *au* auricle; *arv* afferent renal vein; *avv* auriculo-ventricular valve; *b* blood; *c* constriction; *dch* dorsal channel of auricle; *ebv* efferent ctenidial vein; *ef* excretory folds on dorsal wall of kidney; *f* foot; *fc* filtration chamber; *fmc* floor of mantle cavity; *L* left; *lk* lumen of kidney; *lm* longitudinal muscles; *m* muscles crossing lumen of nephridial gland vein; *nf* nerve fibres; *ngl* nephridial gland; *nglv* nephridial gland vein; *om* a strand of the occlusor muscle; *ot* opening of tubule of nephridial gland; *ow* outer wall of vein; *pc* pericardial cavity; *R* right; *rpc* renopericardial canal; *rpo* renopericardial opening; *t* tubule of nephridial gland; *vdi* distal chamber of ventricle; *vpr* proximal chamber of ventricle

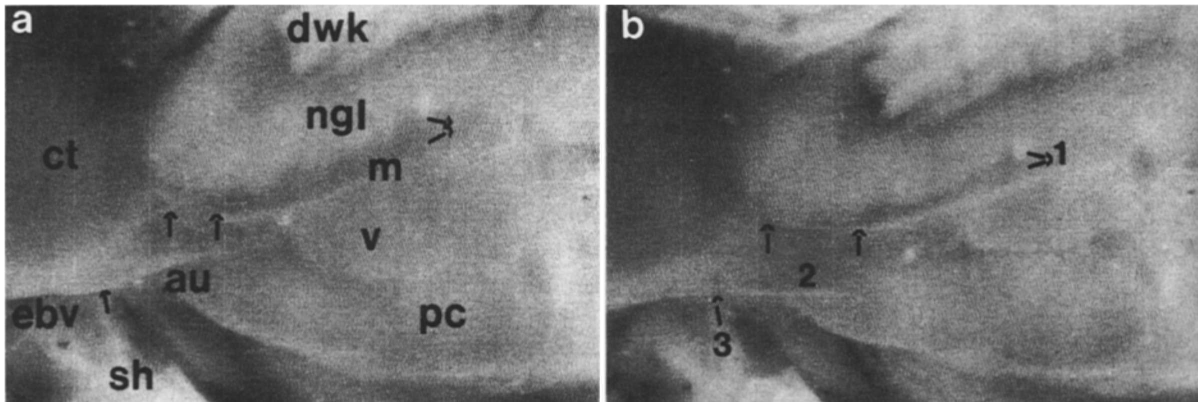

Fig. 5a, b. Video prints of the beating heart of *Littorina* seen in dorsal view by transparency through the pericardium. **a** The auricle in systole. **b** The ventricle in systole. *Arrows* indicate changes in 1: diameter of nephridial gland vein; 2: opening between the vein and auricle; and 3 the junction between the efferent ctenidial vein and auricle. *au* Auricle; *ct* ctenidium; *dwk* dorsal wall of kidney; *ebv* efferent ctenidial vein; *m* trabecular muscles of nephridial gland vein; *ngl* nephridial gland; *pc* pericardial cavity; *sh* shell; *v* ventricle

effect of its contractions on the PCF, and elastic fibres in the arterial wall are believed to confer the property of elastic recoil (Elder 1973).

The pericardium and renopericardial canal

The constant volume theory requires that the pericardial wall is in effect a rigid box or that deformation in one part is compensated for by a complementary change elsewhere. This is not so in *Littorina* (see p. 255). The outer wall, supported by the shell from which it is separated by extra-pallial fluid (itself subject to some fluctuation), and postero-ventral wall, where it is bound by connective tissue to viscera, are the most stable regions. The anterior, median and part of the ventral walls, with only a modest amount of collagen, are more susceptible to deformation. Radial muscle fibres, innervated by the same muscle fibres as the auricular longitudinal muscles, are the most prominent feature of the thin anterior wall abutting the mantle cavity, whilst subepithelial muscles predominate in the medial wall adjacent to the kidney.

The renopericardial canal (Fig. 3, rpc) arising ventrally in the median wall, is shaped like the spout of a teapot, its wide pericardial mouth narrowing to a slit-like lumen guarded distally by a sphincter. Reflux of urine is prevented not only by strong ciliary currents directed towards the kidney, but also by the shape of the canal, a proximal right-angle bend ensuring that increase in pressure in the kidney would compress and close the long spout against the pericardium on the renal side. The muscles of the canal have common innervation with the left lip of the external kidney opening, implying co-ordination of their activity.

Observation of heart in vivo and analysis of video recordings

Snails took some hours to recover from removal of the shell over the pericardium and kidney, during which time multiple contractions of the auricle often occurred between ventricular contractions. After recovery regular heart beat continued for more than 24 h and was best observed when a snail was extended with the foot attached to the dissecting dish.

The sequence of events in the cardiac cycle is shown diagrammatically in Fig. 9.

The most powerful muscles in both auricle and ventricle are longitudinal trabeculae traversing the lumen and their contraction alternately in auricle and ventricle produces not only collapse of one chamber, but also stretching and displacement of the other along this axis.

Contraction of the auricle (Fig. 5a) normally results in pulling the ventricle forwards, accompanied by pulling inwards of the anterior pericardial wall, where the longitudinal muscles originate. The combination of forward displacement of the heart and shortening and thickening of the muscles results in occlusion of the opening of the nephridial gland vein to the dorsal channel of the auricle (shown diagrammatically in Fig. 1). The auriculo-ventricular valve is forced open as auricular pressure rises and blood in the channel escapes into the ventricle. Multiple contractions of the auricle are weaker; however, they do not involve contraction of the longitudinal muscles and do not result in refilling the ventricle. Only the muscles in the ventral chamber contract. Although such contractions normally occur after retraction of the snail,

they give a clear indication that refilling of the ventricle is not the only function of the auricle. The weak contractions are accompanied only by dilatation of the nephridial gland vein, indicating that blood from the auricle is expelled into it. This is substantiated by a recorded rise in pressure in the vein at auricular systole (Fig. 8b). Normal auricular systole is also accompanied by a wave of dilatation from the auricle moving along the vein and its branches (Fig. 5). This implies that the vein receives blood from the ventral chamber of the auricle, the opening from which is not occluded at auricular systole. Back flow of blood into the efferent ctenidial vein is slight, as the occlusor muscle can be seen to constrict the distal end of the vein, trapping blood in the right-angled bend at its opening into the auricle. Contraction of the auricular muscles largely isolates the bend from the auricle.

The ventricle can normally be seen to expand rapidly at auricular systole (Fig. 5a) and ventricular contraction follows immediately, synchronised with some collapse of the walls of the nephridial gland vein, most obvious when the heart is beating strongly. There is no visible muscular contraction in the vein at this time, which suggests that blood is sucked into the auricle by a fall in its pressure (confirmed experimentally, Fig. 8b). Ventricular contraction both stretches the auricular wall and pulls the auricle posteriorly, so creating an opening between the nephridial gland vein and dorsal channel, allowing blood to drain from the vein (Fig. 2b). The efferent ctenidial vein simultaneously dilates and opens to the auricle. At this time the anterior pericardial wall can be seen to flatten again as the longitudinal muscles of the auricle are stretched, perhaps aided by contraction of the subepithelial muscles of the pericardium. This increase in volume appears to be compensated for by a bulging of the bulbus and proximal end of the aorta as blood is expelled from the ventricle, though the rest of the ventral wall of the pericardial cavity remains rigid.

The pericardial cavity is therefore seen to change shape during the cardiac cycle, confirming Sommerville's views (1973) that its walls do not form a rigid box.

When a snail is stimulated by touch to retract during observation, the vessels of the nephridial gland are seen to dilate and open to the plexus in the dorsal wall, which becomes engorged with blood. They collapse again as the blood slowly drains away. The heart loses its normal rhythm and weak multiple auricular contractions occur, as many as four or five between ventricular contractions. This behaviour is therefore further evidence of a short circuit, routing blood through kidney to mantle skirt, rather than the systemic circulation (see p. 251). The weak spot in the wall of the rectal sinus is believed to rupture and provide a safety valve at high pressures.

Table 1. Fluid pressures in the heart and pericardium of *Littorina*

Compartment	Fluid pressure [cm H_2O; mean ± 1 SEM (*N*)]	
	Mean diastolic pressure	Systolic pulse pressure
Ventricle	0.81	$+3.04 \pm 0.54$ (12)
Auricle	0.73	$+1.59 \pm 0.27$ (7)
Pericardium	0.60[a]	-1.64 ± 0.28[b] (16)

[a] At ventricular diastole
[b] Corresponding to ventricular systole

Experimental results

Measurement of hydrostatic pressure. Fluid pressures measured within the renopericardial complex of *Littorina* are summarised in Table 1, and show resting pressure to be slightly above ambient. Pulsatile pressures were detected at all recording sites. Mean pulse pressures of 2.6, 1.2, 0.6 and 0.4 cm H_2O were recorded from the posterior aorta, nephridial gland vein, and afferent and efferent ctenidial veins respectively ($N=2$ in all cases).

Most experimental animals remained quiescent with the foot partly extended during recording periods and heart movements could be clearly seen. Some snails became fully extended and more active. Tactile stimulation of the foot in these individuals caused immediate withdrawal into the shell, with a resultant transient increase in resting fluid pressure throughout the renopericardial complex of up to 12 cm H_2O. The cardiac cycle normally consisted of alternate ventricular and auricular contractions, although there were occasional multiple auricular twitches as observed in video recordings (Fig. 7b). Many animals displayed irregular cardiac activity with prolonged periods of cardiac arrest. At cardiac arrest the ventricle was partly expanded with an internal fluid pressure slightly above that of the auricle; the cardiac cycle was generally restarted by an auricular twitch. Isolated ventricles did not collapse entirely and exhibited a certain elastic resistance to deformation; they contracted spontaneously if filled with sea water from a cannula passed through the aortic stump.

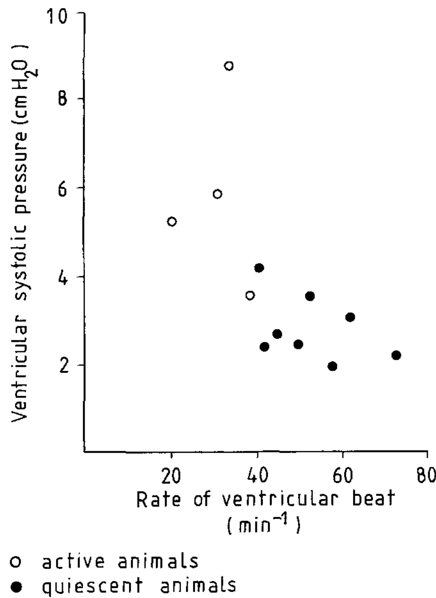

Fig. 6. Graph to show the relationship between systolic pressure and rate of beat in the ventricle of *Littorina*

The mean rate of regular ventricle beating in vivo was 40 ± 3.9 min^{-1} ($N=23$). Maximum pressure at ventricular systole (approximately equal to the circulatory pressure gradient) ranged between 1.9 and 8.9 cm H_2O, and the highest pulse pressures, developed at the lowest regular frequencies of ventricular activity, were recorded in active animals (Fig. 6). Direct observation of beating hearts suggested that the highest pressures are associated with an increase in stroke volume, as reported in other molluscs (Smith 1987).

Fluid pressures in the pericardium and ventricle oscillated in phase with one another (Fig. 7a), peak systolic pressure in the ventricle corresponding to subambient troughs in pericardial pressure. Pressure pulses in the beating auricle were in phase with peaks of pericardial pressure (Fig. 7b). These observations support in broad terms the idea of a constant volume mechanism for heart function in *Littorina*.

Close examination of pericardial pressure waveform, however, often reveals a slight fall in pressure immediately after the peak at auricular systole, particularly evident in single recordings (Fig. 7c). This is not explained by the constant volume hypothesis. It may indicate opening of the renopericardial canal and/or expulsion of blood into the nephridial gland vein.

The aortic pressure waveform (Fig. 8a) showed an inflection on the downslope consistent with closure of the valve in the bulbus aortae (Bourne and Redmond 1977) and an inherent arterial elasticity.

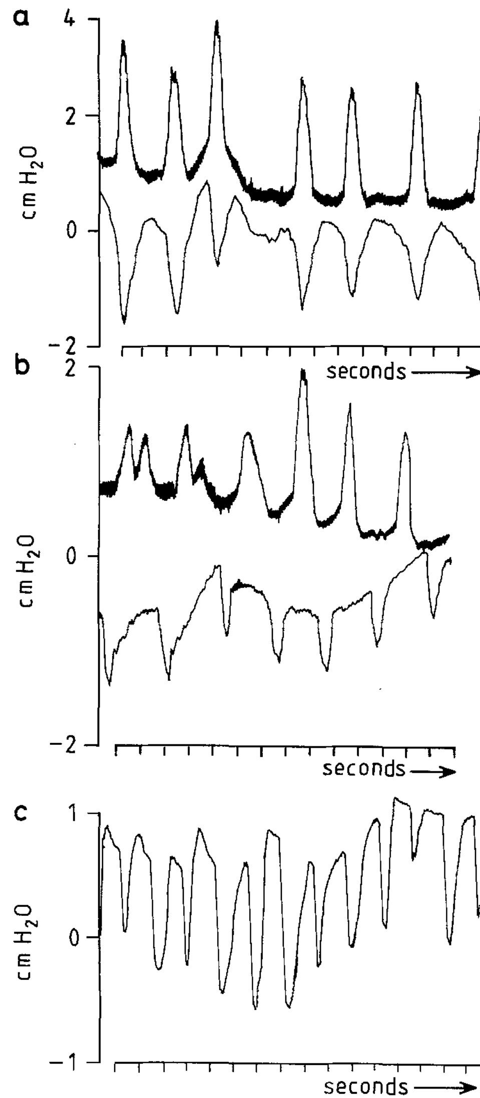

Fig. 7a–c. Simultaneous recordings of fluid pressure in **a** ventricle (*upper trace*, micropipette probe) and pericardial cavity and **b** auricle (*upper trace*, micropipette probe) and pericardial cavity of *Littorina*, the latter showing two contractions of the ventral chamber of the auricle during a single ventricular diastole at the start of the trace. The *arrow* indicates the direction of movement of the traces. **c** A trace of pericardial pressure, showing several peaks followed by a change of slope on the downslope, indicating a slight fall in pressure at auricular systole before the major drop at ventricular systole

Elastic recoil of the arteries may resist collapse and maintain peripheral blood flow during ventricular filling.

Pressure oscillations in the nephridial gland vein (Fig. 8b) were synchronised with those in the auricle, pressure peaks coinciding with auricular systole and with the dilatations observed in the vein, supporting the view that there is tidal flow in the vein. Slight collapse of the walls in the vein coincided with troughs in venous pressure, the

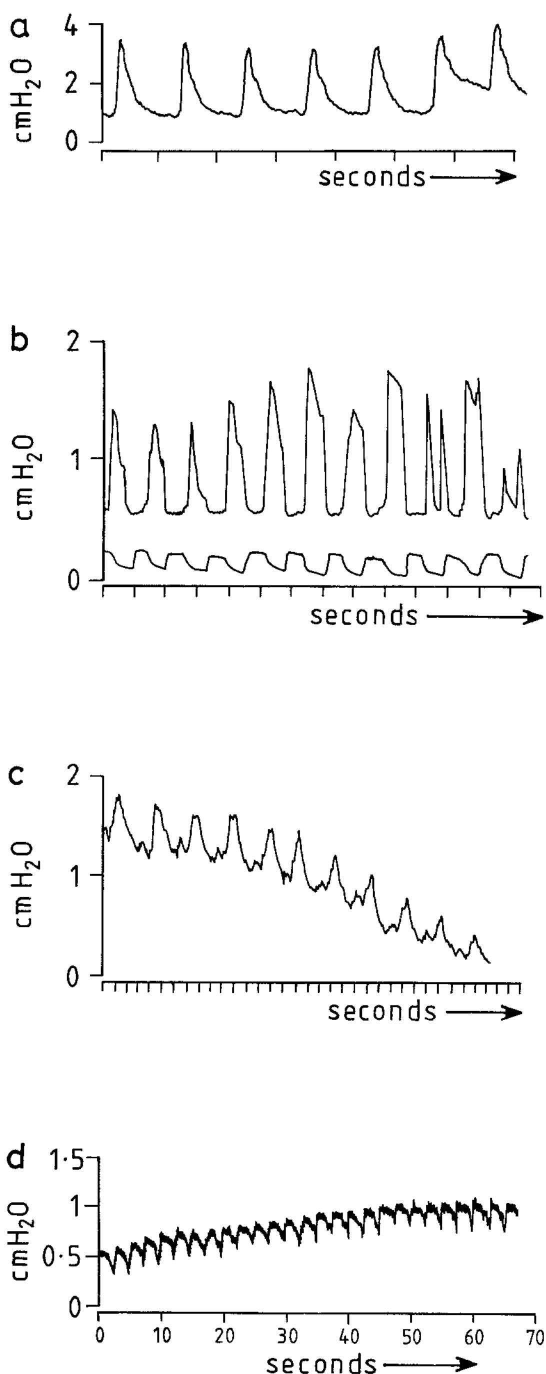

Fig. 8a–d. Pressure recordings from **a** posterior aorta, **b** nephridial gland vein, **c** afferent ctenidial vein and **d** efferent ctenidial vein of *Littorina*, **c** and **d** being recorded sequentially from the same snail. In **b** the lower trace is an event marker visually co-ordinated with ventricular systole (upward deflection). Maximum pressure in the vein coincides with auricular systole, and some double peaks during a single ventricular diastole are apparent as on the auricular recording. In **c** the overall downward slope of the trace reflects a slight extension of the snail from its shell. The alternate high and low pressure pulses (0.5/0.1 cm H_2O) are believed to originate from ventricular and auricular systole respectively. **d** The systemic pressure pulse wave has been attenuated during passage of blood through the ctenidium and the trace appears primarily to reflect auricular systole

Table 2. Protein in the blood and pericardial fluid of *Littorina*

	Blood	Pericardial fluid
Protein concentration ($g\,l^{-1}$)	20.2 ±3.4 (16)	4.5 ±1.6[a] (5)
Colloidal osmotic pressure[b] (cm H_2O)	1.45±0.15 (10)	1.25±0.13 (10)
Average protein MW[c]	354000	92000

Results are presented as the mean ±1 SEM (*N*)

[a] Significantly different from paired blood concentration (paired-sample *t*-test; $P<0.01$)
[b] Pressure across a 10000 Da filter membrane
[c] Estimated assuming 1 cm H_2O pressure is generated by 40 μmol colloid l^{-1} (after Hevert 1984)

mean value of which is the same as minimum auricular pressure (0.7 cm H_2O) (Fig. 1).

There was evidence of little backflow into the ctenidial sinuses since both afferent and efferent ctenidial veins showed pressure pulses corresponding to auricular and ventricular systole alternately (Fig. 8c, d). The rate of propagation of fluid pressure through the blood system of *Littorina* is unknown, so it is impossible to match directly an individual pressure pulse in the traces with a specific cardiac event. Nevertheless, the alternate pulses of greater amplitude appeared to be attenuated across the ctenidial sinuses, indicating that they reflect oscillations of systemic pressure.

The protein concentration of the blood is significantly higher than that of the PCF, although the difference in apparent COP between the two fluids (0.2 cm H_2O) is not significant (Table 2). The gradient in fluid pressure across the auricular epicardium exceeds this difference in COP throughout the cardiac cycle (Fig. 1). The mean effective filtration pressure is 1.5 cm H_2O both at auricular systole and diastole, demonstrating that *Littorina* can form urine by differential filtration of the blood. The molecular weight limit of the auricular filter appears to exceed 92000 Da (Table 2), although a preliminary study with gel electrophoresis indicates that molecules exceeding 60000 Da are excluded from the filtrate to some extent.

The clearance of [^{51}Cr] EDTA from the blood of *Littorina* is described by a single rate constant of $0.0353\pm0.007\,h^{-1}$ ($N=11$). BV is 0.44 ± 0.015 ml g^{-1} wet wt ($N=11$). The mean rate of filtration of urine is therefore 0.0155 ml g^{-1} h^{-1}, equivalent to 0.26 μl g^{-1} min^{-1}. The surface area of the filtration chamber in Fig. 3a and b, from a snail of the order of 1 g wet weight of tissue, is

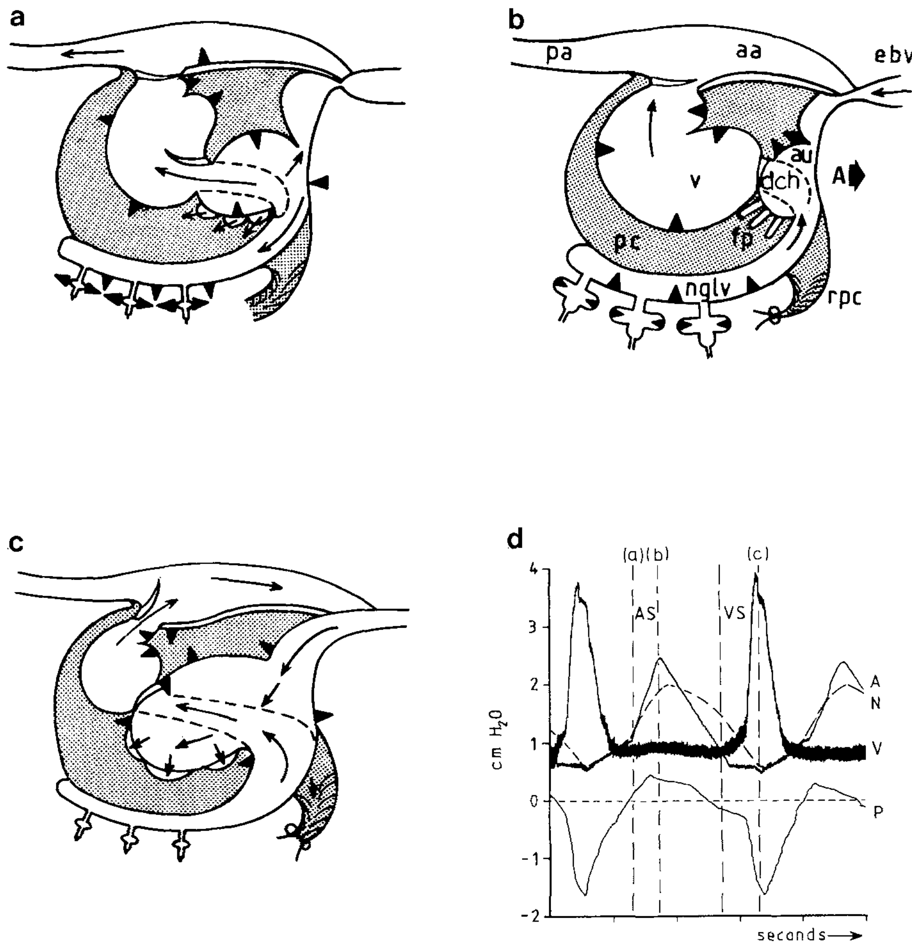

Fig. 9a–d. Diagrammatic representation of events during different phases of heart beat, showing the heart in dorsal view. **a** Beginning of auricular systole; **b** end of auricular systole, abbreviations as in Figs. 1 and 2; **c** ventricular systole; **d** a composite diagram of fluid pressure changes in: the auricle (*A*), nephridial gland vein (*N*), ventricle (*V*) and pericardial cavity (*P*) based on the traces in Figs. 7 and 8. The phases shown in **a–c** are shown by dotted lines on the graph. *AS* Auricular systole; *VS* ventricular systole

0.1509 mm^2, taking it to be approximately hemispherical. This figure must be a considerable underestimate, even of the minimum area, since no allowance has been made for shrinkage or state of contraction, and can only give a crude approximation of a minimum rate of filtration of 0.103 mm^{-2} filtration surface h^{-1}.

Discussion

The generally accepted concept of the gastropod heart as a relatively simple two-stage pump on the systemic circulation, in which emptying of the ventricle is held to be responsible for re-filling the auricle, is no longer tenable in *Littorina*, since it has been shown that filling the ventricle is not the sole function of the auricle. Furthermore, the built-in inefficiency implied by the assumption that post-branchial (arterial) blood is diluted in the auricle with venous blood from the kidney is rejected here with the demonstration of tidal flow of post-branchial blood in the nephridial gland vein (Fig. 9).

Perhaps the most fundamental issue requiring re-appraisal in the light of the hitherto unrecognised complexity of structure and mode of operation of the heart is the validity of the constant volume hypothesis in relation to *Littorina* and other gastropods with an auricular filtration site and a vascular connection with the kidney (Andrews 1988).

The auricle is in effect not one, but two pumps in parallel, the one with a leaky patch for production of primary urine in its wall coupled to the kidney, and with the capacity to pump independently, the other responsible for refilling the ventricle and maintaining a systemic circulation and normally operating together with its partner. It follows that the total volume of blood in the auricle is composed of one aliquot equivalent to the volume of the ventricle, which it refills, a second which is passed to the nephridial gland vein and a third portion amounting to about 0.7% of the ventricular output (see below) from which a blood filtrate is derived. Its capacity is therefore considerably greater than that of the ventricle.

The simultaneous pressure recordings from ventricle and pericardial cavity appear to confirm that a constant volume mechanism operates at ven-

tricular systole, since pericardial pressure is at its minimum then. However, in experimental conditions pressure is subambient at this time, indicating that there is an increase in pericardial volume.

Measurements of some other gastropods and bivalves (Dale 1974; Jones 1971, 1983; Florey and Cahill 1977) have revealed pressures just above ambient in similar experimental conditions (in which part of the shell over the heart has been removed) and it has been argued that slight positive pressure would help to keep the pericardial wall rigid – a requirement for maintenance of constant volume. Whilst the small hole in the shell drilled to gain access to the heart departs from the normal condition and may produce anomalous results in all these experiments, there is no reason to suppose greater inaccuracy in the present observations than in others. Indeed, it is difficult to see how the intact shell, separated from the pericardial wall by extrapallial fluid at ambient pressure, could affect the pressure change. The present result may differ from the earlier ones because modern recording techniques are more sensitive, suggesting that further clarification is required. However, Jones (1970) did record sub-ambient troughs in pericardial pressure in the archaeogastropod *Patella vulgata*.

Furthermore, whilst a constant volume mechanism can account for replacing in the auricle the same volume of blood expelled by the ventricle, maintaining a 'steady state' in which ventricular volume, as well as pericardial volume, remain constant, it does not fully account for refilling the greater volume of the auricle, nor does it satisfy the observed increase in ventricular volume and reduction in rate of beat which accompany increased activity. Values reported here for rate of beat and fluid pressure of the ventricle are similar to those recorded by Kamel (1979). Pulse pressure appears to be highest in an active snail, perhaps reflecting a requirement to overcome possible increases of circulatory resistance in contracting regions such as the foot. Increase in stroke volume would tend to maintain or even increase cardiac output, despite the observed reduction in heart rate. The functional significance of increased volume is therefore understandable, but the means by which this is achieved in a constant volume mechanism is not.

Production of primary urine by filtration of blood through the auricular wall and maintenance of a constant volume of PCF despite this is explicable on a constant volume principle, assuming that opening of the renopericardial canal coincides with auricular systole. Reflux of urine is prevented by a combination of: (1) opening of the canal only when pericardial pressure is at a maximum, (2) closure of the canal by compression if pressure is higher in the renal than in the pericardial compartment, (3) a strong ciliary current towards the kidney. Auricular refilling, when pericardial pressure is at a minimum, is not impaired by reflux, since the canal is closed by a sphincter at this time. Whilst a filtration pressure across the auricular wall has been demonstrated in *Littorina* throughout the cardiac cycle, it follows that filtration is not possible at ventricular systole, when it would impair efficiency of the refilling mechanism, because the auricle is empty then.

The third event at auricular systole, expulsion of blood into the nephridial gland vein, does not appear to be explicable by a constant volume mechanism on the basis of the experimental evidence, although it is theoretically possible. If constant volume were maintained at auricular systole, two events should contribute to a fall in pericardial pressure immediately after its peak: opening of the renopericardial canal and loss of blood to the nephridial gland vein. The latter might be expected to be of the same order as that at ventricular systole and so produce a significant reduction. Indeed, if there were a fall in pressure of this magnitude at this stage in the cardiac cycle, it would enhance the effect of elastic recoil of the spirally striated cardiac muscle in favouring expansion of the ventricle at diastole but could cause premature refilling of the auricle, and ventricular contraction would begin at a low rather than high pericardial pressure, requiring greater effort and resulting in refilling at reduced volume.

There is direct evidence, however, from the video recordings that the pericardial walls are not rigid in *Littorina*, which is consistent with the observations of Sommerville (1973) on *Helix*. The indentation of the anterior pericardial wall at auricular systole in *Littorina* does not appear to be accompanied by a compensatory change of the same magnitude in another part of the pericardium and is therefore believed to reduce pericardial volume. Flattening of the wall at auricular diastole would therefore increase pericardial volume, which offers an explanation of the fall in pressure below zero at this time. Auricular expansion would be assisted by these changes, which also allow replacement of the auricular aliquot passed to the nephridial gland vein.

This mechanism also provides a means of regulating stroke volume of the auricle, and in turn of the ventricle, the extent of volume change in the pericardium and nephridial gland being regu-

lated by the muscles in their walls, which are so richly innervated. The recognition that most of the pericardial wall is not rigid, as required for the constant volume theory, thus offers an explanation for the observed volume changes in the heart and nephridial gland not explicable in a constant volume system. It also overcomes the difficulty in the constant volume hypothesis of requiring the anterior pericardial wall to remain rigid irrespective of the changing pressures in the open mantle cavity and allows for adjustments in response to changing volumes and pressures in the viscera of the snail.

The role of the nephridial gland and its special vascular connection with the heart has long been a matter of debate. It is involved in abstracting molecules of larger molecular weight from the blood, haemocyanin metabolism and resorption of organic solutes from the urine (Delhaye 1974; Martoja et al. 1980; Andrews 1981; Taylor and Andrews 1987), though the dorsal wall also contributes to resorption. On the assumption that a relatively small amount of venous blood returned to the auricle through the nephridial gland rather than across the mantle skirt and gill (the major route), it seemed difficult to reconcile this with the complexity, and obviously important functions of the gland.

Appreciation of its significance, however, is radically changed by recognition that post-branchial blood passes through the gland and is modified by it before release into the systemic circulation. Despite its small volume relative to the dorsal wall of the kidney, its large surface area and 'arterial' blood supply, directly controlled by the heart, clearly make it an efficient organ with a central role in regulating blood composition, indicating that rates of transport and exchange of material in the gland in vivo would be greater than those in the dorsal wall. Indeed, the resorptive capacity of nephridial gland epithelium, lying close to the opening through which primary urine enters the kidney, is likely to be enhanced by rapid flow of a small volume of urine, a relatively small urine: blood concentration gradient and rapid blood flow. The dorsal wall epithelium exposed to urine from which a proportion of organic solutes has already been resorbed, and with a much more sluggish blood supply, is in less favourable conditions for active transport and is likely to abstract remaining solutes slowly, while urine is stored for long periods. All these considerations suggest that the nephridial gland is a major site in regulating blood composition, a major role of the dorsal wall being nitrogenous excretion. The gland and dorsal wall appear in many respects, therefore, to be functionally analogous to the proximal and distal convoluted tubule respectively of a vertebrate nephron.

The vascular connection between the auricle and kidney also appears to subserve a second important function: that of providing a short-circuit for blood forced out of the mantle skirt and foot on retraction of the snail into its shell. The observed dilatation of the nephridial gland veins and efferent renal sinuses, which become engorged, followed by a period of multiple weak auricular twitches and irregular ventricular contractions, result in blood being directed through the kidney to the rectal sinus in preference to the systemic circulation, on which many blood vessels and spaces may be compressed in the tightly packed shell.

Various reports of escape of blood, on retraction of a snail, through blood pores into the mantle cavity or foot, cited by Jones (1983), indicate that in a number of species there is a mechanism for reducing BV. Fretter (1982) reported openings in the wall of the rectal sinus in ten species of *Littorina* other than *L. littorea*, an arrangement allowing for most blood to be accommodated rather than lost, in vessels adjacent to cavities the volume of which can be reduced accordingly – namely the kidney and the mantle cavity. This would not be possible in the confined viscera and foot.

This interpretation differs from that of Depledge and Phillips (1986) on the effects of retraction in the prosobranch *Hemifusus tuba* (Gmelin), in which they recognised two phases of fluid expulsion, the first on rapid retraction of the snail, the second slower, involving drawing up of the operculum. Fluid expelled from the mantle cavity contained blood proteins, particularly in the second phase, indicating blood loss, which they believed to be from the gill. Their observations nevertheless give further corroborative evidence of a "safety valve" on the renal-pallial blood circuit.

The rate of filtration of blood through the auricular wall to produce primary urine is low in a marine prosobranch such as *Littorina*. The rate of primary urine formation, 0.26 $\mu l \cdot g^{-1}$ min^{-1}, is calculated to be only 0.7% of cardiac output, which Kamel (1979) estimated is 66 $\mu l \cdot min^{-1}$ for a 1.7 g snail. Resorption of water from primary urine is known to be negligible in *Littorina* (Taylor and Andrews, unpublished observations), so the difference between the rate of filtration and final urine production should be insignificant in this species. The rate of urine formation in the marine prosobranch *Strombus gigas* is slightly less than in *Littorina*, being 0.08 $\mu l\ g^{-1}$ min^{-1} (Little 1967),

but in the freshwater gastropod *Viviparus viviparus*, in which osmoregulation depends on a high rate, it is 0.42 µl g^{-1} min^{-1} (Little 1965).

The main specialisation of the auricle of *Viviparus* for a higher filtration rate is an increase in the surface area of the filtration site by comparison with *Littorina* (Andrews 1979, 1981), but both retain the podocytes which primitively comprise this part of the epicardium in prosobranchs. The freshwater pulmonate *Lymnaea* also has a high rate of urine production and van Aardt (1968) calculated a rate of flow of 1.9 mm s^{-1} along its renopericardial canal, which clearly plays a major part in maintaining dynamic equilibrium in pericardial volume. The diameter of the canal, reflecting the higher filtration rate, is about 400 µm in *Lymnaea* (Rolle 1908) compared with 80 µm in *Littorina*. The site of filtration has been debated in *Lymnaea*, in which podocytes have been lost, probably during a terrestrial phase in its ancestry, but the whole auricular epicardium is a discontinuous layer and is clearly porous (Andrews 1976).

Dale (1974) argued that such a high rate of filtration and large renopericardial canal in *Lymnaea* would inevitably decrease the efficiency of the heart as a pump, on the incorrect assumption that the ventricle was the site of filtration and that the process would impair auricular refilling. He also assumed that reflux along the renopericardial canal was inevitable which, on the basis of observations on *Littorina* (above) and *Viviparus* (Andrews 1979) is unlikely.

Nevertheless, Dale made some valuable comparative observations on the waveform of auricular pressure traces in *Lymnaea* and the terrestrial pulmonate *Helix pomatia*. A trace from the auricle of *Helix*, which is not a site of filtration, is asymmetrical and similar to a theoretical curve of a system in which there is a single response to a "step-impulse" (ventricular contraction), which is exponential with respect to time. A pressure recording from the auricle of *Lymnaea* (now known to be a filtration site) is symmetrical and not exponential with respect to time, like a theoretical curve in which the "step-impulse" evokes more than one response, the various responses summating to a level equal to the original impulse. There are in this case two responses to ventricular contraction: filtration and auricular refilling. The shape of the auricular pressure curve in *Littorina* is symmetrical, as in *Lymnaea*.

It may also be significant that the pressure gradient across the auricular wall in *Littorina* and *Lymnaea* is constant throughout the cardiac cycle, but in *Helix* and the freshwater bivalve *Anodonta* (Jones 1983) it is steeper at systole than at diastole, suggesting that there is no loss of fluid. Jennings (1984) has shown that in *Anodonta* (as in *Helix*) the auricle is not the site of filtration, which in the bivalve is the pericardial gland.

It has been shown that in *Littorina* any osmotic effect due to blood proteins, which might reduce the net flow of fluid across the filtration membrane, is negligible. The proteins are of high molecular weight; those excluded from the primary urine (15.7 g l^{-1}, generating a COP of 0.2 cm H_2O), appear to have an average molecular weight of the order of 2×10^6 Da. Haemocyanin molecules are likely to form the bulk of this protein, and molluscan haemocyanins are known to exist as aggregates of molecular weight up to several million daltons (Ghiretti and Ghiretti-Magaldi 1972). Subunit aggregation of haemocyanin will tend to lower blood COP, facilitating urine formation whilst maintaining a comparatively high oxygen-carrying capacity (Mangum and Johansen 1975; Andrews 1985). The blood COP (1.45 cm H_2O) reported for *Littorina* is similar in magnitude to that of the neogastropod *Buccinum undatum* (1.34 cm H_2O; Mangum and Johansen 1975).

An estimate of blood volume in the present study of *Littorina* (0.44 ml·g^{-1}) is, however, lower than that reported by Jones and Kamel (1984) (0.63 ml·g^{-1}). The reason for the discrepancy is not clear, as the markers used in the two studies ([^{51}Cr] EDTA and [^{14}C] inulin respectively) have the same distribution space in *Littorina* (Taylor and Andrews, in preparation).

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