Blood pressures and heart rate during larval development in the anuran amphibian *Xenopus laevis*

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Hou, Ping-Chun Lucy, and Warren W. Burggren. Blood pressures and heart rate during larval development in the anuran amphibian *Xenopus laevis*. *Am. J. Physiol.* 269 (*Regulatory Integrative Comp. Physiol.* 38): R1120–R1125, 1995.—Heart rate and blood pressure were measured in lightly anesthetized developing *Xenopus laevis* from hatching (body mass ~3 mg) to the end of metamorphosis (≤1 g). Blood pressures in the conus arteriosus, truncus arteriosus, and ventricle were measured by a servo-null micropressure system. Heart rate was determined from blood pressure recordings, and cardiac cycles were videotaped through a dissecting microscope. Heart rate varied from 50 to 150 beats/min and showed a negative correlation with body mass, with a slope less than predicted from allometric equations based on adult vertebrates. Mean truncus pressures showed a positive correlation with body mass, increasing from 4 mmHg in a 25-mg larva to 9 mmHg in a 1-g larva. The pressure waveform during ventricular systole was similar in all developmental stages examined, whereas those in conus and truncus varied with development. Conus pressures differed distinctly from truncus pressure during diastole in all larvae examined, suggesting the existence of functional valves between conus and truncus as early as stage 46 of the Nieuwkoop-Faber larval staging system. Although the developmental patterns of heart rate and blood pressure in *X. laevis* showed significant correlation with body mass, body mass explained less than one-half of the variation in these variables. Therefore developmental factors other than body mass, such as changes in heart mass and the addition of new resistance vessels, may influence heart rate and blood pressure during development in *X. laevis*.

cardiovascular function; ontogeny; amphibians; heart rate

CARDIOVASCULAR FUNCTION in embryos has been studied most extensively in the domestic fowl (8, 13). The lack of comparable physiological data from lower vertebrates prevents an understanding of general developmental processes shared by all vertebrates as well as the ability to recognize adaptive patterns that might be unique to a particular taxon. Some studies have focused on anuran amphibian larvae (see Ref. 6 for review), and occasional observations have been made on fishes such as the skate (15). These studies have provided important hemodynamic data, but few have comprehensively examined interactions of cardiovascular variables over a very broad range of development. Consequently we have investigated in this and another study (12) the hemodynamics throughout larval development in the African clawed frog *Xenopus laevis*.

The goal of this study was to quantify changes in heart rate and central arterial and ventricular blood pressures from very early developmental stages beginning with larvae weighing only a few milligrams; virtually nothing is known of heart rate or central arterial hemodynamics in such small vertebrates. Unknown effects of allometric scaling, for example, could result in hemodynamics qualitatively or quantitatively different from those of larger animals. Such basic data in unperturbed systems are a necessary prerequisite for subsequent studies examining, for example, the ontogeny of blood pressure regulation and how it responds to internal or external stressors. We began our studies by hypothesizing that *1*) heart rate in larvae would show the same allometric relationship with body mass exhibited by adult vertebrates and *2*) ventricular and central arterial pressures (which are independent of body mass in adult vertebrates) would increase in proportion with body mass during early larval development. Heart rate and hemodynamic measurements were subsequently made on larval *Xenopus* from ~3 mg to ~1 g in body mass.

MATERIALS AND METHODS

Animals. Larvae of *X. laevis* were obtained from adults bred in our laboratory or from commercial suppliers. Eggs and larvae were kept at room temperature (20–22°C) in a 14:10-h light-dark photoperiod in gently aerated water. Larvae were fed *Xenopus* chow ad libitum. Laboratory-bred larvae usually metamorphosed within 2–3 mo. The Nieuwkoop-Faber (NF) larval staging system was used (14). Data were acquired from a total of 65 animals ranging from NF stage 41 (~3 mg, newly hatched) to stage 66 (~1 g, immediately after metamorphosis). Twenty-six animals were used for blood pressure measurements. Heart rates were measured in all 65 animals.

Blood pressure measurement. Larvae were anesthetized with 1:10,000 tricaine methanesulfonate (MS-222, 0.1 g in 1 liter of amphibian Ringer solution buffered at pH 7.4). Ringer solution was used, because blood pressure measurement exposed the heart to fluids external to the body. Larvae bathed in the anesthetic solutions maintained at 20–22°C were placed ventral side up in petri dishes, on the bottom of which was a layer of transparent silicone elastomer cast.

The heart and central vessels were exposed for blood pressure measurements with an incision in the body wall and pericardium. The exposed heart remained at all times under the anesthetic-Ringer solution in the petri dish. The animal was then secured to the bottom of the dish with a 0.2-mm-diameter pin pushed through its snout into the silicone elastomer lining the petri dish. A short length of 0.6-mm-diameter silicone rubber tubing was also pinned to the bottom, so that it ran loosely across the lower abdomen of the larva, to further secure the preparation and prevent movement. Blood pressure was measured ~60–180 min later. At the end of the measurement periods, larvae were killed, gently blotted with tissue paper, and weighed on a Mettler balance.

Blood pressure was measured by a servo-null micropressure system (model 900, World Precision Instrument, Sarasota, FL) as described previously (16). Microelectrodes with 2- to 5-μm tips were made by pulling glass capillaries (1 mm OD) with a vertical puller (model 700C, David Kopf Instrument,

Tujunga, CA). Each microelectrode was beveled and filled with 3 M NaCl solution. A micromanipulator was used to insert the microelectrode into the exposed truncus arteriosus, conus arteriosus, or ventricle and was left in place for continuous recording. Pressure recordings were then made only if the microelectrode caused no significant bleeding.

The trabeculate nature of the ventricle and the presence of valves in the conus arteriosus made continuous recording at these sites especially difficult. Microelectrodes in the ventricle or conus quickly became unstable and often became plugged. However, truncus pressure usually was stable for 30 min, with mean pressure variations $< 10\%$. Therefore most recordings were made in the truncus arteriosus. Nonetheless, in two larvae, blood pressure was recorded sequentially in the truncus, conus, and ventricle.

Zero pressure was determined before and after blood pressure recording by placement of the microelectrode in the saline pool at the same level as the measured blood vessel. Calibration was performed at the end of each recording by setting the micropressure system at 10 or 15 mmHg as appropriate. The precision of the micropressure system was previously verified by calibration against a column of saline solution.

The signals generated by the micropressure system were recorded on chart paper by a Narco MK-IV recorder (Houston, TX) and simultaneously on magnetic tapes by an instrumentation recorder (model 3964A, Hewlett-Packard). The taped pressure signal was later played back to a personal computer with a data acquisition and analysis system (DATACAN IV, Sable Systems, Salt Lake City, UT) for further analysis.

For each animal, phasic truncus pressures were analyzed from four 30-s periods (a total of 100–320 heartbeats, depending on heart rate) separated by 5-min intervals. Mean systolic (P_s), mean diastolic (P_d), and mean truncus pressures were then calculated for each animal by electronic integration and analysis of the recorded pressure signals.

Heart rate measurement. In 26 animals, heart rates were determined from analysis of blood pressure waveforms determined with a micropressure unit (see above). In 39 animals, heart rate was determined from videotape recordings of the heart (see description in Ref. 12). Unless blood pressure measurements were also being made, the beating heart of stage 41–48 larvae was observed directly through the intact transparent body wall and pericardium. Larvae older than stage 49 were prepared as described above for measurement of blood pressure.

Heart rate, determined from blood pressure measurements or videotape, was analyzed from four 30-s periods separated by 5-min intervals (the same analysis protocol used for blood pressure determinations). Mean heart rate was determined for each animal.

Measurements were made 30–180 min after the animals were placed in anesthetic. Animals were then weighed as described previously. For very small larvae (< stage 47), the pooled mass of three to five larvae was measured, and mean body mass was used as the individual mass of each of these animals.

Statistical analysis. Heart rate and blood pressure in developing *Xenopus* are expressed as a function of body mass and plotted on logarithmic scales. A linear regression line and correlation coefficient were generated by the least-squares mean method for each variable (Sigmaplot, Jandel Scientific, San Rafael, CA).

RESULTS

Relationship between body mass and developmental stage. Body mass shows a clear positive correlation with developmental stages during the growth of larval *X. laevis* (Fig. 1). Body mass of *X. laevis* increased dramatically from hatching at stage 35/36 (~ 3 mg) to metamorphic climax at stage 56–58 (~ 1 g). There were two phases in the increase of body mass during this growth period: *1*) from hatching (stage 35/36) to the beginning of feeding (stage 46), the increase in body mass was relatively slow; *2*) from stage 46 to stage 56, the increase in body mass was more rapid. During and shortly after metamorphosis, there was little change in body mass.

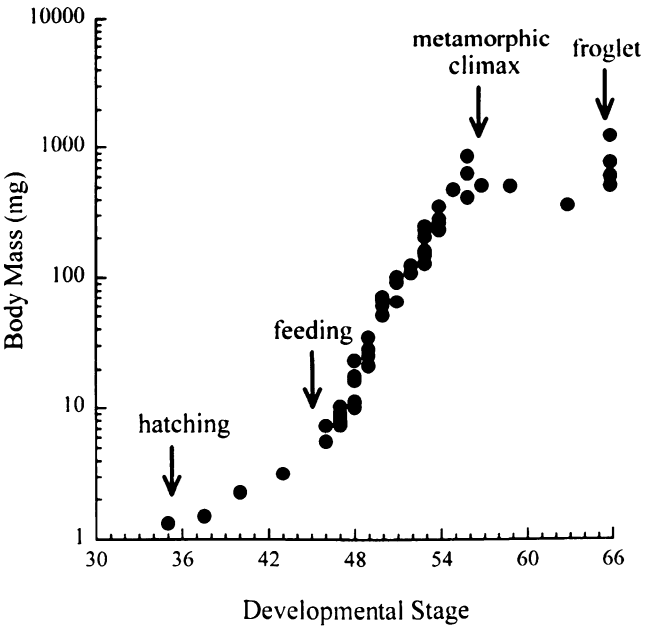

Fig. 1. Relationship between developmental stage and body mass in 65 larval and juvenile *Xenopus laevis*. Body mass is plotted on a logarithmic scale. Developmental stages are based on Nieuwkoop and Faber (14). Major developmental events are labeled.

Heart rate. When expressed as a function of body mass on logarithmic scales, heart rate in developing *X. laevis* decreased significantly ($P < 0.01$) from 117 beats/min at 3 mg to 85 beats/min at 1 g (Fig. 2). However, there were large variations in heart rate between individuals, especially in the metamorphosing larvae. In fact, changes in body mass explain only 25% of the variation in heart rates (r^2, Table 1).

Blood pressure in the truncus arteriosus. The blood pressure waveform recorded in the truncus arteriosus shows qualitative changes (shape) as well as quantitative changes (amplitude) during larval development. Considerable variations also occurred within stages (Fig. 3). Truncus pressure in many early larvae (stage 48–49) showed a biphasic waveform. Visual observation of the cardiac cycles confirmed that the first peak coincided with ventricle contraction and the second peak was due to conus contraction. In older larvae and adults, truncus arteriosus pressure showed a rapid increase to a peak value followed by a slow decline for each cycle. The slow decline was sometimes interrupted by a distinct notch.

Peak P_s and P_d in the truncus arteriosus increased significantly with increasing body mass in developing *X. laevis* (Fig. 4). Calculated P_s and P_d in the truncus from the regression lines are 6.0 and 2.6 mmHg, respectively,

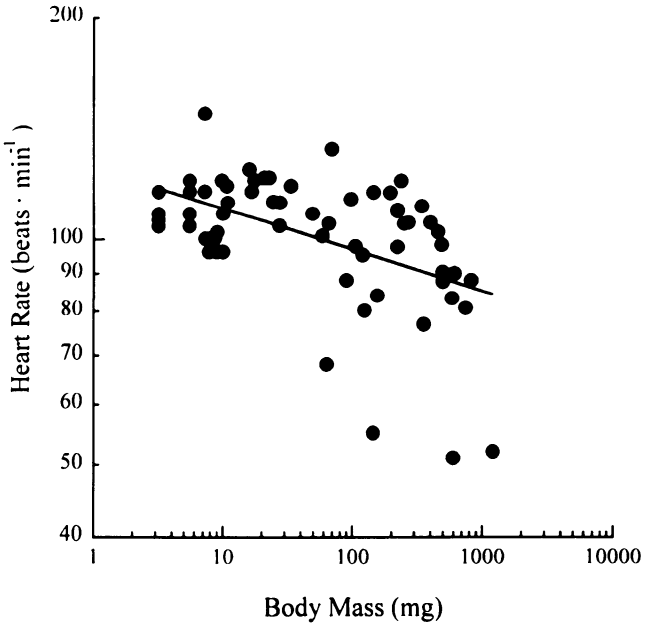

Fig. 2. Heart rate of developing *X. laevis* plotted against body mass. Regression values are given in Table 1.

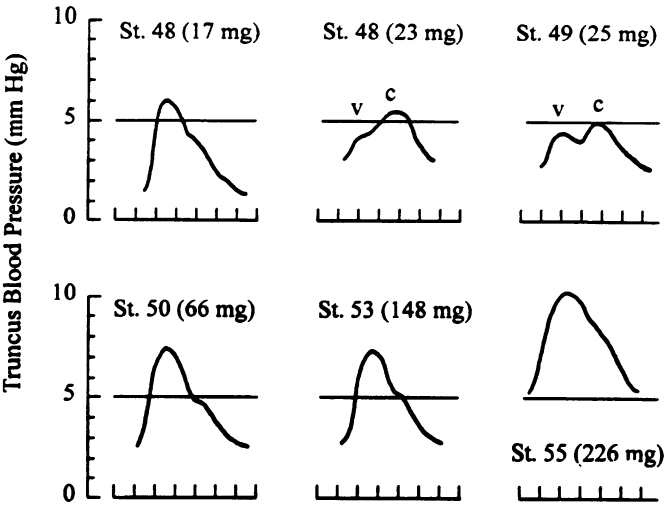

Fig. 3. Pressure recordings in truncus arteriosus of larval *X. laevis*. Developmental stage (St) and body mass of larvae are indicated. V, ventricular contraction; C, conus contraction.

at a body mass of ~ 17 mg (the smallest larvae measured for blood pressure) and 12.6 and 5.9 mmHg, respectively, for a 1-g larva. Changes in body mass explain 59 and 31% of the variations in P_s and P_d, respectively (Table 1).

Pulse pressure ($P_s - P_d$) also increased significantly with increasing body mass in developing *X. laevis* (Table 1). Calculated pulse pressure from the regression line is 3.3 and 6.3 mmHg for a 17-mg and a 1-g larva, respectively. Changes in body mass explain 48% of the variation in pulse pressure.

As would be expected from changes in P_s, P_d, and pulse pressure, mean truncus pressure increased with increasing body mass in developing *X. laevis* (Fig. 5). Changes in body mass accounted for 49% of the variation in mean truncus pressure (Table 1). Mean truncus pressure calculated from the regression line is 4.2 and 9.5 mmHg for a 17-mg and a 1-g larva, respectively.

Blood pressure in the ventricle and conus arteriosus. Blood pressure was successfully measured directly in the ventricle in just two quite large stage 55 larvae weighing 226 and 275 mg. Pressures recorded sequentially from the truncus and conus arteriosus and the ventricle in these two larvae are superimposed for comparison in Fig. 6. P_s resulting from ventricular contraction were closely matched in all three measurement sites. The truncus pressure, however, remained lower than the conus pressure during conus contraction, as indicated by the elevated second peak in the conus pressure contour.

If it is assumed that there was not a significant resistance in the outflow tract from ventricle to conus,

Table 1. *Regression relationship of heart rate and truncus arteriosus blood pressure with body mass in developing Xenopus laevis*

y	n	log a	b	r	r^2
f_h	65	2.10	−0.06 ± 0.01	−0.50	0.25
P_s	26	0.50	0.20 ± 0.03	0.77	0.59
P_d	26	0.11	0.22 ± 0.07	0.56	0.31
$P_s - P_d$	26	0.26	0.18 ± 0.04	0.69	0.48
P_m	26	0.32	0.22 ± 0.05	0.70	0.49

Values for b are means ± SE. Relationships are expressed as $\log y = \log a + b \cdot \log M$, where y is heart rate (f_H, in beats/min) or arterial blood pressure [systolic (P_s), diastolic (P_d), pulse ($P_s - P_d$), mean (P_m), in mmHg) and M is body mass (in mg). n, r, and r^2, sample size, coefficient of correlation, and coefficient of determination for correlation, respectively. All regressions and correlations are significant at $P < 0.01$.

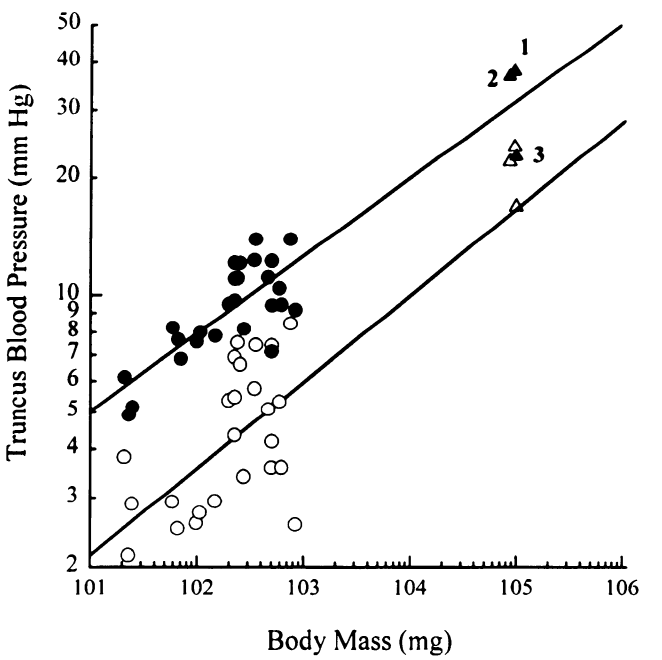

Fig. 4. Systolic (filled symbols) and diastolic (open symbols) pressures in truncus arteriosus of *X. laevis*. Circles, data from present study; triangles, adult *X. laevis* data from Shelton and Jones (19) (*1*), Shelton (18) (*2*), and Emilio and Shelton (10) (*3*). Regression line is based on values of present study; its parameters are given in Table 1.

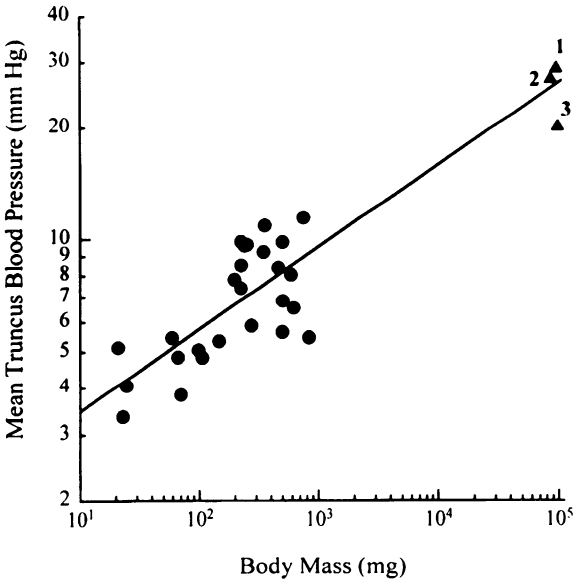

Fig. 5. Mean pressure in truncus arteriosus of *X. laevis*. ●, Larval data from present study; ▲, adult values from other sources as indicated in Fig. 4 legend. Regression line is based on values of present study; its parameters are given in Table 1. Adult values are continuous with extrapolation of larval values.

then P_s in the ventricle could be inferred from the pressures recorded in the conus arteriosus, which could be recorded in much smaller larvae (Fig. 6). The pressure waveform in the ventricle during systole was qualitatively similar in all developmental stages examined (stages 46–66), although the absolute systolic ventricular pressure increased with development. Conus arteriosus blood pressure, however, showed considerable variation in waveform, usually appearing with two peaks in a cycle, as was observed in some readings from the truncus (Fig. 7). Visual observation of the beating heart confirmed that the first peak represented ventricular contraction and the second peak was due to

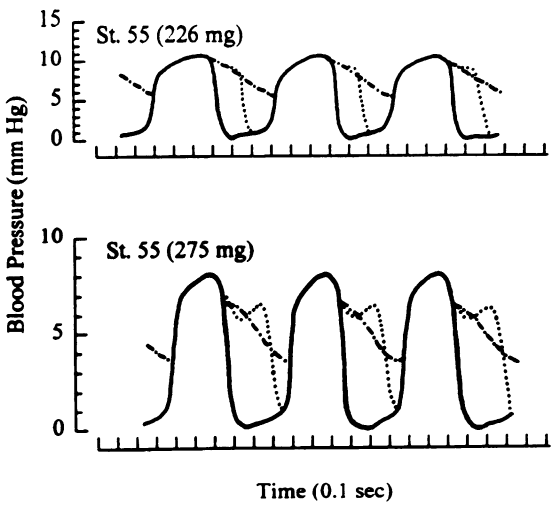

Fig. 6. Superimposed blood pressure recordings from ventricle, conus arteriosus, and truncus arteriosus of 2 stage 55 *X. laevis* larvae. Solid, dotted, and dashed lines, pressure changes in ventricle, conus arteriosus, and truncus arteriosus, respectively.

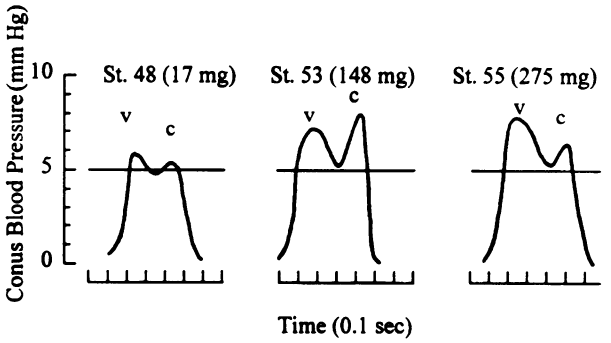

Fig. 7. Representative examples of pressure recordings in conus arteriosus of larval *X. laevis*. Developmental stage (St) and body mass of larvae are indicated. Ventricular (V) and conus contractions (C) were confirmed by visual observation of heart.

conus contraction. P_s increased in the conus arteriosus throughout development, but P_d remained close to zero.

DISCUSSION

Critique of methods. Measurements of blood pressures with a microelectrode servo-null system in small larvae are not possible without immobilization of the animals through the use of anesthetics. MS-222 at 1:1,000 has been shown to increase heart rate and decrease blood pressure in adult toads *Bufo marinus* (20). In the present study, 1:10,000 MS-222 was ample for anesthesia. Feder and Wassersug (11) counted heart rates in unanesthetized and unrestrained *Xenopus* larvae by direct visual observation. They found a heart rate of 55–85 beats/min at 25°C in larvae of Gosner stage 25–42 (equivalent to NF stage 47 at 10 mg and stage 58 at 800 mg, respectively). Their values fall in the range of 50–110 beats/min, which we found using larvae of similar size. Apparently, the low anesthetic concentration of the present study did not significantly disturb heart rate. There was no abrupt difference in heart rate between stage 48 larvae, in which the pericardium was intact, and stage 49 larvae, in which the pericardium was open (Fig. 2). This suggests that heart rate in anesthetized animals was not influenced by the state of the pericardium. Consequently, data from both types of preparations were pooled for the heart rate analysis.

Heart rate. Bride (1) reported a decline of heart rate from 160 beats/min in larval *Xenopus* with internal gills (equivalent to NF stage 47, ~8–10 mg) to 110 beats/min before metamorphosis (presumably at room temperature, but temperature was not reported).

Our experiments extend these observations of heart rate down to about stage NF 41 and a body mass of ~3 mg <24 h after heartbeat begins. From heart rate now obtained over the complete larval series in *X. laevis*, it appears that heart rate decreases progressively but relatively slowly throughout larval development. The slope of the line describing this relationship is −0.06 (from Table 1), which is considerably less than the value of −0.2 to −0.3 measured in a variety of birds and mammals (17). The cause for this discrepancy warrants further investigation. In any event, changes in body mass account for only about one-fourth of the total variation in heart rate during development, indicating

that body mass is a relatively poor predictor of heart rate.

There appears to be no generalized pattern of heart rate change in early development among anuran amphibians (2, 7). For example, in *Rana catesbeiana* (2, 4, 16) and *Pseudis paradoxsus* (3), heart rate falls sharply or shows little change, respectively, as larval development proceeds. In the direct developing frog, *Elutherodactylus coqui*, heart rate initially increases in early development and then declines slowly in later development (5). A decline in heart rate with larval or fetal development has also been recorded in other vertebrates, including fish, frogs, and birds, but there are many exceptions to this pattern (see Ref. 7 for review). Overall there are numerous different patterns of changes in heart rate during vertebrate development, and the linear correlation between heart rate and body mass found in *Xenopus* must be regarded as species specific.

Blood pressure. Arterial blood pressure increases continuously from larvae to the adult in *X. laevis*. Shelton and Jones (19) reported 37.9 and 24.1 mmHg for P_s and P_d, respectively, in the systemic arch of adult *Xenopus*. The mean systemic pressure calculated from these data is 28.7 mmHg by use of the following formula: $\frac{1}{3} P_s + \frac{2}{3} P_d$. These P_s, P_d, and pulse and mean arterial pressures in adult *Xenopus* are similar to our predicted values for adults based on regressions of the larval values (Figs. 4 and 5), suggesting a continuous increase in arterial blood pressure, even after metamorphosis. Body mass is a better predictor of blood pressure (49% of the variation in mean arterial blood pressure, Table 1) than of heart rate. In adult vertebrates, blood pressure does not scale with body mass when intra- or interspecific comparisons are made (17), though rarely do available data extend over four log cycles, as in the present study. Clearly, factors other than allometric scaling influence the cardiovascular system as it develops. Identifying these factors must await additional studies but could result from, for example, the addition of new vascular beds (internal gills, gut, reproductive organs, and eventually limbs).

A progressive increase in arterial pressure occurs during middle and late development in the skate *Raja erinacea* (15), the bullfrog *R. catesbeiana* (16), and the domestic chicken (see Ref. 8 for review). The increase in arterial blood pressure during vertebrate development has been attributed to the growth in ventricular weight, the increase in number of myocardial cells, and the addition of new resistance vessels as body mass increases (8, 9). The developmental pattern of blood pressure in the frog *P. paradoxsus*, however, reveals an increase from larvae (stage 37–40) to juvenile frog with sharply decreasing body mass, but then a constant blood pressure with subsequent increase in body mass from juvenile to adult frog (3). At least in *P. paradoxsus*, body mass does not predict blood pressure during development.

Our central arterial blood pressure measurements (and limited intraventricular pressure measurements) indicate that functional valves must separate the ventricle, conus arteriosus, and truncus arteriosus during diastole in early larvae of *Xenopus*. Examination of videotaped cardiac cycles revealed no backflow of blood from the truncus to the conus or from the conus to the ventricle in these larvae. The distinctly different blood pressures in the ventricle, the conus, and the truncus during diastole (Fig. 6) also indicate the presence of functional valves similar to the synangial and pylangial valves in adult anuran amphibians.

The role of conus contraction in generating arterial blood pressure is greater in young than in older larvae of *Xenopus*. In young larvae, the truncus waveform shows a peak produced by ventricular contraction followed by a similar or higher peak resulting from conus contraction. The second peak produced by conus contraction in the truncus waveform is not seen in the older larvae (Fig. 3). The role of the conus arteriosus in circulation also decreases during development of bullfrog larvae (16). However, at least in bullfrogs, this phenomenon is seasonal and occurs only in fall/winter larvae. In early larval stages of fall/winter bullfrogs, the conus generates arterial blood flow while the ventricle acts as an antechamber that "primes" the conus pump. Our experiments on *X. laevis* show that ventricular contraction alone is capable of propelling blood through the truncus, as indicated by the increase in truncus pressure (the first peak) produced by ventricular contraction (Fig. 3). Furthermore, in older larvae, truncus pressure remained lower than conus pressure during conus contraction (Fig. 6), suggesting that the region of the synangial valves may offer high resistance to blood flow from conus to truncus during a brief period of the cardiac cycle. In bullfrog larvae, changes in blood velocity in the truncus arteriosus follow changes in ventricular pressure, not changes in conus pressure, suggesting that blood flow in the truncus arteriosus is not affected by conus contraction (16).

We thank W. E. Bemis for using his video recorder and image analysis system and E. A. Connor, M. A. Olivo, John L. Roberts, and T. Wang for comments on the manuscript.

This study, which was part of a Ph.D. dissertation at the University of Massachusetts (Amherst, MA), was supported by National Science Foundation Grants DCB-8916938 and IBN-9307832 to W. W. Burggren and NSC 82–0209-B-006–095-T to P.-C. L. Hou.

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Received 21 April 1994; accepted in final form 12 April 1995.

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