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ELEVATED PERICARDIAL PRESSURE AND CARDIAC OUTPUT IN THE LEOPARD SHARK *TRIAKIS SEMIFASCIATA* DURING EXERCISE: THE ROLE OF THE PERICARDIOPERITONEAL CANAL

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Summary

Changes in pericardial pressure, pericardial fluid volume, cardiac stroke volume and heart rate induced by swimming were monitored for *Triakis semifasciata* (Girard). Maximum pericardial pressure (P_{max}, 0.07±0.03 kPa) in resting sharks was typically above ambient, whereas minimum pressure (P_{min}, −0.08±0.03 kPa) was slightly subambient. During swimming, both P_{max} (0.23±0.03 kPa) and P_{min} (−0.02±0.03 kPa) became elevated, as did heart rate (51±2 to 55±2 beats min^{-1}) and fractional cardiac stroke volume (0.49±0.03 to 0.65±0.04 ml). After swimming, all variables fell, except fractional cardiac stroke volume. Estimates of total cardiac output from fractional cardiac stroke volume data during rest, exercise and recovery were 33.1, 56.2 and 60.4 ml kg^{-1} min^{-1}, respectively. The occurrence of both elevated pericardial pressure and cardiac output during swimming argues against a primary role for pericardial-induced *vis a fronte* filling as the principal mechanism responsible for increasing cardiac output with exercise. Pericardial fluid loss *via* the pericardioperitoneal canal (PPC) occurs during swimming as a result of steady-state elevation of pericardial pressure, a series of transient high pericardial pressures, or both. Good general agreement seen for net pericardial fluid loss (0.6 ml kg^{-1}) and the net increase in cardiac stroke volume (0.45 ml kg^{-1}) during swimming establishes fluid displacement as a mechanism for increasing cardiac stroke volume and suggests that this is the primary function of the PPC.

Introduction

In current literature the elasmobranch pericardium is accorded an important role in cardiovascular regulation which, because it is non-compliant and capacious, establishes a subambient pericardial pressure necessary for *vis a fronte* cardiac

Key words: *Triakis semifasciata*, pericardial pressure, cardiac output, heart rate, swimming.

filling. Strongly subambient pericardial pressures in the range of from −0.09 to −0.88 kPa are regarded as typical for normal elasmobranch cardiac function (−0.19 to −0.49 kPa, Schoenlien and Willem, 1894; −0.14 to −0.62 kPa, Sudak, 1963; −0.26 to −0.54 kPa, Sudak, 1965*a*; −0.19 to −0.88 kPa, Sudak, 1965*b*; −0.19 to −0.59 kPa, Johansen, 1965; −0.22 to −0.55 kPa, Satchell, 1970).

There are, however, exceptions to this generally accepted account of pericardial function. Burger and Bradley (1951), for example, were unable to confirm subambient pericardial pressure in the dogfish *Squalus acanthias*. Satchell and Jones (1967) also found that, despite rendering pericardial pressure ambient, pericardotomy did not change cardiac output in the Port Jackson shark *Heterodontus portusjacksoni*. An additional complicating factor is that most of the work that suggests that the subambient pericardial pressure is required for *vis a fronte* filling was done before any physiological investigations of the pericardioperitoneal canal (PPC) had been undertaken (Shabetai *et al.* 1985). The PPC, which connects the pericardial and peritoneal cavities, serves as a unidirectional conduit for egression of pericardial fluid. More recent chronic studies (Abel *et al.* 1986, 1987) have demonstrated that intact, resting and undisturbed elasmobranchs have pericardial pressure close to ambient and, further, that disturbance or handling of these fish causes fluid to be expelled from the pericardium *via* the PPC, thus lowering pericardial pressure and allowing diastolic heart volume to increase.

To our knowledge, no studies of the cardiovascular effects of exercise in elasmobranchs that fully take into account pericardial dynamics and fluid discharge *via* the PPC have yet appeared. The purpose of our study was to establish the relationships of pericardial pressure, pericardial fluid volume, cardiac stroke volume and heart rate as elasmobranchs progressed from rest, through sustained swimming, to recovery. From these relationships we hoped to learn to what extent and by what mechanisms the PPC may function in altering pericardial pressure, and consequently cardiac performance, during exercise.

Materials and methods

Experimental animals and conditions of maintenance

Male and female leopard sharks (*Triakis semifasciata*, 0.6–2.8 kg, N=27), an active species found in the coastal waters of California, were used in this study. They were obtained from the Steinhart Aquarium, San Francisco, or collected in Elkhorn Slough, Moss Landing, CA, and transferred to holding facilities at Scripps Institution of Oceanography, La Jolla. Sharks were maintained in two 5000 l circular tanks filled with running, filtered and aerated sea water at ambient temperature (14–24 °C) and photoperiod. Fish were fed twice weekly on chopped mackerel and squid, but experimental fish were fasted for at least 7 days before study.

Surgical procedures and equipment

Sharks were anaesthetized with tricaine methane sulphonate (MS222, 1:10 000),

placed supine on a V-board, and ventilated at room temperature with aerated sea water containing the same concentration of anaesthetic.

The electrocardiogram (ECG) was recorded with two wire electrodes (36 AWG) percutaneously inserted in the pericardium *via* 25 gauge needles. The needles were then withdrawn from the wires, which were sutured in place. The ECG signal was amplified with a universal amplifier (Gould model 13-4615-55 or Honeywell model V1205B).

The pericardium was cannulated with polyethylene tubing (either PE90 or 240) inserted into the pericardial cavity through the coracoid bar and sealed with tissue adhesive (3M Vetbond). Catheter attachment to the body was reinforced by marine epoxy and sutures. Side ports in the catheter's tip facilitated aspiration and infusion of pericardial fluid and shark saline. Catheters remained patent in actively swimming fish for a minimum of 1 week.

Pericardial pressure was measured using a saline-filled cannula attached to a Statham pressure gauge (Gould model P23ID) or with a Camino transducer-tipped pressure-monitoring catheter (model 110-4). Catheter vibrations in the flowing water prevented monitoring of the signal measured by the Statham gauges during swimming. The signal from the Camino transducer was suitable for analysis of swimming sharks, provided certain precautions were taken. First, large-bore tubing (PE240) was used so that the tip of the Camino transducer would be in free communication with the pericardial space and thus minimize signal attenuation. Also, because Camino transducers measure absolute pressure (i.e. they are sensitive to hydrostatic pressure) and thus reflect changes in swimming depth, a second transducer, mounted on the fish at the same relative vertical position as the heart (i.e. dorsal surface of the pectoral fin) was used as a reference for ambient pressure. To verify that the pressure signal from the Camino transducer did not drift, a Statham gauge connected to the catheter by means of a three-way T-junction was used to check pressure measurements before and after swimming. All cannulae were filled with elasmobranch saline (Smith, 1929). The reference pressure measured by the Statham transducer was zeroed *via* PE tubing connected to the sea water of the swimming tunnel. To maintain this zero reference, it is imperative that the vertical distance between the pressure transducer and the water level in the tunnel remain constant.

Before the start of each experiment, all pressure gauges were calibrated manometrically against a static column of water. Pressure signals were amplified (Honeywell Electronics for Medicine, V2203A, or Gould model 13-4615-50) and recorded on chart paper (Honeywell Electronics for Medicine, AR6, or Gould model 2400). An electromagnetic flow probe (Carolina Medical Electronics) was placed between the innominate and third afferent branchial arteries of the ventral aorta. After surgical exposure of the aorta, optimal positioning of the probe was determined by observing a maximal flow signal. The probe was then secured by filling the space around the wound with silicone foam elastomer (Q-74290, Dow Corning). While the foam was curing, the incision was sutured tightly around the probe and cable.

Placement of the flow probe distal to afferent branchial arteries 3, 4 and 5 meant that only a fraction of the total stroke volume could be measured. Estimation of total cardiac stroke volume from the observed fractional stroke volume data required two calibration steps which were made for each fish after all experiments were completed (Abel *et al.* 1987). The fish was killed, its pericardium opened, and its atrium and ventricle removed. Then, with the ventral aorta and flow probe still in position, a known volume of saline was injected from the conus into the ventral aorta, and the flow signal recorded. Following this, the portion of the ventral aorta with the flow probe was excised and calibrated *in vitro* with a known volume of saline. Assuming that the *in vitro* flow signal represents 100 % flow, the proportion of saline (blood) flowing past the flow probe on the ventral aorta of an intact fish is calculated as:

$$\text{flow proportion} = (A \times b)/(B \times a),$$

where A and B are the *in situ* and *in vitro* flow determined by digitization of the flow signal areas and a and b are the *in situ* and *in vitro* injection volumes.

Water tunnel

The water tunnel, like the system described by Prange (1976), consisted of a closed loop of flowing water separated horizontally and connected at each end by curved metal vanes. The working section (113 cm×30 cm×29 cm) was contained in the upper horizontal channel. Water was driven around the system by a 560 W variable-speed motor (Minarik Blue Chip II) mounted outside the lower channel and coupled to the propeller shaft which entered the tunnel through sealed bearings. Honeycomb collimator material at the front of the working section confined the shark and smoothed flow. Flow probe measurements at different depths in the working section verified rectilinear flow. The maximum velocity of the system was about $1.25\,m\,s^{-1}$. Velocities were calibrated by a flow meter (General Oceanics Inc. model 2035-mk III).

Protocol

After surgery, the shark was allowed several hours to recover in a holding tank before being transferred to the water tunnel for study. Experiments did not begin until at least 24 h after surgery.

Prior to experiments, pericardial capacity and the pericardial opening pressure (POP) of each fish were determined by first aspirating all the pericardial fluid and then infusing fluid into the pericardium until the pressure–volume curve plateaued (Shabetai *et al.* 1985). In all measurements or adjustments of pericardial fluid volume, a 5 ml graduated syringe was used and replicate volume measurements (to the nearest 0.1 ml) were typically made. For the determinations described above, pericardial capacity is defined as the volume of fluid required to reach POP and operational pericardial volume as the volume of fluid instilled into the pericardium just prior to experimentation. Abel *et al.* (1987) established that resting ho[illegible] sharks typically have a pericardial volume of about 50 % of capacity and ou[illegible]

preliminary studies show values similar to this for *T. semifasciata*. Accordingly, most swimming experiments were made with fish at an operational pericardial volume of 50 %. In several cases, however, and for the purpose of testing the effect of operational volume on pericardial fluid displacement during swimming, volumes of 30–70 % were also used.

Data were collected at rest, during swimming and during recovery. Variables measured were pericardial pressure and fluid volume, heart rate and fractional cardiac stroke volume. After resting data had been obtained, each fish was induced to swim in the water tunnel by gradually increasing flow velocity. The speed [0.2–0.5 m s^{-1} or 0.3–0.7 body lengths s^{-1} ($L\,s^{-1}$)] and endurance (up to 30 min) of each fish were affected by catheter drag and body size. During the exercise period, we attempted to keep all the fish swimming steadily for as long as possible while data were taken. As a fish fatigued it either refused to swim or was swept onto the rear grating of the working section. At this point the fish was allowed to recover and data were collected during the first 5–10 min of the recovery phase, when pericardial fluid volume was remeasured. The shark was allowed to rest for several hours, or usually until the next day, before another test was made.

To examine the effects of increased pericardial fluid volume on pericardial pressure, PPC function and swimming capacity, a volume in excess of pericardial capacity was introduced into the pericardium of steadily swimming sharks in which both swimming pericardial pressure and volume had been established. Following this and while the fish swam continuously, pericardial pressure was monitored constantly, and at a later time (up to 17 min later) pericardial fluid volume was determined.

Results

Changes in cardiovascular parameters caused by swimming

Fig. 1 shows the general form of the data. Illustrated are simultaneously recorded pericardial pressures measured by Camino and Statham transducers and a reference (ambient) pressure, ECG and fractional cardiac stroke volume for a leopard shark at rest, during exercise and during recovery. Records taken at rest show that pericardial pressure fluctuates from a minimum (P_{min}) to a maximum (P_{max}) with each cardiac cycle. P_{min} typically occurs just after the onset of electrical ventricular systole (QRS) which slightly precedes peak aortic velocity. P_{max} usually occurs during ventricular diastole. During swimming (Fig. 1B), the fish changed depth and pericardial pressure was increased (as indicated by the greater distance between C_p and C_{ref}). Swimming also increased cardiac frequency and fractional cardiac stroke volume. During recovery (Fig. 1C), pericardial pressures returned to resting level, but with a greater pulse pressure, and both fractional cardiac stroke volume and heart rate remained near exercise levels (in [redacted]st experiments, heart rate fell during recovery, see below).

Fig. 2 summarizes mean pericardial pressure, cardiac frequency and fractional

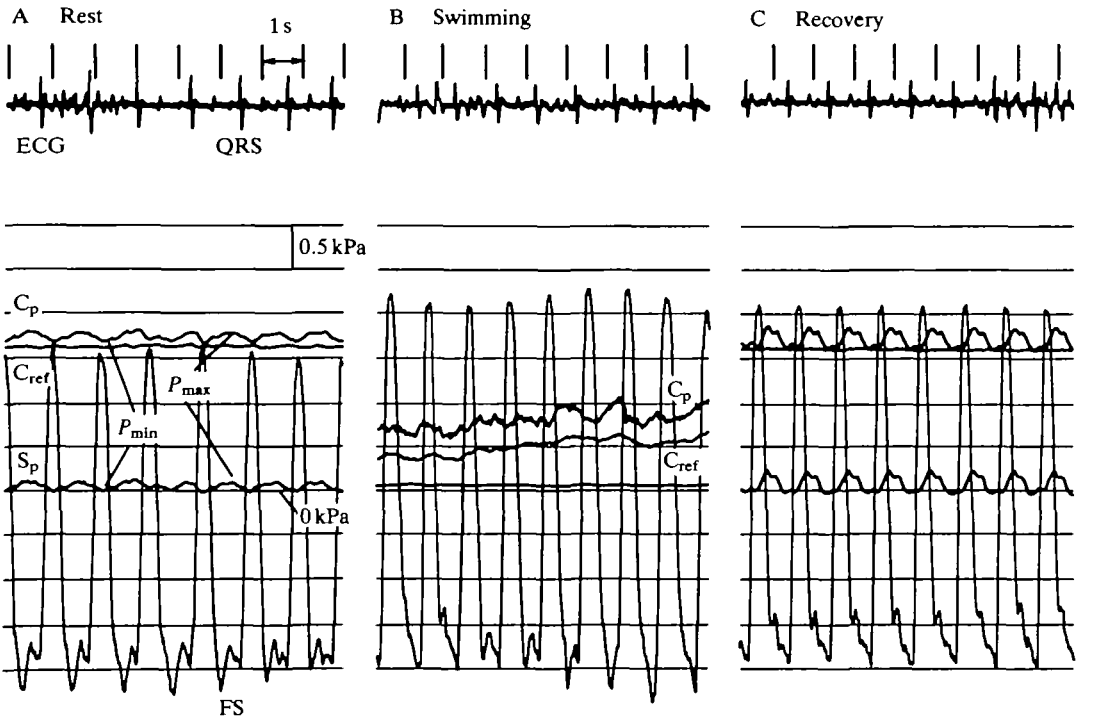

Fig. 1. Electrocardiogram (ECG), pericardial pressure and fractional cardiac stroke flow (FS) records for leopard shark (no. TS061587, 1.8 kg) taken during rest, swimming and recovery. (A) Resting phase showing the pressure records measured by the Statham (S_p) and Camino (pericardial, C_p, and ambient reference, C_{ref}) transducers. (Note that zero pressure for the Statham transducer tracings is shown). P_{max} and P_{min} excursions during a cardiac cycle are indicated for both pericardial pressure records. (B) Swimming record showing a rise in C_p and a change in C_{ref} reflecting a change in depth. Note that S_p is not recorded. (C) Recovery phase showing a return to resting pericardial pressure but with a larger pulse pressure. Pressure calibration is the same for all panels. QRS, electrical ventricular systole.

cardiac stroke volume of 15 sharks during rest, swimming and recovery. The pericardial diastolic pressure of a resting *T. semifasiciata* is typically above ambient and systolic pressure is subambient (Fig. 2A). Mean pressure (not shown) is close to ambient. Resting P_{max} ranged from −0.25 to 0.33 kPa and resting P_{min} ranged from −0.49 to 0.21 kPa. During swimming P_{max} increased significantly ($P<0.05$, regression analysis); P_{max} fell in recovery ($P<0.05$). P_{min} did not change significantly during either swimming or recovery. Swimming P_{max} ranged from −0.09 to 0.68 kPa, P_{min} ranged from −0.33 to 0.43 kPa. At recovery, P_{max} ranged from −0.45 to 0.57 kPa and P_{min} ranged from −0.67 to 0.37 kPa. Heart rate increased significantly during swimming by 7% but declined during recovery. Resting and recovery heart rates were not significantly different. Fractional cardiac stroke volume also significantly increased (33%) during exercise, and a further significant increase (59%) was observed in recovery (Fig. 2B).

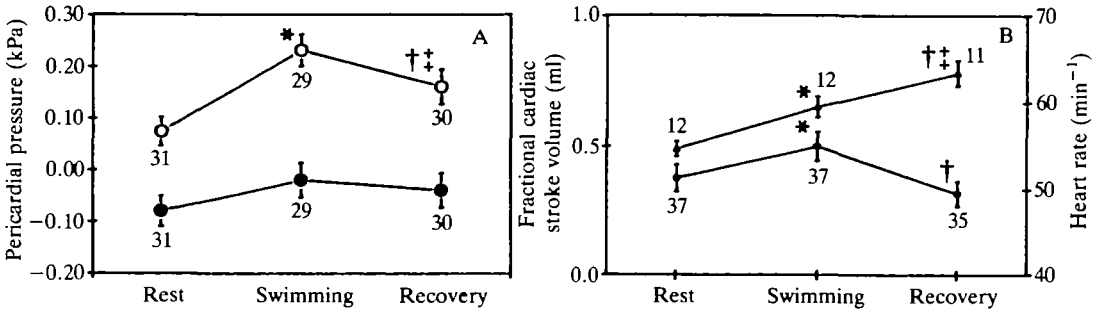

Fig. 2. (A) Changes in mean P_{max} (○) and P_{min} (●), from rest to swimming and during recovery. Error bars are ±S.E.M., and the numbers indicate total number of observations. Symbols indicate significant ($P<0.05$, regression analysis) differences between: rest and swimming (*), swimming and recovery (†), and between rest and recovery (‡). (B) Changes in fractional cardiac stroke volume (▲) and heart rate (○) from rest to swimming and during recovery. Significance symbols as in A.

Flow calibrations indicated that fractional cardiac stroke volume of resting *T. semifasciata* amounted to 33 % of the total cardiac stroke volume. Correction of fractional cardiac stroke volume yielded an estimate of total cardiac stroke volume, which was normalized for mass and multiplied by heart rate to obtain mass-specific cardiac stroke volume and cardiac output (Table 1). The mass-specific cardiac output of *T. semifasciata* increased by 70 % (33.1 to 56.2 ml kg^{-1} min^{-1}) from rest to exercise. Also, despite the drop in heart rate

Table 1. *Changes in mean cardiac parameters of* Triakis semifasciata *prior to, during and following swimming*

	Rest	Swimming	Recovery
Fractional cardiac stroke volume (ml)	0.49	0.65	0.78
N	12	12	11
±S.E.M.	0.03	0.04	0.05
3.03×*in situ/in vitro* correction factor (ml)	1.48	1.97	2.36
Estimated cardiac stroke volume (ml kg^{-1})*	0.77	1.02	1.22
Net increase in cardiac stroke volume (ml kg^{-1})		0.25	0.45
Heart rate (beats min^{-1})	51.3	55.1	49.5
N	37	37	35
±S.E.M.	1.6	1.7	1.5
Total cardiac output (ml kg^{-1} min^{-1})	33.1	56.2	60.4

Average mass 1.93 kg.

during recovery, cardiac output remained elevated (60.4 ml kg^{-1} min^{-1}). The net increase in cardiac stroke volume from rest to recovery was 0.45 ml kg^{-1} (Table 1).

Effects of swimming on pericardial fluid volume

The pericardial fluid loss estimated during an average of 15 min of swimming was 0.6 ml kg^{-1} [i.e. a mean fluid decline from 2.5±0.3 to 1.7±0.2 ml (N=20), average body mass 1.40 kg]. This means that good agreement is seen between the quantity of fluid lost from the pericardium during swimming and the net increase in cardiac stroke volume (0.6 *vs* 0.45 ml kg^{-1}, Table 1).

Table 2 shows the relationship between pericardial fluid volume at rest and the volume lost as a result of swimming. Indicated for each of 11 *T. semifasciata* are body mass, pericardial capacity and the pericardial operating volume experimentally established prior to swimming. Fluid loss occurred in 14 of 20 trials and the volume loss correlated (r=0.53, P<0.05) with the pericardial volume established before swimming. Closer inspection of these data shows that, in cases where fluid

Table 2. *Observed pericardial fluid volume changes following swimming determined for 11* Triakis semifasciata *in relation to total pericardial capacity and operating volume at the onset of swimming*

Shark no.	Body mass (kg)	Pericardial capacity (ml)	Operating volume (% capacity)	Fluid loss (ml)
3	1.8	5.0	30	0.0
3	1.8	5.0	30	0.0
2	1.7	6.5	34	0.6
2	1.7	6.5	34	1.4
7	2.2	7.4	40	1.0
3	1.8	5.0	42	0.0
10	2.6	7.0	43	0.0
9	2.3	6.0	43	1.0
4	1.9	4.6	44	0.0
1	1.0	2.8	45	0.4
1	1.0	2.8	50	0.0
3	1.8	5.0	50	0.2
1	1.0	2.8	50	0.4
10	2.6	7.0	50	1.1
9	2.3	6.0	50	1.4
8	2.2	8.0	54	1.8
11	2.6	9.0	55	0.4
6	2.0	7.0	60	1.8
5	2.0	5.0	60	2.0
6	2.0	7.0	69	1.4
Mean		5.8	46.7	0.7
N		20	20	20
±S.E.M.		0.5	3.0	0.1

Table 3. *Resting and swimming* P_{max}, *POP and the relative occurrence of transient pressure spikes* (P_{tran}) *greater than POP in the 14 observed cases of pericardial fluid loss during swimming in* Triakis semifasciata

Shark no.	Body mass (kg)	Fluid loss (ml)	Resting		Swimming	
			P_{max} (kPa)	POP (kPa)	P_{max} (kPa)	P_{tran} (%)
2	1.7	0.6	0.26	0.33	0.36	50
2	1.7	1.4	0.30	0.33	0.68	100
7	2.2	1.0	0.49	0.25	0.33	11
9	2.3	1.0	0.17	0.17	0.29	33
1	1.0	0.4	0.02	0.32	0.07	10
3	1.8	0.2	0.25	0.40	0.27	24
1	1.0	0.4	−0.01	0.32	0.24	14
10	2.6	1.1	0.09	0.22	0.21	ND
9	2.3	1.4	0.01	0.17	0.17	45
8	2.2	1.8	0.26	0.16	0.34	22
11	2.6	0.4	0.16	0.31	0.50	9
6	2.0	1.8	−0·17	0·16	0·19	50
5	2.0	2.0	0.30	ND	0.44	ND
6	2.0	1.4	−0·07	0.16	0.22	100
Mean		1.1	0.15	0.25	0.31	39
N		14	14	13	14	12
±S.E.M.		0.2	0.04	0.02	0.04	9

ND, no data available.

volume was set at less than 50 % capacity, fluid loss occurred in only five of 10 trials. By contrast, nine of 10 tests made with an operational volume greater than or equal to 50 % resulted in fluid loss.

Table 3 examines pericardial pressure changes during swimming in the 14 trials (Table 2) where fluid loss occurred, by comparing resting P_{max} (averaged over a period just prior to swimming) and POP with swimming P_{max} (averaged over a period of swimming) and the percentage of swimming P_{max} values that exceeded POP (P_{tran}). It is apparent that during swimming P_{max} averaged for all observations was higher than mean POP (0.30±0.04 *vs* 0.25±0.02 kPa). In four trials, however, swimming P_{max} failed to reach POP, which raises the question of how fluid could be lost under such circumstances. Inspection of the pericardial pressure records available for three of these trials revealed that episodic spikes in pericardial pressure to values above POP (P_{tran}) occurred between 10 and 24 % of the time. These spikes, which are unrelated to cardiac cycle, are illustrated in Fig. 3. This observation suggests that even when mean P_{max} is not higher than POP, these transient pressure spikes may open the PPC and thus account for fluid loss.

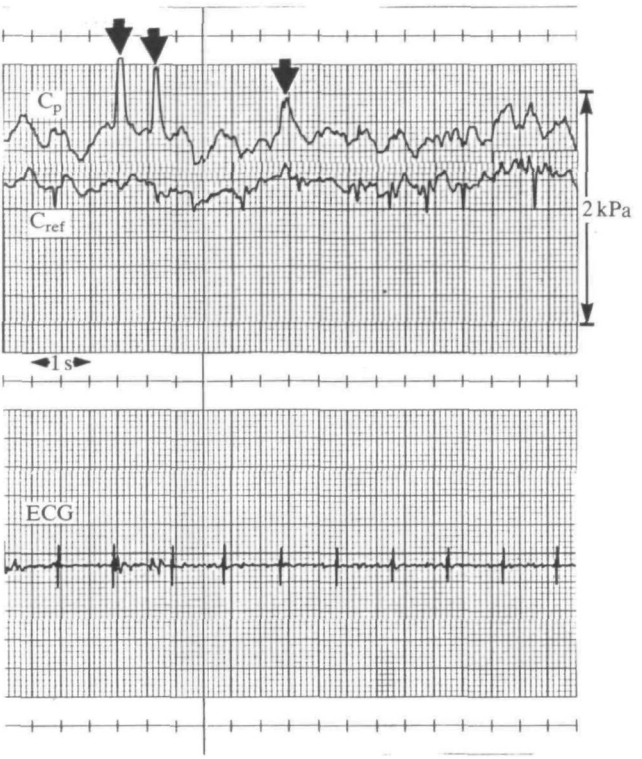

Fig. 3. Recordings of ECG and pericardial pressure during swimming in leopard shark no. TS111887 showing the transient pressure spikes (arrows). Pericardial opening pressure is not indicated because the baseline was fluctuating during depth changes with swimming. Abbreviations as in Fig. 1. Vertical tics at 1 s intervals.

Effects of pericardial infusion in swimming fish

The effects of pericardial fluid infusion on pericardial pressure and fluid loss *via* the PPC were repetitively documented in three swimming sharks. Fig. 4 shows pericardial pressure and volume data for a shark swimming at 0.5 $L\,s^{-1}$. Following a pericardial infusion of 3.2 ml, mean swimming P_{max} rose abruptly off scale and then rapidly declined to a value near POP. Over the next several minutes, P_{max} and pericardial fluid volume gradually returned to the pre-infusion level. A similar pattern was observed in 11 tests where post-infusion volume and pressure were monitored for as long as 17 min. In nine of these studies pericardial volume returned to pre-infusion level and in the other two cases it dropped to within 1.5 ml of the pre-infusion level.

Fig. 4B shows that the P_{max} of a swimming shark was elevated when its velocity increased from 0.3 to 0.8 $L\,s^{-1}$. A subsequent volume infusion (3.0 ml) had the same effect as seen in Fig. 4A and a pericardial volume measurement at about 50 s after infusion indicated that, while the bulk of the infusion (1.8 ml) had been le[illegible] *via* the PPC, an excess volume remained, as indicated by a P_{max} that was still

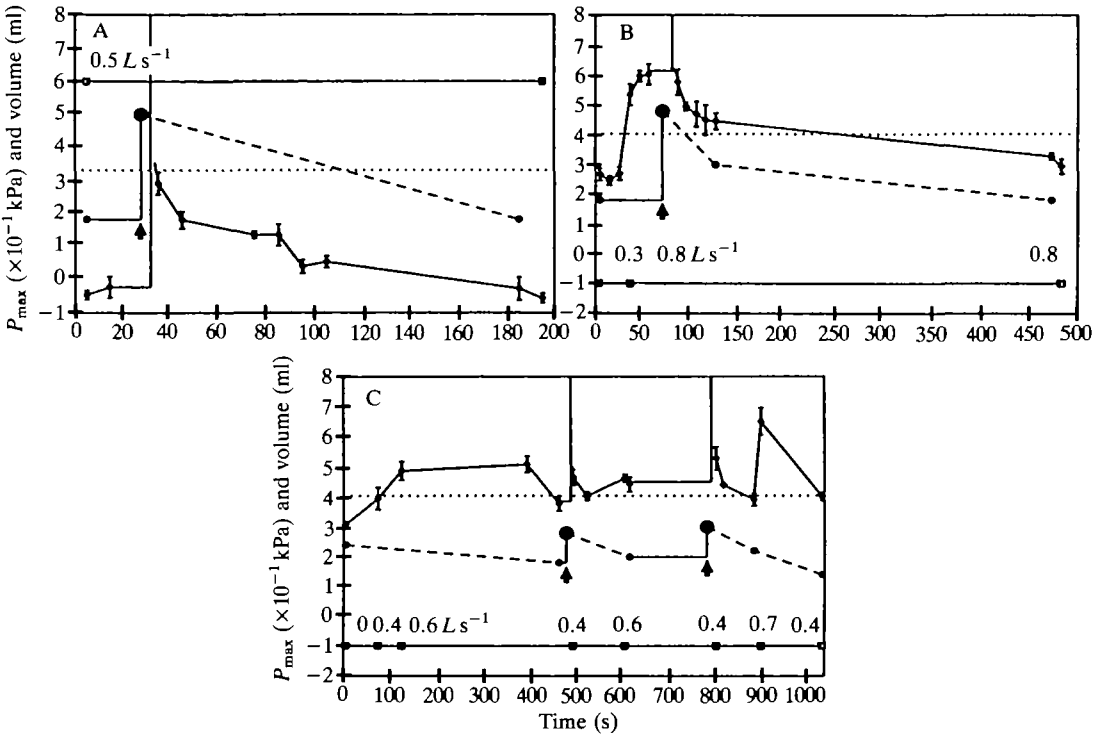

Fig. 4. (A) Effects of pericardial fluid infusion (arrow) on the pericardial pressure of shark no. TS110887 swimming at $0.5\,L\,s^{-1}$ (solid line, □). Other symbols: measured pericardial fluid volume (●); the sum of the initial and added pericardial fluid volume (◉); mean P_{max} over 10 s intervals sampled at the various periods (◆); pericardial opening pressure (dotted horizontal line). Error bars are ±S.E.M. (B) The effects of pericardial infusion and swimming speed on pericardial pressure and volume in shark no. TS113087. Symbols as in A. (C) The effects of multiple pericardial infusions and swimming speed on pericardial pressure and volume in shark no. TS113087. Symbols as in A.

above POP. 420 s after infusion, mean P_{max} had dropped below POP and pericardial volume returned to its pre-infusion level.

A velocity effect on pericardial pressure is also apparent in Fig. 4C, a finding recorded in 11 of 13 instances. Fig. 4C also demonstrates replicate effects of volume infusion on P_{max}, and subsequent volume loss.

Discussion

This study shows that the mean resting P_{max} and P_{min} of leopard sharks range from above ambient to slightly subambient. The pressures are not as profoundly subambient as previously attributed to elasmobranchs, but are more in agreement with values reported for resting horn sharks by Abel *et al.* (1986). This finding is also not compatible with the hypothesis that hydrodynamic flow contours along

the body transmit subambient pressure into the pericardial cavity (Freadman, 1983).

If *vis a fronte* is the principal mechanism for cardiac filling, pericardial pressure would be expected to become even more subambient during swimming. Contrary to this, we found that swimming not only increased fractional cardiac stroke volume in *T. semifasciata* but pericardial pressure also rose. This observation challenges the concept that increasingly subambient pericardial pressure is an important mechanism responsible for augmenting cardiac output during exercise. Cardiac output of the trout heart can be increased by *vis a tergo* filling (Farrell *et al.* 1988), and we postulate that this may also be true for swimming *T. semifasciata*. However, we did not measure venous pressure and ventricular transmural diastolic pressure and, therefore, we cannot estimate the relative contributions of increased *vis a tergo* and *vis a fronte* filling to increased venous return during exercise.

These results and our previous findings (Abel *et al.* 1986, 1987) suggest that the role of subambient pericardial pressure as the dominant mechanism of cardiac filling in elasmobranch fishes (Johansen, 1965; Sudak, 1965*a*,*b*; Hanson, 1967; Satchell, 1971) may have been overstated. Although this concept is still widely held (Johansen and Burggren, 1980; Santer, 1985; Johansen and Gesser, 1986; Butler, 1986; Butler and Metcalfe, 1988), it should be recalled that the data supporting it were derived from studies performed acutely on anaesthetized, supine, emergent elasmobranchs. Under such conditions, pericardial fluid ejection *via* the PPC is likely to have occurred in the course of handling, thus resulting in lower (−0.09 to −0.88 kPa) pericardial pressure (Abel *et al.* 1986).

Concerning the possible effects of the pericardium on swimming performance, our study showed that the moderate swimming effort (from 0.3 to 0.7 $L\,s^{-1}$) of the fish in this study triggered increases in both the fractional cardiac stroke volume (33 %) and heart rate (7 %) that were sufficient to sustain swimming for up to 30 min. These increases suggest that the elevated pericardial pressure measured in swimming sharks did not compromise their cardiac performance. Our estimated 70 % (Table 1) increase in total cardiac output during swimming is based on the assumption that the percentage of cardiac stroke volume proceeding past the flow probe did not change from rest to swimming. This could not be verified. However, data reported by Satchell *et al.* (1970) on *Raja rhina* indicated small and variable changes in regional branchial blood flow during short periods of swimming. Our estimates of the percentage (33 %) of resting cardiac output reaching the segment of the ventral aorta where the electromagnetic flow probe (EMF) was located agree with estimates obtained by other investigators using various methodologies (viz. combined Fick and EMF, *Scyliorhinus canicula*, 37 %, Taylor *et al.* 1977; microspheres, *Squalus acanthias*, 33 %, Kent *et al.* 1971; EMF, *Raja rhina*, 41 %, Satchell *et al.* 1970; *in situ* and *in vitro* calibrations, *Heterodontus francisci* and *Prionace gluaca* both 35 %, Abel *et al.* 1987; radiographic imaging, *H. francisci*, 34 %, Lai, N. C. unpublished data). Finally, agreement between the net loss of pericardial fluid volume and the calculated net increase in cardiac stroke volume

during swimming (Table 2) suggests, assuming end systolic volume did not change during swimming, that increased venous return generated a comparable increase in ventricular end diastolic volume.

Our study suggests that the loss of pericardial fluid can be both the cause and the result of cardiac expansion. On the one hand, mechanisms contributing to increased pericardial pressure during swimming probably include mechanical compression of the pericardium by various contiguous muscle groups, such as those controlling the pectoral fins, branchial apparatus or the locomotory musculature. A higher intensity of contraction by these muscle groups can generate transient pressure spikes forcing ejection of pericardial fluid *via* the PPC, thereby allowing for increased cardiac stroke volume (Fig. 3). This reduction in pericardial fluid volume makes room for heart size to increase without an inordinate increase in pericardial pressure.

On the other hand, elevated venous pressure due to compression of the veins and venoconstriction (swimming caudal vein pressures: from 0.25 to 2.94 kPa, Hanson, 1967; from 2.75 to 2.94 kPa, Satchell, 1965), the presence of venous valves (Birch *et al.* 1969), mobilization of unstressed blood volume from storage organs and large venous sinuses (Johansen and Hanson, 1967; Greenway and Laut, 1986) and the action of caudal hearts (Satchell and Weber, 1987) lead to increased cardiac volume. The resulting increase in heart size would stretch the semi-rigid pericardium and maintain increased cardiac stroke volume *via* the Frank–Starling mechanism. When the pericardium reaches its stressed volume, pericardial pressure would begin to increase. This pressure increase is sufficient to open the PPC, allowing pericardial fluid to be displaced. Further increase in cardiac size thus occurs without raising pericardial pressure.

Experiments in which we simulated alterations of pericardial pressure by direct infusion into the pericardial space of swimming fish yielded results in keeping with the phenomena observed during similar swimming activity. Immediately after infusion pressure fell abruptly, analogous to the response to naturally occurring high-pressure transients. The abrupt decline in pericardial pressure was followed by a gradual fall, analogous to a smaller but more sustained increase in P_{max}. In the experiment depicted in Fig. 4A most fluid loss occurred when pericardial pressure was less than POP: this is analogous to fluid loss caused by pressure transients. By way of contrast, Fig. 4B illustrates pericardial pressure declining by 1 ml over 6 min and gradually falling from above to below POP: this is analogous to fluid loss caused by a sustained increase in P_{max}.

The two principal determinants of cardiac output are heart rate and cardiac stroke volume. In elasmobranchs, absence of sympathetic innervation to the heart explains the minimal chronotropic effects of exercise (Randall, 1968). Cardiac stroke volume is therefore of paramount importance for the cardiac output response to exercise. Shabetai *et al.* (1985), using various scintigraphic agents as markers for pericardial fluid turnover, reported that a portion of the isotopes introduced into the pericardium were adsorbed by the epicardial and pericardial surfaces, demonstrating the membrane-drainage function of elasmobranch peri-

cardium. This drainage system, however, is obviously unsuited for sudden large changes in pericardial volume. The PPC, in contrast, is ideally suited for this function and can vent substantial amounts of pericardial fluid to the peritoneum, creating on demand the space necessary for cardiac expansion. Thus the PPC, in conjuction with the large pericardial volume characteristic of elasmobranchs, may enable instantaneous increases in heart performance, which cannot be achieved humorally or neurogenically (Butler *et al.* 1986). Retention, in this ancient group of fish, of a mechanical system comprising the PPC, a large pericardial fluid volume and contiguous muscles for prompt cardiac adjustments, appears to compensate for the lack of sympathetic innervation of the heart and reliance on inherently slower humoral responses.

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