Central arterial hemodynamics in larval bullfrogs (*Rana catesbeiana*): developmental and seasonal influences

B. PELSTER AND W. W. BURGGREN
Department of Zoology, University of Massachusetts, Amherst, Massachusetts 01003

PELSTER, B., AND W. W. BURGGREN. *Central arterial hemodynamics in larval bullfrogs (Rana catesbeiana): developmental and seasonal influences.* Am. J. Physiol. 260 (Regulatory Integrative Comp. Physiol. 29): R240–R246, 1991.—Central arterial hemodynamics (blood pressure and velocity) as a function of ontogeny and season were determined in larval *Rana catesbeiana* (body mass 0.3–8.7 g, 20–22°C). Ventricular systolic pressure increased from 1.8 mmHg at stage (St) II to 11.9 mmHg at St XIII, while ventricular diastolic pressures usually were <1.0 mmHg. In early stages (up to St V-VII) of fall/winter larvae, the pressure waveform in the conus arteriosus was often biphasic. The first peak was due to weak ventricular contraction (sometimes inadequate to eject blood into the arterial tree), and a stronger second peak resulted from conal contraction. In these young fall/winter larvae, the conus—not the ventricle—was the major circulatory pump. Older larvae (>St X) showed "adultlike" central hemodynamics, with the ventricle ejecting blood through the conus into the central arterial circulation. Systolic blood pressure was considerably higher in young spring/summer (April-June) larvae, and the ventricle rather than the conus was the main circulatory pump at all stages, in contrast to fall/winter larvae. Thus both season and development have profound influences on the central hemodynamics of bullfrog larvae.

circulation; heart; conus arteriosus; blood pressure; ontogeny

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BLOOD PRESSURE represents a crucial measure of cardiac performance in all vertebrates, including embryos. Even in the smallest embryos, once the circulation has taken responsibility for nutrient supply to the tissues and removal of waste products, the blood pressure generated must be high enough to assure sufficient perfusion of all capillaries. In bird embryos, which have historically served as the major model for studying vertebrate embryonic hemodynamics, both heart rate and blood pressure increase significantly with development (5, 6). Very little, however, is known about the cardiovascular dynamics of lower vertebrate embryos and larvae (4).

Of particular interest in this respect are larval amphibians, because of the extensive morphological and physiological changes that they undergo during metamorphosis. Almost all information that is available relates to heart rate and its nervous and humoral control (for review see Ref. 4). In adult amphibians, central arterial hemodynamics, especially the concerted action of ventricle and conus arteriosus in producing blood pressure and flow, have been carefully analyzed (12, 14, 18, 19; for reviews see Refs. 10, 17). The conus usually plays only a small role in arterial blood flow when compared with the ventricle (19), although it has a marked influence on the preferential distribution of oxygenated and deoxygenated blood to the systemic and the pulmocutaneous arch, respectively (see Refs. 1, 17). To what extent the hemodynamics of the metamorphosed adult and the relative roles of the conus and ventricle can be applied to the larval circulation remains completely unknown.

Furthermore, the larval development of many North American anurans (e.g., *Rana catesbeiana*) is not a continuous but rather a seasonal process. Larvae may overwinter once or even twice at higher latitudes, and growth and development may be confined to <6 mo of the year. Cardiovascular physiology of adult lower vertebrates in general and anurans in particular shows profound daily and seasonal rhythms (7; Burggren, unpublished observations), and seasonal differences may well extend to basic aspects of hemodynamics.

The present study investigates central arterial blood pressure and velocity in larvae of the bullfrog *R. catesbeiana*. Hemodynamic measurements were made at various developmental stages to determine ontogenetic changes, and seasonal effects were also assessed.

MATERIALS AND METHODS

Experiments were performed at 20–22°C on larvae (body mass 0.3–8.7 g) and small adults (body mass 17–22 g) of the bullfrog *R. catesbeiana*. Adults were acquired from commercial suppliers, whereas the larvae [stage (St) II-XIII of the Taylor-Kollros staging scheme] were collected in Hampshire County, MA. The animals were maintained in aquaria at 20–23°C on a 12:12 h light-dark photoperiod for at least 1 wk before experimentation and were regularly fed spinach (larvae) or maggots (adults). The first series of experiments was performed with fall/winter animals during the months October through January and a second series with spring/summer animals during the months April, May, and June.

In most experiments, animals were quickly decerebrated, spinally pithed with a fine needle, and then placed in an experimental chamber containing ~1 cm of oxygenated water, which prevented drying of the skin and permitted continued cutaneous gas exchange. The heart and conus arteriosus were exposed by a small incision in the ventral body wall, and the opening was flushed periodically with saline to prevent desiccation of exposed tissues. To test for possible cardiovascular effects of pithing, several animals of different larval stages were

anesthetized with tricaine (MS 222; 0.1–0.15 g/l, pH 7.5), and the results were compared with those of pithed animals.

Blood pressure in the central arterial circulation was recorded with a servo-null micropressure system (model 900, World Precision Instruments, New Haven, CT). The microelectrode (tip diameter 2–5 µm) was filled with 3 M NaCl and mounted in a micromanipulator. With the use of a binocular microscope, the electrode was inserted into the conus arteriosus and left in place to allow for continuous recording. Cardiovascular variables in this preparation usually remained stable for 1–2 h, with a maximum decrease in cardiac frequency or arterial pressure of <10% of the values recorded a few minutes after inserting the electrode.

In some experiments, after recording of conus pressure, brief recordings (3–5 min) were made in the ventricle and truncus arteriosus. In such experiments, measurements from all three regions were obtained within a single 15-min period. Usually the same electrode was used for all three recording sets. Occasionally, a broken electrode had to be replaced during the experiment, but identical pressures were measured at a single recording site when using different electrodes. To assess hemodynamic events, subsequently measured ventricular, conal, and truncus pressure traces were superimposed on a single graph. The onset of ventricular systole, verified in the original recordings by visual observation of the heart, served to align the time bases of the different recording traces.

In some preparations, arterial blood velocity was also measured noninvasively, using a pulsed Doppler flowmeter (Bioengineering, Iowa City, IA). A 1-mm diameter crystal, mounted in a micromanipulator, was maneuvered into the saline pool overlying the heart and central arteries and positioned ~1–2 mm over the anterior end of the conus or, in some preparations, above the origin of the truncus arteriosus. An uncalibrated indication of volume changes in the conus was obtained by looping a thread under the conus and attaching it to a myograph (Narco-Bio-System, Houston, TX). The dorsoventral movements of the conus thus recorded were closely correlated with visually observed changes in its volume. The signal from the myograph, therefore, was referred to as "volume" in arbitrary units. Ventricular volume was similarly recorded with a 50-mg weight suspended from a thread attached to the myograph and carefully rested on the ventral surface of the ventricle. An increase in volume of the ventricle relieved some of the weight's strain on the myograph, producing a clear signal associated with volume changes of the ventricle. All signals were continuously recorded on a physiograph chart recorder (Narco Bio-System).

Statistical analysis was performed using the Biostat I software package of Sigma Soft (Placentia, CA). All data are presented as means ± SD.

RESULTS

Central Vascular Anatomy

The anatomy of the central circulatory system of larval *R. catesbeiana* is shown in Fig. 1. All chambers are
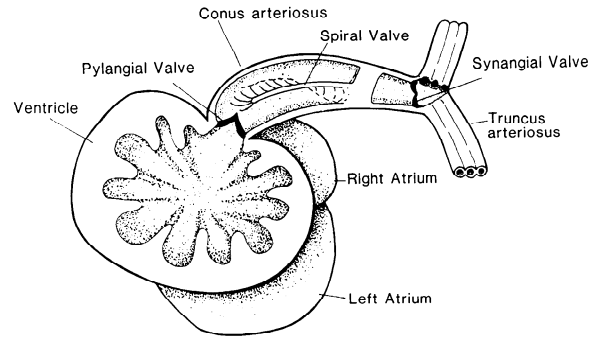

FIG. 1. Schematic drawing of anatomy of central circulatory system of *Rana catesbeiana* larvae. Appearance of spiral valve in conus arteriosus is quite variable depending on fraction of the valve that has been removed with the upper part of conus.

separated by cusped valves. The pylangial valve separates the ventricle and the conus arteriosus, whereas the synangial valve guards the opening from the conus arteriosus to the truncus arteriosus. A prominent spiral valve attached at one edge spirals ~270° along the length of the larval conus arteriosus. This valve was present in St II larvae, the earliest stage examined. The large left and right atria are located dorsal to the ventricle. Visual observation of dissections and of the intact, beating heart revealed that in early larval stages the conus appeared to have a similar or slightly smaller volume than the ventricle, whereas in later larval stages and adults the ventricle had a much greater volume than the conus.

Central Vascular Physiology: Fall/Winter Animals

Experiments were performed in two series at different times of the year. Profound seasonal effects on the cardiovascular system were observed. For clarity of presentation of the data, we first completely describe the results obtained with fall/winter animals and then, in a subsequent section, emphasize the differences observed in the second series performed during spring and summer.

Heart rate. At every developmental stage, tested cardiac frequency and blood pressure of anesthetized animals were not significantly different ($P > 0.05$) from pithed animals. Therefore, the results of both groups were pooled for subsequent analysis. Heart rate was 62 ± 7 beats/min in the youngest larvae (St II) during the fall and winter months, decreasing with development (Fig. 2A). When development is expressed in terms of body mass, heart rate decreased with increasing body mass [heart frequency (f_H) = −0.7mass + 61.1, $P < 0.05$].

Blood pressure. Diastolic pressure in the ventricle was usually <1.0 mmHg in all developmental stages examined during the fall/winter months. Diastolic pressure recorded in the conus was similar to ventricular diastolic pressure in very young larvae but tended to increase slightly in later stages to ~1.5–3.0 mmHg (Fig. 2B). Systolic pressure (P_{sys}) recorded in both the ventricle and the conus increased markedly with larval development. We also analyzed the relationship between body mass and arterial blood pressure, because body mass of larval *R. catesbeiana* increased with development (Fig. 3). In the fall/winter series, the overall relationship in-
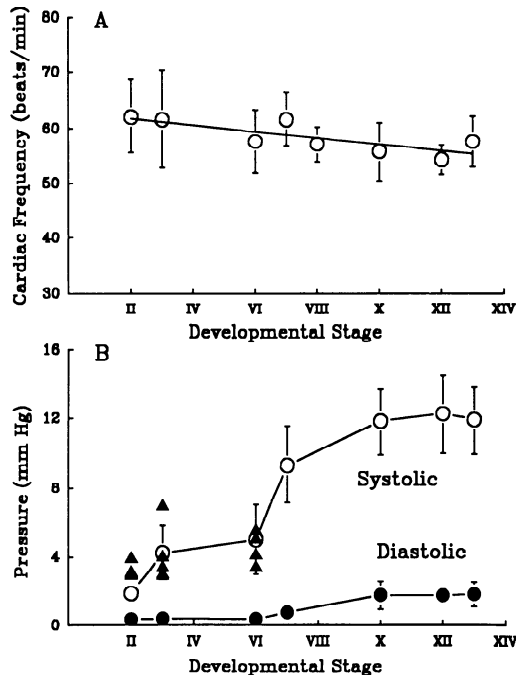
FIG. 2. Central vascular function during development in fall and winter months. *A*: heart rate as a function of developmental stage. Linear regression is represented by equation $y = -0.6x + 62.9$ ($r = 0.8$, $P < 0.05$). *B*: diastolic and systolic blood pressure recorded in conus. Closed triangles represent conal systolic pressure in preparations where conal systolic pressure exceeded ventricular pressure. Values are means ± SD.
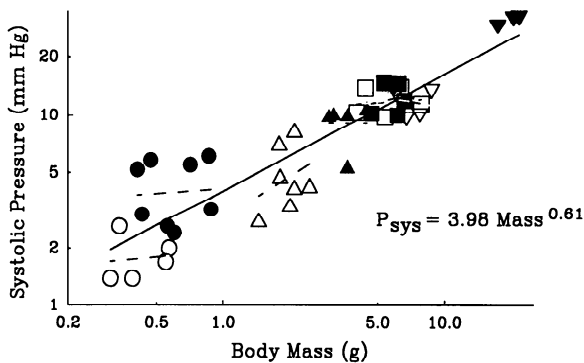

FIG. 3. Relation of systolic blood pressure to increasing body mass in fall/winter animals. Each stage (St) is represented by a separate symbol. Open circle, St II; closed circle, St III; open triangle, St VI; closed triangle,St VII; open square, St X; closed square, St XII; inverted open triangle, St XIII; inverted closed triangle, adult. Dashed lines represent regression lines calculated for each stage; continuous line is overall regression.

cluding all larval stages and small adults is described by the relationship $P_{sys} = 3.98 mass^{0.61}$ ($r = 0.9$; $P < 0.01$).

Central hemodynamics. During the fall and winter months in early larval stages (i.e., many St II, some St III, and few St VI), conal contraction generated a distinct pressure peak that was higher than the mean ventricular pressure in the preparation (triangles in Fig. 2*B*).

Before St VII, the pressure waveform recorded in the conus showed considerable variability between individuals (Fig. 4), but the pattern evident in the youngest larvae became less and less common as development continued. With further larval development, the ventricular systolic pressure increased to become the dominant

feature of the waveform, and the contribution of the conal contraction to the pressure waveform recorded in the conus became less obvious (Figs. 4 and 5). By St X and older, the waveform almost invariably showed a dominant ventricular peak with only a slight contribution produced by the conus during early ventricular diastole.

An overlay of serial pressure measurements from the ventricle, conus arteriosus, and truncus arteriosus of animals of different developmental stages is shown in Fig. 5, demonstrating the changing influence of the conus on arterial blood pressure. In the St II larva, shown in Fig. 5, *top left*, ventricular pressure remained below arterial pressure, whereas the conal pressure surpassed it. In later stages, ventricular contraction clearly generated the highest pressure peak.

Figure 6 shows recordings of pressure and volume changes in the conus and in the ventricle of a St VI larva, representative of the hemodynamics in a young fall/ winter larva in which conus systolic pressure was biphasic. The pressure in the ventricle, recorded a few minutes after measuring in the conus, exactly matched the first peak of the conus pressure signal. However, while ventricular pressure then fell as the ventricle began to relax, the pressure in the conus increased a second time in the cycle, reflecting a contraction of the conus itself. This overlay of serial recordings often was slightly complicated by small changes in frequency. On a beat-to-beat comparison, however, the pressure profiles from the conus and ventricle were almost always superimposable.

The volume changes of the ventricle closely followed pressure changes with filling during the low-pressure phase and emptying during the high-pressure phase of the cycle (Fig. 6). The volume of the conus increased as soon as the ventricle volume decreased, but at the same time the pressure in the conus increased, too. The largest conus volume was observed just after passing the maximum of ventricular pressure where during a slight pressure decrease in the conus the maximum extension of the conus was achieved. At this time, the pressure in the conus started to increase again, thus exceeding the ventricular pressure, and the conus volume decreased to reach the lowest volume at the beginning of the next systole of the ventricle.

Figure 7 shows simultaneously recorded arterial blood pressure and velocity in a St III and a St XIII fall/winter larva. A short time delay of the blood velocity signal compared with the conus pressure recording was used to confirm that the Doppler flow signal arose downstream of the pressure recording site. Although the conus produced a distinct and significant pressure peak in the St III animal, the velocity trace was hardly influenced, which probably has to be attributed to the smaller volume of the conus compared with the ventricle. Unfortunately, the extremely small dimensions of the central arterial system, the changing diameter of the conus along its length, and possible complicating role of the spiral valve in the conus, all obviated calibration of blood velocity and subsequent calculation of blood flow.
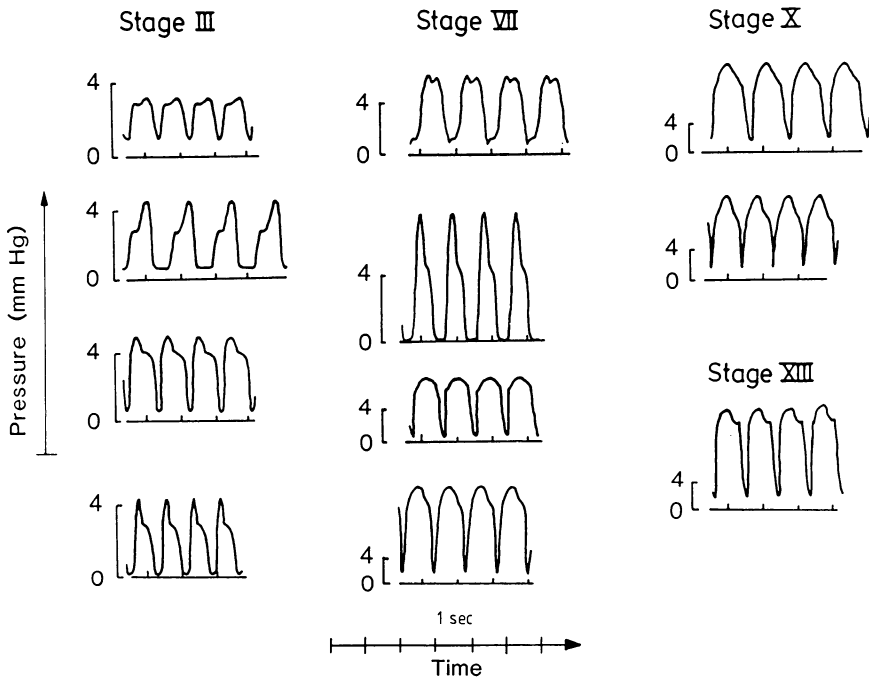
FIG. 4. Examples for pressure signals recorded in conus arteriosus of several fall/winter animals of various developmental stages demonstrating variability of pressure waveform, especially in early stages.

Central Vascular Physiology: Spring/Summer Animals

Profound differences in central hemodynamics were observed between fall/winter and spring/summer larvae (Fig. 8A). Ventricular pressure was much higher in spring/summer larvae from St III to St VII, when compared with fall/winter animals over the same developmental range ($P < 0.05$). There was, however, no seasonal difference in ventricular systolic pressure in larvae of St X and St XIII ($P > 0.1$). The changes become even more obvious when plotting systolic blood pressure vs. body mass (Fig. 8B), where all values for spring/summer larvae clearly exceed the mean value obtained in the fall/winter animals.

In our experiments, no differences were observed in diastolic pressure between fall/winter and spring/summer larvae. The high systolic blood pressures indicate a strong contraction of the ventricle, and accordingly the pressure waveform recorded in the conus was exclusively of the adult type. Heart frequency decreased with development at the same rate in both spring/summer and fall/winter groups. Mean values of heart rate were consist-

ently 6–9 beats/min lower in spring/summer larvae of all stages tested, but because of the individual variation within developmental groups these differences were only significant in two stages.

DISCUSSION

Central Vascular Physiology: Fall/Winter Animals

Heart rate. Although MS 222 acts as a sympathetic stimulus in anurans (20), there was no difference in heart rate between pithed and anesthetized animals, which probably can be attributed to the low dose of the anesthetic. Our values for f_H are similar to those reported for unrestrained larval *R. catesbeiana* of similar developmental stage (2, 3, 22), suggesting that our methods of immobilization did not bias cardiac performance.

Blood pressure. The present study reveals a significant increase in arterial blood pressure with development in *R. catesbeiana*, a phenomenon also occurring in larvae of the frog *Pseudis paradoxa* (W. Burggren, M. Glass, A. Abe, and E. Bicudo, unpublished observations). Moreover, an increase in blood pressure with development has
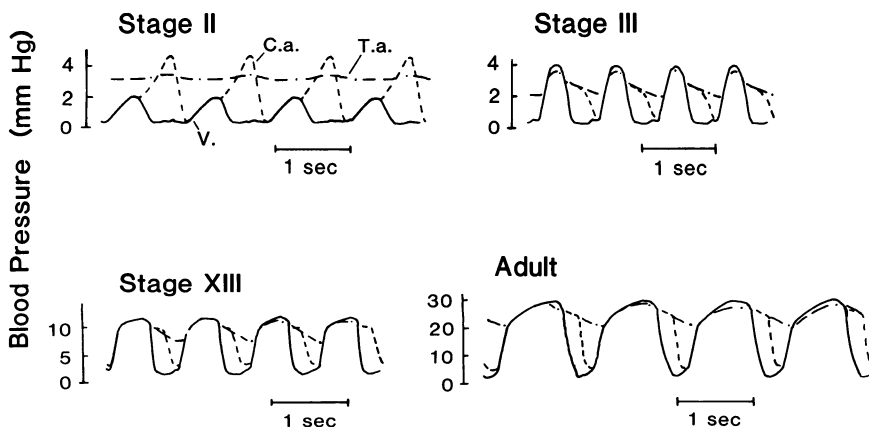

FIG. 5. Superimposed pressure recordings obtained in series from ventricle (continuous line), conus arteriosus (broken line), and truncus arteriosus (broken-dotted line) from 3 larval stages and small adult bullfrogs.
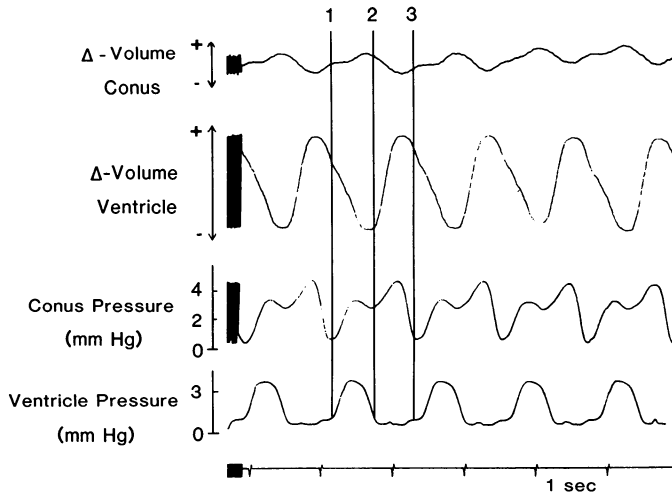
FIG. 6. Original recordings of volume and pressure changes in conus and ventricle of a larva St VI, body mass 1.45 g. Volume changes could not be calibrated, and therefore traces do not allow for volume comparisons between conus and ventricle. Pressure in ventricle was recorded separately with a time difference of ~5 min. *Lines 1, 2,* and *3* indicate onset of ventricular contraction, end of ventricular, and end of conal contraction, respectively.
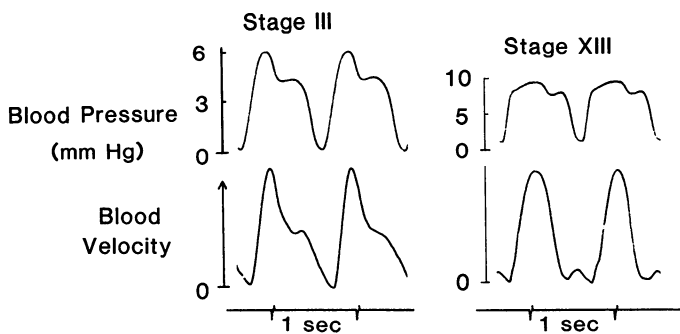

FIG. 7. Original recordings of blood pressure in conus and of blood velocity downstream to micropressure electrode of a St III (0.5 g) and a St XIII (5.8 g) animal.

been documented in vertebrates ranging widely from embryos of chickens (6, 21) to skates (*Raja erinacea*) (B. Pelster and W. Bemis, unpublished observations) and may be a general developmental pattern in vertebrates.

There could be several explanations for the observed correlation between body mass and arterial blood pres-

sure in *R. catesbeiana*. Growth, producing new vessels and enlarged vascular beds, results in both an increase in total cross-sectional area of the vascular bed (tending to decrease vascular resistance) and an increase in total vessel length (tending to increase vascular resistance). If the net effect was an overall increase in vascular resistance (i.e., length increase predominated), then an increase in blood pressure would be required to assure sufficient tissue perfusion as development progresses. However, this presumes constancy of blood flow per gram tissue as development occurs. Although this appears to be the case in chick embryos (5), there has been no experimental verification of this phenomenon during development in any lower vertebrate.

Central hemodynamics: early larval stages. Dissection of bullfrog larvae demonstrated the early presence of valves separating the different chambers of the central vascular system, and the organization of the spiral valve of the conus appears to be similar to that described for adult ranid frogs (17). The physiological functioning of these valves can be assessed by pressure recordings in the various chambers. If pressure remains elevated distal to the valves compared with proximal, then the valves must be closed and, as a result, separate the chambers completely. The overlay of pressure recordings from different sites of the circulatory system (Fig. 5) therefore demonstrates the presence of functional pylangial and synangial valves early in development as well as the changing importance of the ventricle and conus as contractile chambers of the circulation, and both of them have been shown to contain contractile myocytes (8). Examination of Fig. 5, *top left,* shows that the ventricular systolic pressure remained well below diastolic pressure in the truncus arteriosus at all points in the cardiac cycle. The ventricle generates a sufficiently high pressure to open the pylangial valve and generate flow into the conus in early ventricular systole, and, indeed, this was confirmed by observing an increase in conus volume at this point in the cardiac cycle. However, the ventricle cannot be responsible for opening the synangial valve and generating ejection of blood through the conus into the truncus arteriosus. In these early larval stages when conal contraction predominates, the conus therefore must represent the major contractile chamber of the
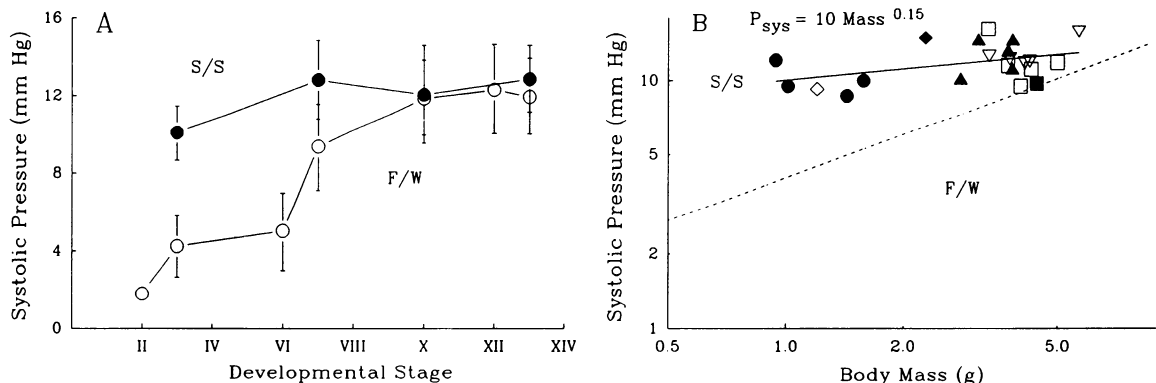

FIG. 8. Systolic blood pressure of various spring/summer (S/S) animals vs. developmental stage (*A*) and vs. body mass (*B*). Open circles in *A* represent fall/winter (F/W) animals (redrawn from Fig. 2*B*). Solid line in *B* represents a linear regression of all data for spring/summer animals. Different stage groups are represented by different symbols (see Fig. 3; open diamonds, St IV; closed diamonds, St V). Dashed line indicates linear regression line obtained for fall/winter animals (redrawn from Fig. 3).

circulation. The ventricle acts as an antechamber that "primes" the conus, and it is the conus that generates arterial blood pressure and flow.

There is, however, no convincing explanation for the relative inactivity of the ventricle in these early stages of fall/winter larvae. A persistent vagal tone resulting from the unlikely event of incomplete pithing could inhibit ventricular contraction. However, it seems unlikely that pithing was incomplete in all animals of only stages II, III, and VI; we would rather expect that incomplete pithing, which would leave part of the nervous system functional, might occur in larger animals with a larger brain and spinal cord rather than in the smaller larvae. Furthermore, vagal tone would result in a reduced heart rate (13, 15), which was not observed in our experiments. Consequently, it seems far more likely that the relative inactivity of the ventricle of young larvae reflected a strong seasonal influence. During fall and winter, the development of the larvae is slowed down or even stopped completely. This might be achieved by hormonal changes, which may also influence the central circulatory system and reduce the activity of the ventricle. In the adult toad *Bufo arenarum*, seasonal variations in the reactivity of the vasomotor system to circulating catecholamines have been correlated with marked seasonal changes in blood pressure (16). Ranid frogs show seasonal effects on heart rate-temperature relationships (9) and in the magnitude of diving bradycardia (11).

Central hemodynamics: older larval stages. The central hemodynamic pattern observed in older larval stages resembled that recorded in adult bullfrogs (Figs. 4 and 5) and in adult *Rana pipiens* (18). Thus, in later larval stages, ventricular contraction opens the pylangial valve first and then the synangial valve and ejects blood through the conus arteriosus into the truncus arteriosus. At St X and later, a large conus contraction comparable with earlier stages was not observed. However, the conal pressure remained elevated during the early phase of ventricular diastole. This could result either from a continued muscular contraction of the conus or from a slow elastic relaxation of the conal walls (a windkessel effect). Either event would act to reduce oscillation in arterial blood pressure. Finally, the very good match of the pressure recordings obtained in series with a very small drop in pressure from the ventricle through the conus to the truncus also indicates a low flow resistance between these three compartments.

Central Vascular Physiology: Spring/Summer Animals

Segura and D'Agostino (16) reported a drop in arterial blood pressure from 67 mmHg during the summer to 37 mmHg during the winter for the adult toad *B. arenarum*, with all pressure measurements being made at 25°C. Taken together with the present findings that indicate that ventricular systolic pressure also shows strong seasonal effects in larval *R. catesbeiana*, it would appear that season should be regarded as an important variable in cardiovascular studies on amphibians.

In conclusion, significant qualitative and quantitative changes in central arterial hemodynamics occur during development in the bullfrog *R. catesbeiana*, and there appears to be a significant seasonal influence on the performance of the central circulatory system. During fall and winter, when development of the larvae is slowed down, cardiac activity appears to be reduced. In early larval stages, the conus arteriosus can even become the main ejection pump for the circulation, establishing both arterial blood pressure and flow. During spring and summer, when development of the larvae proceeds, contraction of the ventricle becomes more powerful, so that the pressure waveform recorded in the central arterial circulation of every stage almost exclusively resembles that in the adult bullfrog, with the ventricle serving as the main ejection pump for the circulation.

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