Title

The high ventricular function in the shortfin mako shark (Isurus oxyrinchus)

Journal

Physiology, 38(S1)

ISSN

1548-9213

Authors

Lai, N Chin
Head, Brian
Lai, Yin
et al.

Publication Date

2023-05-01

DOI

10.1152/physiol.2023.38.s1.5727623

Peer reviewed

Fish Physiology and Biochemistry **24:** 141–148, 2001.

Regulation of cardiac function in the horn shark by changes in pericardial fluid volume mediated through the pericardioperitoneal canal

Brian P. Head[1], Jeffrey B. Graham[2], Ralph Shabetai[3] and N. Chin Lai[1,2,*]

[1]*Marine Science and Environmental Studies, University of San Diego, 5998 Alcalá Park, San Diego, CA 92110-2492, USA;* [2]*Center for Marine Biotechnology and Biomedicine, Scripps Institution of Oceanography, University of California, San Diego, La Jolla, CA 92093-0204, USA;* [3]*Department of Medicine, University of California, San Diego, and Medical Service, Veterans Administration Medical Center, San Diego, CA 92161, USA;* [*]*Author for correspondence (Phone: (858) 534-8044; Fax: (858) 534-1305; E-mail: nclai@ucsd.edu)*

Accepted: June 12, 2001

Key words: cardiac output, cardiac stroke volume, heart rate, pericardial pressure, pericardioperitoneal canal, pericardium, shark

Abstract

The California horn shark (*Heterodontus francisci*), instrumented with a pericardial catheter and a ventral-aortic flow probe, was studied to determine the effect of complete pericardial chamber evacuation on the time course for restitution of pericardial fluid-volume and pressure, and the effects of both fluid removal and its restitution on cardiac output. Prior to evacuation, pericardial pressure was -0.02 ± 0.02 kPa, and cardiac output was 18 ± 2 ml min^{-1} kg^{-1}. Evacuation reduced pericardial pressure to -0.73 ± 0.14 kPa, and increased cardiac output to 23 ± 4 ml min^{-1} kg^{-1}. The time course for restoration of post-evacuation pressure is described by a non-linear asymptotic function. A large percentage of the pericardial pressure and volume recovery occurred within the first hour, while, complete restoration of pre-withdrawal conditions required about 11 h. Pericardial pressure-volume relationships, determined by incremental infusion of small volumes of elasmobranch saline into the pericardium, confirm previous findings that the operating pericardial pressure in the horn shark is at or near ambient pressure and that both pericardial fluid volume and cardiac stroke volume influence horn shark pericardial pressure.

Introduction

Several structural features distinguish the elasmobranch (sharks and rays) pericardium from that of other fishes and these are also considered to have special significance with respect to elasmobranch cardiac function (Shabetai et al. 1985). Elasmobranchs have a relatively non-compliant (rigid) pericardial wall, which was implicated to play an important role in aspirational (*vis-a-fronte*) filling of the heart (Sudak 1965a, b; Johansen 1965; Randall 1968; Satchell 1971). The volume of the elasmobranch pericardium is also much larger than the heart (Shabetai et al. 1985), which appears to have importance in facilitating increased cardiac stroke volume during exercise (Lai et al. 1989a). Elasmobranchs [as well as the jawless fishes (Superclass Agnatha) and some primitive bony fishes (e.g., Subclass Chondrostei)] also have a connecting duct between the pericardium and peritoneum (Romer 1964; Shabetai et al. 1985; Abel et al. 1986; Lai et al. 1989a). This duct, termed the pericardioperitoneal canal (PPC) functions as a one way conduit for the egression of pericardial fluid to the peritoneal cavity which, by decompressing the pericardial pressure, allows for increases in cardiac stroke volume (Shabetai et al. 1985; Abel et al. 1986, 1987, 1994; Lai et al. 1989a).

Elasmobranch pericardial fluid has the chemical properties of plasma (Smith 1929) and thus appears to originate as an ultrafiltrate of myocardial interstitium (Shabetai et al. 1985). The mechanisms of pericardial fluid turnover include its resorption and redistribution to other body regions through the myocardial and pericardial lymphatics, and ejection via

the PPC (Shabetai et al. 1985; Abel et al. 1986; Lai et al. 1989a). In sharks, ejection of fluid via the PPC is a natural consequence of behavior. In the California horn shark (*Heterodontus francisci*), for example, a sudden burst of swimming in response to a startle or to handling results in pericardial fluid loss (Abel et al. 1986, 1994). In continuously swimming leopard sharks (*Triakis semifasciata*), the combination of increased pericardial pressure and increased cardiac stroke volume forces fluid out of the PPC, with the volume of fluid loss corresponding to the increase in cardiac stroke volume (Lai et al. 1989a).

Assuming that myocardial ultrafiltration and the lymphatic drainage are the principal mechanism for pericardial volume compensation following fluid ejection, it can be expected that fluid restoration would be time-dependent. In an earlier study Abel et al. (1994) estimated a pericardial fluid replenishment rate of 0.6 ml kg^{-1} h^{-1} in the horn shark. However, their data also show that restoration rate varies with time after evacuation suggesting a non-linear process. It seems reasonable to assume that, in the early phase of volume recovery, the pre-load effect on the heart (i.e., the increased transmural pressure resulting from a reduced pericardial pressure and volume) could increase myocardial contraction force thereby augmenting ultrafiltration.

This paper reports experiments designed to examine interactions between abrupt and gradual changes in pericardial pressure and volume and heart function. The effects of pericardial fluid removal on pericardial pressure and cardiac output and the effect on these on the natural time course of fluid repletion were determined by continuously monitoring cardiac output and pericardial pressure in conscious, chronically catheterized horn sharks.

Materials and methods

Experimental fish and maintenance

Horn sharks (2.4 ± 0.2 kg; mean ± SEM; n = 9) were obtained by hook and line from local waters and maintained in a 5,000-l holding tank at Scripps Institution of Oceanography, La Jolla, California. The tank received continuously flowing seawater (20 °C) and sharks were fed twice weekly but fasted for at least 24 h prior to surgery.

Surgical procedures

Sharks were anesthetized in a holding tank with tricaine methanesulfonate (MS-222; 1:10,000 Finquel® Argent Chemical Laboratories), placed supine on a surgical cradle, and continuously ventilated with aerated seawater containing the same concentration of anesthetic.

To catheterize the pericardium, a 2-mm skin incision was made just above the mid-ventral aspect of the coracoid bar, and the coracoid and adjacent pericardium were then punctured with a 15 ga hypodermic needle. The needle was removed and a trocar was inserted into the puncture. A polyethylene catheter (PE 240), filled with elasmobranch saline and having a side port near its tip, was inserted through the trocar and into the pericardium. The trocar was withdrawn and the catheter was positioned so that its side port was level with the pericardial floor. This allowed measurement of pericardial pressure (without interfering with heart action) as well as both fluid withdrawal and injection. Tissue adhesive (3M Vetbond) sealed the catheter to the coracoid and the skin was stitched and closed around the tube. The catheter was led to the dorsal body surface and secured to the skin and dorsal fin at various points. The free end was sealed with a stopcock and wrapped around a cork to form a short, floating tether. The shark was placed in a 50-l experimental tank supplied with aerated, continuously flowing seawater and allowed to recover for 24 h. Pericardial studies were carried out on nine sharks.

After the pericardial studies, a perivascular ultrasonic flow probe (Type 2B1336 or 4SB665, Transonic Inc.) was placed on the ventral aortae in four (2.4 ± 0.1 kg) of the nine sharks to enable simultaneous records of pericardial pressure and fractional stroke volume. After completing the procedure for pericardial catheter placement, a ventral midline incision was made in the branchial region and, using blunt dissection, the branchial muscles were displaced to expose the ventral aorta. The flow probe was then fitted to the ventral aorta and the cable secured along the body surface by sutures. The probe connector was sealed in a plastic bag and attached to a foam float to keep it dry. The shark was then placed in the experimental tank and allowed 24 h to recover.

The open span along the ventral aorta between the anterior end of the pericardium and points of origin of combined afferent branchial arteries 3, 4, and 5 are too short for probe placement. The probe was therefore placed between afferent branchial 3 and the

innominate arteries (branchials 1 and 2). Thus, only fractional cardiac output could be monitored and this was corrected to total cardiac output by using the species-specific correction factor of 2.9 (Abel et al. 1987). Although we have validated in elasmobranchs that the *in vivo* flow distribution between the innominate and the afferent branchial 3 was around 30%, we caution that changes in cardiac output may influence branchial blood flow distribution (Lai et al. 1989b). Heart rates were estimated from either the blood-flow signal or the pericardial pressure waveform.

Instrument calibration and data collection

Pericardial pressure was measured using a TruWave^{TM} disposable pressure transducer (Model px600I, Baxter Healthcare Corporation). Prior to each study, the transducer was calibrated against a static water column (0 and 2.0 kPa). A separate fluid-filled PE tube connecting the transducer to water in the experimental tank was used to establish zero pressure (Abel et al. 1986), and a stopcock was used to switch the transducer signal between zero and pericardial pressure. Depth (pressure) artifacts in the pericardial pressure were eliminated by keeping the distance between the transducer and the water level in the experimental tank constant. Pressure signals were amplified (Model 13-4615-50, Gould Inc.), digitally converted (DI 220, Dataq), and stored on a microcomputer for offline processing. The flow probes are factory calibrated for measurements at 37 °C, and the manufacturer's calibration adjustment of 4% was used to correct measurements made at 20 °C.

Pericardial pressure measurement

The first objective was to determine operating pericardial pressure in a quiescent shark, which was done by carefully connecting the catheter to the pressure transducer and recording pressure without disturbing the fish. Next, the operating pericardial fluid volume (PFV) was measured by withdrawing the entire pericardial content into a syringe. Maximum PFV was then measured by infusing a sufficient volume of elasmobranch saline to open the PPC (Shabetai et al. 1985). The opening of the PPC was indicated by a leveling off of the pressure-volume curve (i.e., additional fluid injection no longer increased pericardial pressure). When this point was reached the contents of the pericardium were withdrawn to a syringe and measured. This volume is defined as maximum PFV (Shabetai et al. 1985). Elasmobranch saline was then infused into the catheter to restore pericardial pressure and volume to operating level, and after about 12 to 24 h, the pericardium was again evacuated and the rate of pressure recovery was determined by continuously monitoring (for up to 24 h) pericardial pressure. These pressure recovery experiments were done during night and daylight hours and, at the end of each study the volume of the replenished pericardial fluid was measured.

Post-evacuation pericardial pressure and volume recovery and its effect on cardiac output

For the sharks fitted with blood flow probes, quiescent-state operating pericardial pressure and cardiac output were first determined. Pericardial fluid was then evacuated and both pericardial pressure and cardiac output were continuously monitored during pressure recovery.

A subsequent blood-flow experiment determined the effects of the infusion of fluid into the pericardium on cardiac output. This was done by totally evacuating the pericardium and recording pressure and fractional cardiac output, and then making a series of small volume (0.2 ml) infusions into the pericardium and measuring pericardial pressure and cardiac output. These incremental infusions were continued until PPC opening pressure was reached.

Results

Pericardial pressure recovery rates

Table 1 shows the pericardial pressures, volumes, cardiac outputs, and heart rates for horn sharks in this study. Mass-specific operating PFV was 2.1 ml kg^{-1} (mean operating PFV was 5.1 ml), and the mass-specific maximum PFV was 3.5 ml kg^{-1}. Mean operating pericardial pressure was -0.02 kPa, and this was reduced to -0.73 kPa by total pericardial fluid withdrawal. Heart rates before (32 bpm) and 11.5 h after (36 bpm) total fluid evacuation were not significantly different, and there was no correlation between time and heart rate after evacuation.

Although variation is evident in initial post-withdrawal pericardial pressures, all nine horn sharks showed a similar pattern for pericardial pressure recovery. This pattern featured a steep rise in pericardial pressure over the first hour, followed by a gradual increase to near ambient levels over the next 8–15 h. Figure 1 combines the pressure-recovery data for all

Table 1. Pericardial pressures, volumes, heart rates, cardiac outputs, and pericardial pressure recovery values for *Heterodontus francisci*

	Mean ± SEM (n)	Units
Fish mass	2.4 ± 0.2 (9)	kg
Pericardial fluid volume (PFV)		
Operating	5.1 ± 0.8 (9)	ml
Mass specific	2.1 ± 0.1 (9)	ml kg^{-1}
Mass specific 12 to 24 h post-Evacuation	2.5 ± 0.3 (4)	ml kg^{-1}
Maximum	8.4 ± 1.7 (7)	ml
Mass specific	3.5 ± 0.6 (7)	ml kg^{-1}
Pericardial pressure (PP)		
Operating	−0.02 ± 0.02 (9)	kPa
Post-Evacuation (0 h)	−0.73 ± 0.14 (9)	kPa
Post-Evacuation (11.5 h)	−0.05 ± 0.05 (9)	kPa
Post-Evacuation (19.8 h)	0.00 ± 0.03 (3)	kPa
Heart Rate		
Operating	32 ± 3 (7)	beats min^{-1}
Post-Evacuation (0 h)	36 ± 3 (7)	beats min^{-1}
Post-Evacuation (11.5 h)	39 ± 6 (3)	beats min^{-1}
Cardiac output		
Operating	18 ± 2 (4)	ml min^{-1} kg^{-1}
Post-Evacuation (0 h)	23 ± 4 (4)	ml min^{-1} kg^{-1}
Post-Evacuation (11.5 h)	19 ± 2 (4)	ml min^{-1} kg^{-1}
Estimated time for pressure recovery to −0.02 kPa (Figure 1; Equation 1)	11.0 (9)	h
Observed time for pressure recovery to −0.05 kPa	11.5 (9)	h
Estimated % maximum PFV for pressure return to −0.02 kPa (Figure 2)	37.7 (4)	%
Equation		
(1) Pericardial pressure as a function of time. PP = $-3.50 + 1.35ln(h + 0.5)$	$R^2 = 0.91203$	
(2) Pericardial pressure as a function of % maximum PFV. PP = $-7.96 + 2.18ln(\%vol + 0.5)$	$R^2 = 0.99242$	
(3) Percent maximum fluid volume as a function of time. $\%Vol = 7.72\ (h + 0.5)^{0.622} - 0.5$		

nine fish into an asymptotic function described by Equation 1 (Table 1). Solving Equation 1 for a mean operating pressure of −0.02 kPa yields an estimated post-withdrawal pressure-recovery time of 11 h.

Pericardial pressure and volume inter-relationships and effects on heart function

Changes in mass-specific cardiac output following pericardial evacuation and over the recovery period are shown in Figure 2. Prior to evacuation cardiac output was 17 ml min^{-1} kg^{-1} and heart rate was 36 beats min^{-1}. Post-evacuation cardiac output was 23 ml min^{-1} kg^{-1} and heart rate was 40 beats min^{-1}. Over the interval of pressure restoration, the cardiac output gradually returned to pre-evacuation value (19 ml min^{-1} kg^{-1}; Table 1).

Incremental fluid additions to the evacuated pericardium enabled quantification of the relationships between pericardial pressure and maximum PFV and between PFV and cardiac output. Figure 3 shows that at zero PFV pericardial pressure is −1.2 ± 0.4 kPa, and pressure is 0.25 ± 0.11 kPa at maximum (100%)

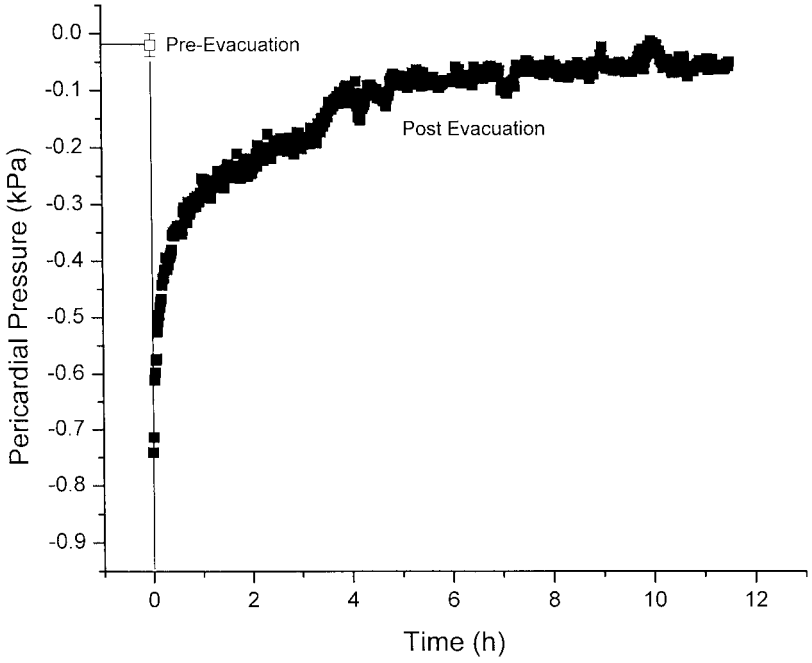

Figure 1. Post withdrawal (time zero) pericardial pressure records for nine horn sharks showing mean pre- and post evacuation pericardial pressures.

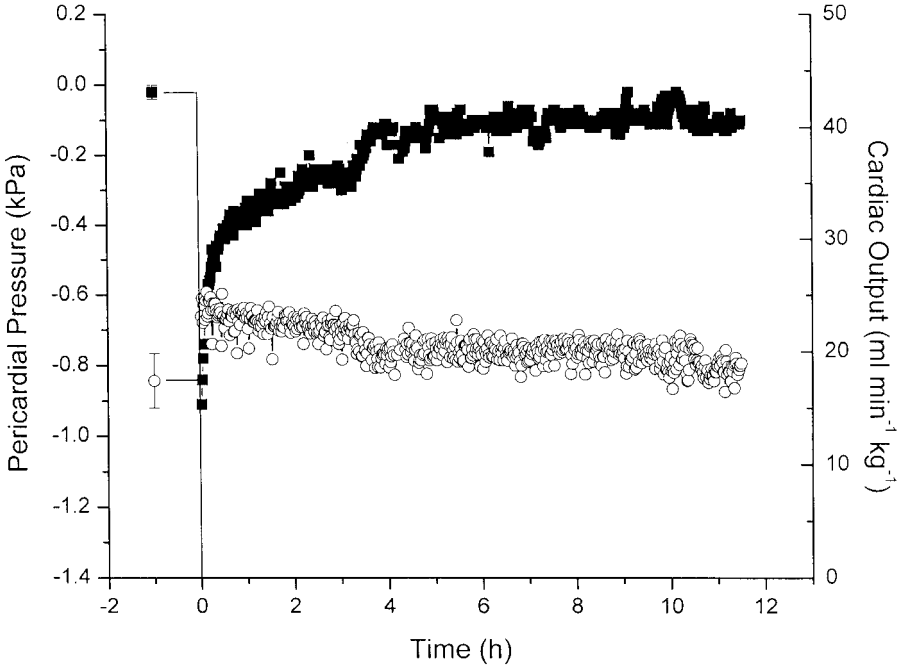

Figure 2. Mean pre- and post evacuation pericardial pressures (squares) and cardiac output (circles) data for four horn sharks.

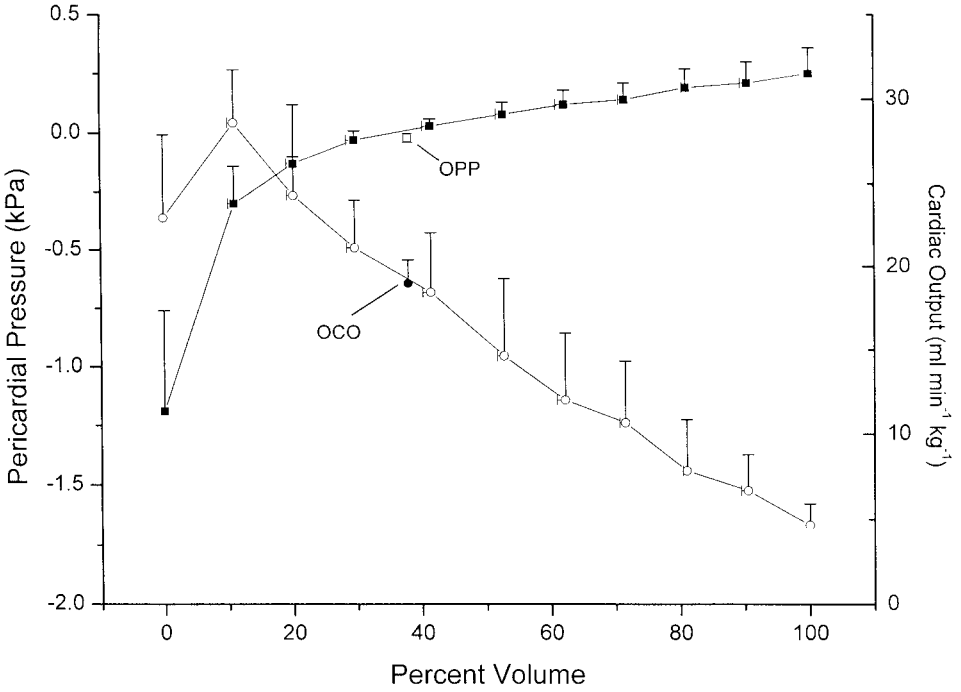

Figure 3. Interrelationships of pericardial pressure (closed squares) and cardiac output (open circles) as a function of percent maximum pericardial fluid volume (PFV) in horn sharks. Both the mean operating pericardial pressure (OPP) determined for the nine sharks and the corresponding operating cardiac output (OCO) are also shown. The relationship between pericardial pressure and maximum PFV curve is described by Equation 2 (Table 1).

PFV. The greatest change in pericardial pressure occurs with volume changes between zero and 11% PFV. Equation 2 (Table 1) describes the relationship between pericardial pressure and PFV and, solving this for the mean operating pressure of quiescent horn sharks (−0.02 kPa), yields an estimated PFV of 37.7% of maximum. These reference values are shown in Figure 3. Also shown in Figure 3 is the inverse effect of PFV on cardiac output. Maximum cardiac output (29 ± 3 ml min^{-1} kg^{-1}) occurs at 11% maximum PFV and decreases both at lower and higher PFVs, ranging from 5 ± 1 ml min^{-1} kg^{-1} at 100% PFV to 23 ± 5 ml min^{-1} kg^{-1} at 0% PFV.

Using Equation 2, the combined pericardial pressure recovery record for the nine horn sharks was transformed into volumes and Figure 4 shows estimated rates of pericardial volume replenishment over the 11 h period of recovery. PFV replenishment rate was maximal during the first 10 min post-withdrawal (6 ml kg^{-1} h^{-1}), and then reduced steadily. At 1 h the rate was 1 ml kg^{-1} h^{-1} and between 6 and 11 h it dropped from 0.21 to 0.12 ml kg^{-1} h^{-1}.

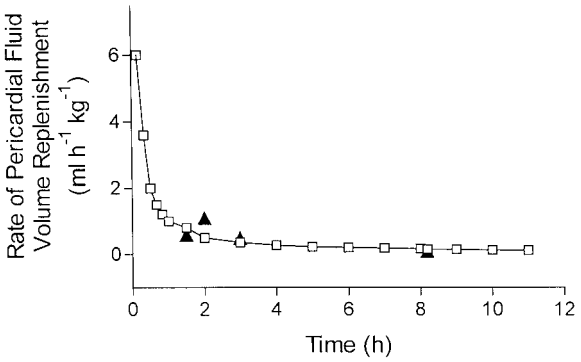

Figure 4. Instantaneous rates of pericardial fluid volume replenishment (ml kg^{-1} h^{-1}) for the nine horn sharks (squares). Triangles show the instantaneous PFV replenishment rates of horn sharks calculated over different time intervals and at different times after evacuation by Abel et al. (1994).

Discussion

The classical interpretation of elasmobranch heart filling is that both a rigid pericardium and a negative pericardial pressure are required for aspirational (*vis-a-fronte*) filling (Schoenlein and Willem 1894; Johansen 1965; Sudak 1965a, b; Hanson 1967; Randall

1968; Satchell 1970; Franklin and Davie 1993). However, the effect of the PPC on heart function had not been considered until Shabetai et al. (1985) showed the effect of pericardial fluid volume on pericardial pressure. Prior to this work, it had not been generally appreciated that handling could eject pericardial fluid, which in turn lowers pericardial pressure and increases cardiac output in elasmobranchs. The present study has confirmed previous findings that the pericardial pressures in quiescent sharks are not strongly negative (Abel et al. 1986; Lai et al. 1989a), but are rather maintained at near ambient pressure, with an operating PFV that is regulated by the PPC. In addition, we have quantified the relationship between PFV and cardiac output, and demonstrated the influence of these parameters on pericardial pressure, as well as the horn shark's capacity to rapidly restore PFV following evacuation.

The mechanism responsible for the rapid reaccumulation of PFV is not known. Pericardial fluid removal, either by aspiration or expulsion through the PPC, lowers pericardial pressure. This increases ventricular diastolic transmural pressure which, by increasing preload, raises stroke volume. We suggest that this increase in preload might augment myocardial ultrafiltration, thereby increasing the rate of fluid return.

Our findings for the rate of fluid restitution into the pericardium of horn sharks are in general agreement with data presented by Abel et al. (1986, 1994). These workers reported a rate of pressure increase of 0.09 ± 0.02 kPa h^{-1} and estimated a PFV replenishment rate of 0.6 ± 0.1 ml kg^{-1} h^{-1}. While this mode of data presentation implies a linear PFV recovery, the data of Abel et al. (1994) actually vary with time after withdrawal. The non-linear recovery models generated by our studies provide a more quantitative description of pericardial pressure and fluid return as a function of time (Figure 4; Equations 1 and 3).

Because pericardial fluid is continually added to the elasmobranch pericardium (Shabetai et al. 1985), the potential exists for pericardial pressure to increase to the point of causing cardiac tamponade (Figure 3). Thus, the PPC serves as an important escape route for pericardial fluid, and by regulating PFV in this manner, also exercises some control over cardiac stroke volume (Shabetai et al. 1985; Lai et al. 1989a; Abel et al. 1994). The importance of the PPC in pericardial decompression is documented by PPC occlusion experiments (Abel et al. 1994). Horn sharks with an occluded PPC had a decreased pericardial pulse pressure (meaning cardiac output was impaired) and a reduced survival time (10.4 ± 2.9 days) compared to a sham operated group (29.6 ± 20.4 days, Abel et al. 1994). Also, observation of the occluded group for eight days following surgery indicated a 245% increase in PFV and this was associated with a higher incidence of coughing behavior (0.5 to 4.0 times min^{-1}), which indicated attempts by these sharks to expel the accumulated pericardial fluid. These findings suggest that, in the absence of normal PPC function, the rate of pericardial fluid removal by the pericardial and myocardial lymphatic systems is inadequate.

In summary, we have shown that a strongly negative pericardial pressure is not required for heart filling, is not a normal condition in quiescent sharks, and that the removal of pericardial fluid as a result of aspiration is rapidly compensated. Horn shark pericardial pressure is influenced by pericardial fluid volume and by cardiac stroke volume, which can in turn be modulated by the action of the PPC.

Acknowledgements

We thank Drs M.S. Lowery and S. Eckert for their generous contributions to this research. This study was supported by a USD student grant to B.P.H., NSF IBN 9316621 to J.B.G., and by a VAMC merit grant to R.S. We also thank R. McConnnaughey, P. Zerofski, D. Bernal, T. Draganski, Y.Y. Lai, and H. Boyle for technical support.

References

Abel, D.C., Graham, J.B., Lowell, W.R. and Shabetai, R. 1986. Elasmobranch pericardial function. 1. Pericardial pressures are not always negative. Fish Physiol. Biochem. 1: 75–83.

Abel, D.C., Graham, J.B., Lowell, W.R. and Shabetai, R. 1987. Elasmobranch pericardial function. 2. The influence of pericardial pressure on cardiac stroke volume in horn sharks and blue sharks. Fish Physiol. Biochem. 4: 5–14.

Abel, D.C., Lowell, W.R. and Lipke, M.A. 1994. Elasmobranch pericardial function. 3. The pericardioperitoneal canal in the horn shark *Heterodontus francisci*. Fish Physiol. Biochem. 13: 263–274.

Franklin, C.E. and Davie, P.S. 1993. The role of the pericardium in cardiac function in the dogfish, *Squalus acanthias*. J. Fish Biol. 43: 213–219.

Hanson, D. 1967. Cardiovascular dynamics and aspects of gas exchange in Chondrichthyes. PhD dissertation, University of Washington.

Johansen, K. 1965. Dynamics of venous return in elasmobranch fishes. Hval. Skr. 48: 94–100.

Lai, N.C., Graham, J.B., Lowell, W.R. and Shabetai, R. 1989a. Elevated pericardial pressures and cardiac output in the leopard shark *Triakis semifasciata* during exercise: the role of the pericardioperitoneal canal. J. Exp. Biol. 147: 263–277.

Lai, N.C., Graham, J.B., Bhargava, V., Lowell, W.R. and Shabetai, R. 1989b. Branchial blood flow distribution in the blue shark (*Prionace glauca*) and the leopard shark (*Triakis semifasciata*). Exp. Biol. 48: 273–278.

Randall, D.J. 1968. Functional morphology of the heart of vertebrates. Am. Zool. 8: 177–189.

Romer, A.S. 1964. The Vertebrate Body. W.B. Saunders Company, Philadelphia.

Satchell, G.H. 1970. A functional appraisal of the fish heart. Federation Proc. 29: 1120–1123.

Satchell, G.H. 1971. Circulation in Fishes. Cambridge University Press, London.

Shabetai, R., Abel, D.C., Graham, J.B., Bhargava, V., Keyes, R.S. and Witzum, K. 1985. Function of the pericardium and pericardioperitoneal canal in elasmobranch fishes. Am. J. Physiol. 248: H198–H207.

Schoenlein, K. and Willem, V. 1894. Observations sur la circulation du sang chez quelques poisson. Bull. Sci. Fr. Belg. 26: 442–468.

Smith, H.W. 1929. The composition of body fluids of elasmobranchs. J. Biol. Chem. 81: 407–419.

Sudak, F.N. 1965a. Intrapericardial and intercardiac pressures and the events of cardiac cycle in *Mustelus canis* (Mitchell). Comp. Biochem. Physiol. 14: 689–705.

Sudak, F.N. 1965b. Some factors contributing to the development of subatmospheric pressure in the heart chambers and pericardial cavity of *Mustelus canis* (Mitchell). Comp. Biochem. Physiol. 15: 199–215.