Remodeling of Chick Embryonic Ventricular Myoarchitecture Under Experimentally Changed Loading Conditions

DAVID SEDMERA,1,2* TOMAS PEXIEDER,2 VLASTA RYCHTEROVA,3 NORMAN HU,4 AND EDWARD B. CLARK4

1Institute of Physiology, Faculty of Medicine, University of Lausanne, CH-1005 Lausanne, Switzerland
2Institute of Histology and Embryology, Faculty of Medicine, University of Lausanne, CH-1005 Lausanne, Switzerland
3Department of Pathology, Third Medical Faculty, Charles University, 100 00 Prague 10, Czech Republic
4National Institutes of Health Specialized Center of Research in Pediatric Cardiovascular Diseases, Strong Children’s Research Center, Department of Pediatrics, University of Rochester School of Medicine and Dentistry, Rochester, New York 14642

ABSTRACT

Adult myocardium adapts to changing functional demands by hyper- or hypotrophy while the developing heart reacts by hyper- or hypoplasia. How embryonic myocardial architecture adjusts to experimentally altered loading is not known. We subjected the chick embryonic hearts to mechanically altered loading to study its influence upon ventricular myoarchitecture.

Chick embryonic hearts were subjected to conotruncal banding (increased afterload model), or left atrial ligation or clipping, creating a combined model of increased preload in right ventricle and decreased preload in left ventricle. Modifications of myocardial architecture were studied by scanning electron microscopy and histology with morphometry.

In the conotruncal banded group, there was a mild to moderate ventricular dilatation, thickening of the compact myocardium and trabeculae, and spiraling of trabecular course in the left ventricle. Right atrioventricular valve morphology was altered from normal muscular flap towards a bicuspid structure. Left atrial ligation or clipping resulted in hypoplasia of the left heart structures with compensatory overdevelopment on the right side. Hypoplastic left ventricle had decreased myocardial volume and showed accelerated trabecular compaction. Increased volume load in the right ventricle was compensated primarily by chamber dilatation with altered trabecular pattern, and by trabecular proliferation and thickening of the compact myocardium at the later stages. A ventricular septal defect was noted in all conotruncal banded, and 25% of left atrial ligated hearts.

Increasing pressure load is a main stimulus for embryonic myocardial growth, while increased volume load is compensated primarily by dilatation. Adequate loading is important for normal cardiac morphogenesis and the

Abbreviations used: HH, Hamburger-Hamilton; LAL, left atrial ligation; LHH, left heart hypoplasia; LV, left ventricle; RV, right ventricle; SEM, scanning electron microscopy; VSD, ventricular septum defect.

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*Correspondence to: David Sedmera, Department of Cell Biology and Anatomy, Medical University of South Carolina, 171 Ashley Avenue, Charleston, SC 29425. E-mail sedmerad@musc.edu

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With increasing size of the organism, the heart architecture is modified accordingly to cope with increased demands. Examples of this relation between structural and functional requirements can be found among species during ontogenesis. In fish, an increasing proportion of the compact myocardium with emergence of coronary supply correlates with the activity and life style of individual species (reviewed by Agnisola and Tota, 1994). The proportion of compact myocardium increases during embryogenesis (Blausen et al., 1990; Pham, 1997; Sedmera et al., 1998). Apart from increasing thickness, the architecture of the myocardial wall undergoes extensive modifications (Sedmera et al., 1997). Rychterova (1971) studied the mechanism of thickening of the right ventricular wall in the chick embryo, and showed that it is partly due to the compaction of the basal parts of the trabeculae. These results show that normal developmental adaptation to physiologically increasing load is an augmentation of proportion and thickness of the compact myocardium.

The influence of hemodynamics on heart morphogenesis is well recognized. Recently, Hogers et al. (1997) demonstrated that there is a close correlation between modified intracardiac streams and abnormal ventricular septation after left vitelline vein clipping. Hemodynamic patterns alter endocardial cells alignment (Icardo, 1989) possibly due to increased shear stress. It is therefore reasonable to expect that changed hemodynamic conditions may also influence cardiomyocyte assembly at the organ level.

The heart has a considerable functional plasticity enabling compensation when the functional demands are changed abruptly. The adult heart responds by hypertrophy and/or dilatation according to the speed and magnitude of the increased load (Hutchins et al., 1978; Castaneda et al., 1994; Rockman et al., 1994). Pressure load is a more powerful stimulus for cardiac growth than volume load (Zak, 1974). Embryonic or fetal heart responds to altered load conditions in a different manner. Increased pressure load induced by outflow tract constriction in the chick embryonic heart (Clark et al., 1989) or ascending aorta banding in the fetal guinea pig (Saiki et al., 1997) results in cardiomyocyte hyperplasia rather than hypertrophy. Similarly, decreased afterload by treating the embryo chronically with verapamil leads to smaller hearts that contain fewer cells of normal size (Clark et al., 1991). Investigations of rearrangement of cardiomyocyte assembly in this model of decreased load conditions revealed decreased compact layer thickness with relatively more trabeculae (Sedmera et al., 1998).

Another approach to study structural adaptations to altered hemodynamic conditions is left atrial clipping or ligation (LAL), which redirects the blood streams from left to right thus resulting in underdevelopment of the left heart structures and compensatory overdevelopment of the right ventricle (Rychter and Lemez, 1964; Rychter et al., 1979). Unlike conotruncal banding in which the heart works against an increased resistance (afterload), this procedure changes the distribution of blood flow thus creating a combined model of increased/decreased preload. At later stages in severe cases of left heart hypoplasia (LHH), the right ventricle has to act as a systemic pump. This model, based on mechanical intervention with normal hemodynamics, is one possible experimental approach to study human hypoplastic left heart syndrome. Production of chick LHH was also achieved by obstructing the left atrioventricular ostium with an insertion of nylon thread (Harh et al., 1973; Sweeney, 1981).

Hypoplastic left heart syndrome is relatively rare congenital pathology occurring in approximately 3.8% of human congenital heart disease but contributing significantly to its mortality. Characterization and description of this syndrome are attributed to Lev (1952), who called it “hypoplasia of the aortic tract complex.” The term hypoplastic left heart syndrome was introduced by Friedman et al. (1951). It occurs sporadically in other species as well—pig (Shaner, 1949), cattle and lamb (van der Linde-Sipman, 1978), minipig (van der Linde-Sipman and Wensing, 1978), and domestic cat (van Nie, 1980) which suggests that its cause lies in perturbation of a generally operating developmental mechanism.

In order to investigate the reactions of embryonic heart to altered hemodynamic conditions, we studied modifications of myocardial architecture in hearts which underwent conotruncal banding (model of increased afterload) or left atrial clipping or ligation (redistribution of preload) by histology and scanning electron microscopy. We hypothesized that 1) increased pressure load leads to accelerated development in compact layer thickness and proportion, and variable degree of dilatation; and 2) increased volume load is compensated mainly by dilatation with only mild and delayed ventricular wall thickening while the volume-underloaded ventricle is thought to show delayed development. We found that increased pressure load accelerated morphogenesis expressed as increased thickness of the compact myocardium, and in addition a precocious trabecular spiraling. Altered volume load accelerated morphogenesis of the volume-underloaded left ventricle (LV), manifested by sooner trabecular compaction and changes of the lumen. The increased volume load in right ventricle (RV) produced dilatation, followed by trabecular proliferation and increased thickness of the compact myocardium. Our results indicate that cardiac growth and morphogenesis are concomitant but distinct processes and that proper pressure and volume load are prerequisites for normal development of ventricular myoarchitecture.

MATERIALS AND METHODS

Conotruncal Banding

Fertilized White Leghorn chicken eggs were incubated blunt end up in a 38°C forced-draft incubator to Hamburger-Hamilton (HH) stage 21 (3.5 days, Hamburger and Hamilton, 1951). The embryo was exposed via a window in the shell and an incision of the inner shell membrane. A
10–0 nylon suture was passed around the mid-portion of the conotruncus, and tied in an overhand knot snug against its wall, but without any constriction to the blood flow. The window in the shell was then sealed with parafilm, and the eggs were reincubated to HH stages 24, 27, and 29 (n = 5 per stage). Sham controls had the suture passed and removed. Normal embryos were unoperated. These stages were selected because they represent approximately twofold increase in embryo mass. Additional sampling was done at HH stage 34 in order to evaluate morphological anomalies after the completion of cardiac organogenesis and the rearrangement of embryonic to fetal ventricular wall structure.

**Left Atrial Ligation**

The eggs were incubated to HH stage 21 as described above. The egg was positioned under the photomacroscope, and the egg shell and its membrane were removed to expose the embryo. The embryo was gently turned to the left side up position, and a slit-like opening was made in its thoracic wall using a pair of forceps. An overhand knot of 10–0 nylon suture loop was placed across the left atrium and tightened, constricting the left atrioventricular orifice and decreasing the effective volume of the left atrium. The embryo was then gently flipped to its original right side up position. The opening in the egg shell was sealed with parafilm, and the eggs were returned to the incubator for reincubation until HH stages 29 (n = 18) and 34 (n = 34). Sham-operated embryos (n = 9) had the suture tied adjacent to the left atrial wall. Normal embryos (n = 9) were unoperated.

**Left Atrial Clipping**

In the first experimental series (Rychter et al., 1979), left heart hypoplasia was produced by clipping the left atrial anlage at day 4 of incubation using a custom designed microclips from electrolytically purified silver wire. The aim of this study was to make an initial description of the chick heart left hypoplasia (Rychter and Rychterova, 1981). Sampling for histology was done in 48 hr intervals at incubation days 6, 8, 10, 12 and 14 (n = 6 per stage).

**Histological Sections**

The isolated hearts were fixed in 10% neutral formalin, embedded into paraffin, and serial sections of 7 µm thickness were cut in frontal plane. Measurements of compact myocardium and trabecular thickness, ventricular cross section area and diameters were performed using ocular micrometer and planimetry on representative sections (one per heart) running through the atrioventricular junction (“four chamber view”). Quantitative analysis was based on four normal and four experimental (presenting LHH of different severity but all having left atrium to right atrium ratio 1:4 or less) hearts per stage.

**Scanning Electron Microscopy (SEM)**

After removal of the embryos from the shells, the hearts were perfusion-fixed at high flow low pressure (Pexieder, 1981) with 2% glutaraldehyde–1% formaldehyde and 2 × 10⁻⁵% verapamil in isotonic 0.1 M cacodylate buffer. The hearts were then postfixed in 1% osmium tetroxide, and frontal views were taken under a M 400 photomacroscope (Wild, Switzerland) for the purposes of tissue shrinkage estimation and ventricular length measurement. The specimens were cut transversely to the long axis with thinned microdissection scissors into 0.25–0.50 mm thick slices. Additional specimens were dissected in frontal plane. The slices were ethanol-dehydrated, and critical point dried using freons in a CPD 030 critical point dryer (Balzers, Liechtenstein). After mounting on stubs with colloidal silver, the specimens were coated with 300 nm of gold in a S150 sputter coater (Edwards, United Kingdom), and photographed in a J SM 630 OF scanning electron microscope (JEOL, Japan). At minimum, five embryos per stage and group were transversely dissected, and four were selected for quantitative evaluation performed on SEM micrographs.

**Quantitative Analysis**

We analyzed the SEM micrographs of transverse slices (n = 4 per group). Compact myocardium thickness was measured using a digimatic calliper (Mitutoyo, Japan) on the midportion slice which contained both ventricles at standard points (at the intercepts of lines drawn at 0 deg., +45 deg. and −45 deg. from the center of the interventricular septum with the lateral wall). To determine the ventricular wall composition, cross-sectional areas contributed by the compact myocardium, trabeculae and intertrabecular spaces were measured using user-developed planimetric macroprograms on Quantimet 970 (Leica, Cambridge, England) in various locations. Trabecular orientation was evaluated visually and verified by measurements performed on intertrabecular spaces using techniques of polar histograms, mean linear intercept and color classification according to orientation (Cowin, 1986; Usson et al., 1994; Sedmera and Pexieder, 1995; Sedmera et al., 1998) on Quantimet 970. For volume estimations performed on HH stage 34, cross-sectional areas of the ventricles and their different components (compact myocardium, trabeculae, intertrabecular spaces, trabecula-free lumen) were estimated by unbiased point counting technique on digitized SEM photographs using Analyze 7.1 (Cytovision, London, United Kingdom) image analysis system run on HP UNIX platform. Volumes were calculated using a formula derived from Cavalieri’s principle (Cavaleri, 1635), according to which the volume of a feature is a product of its length perpendicular to slicing direction and its mean cross sectional area. Typically, over 1,000 points per slice were counted. Theoretical discussion and precision estimation of this methodology were given by Gundersen and Jensen (1987). Lengths of ventricles were measured on frontal macrophotographs of undiseased specimens. The values were corrected for tissue shrinkage during dehydration and critical point drying, which caused diminution of linear dimensions to approximately 59% of the initial values. All volumes are expressed in dry state; to approximate the in vivo diastolic values they could be multiplied by a factor of 4.87.

**Statistical Analysis**

Statistical analysis of all quantifiable data (compact layer thickness, myocardial volumes, ventricular diameters, trabecular counts) was performed by non-parametric Mann-Whitney test. Results P < 0.05 were considered significant.
RESULTS

Conotruncal Banding

Discrete changes of myocardial architecture were detected at HH stage 24 (12 hr after banding). The whole heart was dilated, and the thickness of the compact myocardium (Fig. 1) was increased. The trabeculae appeared thickened as well. The conotruncus was markedly dilated downstream after constriction, and the conotruncal ridges were hypoplastic.

By HH stage 27, the dilatation progressed, and the heart shape became more spherical (Figs. 2, 3). The experimental heart was larger and distinctly elongated compared to control. The trabeculae in the LV appeared coarser than normal (Fig. 3), and the intertrabecular spaces in the RV were smaller and more rounded. The trabeculae in the supraapical part of the LV had started to spiral in some cases counterclockwise when viewed from the base towards apex. The compact layer was thickened in both ventricles, but its ratio to trabecular myocardium was not significantly different from controls. The dilatation of the conotruncus distally from the banded (stenotic) part was even more distinct, as was the hypoplasia of the conotruncal ridges.

By HH stage 29, the differences between the sham control and banded hearts were pronounced. In the banded group, the LV lumen expanded, and extended as far as to the apex (Fig. 4). The trabeculae in the supraapical portion followed an evidently spiral course (Figs. 4, 5). Such spiraling was never seen in control hearts at this stage, though it was the characteristic of the definitive trabeculation pattern, and apparently started from HH stage 36 (Sedmera et al., 1997). The thickness of the compact myocardium was increased (Fig. 1), but its ratio to the trabeculated myocardium was augmented only in the basal part of the LV, suggesting starting compaction. In the RV, the external shape was markedly globular, consistent with some degree of dilatation, and the interconnecting segments between the trabeculae were more abundant. The proportion of intertrabecular spaces was decreased, and they appeared smaller than those of controls as a result of trabecular coarsening and starting compaction. Similarly to previous stages, the ratio of intertrabecular spaces to trabeculae was slightly decreased but not substantially different from control in any ventricular region. As a result of the increased number of cell layers (approximately 4–5 in control and 7–8 in banded), the contribution of the compact layer augmented moderately in the apical part, and its thickness was significantly increased. The size of myocytes (as appreciated on high power view in scanning electron microscope) was not different from controls. Coarsening of the atrial relief (pectinate muscles) was noted, and the atrial wall appeared thicker than control as well.

By HH stage 34, a ventricular septum defect (VSD) was present (Fig. 6) with either double outlet right ventricle or persistent truncus arteriosus, and trabecular compaction was more advanced than in controls, resulting in increased compact layer thickness (Fig. 1) and expansion of trabecula-free lumen. In some cases, morphology of right atrioventricular valve was changed from normal muscular flap-like to bicuspid structure, similar to the mitral valve.

Left Atrial Ligation

In both series of experiments, qualitative and quantitative changes found at HH stage 29 (60 hr after ligation) were generally mild. In the LV, the principal trabecular sheets were more closely packed together (Fig. 5), while in the RV, they were more widely spaced and thinner than normal. In extreme cases, the normal predominantly radial arrangement of RV trabeculae was changed to parallel to the compact layer (Fig. 7) together with severe dilatation and expansion of trabecula-free lumen. The thickness of compact myocardium in either RV or LV was similar to controls.

At HH stage 34, the changes in survivors (25–30% of the operated embryos) were more pronounced. The trabeculae in both ventricles were thinner than normal. In the LV, they started to adhere to the lateral wall. The RV was dilated and its trabeculae shifted toward the compact layer (Fig. 8). Compact layer thickness was slightly decreased in both ventricles in comparison with controls but not significantly due to considerable variability. In six of 20 examined hearts from the second series a VSD was observed in subaortic position (Fig. 6), typically in moderate cases of LHH.

Quantitative volume estimations performed at HH stage 34 revealed some significant differences between sham and LAL hearts. Total volume of the LV was decreased (0.691 ± 0.143 vs. 1.107 ± 0.185 mm³, P < 0.05) (mean ± SD) while total volume of the RV was increased though not significantly (1.488 ± 0.517 vs. 1.075 ± 0.418 mm³, P = 0.16). This resulted into inversion of LV to RV ratio from 1.103 ± 0.245 (sham) to 0.531 ± 0.279 (LAL, P < 0.05) while the volume of the entire heart remained similar (sham 2.641 ± 0.803, LAL 2.522 ± 0.613 mm³, P = 1). The volume changes differed by different ventricular compo-
Most pronounced was the absolute volume decrease of LV trabeculae compensated by increase of RV trabeculae. In relative numbers, a significant decrease of LV trabecular volume ($P < 0.05$) was accompanied by relatively increased proportions of compact myocardium ($P = 0.16$) and lumen ($P < 0.05$). Local porosity (percentage of intertrabecular spaces in the trabeculated myocardium) was slightly higher in LAL hearts but not significantly different between sham and LAL embryos in neither ventricle. In the RV, the proportion of the compact myocardium
Fig. 3. Transverse dissection of banded heart at HH stage 27. Note ventricular elongation and shape change, most spectacular in the RV. The LV trabeculae are remarkably thicker when compared with control (Fig. 2). The dilatation of conotruncus (entry marked by a star) is remarkable as well as the hypoplasia of the conotruncal ridges. Scale bars = 100 µm.
increased suddenly above control values at day 12 (82.65 ± 0.05) in the RV and continued to be higher on day 14, but the process of trabecular compaction was delayed, and dilatation persisted.

**DISCUSSION**

The selection of suitable quantitative parameters to describe the complex patterns of embryonic myocardial architecture is not easy. Quite straightforward solution is the measurement of compact myocardium thickness, which is altered under changed pressure loading (Sedmera et al., 1998), and in some mutant mice with cardiac phenotype ("thin myocardium syndrome") where it is usually associated with poor survival in later fetal development (e.g., Sucov et al., 1994; Kastner et al., 1994; Jaber et al., 1996). Estimation of myocardial volumes in different compartments is informative and reliable, but requires time-consuming point counting (Bouman et al., 1997). The value of simple length and area measurements giving a general idea about heart shape and dimensions should not be underestimated. All these techniques provide us with statistically comparable values, but do not tell much about trabeculation patterns.

The proportion of compact to trabeculated myocardium and ventricular wall porosity give us some idea about the myocardial fabric, but the planimetric approach employed on SEM slices needs correcting for different slicing level (Sedmera et al., 1998) thus preventing a rigorous statistical comparison. It provided two interesting results. First, there is a gradient of increasing proportion of compact myocardium from LV apex towards the base, which is accentuated in the conotruncal banding group. Second, the local porosity of the trabeculated myocardium did not show substantial modifications under altered loading conditions, suggesting that this ratio must be maintained in relatively narrow limits to ensure an efficient blood pumping in the trabeculated heart without coronary circulation.

The computer-based classification of intertrabecular spaces does clearly demonstrate the anisotropy of the trabeculated myocardium, but is not of much help for quantification of differences between different groups and does not add new information to visual appreciation of trabecular patterns. There is probably no simple mathematical formula that could describe the complex pattern of ventricular trabeculation, and careful dissection of well-perfused matched specimens stays the method of choice.

A topic deserving discussion is the exchange of material between the compact layer and trabeculae. Initially, the early compact layer is the source of cells for the trabeculae, as it presents two to three times higher proliferative activity and less cardiomyocyte differentiation (Jeter and Cameron, 1971; Tokuyasu, 1990; Franco et al., 1997). On the other hand, the compacting trabeculae start to add volume to the free wall from approximately HH stage 31. This process should be kept in mind when comparing the values between different experimental groups. The increased thickness of the compact myocardium can come either from accelerated or more abundant compaction (probably the case for HH stage 34 banded hearts, and RV at later stages of LHH), or increased proliferative activity in stages preceding compaction. The importance of compaction for force generation by the definitive multilayered compact myocardium is suggested by the lethality of mutant mice where it is impaired (e.g., Sucov et al., 1994; Kastner et al., 1994; Jaber et al., 1996). Delay of this

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**Fig. 4.** Slices from normal (a) and banded (b) HH stage 29 hearts at the apex level. Note the honeycomb arrangement of the LV trabeculae in this area and presence of the RV on the cross section in the normal heart. In the banded one, only LV with more circular cross section is present in this location, its trabeculae show spiral course and there is a distinct trabecula-free lumen. Scale bars = 100 μm.
process might be responsible for impaired survival of LAL embryos, as the trabeculated RV might not be able to generate enough pressure to meet the increasing circulatory demands of the growing embryo at later stages. On the other hand, acceleration of this process in the conotruncal banding group might not be too advantageous in the absence of properly established coronary circulation (despite the normal capillary density, Tomanek et al., 1996), and could account, together with increasing severity of obstruction, for some later lethality in this group.

Conotruncal banding has, apart from increasing pressure load, also pronounced effects on morphogenesis, producing cardiovascular defects in survivors (89% lethality until hatching; Clark et al., 1984, 1989). These can be characterized by alignment problems of the outflow tract, expressed as increased aortic-mitral separation with inevitable occurrence of VSD, with either double outlet right ventricle or persistent truncus arteriosus. This might well be due, apart from mechanical intervention of the suture, to changes in blood flow patterns (Hogers et al., 1997), and hypoplasia of the conotruncal ridges. The influences of this procedure on myocardial architecture were rapid and pronounced. Initially, the heart dilated and became more spherical, similar to changes in the adult heart (Hutchins et al., 1978). Trabeculae normally fill the ventricular cavities almost entirely between HH stages 21 and 31 (Ben-Shachar et al., 1985; Sedmera et al., 1997), and the precocious reappearance of a trabecula-free lumen is a sign of dilatation and accelerated trabecular compaction. Consistent with this is slightly decreased proportion of intertrabecular spaces. Together with relatively small magnitude of this change (similar to insignificantly increased porosity in LHH model), it suggests that there are probably certain limits necessary for adequate myocardial nutrition and efficient blood pumping that cannot be overcome and define survival.

Increased pressure load is a powerful stimulus for cardiomyocyte proliferation in the developing heart (Zak, 1974; Saiki et al., 1997). Indeed, purely hyperplastic response was reported in this model by Clark et al. (1989). This response occurs both in the compact myocardium and trabeculae, as indicated by their increased thickness and relatively minor changes in their ratio and supported by our preliminary data on the proliferative structure. The patterns of trabeculation are modified: the intertrabecular spaces are smaller and more circular in the RV, and trabecular spiraling occurs in the LV. This is an interesting phenomenon, since it mimics the orientation of definitive trabeculation in this location (Sedmera et al., 1997), and is similar to the course of muscle fibers in the compact layer (Streeter, 1979; Jouk et al., 1995). These are normal features of development which likely reflect adaptation to gradually increasing functional demands. We speculate that spiraling is a mechanism of increasing the pumping efficiency of the pressure-overloaded embryonic heart. In unperfused contracted hearts from HH stages 24 to 31, slight spiraling can be observed in the apical trabeculation (Harh and Paul, 1974; our unpublished observations), suggesting that the roughly orthogonal alignment is the relaxed state, and the contraction is effected by an anti-clockwise twist similar to the adult heart (Gibbons Kroeker et al., 1993; Fig. 12). Accelerated trabecular compaction with increased compact myocardium proportion and thickness between HH stages 29 and 34 is another phylogeneti-
cally well established mechanism that increases heart performance (Tota et al., 1983; Agnisola and Tota, 1994). If only one ventricle has to function against increased resistance, changes in the interventricular topography take place (e.g., shift of the interventricular septum to the left in an isolated RV pressure overload), as was demonstrated both mathematically and experimentally by Beyar et al. (1993). However, both ventricles in conotruncal banding have to act against increased resistance, so appropriate changes in shape occur in both, approaching to the “ideal” shape (spherical; Hutchins et al., 1978), and the relative position of the interventricular septum remains the same. On the other hand, shifting of the interventricular septum was observed in LHH or right ventricular hypoplasia by Rychter et al. (1979), where uneven volume loading occurs.

The phenotype of LAL hearts shows considerable variability from almost normal to extreme involution of the LV. The results from different techniques (clipping or ligation) are comparable. The selection of only distinct phenotypic cases for quantitative analysis was essential, because otherwise the already considerable variability of measured parameters would completely obscure any significant differences. Relatively higher variability of LAL data reflects the different penetrance of the phenotype. This continuous spectrum of cases allows qualitative perception of RV response to a graded overload. Generally small differences between sham and LAL hearts at HH stage 29 could be explained by persistent interventricular communication which allows some blood to pass to the LV. The occurrence of VSD (ca. 25%) is similar to results obtained recently by Hogers et al. in left vitelline vein clipping model (Rychter et al., 1979; Hogers et al., 1997), and might be likewise attributed to changes of intracardiac blood stream patterns. Other associated abnormalities were described in chick embryo LHH model (Rychter et al., 1979; Rychter and Rychterova, 1981) and resemble features reported from human pathology (e.g., Noonan and Nadas, 1958). Not surprisingly, there is generally altered atrial morphology with an abnormal development of the interatrial septum. In extreme cases, right atrioventricular valve loses its typical muscular flap-like morphology and looks more like a bicuspid fibrous valve, similar to the left atrioventricular (mitral) valve. This resembles changes observed under increased pressure load conditions, suggesting that loading is an important determinant of morphology of the developing valvular structures. The structure of atrioventricular valves is often abnormal in congenital heart disease. The myocardialization of the right atrioventricular valve is specific for birds, and does not normally occur in mammals; but abnormal muscularization of the antero-superior leaflet is a relatively frequent finding in Ebstein’s malformation (e.g., Zubereuiler et al., 1979), indicating that this potential is existing as well. The ascending aorta is hypoplastic, and the blood is conducted by largely dilated ductus arteriosi. This led Rychter and Lemez (1964) to propose it as a model of aortic coarctation. The development of coronary circulation is delayed by approximately 2 days at day 14 (Dbaly and Rychter, 1967). Lymphatics are also abnormal, being absent in the hypoplastic LV and showing dysplastic valves in the basal

![Fig. 6. Frontal dissection of HH stage 34 sham (a), banded (b) and LAL (c) hearts. Note distorted atria, changed proportions of the ventricles and defect of interventricular septum (star) in the LAL one. The trabeculae in the LV show precocious adherence to the lateral wall and changed orientation with circumferential alignment in the right. The muscular flap-like morphology of the right atrioventricular valve is changed to a bicuspid, mitral-like structure. Left atrioventricular valve is behind the section plane because of the dorsal shift of the whole ventricle (compare with Figure 8). Venticular septum defect is seen also in banded heart, where the trabecular compaction is distinctly advanced in both ventricles resulting in thickening of the compact myocardium and interventricular septum and similar modification of right atrioventricular valve morphology. Dorsal halves, scale bar = 1 mm.](image-url)
Fig. 7. Transverse midportion slices of sham (a) and LAL (b) HH stage 29 hearts. Note marked dilatation, and changed proportion of left to right ventricle. In this rather extreme case of LHH, RV trabeculae changed their normal radial arrangement to parallel with the outer compact layer. Scale bar = 100 µm
Fig. 8. Midportion slices of HH stage 34 sham (a) and LAL (b) hearts. Note shrinkage and dorsalwards shift of the LV and dilatation of the RV. Scale bar = 100 µm
portion of the hyperplastic RV (Rychter and Rychterova, 1981).

Adaptation of the RV to gradually increasing volume load occurs in three steps. First, dilatation becomes evident as slight thinning and decreased proportion of the compact myocardium. In extreme cases the trabeculae even change their radial orientation (Rychter and Rychterova, 1981; Ben-Shachar et al., 1985; Sedmera et al., 1997) to parallel with the outer compact layer, indicating increased circumferential strain (Cowin, 1986). Second, proliferation of trabeculae (starting from HH stage 34) is suggested by their increased number and relative and absolute volumes, but they are finer and their orientation in some cases remains altered. Also diminished compact layer thickness and increased local porosity (proportion of the intertrabecular spaces within the non-compact myocardium) give evidence of persisting dilatation. Third, the compact myocardium appears thicker, a finding we interpret as an acceleration of normal course of development (Rychterova, 1971) and probably necessary for more efficient force generation (e.g., Greer Walker et al., 1985). Delayed trabecular compaction underscores the more important role of RV trabeculae in contractile function than in the LV, which starts to rely on its compact myocardium sooner (Sedmera et al., 1997). Significant decrease in the volume of RV free wall (compact myocardium) was reported from the most severely affected hearts (double outlet right ventricle) of HH stage 34 chick embryos after treatment with retinoic acid (Bouman et al., 1997) and seemed to correlate with decreased heart performance. Although volume estimates were made from serial histological sections, their data correspond quite well with our results.

In pediatric pathology, coarsening of the trabecular relief and thickening of the RV compact myocardium is a common finding in cases where the RV functions as a systemic pump (e.g., Oren et al., 1994). This thickening thus seems to be a secondary effect of increase in the pressure load. The relatively slow nature of these changes contrasts with fairly rapid response (increased cell number, compact layer and trabecular thickening) observed as soon as 24 hr after increased pressure loading (Clark et al., 1989). The proliferative structure of LAL hearts is altered (Rychter et al., 1979; Rychter and Rychterova, 1981) with overall decrease in mitotic counts. It shows that increased pressure load in the chick embryonic heart is a powerful growth stimulus for hyperplastic cardiomyocyte growth while increased volume load is compensated preferentially by cardiac dilatation.

The changes in myocardial architecture of the under-loaded LV are characterized by precocious adherence of trabeculae to the lateral wall and their compaction, perhaps resulting from their closer packing in diminished space. This is illustrated by shift of LV composition at HH stage 34, where proportion of trabeculae is decreased in favor of compact myocardium and lumen. This indicates an acceleration of the normal developmental processes which shows that growth and morphogenesis are two concomitant but distinct processes. On the other hand, decreased pressure loading achieved by chronic verapamil suffusion results in hypoplastic hearts containing fewer cells of normal size (Clark et al., 1991) with decreased proportion and thickness of the compact myocardium (Sedmera et al., 1998). It would be interesting to extend the pilot experiments of Rychter et al. (1979) of inverted procedure (right atrial clipping), which results in hypoplasia of the RV and hyperplasia of the LV. The authors reported changes in inner heart relief and altered heart form but detailed analysis was prevented by poor survival of operated embryos, perhaps due to problems with outflow tract alignment. In addition, the higher occurrence of VSD...
(60% of cases) made the search for consistent rearrangements of ventricular myoarchitecture more difficult.

We have shown differential response of the embryonic heart to changes in pressure and volume loading (summarized in Fig. 12). Increased pressure load induced first dilatation, compensated rapidly by cardiomyocyte proliferation, trabecular and compact myocardium thickening, and precocious spiraling and compaction of the trabeculae, all
of which can be regarded as an acceleration of normal developmental processes. Decreased volume load resulted in shrinkage and accelerated morphogenesis of the LV. Increased volume load to the RV led to its dilatation, only later followed by trabecular proliferation and thickening of the compact myocardium. The slow rate of compensatory processes with high mortality of operated embryos may reflect the poor ability of the RV to function as a systemic pump. Its morphologic (Hirokawa, 1972; Sedmera et al., 1997) and molecular (Zimmer, 1994) differences seem to be the factors responsible for the invariably fatal outcome of unoperated LHH in humans. Our study indicates the importance of analysis of myocardial architecture in the normal hearts, as it is of considerable importance for the performance. Such data, supplemented by functional data, are of great importance for the development of treatment of congenital cardiovascular anomalies such as transposition of great arteries (Castaneda et al., 1994).

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LITERATURE CITED


Fig. 12. A diagram summarizing the mechanics of trabeculated heart contraction at HH stage 29 in normal and experimental hearts. In banded heart, there is a thickening of the compact myocardium and trabecular, and spiralled trabecular course even in diastole. LAL heart presents dilatation of the right ventricle with alteration of preferential orientation of its trabeculae from radial to circumferential. The left ventricle is shifted dorsally.


