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Mechanical performance of the isolated and perfused heart of the haemoglobinless Antarctic icefish *Chionodraco hamatus* (Lönnberg): effects of loading conditions and temperature

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SUMMARY

Scaling of heart ventricle mass and body mass in the haemoglobinless Antarctic fish *Chionodraco hamatus* Lönnberg shows a relationship similar to those reported for other 'cardiomegalic' icefish (*Chaenocephalus aceratus* and *Channichthys rhinoceratus*). An *in vitro* preparation of the heart of *C. hamatus* was set up to investigate the mechanical performance of this heart at different preloads and afterloads. It appears that this heart is well adapted to working within a range of preloads varying from −0.07 to −0.04 kPa, while it is unable to sustain increases of afterloads higher than 3.0 kPa. As in other teleosts, heart rate is unaffected by changes in preload and afterload. Increase in temperature from 0.5 to 5.8 °C affects heart rate whereas stroke volume is unaffected. On the whole, the *in vitro* data are similar to those *in vivo* measured in another icefish, *C. aceratus* and show that the heart of *C. hamatus* works as a typical volume pump. This is discussed in relation to both the structural constraints related to the cardiac design of this icefish and the biology of this unique vertebrate.

1. INTRODUCTION

A universal response of the heart to an augmented load is to increase myocardial mass. It is well known from mammalian and medical literature that this increase is primarily due to an increase in wall thickness under pressure overload, and to an increase in cavity size under volume overload (Braunwald 1980). Morphofunctional studies have also shown that the architecture of the heart ventricle differs in accordance with the tasks of pumping blood through a relatively high pressure and high resistance circulatory system or ejecting large volumes of blood against a very low outflow pressure (pressure pump or volume pump design, respectively, Rowlatt (1968) and Poupa & Lindstrom (1983)). Teleosts, the most numerous and diverse of vertebrate groups, have succeeded in almost every kind of living space in the hydrosphere, often facing extreme environmental conditions. It is to be expected, therefore, that the study of the teleost heart will disclose an underexploited number of natural cardiac models particularly useful for exploring the relationships between life style and patterns of cardiac growth and ventricular architecture.

The antarctic icefish of the family Channichthyidae provide an extraordinary opportunity for studying this relationship, since they exhibit a remarkable cardiac enlargement which is caused by volume overload. In fact, these teleosts, unique among adult vertebrates in that they lack haemoglobin in their blood (Ruud 1954, 1965), have developed extraordinary circulatory adaptations to their lack of oxygen-carrying pigment. The main compensations consist of increases in blood volume and blood flow coupled with a lowered oxygen demand (Hemmingsen & Douglas 1970, 1972; Holeton 1970). The blood volume is from two to four times larger than that in many teleosts: *C. aceratus* has a blood volume of about 9% of its body mass (Hemmingsen & Douglas 1972). The corresponding increase in cardiac output (Holeton 1970; Hemmingsen & Douglas 1972) is achieved by large stroke volumes (6–15 times greater than in other teleosts) at low heart rate and at relatively low ventral aortic pressure. This is reflected in an impressive increase in heart size: the relative heart mass of icefish is similar to that of small mammals (Johnston *et al.* 1983; Feller *et al.* 1985). This cardiomegaly is of the spongy type and shows ultrastructural features that may be related to the way the icefish heart pumps blood (Tota *et al.* 1988). However, the basic mechanisms of cardiovascular adaptation in this teleost cannot be understood until the intrinsic mechanical properties of the icefish heart (which have not yet been studied) have been characterized. The availability of specimens of *Chionodraco hamatus* (Lönnberg), the most common icefish in Terranova Bay, Antarctica, has made it possible to

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examine these. Accordingly, an isolated working heart preparation was set up. This represents the most suitable approach, since the heart is removed from extrinsic neural and humoral influences, so that its intrinsic mechanical properties can be more easily defined. Studies with such preparations both in mammals (Sonnenblick 1962) and fishes (Farrell 1985) have suggested that the four primary parameters of cardiac muscle performance are heart rate, preload, afterload and contractile state. The main aim of the present work was to collect basic information on the mechanical performance of this heart under different preload and afterload values and at different temperatures.

2. MATERIALS AND METHODS

(*a*) *Animals*

The study was conducted on 14 specimens of the icefish *Chionodraco hamatus* Lönnberg of both sexes. Fish were caught by nets in the Terranova Bay, Ross Sea, Antarctica, during January 1990. Identification was based on descriptions of Fisher & Hureau (1985).

After capture, the animals were maintained without feeding for at least three days in aerated, running sea water at temperatures between 0 and 2 °C. Only fish that appeared to be healthy were used in the experiments. Their masses ranged from 290 to 456 g.

All the experiments were done in the laboratories of the Italian Antarctic Base, Terranova Bay (74° 42′ S, 164° 06′ E), during the Fifth Italian Antarctic Expedition (December 1989–February 1990).

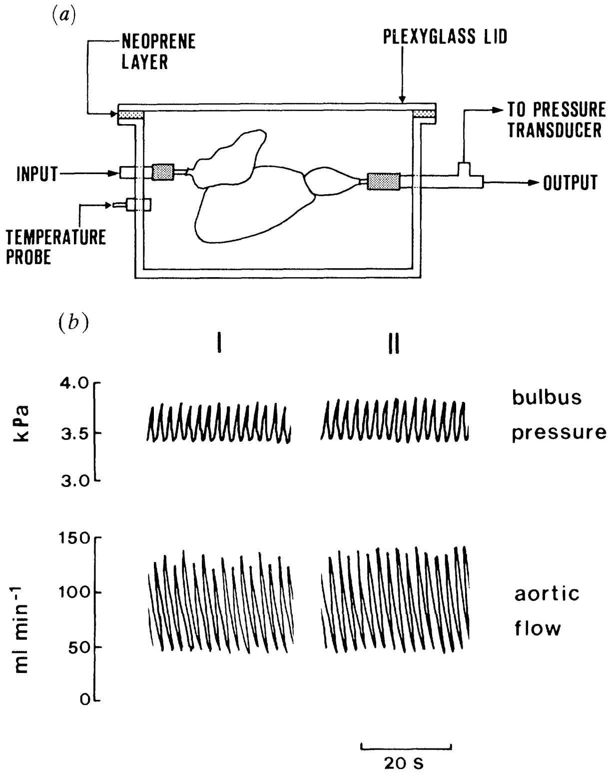

Figure 1. (*a*) Scheme of the perfusion chamber used for the isolated heart of *C. hamatus*. Reservoirs and general circuit were as in Houlihan *et al.* (1988). (*b*) Typical records of bulbus pressure and aortic flow in the isolated heart of *C. hamatus*, made 30 min and 180 min after the onset of perfusion.

(*b*) *Saline*

The perfursate was a modified version of the Cortland saline (Wolf 1963), with an increased NaCl content to bring the ionic concentration up to the level found in channichthyid blood (Holeton 1970). Its composition (in millimoles per litre) was: NaCl 252.4, KCl 5.0, $MgSO_4 \cdot 7H_2O$ 2.0, Dextrose 5.56, $CaCl_2$ 2.3, $NaH_2PO_4 \cdot H_2O$ 0.2, $Na_2HPO_4 \cdot 2H_2O$ 2.3. The saline was aerated and pH was adjusted to 7.84 at 1 °C, by using about 0.5 g of $NaHCO_3$. The pH used was chosen on the basis of the averaged pH value of nine freshly collected blood samples (7.84 ± 0.04, mean ± s.e.). The oxygen content of this saline was clearly higher than that of the venous icefish blood (P_{O_2} about 20 torr ($\approx$ 2666 Pa), Hemmingsen *et al.* (1972); Hemmingsen & Douglas (1977)). This means that oxygen can be considered a non-limiting factor in the experiments with changing temperature, when a change of oxygen solubility occurs (Graham 1987).

(*c*) *Isolated heart preparation*

The animals were heparinized with a caudal vein and artery injection of 0.7 ml kg^{-1} of sodium heparin in saline (50 U.I. ml^{-1}) and killed after a few minutes by a sharp blow on the head and spinectomy. The animal was opened ventrally behind the pectoral fins. The ventral aorta was cannulated and the heart was removed without the pericardium and placed in a ice-chilled dish filled with saline for the atrium cannulation procedure. A polyethylene cannula was secured in the atrium at the junction with the sinus venosus. Heart perfusion was immediately started from a reservoir of saline and rapidly emptied the heart of blood. Preparation time was 5 min.

The isolated heart was placed in the perfusion apparatus, modified from that described by Houlihan *et al.* (1988). In these experiments the chamber was designed to allow the development of subambient pressures during ventricle contraction, thus permitting suction filling of the atrium which is an important mechanism operating in teleosts (Lai *et al.* 1986; Farrell *et al.* 1988). The chamber was completely filled with perfusate and covered with an unsecured Plexiglas lid (figure 1*a*). A thin layer of neoprene, placed between the large upper lip of the main part of the chamber and the lid, allowed slow capillary movements of the medium into and out of the chamber in response to the volume changes of the heart during its cycle. It is important to use this type of chamber for isolated heart preparations in those teleosts where it is difficult to isolate the heart with an intact pericardium in a reasonably short time. The parietal pericardium in the icefish is firmly connected to the perimisium of the muscles surrounding the heart.

Two litres of aerated recirculating saline were used in each experiment. Hearts that were not stabilized within 10 min of perfusion were not used. When perfused at constant temperature the isolated heart performance was generally stable for at least three hours (figure 1*b*). Each heart generated its own rhythm.

All the experiments were performed in a cold thermostatted cabinet (LKB 2021 Maxicoldlab), which allowed the heart and perfusion system to be maintained at near zero temperatures.

The fish were weighed before each experiment, and the blotted wet weights of whole hearts (atrium, ventricle and bulbus) and ventricles were determined at the end.

(*d*) *Protocols*

Volume and pressure loading on the isolated heart

Pressure loading on the isolated heart was obtained by increasing the static output pressure, while volume loading was varied by changing the input pressure. Three fixed levels in the input reservoir of 0.0, 0.5 and 1.0 cm, respectively above the level of saline in the perfusion chamber were used on each heart. At each input level the height of the output reservoir was slowly increased to change the afterload from 2.0 to 4.0 kPa by 0.2 kPa steps. Bulbus pressure and cardiac output were continuously recorded.

The experiments were done at 3 °C and were completed within 2 h. In preliminary experiments repeated determinations of mechanical heart performance–afterload relation at constant preload were not significantly different.

Temperature effects on the isolated heart performance

We have assayed the effect of an increase in the perfusion temperature on performance of the isolated heart. The temperatures used were within the tolerance range in icefish (from −1.9 to 6 °C, Macdonald *et al.* (1987)). The experiments were done by keeping the input reservoir at the height of 0.5 cm above that of the saline in the perfusion chamber. The static afterload was 2.1 kPa. After stabilizing cardiac parameters at a temperature between 0 and 1 °C, the heart was subjected to a temperature increase of up to 5.5–6.0 °C by switching off the refrigerator system of the thermostatted cabinet. The initial rate of temperature change varied between experiments. Twenty minutes after the onset of the temperature change, the thermostatted cabinet was set up at 6 °C and the heart was allowed to equilibrate to this temperature for 10 min. Finally, the temperature was again decreased to the basal value. Bulbus pressure and cardiac output were continuously recorded.

In a few experiments, the temperature was allowed to increase to above 10 °C, to obtain a qualitative evaluation of the effect of relatively high temperatures on heart performance.

The pH of the saline decreased by 0.05 pH units when the temperature was increased from 0.5 to 11 °C. However, this change, which was not statistically significant, was within the range of variability of physiological pH. The experiments were performed within 2–4 h.

(*e*) *Measurements and calculations*

The preload was defined as the mean input pressure and was calculated from the static input pressure and the fall in pressure due to the input tube and cannula resistances. The afterload was defined as the mean value of output pressure, measured in the bulbus, and was calculated as: $\frac{1}{3}$ (minimum bulbus pressure) + $\frac{2}{3}$ (maximum bulbus pressure). Pressure measurements were made with a Bentley Trantec 800 pressure transducer connected to a Gemini 7070 2-channel recorder (UGO BASILE) and were referred to the saline level in the open perfusion chamber. All pressure values are expressed in kPa.

The heart rate was calculated from the pressure recordings. The cardiac output was measured by using a Gould–Statham SP2202 Flowmeter and was recorded simultaneously with the bulbus pressure by using the Gemini 7070 recorder.

Cardiac output and stroke volume were normalized per kilogram of wet body mass. Ventricular stroke work was calculated as (mJ g^{-1}) = (afterload − preload) (kPa) × stroke volume (ml)/ventricle mass (g). Power output was calculated as (mW g^{-1}) = (afterload − preload) (kPa) × cardiac output (ml min^{-1})/60/ventricle mass (g).

Temperature in the perfusion chamber was continuously measured with a YSI 42SC tele-thermometer.

All the results are expressed as mean value ± standard error (s.e.). Statistical differences ($p < 0.05$) were determined by using Student's *t*-test.

3. RESULTS

(*a*) *Effect of volume and pressure loading*

The same 'cardiomegaly' occurs in *C. hamatus* as in other icefish. We have found a mean RHW (= heart mass/body mass × 100) of 0.535 ± 0.022, and a mean RVW (= ventricle mass/body mass × 100) of 0.390 ± 0.017. A RVW value similar to that found in *C. hamatus* has been reported by Holeton (1970) for *C. aceratus* (table 1). These values are higher than the typical values of poikilotherm vertebrates, including fish (see Poupa & Lindstrom 1983; table 1).

Figure 2 shows the results of volume and pressure loadings on the isolated and perfused heart of *C. hamatus*. The heart rate was independent of both preload and afterload. The mean heart rate found in these experiments was 26.3 ± 1.94 beats min^{-1}. This was higher than the typical *in vivo* values of other icefish, reported in the literature and listed in table 1.

In *C. hamatus*, the increase of preload in a relatively narrow range (from −0.07 to −0.04 kPa) caused increases in stroke volume (figure 2) of the *in vitro* perfused heart up to two- or threefold, depending on the afterload used. The stroke volume is negatively affected by afterload, particularly at the lowest preload. As the heart rate is unchanged in all loading conditions used, the decrease of stroke volume with afterload is reflected in the behaviour of cardiac output (figure 2*b*). Over the full range of afterloads used, decreases were found in both stroke volume and cardiac output of 90, 60 and 55% at −0.07, −0.05 and −0.04 kPa of mean preload, respectively. In some experiments a decrease to almost zero flow at all preloads was found when afterloads were increased up to values similar to those of temperate sea fish (> 4.0 kPa, table 1), although the heart rate remained almost constant (data not shown).

The pulse pressure recorded in the bulbus ranged

Table 1. In vivo *cardiovascular parameters of icefish compared with those of some other antarctic and temperate teleosts* (*RVW, relative ventricular weight*; P_{va}, *ventral aorta pressure*; *CO, cardiac output*; *HR, heart rate*; *SV, stroke volume*; *SW, stroke work*; *PO, power output.*)

species	RVW %	P_{va} kPa	CO ml min^{-1} kg^{-1}	HR beats min^{-1}	SV ml kg^{-1}	SW mJ g^{-1}	PO mW g^{-1}	T/°C	Reference
Icefish									
Chaenocephalus aceratus	—	3.0/2.0^1	104^2, 66^3	16.4	4.02	3.57^4	0.98^4	0.5–2	(a)
Pseudochaenichthys georgianus	—	2.1/1.6^1	94^2, 67^3	12.6	5.32	3.43^4	0.72^4	0.5–2	(a)
Chaenocephalus aceratus	—	—	—	17.5	—	—	—	1–2	(b)
Chaenocephalus aceratus	0.300	2.7/1.6^1	—	13.8	—	—	—	0.5	(c)
Chaenocephalus aceratus	—	3.1/2.0^1	119^2	18	6.60	6.01^4	1.80^4	1.2	(d)
Red-blooded Antarctic fish									
Notothenia gibberifrons	0.061	—	—	16	—	—	—	0.5	(c)
Notothenia neglecta	0.100	—	—	15	—	—	—	0.5	(c)
Parachaenichthys charcoti	0.082	—	—	10.5	—	—	—	0.5	(c)
Temperate fish									
Hemitripterus americanus	0.080	4.7	13.4	39.4	0.34	1.76	1.16	10	(e)
Ophiodon elongatus	0.080	5.1	11.2	29.4	0.38	2.41	1.18	10	(e)
Gadus morhua	0.08	5.0	29.1	41.0	0.71	3.89	2.66	10	(e)
Anguilla australis	—	6.0	11.4	51.0	0.22	—	—	17	(f)

[a] Hemmingsen & Douglas (1977); [b] Hemmingsen & Douglas (1972); [c] Holeton (1970); [d] Hemmingsen *et al.* (1972); [e]Farrell (1984); [f] Hughes *et al.* (1982).

[1] Maximum/minimum; [2] determined by Fick method; [3] determined by direct measurement; [4] estimated from tabulated values from SV × (P_{va}-preload) and CO × (P_{va}-preload) for SW and PO, respectively; preload is assumed to be zero where preload is unknown.

between 0.3 and 0.7 kPa. No correlation with the afterload was found, although it increased significantly from 0.45 ± 0.02 kPa to 0.53 ± 0.02 kPa when the preload was increased from -0.07 to -0.05 kPa. Further increase in preload did not significantly affect the bulbus pulse pressure. The data reported in table 1 show that the *in vivo* values of pulse pressure obtained in *C. aceratus* by several authors is about 1 kPa, while a value similar to those *in vitro* reported here has been recorded in *Pseudochaenichthys georgianus* by Hemmingsen & Douglas (1977).

The isolated heart of *C. hamatus* is able to maintain constant stroke work and power output only within a relatively narrow range of afterloads (figure 2). This range includes the physiological values of ventral aorta pressure found in icefish (from 1.8 to 2.7 kPa, table 1). The mean output pressure of 3 kPa seems to be a very critical upper limit, above which the work and power production of the heart is quickly impaired. This is particularly evident at the lowest preload.

Because of the limited number of animals available, it was not possible to obtain significant *in vivo* determinations of preload. As the caudal vein pressure in other icefish is considered to be practically zero (Hemmingsen *et al.* 1972; Hemmingsen & Douglas 1972; Hemmingsen & Douglas 1977), we have supposed that the preload in icefish is sub-ambient as in other teleosts (Farrell *et al.* 1988). In the isolated heart of *C. hamatus*, stroke volume and stroke work values comparable with those reported for other icefish (4–7 ml kg^{-1} and 3.5–6 mJ g^{-1}, respectively; table 1) have been obtained by using a preload of -0.09 kPa and afterloads of 2.6–3.0 kPa.

(*b*) *Temperature effects on heart performance*

Increasing the temperature from 0.6 to 5.8 °C causes a significant increase in heart rate (21%), cardiac output (18%) and power output (24%). These increases are reversed by returning the temperature to basal values (figure 3). In contrast, the effects on stroke volume and stroke work were not significant.

During the temperature increase there is a temporary decrease in heart performance. This effect is shown in figure 4, and is related to the initial rate of temperature increase. Specifically, it appears that the stroke volume is highly dependent on the rate of temperature change. Decreases of up to 70% have

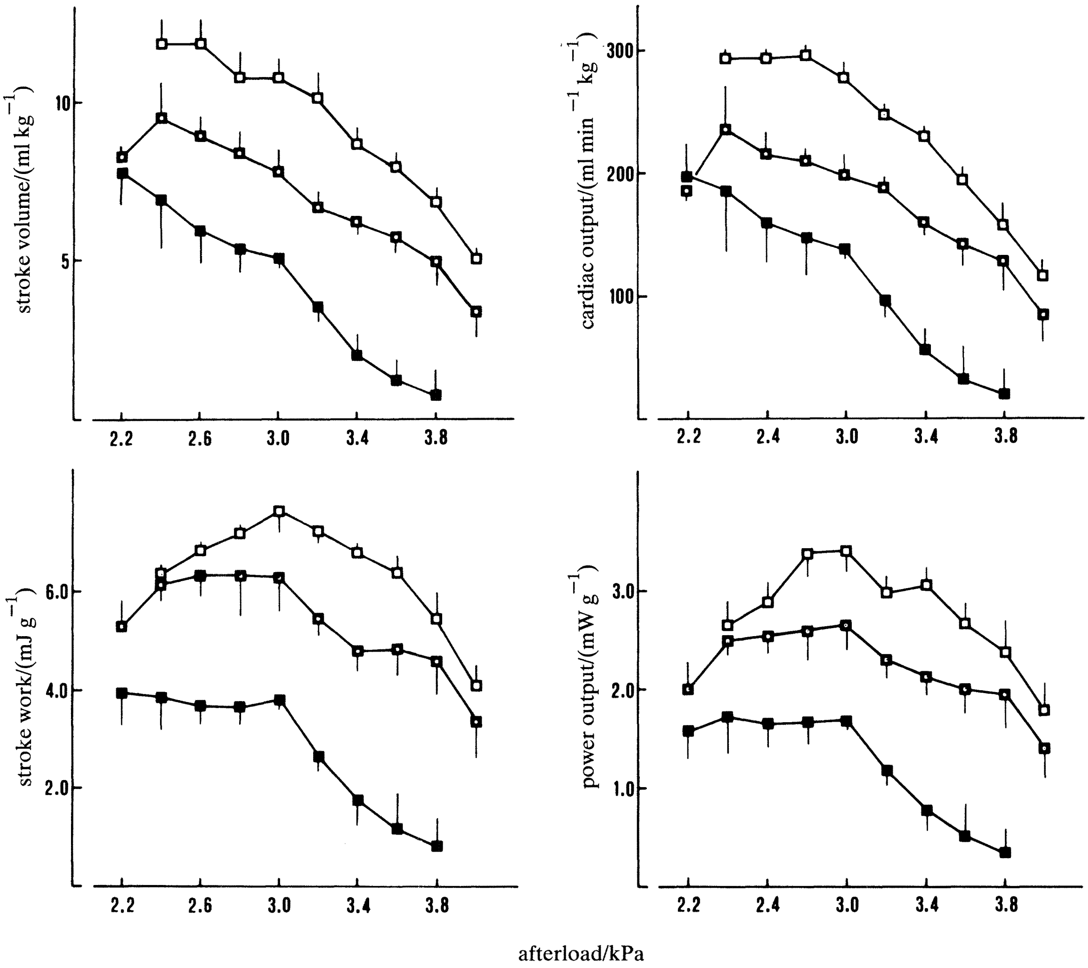

Figure 2. Relationship between stroke volume, cardiac output, stroke work and power output and afterload at the following values of preload (mean value ± s.e. for each input level): ■, −0.068 ± 0.006 kPa (input level 0.0 cm); ▣, −0.054 ± 0.012 kPa (input level 0.5 cm); □, −0.043 ± 0.002 kPa (input level 1.0 cm). Each data is the mean of four determinations ± s.e. All the experiments were performed at 3 °C. The heart rate was independent from the afterload. At each preload the mean heart rate was: ■, 26.90 ± 0.71 beats min^{-1} ($n = 32$); ▣, 25.10 ± 1.17 beats min^{-1} ($n = 40$); □, 26.80 ± 1.30 beats min^{-1} ($n = 29$). No significant differences were found between these values. The mean mass of animals was 409 ± 24.4 g ($n = 4$).

been observed. The effect on the stroke volume is reflected in cardiac output, stroke work and power output. In contrast, heart rate is hardly affected.

When the temperature of the perfusate was increased above 10 °C the hearts became arhythmic and the general performance was impaired.

4. DISCUSSION

(*a*) *The mechanical properties of the heart of* C. hamatus

The present study on the perfused working heart represents the first attempt to analyse the intrinsic mechanical characteristics of cardiac performance in an icefish. The important functional parameters of the curves generated in figure 2 are stroke volume and stroke work used as measures of systolic function, and power output used as a measure of the heart's ability to generate sufficient pressure to overcome vascular resistance and to supply the tissues with sufficient blood flow (cardiac output) to meet their metabolic requirements. The *in vitro* values of these parameters found in *C. hamatus* compare well with the *in vivo* values found in other icefish and reported in table 1.

Two conclusions arise from the data shown in figure 2. First, the heart of *C. hamatus* is able to achieve remarkable increases in stroke volume, and hence stroke work and cardiac output, in response to small changes within the physiological range of preloads (corresponding to end diastolic volumes). In common with other teleosts (see Farrell 1984; Houlihan *et al.* 1988), the heart of *C. hamatus* also displays the Frank–Starling mechanism, and it does so without significant changes in heart rate with preload and afterload. Secondly, and more specifically, this heart is unable to maintain a constant systolic function over a range of afterloads higher than 3 kPa; this value corresponds to the upper limit of the blood pressure values found in the ventral aorta by Holeton (1970) and Hemmingsen *et al.* (1972) on unrestrained specimens of *C. aceratus*. This behaviour contrasts with that of heart preparations from temperate teleosts, which are able to maintain their stroke volume over a wider range of afterloads (up to 5 kPa (Farrell 1984)). Clearly, this intrinsic response to afterload, i.e. to

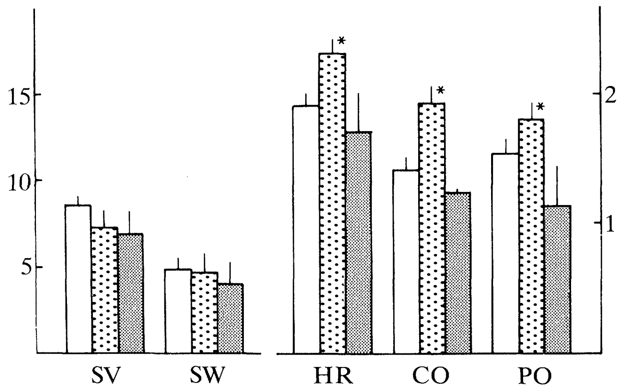

Figure 3. Effect of perfusion temperature on the performance of the isolated heart of *C. hamatus*. Temperatures: □, 0.6 ± 0.2; ▨, 5.8 ± 0.7; ▩, 0.8 ± 0.4 °C. The data are the mean of nine experiments ± s.e. The mean preload was -0.033 ± 0.006 kPa; the mean afterload was 2.29 ± 0.04 kPa. Units: stroke volume (SV) = ml kg^{-1}; stroke work (SW) = mJ g^{-1}; heart rate (HR) = × 10 beats min^{-1}; cardiac output (CO) = $\times 10^2$ ml min^{-1} kg^{-1}; power output (PO) = mW g^{-1}. Asterisks indicate differences statistically significant between 0.6 and 5.8 °C ($p < 0.01$). When this happens, the return to low temperature also displays a significant change ($p < 0.01$). In this figure and in Figure 4, abbreviations are as in table 1. The mean mass of animals was 369.1 ± 18.0 g ($n = 9$).

ventral aorta pressure, must set a critical limit in the cardiac homeostasis of the icefish, because it appears that normal myocardial function is impossible unless the blood pressure in the ventral aorta is kept below values of about 3 kPa. And indeed, as previously suggested by Holeton (1970) concerning *C. aceratus*, the morphological features of the icefish vascular tree seem well suited to maintain a low resistance: both the bulbus and the ventral aorta are very large, and gill blood vessels have large diameters compared with other teleosts (see Steen & Berg 1966; Vogel & Koch 1981).

Thus the icefish heart is a very specialized high volume pump, able to displace relatively high volumes of blood at high flow rates and low pressures. This ability results not only from the characteristics of the circulatory tree which establish the load on the heart, but also from the intrinsic contractile behaviour of the cardiac pump itself.

The mechanical behaviour of the heart of *C. hamatus*, to some extent, may be attributed to the architectural constraints of the ventricular chamber of the icefish. Table 1 shows that icefishes exhibit higher relative heart weights than other marine fishes, including notothenioids with haemoglobin. Our data show that the heart of *C. hamatus* is as large as that of *C. aceratus* (Johnston *et al.* 1983) and *C. rhinoceratus* (Feller *et al.* 1985). The histological arrangement of the ventricular wall of *C. hamatus* is very similar to that described previously in *C. aceratus* (Tota *et al.* 1988); it consists of a spongy trabecular myocardium with a very extensive and diffuse network of lacunae (unpublished observations). As pointed out by Tota *et al.* (1988) and Harrison *et al.* (1991), the cardiomegaly of the spongy type of ventricular musculature, by subdividing the ventricular wall into a multichambered trabeculum, is

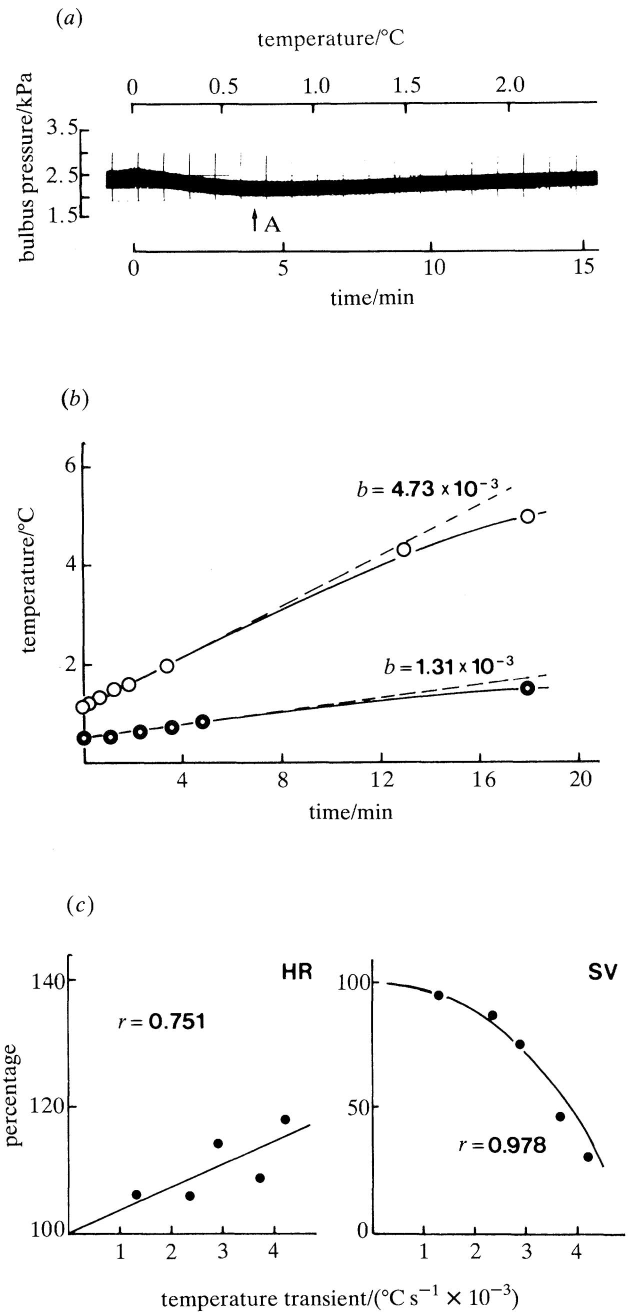

Figure 4. (*a*) Transitory effect of the increase of temperature on the pressure generated by the isolated working heart of *C. hamatus*. (*b*) The maximum and minimum rates of experimentally induced temperature changes used in these experiments (solid lines). The extent of the effect shown in (*a*) was found to be related to the initial rate of change in temperature (the temperature transient through time 0, dashed lines). (*c*) The influence of the initial temperature transients on heart rate (HR) and stroke volume (SV). Both parameters were measured at time points at which the apparent effects were maximal (e.g. point A in (*a*)). They are represented as a percentage of control values obtained before the temperature changes. Mean control temperature: 0.6 ± 0.2 °C. Mean control values of cardiac parameters: HR, 19.0 ± 1.6 beats min^{-1}; SV, 8.23 ± 1.06 ml kg^{-1}. r, correlation coefficient for the equation: $\ln Y\,(\%) = a + b \ln X$ (transient). The reported value of r for SV was statistically significant ($p < 0.01$). Other parameters (CO, SW and PO) followed the behaviour of SV, and their variations were statistically significant ($p < 0.01$). The mean mass of animals was 357 ± 23.6 g ($n = 5$).

suitable for the displacement of large volumes of blood with relatively low tension (stress) in the myocardial trabeculae. The myocytes of icefish have remarkably few myofibrils (Johnston *et al.* 1983), although the interstitium is very rich in microfibrils, tubular structures that may increase the elastic modulus of the cardiac wall (Tota *et al.* 1988). By virtue of these features, the ventricle displays both a cavity with a relatively larger surface area for diffusion with the luminal blood and a wall with a higher adaptability and distensibility (compliance) than a chamber with greater wall muscularity (increased thickness), as in the case of a high pressure pump like the pyramidal type of heart ventricle (scombroids, salmonoids, etc., which possess an outer layer of compact myocardium (Tota 1978, 1989; Santer *et al.* 1983)). By the same token, it is evident that a volume pump design has its disadvantages, in that it is unable to compensate for increased afterloads, as shown by the data in figure 2.

Recent studies have shown the importance of the pericardium on heart performance in teleosts. The subambient intrapericardial pressure generated during ventricular contraction is the basis for a *vis a fronte* filling of the heart (Farrell *et al.* 1988). The type of perfusion chamber used in the present work seems suitable for atrial filling with a suction mechanism similar to the *vis a fronte* mechanism operating in the intact fish, allowing the heart to work at physiological levels with subambient preloads (see figure 2 legend). Another advantage of this preparation concerns the functioning of the bulbus. This extremely distensible structure (Licht & Harris 1973) lies within the pericardial cavity, and its *in vivo* distensibility is influenced by the intrapericardial pressure and by the rigidity of the parietal layer of the pericardium. In this respect, the use of a perfusion chamber with an unsecured lid allows the very large and distensible bulbus of icefish to function as *in vivo*. This is confirmed by the values of the bulbus pulse pressure which are similar to the *in vivo* values reported for other icefish (see table 1).

(*b*) *Temperature effects on the heart performance*

The effects of temperature on heart rate and stroke volume in teleosts consist of positive chronotropic and inotropic responses that probably represent a compensatory mechanism by which cardiac output is intrinsically increased to meet the metabolic needs of the tissues, which are increased by temperature (Farrell 1984). Accordingly, Hemmingsen & Douglas (1972) found that in the icefish *C. aceratus* the mean resting heart rate for four specimens at 2 °C was 17.5 beats min^{-1}, and this almost doubled when one animal was exposed to 10 °C. Our data on the cardiac response to temperature of *C. hamatus* are in agreement with these findings and indicate that the response is basically independent from extrinsic nervous control. Moreover, the results reported in figure 4 are somewhat unusual, in that the increased cardiac output in *C. hamatus* at the highest temperature is attained only by increasing heart rate, whilst the positive inotropic response is absent. Important neuro-humoral modulations, including the role of catecholamines and the occurrence and availability of α- and β-adrenoceptors, are implicated in both acute and long-term adaptations to temperature of the teleost hearts (Laurent *et al.* 1983; Ask 1983; Farrell 1984, 1985). Nothing is known about the innervation and occurrence of adrenergic receptors in the icefish heart.

The deterioration of cardiac performance evident at temperatures above 10 °C, mirrors the narrow limits that separate the lower and upper incipient lethal temperature in the icefish (Macdonald *et al.* 1987).

Rapid temperature changes are of interest in the vertebrate myocardium (Langer & Brady 1968; Reiser & Lindley 1990). Figure 4 serves to emphasize that in the icefish there is, in relation to temperature transients, a striking divergence between the increased heart rate and the decreased stroke volume. It is likely that this divergence mirrors different physical (pacemaker activity) and chemomechanical processes (force generation). Moreover, the energy cost of cardiac output in *C. aceratus* represents a major fraction, nearly 30%, of the total energy production (Hemmingsen *et al.* 1972); at the same time the 'pale' myocardium of the icefish is characterized by a remarkably limited oxygen transport capacity both outside the myocytes (owing to the lack of haemoglobin) and inside (owing to the low endowment of myoglobin; Douglas *et al.* 1985; Johnston & Harrison 1987). The behaviour of stroke volume seen in figure 4 may reflect, to a certain extent, the difficulty of the icefish heart in instantaneously matching its dynamic performance with the changing oxygen demands of its metabolic machinery.

(*c*) *Conclusions*

This study shows that in the icefish many of the cardiocirculatory adaptations that make life without haemoglobin feasible are related to the intrinsic characteristics of the heart which functions as a highly specialized volume pump. In turn, this functional design depends on the opportunities offered by the spongy type of ventricular myoarchitecture that allows an increase in the whole ventricular cavity without dilation and at the same time an increase in cardiac mass without hypertrophy and hence reduced wall distensibility.

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