Residual strain and strain, i.e., the stress and strain remaining in a solid when all external loads are removed, may be produced in biological tissues by differential growth. During cardiac development, residual stress and strain may play a role in cardiac morphogenesis by affecting ventricular wall stress.

After a transmural radial cut, a passive ventricular cross section opens into a sector, and the size of the opening angle provides a measure of the circumferential residual strain. Residual strains were characterized in this manner for the apical region of the diastolic embryonic chick heart for Hamburger-Hamilton stages 16, 18, 21, and 24 (approximately 2.5, 3.5, 4.0, and 4.5 days, respectively, of a 21-day incubation period). The average opening angle at these stages was $107\pm10^\circ$, $79\pm10^\circ$, $73\pm11^\circ$, and $74\pm7^\circ$, respectively ($n\geq5$ for each stage). These measured angles were correlated with changes in ventricular morphology. Scanning electron micrographs of the apex revealed that the wall of the ventricle is smooth at stage 16. Then at stage 18, myocardial trabeculae develop, forming ridges with primarily a circumferential orientation. By stage 21, the trabeculae develop into a mesh, giving the ventricular wall a spongeliike appearance, and the preferred orientation is lost by stage 24. The large decrease in opening angle between stages 16 and 18 corresponded to the onset of trabeculation, which is the greatest change in form during the studied stages. We speculate that residual strain is an important biomechanical factor during cardiac morphogenesis. (Circulation Research 1993;72:455–462)

**KEY WORDS** • cardiovascular development • cardiac mechanics • myocardial strain • chick embryos

**During** the past decade, led primarily by the efforts of Professor Y.C. Fung, University of California, San Diego, researchers have begun to realize the significance of residual stress in cardiovascular mechanics. Residual stress, defined as the stress remaining in a solid when all external loads are removed, can be generated by differential growth. Consider, for example, a cube of cells in mechanical equilibrium with no applied loads. If a single cell in the center of the cube grows larger, it pushes against the surrounding cells, which push it back with equal stresses. Thus, a local concentration of residual stress forms near the growing cell, even though the cells remain free of external loads and any changes in form may be difficult to detect. Since the cube remains in equilibrium, the residual stresses are self-equilibrating.

The residual stress can be relieved by cutting the cube, e.g., by cutting out the growing cell, as the tissue deforms into the zero-stress state. We define residual strain as the strain in the intact cube relative to its zero-stress configuration. Measurements of the residual strain correspond to the growth that has taken place.

In the absence of residual stress and strain, the combined effects of large deformation and the highly nonlinear constitutive relations that characterize the behavior of many soft tissues can create severe stress concentrations, even under normal loading conditions. Since efficient use of load-bearing material requires more or less uniform stresses, stress concentrations in man-made structures are often reduced by building in residual stresses that oppose the stresses due to loading. Living tissues generate their own residual stresses through differential growth, and because of the characteristic strain-stiffening behavior of many tissues, even small residual strains can dramatically reduce stress concentrations, producing a more efficient biomechanical design. (The terms strain stiffening and strain softening refer, respectively, to an increase and decrease in the material elastic modulus with increasing strain.) In the embryo, these effects likely influence cardiac morphogenesis, as the heart transforms from a single muscle-wrapped tube into a four-chambered pump.

In studies of the mature cardiovascular system, Fung and coworkers and Vaishnav and Vossoughi have quantified residual strains in arteries, and Omens and Fung have measured these strains in the passive rat left ventricle. In arteries subjected to normal blood pressures, residual strains greatly decrease stress concentrations. In the mature left ventricle, however, material anisotropy reduces transmural stress gradients to a large extent, with the effects of residual strain being less notable. Our model for the stage 16 embryonic chick
ventricle shows similar behavior.\textsuperscript{11} In this case, however, the lesser effects of residual strain are due to the strain-softening behavior of the primitive cardiac tissues, which are essentially isotropic and contain little collagen.\textsuperscript{12,13} Although these studies indicate a minor role for residual strains in basic cardiac mechanics, we speculate that even small changes in stresses due to residual strain may be important in adaptive growth and morphogenesis. In the mature heart, this speculation is supported by the sensitivity of morphology to wall stress; e.g., the ventricular wall thickens in response to pressure overload to lower wall stresses back to normal.\textsuperscript{14}

The relation between residual strain and loading conditions in the heart is currently unknown. In the aorta, however, hypertension changes residual strains. On banding (constricting) the distal aorta in rats, Fung and Liu\textsuperscript{4,6} found that the increased resistance to flow raises the proximal aortic blood pressure. In response to this increased loading, the residual strain in the aorta, as characterized by the opening angle of a radially cut cross section, increases to a peak value in a few days, and then decreases toward an asymptotic value in a few weeks, as the artery remodels. The opening angle, which is the circumferential angle between the cut edges, corresponds to the circumferential residual strain in the unloaded intact state relative to the cut state of the artery. In theory, the increased pressure acutely produces abnormally large stress concentrations, which are then alleviated by increased residual strain due to adaptive growth. In the embryonic chick heart, adaptive growth has been demonstrated as early as stage 21. When the outflow tract is banded, ventricular pressure increases, and the heart grows larger than normal, but its rate of development remains unchanged.\textsuperscript{15} As in arteries, changes in residual strains likely accompany this growth.

We characterized residual strains in the apical region of the diastolic embryonic chick heart at stages 16, 18, 21, and 24 (approximately 2.5, 3.5, 4.0, and 4.5 days, respectively, of a 21-day incubation period). During this period of development, beginning at the apex, the ventricle transforms from a smooth-walled to a trabecular chamber. Furthermore, between each of the studied stages, the embryo doubles in weight, and ventricular pressure increases markedly.\textsuperscript{12,16} We hypothesized that residual strains change in the embryonic heart as its form and work load change. Residual stress was relieved by cutting a cross section radially, after which the cross section opened immediately. Circumferential residual strains, as characterized by the opening angle of the resulting sector, changed during trabeculation. Since residual strains are likely related to tissue growth, these data lay the foundation for studies of the connection between function and growth in the embryonic heart.

Materials and Methods

Fertile white Leghorn chicken eggs were incubated blunt end up in a forced-draft 38.5°C incubator to Hamburger-Hamilton\textsuperscript{17} stages 16, 18, 21, and 24. The embryo was exposed by opening a small window in the egg shell and removing the inner shell membrane adjacent to the embryo. We acquired video images of the embryo using a Dage 70 series video camera (Dage-MTI, Inc., Michigan City, Ind.) mounted on an M400 Wild photomicroscope.\textsuperscript{16} The video field was superimposed with real time ±0.005 second using a time-date generator (model V TG-33, FOR.A, West Newton, Mass.) and recorded on a videocassette recorder.

Krebs-Henseleit perfusate\textsuperscript{8} containing the calcium chelator EGTA (10 mM) and the calcium channel blocker verapamil (2×10⁻⁴ µg/µl) was oxygenated with 95% O₂ gas. We perfused the embryo at low pressure and high flow to arrest the ventricle in diastole. We then removed the entire heart and sliced the ventricle with a microdissecting blade on both sides of the apex to generate a cylinder approximately 100 µm in length (Figure 1). The cross section of the slice was imaged (Figure 2, top panel), and then the cylinder was cut once radially with a blade (Figure 2, middle panel) at the inner curvature of the curved tube (“inner cut”) in stage 16, 18, 21, and 24 embryos or at the outer curvature (“outer cut”) in stage 16 and 21 embryos (see Figures 1 and 2). The time from isolation to transection of the heart was less than 3 minutes. We observed the appearance of the myocardium, cardiac jelly, and endocardium before perfusion, after perfusion, and after transection to ensure that the sliced segment was not damaged or distorted. The cross section of the cut slice was imaged intermittently for 10 minutes.

In making the geometric measurements, we modified the method used by Omens and Fung.\textsuperscript{9} The procedure for an inner cut is described in the Figure 2 legend; the procedure for an outer cut is similar. Individual video fields were analyzed at a work station that included a personal computer, a frame-grabbing board (model M8, TARGA), JAVA video analysis software (Jandel Scientific, Corte Madera, Calif.), a Microsoft mouse, and a multipurpose video monitor.

Five or more embryos were analyzed at each stage. Using the digitizing software, we measured inner, midwall, and outer opening angles (θ, θ, and θ) (see Figure 2, bottom panel). Each minute for 9 minutes, we measured only the midwall opening angle θ, which corresponded to that measured by Omens and Fung.\textsuperscript{9} At 10 minutes, all three opening angles were measured, and the average opening angle (θ) was computed as θ/3. Data are reported as mean±SEM, with statistical comparison done by two-way analysis of variance and regression analysis. The significance level was defined as \( p<0.05 \).

In separate studies, stage 16, 18, 21, and 24 embryonic chick hearts were arrested in diastole as described above. The hearts then were perfusion-fixed with 2% glutaraldehyde and 1% formalin using the verapamil and Krebs-Henseleit-supplemented buffer. The apex of each heart was sectioned in preparation for scanning electron microscopy.

Results

We first examined the sensitivity of the opening angle to the location of the radial cut and the time of measurement. In the stage 16 ventricle, an outside cut produced an opening angle slightly larger than but statistically similar (\( p>0.05 \)) to that of an inside cut (Figure 3). For the stage 21 ventricle, the trend reversed, with an outside cut yielding a slightly smaller but statistically similar (\( p>0.05 \)) angle relative to that of an inside cut. In addition, midwall opening angles (Figure 3) changed little with time during the recorded 10 minutes after the cut. Since the differences between
inside and outside cuts were relatively small and since the deformation was virtually time independent, we used data from inside cuts at the 10-minute mark to characterize each heart (Figure 4).

The average opening angle (Figure 4) was significantly different from the midwall opening angle for the stage 21 and 24 ventricles. Because of the irregularly shaped edges of the cut (Figure 2, middle panel), we used the average angle as a representative measure of the circumferential residual strain. As development progressed, the average opening angle (θ at 10 minutes) decreased from a value of \(107 \pm 10^\circ\) at stage 16 to \(79 \pm 10^\circ\) at stage 18 and then remained fairly constant with values of \(73 \pm 11^\circ\) at stage 21 and \(74 \pm 7^\circ\) at stage 24.

During the studied stages, the embryonic chick ventricle is a looped tube without formed valves, coronary arteries, or innervation. At stage 16, the smooth-walled ventricle is composed of three layers: an inner layer of endocardium, a middle layer of cardiac jelly (extracellular matrix), and a relatively thin outer layer of myocardium (Figure 5a). Trabeculation of the wall begins in the apical region at stage 17, as myocardial ridges form with a prevailing anteroposterior circumferential orientation. At stage 18, while still restricted to the apex, the myocardial ridges grow more prominent and become interconnected (Figure 5b). As trabeculation progresses, the relative amount of cardiac jelly decreases. By stage 21, the body of the embryonic ventricle is trabecular, and the wall now resembles a sponge (Figure 5c). At stage 24, the anteroposterior orientation of the trabecular sheet disappears, with the wall assuming a more randomly oriented honeycomb appearance (Figure 5d).

**Discussion**

The current study revealed two fundamental results related to the maturation of the embryonic heart. First, residual strain is present in the diastolic embryonic chick heart as early as stage 16. Second, the magnitudes of the opening angles and, therefore, the residual strains change during development. These changes affect ventricular wall stresses during the cardiac cycle and may influence morphogenesis.

The embryonic heart undergoes morphogenesis from a muscle-wrapped tube to a four-chambered heart while providing circulatory support for the rapidly growing, metabolically active embryo. In this initial study of residual strains in the embryonic heart, we focused on the apical region because it is a site of rapid morphological changes during the studied stages (Figure 5). Myocardial trabeculation initiates at the apex at stage 17, and the interventricular septum begins to form there at stage 21.

**Biomechanical Interpretation of Results**

The method for relieving residual stress has important implications for the analysis of the results. Ideally, after a single radial cut, the ventricular segment assumes a state of zero stress. If the cardiac tissue is pseudoelastic, reclosing the cross section would restore the original residual stresses and strains. Therefore, we can interpret our results by examining the deformation from the cut to the uncut configuration. Since the uncut ventricle has no external loads, the forces needed to close the ring must be self-equilibrating; i.e., they alone must satisfy equilibrium. To a first approximation, equal and opposite moments applied to the cut edges are adequate and produce residual bending stresses and strains in the closed cross section. For this thick “curved beam,” the (circumferential) bending strains vary nonlinearly but monotonically across the wall from peak tension at the epicardium to peak compression at the endocardium. The values of the residual strains depend primarily on the opening angle and the ratio of radius to thickness of the cross section, i.e., on the geometry. On the other hand, the values of the bending...
moments and stresses depend not only on these strains but also on the material properties of the ventricle.

The ratio of radius to thickness in the embryonic chick ventricle is similar (approximately 1.5 at end diastole) at each of the studied stages. According to the above arguments, therefore, the decrease in the opening angle between stages 16 and 18 corresponded to a decrease in residual strains. However, since first-approximation constitutive (stress-strain) relations indicate that the modulus of the myocardium is higher than that of the cardiac jelly and increases from stage 16 to stage 18, the residual stresses may actually increase. Likewise, the similar opening angles from stage 18 to stage 24 indicate similar residual strains. However, since material properties likely change, the residual stresses probably are different.

The small differences in opening angles due to inside and outside cuts at the same stage (Figure 3) suggest...
slightly nonhomogeneous residual strains around the circumference. In the aortic arch of the rat, Liu and Fung found that an inside cut yielded a larger opening angle than an outside cut. They speculated that the circumferential variation in stress that is present in the wall of a pressurized curved tube like the aortic arch induces differential growth. In stage 16 and 21 chick hearts, the differences in opening angles due to inside and outside cuts were similar to those found in the aorta (Figure 3), but in the stage 16 ventricle, the outside cut produced the greater opening angle, whereas the reverse was true in the stage 21 heart. Since the curvatures of these ventricles are not substantially different, this behavior is likely due to different in vivo wall-stress distributions between the smooth-walled and trabecular ventricles.

Potential Biological Sources of Residual Strain

As discussed earlier, constrained growth produces residual strain. Consider a heart tube with no residual strains, such that it would not open when cut. Endocardial growth would increase the unstressed circumference of the endocardium. If the outer layers of the tube do not also grow, they would inhibit the length change of the endocardium, producing residual compressive strain. Cell death, which occurs during cardiac development, would have the opposite effect in this region, with tensile strain possibly being created near the endocardium.

The residual stresses that produce the opening after a radial cut could be produced by a higher growth rate of the inner layers relative to the outer layers (differential growth), by a greater cell death rate in the outer layers (differential death), or by a combination of these processes. The first of these mechanisms would produce compression in the inner layers to push the cut ventricle open; the second would produce tension in the outer layers to pull it open. During early cardiac development, growth is due to hyperplasia (more cells) rather than hypertrophy (bigger cells). Thus, changes in the transmural distribution of cell number may alter residual stress and strain. The available quantitative cell growth and death rate data are not suitable for direct correlation with our results. The morphology (Figure 5) suggests that tension in the compact layer is a likely source of the residual stress; however, relatively little epicardial cell death occurs in the embryonic heart.

Before ventricular trabeculation, i.e., at stage 16, interstitial pressure also may contribute to the residual strains. Manasek et al measured swelling pressures of 0.1–0.2 mm Hg in the cardiac jelly of chick embryos; these pressures are similar to end-diastolic pressures in the tubular ventricle. Recall that the cardiac jelly is extracellular matrix sandwiched between the endocardium and myocardium (Figure 5a). Since the myocardium is thicker than the endocardium, the jelly pressure would produce a residual compression, centered toward the endocardial layers, with a consequent increase in tension in the myocardium. Indeed, Nakamura and Manasek observed that, when the cardiac jelly is digested by hyaluronidase, the myocardium takes on a flaccid appearance. This added effect may have been partly responsible for the greater opening angle of the stage 16 ventricle relative to those at the other stages (Figure 4).

Physiological Implications

When correlated with those of other studies, our results suggest that residual strains are important in cardiac development. First, the opening angles measured in the embryonic ventricle were larger that those measured in the mature rat left ventricle (45 ± 10°). Second, in our model for the stage 16 chick ventricle, residual strains increase the peak wall stresses, which occur in the outer layer of myocardium during systole. Beginning at stage 17, the highest growth rate occurs in this outer layer; thus, if this growth is stress-modulated, residual strains may influence this process. (In arteries and the mature left ventricle, the highest stresses occur in the inner layers, and residual strains decrease the peak stress.) Finally, changes in cell shape accompany some morphogenetic processes, and these shape changes may reflect residual strains. For example, during looping of the early cardiac tube (stages 12–14), cells in some portions of the ventricle become elongated, whereas other cells become flat. It is not known whether these changes produce or are caused by the...
FIGURE 5. Scanning electron micrographs of apical cross sections of embryonic chick ventricles arrested in diastole. Panel a: Stage 16. The ventricle is smooth-walled and composed of endocardium, cardiac jelly, and a relatively thin outer myocardial sleeve. Panel b: Stage 18. Myocardial trabecular ridges are present with primarily an anteroposterior circumferential orientation.

looping. The analysis of cell shapes before and after cutting a ventricle would provide insight into the role of cell shape change on the looping mechanism.

For several reasons, we speculate that stress, not strain, modulates growth and morphogenesis. First, according to our model for the stage 16 chick heart, the peak "operating" wall stresses during the cardiac cycle occur in the outer layer of myocardium, but the peak strains occur in the endocardium. The large myocardial stresses may induce the growth of myocardial trabeculae at stage 17. Second, strain must be computed relative to a chosen reference configuration, whereas stress depends only on the current geometry (and the loading). Since the heart never actually "sees" the
zero-stress configuration in vivo, the choice of a reference state for strain is not clear. Third, research on the mature cardiovascular system has shown that, in response to abnormal loading conditions, arteries and hearts often grow in ways that return average wall stresses to near normal levels.

If stress is the biomechanical factor that regulates growth in the embryonic heart, then residual strains affect the process indirectly by altering the stresses. We speculate that the relatively small residual strains in the mature heart keep wall stress at "growth equilibrium" levels. In the embryonic heart, the elevated residual strains would boost the peak stresses in the outer myocardial layer, inducing growth or morphological changes. Indeed, the residual strains that correspond to the remarkably similar opening angles from stage 18 to

FIGURE 5.  Panel c: Stage 21. The wall, now composed of thin trabeculae, resembles a sponge with an outer compact layer of myocardium. Panel d: Stage 24. The orientation of the trabeculae becomes more random. Bars, 100 µm.
stage 24 (Figure 4) may increase peak wall stresses just enough to maintain the process of trabeculation. In spite of the enormous changes in form that occur during these stages (Figure 5), it appears that the ventricle grows in a way that keeps the residual strains nearly constant.

**Sources of Error**

The perfusate used to arrest the hearts in diastole was modeled after the calcium blocking and chelating solution used by Omens and Fung. Similar to their results for the mature rat left ventricle, the midwall opening angles (θ) in stage 16–24 chick ventricles changed little with time after cutting (see Figure 3). These data indicate negligible contracture, which would produce a gradual increase in θ with time. It is possible, however, that θ increased during the short time between cutting and the start of image recording. If so, this response was likely due to viscoelasticity, especially for the stage 16 heart with its high cardiac jelly content, rather than the longer time contracture mechanism.

The irregular shapes of the ventricular slices may have led to measurement errors. For example, the point O, as defined in the cut cross section (Figure 2, bottom panel), was not the center of an idealized circular sector. Determining the location more accurately would have required well-defined boundaries, which were difficult to see if the slice was slightly tilted. Since small changes in the position of O should not affect the results significantly, we used the measurement procedure of Omens and Fung.

Interpreting the results of this study depend on how well a single radial cut produces the actual zero-stress state. By showing that a second radial cut produces little additional deformation, Omens and Fung demonstrated that one radial cut is adequate to produce the stress-free state in the mature rat left ventricle. Since the small size of the stage 16–24 chick heart prohibits successive cuts without damaging the myocardium, determining the adequacy of a single cut in these early stages of development may require other techniques.

**Conclusions**

We found that residual strains are present in the diastolic embryonic chick ventricle during stages 16–24 and that these strains change during development. Our results suggest that residual strains, through their influence on wall stress, are one mechanism the developing heart uses to modulate growth during primary morphogenesis. Experiments that quantify changes in residual strain due to altered loading conditions are needed to define their precise role in cardiac development.

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