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Cardiovascular control in Mollusca

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Introduction: Comparative physiology of cardiovascular control

by R. B. Hill

Department of Zoology, University of Rhode Island, Kingston (Rhode Island 02881, USA)

This 'Multi-author Review' contains papers on *cardiovascular control in Mollusca*, together with a comparative review of cardiovascular control in Crustacea. These papers were presented at a symposium of the XXXth International Congress of Physiological Sciences, Vancouver, July 13–18, 1986. The Introduction is based on the chairman's brief introduction at the Symposium. The Conclusion emphasizes the main points of the presentations and discussions, with particular regard to the emergence of future converging directions for research. The main body of the multi-author review contains six chapters, all dealing with aspects of neural control of cardiac function in Mollusca. Finally, the multi-author review concludes with a chapter by the co-chairman on cardiovascular control in Crustacea, with comparisons to molluscs.

The ultrastructural basis for electrophysiology of molluscan hearts

Several aspects of the ultrastructure of molluscan hearts are particularly advantageous for electrophysiological recording with suction electrodes, sucrose gap, microelectrodes, and patch-clamping electrodes. In hearts of bivalves and gastropods, the surface of the heart facing the pericardial cavity is furnished with an external lamina and basement membrane which form a strong substratum for suction electrode recording, even though the wall of the heart is a loose basketwork of trabeculae. The trabeculae of certain hearts assume a longitudinal orientation when stretched, which is very advantageous for sucrose gap recording (together with the long fibers, strong intercalated discs, and extensive coupling by nexuses). Microelectrode recording is usually accomplished in preparations dissected to expose the inner luminal surface, normally directly bathed in hemolymph, where there is no external lamina or endothelium. Since the pioneering work of Irisawa and his colleagues in 1961[12–14], microelectrode recording from the luminal surface has become the conventional technique for studying postjunctional responses of molluscan cardiac muscle, but now this technique may be supplemented with patch-clamp recording from single ionic channels of molluscan myocytes, dissociated[5, 6, 25] or in situ. The interrelationship of ultrastructure and electrophysiology was recognized in two previous symposia, which may be considered ancestral to the Vancouver 1986 symposium from which this review stems. In 1968, F. V. McCann organized a symposium on 'Comparative Physiology of the Heart: Current Trends', which was published as *Experientia Supplementum 5*, in 1969. Seven of the contributions dealt with molluscan heart, covering both electrophysiology and correlation of function with ultrastructure (the latter in a contribution by Nisbet and Plummer).

In 1977, Hill and Lang organized a symposium on 'Comparative Physiology of Invertebrate Hearts', which was published in the American Zoologist, vol. 19, in 1979. Six of the contributions dealt with molluscan heart, again covering electrophysiology and ultrastructure (the latter in contributions by J. W. Sanger and K. Kuwasawa).

The literature describing the morphology of molluscan hearts has been briefly summarized[11, 23]. In general gastropod and bivalve hearts have an epicardium toward the pericardial space, no endocardium toward the hemolymph, and a branching, anastomosing network of muscle bundles loosly arranged toward the lumen and more densely arranged toward the epicardium. Brunet and Jullien[7] published a comparative study of trabecular architecture in gastropods and bivalves and a light micrograph of ventricular trabeculae in *Busycon canaliculatum* appears as figure 6, p. 132 in a review by Hill and Welsh[11].

Ultrastructure of cardiac muscle of *Busycon canaliculatum* has been reviewed[23]. Transmission electron micrographs (TEMs) in transverse section show gap junctions between ventricular muscle cells. In longitudinal sections, sarcoplasmic reticulum (SR) elements can be seen associated with the plasmalemma by feet at the terminal cisternae, and extending into longitudinal tubular channels. The surface of molluscan cardiac cells is not deeply invaginated, as in arthropods, and does not extend into the interior of the cells in a t-tubular system. Instead, sarcolemmic tubules of *B. canaliculatum* penetrate 0.5 µm from the cell surface. However, the SR is coupled to the uninvaginated plasmalemma. Ventricular cells of *B. canaliculatum* are mechanically coupled by intercalated discs, and electrically coupled by gap junctions. Nerve endings with clear and dense-cored vesicles lie next to ventricular cells of *B. canaliculatum*.

The ultrastructure of muscle of the atrio-ventricular (AV) valve of *Dolabella auricularia* has been compared to ventricular muscle ultrastructure[15]. Ventricular muscle bundles are arrayed in parallel, with numerous cell contacts and intercalated discs, while valve muscle bundles are interwoven in a basketwork, with cell contacts on sarcolemmic projections. Ventricular cells have transverse and longitudinal SR tubules, while valve cells have deeply penetrating sarcolemmal folds, but no SR.

Particular attention has been paid to the ultrastructure of molluscan hearts with regard to ultrafiltration. In general, ultrafiltration takes place through a basement membrane which separates the cardiac muscle, lining the lumen, from specialized epithelial structures which face the pericardial cavity. Generally, ultrafiltration may occur mainly through the auricular wall[18, 22]. However, ultrastructural studies of a number of molluscan species have indicated that the myocardium of both ventricle and atrium is naked toward the car-

diac lumen, with an external lamina or basement membrane only on the surface of the myocardium towards the pericardial space. For instance, transmission electron micrographs show a basement membrane underlying the outer epithelium of the heart of *Mytilus edulis*[22], the epicardium of the heart of *Crassostrea virginica* rests on a basement membrane 0.5 µm

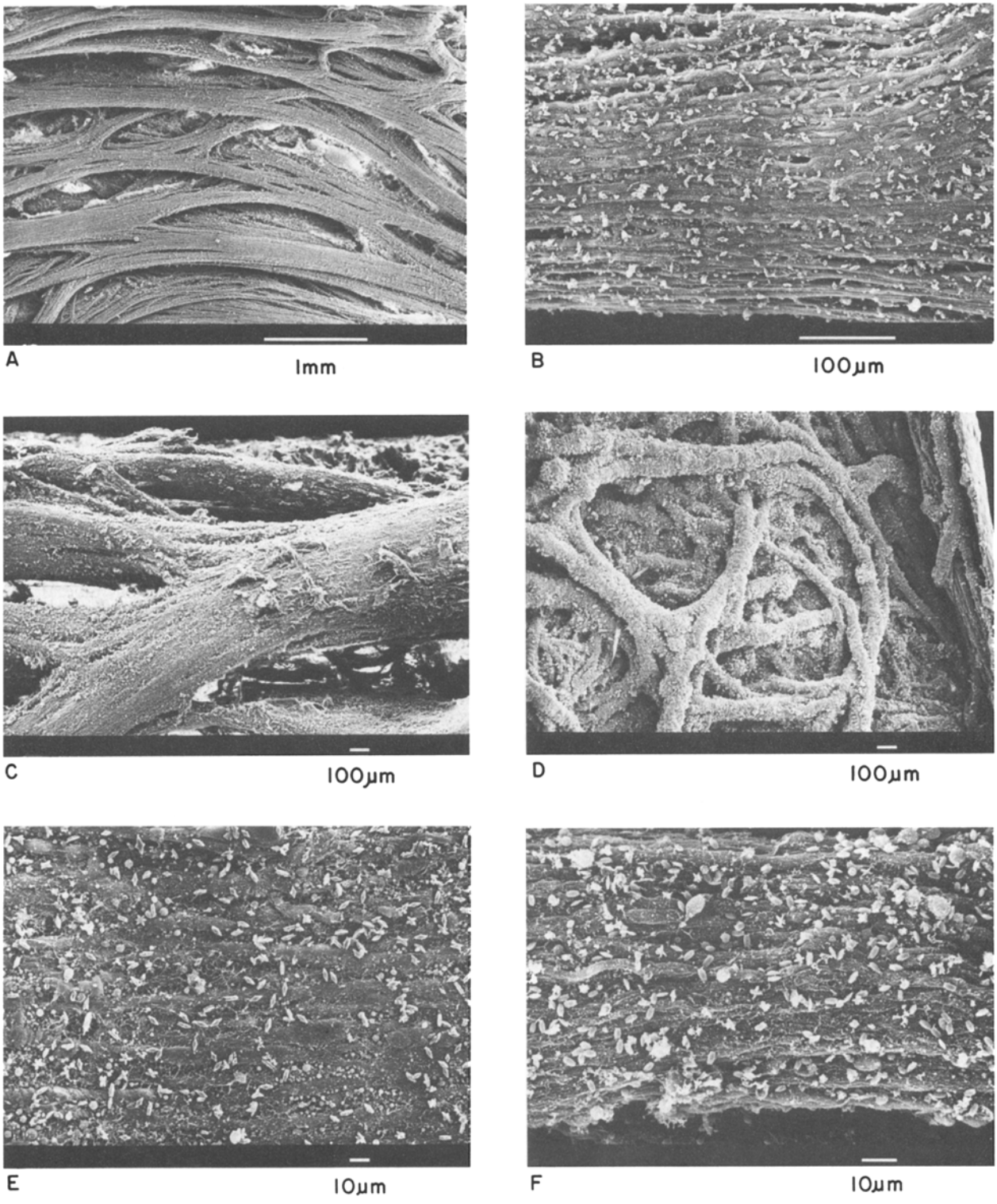
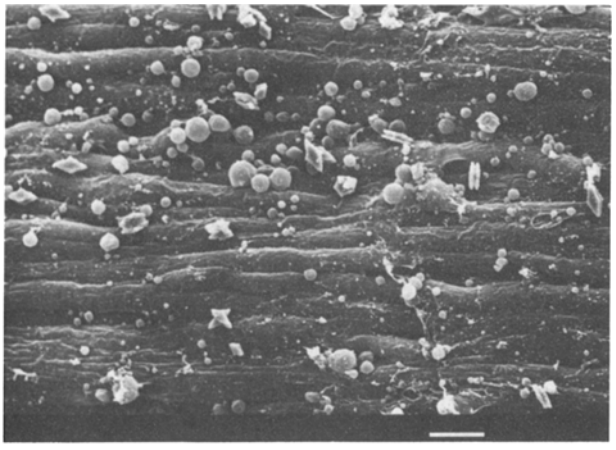


Figure 1. Scanning electron micrographs (SEM) of the myocardium of *Busycon canaliculatum*, viewed from the lumen of the heart. *A* Many of the major trabeculae run in parallel longitudinally, oriented from AV junction to aorta. The gritty surface of the trabeculae reveals adhering percipitate and blood cells. Perfused with EDTA and protease. *B* Individual fibers – (muscle cells) are densely arrayed, running longitudinally in a ventricular trabeculum. Perfused with EDTA. *C* Ventricular trabeculae appear much more massive than auricular trabeculae, at the same magnification. Not perfused. *D* Auricular trabeculae form a looser, more open network than ventricular trabeculae. Perfused with trypsin and collagenase. *E* Ventricular fibers are evenly covered with blood cells. Not perfused. *F* Some adhering flattened cells can be seen among the blood cells covering ventricular fibers. Perfused with trypsin and collagenase. SEM by Colin Leech.



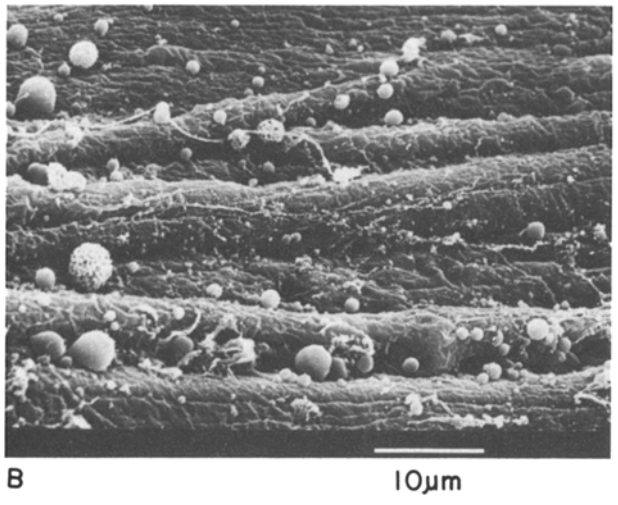


Figure 2. SEM of ventricular myocardium of *B. canaliculatum* at higher magnification than in figure 1. *A* Cells which appear to be hemocytes adhere to longitudinally arrayed fibers. (Numerous crystals also adhere after preparation for SEM). Perfused with seawater and then EDTA solution. *B* In addition to the hemocytes, perforated cells and cells giving rise to ramifying processes adhere to the fibers. SEM by C. Leech. Perfused with seawater for 3 h.

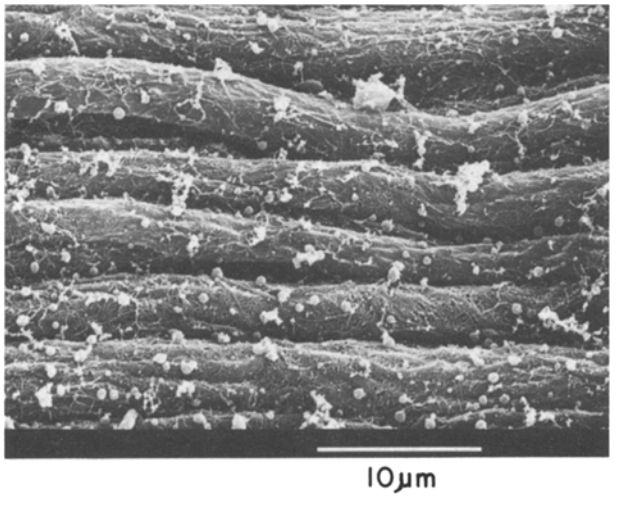


Figure 3. A fine network of filaments appears to cover ventricular myocardial fibers of *B. canaliculatum*. SEM by C. Leech. Perfused with EDTA and protease.

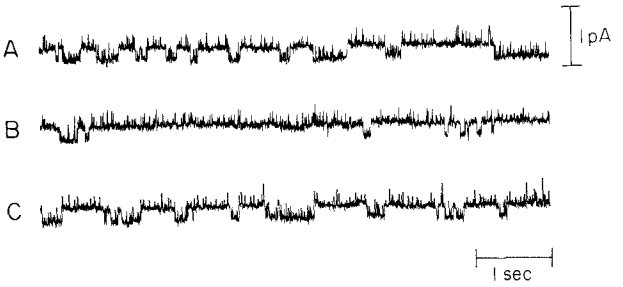


Figure 4. A nearly continuous set of records obtained from a cell-attached patch on a trabeculum of an entire ventricle of *B. canaliculatum*. Pipette voltage = 0 mV. Bath solution = modified artificial seawater containing 1 mM Ca^{++}, 1 mM Mg^{++}. Electrode internal solution = 10% (hypotonic) normal artificial seawater. The heart had been washed by internal perfusion with seawater and was still beating when cut open and pinned out for patch-clamp recording. A period of quiescence appears at (*B*) between periods of activity at (*A*) and (*C*). Records obtained by Colin Leech.

thick[9], the peripheral epithelium of *Geukensia demissa* is separated form the myocardium by a 0.3–0.7-μm collagen-filled space[26], podocytes on the external surface of the atrium of *Viviparus viviparus* rest on a basement membrane[4], there is a basal lamina over the atrium and under the epicardium of the ventricle of *Cyclotus canaliculatus*[3], pericardial gland tissue rests on the basal lamina in *Acila castrensis*[19], and a basal lamina separates muscle cells and epicardium in *Littorina littorea*[2]. Even in the cephalopods, *Sepia officinalis* and *Octopus vulgaris*, TEM shows a route for ultrafiltration through a basal lamina which forms the external limit of a blood space[24]. The pathway for ultrafiltration begins with passage from the hemocoel through the basal lamina, in 3 taxa of bivalves; protobranchs, filibranchs, and heterodonts, even though the subsequent pathways differ. Thus the basal lamina separates the hemocoel from the pericardial cavity[19]. There seem to be no cases where an external lamina lies between bivalve or gastropod myocardium and the blood space of the lumen. TEM shows that there is no endothelium in the heart of *Geukensia demissa*[26], but the myocardial trabeculae have a connective tissue layer in the heart of *Crassostrea virginica*[9]. This layer appears as fine interwoven filaments in SEM, which were identified as collagen fibrils in TEM. Ruthenium red staining demonstrated a cell coat about 0.1 μm thick on individual myocardial cells of *Crassostrea virginica*[9], but no overlying endocardium. Hawkins et al.[9] suggest that the connective tissue layer on the trabeculae takes the place of an endocardium.

SEM of ventricular and atrial myocardium of *Crassostrea virginica* shows a branching network of muscular trabeculae[9] which are studded with several types of small cells on the surface of or trapped among the muscular trabeculae. A distinctive type of perforated cell adheres to the surface of the trabeculae and gives rise to ramifying thin processes, which appear to entrap some of the numerous blood cells which appear in scanning electron micrographs of atrium and ventricle of *Crassostrea virginica*[9]. These perforated adhering cells do not appear to be circulating hemocytes[8].

SEM of the myocardium of *Busycon canaliculatum* reveals a structure very much like that reported by Hawkins et al.[9] and by Hawkins and Howse[8]. There is no endocardium, branching trabeculae being directly exposed to the hemolymph compartment (fig. 1). As in *C. virginica*, cardiac trabeculae are studded with hemocytes and adherent perforated cells (fig. 2). Fine fibers adhere to the surface of the muscle cells (fig. 3).

The ultrastructure of molluscan hearts allows comparable electrophysiological measurements with a number of techniques. The time-course of the action potential can be fol-

lowed with suction electrode, sucrose gap, or microelectrode recording[10]. The resting potential of gastropod ventricular muscle fibers has been determined by microelectrode methods to be 65.6 ± 7.0 mV for *Dolabella auricularia*[21] and 61.2 ± 3.5 mV for *Lymnaea stagnalis*[5], which is very close to resting potential values determined by the sucrose gap method[10]. Recently, Brezden et al.[6] have also demonstrated that the membranes of gastropod myocardial cells provide an opportunity for gigohm-seal patch-clamp recording, after enzymatic isolation of ventricular muscle cells. Using this technique, a stretch-activated K^+ channel has been discovered in dispersed ventricular muscle cells of *Lymnaea stagnalis*[25]. Since the proteolytic enzymes used in dissociation may affect the electrophysiological activity of tissues[1] it was hoped that the lack of an external lamina might indicate that the *Busycon* preparation (figs 1–3) would be suitable for patch-clamp recording without dissociation. In fact, gigohm seals and channel recordings can be obtained from trabeculae in a ventricular preparation washed only with seawater (personal communication, Colin Leech) but the abundant fibers and blood cells, seen adhering to the muscle fibers (figs 1–3), seem to make it difficult to obtain good seals or large currents (fig. 4).

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Cardiac output in the Mollusca: Scope and regulation

by P.J.S. Smith

Department of Zoology, Downing Street, Cambridge CB2 3EJ (England)

Summary. Different molluscan groups have evolved functionally specialised cardiovascular systems in response to varied behavioural and environmental demands, making the study of cardiovascular regulation in these animals a fascinating area for research. Currently, such research is frustrated by the lack of data on the in vivo performance of these systems, although, where examined, increased cardiac output appears to be accommodated by a change in stroke volume. This paper considers the in vivo regulation of cardiac output, primarily by extrapolating from in vivo experiments, and proposes the following three hypotheses for future study.
1. The increase in stroke volume is critically dependent on the phasic action of acetylcholine, expanding the end-diastolic volume of the ventricle for the same returning venous pressure.
2. Circulating cardioactive peptides will set the level of myocardial tone on a sliding scale, against which the action of both intrinsic and extrinsic factors are expressed.