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Body, eye, and chorioallantoic vessel growth are not dependent on cardiac output level in day 3–4 chicken embryos

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Burggren, Warren, Sheva Khorrami, Alan Pinder, and Tiffany Sun. Body, eye, and chorioallantoic vessel growth are not dependent on cardiac output level in day 3–4 chicken embryos. *Am J Physiol Regul Integr Comp Physiol* 287: R1399–R1406, 2004. First published August 19, 2004; doi:10.1152/ajpregu.00086.2004.—Normal aerobic metabolic rates persist in the early chicken embryo after elimination of cardiac output, but the dependence of tissue growth and differentiation on blood flow is unknown in these early stages. We partially ligated (25–50% occlusion) the ventricular outflow tract of Hamburger-Hamilton stage (HH) 16–18 embryos, producing a wide range of cardiac output. For the next ~48 h (to HH 24), we measured heart rate (HR), stroke volume (SV), and cardiac output (CO), as well as these growth indicators: eye diameter, chorioallantoic vessel density, and body mass. Acutely, HR declined with partial ligation (from 108 to 98 beats/min). Paradoxically, SV and CO decreased sharply in most embryos but increased in others, collectively producing the desired large variation (up to 25-fold) in CO and permitting assessment of tissue growth over a very large range of blood perfusion. Eye diameter doubled (from 0.6 to 1.2 mm) with development from HH 16 to HH 24, but within a developmental cohort there was no significant correlation between eye diameter and CO over a 25-fold range of CO. Similarly, chorioallantoic membrane vessel index was independent of CO over the CO range at all stages. Finally, body mass increase during development was not significantly affected by partial conal truncal ligation. Collectively, these data suggest that normal eye and vessel growth and body mass accumulation occur independent of their rate of blood perfusion, supporting the hypothesis of prosynchronotropy—that the heart begins to beat and generate blood flow in advance of the actual need for convective blood flow to tissues.

embryo development; blood flow; heart rate; vascular growth

CONSIDERABLE FOCUS NOW EXISTS on how the embryonic vertebrate heart first becomes regulated in its emerging task as a pump generating convective blood flow (see multiple reviews in Ref. 6). Typically, these studies of the early embryonic circulation operate from two basic assumptions: *1*) that the purpose of the embryonic heart is to create a convective blood flow for mass transport of nutrients and waste and *2*) that this transport is an absolute requirement for life. Both assumptions seem to be truisms, accurately reflecting the reality in more mature circulations. Yet these assumptions are inconsistent with early but largely ignored observations showing that elimination of cardiac output by surgical ligation is initially nonlethal in very early chicken embryos (10, 43). More recent observations have shown that complete elimination of blood flow by cardiac ligation has no significant acute effect on embryonic oxygen consumption from day 3 to day 5 in chicken embryos (8). Similarly, elimination of blood oxygen transport in adult trout (20), embryonic chickens (11), and larval zebrafish (35) or ablation of the heart in salamander larvae (33) is also nonlethal. Collectively, these observations have lent credence to the hypothesis of "prosynchronotropy," namely, that the heart begins to beat and convey blood well in advance of the absolute need for convective transport (7, 42).

Although the evidence to date in support of prosynchronotropy is compelling, the techniques of complete conotruncal ligation or, even less subtle, complete cardiac ablation, do not provide an opportunity to assess whether there is a functional relationship between cardiac output (and the associated nutrient delivery and waste removal) and tissue growth and development. That is, although embryos may survive complete elimination of cardiac output, under less severe situations is there a graded effect whereby there is a threshold level of blood flow below which embryonic development and growth begins to be retarded? To answer this question, we have measured eye growth and chorioallantoic vessel density index in a series of early-stage chicken embryos [Hamburger-Hamilton stage (HH) 16–24] that, through surgical intervention and partial conotruncal ligation, exhibit a wide range in heart rate, stroke volume, and cardiac output during critical developmental stages.

MATERIALS AND METHODS

Egg storage and incubation. Medium-sized chicken eggs (*Gallus gallus*) ranging from 55.4 to 73.2 g were obtained from a local breeder and shipped to Denton, TX. Fertilized eggs were stored at 6–10°C and 75–80% humidity for a maximum of 14 days before incubation. The chicken embryo heart begins to beat at HH 10 (10–12 somites), ~33 h after incubation. However, the contractions are not strong enough to begin blood flow until HH 12. Our intent was to work on relatively early stages with established, vigorous blood flow. Consequently, eggs were incubated in a commercial incubator (Lyon Electric, Chula Vista, CA) at 37.5°C and 55–65% humidity for ~3–4 days, yielding embryos of HH 17–19. Subsequent incubation for an additional ~24–36 h yielded HH 24 embryos.

Experimental manipulations. For surgery, eggs were removed from the incubator and placed air cell end upward in a thermostated chamber containing sand (38°C), which temporarily covered the lower half of the egg. The egg was opened with a 20-gauge needle at the blunt end where the air sac is located. A circular window of ~3-cm diameter was created to allow access to the underlying embryo. The air sac membrane was then carefully removed with fine forceps, ensuring that no chorioallantoic membrane (CAM) vessels were disturbed. Next, it was confirmed that the embryo was alive and at HH 17–19, and that the heart was beating strongly, which con-

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cluded the experimental treatment of this control group. A small thermistor was then inserted into the egg near the embryo to monitor temperature.

The first group of eggs consisted of "controls," in which the heart was exposed as described above but otherwise not disturbed. Controls were videotaped at ×400 with a video camera (Oscar 40-D) mounted on a Leica Wild M3Z dissecting microscope. The beating heart, the extraembryonic CAM vessels, and the eye were all recorded for measurements of heart rate, stroke volume, cardiac output, blood vessel index, and eye diameter (see below).

In the second group, videotaping for heart rate and cardiac output was carried out before (preligation) and at specified times after partial ligation of the conotruncal outflow tract (the arterial outflow tract from the ventricle). For the "preligation" treatment of the second group, the amnion was carefully bisected and removed to expose the conotruncus. A 10-0 monofilament nylon suture (Ethicon) was carefully placed around the outflow tract of the heart but was not tightened until recordings were made of heart rate and stroke volume. The subsequent "partial ligation" treatment consisted of gently tightening the ligature to partially occlude the outflow tract from the heart (25–50% reduction in cross-sectional area) without actually stopping cardiac output. Another set of video recordings were then made of heart rate and stroke volume.

Immediately after initial treatments and videotaping, the opening in the eggshell was covered with a piece of plastic film to ensure that the humidity remained high within the eggshell. All embryos were then returned to the incubator.

After a postsurgery incubation period of 4 h, partial-ligation embryos were removed and videotaped again. Partial-ligation embryos were then returned to the incubator once again. After ~12 h of further development, the partial-ligation embryos were removed from the incubator and staged, typically having developed to HH 18–20. These embryos were then once again videotaped, after which they were returned again to the incubator. A final set of measurements was made ~24–36 h later, corresponding to HH 21–24. The wet weight of embryos was measured at the end of measurements.

Video data analysis. Videotape of each embryo was analyzed with an imaging and analysis system (ImagePro). Stroke volume was determined from the difference between calculated end-diastolic and end-systolic volume of the ventricle, determined through optical imaging of the beating heart (see Refs. 5, 9, 17, 21, and 26 for detailed discussion of technique). Essentially, end-systolic and end-diastolic volume were determined from the formula $V = 4/3\pi ab^2$, where V is ventricle volume and a and b are the long and short radii of the ventricle, respectively.

Heart rate was determined by counting beats from examination of 1 min of videotape. The product of heart rate and stroke volume yielded cardiac output, in microliters per minute.

Embryonic eye diameter is an excellent indicator of growth because it shows large increases during days 3–5 (36) and its growth is easily quantified (8). Eye diameter (mm) was recorded for each embryo in each group for each indicated treatment.

A 4 × 5-mm section of the CAM was videotaped close to the dorsal-cephalic region of the embryo (×400). An index of CAM blood vessel density was determined by creating a 1-mm line perpendicular to the long axis of the embryo in the video image. CAM blood vessel density, 0.5 mm from the embryo, was determined by counting the number of all visible vessels intersecting this line and was expressed as the number of vessels per millimeter.

Statistical methods. Linear regressions, yielding correlation coefficients and P values, were performed to quantify the relationships among basic cardiovascular variables and developmental stage. One-way ANOVAs were performed to determine the significance of changes in variables with experimental treatment (acute and chronic ligations). Pairwise multiple comparisons (Student-Newman-Keuls) were carried out once a significant treatment effect had been demonstrated. A significance level of $P < 0.05$ was adopted for all statistical tests.

RESULTS

Basic cardiovascular variables—HH 16–24. Basic cardiovascular characteristics of control chicken embryos, and their relationship to developmental stage, are shown in Fig. 1. Heart rate was <100 beats/min at HH 16, increasing significantly to >135 beats/min by HH 24 (Fig. 1*A*). Over this same developmental period, stroke volume increased fivefold from ~0.05 μl/min at HH 16 to 0.25 μl/min at HH 24 (Fig. 1*B*). Not surprisingly, then, cardiac output—the product of heart rate and stroke volume—increased dramatically from ~2 μl/min at HH 16 to >35 μl/min by HH 24 (Fig. 1*C*). Figure 1*D* shows the relationship between heart rate and cardiac output. Although this relationship is highly significant ($P < 0.001$), the relatively low r^2 value of 0.314 indicates that there are multiple sources for the observed variation in cardiac output.

Cardiovascular effects of surgery and partial ligation. In 9 of 10 HH 17 embryos, the heart rate (~105–110 beats/min before surgery) decreased significantly to <100 beats/min with placement of the suture around the cardiac outflow tract and then decreased additionally with constriction of the outflow tract produced by partial ligation (Fig. 2, *A* and *B*). However, within 24 h of partial ligation and further development to HH 18–20, heart rate increased by ~20 beats/min to a value slightly but not significantly higher than that of undisturbed control embryos at that stage. Heart rate increased significantly from ~120 beats/min to nearly 130 beats/min after further development to HH 21–24. However, as at HH 18–20, the heart rate of embryos with ligations was not significantly different from that of controls at HH 21–24.

Mean values of stroke volume did not change significantly between preligation and partial-ligation treatments, but large interindividual variation was induced by partial ligation (Fig. 2*C*). Both preligation and partial-ligation treatment altered stroke volume in almost every HH 17 embryo, but, paradoxically, the changes were a combination of either large increases (3 embryos) or large decreases (7 embryos) (Fig. 2*D*). Similar to heart rate, stroke volume in HH 18–20 embryos with partial ligation had increased back to levels not significantly different from HH 17 controls and by HH 21–24 was significantly higher than at HH 17. At HH 18–20 and HH 21–24, the values in ligated embryos were not significantly different from control embryos at these stages.

Cardiac output followed the trend of stroke volume, with preligation and partial ligation having little effect on mean values but large effects on the interindividual variation in heart performance. Three of ten embryos showed increased cardiac output in the preligation state, but the remaining seven showed moderate to severe reduction in cardiac output (Fig. 2*F*). Not surprisingly, most (8 of 10) embryos showed a reduced cardiac output on partial ligation of the heart outflow tract. Cardiac output had increased back to control levels for this developmental group by HH 18–20, and by HH 21–24 had increased nearly fourfold as a consequence of joint increases in both heart rate and stroke volume. Just as for heart rate and stroke volume, ligated embryos at both HH 18–20 and HH 21–24 were not significantly different from controls at each of these stages (Fig. 2*D*).

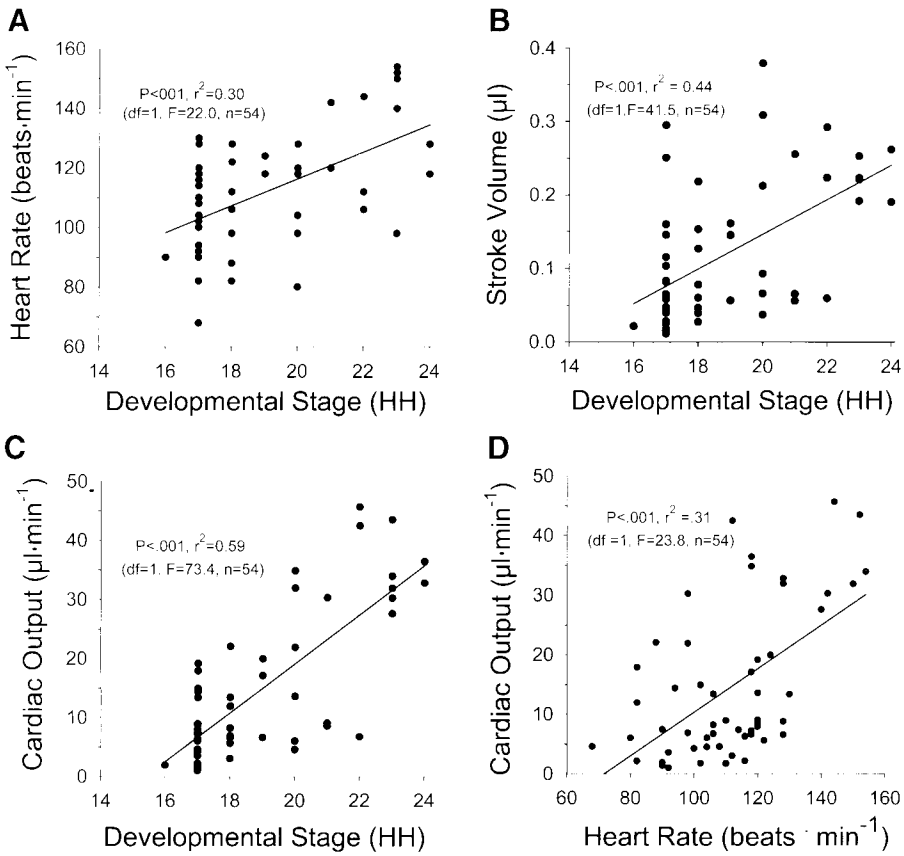

Fig. 1. Basic cardiovascular characteristics in chicken embryos. Heart rate (*A*) stroke volume (*B*), and cardiac output (*C*) as a function of developmental stage are shown. *D*: cardiac output as a function of heart rate. HH, Hamilton-Hamburger stage (see text); df, degrees of freedom.

Eye growth. Mean eye diameter in control populations increased significantly [$P < 0.001$, degrees of freedom (df) = 2, $F = 42.41$] from 0.6 mm at HH 16–17 to nearly 1.2 mm at HH 21–24 (Fig. 3*A*). Eye growth in the partial-ligation group followed an identical rate of growth. At no developmental stage were the means of the eye diameters of the control embryos significantly different from the means of those of the partial-ligation embryos (ANOVA, $P > 0.1$).

Highly noteworthy, however, is the fact that within a developmental group, there was no significant correlation between eye diameter and cardiac output even though there were very large differences in cardiac output at each stage (Fig. 3*B*). Embryos at HH 16–17, HH 18–20, and HH 21–24 showed 12-fold, 17-fold, and nearly 4-fold intragroup variation in cardiac output, respectively. Despite these enormous cardiac output differences within any given developmental group, there was no significant correlation ($P > 0.1$) between eye diameter and cardiac output within any of three groups. Thus it appears that although the eyes certainly get larger with development, and the heart gets larger with development (as evident in the larger stroke volume), the growth of the eye within any given state is not dependent on any particular level of cardiac output.

Vessel density index. Vessel density index of the CAM vessels in control embryos ranged from ~8 to 10 vessels/mm, with no significant change ($P = 0.124$, df = 2, $F = 2.27$) in CAM vessel density index from HH 17 to HH 24 (Fig. 4*A*). This suggests that although the CAM certainly grows and extends over the inner surface of the egg shell during early embryonic growth, the actual density of vessels within established areas of the CAM does not change. At no developmental stage was the CAM vessel index of the partial-ligation group significantly different from that of the control embryos ($P > 0.1$). As with eye growth, CAM vessel density was not significantly correlated with cardiac output at any developmental stage (Fig. 4*B*).

Body mass. Body mass in control embryos increased significantly ($P < 0.001$) and dramatically (~20-fold) from HH 16 to HH 24 (Fig. 5). The relationship between body mass and development was not significantly different between control embryos and those with partial conotruncal ligation over the development range of HH 21–26.

DISCUSSION

Basic cardiovascular variables—HH 16–24. Considerable cardiovascular growth occurs between HH 16 and HH 24. Although the increase in heart rate was relatively modest over this developmental period, the attendant increase in stroke volume was nearly 5-fold, resulting in the dramatic 10- to 15-fold increase in cardiac output over this same developmental period. Even though the correlation was highly significant ($P < 0.001$) between developmental stage and each of heart rate, stroke volume, and cardiac output, the relatively low correlation coefficients clearly indicate that unidentified factors [e.g., variation in epicardial surface area measurement, aortic valve regurgitation] serve as major sources of additional variation.

Mean values of these basic cardiovascular values—along with the considerable attendant variation—are in general ac-

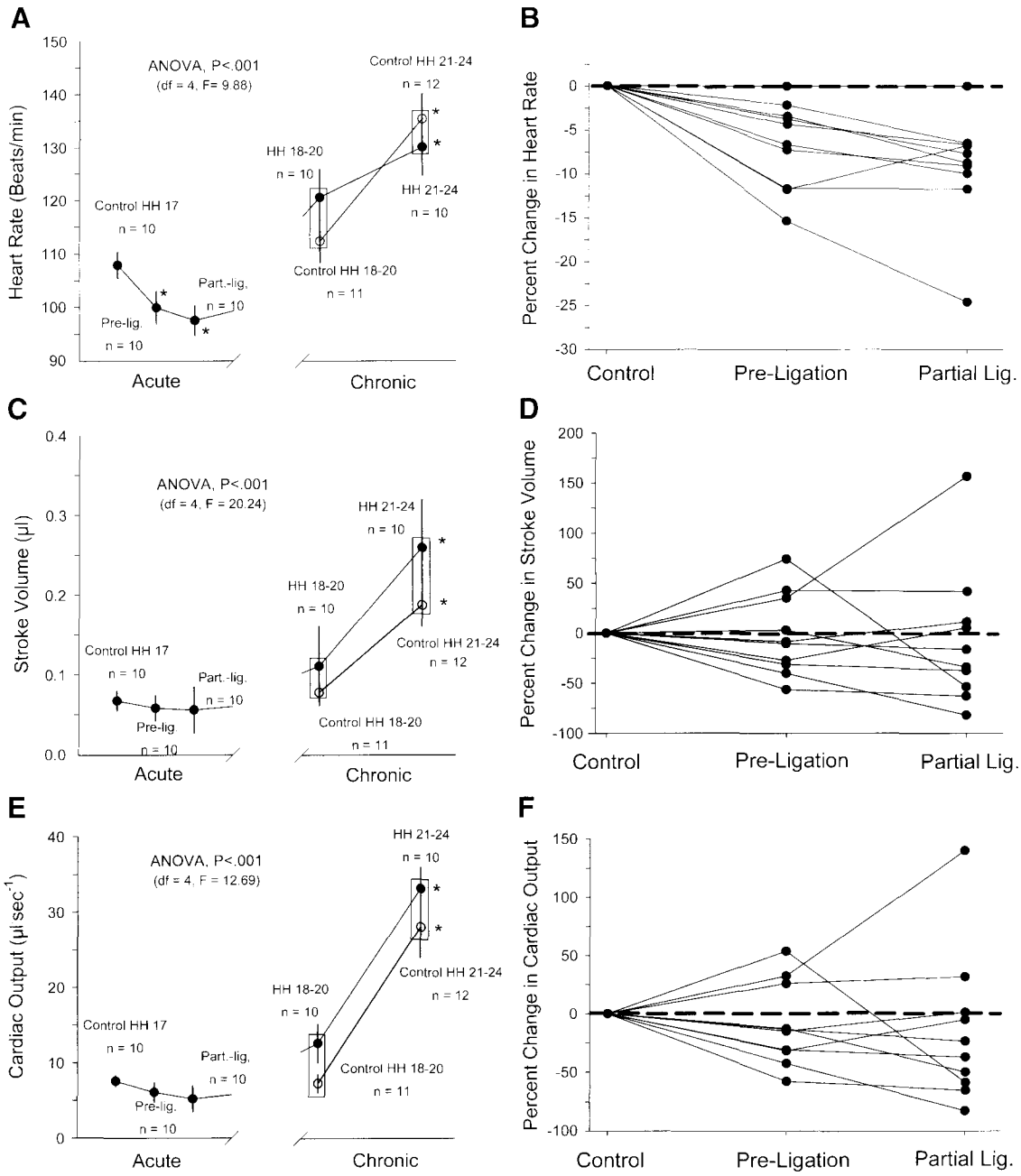

Fig. 2. Effect of experimental treatment on heart rate, stroke volume, and cardiac output in chicken embryos. *A*, *C*, and *E*: actual values of these variables under initial control conditions, immediately after preligation, and 5 min later after partial ligation—all in HH 17 embryos—and then at HH 18–20 (~20–28 h after ligation) and at HH 21–24 (~45–55 h after ligation) after partial ligation. Data points enclosed within a box are not significantly different. *Significant difference within a treatment group from the corresponding HH 17 control value (ANOVA, $P < 0.001$). To compare the highly variable responses of individuals, *B*, *D*, and *F* show the corresponding % changes in heart rate, stroke volume, and cardiac output that occurred during the acute phase of experimentation. Values are means ± SE.

cordance with those measured in chicken embryos at specific stages (9, 17, 22, 25, 26, 27, and 39) with a variety of techniques including optical and pulsed Doppler velocity determinations of stroke volume and optical or impedance determinations of heart rate. However, previous studies have not reported concurrent measurements of heart rate, stroke volume, and cardiac output over the HH 16–24 developmental range as in the present study.

Cardiovascular effects of surgery and partial ligation. The procedure used to open the egg, expose the embryo, and prepare it for ligation was initially intended to serve as a "sham" procedure. It is quite clear from Fig. 2, however, that this procedure was anything but a sham. Particularly susceptible was heart rate, which showed a significant decrease of nearly 10 beats/min merely from the act of placing an untied ligature inferior to the cardiac outflow tract. Although heart

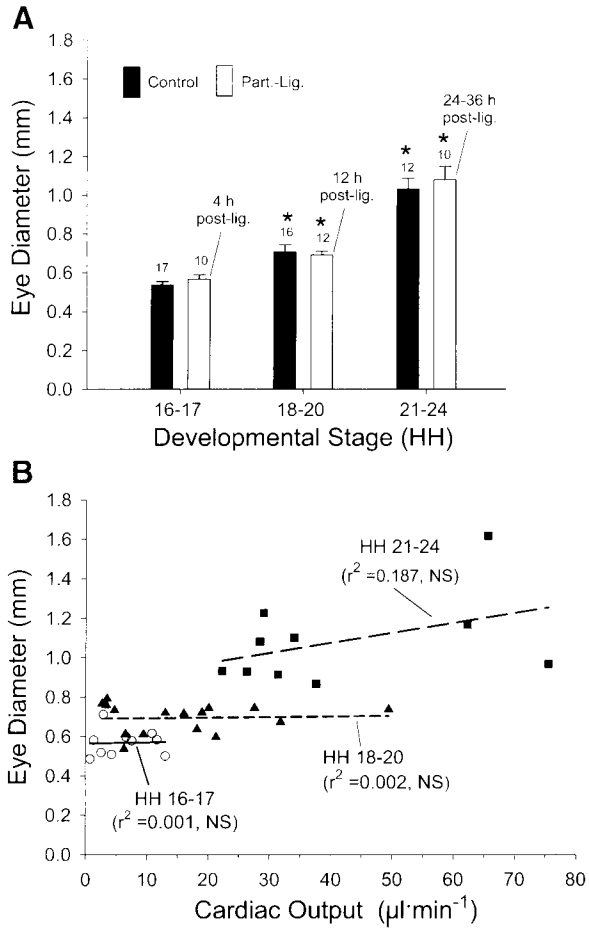

Fig. 3. *A*: eye diameter in control and partial-ligation embryos at HH 16–17, HH 18–20, and HH 21–24. Approximate postligation hours are indicated for each partial ligation population; *n* values are indicated above each bar. There were no significant differences between treatments in any of the 3 developmental groups measured ($P > 0.1$). However, within a treatment, there was a significant effect of development in both groups. (ANOVA, df = 2, $F = 42.44$, $P < 0.001$). *Significant difference from the corresponding value at HH 16–17 within each treatment group. *B*: eye diameter as a function of cardiac output in partial-ligation embryos. Separate linear regressions are shown for each developmental group. Although eye diameter increased with stage (*A*), there was no significant correlation ($P > 0.1$) between cardiac output and eye diameter within any developmental grouping. NS, nonsignificant.

rate in the chicken embryo is highly susceptible to embryonic temperature change, a phenomenon likely accounting for much of the heart variation for chicken embryos in the literature, we went to great lengths to ensure that the embryonic temperature was constant throughout the measurements. Subsequent partial ligation of the outflow tract caused an additional significant decrease in heart rate. These combined chronotropic effects were transient, however, because ~24 h after ligation, heart rate was not significantly different from embryos that had received no surgical preparation for ligation. Why placement and tightening of the ligature each caused a mild bradycardia is uncertain. At this early stage, the chick heart is devoid of innervation, eliminating reflex responses as a cause. Perhaps the pacemaker region of the heart is routinely disturbed by opening of the body wall and developing pericardium. As discussed below, a functional Frank-Starling mechanism is evident as early as HH 24 in chicken embryos. Although little cited, changes in cardiac wall stretching can also elicit direct chronotropic effects on the vertebrate heart, and this may account in whole or in part for the mild bradycardia associated with preligation and partial ligation. Notably, Keller et al. (26) found no chronotropic effects of acute conotruncal ligation in HH 21 chicken embryos, at variance with our own findings.

The effects on stroke volume of merely preparing the embryo for ligation were not as dramatic as heart rate, and there was no significant change in stroke volume in preligation embryos. Paradoxically, there was also no significant change in stroke volume after ligation, even though the diameter of the cardiac outflow tract was reduced by an estimated 25–50%. The result was that while the mean values of stroke volume and cardiac output of postligation embryos were not significantly different from controls at subsequent stages of HH 18–20 and 21–24, the large variation among similarly staged postligation embryos was maintained during the course of the experiments. The net effect, then, was that we were able to record eye and

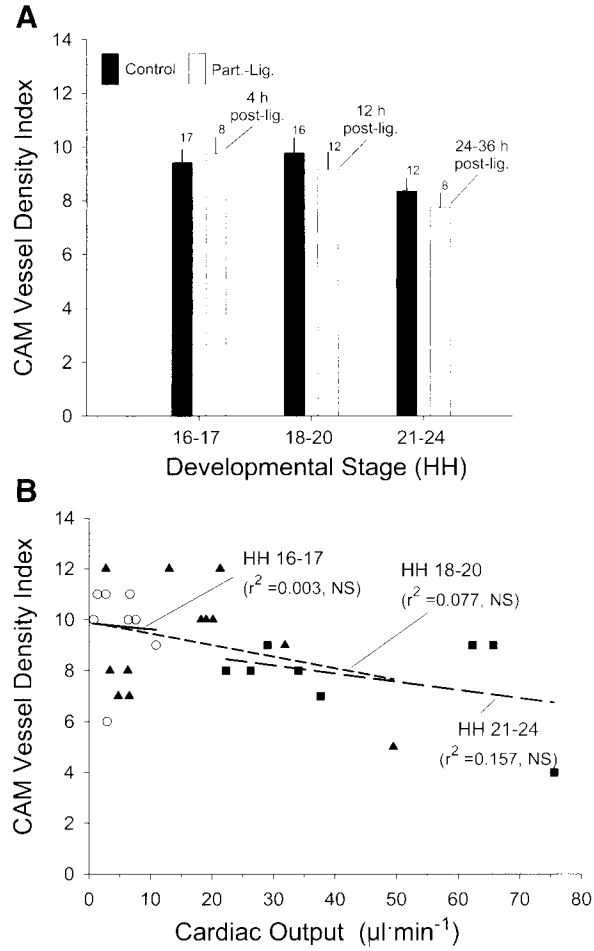

Fig. 4. *A*: Chorioallantoic membrane (CAM) vessel density index in control and partial-ligation embryos at stages HH 16–17, HH 18–20, and HH 21–24. Approximate postligation hours are indicated for each partial ligation population; *n* values are indicated above each bar. There were no significant differences either within or between treatment groups at any developmental stage (ANOVA, df = 2, $F = 2.27$, $P > 0.1$). *B*: CAM vessel density index as a function of cardiac output in partial-ligation embryos. Separate linear regressions are shown for each developmental group. There was no significant correlation ($P > 0.1$) between cardiac output and CAM vessel density index within any developmental group.

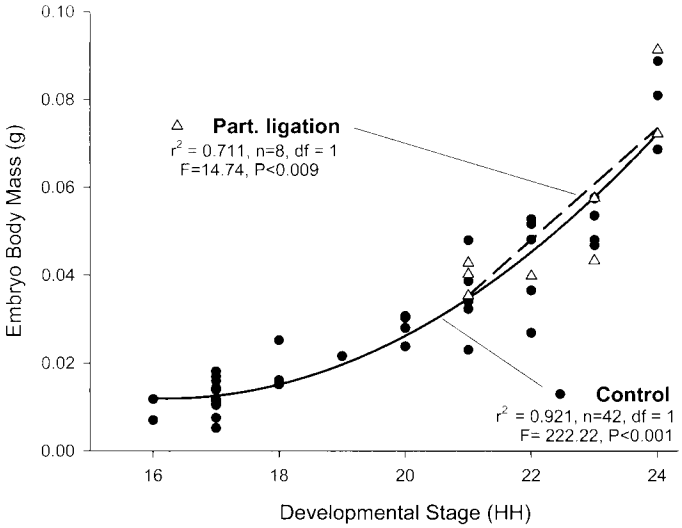

Fig. 5. Body mass as a function of developmental stage in embryos under control conditions or after partial conotruncal ligation for ~24 h (generally 4 stages). The slopes of the lines describing each relationship are not significantly different ($P > 0.05$)—that is, there is no significant effect of partial conotruncal ligation on body mass.

CAM vessel growth over very large ranges of cardiac output in similarly staged animals—the original intention of the study. If anything, the technique we used is likely to have overestimated forward stroke volume and thus overestimated cardiac output, because there is likely to be some AV regurgitation. Thus the lowest values of cardiac output that we recorded may well have been even lower, strengthening our findings.

Why partial ligation caused such large variation, including increases, in stroke volume and cardiac output in these experiments is unclear. Great care was taken to ensure consistency in procedure, and visual inspection suggested a similar degree of outflow tract occlusion. A point constriction of a blood vessel of <25% of its cross-sectional area should not have hemodynamic implications (32), although admittedly this observation comes from high-pressure, high-flow, mature mammalian systems. Perhaps the ligation was not severe enough to mechanically obscure flow through the outflow tract. Alternatively, it is possible that in a few of the embryos there might actually have been a compensatory increase in blood pressure to maintain and even increase flow after ligation, although this suggests a level of reflex adjustment that would seem unlikely at this early stage of development (see below).

It is noteworthy that large decreases in mean heart rate produced by preligation and partial ligation did not evoke corresponding increases in mean stroke volume, which one might expect if either or both of a Frank-Starling mechanism or a baroreceptor reflex were routinely in place by this point in development. Elements necessary for baro- and chemoreceptor reflexes actually develop surprisingly late in avian embryos—generally in the last third of incubation (4, 12, 13, 40, 41). However, the intrinsic characteristics of cardiac muscle responsible for Frank-Starling reflex might be anticipated to show up much earlier in avian development. In fact, a Frank-Starling mechanism is in place at least by HH 24 in the chicken embryo (1, 45). Additional experiments are warranted to determine the length-force relationships of very early embryonic cardiac muscle.

Impact of cardiac output on eye and vessel growth. Although partial ligation did not achieve the anticipated predicted universal decrease in stroke volume, it had the fortuitous effect of creating a very large range of cardiac outputs within a given developmental grouping. As evident in Figs. 3 and 4, within any given developmental grouping there were manyfold differences in cardiac output accompanying eye diameter and chorioallantoic vessel index. Eye diameter—a sensitive indicator of embryonic viability and growth—increased markedly during development, approximately doubling over the developmental periods considered. At the same time, cardiac output also increased with development. Importantly, however, there was no significant correlation between eye diameter and cardiac output within any given stage. Thus HH 18–20 embryos with highly reduced cardiac outputs of only 2–3 μl/min nonetheless still managed to achieve eye diameters not statistically different from those embryos with vastly higher cardiac outputs of 40–50 μl/min.

CAM vessel index, unlike eye diameter, showed no significant change over the developmental period measured (Fig. 4). Similar to eye diameter, however, within each of the three developmental cohorts there was no significant correlation between cardiac output and CAM vessel density despite the enormous cardiac output ranges.

Collectively, these data suggest that convective transport of respiratory gases, nutrients, and wastes provided by blood flow is not an absolute requirement for organogenesis and growth in early chicken embryos. It could be argued that convective transport is indeed required for eye growth and vessel proliferation, but that the experimental reduction in cardiac output in our experiments was still not small enough to retard their development. However, in those embryos suffering the greatest reduction in blood flow to 1/10th to 1/15th of the control value for embryos of that stage, there were no reductions in our indicators of development and growth. Even granting that such low flow was still sufficient to maintain some minimal required convective transport to and from the eyes and CAM vessels, it would appear that the great majority of blood flow in stages HH 16–24 is completely superficial to any need for growth and development.

Implications for prosynchronotropy hypothesis. The conventional view of embryonic blood flow is that it plays the fundamental "adult" role of tissue nutrient and waste transport. A secondary role for blood flow appears to be cardiovascular morphogenesis (26, 34, 37, 38, 47). The alternative hypothesis of prosynchronotropy suggests that the heart starts beating and delivering a convective flow of blood to the embryonic tissues well in advance of the tissues' actual need for convective transport of oxygen and nutrients. This "early" flow is thought to serve in angiogenesis rather than convective transport (7, 8, 42). The data from the present study indicate that levels of blood flow far below normal, approaching zero flow in some instances, have no significant effect on eye or CAM blood vessel growth. That is, in the early chick embryo these organs appear to be "hyperperfused" with respect to their need for nutrient and waste transport (which can be supplied by diffusion). The lack of dependence on blood flow by these organs would not occur if the early embryonic blood flow had an important convective function. Consequently, these data support the hypothesis of prosynchronotropy—at least for eyes and blood vessels.

Several possible explanations exist for our finding that highly reduced blood flow has no effect on growth of the eye, growth of the CAM vessels, or overall body mass. The first explanation is that the early embryo enjoys an enormous "safety factor" in cardiovascular design, with the developing heart generally hyperperfusing the circulation by a factor of 10–15. Although possible, this explanation is unlikely, because physiological safety factors resulting from even extreme natural selection are rarely greater than two- to fivefold given their inherent expense in creation and maintenance (14, 46). However, the whole concept of safety factor as it applies in a developing animal is certainly worthy of further study.

A second explanation for the seemingly high rates of perfusion is that even though the eye and CAM vasculature may require only a fraction of the perfusion rate normally provided under control situations, there may be some other unidentified tissue or tissues that require a much higher blood flow to differentiate and grow adequately. Nervous tissue is generally regarded as oxygen sensitive, but our unpublished data on the growth of the fore- and midbrain of the embryonic chicken during highly reduced blood flow show no gross morphological retardation apparent by visual microscopic examination. Moreover, the eye is generally regarded as one of the more oxygen-sensitive organs of the body, requiring a constant, large blood supply. At these early developmental stages the eye is easily one of the largest and most dominant structures with one of the most well-developed vascular beds, and as such would seem to have among the very greatest needs for a convective transport of materials to the eye's interior. Thus the eye would seem to be an excellent barometer for indicating whether cardiac output was suboptimal for any tissue.

This second explanation—that some other vascular bed needs a far higher perfusion rate for development—assumes that there must be a more or less equivalent rate of blood flow to all vascular beds, and that vascular beds with low nutrient demand are thus necessarily "hyperperfused" to ensure adequate blood flow to the more nutrient-dependent tissues. More efficient, of course, would be differential distribution among the numerous distinct vascular beds of the embryo. Unfortunately, we know very little about the potential for redistribution of blood flow during early development in vertebrate embryos. In one of the few studies addressing this question, Hu et al. (23) report that during chicken embryo development there is a gross redistribution of blood flow between the extraembryonic and embryonic circulation from 81%/19% in HH 18 chick embryos to 66%/34% by HH 24. The most likely explanation of this redistribution is that it reflects changes in the basic impedance of the various growing vascular beds rather than active vasomotion designed to redirect blood flow. Although such active distribution occurs in later-stage avian embryos (15), studies on a variety of baro- and chemoreflex arcs suggest that the maturation of the necessary sensory and motor components for these reflexes occurs in the last one-half to two-thirds of development (4, 12, 13, 39). Thus it would be surprising to find any capability for active—even local—autoregulation in the first few days of blood flow generation.

A third explanation for what would appear to be the gross hyperperfusion of the eye and CAM relative to their needs for growth is that the heart begins to beat for some purpose other than generating convective blood flow for transport of gases, nutrients, and wastes. Several possibilities exist here. In addition to these blood-borne substances, blood flow transports heat and pressure. The ability of the microscopic circulation to transport heat from one region to another is near-infinitesimal, given the relatively huge heat sink that is formed by the surrounding albumen and yolk. Moreover, the tiny caloric heat generation by the embryo does not create any regional thermal gradients within the egg until very late in development (2). Consideration of the transmission of pressure (energy), however, is more intriguing and offers additional possibilities. The role of the circulation in shaping the developing heart has long been investigated—and debated (19, 24). Although blood flow and centrifugal forces play a role in normal heart development, the heart will nonetheless develop its essential structures without hemodynamic sculpting (18, 24, 30, 31).

Another "pressure-related" reason for early generation of pressure and flow involves angiogenesis. Endothelial cells respond to shear stress and strain by secreting VEGF and other vasculogenic factors [e.g., angiopoietins, fibroblast growth factors (FGF-2), transforming growth factors (TGF-β1), ephrin, and integrins] that, through a paracrine effect, stimulate endothelial cell division and proliferation (16, 28, 29, 38, 44). Could the major purpose of the early embryonic heartbeat be to create a pulsating "blood pressure wedge" that helps stimulates endothelial cell division in the very most peripheral tips of the growing circulation? If the purpose of the heartbeat is to promote vasculogenesis, then embryos with reduced cardiac output might be expected to see reduced blood vessel proliferation and growth. Unfortunately, we could not accurately visualize the sprouting microcirculation leading to capillary bed formation—the region of the circulation where vasculogenesis in the embryo is most active. However, future experiments will focus on testing the prosynchronotropy hypothesis by examining the role of blood pressure and flow in the vascular sprouting and proliferation of embryonic vascular beds.

Whether we ultimately determine that embryos have very large cardiovascular safety factors, have very large differential demands for convective transport by blood among embryonic tissues, or have a need for the heartbeat to create pressure fluctuations that stimulate blood vessel growth, it is clear that the generally close correlation between cardiac output and tissue/organ function that we observe in perinatal and adult animals simply does not hold for the early embryo.

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