

**CARDIAC PHYSIOLOGY
OF
THE SPINY DOGFISH
(*Squalus acanthias*)**

by

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ABSTRACT

The elasmobranch heart is comprised of four sequentially arranged chambers, the sinus venosus, the atrium, the ventricle and the conus arteriosus. The heart lies within a semi-rigid, capacious pericardial cavity which is provided with a communicating duct to the abdomen, the pericardio-peritoneal canal. The first part of this investigation focused on clarifying the role of the conus arteriosus on output pressure and flow. Satchell & Jones (1967) proposed that the contraction of the conus serves to prolong cardiac contraction so that the upper tier of valves of the conus do not face the extreme negative intrapericardial pressure, created due to ventricular ejection. They further concluded that the conus does not contribute to either pressure or flow in the ventral aorta. The present work does not confirm these conclusions. Pressure and flow records from the ventral aorta of anaesthetised and unanaesthetised spiny dogfish (*Squalus acanthias*) show conclusively that conal contraction contributes to output pressure and flow, prolongs the ejection phase of the heart and may serve a depulsator function.

Past research has emphasised that a semi-rigid pericardium allows development of negative intrapericardial pressure during ventricular ejection (Sudak 1965a, Johansen 1965, Satchell 1971, Shabetai *et al* 1985) which in turn aids in filling of the heart through suction (*vis-à-fronte* filling). However, recent studies on leopard sharks (Lai *et al* 1990) suggested that filling of the heart through suction is not important. Instead the heart is filled by positive venous pressure (*vis-à-tergo*

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GENERAL INTRODUCTION

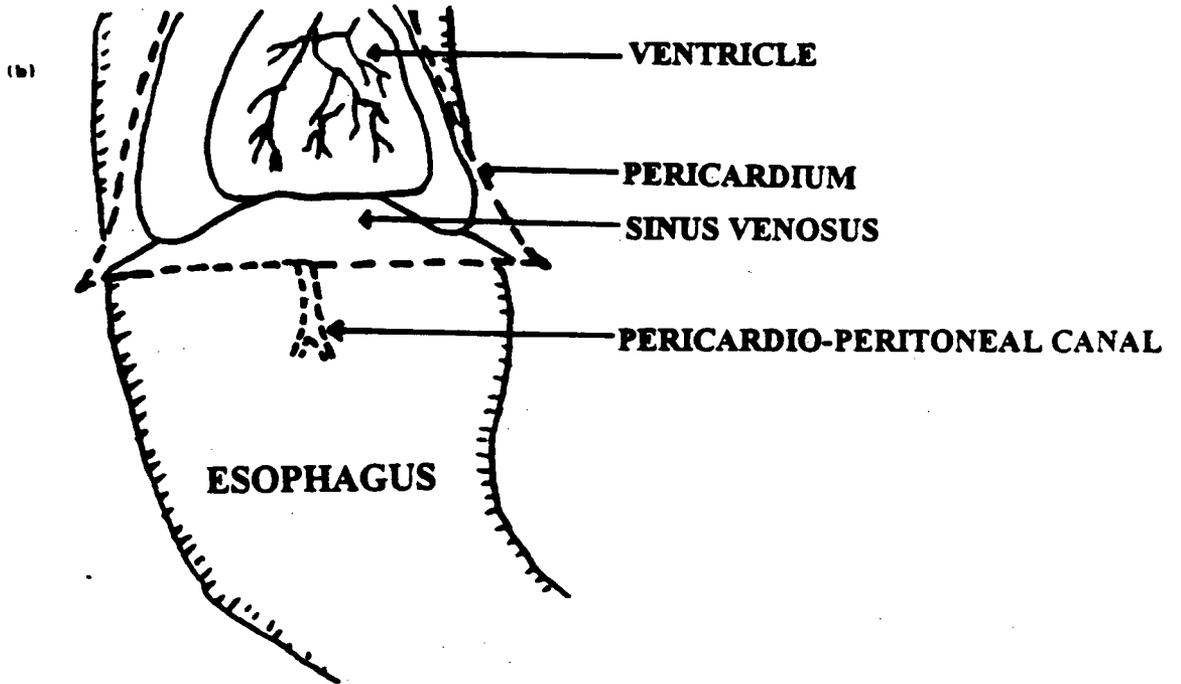
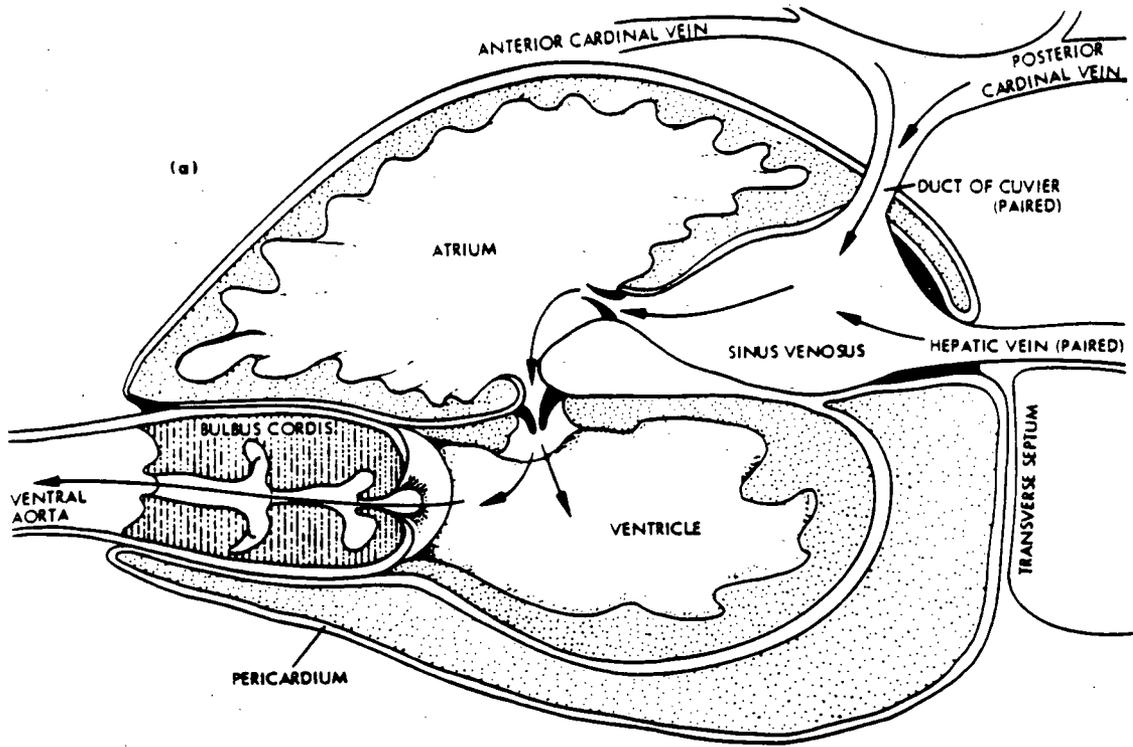
The circulatory system functions to maintain an optimal environment in the tissues through supply of oxygen & nutrients for metabolic activities and removal of carbon-dioxide & waste products. All vertebrates possess a closed circulatory system, that is, blood flows in a continuous system of interconnected tubes the arteries, the capillaries, the veins and never leaves these tubes. Such a system is advantageous because it makes possible the development of high pressures and therefore efficient regulation of blood flow to different organs. A head of pressure is required to make the blood flow which is provided by a pump, the heart. The vertebrate heart is a multi-chambered pump with contractile walls and valves to impart directionality to flow.

The elasmobranch heart comprises four sequentially arranged chambers, namely the sinus venosus, the atrium, the ventricle and a conus arteriosus (formerly called as bulbus cordis) (figure 1a). The heart is enclosed within the pericardium. Visceral pericardium, the inner layer of pericardium, covers the heart and forms the epicardial layer of the heart while parietal pericardium, the outer layer, is attached to the surrounding skeletal structures. The pericardial cavity so formed is conical in shape. In elasmobranchs, the rostral end of the cavity is formed by forwardly slanting septa and supported by coraco-branchial muscles (Satchell 1971). The caudal end is formed by a fibrous transverse septum which separates the peritoneal

Figure 1. (a) Schematic representation of the elasmobranch heart showing the semi-rigid pericardium surrounding the cardiac chamber.

(from "Chordate structure & function" edited by Waterman *et al* 1971)

(b) ventral view, showing the location of the pericardioperitoneal canal (PPC) in the dogfish, *Squalus acanthias*. (modified from Shabetai *et al* 1985)



and pericardial cavities. The posterior ventral and lateral surfaces of the cavity are formed by the cartilage of the pectoral girdle while the triangular roof is formed by the basibranchial plate of the pharyngeal skeleton. These surrounding supporting structures make the pericardial cavity of elasmobranchs extremely rigid. Compared with elasmobranchs, the pericardial cavity of teleosts is much more pliant as it is not so extensively supported by the surrounding skeletal structures and the transverse septum is not fibrous (Santer 1985).

In the sixteenth century Rondelet first observed that the transverse septum was complete in scaly fishes (teleosts) but perforated in cartilaginous fishes (cited in Cole 1944). This dorsal opening through the transverse septum, called the pericardioperitoneal canal (PPC), provides communication between the pericardial and the peritoneal cavities (figure 1b). Besides elasmobranchs, a PPC is also present in a few primitive bony fishes such as *Acipenser*, *Lepisosteus* and *Polypterus* (Satchell 1971).

It is widely believed that elasmobranchs lack (Weidenreich 1933, Ruzsnyak *et al* 1960 cited in Casley-Smith and Mart 1969) or only have a poorly developed lymphatic system (Abel *et al* 1994). Consequently, fluid accumulates inside the pericardium as the rate of secretion exceeds the rate of removal. The PPC is supposed to function as an outflow conduit for pericardial fluid into the peritoneal cavity. Ejection of pericardial fluid via the PPC will also allow the heart to increase

in size when and if stroke volume increases are required, such as during exercise (Lai *et al* 1989). Otherwise the heart could potentially be constrained from increasing its volume. The PPC is a collapsible tube and serves as a one way conduit for the pericardial fluid (Satchell 1971).

In elasmobranchs, the sinus venosus is a thin walled chamber that is triangular in shape (figure 1a). The two lateral corners communicate with the cuvierian ducts at the postero-lateral borders of the pericardium. The posterior side of the sinus-venosus is attached to the fibrous transverse septum. Two openings in the middle of the septum provide for the entry of the hepatic veins. Hence, the sinus venosus receives venous blood from the paired hepatic veins and cuvierian ducts. Elasmobranch fishes have a prominent sphincter surrounding the opening of each hepatic vein into the sinus venosus (Johansen & Hanson 1967), but the cuvierian ducts communicate with the cardinal veins and lack valves. That part of the cardinal veins communicating with the cuvierian ducts is remarkably capacious and is referred to as the cardinal sinus.

The sinus venosus opens anteriorly into the atrium through a sino-atrial aperture which is guarded by a pair of sino-atrial valves. The atrium of elasmobranchs is an extremely thin walled, very compliant, roughly triangular chamber lying anterior to the sinus venosus and dorsal to the ventricle (figure 1a). The atrium opens into the ventricle through the atrio-ventricular aperture. A set of

atrio-ventricular valves guards this aperture and prevents backflow of blood during ventricular contraction.

The ventricle is a muscular, sac-like chamber lying ventral to the sinus venosus and the atrium (figure 1a). It is the main pumping chamber of the heart.

In elasmobranchs, the most anterior chamber of the heart is the conus arteriosus (figure 1a). It is torch-shaped or cylindrical in *Squalus acanthias* and is rhythmically contractile by virtue of being invested with cardiac muscle. It traverses the anterior pericardial boundary to continue as the ventral aorta. Internally, the conus bears a variable number of tiers of valves arranged longitudinally. There are four tiers of valves in *Squalus acanthias* (O'Donoghue 1928), seven in *Squatina squatina* (Marples 1936, cited in Santer 1985) and two in *Scyliorhinus canicula* (Sans-Coma *et al* 1995). The most anterior or upper tier has large valves which are capable of occluding the lumen of the relaxed conus and separate the conus from the ventral aorta. The remaining tiers of smaller valves have to depend on the active contraction of the conus in order to occlude the lumen. According to Satchell and Jones (1967), an anteriorly directed wave of contraction ensures successive closure of each tier of valves.

The conus arteriosus is also present in certain primitive bony fish such as *Amia calva*. In the majority of teleosts, a narrow muscular ring with two valves at

the ventriculobulbar junction might represent a vestige of the ancient conus (Farrell & Jones 1992).

The purpose of this thesis is to re-examine the inflow and outflow dynamics of the elasmobranch heart. The capacious, relatively non-compliant, fluid-filled pericardium, which encloses the heart, must affect the performance of the heart. Negative pressure can be maintained in the pericardial cavity and may be accentuated by the volume changes during cardiac contraction. These negative pressures would have greater effect on the thin-walled inflow chambers (i.e. sinus venosus and atrium) than the thick-walled ventricle and conus. During the cardiac cycle a decrease in the volume of one chamber must be reciprocated by an compensatory increase in another or else extremely negative intrapericardial pressures will develop during the ejection phase of the heart. Since, the elasmobranch heart is four-chambered, the compensatory volume-related cardiac interactions are complex.

The performance of the heart can also be affected by the volume of pericardial fluid. A sudden loss of pericardial fluid via the PPC will result in a reduction in the intrapericardial pressure which would be transmitted to the thin-walled veins, sinus venosus and the atrium. This in turn would increase the pressure gradient for venous return. Conversely, an increase in secreted pericardial

fluid volume would have an opposite effect, raising intrapericardial pressure and reducing pressure gradient for filling the heart.

Hence, the short term (each cardiac cycle) and the long term (changes in pericardial fluid volume) changes in intrapericardial pressure would affect the inflow and outflow dynamics of the heart. To understand these complex interactions the present thesis focuses on 2 major aspects:

1) effect of contraction of the conus arteriosus on pressure and flow in the ventral aorta.

2) effect of change in intrapericardial pressure on the input and output pressures and flow.

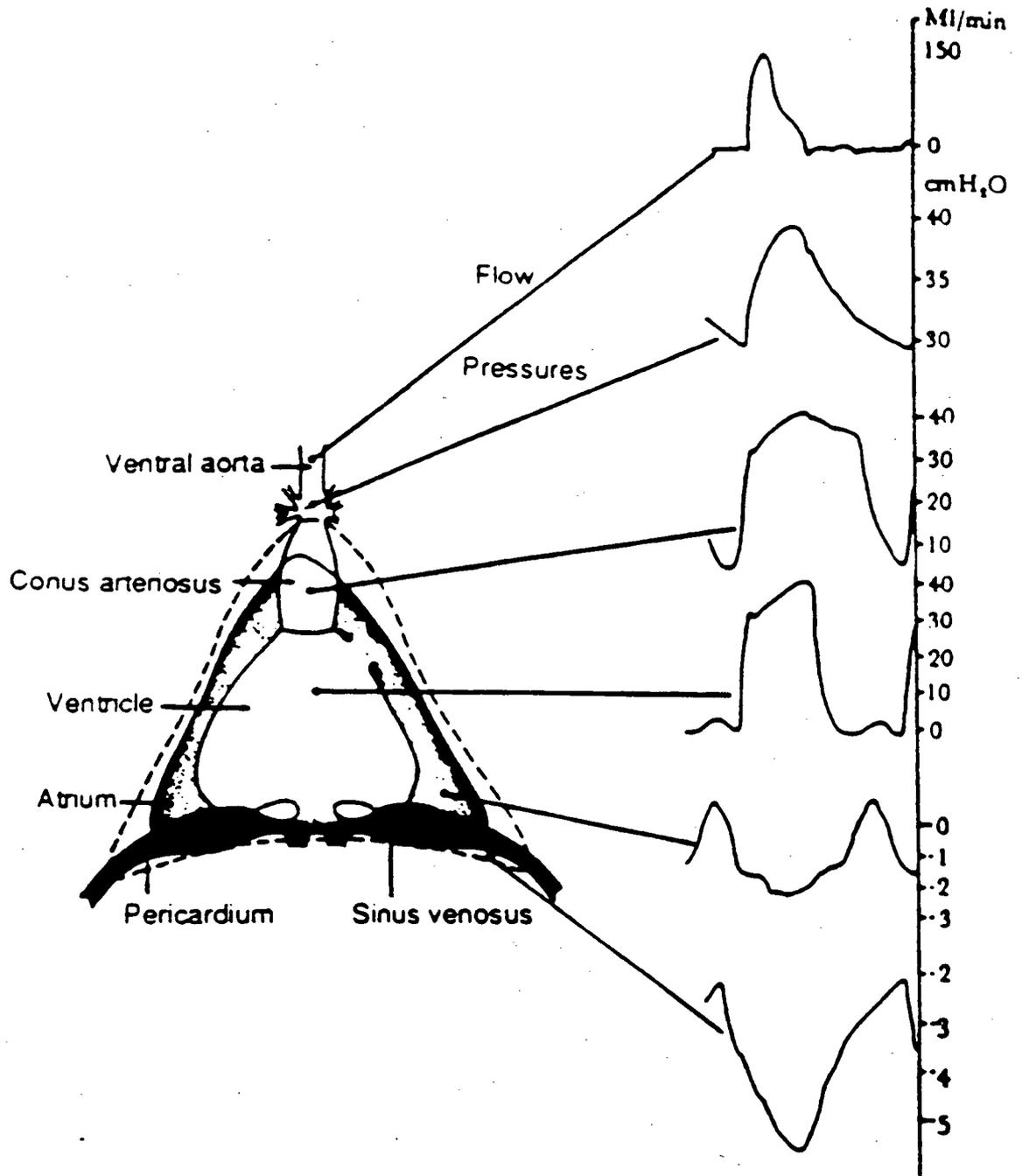
**CHAPTER ONE : Effect of contraction of the conus arteriosus on
pressure & flow in the ventral aorta.**

INTRODUCTION

The name conus arteriosus was coined by Gegenbaur in 1866 (cited in Parsons 1930). Since the early seventeenth century numerous functions have been assigned to this mysterious chamber. Duverney (1702) suggested that the conus might function as an extra ventricle (cited in Satchell and Jones 1967). Keith (1924) compared the conus to a shock absorber. He proposed that at times of extreme exertion by the ventricle, the conus might function as an elastic chamber to reduce the peak systolic pressure thus protecting the delicate gill vasculature. March *et al* (1962) suggested that the conus functions as a powerful musculovalvular accessory in the delivery and regulation of blood flow from ventricle to the gills. Satchell and Jones (1967) working on anaesthetised Port Jackson shark, *Heterodontus portusjacksoni*, concluded that the conus does not contribute to either ventral aortic pressure or flow (figure 2). They suggested that the conal contraction, instead, serves to postpone the closure of the most anterior tier of valves until the negative intrapericardial pressure, caused by ventricular ejection (figure 2), had decayed to a lower value. They argued that extreme negative pressures inside the pericardium would lead to incompetency of valves and hence considerable backflow. This was based on the presumption that a negative pressure outside the conus is more likely to make the valves incompetent than an equivalent positive pressure in the ventral aorta (Satchell and Jones 1967).

Figure 2. Pressures & flow recorded by Satchell and Jones (1967) from anaesthetised Port Jackson shark, *Heterodontus portusjacksoni*. Not all the traces are from the same fish.

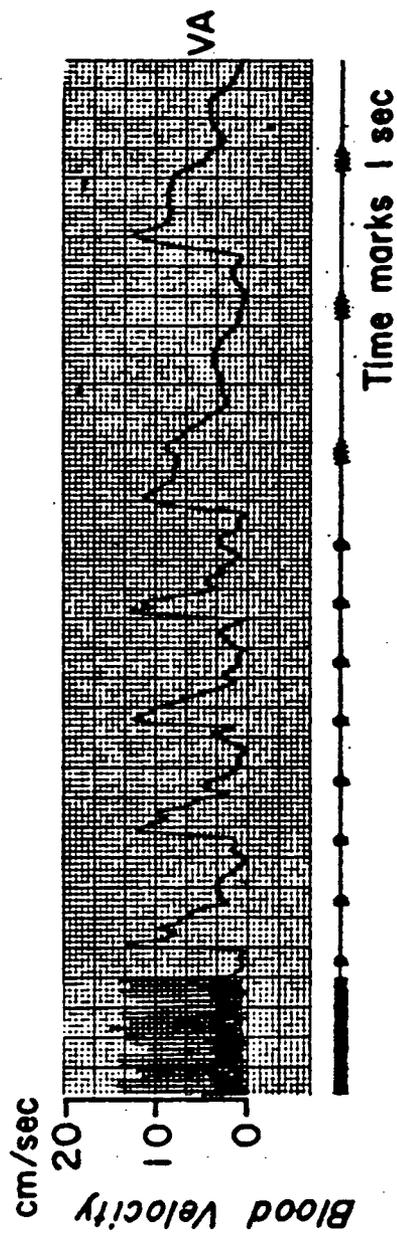
(from "Circulation in fishes" by Satchell 1971)



At about the same time when Satchell and Jones were experimenting, Johansen *et al* (1966) recorded ventral aortic blood velocity traces from freely swimming California horn shark (*Heterodontus francisci*) (figure 3). They reported oscillations in their ventral aortic blood velocity traces which they ascribed to contraction of the conus arteriosus. Later, Hanson (1967) also reported a conal contribution to ventral aortic pressure and flow in the dogfish (*Squalus acanthias*) and the large pacific skate (*Raja binoculata*).

In light of the above discrepancies in reported results (i.e. Satchell and Jones 1967; Johansen *et al* 1966; Hanson 1967) on the function of the conus arteriosus, one purpose of the present investigation was to re-investigate the effect of contraction of the conus arteriosus on pressure and flow relationships in the ventral aorta of anaesthetised and unanaesthetised spiny dogfish (*Squalus acanthias*).

**Figure 3. Ventral aortic blood velocity recorded by Johansen *et al* (1966)
from freely-swimming California horn shark, *Heterodontus francisci*.**



Ventral aortic (VA) blood velocity telemetered from a free-swimming California Horn shark (*Heterodontus francisci*).

METHODS

Spiny dogfish (*Squalus acanthias*) of either sex were collected from the coastal waters of British Columbia by commercial fishermen and temporarily held at Bamfield Marine Station, Vancouver Island. Healthy, uninjured animals were transported to holding facilities at the Department of Zoology, U.B.C., in a large tank with oxygen bubbled, chilled, anaesthetic (Tricaine Methane Sulphonate, MS-222, Syndel laboratories, 0.05g/l of sea water) containing sea water.

Occasionally, dogfish were purchased directly from a local fisherman. Dogfish were held in three circular tanks (2m in diameter and 1.2m deep) provided with filtered, aerated, running sea water. The temperature was kept in the range of 9-11 °C.

Usually not more than four animals were kept in one tank at a time. The length of the animals ranged from 60-90 cm long.

Captive animals were fed fresh herring at least twice a week. They initially refused to eat but variable success was achieved once the fish were acclimated to their new environment (2-3 weeks). All animals were allowed at least two weeks of recovery from effects of transportation before they were used in experiments.

Tricaine Methane Sulphonate at a concentration of 0.1 g/l of sea water was used for anaesthetising the animals. The anaesthetised fish was then placed on the surgery table. A flat PVC board (1m long and 0.25m wide) with a narrow strip cut

out of the middle along the entire length, to allow the dorsal fins and spines to pass through it, was used for holding the fish. Fish were ventilated artificially with air equilibrated anaesthetic solution (0.005g/l of sea water) alternating with sea water without anaesthetic.

Pressures from the pericardium, ventral aorta and ventricle were recorded while flow was recorded from the ventral aorta.

For pericardial cannulation, a hypodermic needle (15 gauge) was advanced through the coracoid bar, into the pericardial cavity (Shabetai *et al* 1985). Elasmobranch Ringer's (Saline) was injected into the pericardial cavity to avoid introducing air during these procedures or, alternatively, the cannulations were performed under sea water. A polyethylene tube (P.E.90) flared at the tip was inserted through the hypodermic needle, into the pericardium, and the area around the entry site sealed with tissue adhesive (Vetbond 3M). The correct placement of pericardial catheters was confirmed by being able to infuse and remove pericardial fluid (Shabetai *et al* 1985).

The ventral aorta was cannulated through the floor of the mouth. A small incision was made in the skin and the ventral aorta was exposed. The flow to the two innominate arteries was stopped by placing an artery clamp between the innominate and third afferent branchial artery. A small nick was made in the

ventral aorta and a polyethylene cannula (P.E.90 or 50) was then inserted into the ventral aorta. The tubing was secured at the site of entry by tissue adhesive. The artery clamp was removed and the cannula was further secured by silk sutures (size 0) inside the mouth. The incision was then closed with silk (size 0) and the free end of the cannula was passed through the spiracle and sutured (size 2-0 silk) to the dorsal side of the animal. For ventricular cannulation, the same procedure was used except that the cannula (P.E.50) was advanced along the ventral aorta through the conus and into the ventricle.

Partial stroke flow (the blood flow through paired afferent branchial arteries 1,2 and 3 during each cardiac cycle) was recorded by means of a Directional Pulsed Doppler flowmeter (Bioengineering, Iowa, model 545C-4) connected to a cuff-type flow transducer (Iowa Doppler Products, Iowa) surgically implanted around the ventral aorta between the innominate and third afferent branchial arteries. The cuff was approximately the diameter of the ventral aorta and was secured in position with tissue adhesive. The incision was then closed, and the probe wire passed out through the spiracle. The flow probe was calibrated at *post-mortem* by pumping known volumes of blood into the ventral aorta. For recording total stroke flow (the amount of blood pumped by the ventricle and conus during each cardiac cycle), the 4th and 5th afferent branchial arteries on both sides were ligated and the flow transducer implanted between the 4th and the 3rd afferent branchial arteries.

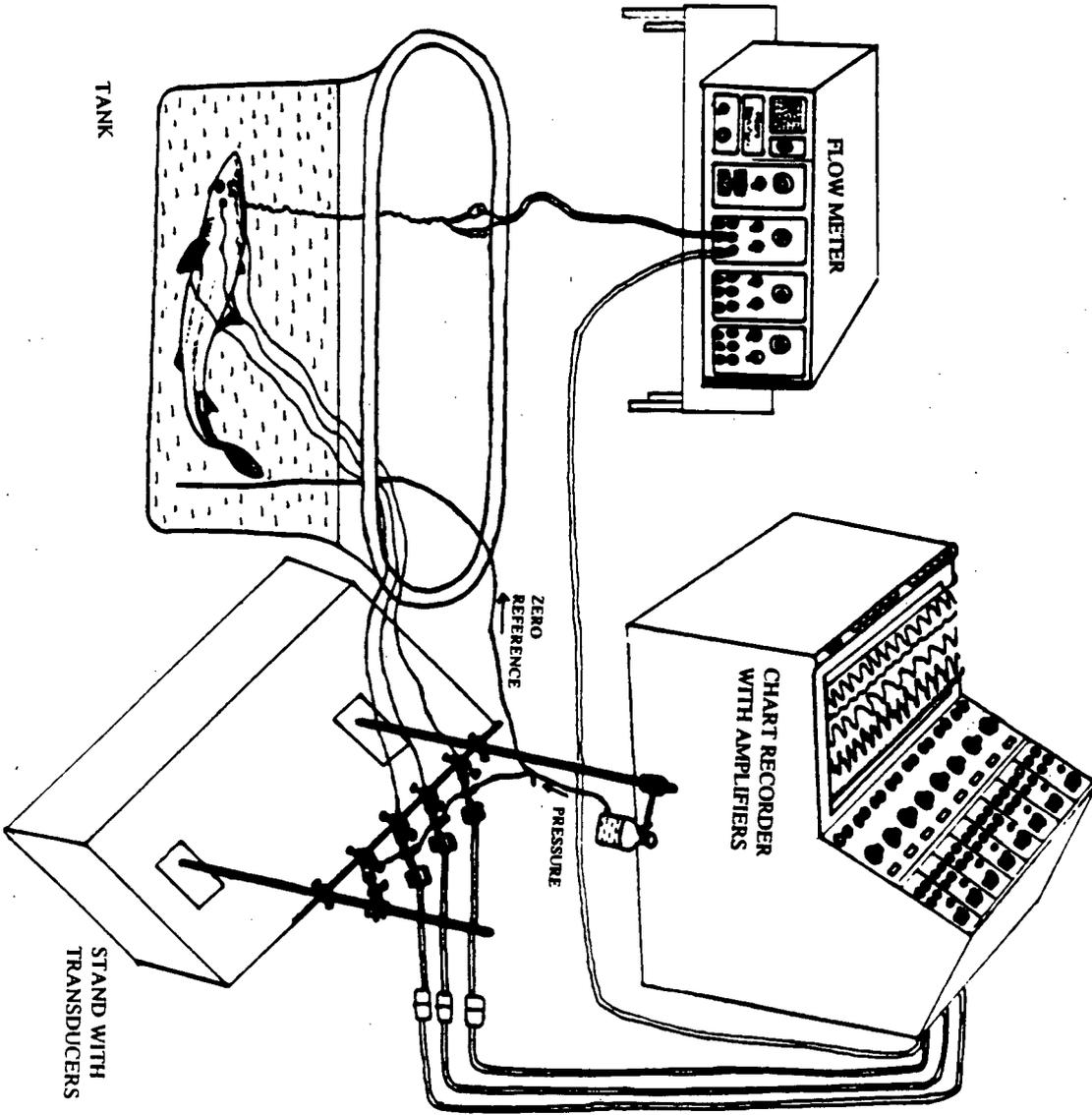
This ensured that the entire cardiac ejectate would pass through the flow transducer.

All cannulae were filled with heparinised (20 I.U./ml of Ringer's) elasmobranch Ringer's (NaCl 16.36 g/l, KCl 0.447 g/l, CaCl₂ 0.277 g/l, MgCl₂ 0.285 g/l, Na₂SO₄ 0.07 g/l, Urea 21.02 g/l, NaHCO₃ 0.67 g/l). For the pericardial cannula unheparinised elasmobranch Ringer's (saline) was used.

Animals prepared for chronic experiments, were returned to the holding tank after surgery and allowed at least 24-48 hours to recover before any experiments were conducted. The animal was then transferred to a small rectangular tank (1.4m long, 0.7m wide, 0.6m deep) with aerated running seawater. The cannulae were attached to disposable Deltran transducers (DPT 100, Utah Medical Products, U.S.A.). The seawater surface in the tank was used for obtaining zero pressure. The transducers were calibrated statically against a column of water or saline. The signals were amplified (Gould Universal Amplifiers, model 13-4615-58) and recorded on an electrostatic chart recorder (Gould, ES1000)(figure 4).

For recording the ventricular and conal depolarisation signals, the fish was anaesthetised and placed on the surgical table as described earlier. The heart was exposed by cutting open the pericardium. Two pairs of ECG recording electrode wires (40 gauge) were placed directly on the surface of the heart. The depolarisation

Figure 4. A diagrammatic sketch of the experimental setup used to record data from chronically instrumented dogfish in the present study.



signal was isolated (Gould Isolated Preamplifier, model 11-5407-58), amplified (Gould Universal Amplifiers, model 13-4615-58) and recorded on an electrostatic chart recorder (Gould, ES1000) at fast speeds. By measuring the distance between the two pairs of electrode wires and the time taken by the depolarisation wave to pass these electrode pairs, conduction velocities within and between the heart chambers could be calculated.

Scanning electron microscopy was employed to study the position of valves in the conus arteriosus of the spiny dogfish. An animal was anaesthetised and placed on the surgical table. The pericardial cavity was opened and the heart exposed. The conus arteriosus was excised from the ventricle and the ventral aorta and quickly washed in elasmobranch Ringers. The conus was then transferred to freshly prepared fixative (2.5% glutaraldehyde in 0.1M sodium cacodylate buffered to pH 7.3-7.4 was used as a fixative). After eight hours in the fixative at room temperature the conus was then washed in 0.1M sodium cacodylate buffer and osmicated, for one hour, in 1% OsO₄ in 0.1M sodium cacodylate buffer. After dehydration in increasing grades of ethanol (70%, 80%, 90% and 100%), the tissue was critical point dried (Belzers, CPD 020) and then sputter coated (Nanotech, Sempreg II). The conus was then viewed under a Cambridge 250T scanning electron microscope at 20 kV.

RESULTS

Upon direct observation of a beating heart, it was seen that conal contraction followed ventricular contraction. The same was confirmed by the ECG recorded by placing recording electrodes directly on the surface of the ventricle and conus (figure 5).

The intraventricular pressure showed two components in the rising phase (figure 6), an isovolumic phase and an ejection phase. It took approximately 0.9-1.0 seconds for the intraventricular pressure to reach its peak value. The intraventricular pressure then began to fall, signalling ventricular relaxation, and reached its baseline value within 0.4 seconds.

Ventral aortic pressure recorded from anaesthetised and unanesthetised spiny dogfish (N=10) showed two distinct peaks (figures 7a,7b,8). The first peak, which can be attributed to ventricular systole, was higher. The second, later and smaller peak can be ascribed to the contraction of the conus arteriosus. If the ventral aortic pressure was recorded from the proximal region of the ventral aorta, a conspicuous notch in the trace was observed (figure 8b). Also, this notch was not observed if a chart recorder with a lower frequency response was used (figure 8a). This notch signalled the initiation of contraction of the lower part of the conus and hence the isolation of the ventricle from the output circulation. As the conus

Figure 5. ECG traces from a dogfish recorded by placing the recording electrodes directly on the surface of the heart showing the time delay between ventricular and conal depolarisation signals.

(Room temperature 16°C, ventilating sea water temperature 10°C).

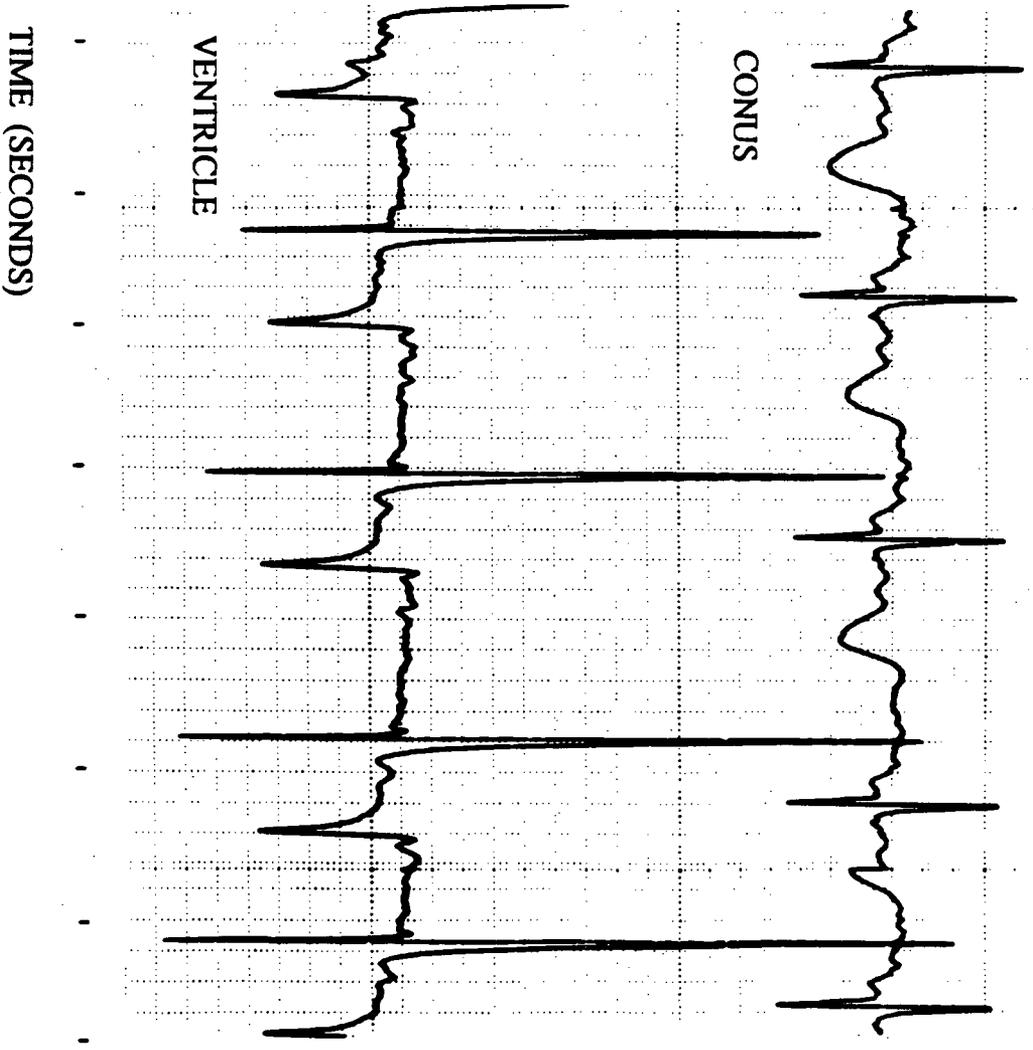


Figure 6. Simultaneous recording of ventricular pressure (VP), partial stroke flow (PS), abdominal pressure (ABP) and intrapericardial pressure (P) in an unanaesthetised, chronically instrumented, resting spiny dogfish.

(SDF 180894, 1.0Kg, female, 68cm long)

TIME (seconds)

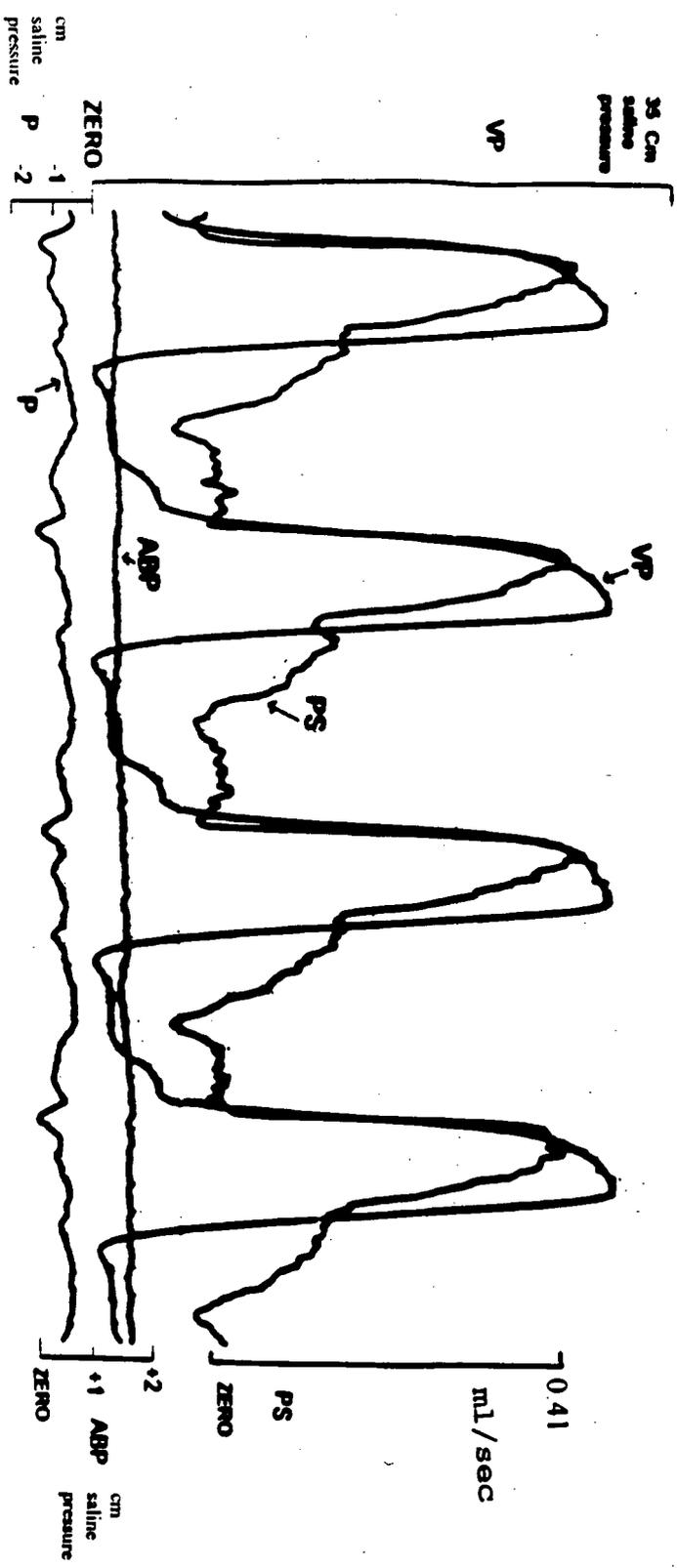
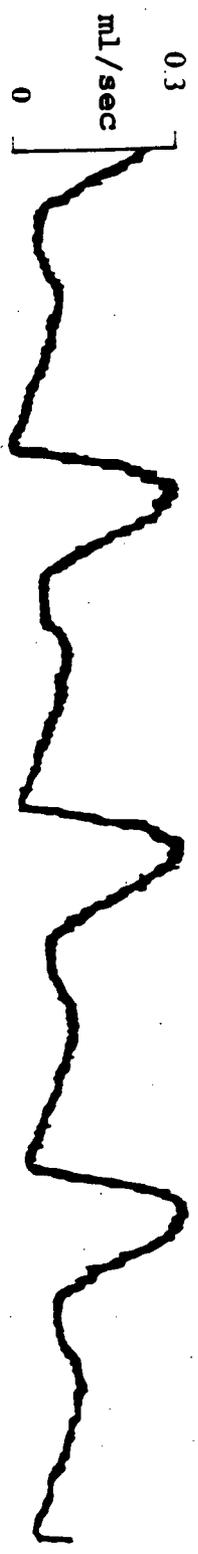


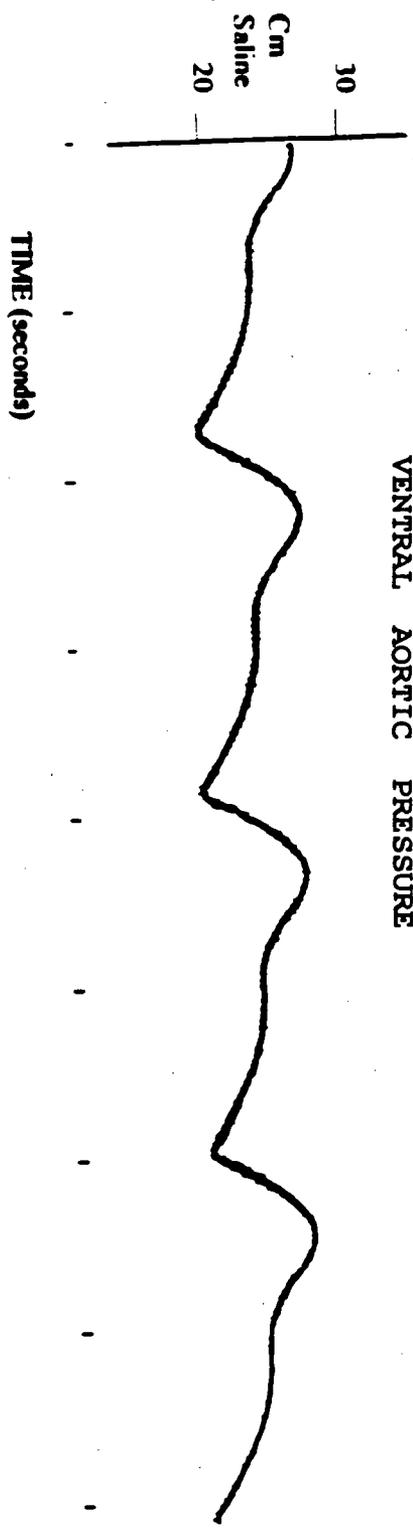
Figure 7(a). Partial stroke flow and ventral aortic pressure recorded from an anaesthetised, spiny dogfish.

(SDF 171292, 0.75 Kg, female, 70cm long)

PARTIAL STROKE FLOW



VENTRAL AORTIC PRESSURE



contracted, the pressure in the ventral aorta rose and then gradually began to fall indicating conal relaxation.

The ventral aortic flow records also showed two distinct pulsatile phases (figures 6,7a,7b,8) which corresponded to contributions by the ventricle and the conus respectively. The first peak represented the ventricular contribution and the second smaller peak corresponded to the conal contraction. Simultaneous measurements of ventricular pressure and ventral aortic flow confirmed that the distinct second component observed in the flow was not due to the ventricular contraction.

Conduction velocities within and between the heart chambers were calculated (N=4) as described in the methods. There was only a very slight time delay of the depolarisation signal between the two pairs of electrodes placed on the ventricle hence, the conduction velocity within the ventricle was quite rapid (1.2-1.6 meters/second). However, there was considerable time delay of the depolarisation signal between the ventricle and the conus as compared with that in the ventricle (figure 5) and was around 0.01-0.02 m/s. The depolarisation signal also showed a time delay between recording sites on the conus (figure 9). The conduction velocity within the conus was slow compared to that within the ventricle, and ranged between 0.04-0.13 m/s.

Figure 7(b). Partial stroke flow and ventral aortic pressure recorded from an anaesthetised, spiny dogfish.

(SDF 220693, 2.0Kg, female, 80cm long)

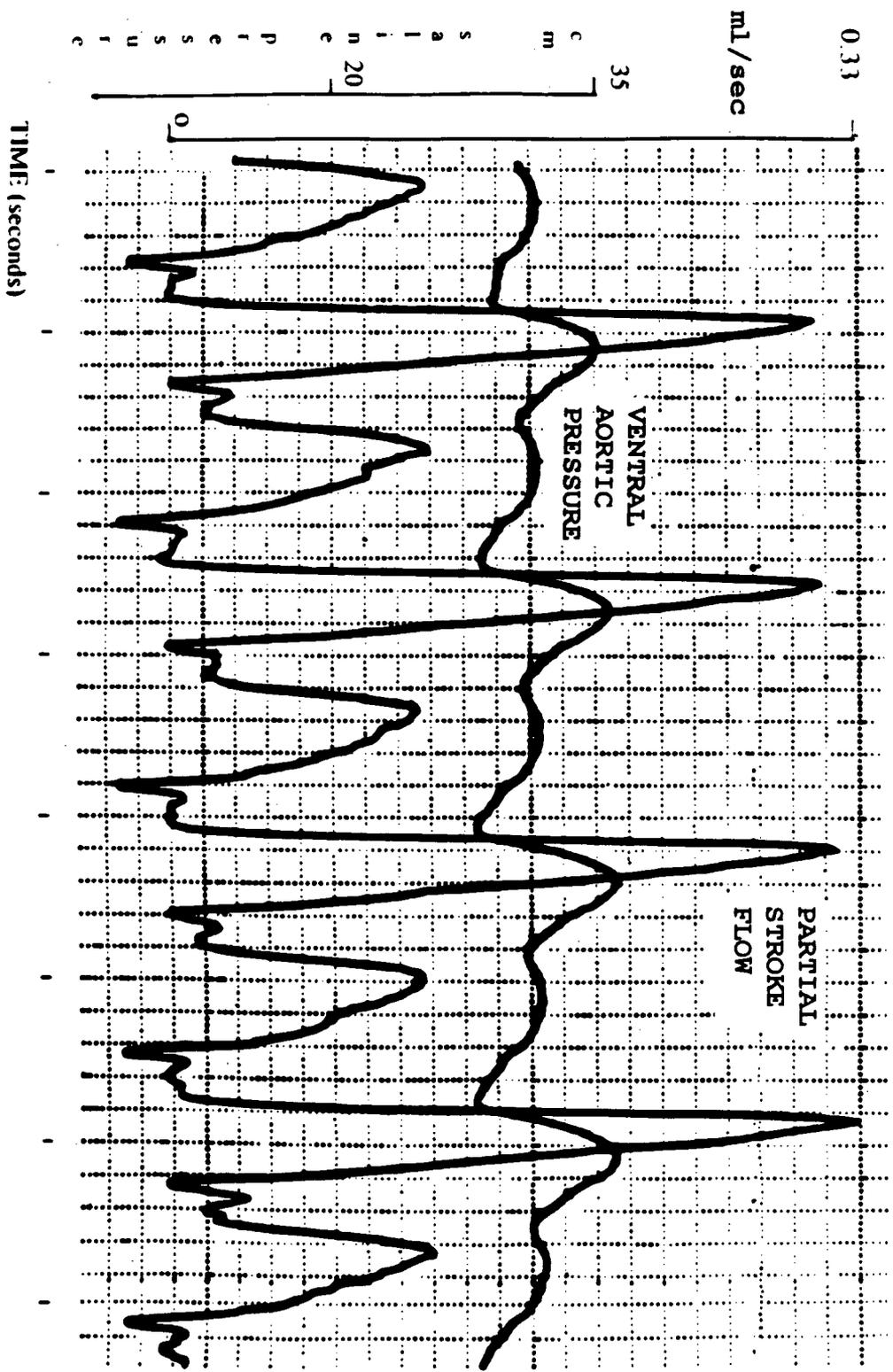


Figure 8. Total stroke flow and ventral aortic pressure from an unanaesthetised, chronically instrumented, resting spiny dogfish (SDF 020394, 3.0 Kg, female, 85cm long). Note that both traces are from the same animal but recorded on different chart recorders. Figure 8(a) recorded on 2-channel Technirite chart recorder (model TR722), while in figure 8(b) the ventral aortic pressure is recorded on Gould electrostatic chart recorder (model ES1000).

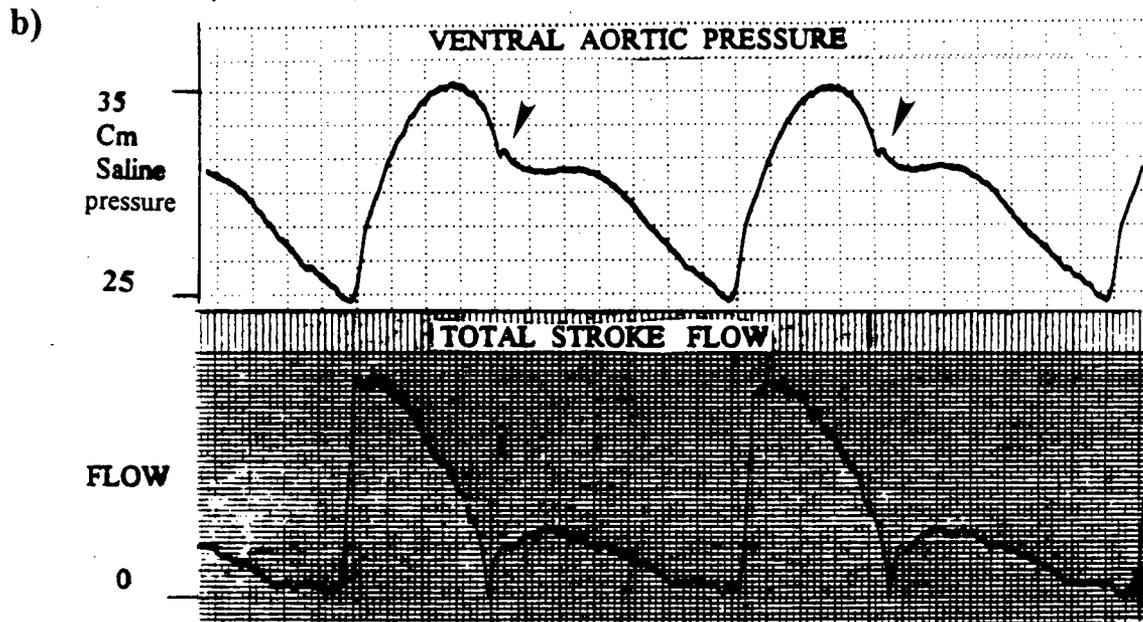
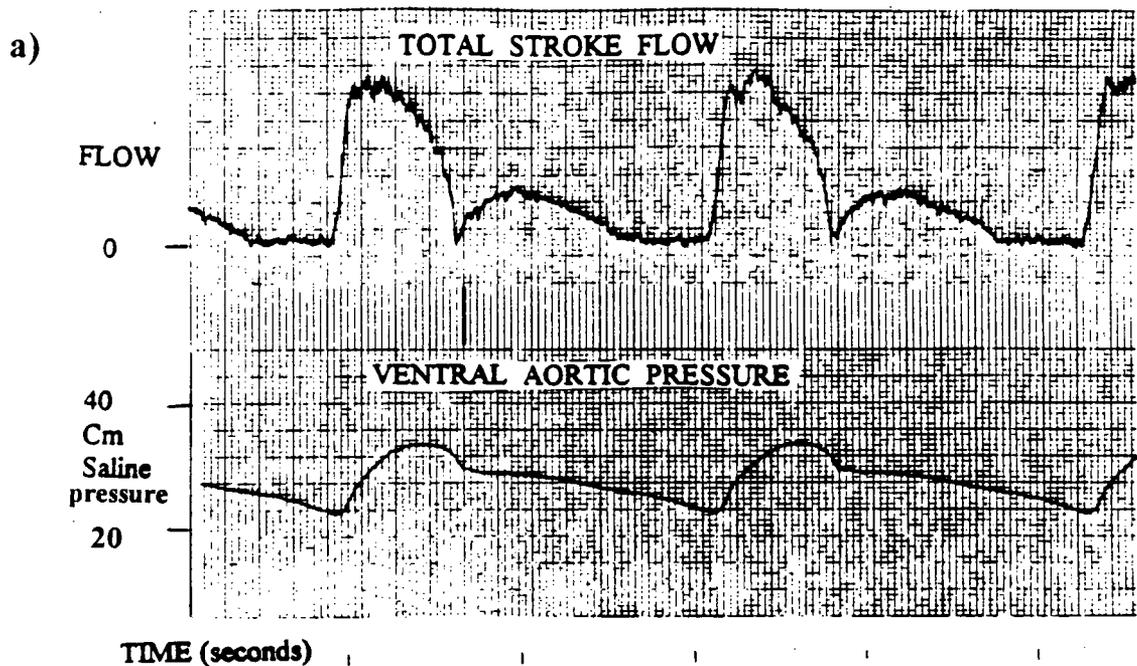
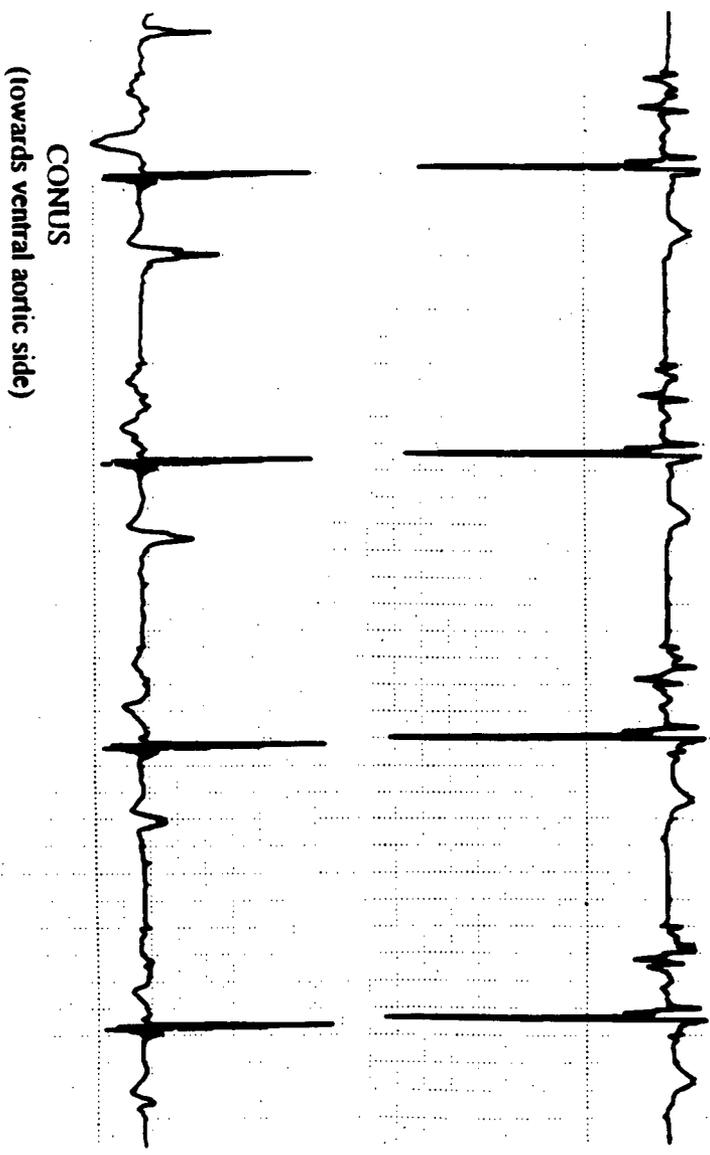


Figure 9. ECG traces from a dogfish recorded by placing recording electrodes directly on the surface of the heart showing time delay of depolarisation signal between two reference points on the conus arteriosus.

(Room temperature 16°C, ventilating seawater temperature 10°C).

CONUS
(towards ventricular side)



TIME (SECONDS)

Scanning electron micrographs showed that the valves were arranged in 4 tiers (figure 10). Each valve was composed of two parts: a central body, the leaflet, and the space enclosed by the leaflet, the sinus. Each leaflet was attached laterally and proximally to the conus wall while the distal portion was free. Adjacent leaflets joined together at their latero-distal edges and were connected to the conus wall (see Sans-Coma *et al* 1995 for review). The anteriormost tier (closest to the conus-ventral aortic junction) consisted of three large valves of similar size which extended almost half the length of the conus. The leaflet component in the anteriormost valves was much thickened compared with the valves in the posterior tiers. It was wider towards the conus-ventral aortic junction and became narrower as it reached the mid-length of the conus.

The remaining posterior three tiers of valves were positioned very close to each other. The valves in these tiers, in contrast to the anteriormost tier, had a smaller leaflet. A peculiar feature was that the free edges of these valves were connected to the base of the leaflet above it by a variable number of *chordae tendinae*. Further, the number of valves in these posterior tiers was also variable, with a range of 3-5 valves in each tier (figure 11). Also, the shape and size of the valves in the posterior tiers were variable.

Figure 10. A scanning electron micrograph montage of the conus arteriosus of spiny dogfish to show the position of various tiers of valves and the number of valves in each tier.

(R= right side, D= dorsal side, L= left side)

(1,2,3,4 refers to the tier number)

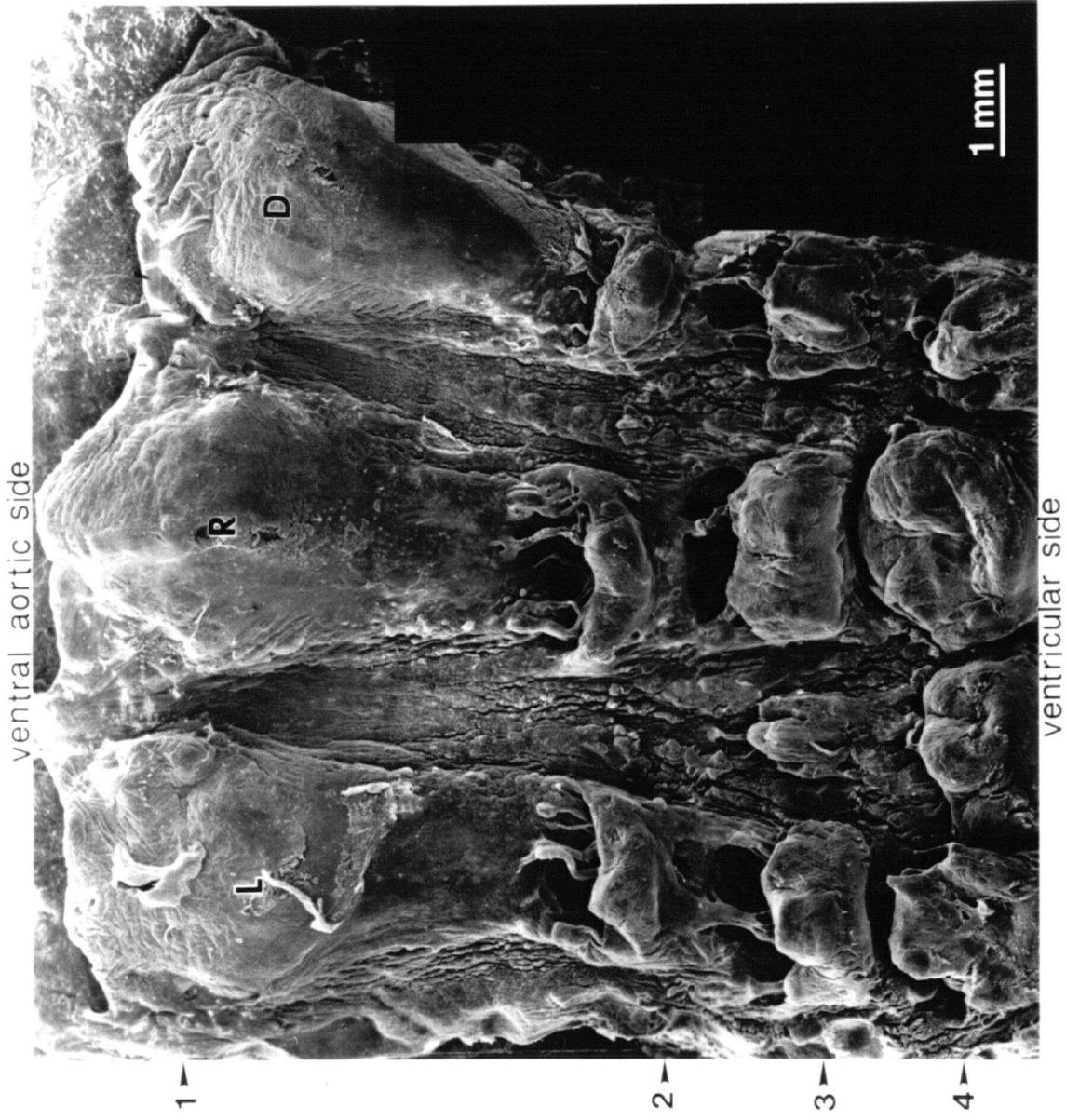
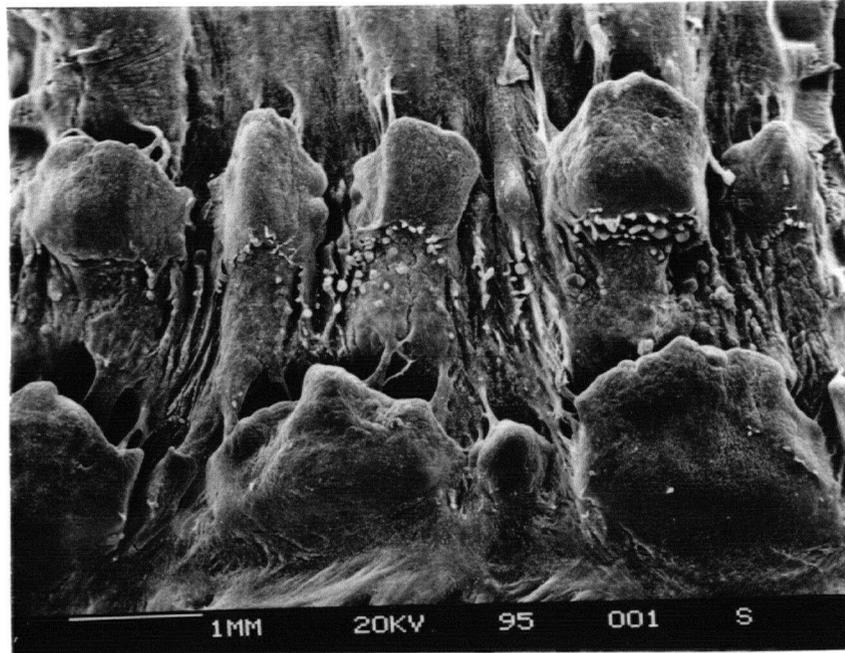


Figure 11. Scanning electron micrographs of the conus arteriosus of spiny dogfish from two different animals showing the variation in the number of valves in the lowermost tiers (i.e. 3rd & 4th).

(3,4 refers to the tier number).

VENTRAL AORTIC SIDE

3



4

VENTRICULAR SIDE

VENTRAL AORTIC SIDE

3



4

VENTRICULAR SIDE

DISCUSSION

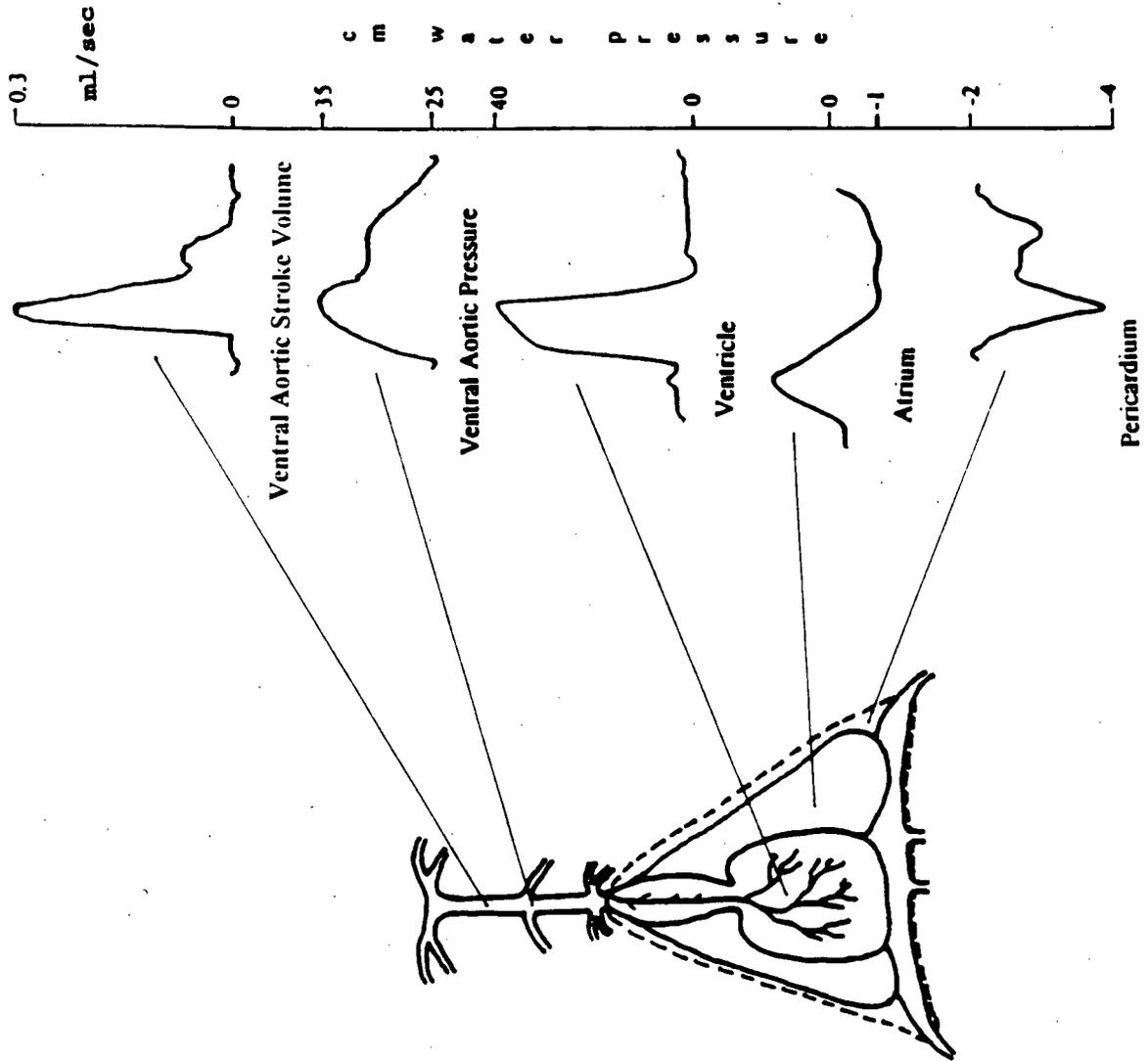
The results from the present study clearly show a distinct second component in the ventral aortic pressure and flow. Based on direct observation of a beating heart, the ECG traces and the simultaneous recording of the ventricular pressure and ventral aortic flow, this second component in the ventral aortic pressure and flow can be ascribed to contraction of the conus arteriosus. Hence, the presented results do not support the conclusions made by Satchell and Jones (1967) that the ventral aortic pressure declines steadily when the conus and the ventral aorta are in communication; and that the blood flow in the ventral aorta is at or near zero during conal contraction.

The addition of conal contraction on to ventricular contraction, besides contribution to ventral aortic pressure and flow, also serves to prolong the ejection phase of the heart. Further, conal contraction is slow, as evident by slow conduction of the depolarisation signal, possibly due to the high resistance of the conal muscle fibers.

The contribution of the conus to the total flow in comparison to ventricle was small but not insignificant. This was not unexpected because the volume of the conus is about one-quarter of that of the ventricle.

Figure 12. Pressures from various heart chambers, pericardium, ventral aorta, and flow from the ventral aorta of dogfish in the present study, arranged to represent one cardiac cycle. Not all the traces are from the same animal.

(layout adapted from a figure in "Circulation in fishes" by Satchell 1971).



Satchell and Jones (1967) suggested that the prime function of conal contraction was to postpone the time when the anteriormost tier of valves would close, to an instant when the negative pressure inside the pericardium, caused by ventricular ejection, was at its minimum. In their experiments they changed the intrapericardial pressure and observed its effect on ventral aortic flow and pressure. They concluded that with negative intrapericardial pressure, the backflow in the ventral aorta increased. However, they did not control the ventral aortic and intrapericardial pressures independently. Hence, the only inference that can be drawn from their work is that as the transmural pressure increases, backflow increases (Farrell and Jones 1992). Surprisingly, some of their traces show backflow even when the intrapericardial pressure was positive throughout the cardiac cycle. Furthermore, backflow in their traces is associated with the closure of the lower tier of valves not the upper tier of valves (figure 2), which are competent even when the conus is relaxed. Moreover, the ventral aortic flow records in the present study show that the backflow always occurred after, and not before, conal contraction.

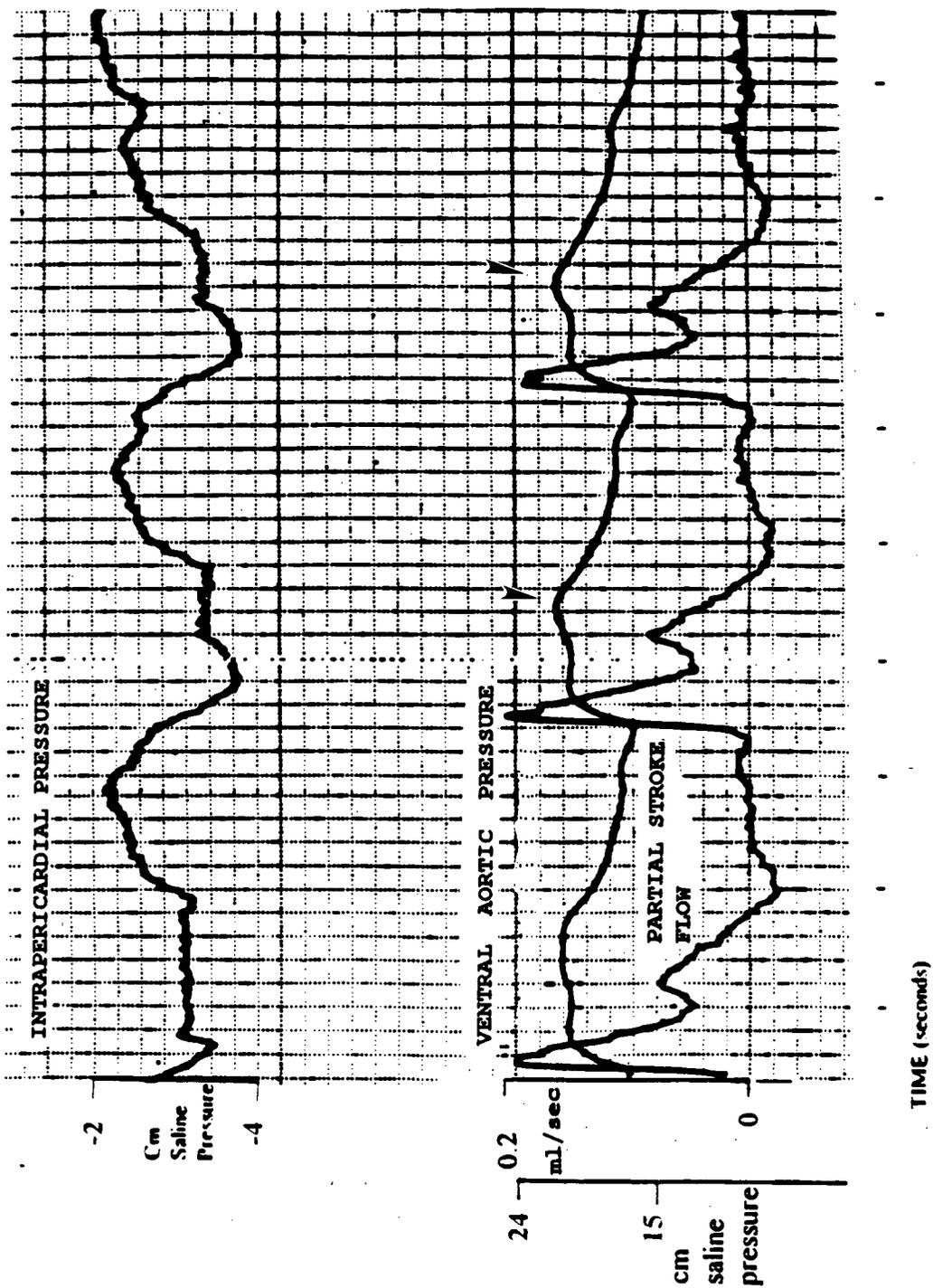
Satchell and Jones (1967) presumed that a negative pressure outside the conus is more likely to make the valves incompetent than an equivalent positive pressure in the ventral aorta. This presumption is unreasonable because the effect of a negative intrapericardial pressure of, for example, 2cm H₂O pulling apart the valves is no different than a positive pressure of 2cm H₂O in the ventral aorta pushing the valves apart.

The present results are in agreement with those reported by Johansen *et al* (1966) and Hanson (1967). However, Johansen *et al* (1966) and Hanson (1967) used an ultrasonic frequency shift flowmeter to record ventral aortic flow which could lead one to question their results because such equipment displays reverse and forward flow as forward flow, i.e. it is unidirectional in its output. Hanson (1967) reported conal contribution to flow in the dogfish (*Squalus acanthias*), but his blood velocity traces cannot be interpreted conclusively due to the nature of the recording equipment, i.e. the second phase of positive flow could be backflow. However, his blood velocity traces in the skate (*Raja binoculata*) show an uninterrupted second phase of flow that must be attributed to conal systole. This is because in order for flow to reverse it must first decline to zero. Hence, the oscillations in the ventral aortic flow associated with the conal contraction, as reported by Johansen *et al* 1966, should represent forward flow.

A recent study on the study on embryos of the little skate (*Raja erinacea*) reported ventral aortic pressures with two distinct peaks corresponding to ventricular and conal systole (Pelster and Bemis 1990). Johansen's (1965) work on the skate (*Raja binoculata*) showed conal pressure to be greater than the ventricular pressure. Such an observation has not been reported by other researchers. In the present study only in one case, transiently, was such a pattern observed (figure 13) however, within minutes the ventral aortic pressure reversed suggesting this transient phase may have been an artefact.

Figure 13. Intrapericardial pressure, partial stroke flow and ventral aortic pressure from an anaesthetised, spiny dogfish. Please note the second peak in the ventral aortic pressure.

(SDF 100693, 1.8Kg, male, 75cm long)



The question whether or not the conus can function as a depulsator (reducing peak systolic pressure) has long been debated (see Bushnell & Jones 1992). It was argued that for the conus to assume such a function elasticity would be the main requirement. This elastic component was thought to be lacking because the conus was contracting during ventricular ejection (March *et al* 1962; Sudak 1965a,b; Satchell & Jones 1967; Bushnell & Jones 1992), therefore, its wall would be stiff. On the contrary, the electrocardiogram (ECG) shows a considerable time delay between the ventricular and conal depolarisation (Tebecis 1967; Satchell 1991; this study fig. 5) thus, favouring the idea that the conus may not be stiff early in the ventricular systole and therefore may be capable of a depulsating function. A long conduction delay implies the presence of nodal tissue at the ventricular-conal junction (Satchell 1991; Bushnell & Jones 1992). Further work needs to be done using electron microscopy techniques to identify this nodal tissue.

A strange aspect of the conus is the presence of numerous tiers of valves. The significance of the anteriormost tier of valves is understandable, however, the presence of numerous smaller tiers of valves (figure 10) remains a mystery. The problem is further compounded by the variation in the number of smaller tiers of valves and the number of valves in each tier. This variation could be inter-generic, inter-specific or surprisingly even intra-specific. For example, in *Squalus acanthias* transverse rows 2-4 are reported to have four valves each (O'Donoghue 1928, Hanson 1967). However, O'Donoghue (1928) also reported that the number of

valves could vary due to the existence of smaller valves in between those normally present. He called these valves supernumerary valves. The present scanning electron micrographs support O'Donoghue's view (figure 11). Satchell & Jones (1967) proposed that an anteriorly directed wave of contraction ensures successive closure of each tier of valves. It is quite possible that the smaller conal valves in each posterior tier ensure that there is no backflow into the ventricle during conal contraction. Further, the presence of an increased number of tiers of valves in the conus may imply a prolonged conal contraction which in turn would prolong the ejection phase of the heart and at the same time delaying the decline in the ventral aortic pressure. This would permit increased diastolic filling of the ventricle and hence cardiac output.

Hence, the present study proposes multifaceted functions for the elasmobranch conus arteriosus, such as prolongation of the ejection phase of the heart, contribution to ventral aortic pressure and flow, patency of smaller tiers of valves and perhaps depulsation.

**CHAPTER TWO : Effect of change in intrapericardial pressure
upon input & output pressures and flow.**

INTRODUCTION

Reports of subambient pressures inside the pericardium of elasmobranchs date back to the late nineteenth century. Schoenlein 1895 reported negative intrapericardial pressures in the elasmobranch *Torpedo ocellata* (cited in Johansen 1964). Subsequent work on different elasmobranch species has confirmed the presence of subambient intrapericardial pressures (Sudak 1965a,b, Johansen 1965, Satchell 1971, Shabetai *et al* 1985). The cause of subambient intrapericardial pressures is attributed to the attachment of the pericardium to the surrounding skeletal structures which imparts semi-rigidity to it. Ventricular ejection of blood results in a sharp reduction in the volume of the ventricle inside the pericardium that results in a sharp fall in intrapericardial pressure. This decrease in intrapericardial pressure is transmitted to the thin-walled atrium and sinus venosus and provides the necessary pressure gradient for atrial filling. This type of filling is known as *vis-à-fronte* or aspiratory filling. The integrity of the pericardium is essential for the development of subambient intrapericardial pressures. Rupturing the pericardium results in equilibration of intrapericardial pressure with ambient and a consequent decrease in cardiac output of the heart (Johansen 1964, Sudak 1965a,b, Satchell 1971, Abel *et al* 1987). Even in perfused dogfish hearts, rupturing the pericardium causes a marked reduction in cardiac output, suggesting that *vis-à-fronte* is the important filling mechanism under resting conditions (Franklin and Davie 1993).

Despite the widespread agreement about the presence of subambient intrapericardial pressures in elasmobranchs, Burger & Bradley (1951) could not confirm subambient intrapericardial pressures in the dogfish *Squalus acanthias*. Also, Satchell & Jones (1967) did not observe any changes in blood pressure & flow upon opening the pericardium in *Heterodontus portusjacksoni*. Further, a recent report on horn sharks (*Heterodontus francisci*) indicated that chronic resting intrapericardial pressures may not be negative (Abel *et al* 1986). Abel *et al* (1986) argued that most of the previous pressure measurements were taken on supine, anaesthetised fish, and the handling stress involved in preparing the animals for experiments may have forced pericardial fluid into the peritoneal cavity, via the PPC, thus making the intrapericardial pressure subambient.

A consequence of *vis-à-fronte* filling is that central venous blood pressures will be subambient (Satchell 1971). Lai *et al* (1989) recorded positive intrapericardial pressure and elevated cardiac output during swimming in leopard sharks (*Triakis semifasciata*) and argued that the filling mechanism in elasmobranchs was *vis-à-tergo* or filling by force (an implication of *vis-à-tergo* filling is that central venous blood pressure must be positive). They later showed that cardinal sinus pressure was positive during rest and increased during swimming, suggesting that increased cardiac filling was by a *vis-à-tergo* mechanism in leopard sharks (Lai *et al* 1990).

Some reported experimental results (Abel *et al* 1987) suggest that the responses exhibited to certain experimental situations may not be the same in different elasmobranch genera for there are major differences in stroke volumes at positive intrapericardial pressures in horn sharks & blue sharks (*Prionace glauca*). Abel *et al* (1987) reported that in horn sharks at intrapericardial pressures above 0.5 cm H₂O, stroke volume was greatly reduced. However, in blue sharks, large stroke volumes were recorded at intrapericardial pressures of 1-2 cm H₂O (Abel *et al* 1987).

Hence, it was the intention of this research to re-investigate the role of pericardium in cardiac function in elasmobranchs using spiny dogfish (*Squalus acanthias*). Simultaneous measurement of venous, intrapericardial and arterial pressures and flow have been made from chronically instrumented dogfish. Further, the effect of changing intrapericardial pressure on the above mentioned variables was recorded. It has been suggested that the PPC permits increases in heart size by allowing the pericardial fluid to escape into the peritoneal cavity (Lai *et al* 1989). However, it is not known if the PPC could regulate the movement of pericardial fluid, i.e. by means of either valves or smooth muscle cells. To my knowledge no studies on the ultrastructure of PPC have been reported. So, finally the ultrastructure of the PPC was studied using transmission electron microscopy.

METHODS

Pressures from the pericardium, ventral aorta, cardinal sinus, peritoneum, and atrium were recorded, while flow was recorded from the ventral aorta. Not all the pressures were recorded from the same fish.

For pericardial and ventral aortic cannulation methods the reader is referred to the methods section of chapter 1. Double cannulation of the pericardial cavity was done to allow uninterrupted pressure recording while making simultaneous saline infusions or withdrawals. The tip of the second cannula was positioned as close to the bottom of the pericardial cavity as possible to allow complete aspiration of the pericardial fluid.

For atrial cannulation, a midline incision (4-5 cm long) was made in the abdominal region just posterior to the coracoid bar, opening the abdominal cavity. The hepatic vein on either side was located and an angiocath (20 gauge) advanced through it, into the atrium. The angiocath was secured in place using tissue adhesive and the incision was closed with silk (size 2-0). The cardinal sinus was cannulated with a P.E. tubing (P.E.90) after opening the abdominal cavity, or cannulated from the dorsal side of the animal close to the subscapular cartilage, using a 15 gauge hypodermic needle as described by Lai *et al* (1990). The peritoneal cavity was cannulated ventrally using a 15 gauge hypodermic needle. In

cases when the abdomen was opened, the cannula (P.E.90) was glued to the body wall or liver using tissue adhesive.

Transmission electron microscopy was employed to study the detailed structure of ventral wall of the PPC. An animal was anaesthetised and placed on the surgical table. The abdomen was opened medially just posterior to the coracoid bar and the PPC located. The ventral wall of the PPC was dissected free and transferred to freshly prepared fixative (2.5% glutaraldehyde in 0.1M sodium cacodylate buffered to pH 7.3-7.4 was used as a fixative) and cut into small pieces less than or close to 1mm^3 . The samples were transferred to small vials containing the fixative. After four hours at room temperature the samples were washed in 0.1M sodium cacodylate buffer and osmicated, for one hour, in 1% OsO_4 in 0.1M sodium cacodylate buffer. After dehydration in increasing grades of ethanol (70%, 80%, 90% and 100%), the samples were embedded in spurr resin. Fine sections showing gold-silver interference colours (70-150 nm) were stained with uranyl acetate and lead citrate and viewed on Zeiss 10C electron microscope at 60 kV.

RESULTS

To assess the effect of changes in intrapericardial pressure upon input and output pressure and flow, volume loading and unloading of the pericardium with saline was performed (N=10). As saline was gradually infused into the pericardium, the intrapericardial pressure increased, but the ventral aortic pressure and flow gradually decreased and vice versa (figures 14a,14b). Ventricular pressure also decreased with an increase in intrapericardial pressure and increased with decreasing intrapericardial pressure.

The maximum intrapericardial pressure achieved through volume loading ranged from 1.5 to 2.6 cm H₂O. Further saline infusion led to a temporary increase in intrapericardial pressure which soon returned back to the pre-infusion level, suggesting that the maximum pericardial fluid volume had been reached and excess saline was being vented through the PPC. At maximum intrapericardial pressures the characteristic oscillations associated with each cardiac cycle were very much reduced or lost.

When all the fluid had been aspirated from the pericardium, the pericardial pulse pressure was maximum. The intrapericardial pulse pressure decreased as fluid was infused into the pericardium (figures 14a,14b.).

Figure 14(a). Effect of saline infusion into the pericardium on the cardinal sinus pressure, intrapericardial pressure, ventral aortic pressure and ventral aortic flow from an unanaesthetised, chronically instrumented, resting dogfish.

Downward arrows indicate saline infusion and numbers on top of the arrows indicate the volume of saline infused into the pericardium. The infusion begins at zero pericardial fluid volume.

(SDF 190795, 4.0 Kg, female, 90cm long).

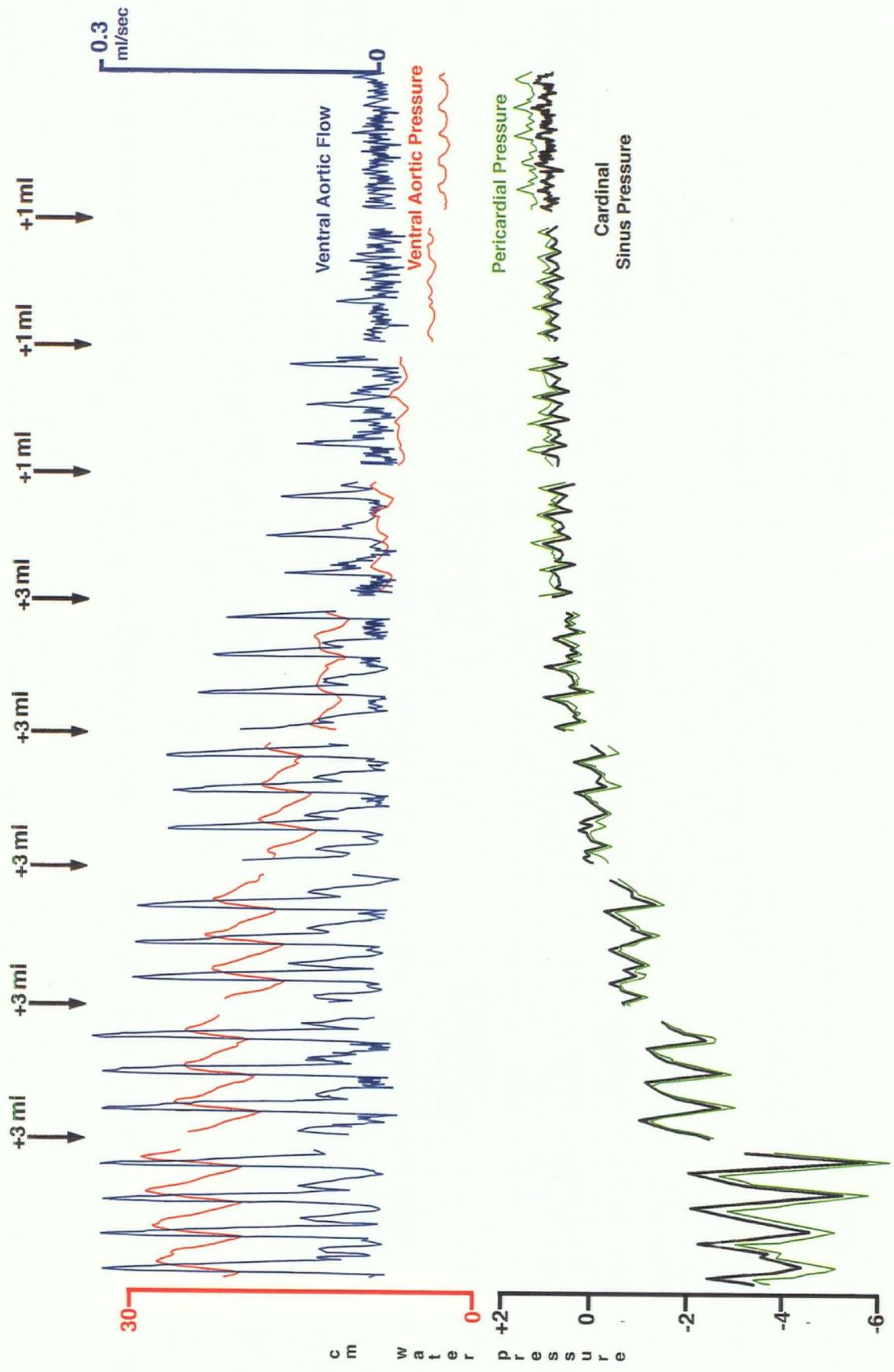
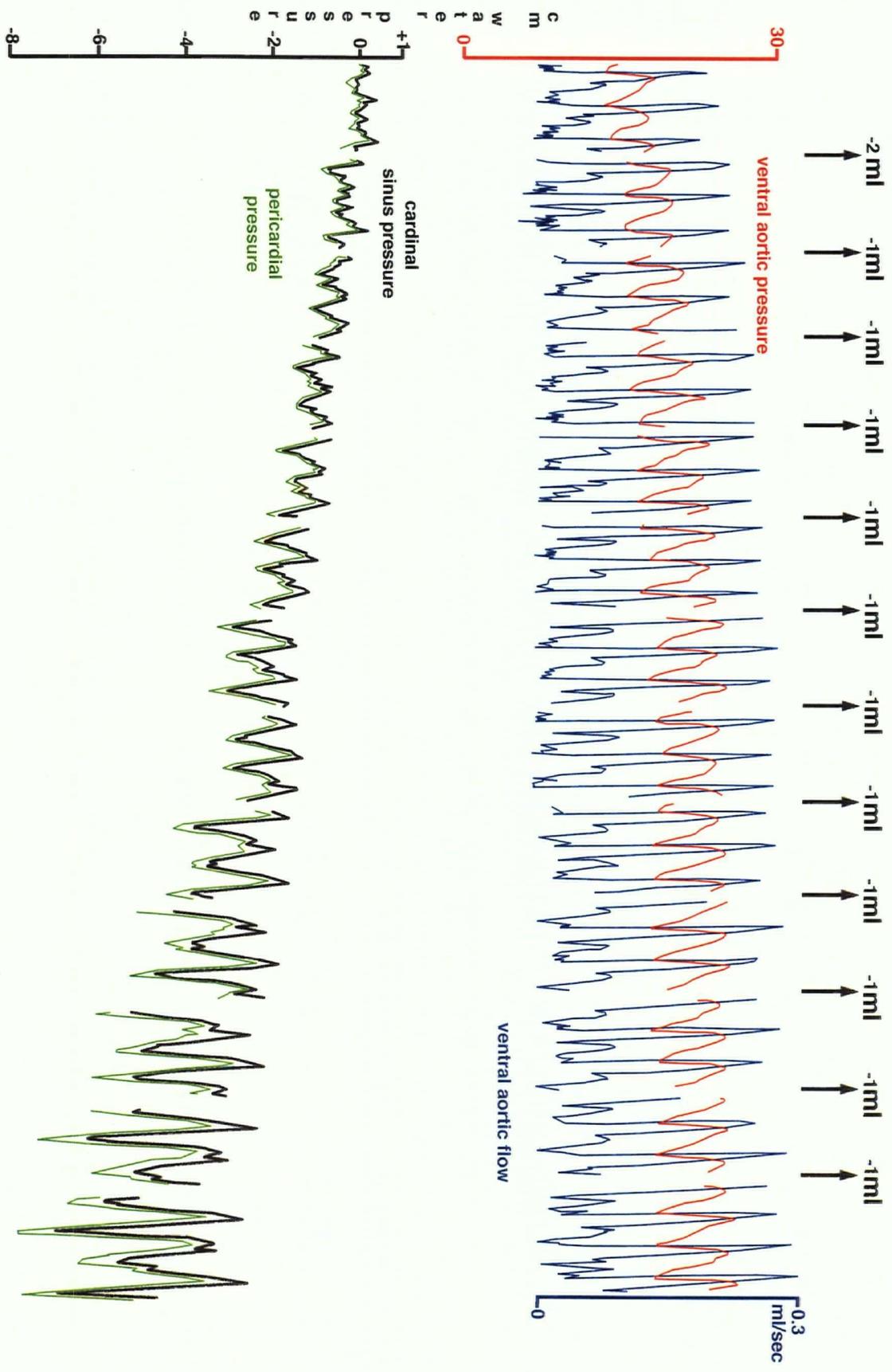


Figure 14(b). Effect of saline removal from the pericardium on the cardinal sinus pressure, intrapericardial pressure, ventral aortic pressure and ventral aortic flow from an unanaesthetised, chronically instrumented, resting dogfish. Upward directing arrows indicate saline removal and the numbers on top of them indicate the volume of saline removed from the pericardium. The infusion begins at zero pericardial fluid volume.

(SDF 190795, 4.0 Kg, female, 90cm long).



For pericardial fluid to be voided through the PPC, the intrapericardial pressure must exceed that in the abdominal cavity. Peritoneal pressure recorded from chronically instrumented dogfish (N=5) was usually at or above ambient (figure 6). Occasionally it was slightly subambient. The pressure trace often showed abrupt changes which may have been due to the movement of the internal organs against the recording cannula. Otherwise the peritoneal pressure remained constant.

Chronic intrapericardial pressure recorded from the instrumented dogfish was observed to be close to ambient (N=4). Usually, the maximum intrapericardial pressure was slightly positive while the minimum slightly negative, but on occasions both could be positive or negative (figure 15).

Simultaneous pressure recordings showing the effect of saline infusion/removal from the pericardium on cardinal sinus, atrial, ventral aortic pressure and/or flow were recorded from chronically instrumented dogfish (N=8). Figure (16) summarises the events from one dogfish. Extremely negative intrapericardial pressures did not produce any marked changes in either ventral aortic pressure or flow. When saline (1ml) was added to the pericardium at maximum negative intrapericardial pressure, the ventral aortic pressure rose and reached its maximum value. Further infusions of saline caused ventral aortic pressure and flow to decline. This sequence of events was reversed when saline was

Figure 15. Chronic intrapericardial pressure (maximum and minimum) recorded from unanaesthetised, resting dogfish and plotted against the number of days the measurements were recorded. Day 1 refers to first day after the surgery.

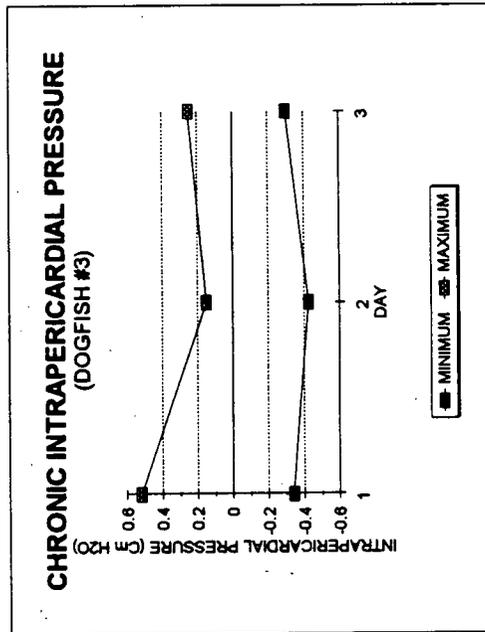
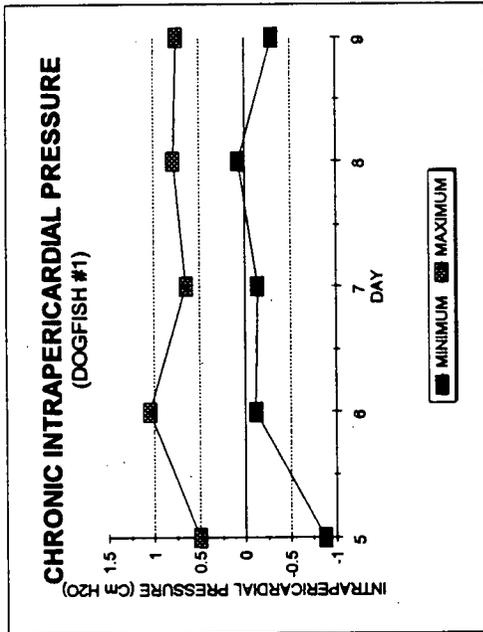
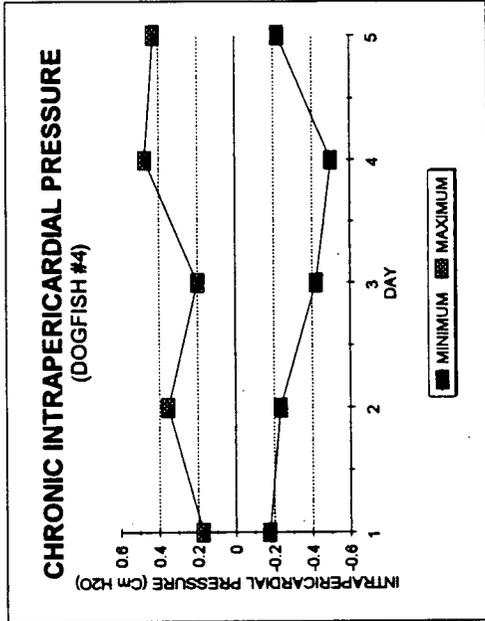
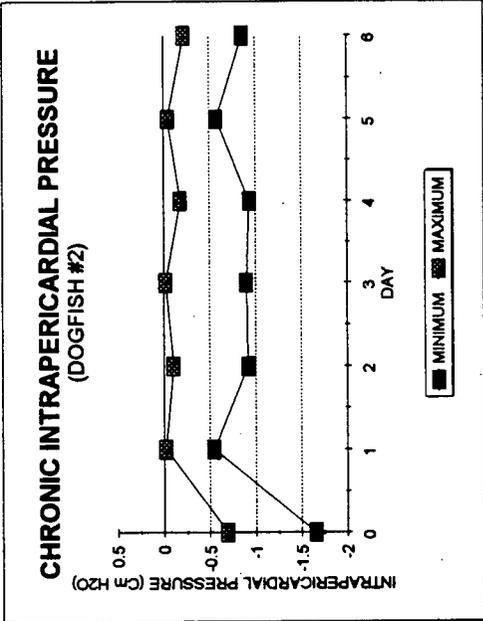
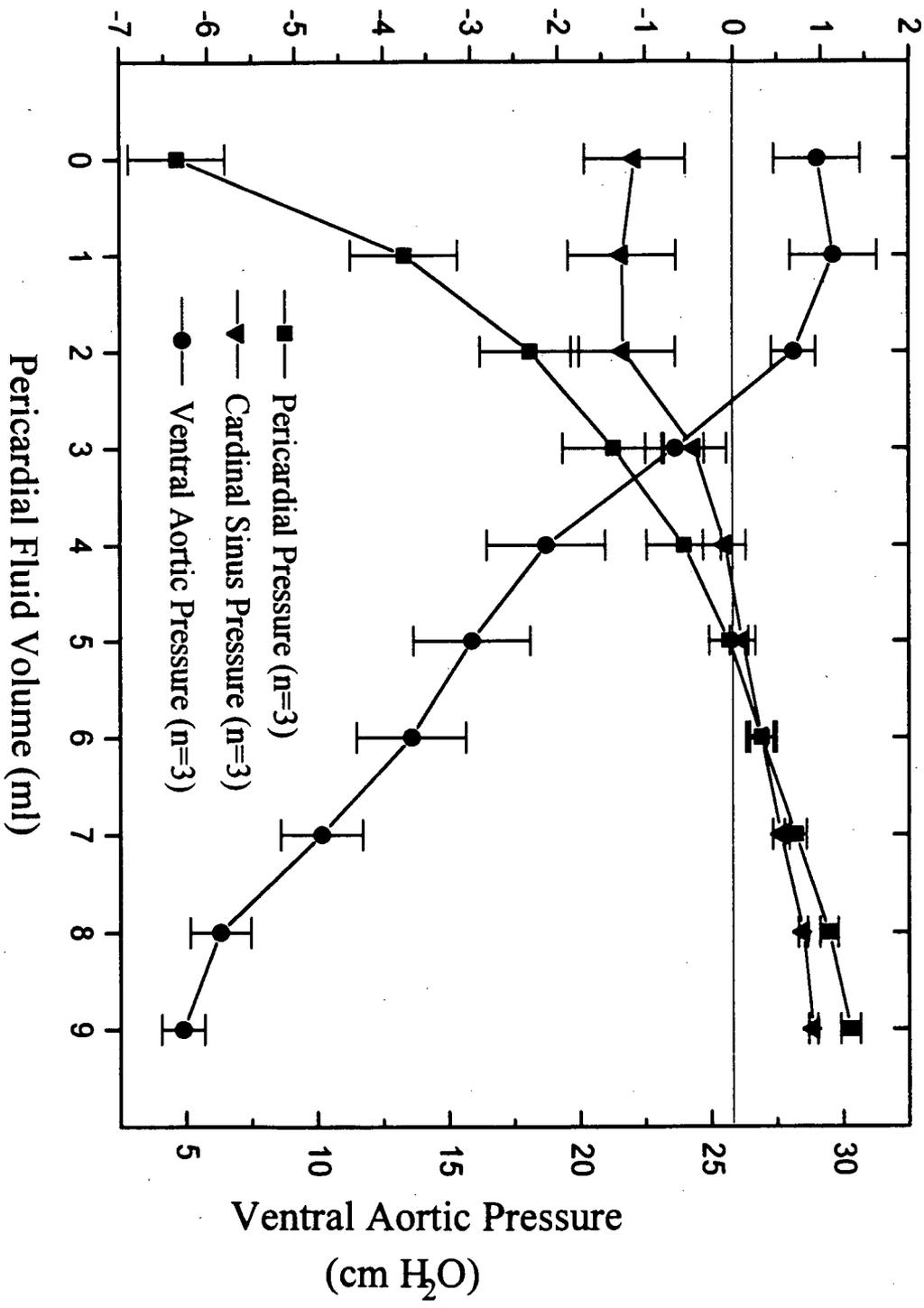


Figure 16. Effect of saline infusion/removal from the pericardium on the cardinal sinus pressure, intrapericardial pressure and ventral aortic pressure from an unanaesthetised, chronically instrumented, resting dogfish. X-axis represents the volume of saline infused or removed from the pericardium. At zero on the X-axis no more saline could be withdrawn. Data points are means of 3 cycles of infusion and withdrawal. Error bars are standard error of mean.

(SDF 300695, 0.6Kg, female, 72cm long).

(n = number of trials)

Pericardial & Cardinal Sinus
Pressure (cm H₂O)



removed from the pericardium. As the intrapericardial pressure was gradually increased, both atrial and the cardinal sinus pressures also increased and vice versa (figure 17). The maximum cardinal sinus pressure observed in resting dogfish ranged from 1.4 to 2.0 cm H₂O. When the intrapericardial pressure exceeded the cardinal sinus pressure, ventral aortic pressure reached its lowest value (figure 16) resulting in cardiac arrest. A brief period after the cardiac arrest the fish began to swim, and on cessation of movement it was observed that the intrapericardial, cardinal sinus pressures, ventral aortic pressure and flow had returned to pre-infusion levels (figure 18).

Figure (19) shows the mean of all the data, and it can be seen that the events depicted are similar to those described for the individual dogfish above. These data confirm that the maximum output of the heart occurs over a narrow range of intrapericardial pressure i.e. from -2 to 0 cm H₂O pressure.

PPC Ultrastructure

The PPC was an extremely thin, Y-shaped, normally closed, transparent canal in the dogfish. In *Squalus acanthias*, the transparent PPC was stretched sideways and attached to the ventral side of oesophagus. A physical examination of the PPC ruled out the existence of valves in or at its openings. The structure of the ventral wall of the PPC, with some exceptions, was quite similar to that described for the mammalian pericardium. The following account is compared with Reddy *et*

Figure 17. Effect of saline infusion and removal from the pericardium on the cardinal sinus pressure, atrial pressure and intrapericardial pressure from an unanaesthetised, chronically instrumented dogfish. Downward directed arrows indicate saline infusion while upward directed arrows indicate saline removal from the pericardium. The numbers on top of the arrows indicate the volume of saline infused or removed.

(SDF 260994, 1.3Kg, female, 80cm long).

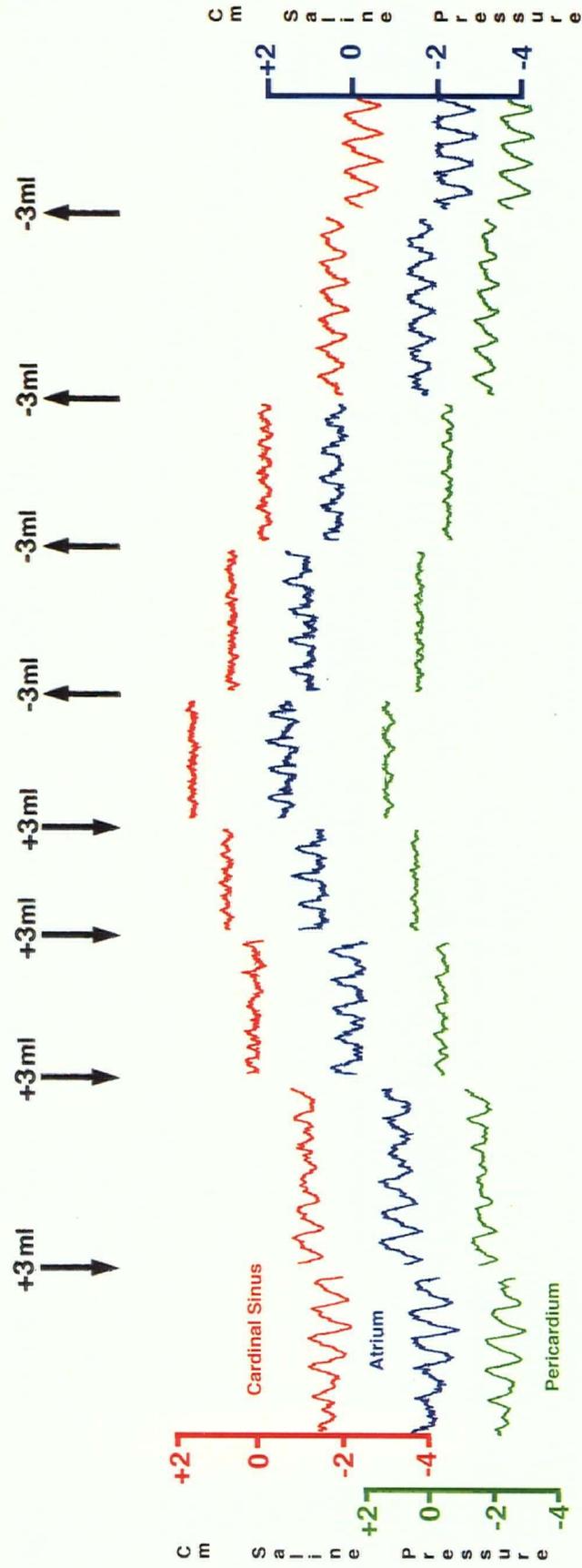
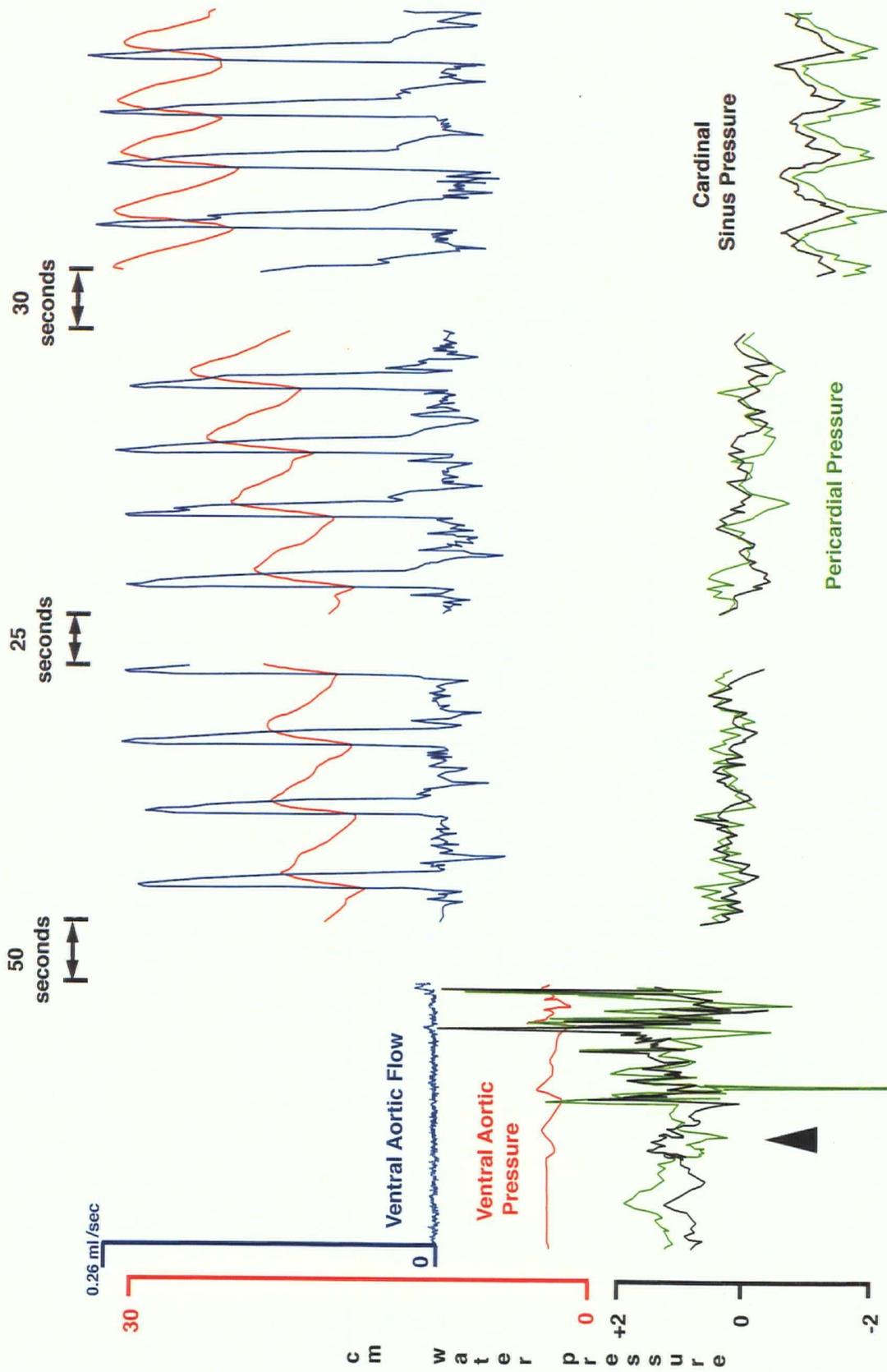


Figure 18. Effect of fish's movement in response to maximum saline infusion into the pericardium leading to a loss of cardiac activity, on the cardinal sinus pressure, intrapericardial pressure, ventral aortic pressure and ventral aortic flow. The fish's movement (arrowhead) probably resulted in opening of the PPC and a resultant loss of saline from the pericardium. Note the time taken to reach its final value.

(SDF 190795, 4.0 Kg, female, 90cm long)



cm water pressure

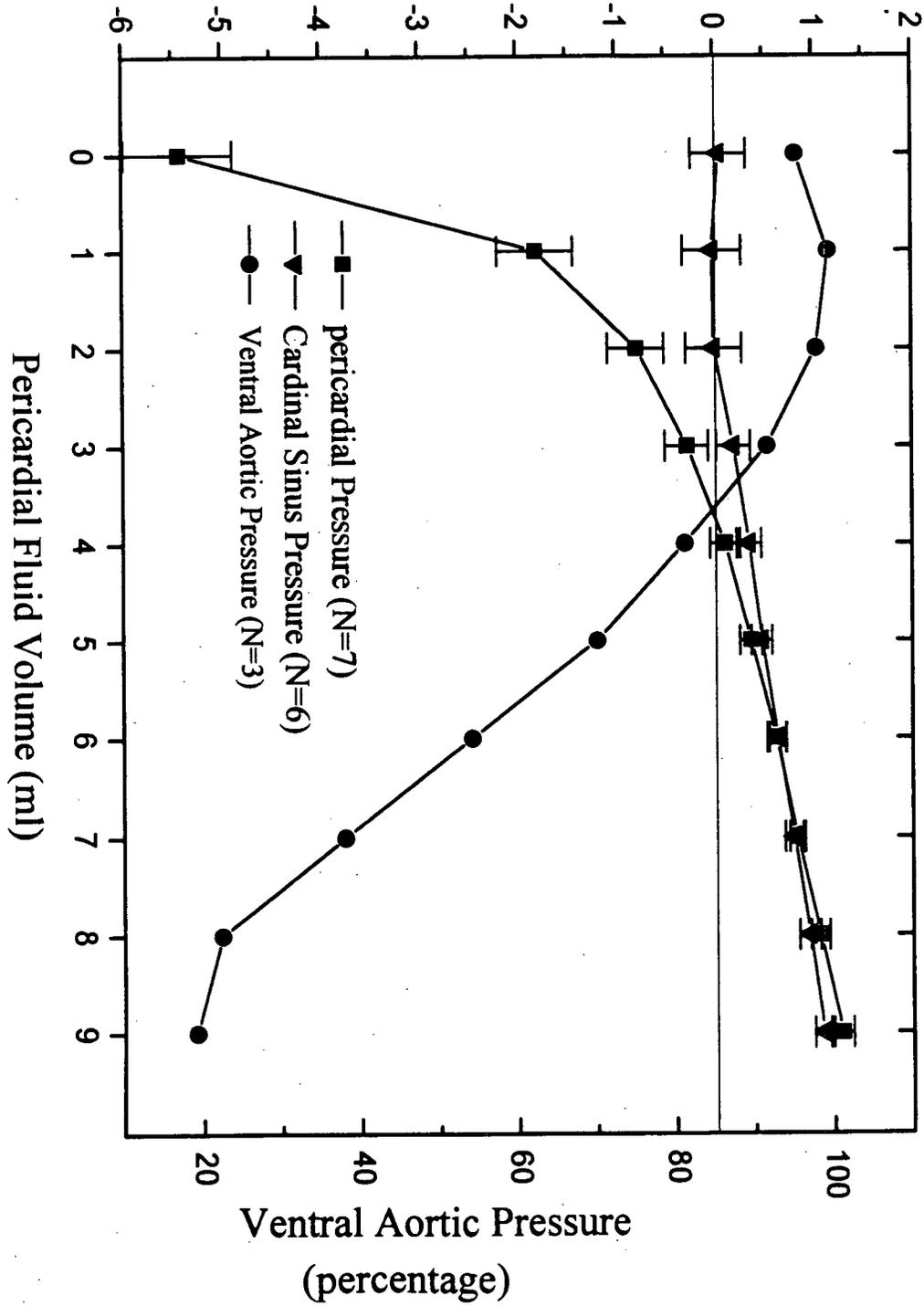
Cardinal Sinus Pressure

Pericardial Pressure

Figure 19. Effect of saline infusion/removal from the pericardium on the mean cardinal sinus pressure, mean intrapericardial pressure and mean ventral aortic pressure from unanaesthetised, chronically instrumented, resting dogfish. X-axis represents the volume of saline infused or removed from the pericardium. At zero on X-axis no more saline could be withdrawn. Error bars are standard error of mean.

(N= number of animals)

Pericardial & Cardinal Sinus Pressure (cm H₂O)



al (1984) on the ultrastructure of the mammalian parietal pericardium. In dogfish PPC two main histologic layers: the serosa and the fibrosa may be differentiated. In mammalian pericardium a third outer layer of epipericardial connective tissue is also seen. In dogfish PPC (N=4), the serosa was present on both the luminal and abluminal sides and sandwiched the fibrosa which constituted the major area of the PPC (figure 20).

The serosa consisted of a layer of mesothelial cells that were characterised by numerous microvilli on their apical surface. Two types of intercellular junctions namely *zonula occludens* (or tight junctions) and *macula adherens* (or desmosomes) connected the lateral surfaces of adjacent mesothelial cells (figure 21). A peculiar feature of the mesothelial cells was that their lateral surfaces were highly folded suggesting that these cells had the potential to increase their volume (figure 22). Each cell had a single, centrally located nucleus. The cytoplasm contained mitochondria and multivesicular bodies. The basal surface of the cell showed a distinct basal lamina. Vesicles were present both close to the basal surface as well as towards the apical surface.

The fibrosa was mainly composed of layers of collagen which ran in every possible direction, though the majority were oriented either parallel or perpendicular to the long axis. In between the layers of collagen could be found oblong shaped fibroblasts with slender long processes. These fibroblasts had very

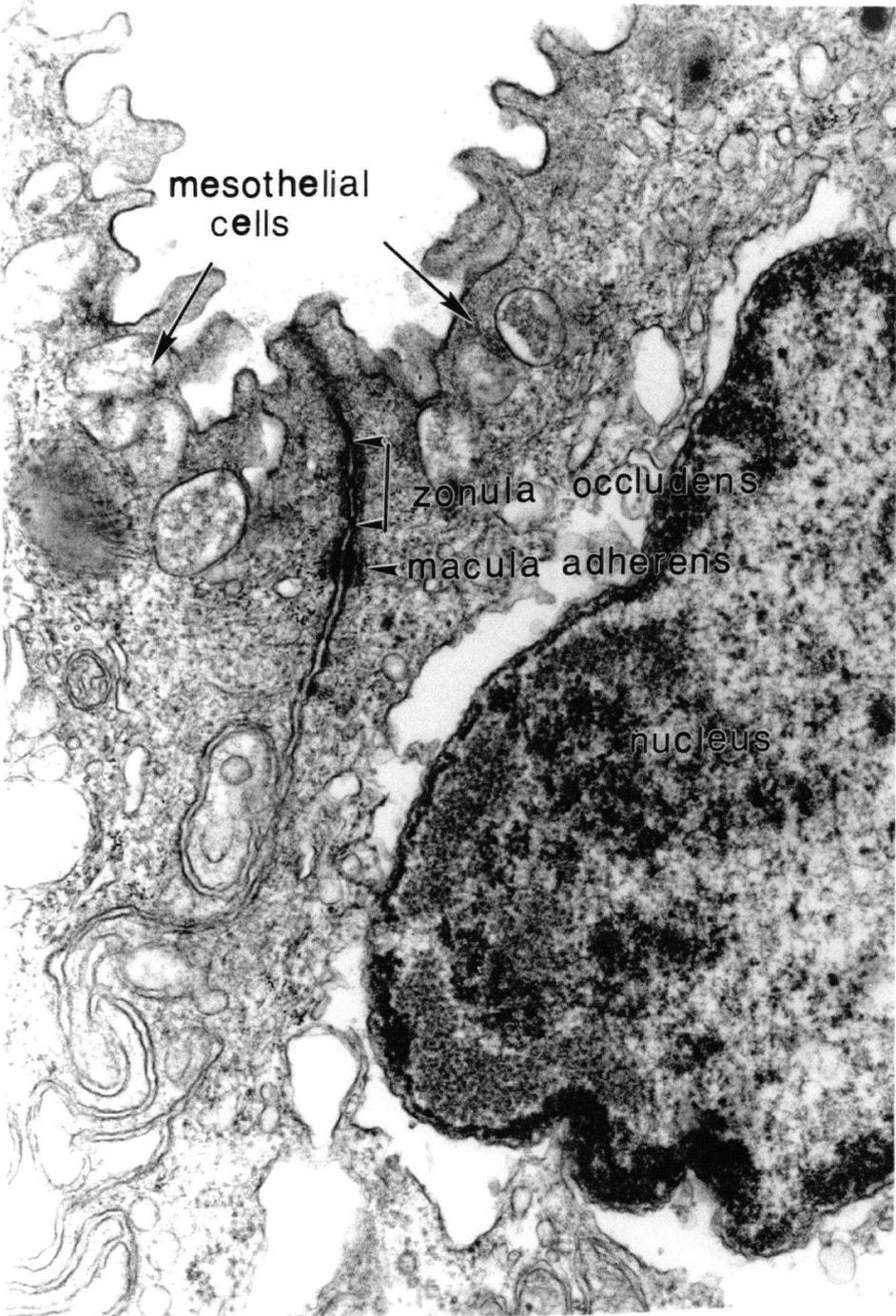
Figure 20. A transmission electron micrograph montage of ultrastructure of the ventral wall of the pericardioperitoneal canal (PPC) showing the entire cross-section of the wall.

(Magnification 4,150 X)



Figure 21. A high magnification transmission electron micrograph showing the lateral intercellular junctions of the mesothelial cells in the ventral wall of the PPC of spiny dogfish.

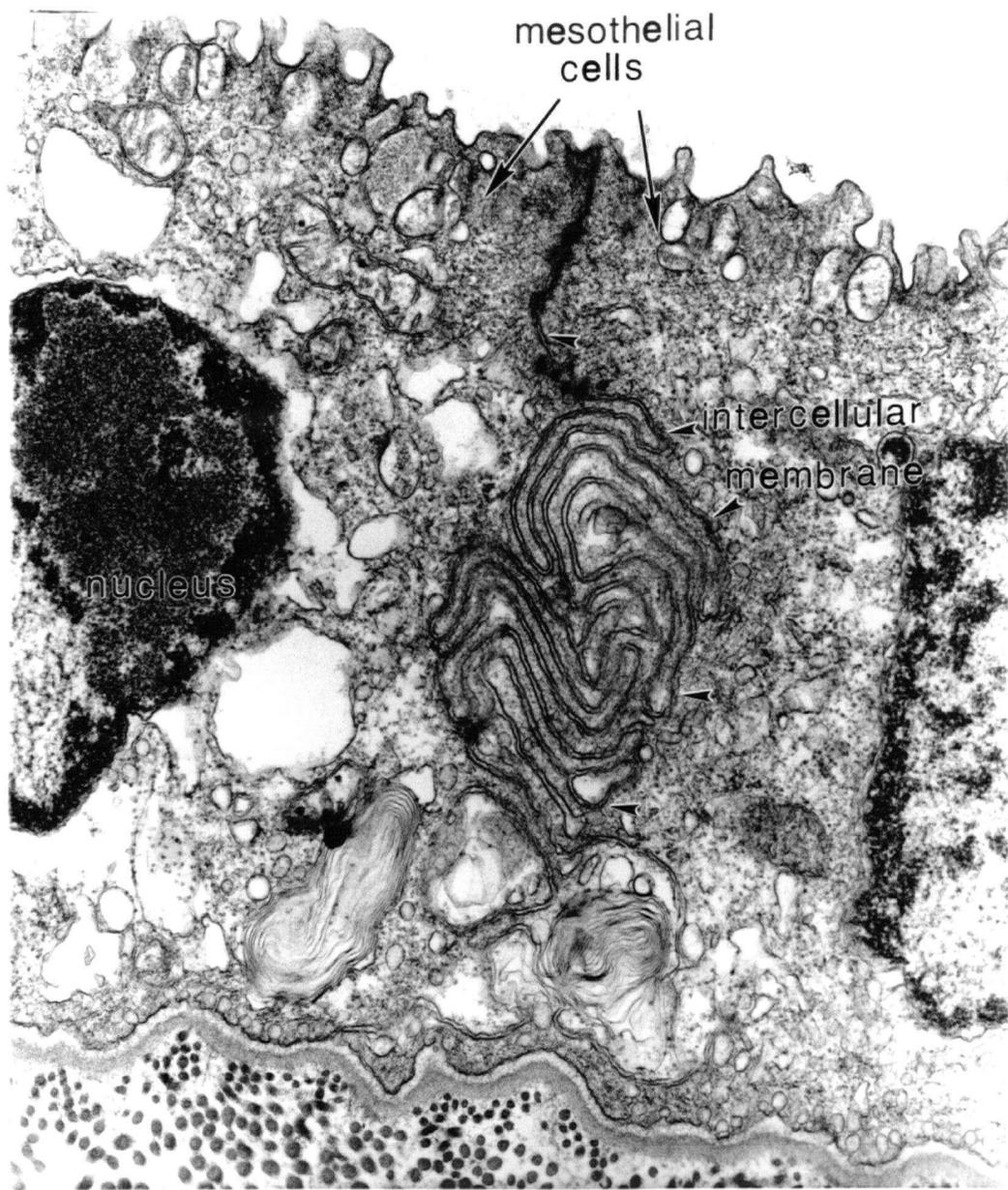
(Magnification 36,000 X).



little cytoplasm compared to the size of the nucleus. High magnification transmission electron micrographs of the cytoplasm of the fibroblasts confirmed that there was no contractile component in the cytoplasm. Hence, the ultrastructure of ventral wall of the PPC was devoid of smooth muscle cells. Relative to collagen, elastin was minimally present.

Figure 22. A high magnification transmission electron micrograph showing the highly folded lateral intercellular membrane of a mesothelial cell in the ventral wall of the PPC of spiny dogfish.

(Magnification 21,800 X).



DISCUSSION

Simultaneous records of the effect of saline infusion/removal on cardinal sinus pressure, intrapericardial pressure, atrial pressure, ventral aortic pressure and flow (figures 14a,14b,17) show that the heart of a resting dogfish fills by both *vis-à-fronte* and *vis-à-tergo* mechanisms. The effect of changes in intrapericardial pressure upon mean cardinal sinus (venous input pressure) and mean ventral aortic pressures (arterial output pressure) is shown in figure (19), and it can be seen that ventral aortic pressure is maximal at a negative intrapericardial pressure. The presence of a negative intrapericardial pressure is suggestive of an aspiratory (*vis-à-fronte*) effect on blood in the systemic veins. As soon as the intrapericardial pressure approached the cardinal sinus pressure, a fall in the ventral aortic pressure was observed. If the intrapericardial pressure was increased further, a concomitant rise in cardinal sinus pressure was seen, while ventral aortic pressure continued to fall. Finally, when intrapericardial pressure exceeded the cardinal sinus pressure, the ventral aortic pressure fell to its lowest value, indicating restricted cardiac function (figures 14a,14b).

The above observation can be explained as follows. The pericardium is a fluid-filled cavity, and any fluid loss from it results in more space for the heart. This in turn promotes increased filling of the heart and a subsequent increase in ventral aortic pressure and flow due to Starling's law. Conversely, infusion of saline into

the pericardium begins to compress the heart, with the thin-walled chambers like the sinus venosus and atrium being more susceptible to this compression. As the intrapericardial pressure is increased further, the heart is unable to fill adequately due to restraint on its relaxation imposed by saline infusion. Hence, both stroke volume and the force of contraction decrease, resulting in a fall in ventral aortic pressure.

Another factor affecting the filling of the heart is the pressure gradient between the input veins and the cardiac chambers. Once the intrapericardial pressure exceeds the cardinal sinus pressure the pressure gradient for flow is reversed, and the filling of the atrium is reduced because the thin-walled sinus venosus and atrium reflect the intrapericardial pressure.

A negative venous pressure is indicative of a *vis-à-fronte* mode of filling. In the present study, in 8 out of 10 chronically instrumented dogfish the cardinal sinus pressure went negative at some point when intrapericardial pressure was made negative. Under these circumstances, however, cardinal sinus pressure was always less negative than the intrapericardial pressure, implying *vis-à-fronte* filling. Further, in resting dogfish, maximum ventral aortic pressure and flow occurred at a negative intrapericardial pressure. Moreover, the first three mean posterior cardinal sinus values in figure (19) are either at ambient or slightly subambient, implying that the early diastolic value would definitely be negative and hence the

heart will be filled by *vis-à-fronte*. Moreover, the chronic, resting intrapericardial pressures usually oscillate between ambient and subambient (Abel *et al* 1986, present study).

Hence, *vis-à-fronte* does operate, but what needs to be ascertained is its contribution relative to *vis-à-tergo*. Franklin and Davie (1993) concluded that 100% filling of the heart under resting conditions occurred due to *vis-à-fronte*. It has been suggested that elasmobranchs and active teleosts employ *vis-à-fronte* under resting conditions and switch to *vis-à-tergo* during exercise (Farrell & Jones 1992). Farrell & Jones (1992) reason that *vis-à-fronte* filling has a finite volume that should limit any increases in stroke volume. Therefore, a *vis-à-tergo* mechanism would prevail during exercise. In fact, a switch from *vis-à-fronte* to *vis-à-tergo* is evident in the present records. If one examines the atrial and the cardinal sinus pressure waveform when the intrapericardial pressure is extremely negative (figure 16), it is very similar to that of the intrapericardial pressure which is suggestive of *vis-à-fronte* filling. However, as the intrapericardial pressure approaches ambient pressure, the atrial waveform takes a different shape with a longer, somewhat flatter diastolic period suggestive of *vis-à-tergo* filling.

The animal does not appear to benefit at extremely negative intrapericardial pressure, in terms of ventral aortic pressure (figures 16,19). There may be three reasons for this. First, it is quite possible that these extremely negative

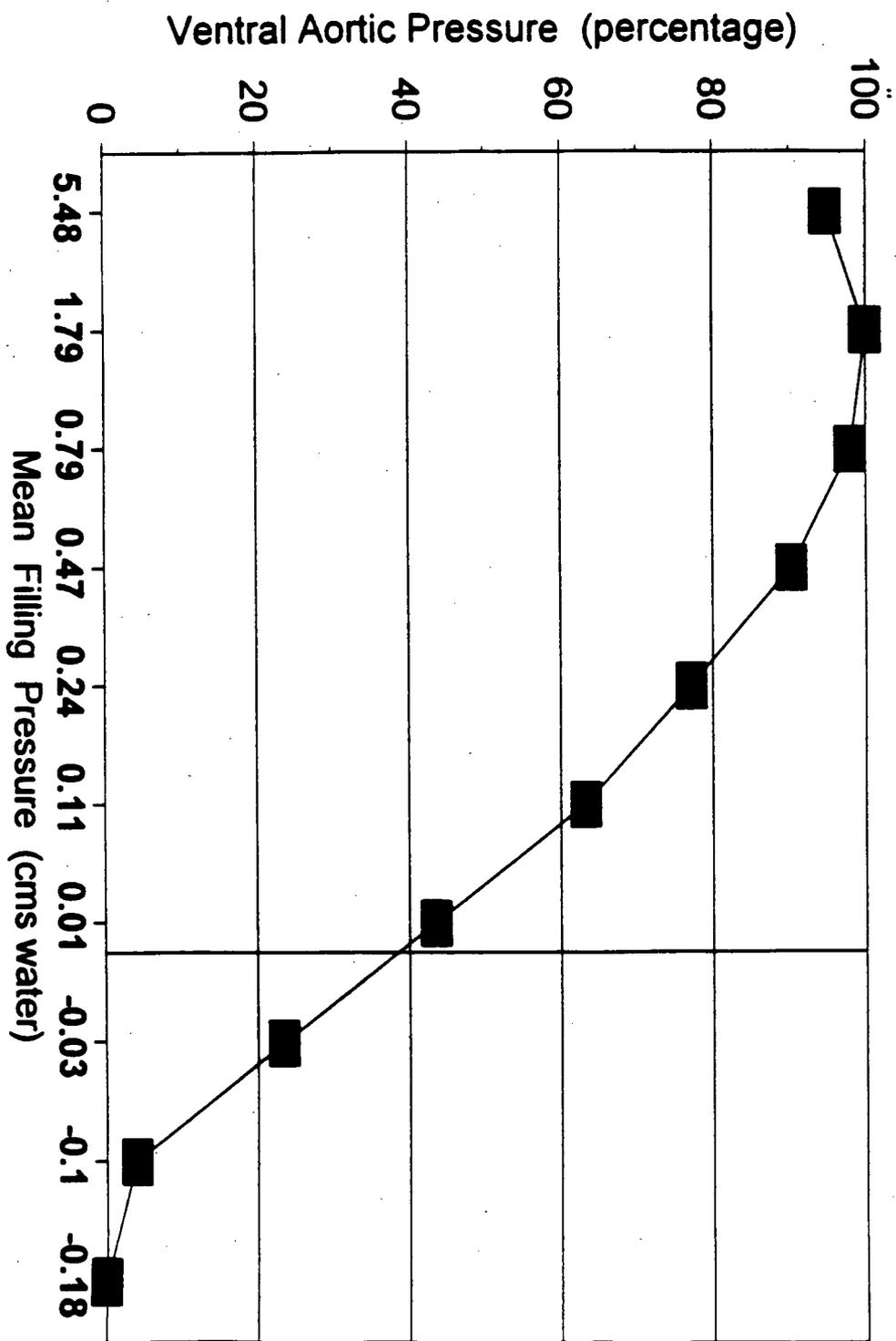
intrapericardial pressures are an artefact because adding a small volume of saline (1ml) into an empty (dry) pericardium results in a huge intrapericardial pressure change. Second, it may be that the heart is already fully distended at near ambient pressures. Hence, extremely negative intrapericardial pressures cannot distend the heart any further. This suggestion may be supported by the fact that chronic intrapericardial pressures in horn sharks (Abel *et al* 1986) and spiny dogfish (present study) are at ambient or slightly negative. Third, it is possible that the cardinal sinus collapses due to the extremely negative atrial/sinus venosus pressures (due to extremely negative intrapericardial pressures being transmitted to thin-walled atrium and sinus venosus), and hence after a certain critical value no further increase in venous return is observed.

Besides the effect of venous pressure, atrial filling is also influenced by intrapericardial pressure. When the intrapericardial pressure is negative, the atrial transmural filling pressure is maximum, promoting maximum ventral aortic pressure and flow. As the intrapericardial pressure is gradually made positive through saline infusion, the atrial filling pressure is reduced. At this stage atrial filling is affected by two factors, first a space limitation which does not allow it to relax completely and second a reduction in the cardinal sinus and atrial pressure difference, resulting in decreased inflow and therefore, a gradual decline in cardiac output. The second factor could be compensated by a decrease in the gill and/or peripheral resistance raising first arterial and then venous pressure. When

intrapericardial pressure exceeds that in the input veins (i.e. reversing the normal gradient for flow) (figure 23) there can be no cardiac output since the heart does not fill. At this point the fish's overwhelming need is to resuscitate its heart, and it swims vigorously (Abel *et al* 1986, present study). This results in opening of the pericardioperitoneal canal, and the excess pericardial fluid is expelled. This loss of pericardial fluid lowers the intrapericardial pressure relative to the cardinal sinus pressure. This restores the cardiac output to normal levels (figure 18).

Franklin and Davie (1993) found in perfused dogfish hearts that upon opening the pericardium to ambient pressure, cardiac output dropped drastically. However, it required only an increase in venous pressure of 0.2 cm H₂O to bring the cardiac output back to its normal level, suggesting that the heart is responsive to extremely small changes in the filling pressure. A similar response could be seen in the present records, for changes in ventral aortic pressure and flow occurred within a narrow range of filling pressure (figures 14a,14b). It is difficult to control the input pressures *in vivo*, so a precise value for filling pressure is difficult to specify. However, what is clear from Franklin and Davie's (1993) study and the present study is that for any significant change in output pressure and flow to occur, only a small change in mean filling pressure is required. This is further supported by the fact that the venous pressures are quite low in the dogfish.

Figure 23. A plot showing the effect of change in the mean filling pressure of the heart on mean ventral aortic pressure. The values are derived from figure 19.



The fact that an infused volume can be removed from the pericardium without any fluid loss is an indication that the PPC is normally closed. However, when the intrapericardial pressure is raised high enough, the PPC opens and the fluid escapes into the abdominal cavity (Satchell 1971, Shabetai *et al* 1985 , present study).

Lack of valves and absence of smooth muscle cells in the ventral wall of the PPC means that flow of fluid in either direction would depend on the existing pressure gradient between the pericardial and peritoneal cavities. Passive control of PPC function is unlikely as the peritoneal pressures are either at ambient or above ambient (present study). This would necessitate above ambient intrapericardial pressures to exist all the time to create a pressure gradient for flow, and heart function is impaired at above ambient intrapericardial pressures (Abel *et al* 1987, 1994, present study).

A collapsible, thin canal ensures that any backflow of fluid into the pericardium from the peritoneal cavity would not occur (Satchell 1971, present study). Instead flow through the PPC appears to be from pericardial to peritoneal cavity only and is controlled by both vigorous body movements and contraction of respiratory muscles attached to the pericardium. These actions compress the pericardium, raising the intrapericardial pressure and thus, force open the PPC (Lai *et al* 1989). In a resting fish, once the PPC is opened, the movement of the heart

itself might be able to force some fluid out gradually via the PPC with each heart beat. This may explain why once the PPC has opened, the intrapericardial pressure declines gradually rather than abruptly (Abel *et al* 1986,1994, present study). Since the dorsal side of the PPC is attached to the oesophagus, which is made up of smooth muscle cells, the contraction of the oesophagus could exert partial control on the size of the lumen.

Lai *et al* (1989) suggest that the PPC may enable instantaneous increases in heart performance which cannot be achieved humorally or neurogenically. Elasmobranchs lack sympathetic innervation of the heart (Lutz 1930) and humoral mechanisms are too slow (Butler *et al* 1986, Lai *et al* 1989) to account for the instantaneous changes in the cardiac output which are seen during exercise. Hence, if fluid is vented through the PPC, then the heart will have more room to expand in the pericardial cavity and the output of the heart will be increased by the Starling's mechanism.

Vis-à-fronte filling has its own advantages and disadvantages. It offers the advantages of keeping the stressed venous blood volume to a minimum, possible development of high ventral aortic pressures and more rapid filling than *vis-à-tergo* (see Farrell & Jones 1992 for review). Farrell & Jones (1992) suggest that for increasing the stroke volume the pericardium may restrict *vis-à-fronte*, compelling the animal to switch to *vis-à-tergo*. However, they also suggest that the PPC in

elasmobranchs helps to overcome this problem by displacing the pericardial fluid, thereby creating more room for the heart and hence operation by *vis-à-fronte*. Though *vis-à-tergo* does operate, it is against a background of reduced ventral aortic pressure and flow which raises doubts about it being the method of choice in elasmobranchs. Further, chronic resting intrapericardial pressures usually oscillate between ambient and subambient pressures (Abel *et al* 1986, present study). The present findings coupled to the structural adaptations of elasmobranchs (semi-rigid, capacious pericardium; sinuses as veins; a PPC) confirm that at least in resting dogfish *vis-à-fronte* is the most important mechanism for cardiac filling.

GENERAL DISCUSSION

The presence of a semi-rigid, capacious pericardium makes development of negative pressures possible during each cardiac cycle. These negative intrapericardial pressures have long been thought to play an important role in modulating the performance of the heart. In fact, Satchell and Jones (1967) presumed negative pressures to be more effective than the equivalent positive pressures. Based on their experiments, they suggested that negative intrapericardial pressures could be disadvantageous (i.e. increasing backflow from the ventral aorta), while conal contraction alleviates this problem. However, their anaesthetised animals had extremely negative intrapericardial pressures (upto -6 cm H₂O) which are never observed in chronically instrumented animals (Abel *et al* 1986, present study). The approach by Johansen *et al* (1966) to record ventral aortic flow by telemetry from freely-swimming animals was definitely more innovative, but as the present study shows ventral aortic pressure and flow patterns remain unchanged both in anaesthetised and unanesthetised animals.

It is difficult to speculate why Satchell and Jones (1967) did not observe any secondary rise in ventral aortic pressure and flow. The suggestion that the pumping action of the conus may be less important in bottom-living species such as *Heterodontus portusjacksoni* (Satchell 1971) is questionable, as the dogfish shows a distinct secondary rise in ventral aortic pressure and flow (present study).

The experiment by Satchell and Jones (1967) demonstrating that increasing the negative intrapericardial pressure caused a large backflow from the ventral aorta was compromised by the fact that they failed to control ventral aortic pressure and flow. In fact, ventral aortic pressure and flow increased markedly. Logic suggests that an extremely negative intrapericardial pressure would be no more effective in making the conal valves incompetent than extremely positive ventral aortic pressure. It is strange that Satchell and Jones (1967) would associate negative intrapericardial pressure with incompetency of conal valves and choose to ignore the effect of negative intrapericardial pressure on the venous return to the heart, though numerous previous studies had shown that abolishing the negative intrapericardial pressure results in lowering of ventral aortic pressure (Johansen 1965, Sudak 1965a).

The present study concludes that the conus could serve the following functions: prolonging the ejection phase of the heart, contributing to ventral aortic pressure and flow, making the smaller conal valves patent and perhaps depulsation. However, the effect of inactivation of the conus was not investigated. Inactivation of the conus in an unanesthetised animal, though difficult, would give a better insight into the role of conus arteriosus in elasmobranchs.

The beneficial effect of negative intrapericardial pressure has been associated with the return of blood to the heart (*vis-à-fronte* filling). This concept fits with the

structural modifications present in elasmobranchs. However, the data presented by Lai *et al* (1989,1990) argues for a *vis-à-tergo* mode of filling. But *vis-à-tergo* filling fails to explain the significance of a semi-rigid, capacious pericardium which appears to be designed specifically for the development of negative intrapericardial pressures. The present data and a recent study on dogfish (Franklin and Davie 1993) provide evidence for *vis-à-fronte* filling.

The dogfish heart can also operate *vis-à-tergo* when the intrapericardial pressure is at ambient or above ambient (present study). However, the output pressure and flow are reduced. This observation is in contrast to that reported by Lai *et al* (1989) where they observed no deleterious effect on the performance of the heart due to positive intrapericardial pressure.

An aspect that warrants further investigation in elasmobranchs relates to the secretion of pericardial fluid. Though elasmobranchs have been shown to have lymphatic channels comparable to mammals (Shabetai *et al* 1985), an excess of pericardial fluid accumulates. This increased volume of fluid would imply that the rate of secretion was greater than the rate of removal. It would be interesting to study if there is any control over this secretory process. Otherwise the only relief with respect to an increase in heart size is achieved by expelling the pericardial fluid via the PPC (Lai *et al* 1989). The fate of this expelled fluid is not known, but it may

either be absorbed or may be vented via the abdominal pores (Abel *et al* 1994) which connect the abdominal cavity to the environment.

The present study looked at the presence of smooth muscle cells in the ventral wall of the PPC using transmission electron microscopy (TEM). The length of the PPC precluded a comprehensive TEM study. Hence, the possibility of smooth muscle cells occurring in the non-sectioned PPC, though small, does exist. Furthermore, although no valves are obvious on visual inspection, a search of the wall for sphincter like structure at the pericardial-PPC junction might be worthwhile.

The elasmobranchs, though considered to be quite primitive in their overall organisation, display very unusual and complex physiological and structural adaptations. Recent advances in recording equipment have helped answer many questions, but further research is definitely needed for better understanding of the elasmobranch cardiac physiology.

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APPENDIX I

Collagen biosynthesis involves intracellular and extracellular steps. In the intracellular step a folded, triple helical, soluble, procollagen molecule is formed and secreted from the cell (Kadler 1994). In the extracellular step the procollagen is converted to collagen through proteolysis. The collagen molecules so formed self-assemble into fibrils. These fibrils then are covalently cross-linked (Kadler 1994).

However, there are numerous reports which provide evidence for the presence of intracellular collagen suggesting that collagen-fibril formation can take place intracellularly. But results from *in vitro* studies have shown collagen-fibril formation to be extracellular (Kadler 1994). The reported intracellular collagen-fibrils have been shown to be enclosed within a lysosomal vacuole and hence considered to represent phagocytosed collagen-fibrils rather than recently synthesised (Dr. Vincent Everts, personal communication). To my knowledge no studies have been reported on the occurrence of intracellular collagen-fibrils in dogfish tissues.

Figure 1.1 A transmission electron micrograph of the ventral wall of the PPC of an adult spiny dogfish showing intracellular collagen-fibrils.

(Magnification 40,000 X)

(C=cytoplasm, CO=collagen fibrils, E=elastin, EM=extracellular matrix,

F=fibroblast, N=nucleus)

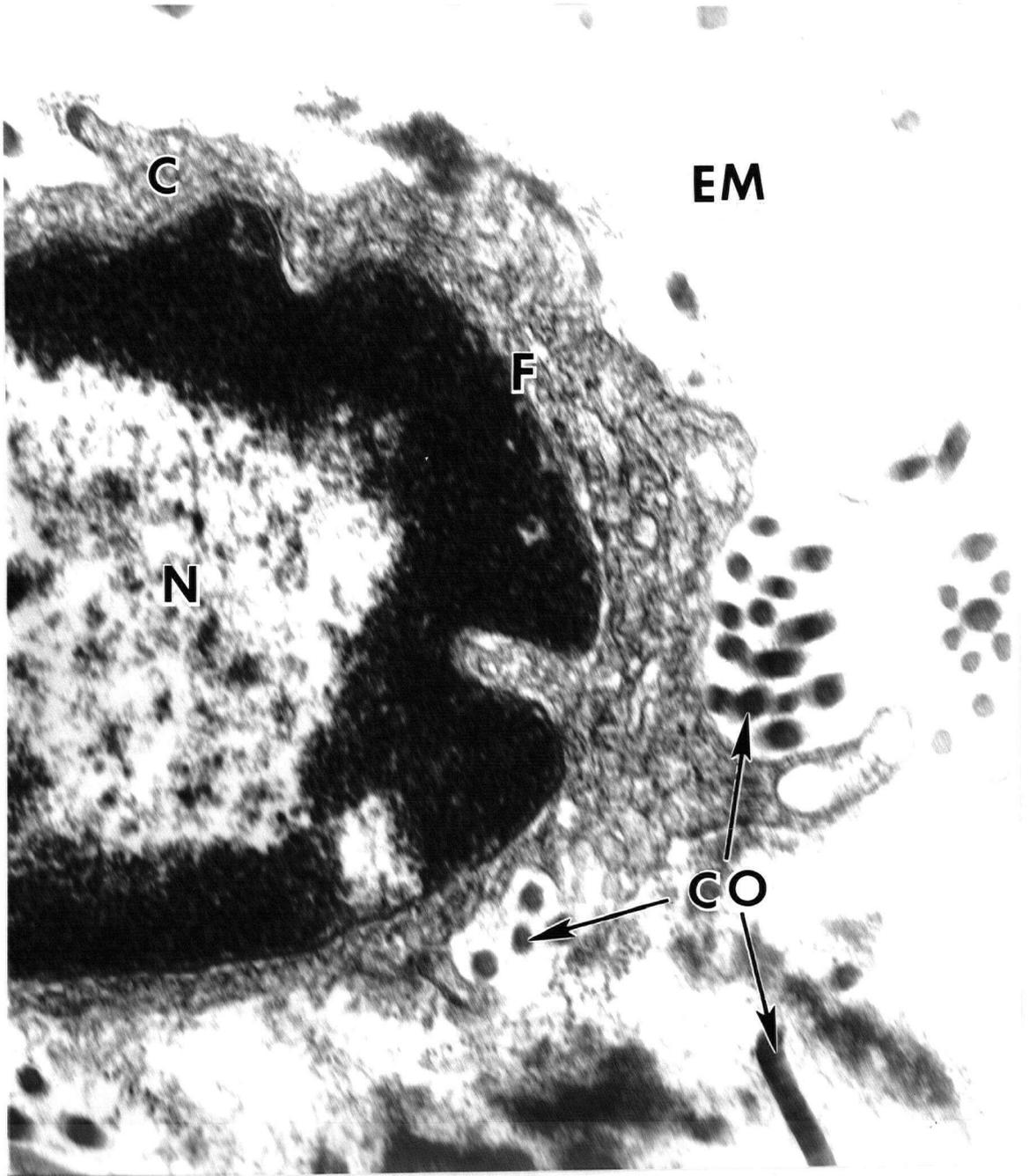


Figure 1.2 A transmission electron micrograph of the ventral wall of the PPC of an adult spiny dogfish showing intracellular collagen-fibrils.

(Magnification 50,000 X)

(C=cytoplasm, CO=collagen fibrils, E=elastin, EM=extracellular matrix,

F=fibroblast, N=nucleus)

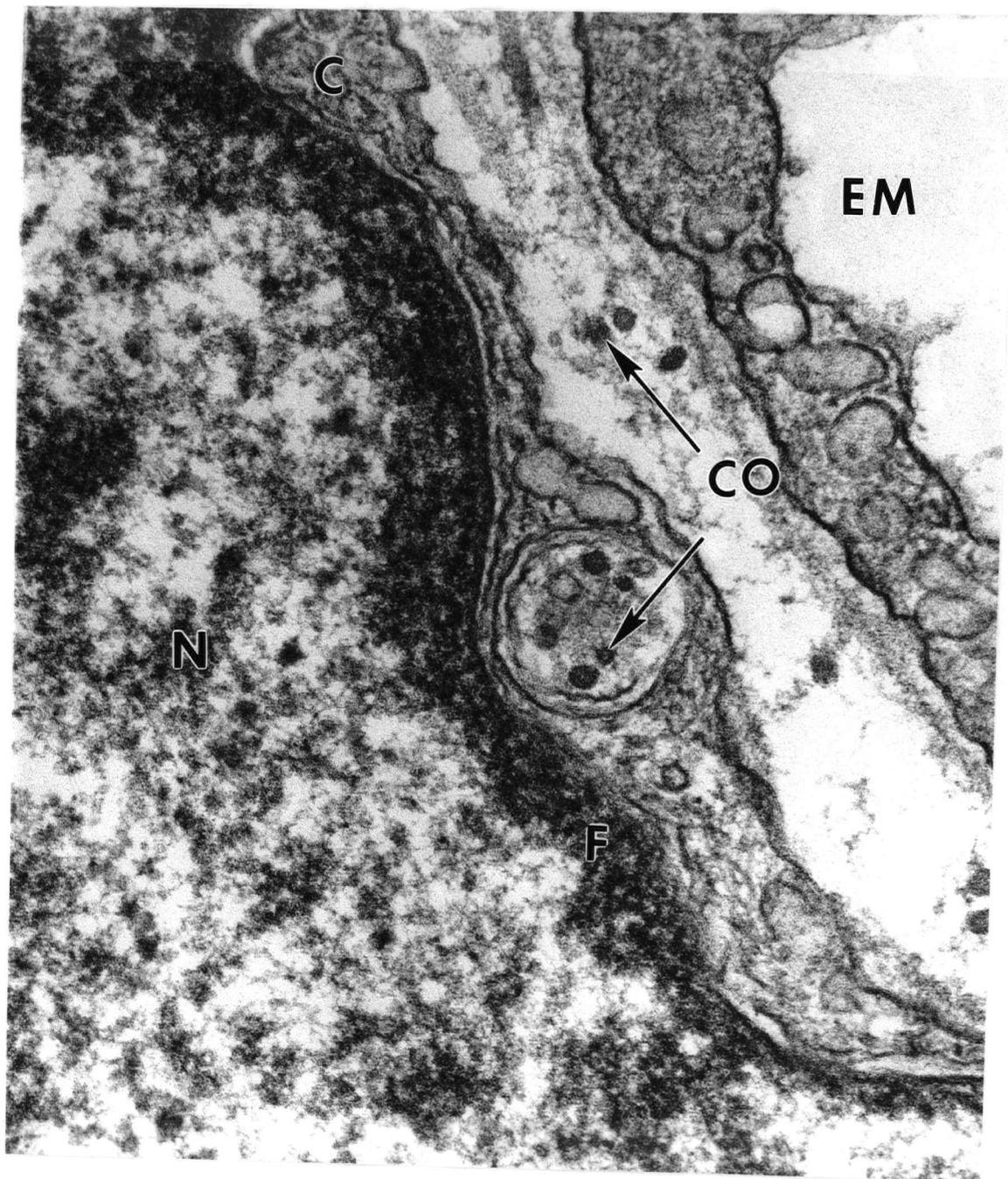


Figure 1.3 A transmission electron micrograph of the ventral aorta of an adult spiny dogfish showing intracellular collagen-fibrils.

(Magnification 6,300 X)

(C=cytoplasm, CO=collagen fibrils, E=elastin, EM=extracellular matrix,

F=fibroblast, N=nucleus)

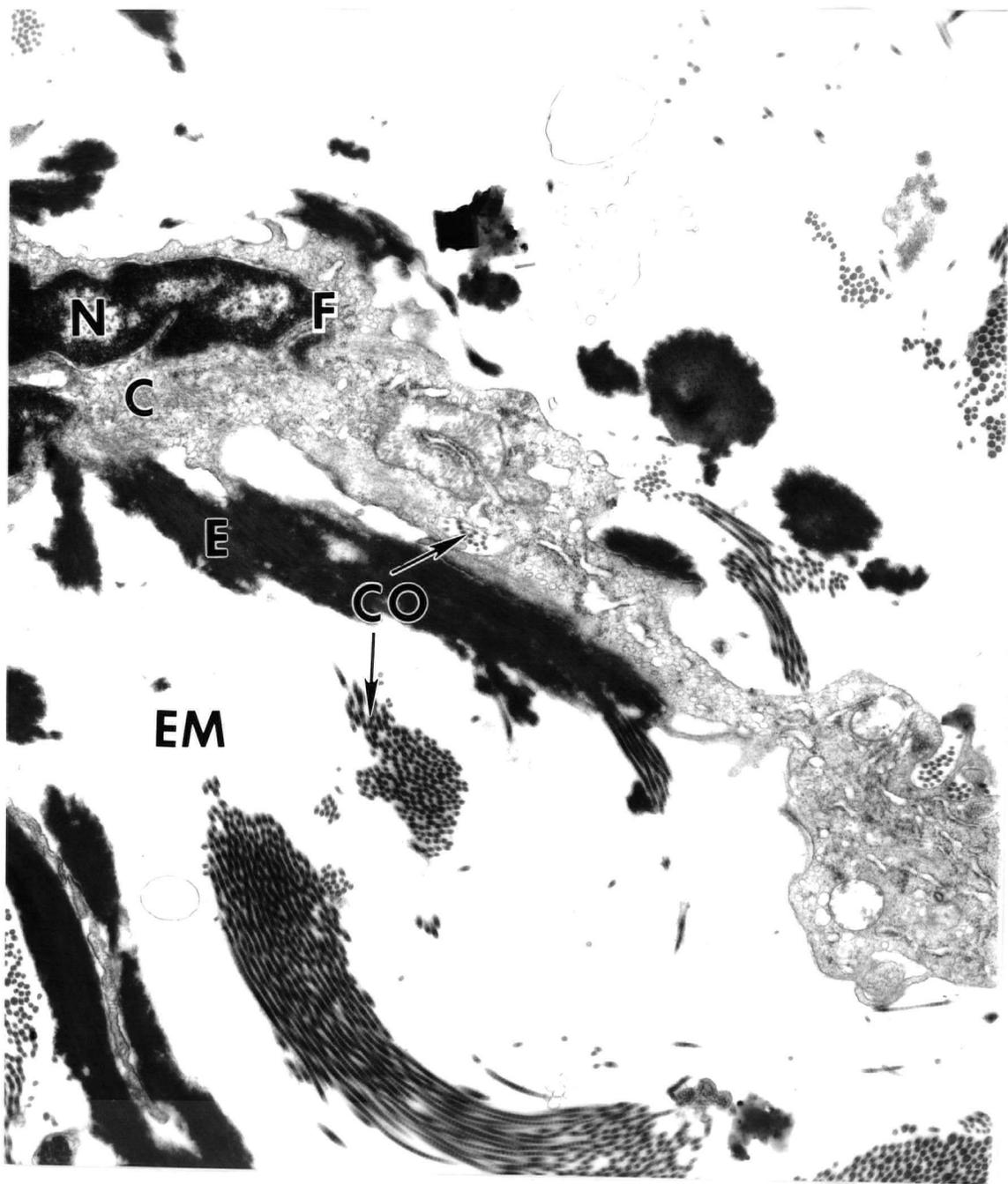


Figure 1.4 A transmission electron micrograph of the ventral aorta of an immature spiny dogfish showing intracellular collagen-fibrils.

(Magnification 4,000 X)

(C=cytoplasm, CO=collagen fibrils, E=elastin, EM=extracellular matrix,

F=fibroblast, N=nucleus)

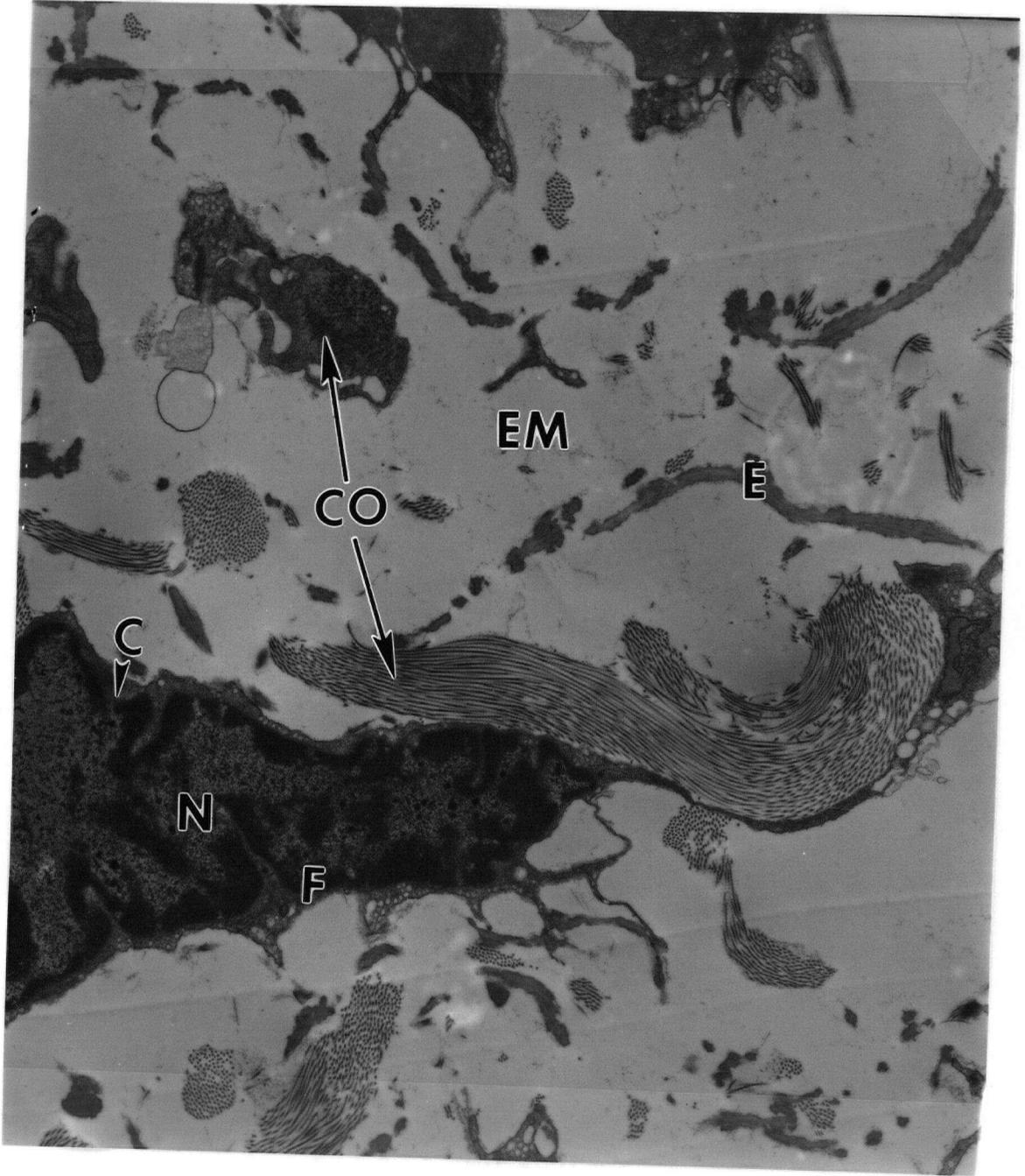
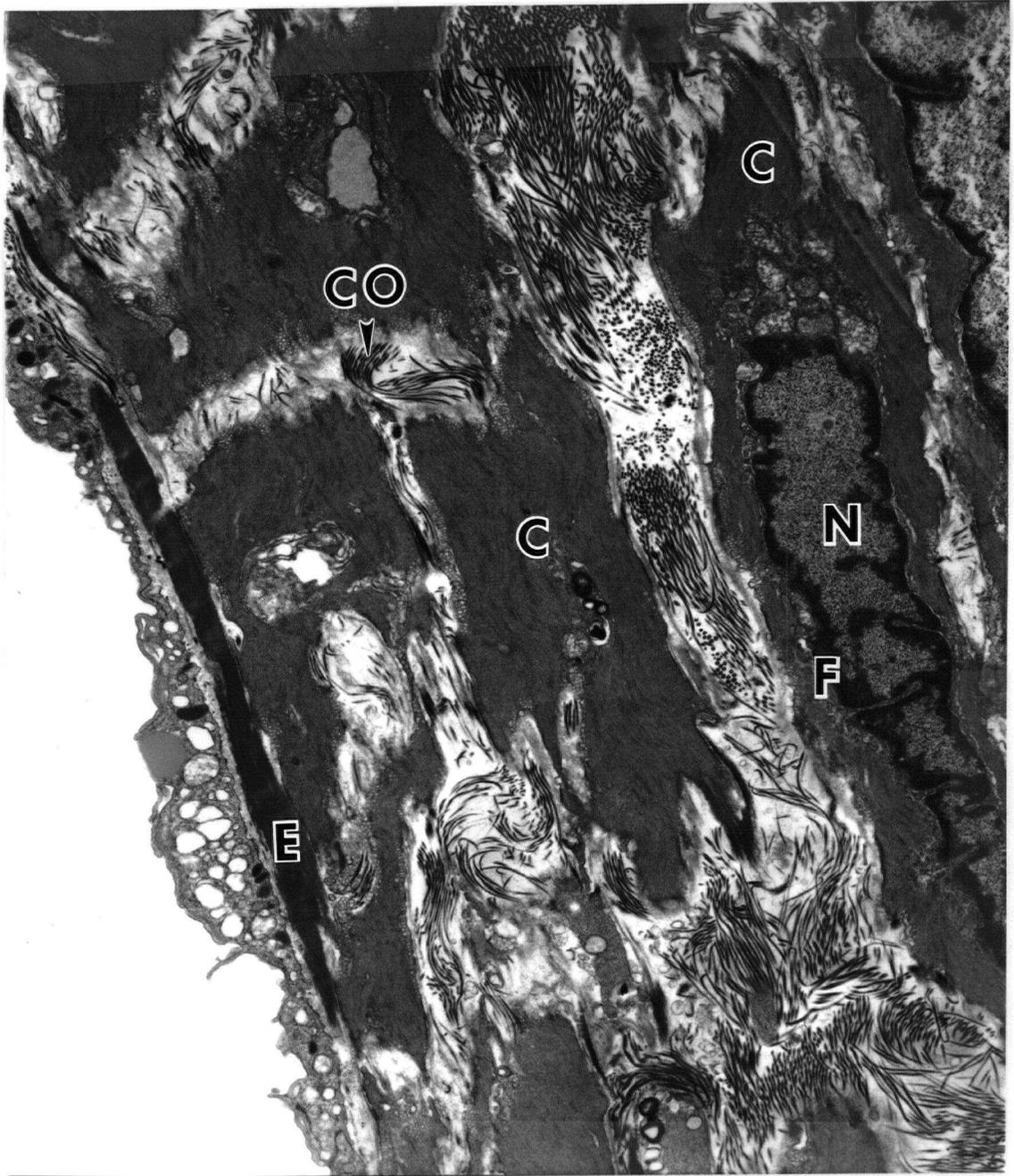


Figure 1.5 A transmission electron micrograph of the dorsal aorta of an adult spiny dogfish showing intracellular collagen-fibrils.

(Magnification 3,150 X)

(C=cytoplasm, CO=collagen fibrils, E=elastin, EM=extracellular matrix,

F=fibroblast, N=nucleus)



APPENDIX II

Parsons (1930) reported that the myocardium of conus arteriosus in *Squalus acanthias* extends all the way to the anterior pericardial boundry. However, the present study found that the myocardial covering does not extend the entire length of the conus. About one-fifth of the conal area next to the anterior pericardial boundry is white in color and hence devoid of myocardium. This appendix reports measurements of the conal lenth with and without the cardiac muscle.

Dogfish Weight (in Kg)	Dogfish length (in cm)	Total conal length (in cm)	Conal length with cardiac muscle (in cm)	Conal length with cardiac muscle (%)	Conal length without cardiac muscle (in cm)	Conal length without cardiac muscle (%)
1	67	1.9	1.5	79	0.4	21
1.3	76	1.75	1.5	86	0.25	14
0.5	75	1.75	1.35	77	0.4	23
-	64	1.35	1.15	85	0.2	15
1	75	1.8	1.3	72	0.5	28
0.25	60	1.3	1.1	85	0.2	15
0.6	73	1.4	1.2	86	0.2	14
0.5	70	1.5	1.3	87	0.2	13
N=	7	8	8	8	8	8
MEAN=	0.73	70	1.3	82.12	0.29	17.87
S.E.=	0.14	2.07	0.05	1.93	0.04	1.93