

## BLOOD PRESSURE CONTROL IN A LARVAL AMPHIBIAN, *XENOPUS LAEVIS*

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### Summary

The regulation of arterial pressure in early vertebrate embryos and larvae with non-innervated hearts is poorly understood. We used nanoliter intravascular injections in anaesthetized *Xenopus laevis* larvae (stage 49–51) to assess their ability to maintain arterial pressure in the face of a volume load. Injections of saline and hetastarch (a volume expander) were made into the ventricle. Arterial pressure, end-diastolic ventricular volume, end-systolic ventricular volume and heart rate were measured. Injection of 800 nl caused a rapid rise in arterial pressure and stroke volume. There were no changes in heart rate, indicating the absence

of an arterial baroreflex. Blood pressure in saline-injected animals recovered quickly (within 5 min), whereas hetastarch injections caused hypertension to be maintained for much longer, for over 40 min in the most extreme case. We surmise that Starling forces at the capillary play an important role in pressure regulation but are not adequate to explain the entire response. Finally, there was ample evidence for a Frank–Starling relationship in the ventricle.

Key words: *Xenopus laevis*, ontogeny, blood pressure, Frank–Starling.

### Introduction

The vertebrate heart begins to pump blood very early in development, typically long before cardiac innervation occurs. Despite the lack of the adult baroreceptor-mediated control system, blood pressure at any point during development is quite predictable, suggesting that some form of control or regulation exists (see, for example, Clark and Hu, 1982; Pelster and Burggren, 1991). Several non-neural antihypertensive control mechanisms can be imagined, including reduction of vascular volume *via* Starling transcapillary forces resulting in Frank–Starling-mediated reductions in stroke volume and cardiac output. A second mechanism might be the release of local vascular mediators in response to increased shear stress or flow velocity, with a resultant vasodilation (decreased peripheral resistance) or venodilation (decreased preload), both ultimately resulting in decreased arterial pressure.

Previous studies on the regulation of blood pressure in early embryos have been limited because of the difficulty of maintaining blood pressure recordings for useful periods. Blood pressure recordings in early embryos are accomplished using micro servo-null techniques, which are very sensitive to the location of the recording electrode. Experiments on chicken embryos are hampered by the tendency of the embryo to drift slowly in the allantoic fluid, causing the signal to be lost. To study blood pressure regulation in response to an imposed volume load, we studied *Xenopus laevis* larvae to capitalize on our ability to maintain pressure recordings for over 40 min. Specifically, we wished to investigate the ability of stage 49–51 larvae to correct a volume-load-induced hypertension.

### Materials and methods

#### *Experimental animals*

*Xenopus laevis* Daudin adults were bred in the laboratory, and the larvae were reared at 20 °C and fed daily with commercial *Xenopus* meal (Blades Biological, Kent, UK). All studies were performed on Nieuwkoop–Faber stages 49–51 larvae (Nieuwkoop and Faber, 1994).

#### *Surgery and experimental apparatus*

Prior to surgery, larvae were immersed in 0.05–0.1% tricaine methanesulphonate (MS-222; Sigma) buffered to pH 7.4 with 1 mol l<sup>-1</sup> NaOH. We removed the larvae from the anaesthetic solution when they lost the righting reflex and placed them ventral side up in a clear glass dish with a transparent silastic bottom layer. Amphibian Ringer's solution at 20 °C equilibrated with 100% oxygen and containing 0.05–0.1% MS-222 was continuously flowed over the larvae for the duration of the experiment. Each larva was held in place using a 'cage' of insect pins, and the thoracic cavity was surgically opened in a caudal–cranial direction. The pericardium was similarly opened. This open thoracic preparation is the reason that the animal is maintained under Ringer's solution rather than under tapwater.

Blood pressure was measured using an IPM (Instrumentation for Physiology and Medicine) micro servo-null pressure system and techniques described in earlier papers (see, for example, Fritsche and Burggren, 1996). Briefly, the servo-null pipette was zeroed in the open body cavity at the level of the truncus arteriosus and then inserted into the truncus arteriosus. Appropriate pressures and the characteristic

Table 1. *Haemodynamic data from Xenopus laevis indicating the control value (preinjection), the value at the time of maximal change from the control value and the value of maximum change (%) from the control values*

Variable	Group	Control value	Maximum value	% Control	Time (min)
$P_{MA}$ (kPa)	Saline	1.01±0.08	1.07±0.06	105.9±3.1	2
	Hetastarch	0.94±0.04	1.09±0.05	116.0±3.0	2
$f_H$ (beats $\text{min}^{-1}$ )	Saline	120.1±3.4	119.7±3.2	99.7±0.4	4
	Hetastarch	131.7±2.2	129.5±1.8	98.3±1.0	1
$V_{EDV}$ ( $\mu\text{l}$ )	Saline	1.76±0.17	1.97±0.14	111.9±3.9	1
	Hetastarch	1.86±0.36	2.27±0.38	122.0±6.5	2
$V_{ESV}$ ( $\mu\text{l}$ )	Saline	0.65±0.06	0.72±0.06	110.8±4.0	2
	Hetastarch	0.81±0.14	0.95±0.16	117.3±4.0	2
$V_S$ ( $\mu\text{l}$ )	Saline	1.13±0.11	1.28±0.11	115.3±4.4	1
	Hetastarch	1.05±0.22	1.34±0.23	127.7±9.6	4
$\dot{Q}_{tot}$ ( $\mu\text{l min}^{-1}$ )	Saline	133.2±13.9	150.1±11.3	112.7±4.1	1
	Hetastarch	136.9±28.5	174.9±29.5	127.8±9.2	4
$R_{TP}$ (kPa $\text{ml}^{-1} \text{min}^{-1}$ )	Saline	13.5±1.3	12.3±0.9	91.1±3.0	1
	Hetastarch	17.0±3.7	13.4±2.9	78.8±3.7	4

Values are mean  $\pm$  S.E.M.

All variables changed significantly with time ( $P<0.05$ ) with the exception of heart rate (see Results).

$P_{MA}$ , mean arterial pressure;  $f_H$ , heart rate;  $V_{EDV}$ , end-diastolic ventricular volume;  $V_{ESV}$ , end-systolic ventricular volume;  $V_S$ , stroke volume;  $\dot{Q}_{tot}$ , total cardiac output;  $R_{TP}$ , total peripheral resistance.

diastolic curve verified successful insertion of the pressure pipette. Blood pressure data were continuously recorded on chartpaper, and mean values over periods of 30 s were recorded on computer. Heart rate was similarly recorded from the output of an electronic tachograph (Grass model 7D) triggered from the pressure signal. Stroke volume was determined by video image analysis. Briefly, video images at end-diastole and end-systole were captured, and ventricular volumes were calculated assuming a prolate spheroid ventricle (Hou and Burggren, 1995; Fritsche and Burggren, 1996). Thus, volume estimates include myocardial volume as well as chamber volume. Assuming that myocardial volume remains constant, differences in end-diastolic ventricular volumes ( $V_{EDV}$ ) and end-systolic ventricular volumes ( $V_{ESV}$ ) must reflect changes in chamber volume or stroke volume. Importantly, end-diastolic and end-systolic volumes as reported here must include myocardial volumes and are therefore reported as cardiac volumes. Volume loading was achieved using a nanoliter injector (WPI model Micro-1) fitted with a borosilicate glass micropipette to inject 800 nl of fluid directly into the ventricle.

#### Experimental protocol

Preliminary experiments with dyed injectates confirmed that successful injection of 800 nl caused an immediate increase in truncus pressure, and thereafter this increase was used to confirm successful injection. Injection occurred at a nominal rate of  $10 \text{ nl s}^{-1}$ . Following the injection, blood pressure was monitored for 20 min or until it returned to control values. Animals were then killed and weighed to the nearest 0.1 mg.

Larvae were arbitrarily assigned to one of two injectate groups: 0.9% NaCl ( $N=9$ ; mean body mass 124.6 mg) or 6%

hetastarch in 0.9% NaCl ( $N=8$ ; mean body mass 142.8 mg) (means  $\pm$  S.E.M.). Hetastarch (Sigma H2648) is a plant-derived crystalloid volume expander with a longer retention time in circulatory systems than albumin and was used to assess the importance of transcapillary Starling forces.

#### Data analysis and statistical analyses

Data were analyzed at 1 and 2 min prior to injection and at 0.5, 1, 2, 4, 6, 10 and 15 min after completion of the injection. Statistical evaluation of time course data was *via* repeated-measures analysis of variance (ANOVA) using simple contrasts of each time point against the initial time point (SPSS 7.5, SPSS Inc.). Only data for the first 10 min post-injection were used in this analysis. Analysis of Frank–Starling relationship was *via* linear regression (SigmaStat 1.0, Jandel Scientific). In all cases, a fiducial value of 5% was chosen for statistical significance. Summarized data are presented as mean  $\pm$  S.E.M. Data in graphs are presented as a percentage of the mean control (i.e. preinjection) values. Total peripheral resistance was calculated assuming that central venous pressure was 0 kPa.

#### Results

The data are summarized in Table 1. Injection of 800 nl caused an immediate increase in systolic blood pressure, indicating successful injection into the vascular space (Fig. 1). Infusions of both saline and hetastarch resulted in significant increases in blood pressure ( $P<0.001$ ; Fig. 2A). For no variables were the results obtained from saline and hetastarch infusion significantly different from each other. Infusion of saline caused a 6% increase in mean arterial pressure from

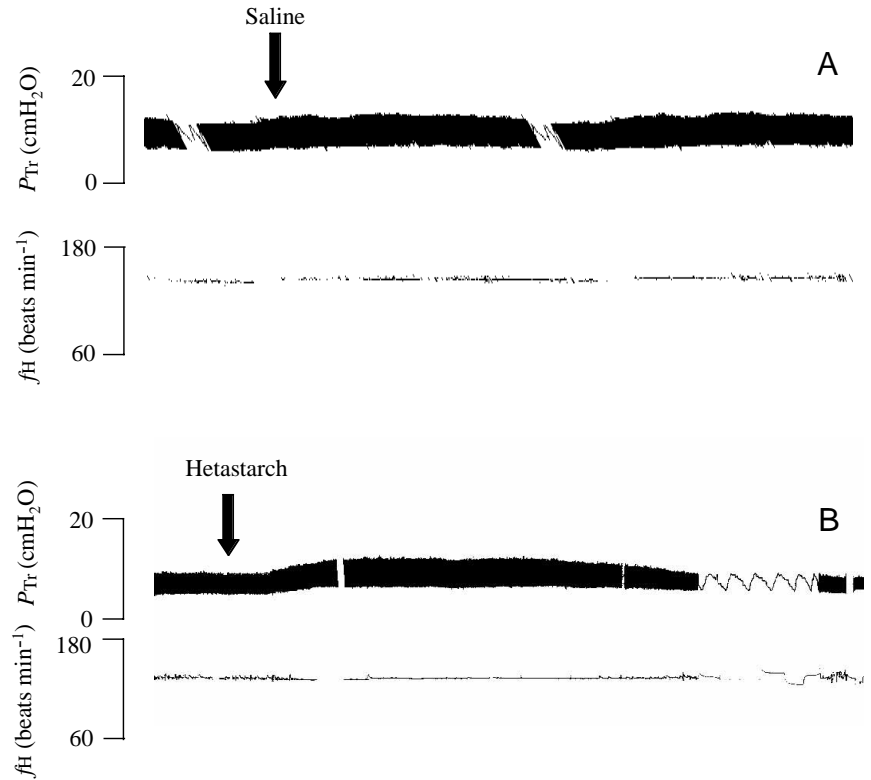


Fig. 1. Representative trace recordings of truncus pressure ( $P_{Tr}$ ) and heart rate ( $f_H$ ) from Nieuwkoop–Faber stage 50 *Xenopus laevis* larvae in response to (A) saline injection and (B) hetastarch injection. The arrow indicates the start of injection. 1 cmH<sub>2</sub>O=98.1 Pa.

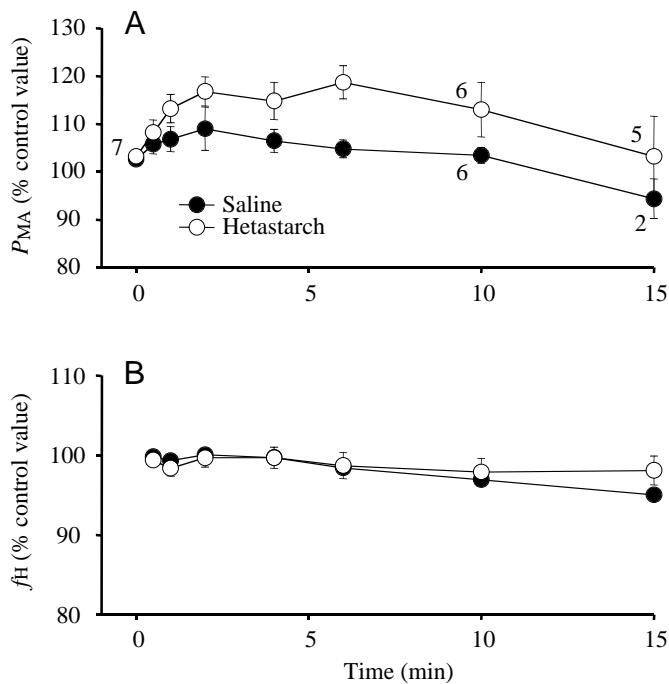


Fig. 2. (A) Mean arterial pressure ( $P_{MA}$ ) expressed as a percentage of preinjection (control) values. (B) Heart rate ( $f_H$ ) expressed as a percentage of preinjection values. Values are expressed as mean  $\pm$  s.e.m. Filled circles, saline-injected animals; open circles, hetastarch-injected animals. The number of animals is indicated (in A) when it is less than the number indicated in the Materials and methods section.

1.01 $\pm$ 0.08 to 1.07 $\pm$ 0.06 kPa, while infusion of hetastarch caused a 15% increase from 0.94 $\pm$ 0.04 to 1.08 $\pm$ 0.05 kPa. There were no significant changes in heart rate ( $f_H$ ) at any time ( $P=0.142$ ; Fig. 2B).

In both groups, injection resulted in significantly increased  $V_{EDV}$  ( $P<0.001$ ; Fig. 3A). Saline injection increased  $V_{EDV}$  by 12% from 1.76 $\pm$ 0.17  $\mu$ l to a maximum of 1.97 $\pm$ 0.14  $\mu$ l. Hetastarch increased  $V_{EDV}$  by 22% from 1.86 $\pm$ 0.36 to 2.27 $\pm$ 0.38  $\mu$ l. End-systolic cardiac volume ( $V_{ESV}$ ) also increased significantly with injection ( $P<0.001$ ; Fig. 3B). Saline injection increased  $V_{ESV}$  by 11% from 0.65 $\pm$ 0.06 to 0.72 $\pm$ 0.06  $\mu$ l. Hetastarch injection increased  $V_{ESV}$  by 17% from 0.81 $\pm$ 0.14 to 0.95 $\pm$ 0.16  $\mu$ l. Stroke volume ( $V_S$ ) also increased ( $P<0.001$ ; Fig. 3C). The maximum increase in hetastarch-injected animals was at 4 min post-injection, rising by 28% from 1.05 $\pm$ 0.22 to 1.34 $\pm$ 0.23  $\mu$ l, while the increase in saline-injected animals was maximal at 1 min, rising by 13% from 1.13 $\pm$ 0.11 to 1.28 $\pm$ 0.11  $\mu$ l. Predictably, given the constant  $f_H$ , total cardiac output ( $\dot{Q}_{tot}$ ) mirrored the  $V_S$  results ( $P<0.001$ ; Fig. 4A). Finally, total peripheral resistance ( $R_{TP}$ ) declined significantly ( $P=0.01$ ; Fig. 4B). In saline-injected animals,  $R_{TP}$  fell from 13.5 $\pm$ 1.3 kPa ml<sup>-1</sup> min<sup>-1</sup> to a minimum of 12.3 $\pm$ 0.9 kPa ml<sup>-1</sup> min<sup>-1</sup> at 1 min post-injection, and in hetastarch-injected animals,  $R_{TP}$  fell from 17.0 $\pm$ 3.7 to 13.36 $\pm$ 2.9 kPa ml<sup>-1</sup> min<sup>-1</sup> at 4 min post-injection.

A positive linear correlation was found between  $V_{EDV}$  and stroke volume ( $r^2=0.821$ ;  $P<0.0001$ ), indicating a functional Frank–Starling relationship in these larvae. The relationship was described by  $V_S=0.236V_{EDV}-0.484$  (Fig. 5).

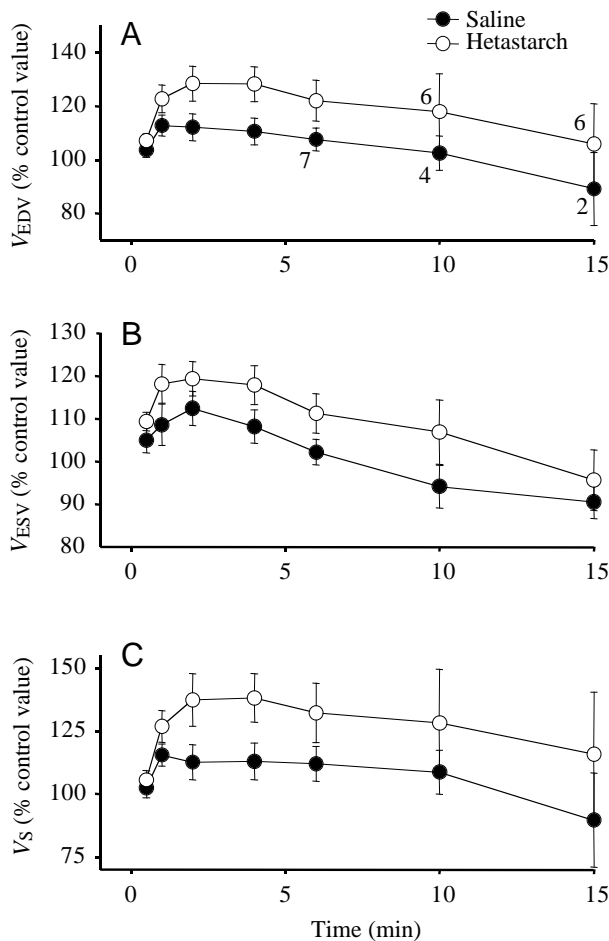


Fig. 3. (A) End-diastolic ventricular volume ( $V_{EDV}$ ) expressed as a percentage of preinjection (control) values. (B) End-systolic ventricular volume ( $V_{ESV}$ ) expressed as a percentage of preinjection values. (C) Stroke volume ( $V_s$ ) expressed as a percentage of preinjection values. Values are expressed as mean  $\pm$  S.E.M. Filled circles, saline-injected animals; open circles, hetastarch-injected animals. The number of animals is indicated (in A) when it is less than the number indicated in the Materials and methods section.

### Discussion

Our ability to manipulate arterial pressure in stage 49–51 *Xenopus laevis* larvae coupled with blood pressure recordings lasting up to 40 min allows us to follow barostatic responses in animals lacking a classical baroreflex, i.e. baroreceptor-activated alterations in heart rate mediated by the central nervous system. It is not known whether these animals possess functional baroreceptors at this stage of development, but there is ample evidence that some portion of the negative feedback system is lacking. Adrenaline-induced increases in blood pressure do not trigger bradycardia at these stages (Fritsche and Burggren, 1996; Fritsche, 1997). In the present study, we never witnessed changes in heart rate, regardless of the degree of hypertension, indicating that neither a baroreflex nor the Bainbridge reflex exists at these developmental stages. This lack of heart rate response may seem surprising given the potential presence of stretch-sensitive channels in the

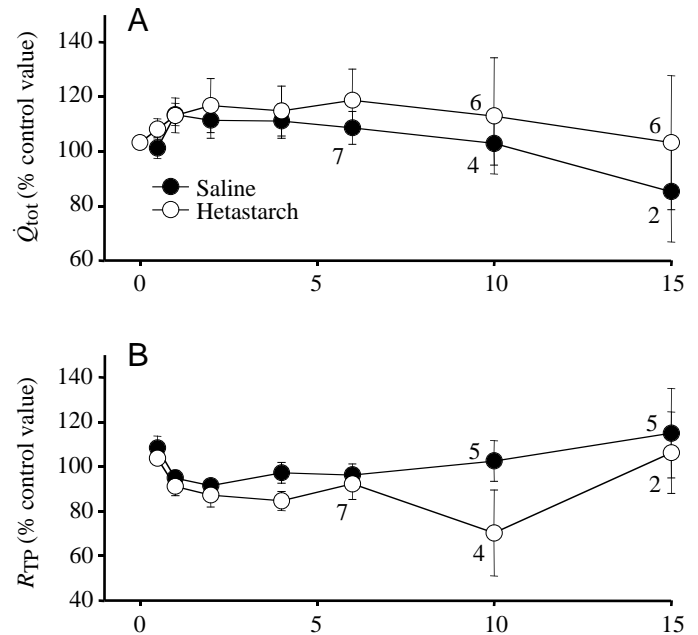


Fig. 4. (A) Cardiac output ( $\dot{Q}_{tot}$ ) expressed as a percentage of preinjection (control) values. (B) Total peripheral resistance ( $R_{TP}$ ) expressed as a percentage of preinjection values. Values are expressed as mean  $\pm$  S.E.M. Filled circles, saline-injected animals; open circles, hetastarch-injected animals. The number of animals is indicated when it is less than the number indicated in the Materials and methods section.

pacemaker cells (Lab, 1982; Kohl et al., 1992; Hu and Sachs, 1996). This same issue also raises questions about the appropriateness of the ventricle as the injection site. However, acute responses would not be expected because the volume load occurred over 80 s and, thus, with stable heart rates of approximately  $120 \text{ beats min}^{-1}$ , our injection spanned 160 beats. With stroke volumes of greater than  $1 \mu\text{l}$  and an injection volume of 800 nl, this means an approximately 0.5% increase in stroke volume with each beat. Stretch-sensitive channels in adult frog hearts have a threshold of approximately 15% length increase (Fasciano and Tung, 1999), suggesting that the 0.5% increase induced by our volume load is not likely to have a large effect on these channels. In addition, stretch-sensitive channels in frog ventricular cells show a diminished sensitivity with frequency of contraction (Fasciano and Tung, 1999), and the high heart rates of embryos relative to adult rates suggest a diminished importance of these channels. It is unclear, however, whether larval channel populations differ from adult channel populations. With our current data, it is not possible to rule out the possibility that exactly equal and opposite tachycardic and bradycardic reflexes with equal gain and equal time constants were invoked, although we consider this unlikely.

Despite the stable heart rate, all animals were capable of attenuating the initial hypertension, although not all were successful in restoring blood pressure to preinfusion levels. The degree of perturbation in this study was sufficient to obtain significant increases in blood pressure. The hypertension was

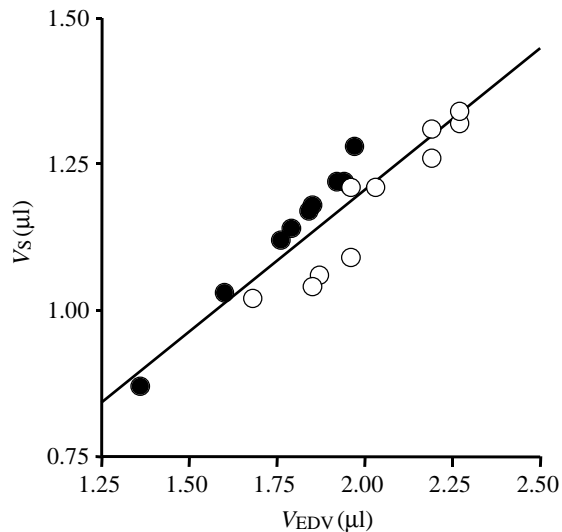


Fig. 5. Frank–Starling relationship of larval *Xenopus laevis* hearts. Each symbol represents an individual animal either before or after intraventricular injection of 800 nl. There was a strong linear relationship ( $r^2=0.821$ ;  $P<0.0001$ ) between end-diastolic ventricular volume ( $V_{EDV}$ ) and stroke volume ( $V_S$ ):  $V_S=0.236 V_{EDV}-0.484$ . Filled circles, saline-injected animals; open circles, hetastarch-injected animals.

a result of increased flow induced by increased preload. The direct measurement of arterial pressure together with evidence of increased central venous pressure, as suggested by increased  $V_{EDV}$ , indicates an increase in mean circulatory filling pressure. Reliable estimates of blood volume in larval *Xenopus laevis* are lacking, but blood volume estimates for adult amphibians range from 3.5 to 9.5 ml 100 g<sup>-1</sup> body mass (Prosser and Weinstein, 1950; Carmena-Suero et al., 1980). Extrapolation of these values to these larvae indicates that our injections increased blood volume by between 5.5 and 14%.

Blood pressure recovery can be accomplished in the absence of cardiac control either by reduced preload or by reduced peripheral resistance. Both mechanisms are presumably available to these larvae. Reductions in preload can be accomplished by several mechanisms, including a dilation of the volume reservoir, a reduction in vascular volume or an increase in resistance to venous return. In adult animals, it is thought that the muscular arteries normally serve as pressure reservoirs, while the more flaccid veins serve as volume reservoirs; however, this assumption remains unproven in embryos or larvae in which blood pressures are quite low and vascular differentiation may not be dramatic. It seems entirely possible that the entire vascular system may function as a volume reservoir and thereby contribute to pressure maintenance *via* alterations in mean circulatory filling pressure and thereby preload.

Reductions in vascular volume may also occur either *via* the developing nephric system or *via* Starling exchanges in the capillaries. At the stages used in this study, both the pronephros and mesonephros are functional in ionoregulation (Nieuwkoop and Faber, 1994; Taraha et al., 1993), although their function

in volume regulation is unclear. Active volume regulation could also occur across the skin or newly forming gills, but this possibility remains highly speculative. Volume adjustment *via* fluid loss across the capillary endothelium requires no neural control and presumably operates upon initial formation of a true vascular space. However, attempts to estimate transcapillary forces are hampered by our almost complete lack of essential information. We know nothing of plasma colloid osmotic forces early in development and equally little about interstitial hydrostatic or osmotic forces. The addition of a crystalloid volume expander, hetastarch, to the injectate may have increased the magnitude and duration of the responses, although the differences in this study were not significant. However, it is possible that infusion of hetastarch expanded the vascular volume extensively beyond the 800 nl injectate volume. If this were the case, the increased duration of the response may merely be the extra time required to correct a larger initial disturbance.

A decrease in peripheral resistance was also observed. In the absence of a baroreflex, we assume that such a response is mediated by a local vasodilator. Our measurements of total peripheral resistance are based on arterial pressure and cardiac output. Changes in central venous pressure were not included because we did not determine venous pressure. However, given the increase in preload (as indicated by  $V_{EDV}$ ), we assume that central venous pressure increased. Thus, the driving pressure gradient was actually smaller than our calculations would indicate, with the error being larger after volume load. Thus, our values underestimate  $R_{TP}$ , especially during volume load. This indicates that our calculation of peripheral vasodilation underestimates the magnitude of change that occurs with volume loading. Potential mediators of the resistance change include, but are not limited to, nitric oxide, atrial natriuretic peptide and passive stretch due to increased pressure.

In summary, early *Xenopus laevis* larvae (stages 49–51) display a Frank–Starling response to volume load and, despite the lack of a baroreflex, are able to correct the hypertension induced by volume load. A prompt increase in mean arterial pressure upon injection of 800 nl, from increased flow (due to increased stroke volume), was partially ameliorated by a lesser increase in peripheral conductance. Recovery was virtually complete in most animals by 10 min post-infusion. Osmotic expanders amplify the hypertension and impair the ability of the animals to correct it.

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