Echocardiographic localization of the sites of left ventricular outflow tract obstruction. Am J Cardiol 37: 250, 1976

Models of Congenital Heart Disease in Fetal Lambs

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SUMMARY Intracardiac flow patterns were chronically altered by partially obstructing left ventricular (LV) inflow or outflow in midgestational fetal lambs. Physiological measurements of the fetal circulation were made serially through indwelling catheters and the use of radioactive microspheres.

With LV inflow obstruction, mean LV output (LVO) decreased to 30% of control (P < 0.01). Within seven days, the LV/right ventricular (RV) weight ratio decreased to 70% of control (P < 0.01), and the mean LV/RV chamber volume decreased to less than one-half of control (P < 0.001), simulating an early form of the hypoplastic left heart syndrome.

With LV outflow obstruction, mean LVO decreased to 64% of control (P < 0.05). Mean LV/RV wall thickness doubled (P < 0.0001) and mean LV/RV chamber volume decreased to less than one-half of control (P < 0.0001). Within four to ten days after increasing LV afterload, a large increase in LV mass occurred, which was demonstrated by morphometric analysis to be due to hyperplasia of ventricular myocytes. LV chamber volume decreased somewhat, simulating moderately severe congenital aortic stenosis. Over the long term (30–36 days), the mean LV/RV weight ratio decreased and the LV chamber was nearly obliterated, simulating very severe congenital aortic stenosis.

The results suggest that by varying preload and afterload in both ventricles of the fetus, various forms of congenital heart disease may be simulated.

IT IS NOT KNOWN WHY the massive ventricular enlargement associated with severe congenital aortic or pulmonic stenosis cannot be duplicated experimentally in postnatal animals, nor why the structure of blood vessels is altered in fetuses with congenital heart disease. Improved understanding of early adaptation to a mechanical cardiovascular lesion should come from development of fetal animal models in which myocardial and vascular structure and function as well as circulatory reflexes can be measured.

The possibility that non-genetic models of congenital heart disease could be induced in fetuses was suggested by the observations of pathologists and experimental embryologists. Lev et al. noted that the hypoplastic left heart syndrome in human fetuses is often associated with premature closure of the foramen ovale, and postulated that a decreased venous return was responsible for arrest of the left ventricle in those cases. Hahr et al. induced various forms of the hypoplastic left heart syndrome in chick embryos by obstructing flow through the left atrioventricular canal with a thin nylon plug. Subsequently, Shapiro et al. showed that constriction of the pulmonary artery leads to marked thickening of the right ventricular (RV) wall in the fetal lamb. We selected the fetal lamb as the experimental animal in which to attempt to induce models of congenital heart disease for several reasons. Fetal lambs and humans are similar in weight and have similar blood pressures, oxygen tensions, ventricular stroke volumes, and internal distribution of blood flows at corresponding stages of gestation. It is possible to operate on lamb fetuses without precipitating abortion, and physiological measurements of circulatory function can be made in utero for days or even weeks under normal physiological conditions through indwelling catheters.

A great deal of data has been accumulated from chronically catheterized fetal lambs concerning nor-
mal circulatory physiology, and the development of alpha and beta sympathetic receptor activity.\textsuperscript{12, 13} Baroreflexes,\textsuperscript{14} and carotid and aortic chemoreflexes.\textsuperscript{15} The effects of rapidly altered preload, afterload, and heart rate on ventricular stroke volume,\textsuperscript{16-18} and the acute effects of hypoxemia, acidemia, and hemorrhage on the cardiac output, arterial pressure and regional distribution of blood flow,\textsuperscript{19, 20} have also been studied in the in utero fetal lamb preparation.

**Methods**

The investigations described in this report were carried out in three parts in time-dated, pregnant ewes (90–120 days; 0.6–0.8 gestation) that were operated on under halothane anesthesia.

Vinyl catheters 0.038 inches internal diameter, 0.070 inches in outside diameter were introduced through a femoral artery and vein, and the carotid artery and jugular vein, of the fetus. The tips of the catheters were advanced to lie in an intrathoracic or intra-abdominal position. Another vinyl catheter was placed in the amniotic fluid space. The catheters were filled with heparin, plugged and exteriorized through the uterus and lateral abdominal wall of the mother. A left thoracotomy incision was used to insert a catheter directly into the pulmonary trunk of the fetus, and to perform one of two additional procedures which would either impede flow into the left ventricle, thereby decreasing left ventricular (LV) preload, or impede flow out of the left ventricle, thereby increasing LV afterload.

Partial LV inflow obstruction (decreased preload) was produced in nine fetal lambs (Group I) by inserting a balloon catheter through a pursestring (butressed with strips of pericardium) in the wall of the left atrium and filling the balloon with 0.3–0.6 ml of silicone rubber until it was easily palpated through the atrial wall. Once the rubber hardened the catheter was cut short, leaving a few millimeters of catheter projecting from the atrial surface.

Partial LV outflow obstruction (increased afterload) was produced in 12 fetal lambs (Group II) by encircling the ascending aorta with a tubular vinyl band 1.2 mm in thickness. The band was placed between the origin of the coronary arteries and the brachiocephalic vessels. There was very little dissection performed between the great vessels and none at all beneath the aortic arch, thus preserving the chem- and baroreceptors and afferent nerves. The band was tightened until a 20–25 torr peak systolic LV-to-aortic pressure difference was achieved on withdrawal tracings. The band was loosened if significant alterations in femoral arterial pressure or heart rate occurred.

Group III (increased LV afterload) consisted of 13 fetal twins. In one fetus of each pair, the ascending aorta was banded and only the carotid artery was catheterized. The other fetus in each pair was left completely undisturbed and was used only as a morphological control. The fetuses were arbitrarily sacrificed four to ten days after aortic banding because the Group II experiments suggested the high likelihood of LV enlargement during that interval.

**Physiological Studies**

A minimum of 24 hours was allotted for recovery of the mother and fetus from the effects of anesthesia and surgery before physiological measurements were made. Each day thereafter, the mother was allowed to stand quietly while arterial, venous and pulmonary arterial and amniotic fluid pressures were measured with Statham P23Db(1) transducers and recorded on a Beckman R 411 direct writing oscillographic recorder. The femoral and carotid arterial pH, PO\(_2\) and PCO\(_2\) were measured with a radiometer blood gas monitor with appropriate electrodes. The hematocrit was measured daily. Occasionally, the hematocrit decreased postoperatively. In those instances, 5–15 ml of settled maternal red cells were transfused to increase the hematocrit of the fetus from 30–34%. No studies were performed within four hours of transfusion.

Combined ventricular output (CVO) and blood flow to each fetal organ were measured at intervals in Groups I and II by a previously described method.\textsuperscript{11} Microspheres (15 μ in diameter), labeled with one radionuclide (either \(^{88}\)Sr, \(^{125}\)I, \(^{51}\)Cr, \(^{86}\)Sr or \(^{141}\)Ce), were injected into the inferior vena cava, while microspheres labeled with one of the other radionuclides were injected into the superior vena cava. Reference blood samples were drawn simultaneously at a constant rate from the carotid, femoral and pulmonary arteries during the period of injection and circulation of the microspheres within the fetus. Enough microspheres were injected to yield at least 400 spheres in every fetal organ. A special injection chamber was used to disperse the spheres in the diluent during delivery into the circulation.\textsuperscript{11} The presence of a steady state was ascertained by observing the heart rate, arterial pressures, pH and blood gas tensions for one hour before and after each study. The concentrations of microspheres in the reference blood samples were used to calculate the percentage of the CVO flowing through the ductus arteriosus, the aortic istmus and descending thoracic aorta. Whenever carotid, femoral and pulmonary arterial reference samples were simultaneously obtained, it was possible to calculate the percentage of the CVO ejected by each ventricle; i.e., left ventricular output (LVO) and right ventricular output (RVO). The data were compared to normal fetal lambs of the same age in which LVO = 40% and RVO = 60% of CVO.\textsuperscript{21}

After death, the fetuses were dissected and the organs weighed. The quantity of each isotope in the reference blood samples and various fetal structures was measured by gamma spectrometry with the use of a sodium iodide crystal, a pulse height analyzer (512 channel Nuclear Chicago) with a region of interest module, vial changer, and card punch machine (IBM 029). CVO and the blood flow to each organ were calculated using an IBM 360/50 computer and compared by an unpaired t test to the values obtained in
normal fetal lambs of similar gestational age by Rudolph and Heymann.\textsuperscript{21}

Postmortem Morphological Studies

The position of all arterial and venous catheters and of the left atrial balloon (Group I) was verified at autopsy. In Group II, a cast was made to define the internal anatomy of the great vessels by injecting rapidly polymerizing silicone rubber in the aorta.

In Groups I and II, the heart was weighed, then fixed in formalin after cotton had been inserted into the ventricles to maintain the ellipsoidal shape of the chambers during fixation. The volume of each ventricular chamber was measured by the amount of fluid required to fill it. The thickness of the free walls of the left and right ventricles was measured at a point halfway between the apex and atrioventricular (AV) valves, avoiding the areas occupied by the insertions of papillary muscles. The free walls of the ventricles were dissected from the septum, using the anterior and posterior descending coronary arteries as the dividing line between left and right ventricles, and weighed. The weight of the heart changed less than 1% during formalin fixation. To obtain control values of ventricular morphology, the hearts of 11 normal fetal lambs used in other acute experiments and of equivalent gestational ages to the experimental group, were obtained and prepared in precisely the same manner. Comparisons between experimental and control values were made using an unpaired \( t \) test.

In Group III, at the time of reoperation, the heart and great vessels of the fetuses were approached through a midline sternal-splitting incision. A large catheter was passed retrograde through the carotid artery into the ascending aorta, and tourniquet. Perfusion of the coronary circulation with fixative was begun with the heart beating. The perfusion pressure equaled normal aortic pressure during life. The perfusion medium consisted of 3% glutaraldehyde in a 0.12 M cacodylic acid, 0.002 M polyvinyl pyrrolidinone buffer (pH 7.3). The perfusate also contained 0.2% hydrogen peroxide during the first half of the perfusion. The heart became firm and brown within a few minutes, indicating complete and homogeneous fixation of the myocardium. The hearts of the banded and control fetuses in each pair were perfused in the same way.

In one pair of Group III twins, the myocardial cellular changes were evaluated by an extensive morphometric analysis. Thirty minutes before the analysis, sacrifice horseradish peroxidase (50 mg/kg) was injected intravenously into each fetus. Following perfusion fixation with buffered glutaraldehyde, both hearts were completely immersed in the fixative for four hours at 4°C. The LV papillary muscles were removed and cut into 3 mm cubes. The tissues were then cut in 60 \( \mu \)m sections with a Sorvall TC-2 tissue sectioner and incubated with 3,3 diaminobenzidine with hydrogen peroxide in a dark room at room temperature until the brown peroxidase reaction was evident. The tissues were then post-fixed in 1.5% osmium tetroxide for 60 min at 4°C. After dehydration in acetone the tissue slices were imbedded in epoxy resin (Epon-Araldite). Sections (1.0 \( \mu \)m) were cut on a Sorvall JB4 microtome and viewed with a microscope under an oil-immersed lens at 1,000 magnification. The peroxidase reaction product outlined the myocardial cells, and they were easily measured (see Discussion). Maximum cross-section diameters of LV papillary myocytes were measured in five sections, each from the banded and control fetuses, and compared by unpaired \( t \) tests.

Results

Group I

Nine LV inflow obstruction (decreased preload) experiments were carried out. Survival time was two to seven days (mean four days). The following range of measurements was obtained from the femoral artery of all of the fetuses 24 hours after the initial operation: pH, 7.37-7.42; PO\textsubscript{2}, 19-30 torr; PCO\textsubscript{2}, 37-48 torr; hematocrit, 30-37%; heart rate, 180-220 beats/min; systolic pressure, 57-70 torr; and diastolic pressure, 30-50 torr. Pressures in the pulmonary artery were within 1-2 torr of femoral arterial pressures. IVC and SVC pressures varied from 1-3 torr and were within 1 torr of each other. These values were all within the normal range for fetal lambs.\textsuperscript{21}

Physiological Changes

The mean femoral arterial PO\textsubscript{2} decreased progressively (\( r = -0.615 \)) from 22 torr on the first postoperative day to 12 torr on the sixth day after surgery. Arterial pressures remained within normal limits throughout the period of observation. Venous pressures ranged from 3-5 torr on the last day of life.

\( CVO \) was measured in 19 studies in nine fetuses, and found to be decreased significantly (\( P < 0.05 \)) to 77 ± 6% (mean ± SEM) of control. Blood flows to the individual fetal organs remained within normal ranges (table 1), but fetal placental blood flow (FPBF) decreased significantly (\( P < 0.001 \)) to 54 ± 5% of control. In 11 of 19 studies, it was also possible to calculate LVO and RVO. Mean RVO was 104 ± 9% of control, or not significantly increased (\( P > 0.5 \)), but mean LVO was decreased significantly (\( P < 0.01 \)) to 30 ± 11% of control (fig. 1).

Morphological Changes

The balloon was found at autopsy to be within the left atrium in all cases. In two fetuses, the valve of the foramen ovale was closed by the tip of the balloon catheter. Four of the nine fetuses were extremely edematous, exhibiting anasarca, with ascites and engorgement of the liver.

The right and left ventricles of the fetal lamb are normally similar, morphologically, as shown by the LV/RV ratios of weight, wall thickness and chamber volume in controls (table 2). In response to decreased LV preload, the mean values of all the RV morphological characteristics remained normal. The
left ventricle, however, appeared smaller (fig. 2). Mean LV/RV weight decreased significantly ($P < 0.05$) to 83 ± 6% of control, (table 2), and the LV/RV weight ratio decreased significantly ($r = -0.798$) with the duration of LV inflow obstruction (fig. 3). Both mean LV chamber volume and LV/RV chamber volume decreased significantly ($P < 0.025$ and $< 0.001$) to nearly one-third of control (table 2). LV/RV wall thickness was $10^7$ ± 6% of control, which is not a significant difference ($P > 0.4$).

**Group II**

Twelve LV outflow obstruction (increased afterload) experiments were carried out. Survival time was 2–36 days (mean 11.3 days). Nine fetuses lived 2 to 10 days, and three lived for 30–36 days. The femoral arterial pH, PO$_2$, PCO$_2$, the hematocrit, heart rate and arterial systolic and diastolic pressure and the central venous pressure were all normal 24 hours after the operation. Pressures in the pulmonary artery were within 1–2 torr of femoral artery pressures. IVC and SVC pressures varied 1–3 torr and were within 1 torr of each other.

**Physiological Changes**

Femoral arterial PO$_2$ remained within the normal range throughout the entire period of observation in six fetuses and until 48 hours before death in the other six. Venous pressures ranged from 3–5 torr and arterial pressures remained within normal limits in all of the fetuses until a few hours before death.

CVO was measured in 13 studies in eight fetuses and was found to be significantly decreased ($P < 0.05$) to 80 ± 6% of control (mean ± SEM). Blood flows to the organs of the fetus remained within normal ranges (table 3), but fetal placental flow decreased significantly ($P < 0.025$) to 64 ± 8% of control.

In eight of the 13 studies, we were able to separately calculate LVO and RVO. Mean RVO was 90 ± 8% of control or not significantly decreased ($P > 0.5$), but LVO was significantly decreased ($P < 0.05$) to 64 ± 9% of control (fig. 4).

![FIGURE 2. Group I: Fetal heart seven days after initiation of partial left ventricular inflow obstruction (decreased LV preload). Atria and valves removed to expose left ventricle (LV) and right ventricle (RV). Left ventricular chamber volume was one-fourth right ventricular chamber volume in this heart. In a normal fetal lamb, the chamber volumes would have been equal.](image-url)
Morphological Changes

The ascending aorta was narrowed to less than 25% of its normal cross-sectional area by the aortic band by 10 days (fig. 5). Three of the 12 fetuses had severe edema, ascites and hepatic engorgement at autopsy. Ventricular wall thickness and chamber volume were measured in five fetuses retrieved immediately after death. Five fetuses, however, died in the interval between daily observations, and although ventricular weights were measured, wall thickness and chamber volume were not, since these parameters of ventricular geometry might have been affected asymmetrically by rigor mortis.

In the hearts subjected to increased afterload (table 4), the left ventricle was twice as thick and the chamber volume was one-half as large as the right ventricle ($P < 0.001$). The right ventricle remained essentially normal in all morphological characteristics. In the nine fetuses that survived 10 days or less, the left ventricle was significantly heavier ($P < 0.025$) than the right ventricle (4.85 ± 0.53 vs 4.13 ± 0.34 g, mean ± SEM). In the three fetuses that lived longer than 30 days, however, the left ventricle weighed significantly less ($P < 0.05$) than the right (6.9 ± 0.7 vs 8.5 ± 0.8 g), suggesting that growth of the left ventricle had been arrested in the interval between 10–30 days after banding. In the long-term survivors, the left ventricle was reduced to a thick-walled, slit-like cavity resembling that of severe aortic stenosis in human fetuses.

Group III

The LV/fetal body weight ratio (g/kg) was significantly greater ($P < 0.02$) in the banded fetuses than in the controls (2.46 ± 0.20 vs 2.14 ± 0.16; mean ± SEM, n = 13). The LV/RV/body weight ratio (per kg) was also significantly greater ($P < 0.05$) in the banded fetuses than in the controls (0.815 ± 0.074 vs 0.713 ± 0.045). The degree of LV enlargement at the time of sacrifice was most pronounced in the fetuses with the tightest aortic bands. In one fetus the

<table>
<thead>
<tr>
<th>Organ</th>
<th>Percent of control</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gut</td>
<td>94</td>
<td>&gt;0.5</td>
</tr>
<tr>
<td>Spleen</td>
<td>77</td>
<td>&gt;0.2</td>
</tr>
<tr>
<td>Brain</td>
<td>103</td>
<td>&gt;0.5</td>
</tr>
<tr>
<td>Heart</td>
<td>107</td>
<td>&gt;0.5</td>
</tr>
<tr>
<td>Lungs</td>
<td>113</td>
<td>&gt;0.5</td>
</tr>
<tr>
<td>Carcass</td>
<td>76</td>
<td>&gt;0.4</td>
</tr>
<tr>
<td>Kidneys</td>
<td>140</td>
<td>&gt;0.2</td>
</tr>
<tr>
<td>Placenta</td>
<td>64</td>
<td>&lt;0.025</td>
</tr>
</tbody>
</table>

Table 4. Group II: Morphological Data

<table>
<thead>
<tr>
<th></th>
<th>Controls (n = 11)</th>
<th>Left Ventricular Outflow Obstruction (n = 10)</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fetal weight (g)</td>
<td>2061 ± 278</td>
<td>2304 ± 229</td>
<td>&gt;0.5</td>
</tr>
<tr>
<td>L/R ventricular weight</td>
<td>1.05 ± 0.07</td>
<td>1.10 ± 0.05</td>
<td>&gt;0.5</td>
</tr>
<tr>
<td>L/R ventricular wall thickness</td>
<td>1.16 ± 0.07</td>
<td>2.30 ± 0.11</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>L/R ventricular chamber volume</td>
<td>0.90 ± 0.03</td>
<td>0.45 ± 0.05</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Abbreviations: L = left; R = right.
MODELS OF CONGENITAL HEART DISEASE/Fishman et al.

FIGURE 5. Group II: Injection cast of the great vessels of a fetal lamb in which the aortic band had been in place for ten days. The band is situated just above the aortic valve (v) and coronary arteries (Cor) and below the origin of the brachiocephalic trunk (bc). The ductus arteriosus (da) is seen joining with the aorta (Ao).

LV/fetal body weight ratio was 1.5 times greater than in the control twin (fig. 6).

A morphometric analysis utilizing the horseradish peroxidase technique was carried out in twins sacrificed 10 days after operation. The left ventricle in the banded fetus weighed 4.56 g compared to 3.88 g in the control twin. The LV/RV ratio was 1.63 in the banded fetus, compared to 1.12 in the control. The LV papillary muscle of the banded fetus was nearly twice as thick as the control. The maximum cross section diameters of the LV papillary myocytes were similar ($P > 0.40$) in the banded fetus and in the control ($8.87 \pm 0.09 \ \mu m$, $n = 671$ vs $8.77 \pm 0.08$, $n = 751$; mean $\pm$ SEM).

The distribution of myocardial cell types was similar in the banded fetuses and the controls: myocytes 66.2 vs 64.2%; vascular endothelial cells 21.5 vs 19.3% and fibroblasts 12.3 vs 16.5%. Selective proliferation of interstitial cells (vascular endothelium and fibroblasts), such as occurs in adult forms of ventricular hypertrophy, did not occur. Ventricular myocytes are several times larger than interstitial cells, and since the diameter of myocytes was similar in both fetuses, the enlarged muscle of the banded fetus must have contained a greater number of myocytes. We concluded, therefore, that the increase in LV weight resulted from hyperplasia rather than hypertrophy.

Discussion

Physiological Changes

There was an immediate and profound reduction in LVO with LV inflow obstruction. Since LVO normally contributes 40% of CVO and since RVO cannot be increased acutely, CVO also decreased when LVO

FIGURE 6. Group III: Free walls of left (LV) and right (RV) ventricles from twin fetal lambs of equal weight, one of which was subjected to increased left ventricular afterload by partial left ventricular outflow obstruction (BANDED) for four days. The other (CONTROL) was left undisturbed. Both RVs were equal in size and weight. The Banded LV, however, weighed 50% more than the control LV. Control LV and RV were also equal in weight.
ventricular weight, changes in muscle compliance, and the stroke volume does not respond significantly to increased filling pressures acutely. For example, a transient increase in central venous pressure of 20 torr induced by rapid transfusion may increase RV stroke volume by only 10–20% in fetal lambs. The normal range of venous pressures in the fetus is 1–3 torr, and chronic elevations above 5 torr in some of the fetuses in the current experiments were associated with massive edema.

LVO also decreased with LV outflow obstruction, but more gradually, so that more time was available for a compensatory increase in RVO to develop. The placental vessels are not innervated by the sympathetic nervous system. Therefore, fetal placental blood flow is directly proportional to aortic pressure. During low output states normal aortic pressure can be maintained by peripheral vasoconstriction, thereby preserving higher placental flows and arterial oxygen levels. This mode of adaptation has been demonstrated during the acute phase of hypoxia in fetal lambs. Chronic vasoconstriction, however, causes tissue oxygen levels to be decreased and leads to progressive acidosis, which is rapidly fatal in fetal lambs. Instead of vasoconstriction, the fetuses in the current experiments adapted to chronically reduced flows by vasodilatation. Aortic pressures, placental blood flow and arterial oxygen tensions were all decreased, therefore, but blood flows to systemic organs remained normal for several days. The arterial oxygen tensions decreased progressively, but the pH remained normal until the PO2 fell into the range of 10–15 torr. Only then did acidosis and death ensue.

The survival of fetuses with decreased LVO for longer periods depends upon an increase in RVO which can only be accomplished by an increase in the muscle mass and chamber volume of the right ventricle in response to slightly increased preload. Our observations suggest that stimulation of accelerated RV growth by increased preload may require one to two weeks. No increase in RV size or output was observed in the fetuses with LV inflow obstruction (all of which died within one week) or in the short-term survivors of LV outflow obstruction (those that died by 12 days). However, in the long-term survivors of LV outflow obstruction, the right ventricle was capacious and outweighed the left ventricle in each instance. Future attempts to maximize compensatory enlargement of the right ventricle should utilize methods that result in a more gradual decrease in LVO. To do this, an indwelling left atrial balloon might be inflated incrementally over several days or the ascending aorta might be constricted by an aortic band with elastic properties so that the overwhelming stress of abruptly reduced LVO might be avoided.

Morphological Changes
The results of these experiments indicate that ventricular weight, chamber volume, and wall thickness are profoundly influenced by the forces of volume and pressure loading during the last trimester of gestation in the fetal lamb. LV chamber volume decreased as LV stroke volume decreased, whether as a result of decreased preload or increased afterload. In some manner, the chronic reduction in distending force during diastole may have led to a structural rearrangement of muscle cells or sarcomeres.

Although the ratio of LV/RV weight decreased rapidly with LV inflow obstruction (Group I), there is no evidence that an absolute decrease in LV weight occurred. Actually, since the fetal lamb nearly doubles in weight between 100–120 days gestation, the 30% decrease in LV/RV weight that occurred by the end of one week of LV inflow obstruction can be accounted for almost entirely by the cessation of LV growth and the continuation of RV growth at a normal rate. Ventricular preload, therefore, appears to be a required stimulus for normal ventricular growth; a reduction in preload leads to growth arrest.

The changes in LV weight with LV outflow obstruction (Group II: increased LV afterload) are more complex. When first applied, the aortic band created only a small pressure gradient. With rapid growth of the fetus, the banded portion of the aorta became progressively more stenotic because the diameter of the band was fixed. At first, the left ventricle tended to overcome the increasing impedance to outflow by increasing its muscle mass and its pumping pressures through the mechanism of myocardial hyperplasia. Inevitably, however, with continuing growth of the fetus, the aortic channel became too small for adequate flow at any conceivable ventricular pressure (fig. 7). At some point, LV stroke volume did not increase further, the preload stimulus for ventricular growth was lost and growth of the left ventricle was arrested. As LVO decreased, CVO and fetal placental blood flow also decreased, resulting in progressive hypoxemia. As discussed above, the difference between the fetuses that died when confronted by a decreasing LVO and those that did not and became long-term survivors was due to enlargement of the right ventricle in time to compensate for the deficiency.

The massive increase in LV wall thickness with LV outflow obstruction (Group II) was subsequently shown by the Group III experiments to be associated with a real increase in LV mass. The increase in wall thickness with LV inflow obstruction (Group I), however, was minimal. It simply reflected the obligatory geometrical rearrangement of mass around a contracted chamber.

Models of Congenital Heart Disease
Despite the limitations of these initial experiments, an early form of the hypoplastic left heart syndrome developed in a very short time in response to LV inflow obstruction (fig. 2), and LV outflow obstruction resulted in two models of congenital aortic stenosis. The left ventricle of the short-term survivors resembled that of the moderately severe form of congenital aortic stenosis, in which the valve commissures are partially fused but the annulus is of normal size. In
FIGURE 7. Group II. A) Injection cast of the great vessels and B) heart of a fetal lamb subjected to increased left ventricular afterload for 36 days. The aorta (Ao) has been constricted 95% between the valve (v) and the origin of the brachiocephalic trunk (bc). The pulmonary artery (PA) is enlarged. The right ventricular (RV) chamber is enlarged. The chamber of the left ventricle (LV), visualized by cutting a wedge out of the thickened wall, is only a slit. The location of the ventricular septum is shown (spt). The RV weighed more than the LV in this heart.

FIGURE 8. Photomicrograph of myocardium (× 1,000) fixed immediately after death by immersion in formalin and imbedded in paraffin. The section is 6 μ thick. Note shrunken ventricular myocytes (Myo), edematous interstitial space (is) and contracted capillaries (Cap) within some of which crenated erythrocytes are present.
such cases, the LV chamber is smaller than the RV chamber. The wall of the left ventricle, though quite thick, is still compliant enough to fill and eject an adequate volume after satisfactory relief of obstruction by commissurotomy. On the other hand, in the long-term survivors of aortic banding, the left ventricle (fig. 7B) resembled that of the very severe form of congenital aortic stenosis in which the aortic annulus and leaflets are hypoplastic; the wall of the left ventricle is extremely thick and the chamber diminutive. Even if the valvular obstruction could be relieved in such cases, the left ventricle would be too small to handle an adequate volume. The earlier aortic stenosis occurs in fetal development, the more likely is the obstruction to be progressive and, therefore, more severe.

Myocardial Hyperplasia

During fetal life, growth of the myocardium takes place primarily through mitosis of all cellular elements. The mitotic index in ventricular myocytes decreases toward term and ceases completely after birth.25, 24 Thereafter, growth of the heart occurs entirely by an increase in the size of muscle cells and a proliferation of the interstitial tissue. A greater than normal increase in ventricular mass in adults involves a greater than normal enlargement of muscle cells (hypertrophy). In fetuses, it has previously not been known what process is responsible for the enlargement in ventricular mass seen in congenital heart disease. Although an increased rate of mitosis (hyperplasia of myocytes) had been considered a possibility in fetuses, it had not been demonstrated previously. Distinguishing between hyperplasia and hypertrophy of myocytes is difficult because both processes may be accompanied by hyperplasia of interstitial cells.26 The DNA of myocytes, vascular endothelium and fibroblasts is biochemically identical. The same problem exists when attempting to distinguish between myocardial hypertrophy and hyperplasia by differences in RNA, RNA polymerase and other cellular enzymes.26

It is conceptually easy to imagine that a difference in the size or number of muscle cells between normal and enlarged ventricles could be readily determined histologically. Inherent in the use of histological techniques, however, is the assumption that normal geometric relationships are maintained throughout the preparation of the tissues. Unfortunately, with standard histological techniques, a great deal of distortion occurs during fixation and imbedding. During formalin fixation and paraffin imbedding, for example, uncontrolled cellular dehydration and interstitial edema occur (fig. 8). This type of distortion may be avoided by rapid fixation techniques in which glutaraldehyde is perfused through the arteries of the organ and the tissue is then imbedded in plastic. Without interstitial edema to separate cells, however, cell boundaries are not visible by light microscopy (fig. 9). The thickness of two adjacent cell membranes is beyond the resolving power of the light microscope. Resolution of cell membranes by electron microscopy is too cumbersome a technique for analyzing hundreds of muscle cells. Fortunately, the problem of identifying cell boundaries by light microscopy in glutaraldehyde perfused myocardial tissue was solved by the use of the peroxidase reaction (fig. 10).

The demonstration of myocardial hyperplasia in response to increased LV afterload is important, as it helps explain the differences in heart size and cardiovascular performance between congenital and acquired cardiac disease. The degree to which a muscle cell may enlarge by hypertrophy is limited. The average adult ventricular myocyte is 14 μm and can grow to a maximum of 30 μm in diameter. This limitation in growth is thought to be related to the transcriptional capacity of the DNA within the single nucleus of a myocardial muscle cell.27 By comparison, a skeletal muscle cell which is multinucleated, can hypertrophy to a diameter of 90 μm. During hyperplasia, nuclear DNA and cytoplasm are replicated proportionately. The degree of hyperplastic enlargement is, therefore, theoretically unlimited by nutritional constraints. Also, the muscle cells in a hyperplastic ventricle may secondarily hypertrophy after birth if the stimulus of increased afterload persists, thereby extending the ultimate potential for enlargement of the ventricle.

In summary, surgically induced lesions which alter patterns of blood flow within the heart and great vessels profoundly affect the systemic flow, blood pressures and gas tensions and the development of the myocardium of the fetus. Ventricular preload is clearly a prerequisite for continued growth of the ventricle at a normal rate and for maintaining a normal

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**Figure 9.** Photomicrograph of a fetal myocardium: cross-section of left ventricular (LV) papillary muscle (×1,000) fixed by perfusing glutaraldehyde through the coronary circulation of the beating heart, postfixed in O₂O₂ and imbedded in epoxy resin. Section is 1 μ thick. Open spaces are capillaries (Cap) in which the nuclei of the endothelial cells and relatively undistorted erythrocytes are visible. There is no visible intercellular space, nor are cell boundaries discernible.

**Figure 10.** Photomicrograph of fetal myocardium: cross-section of left ventricular papillary muscle (×1,000) showing cells outlined by the peroxidase reaction. Perfusion of the coronary circulation with glutaraldehyde was begun while the heart was beating. Tissue was imbedded in epoxy resin. Section is 1 μ thick. The open spaces are capillaries (Cap) in which the nuclei of the endothelial cells are visible and in some of which undistorted erythrocytes are also visible. Interstitial space is not visible.
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Cap

Cap
ventricular chamber size. Increased ventricular preload may, in the long run, also contribute to enlargement of the chamber volume. Increased ventricular afterload stimulates hyperplasia of ventricular myocytes, and within a very short period of rapid increase in ventricular mass may occur. We believe that the search for techniques by which to develop models of specific congenital heart disease in fetuses has been advanced by the observations made in these experiments.

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