Regression of Left Ventricular Dilation and Hypertrophy after Removal of Volume Overload

MORPHOLOGICAL AND ULTRASTRUCTURAL STUDY

By John M. Papadimitriou, Barry E. Hopkins, and Roger R. Taylor

ABSTRACT

Gross and ultrastructural changes occurring in the left ventricle in response to chronic volume overload were studied in dogs. One group of dogs was examined 28-43 days after an aortocaval fistula had been created; congestive heart failure had developed at the time of the examination. Two other groups of dogs were investigated 78 ± 17 (SD) days and 178 ± 4 days after similar fistulas had been closed. A group of control dogs was also studied. Aortocaval fistulas produced significant left ventricular dilation and hypertrophy. Ultrastructural changes included enlargement and distortion of intercalated disks, increase in number but decrease in size and relative volume of mitochondria, and loss of lateral alignment of sarcomeres. Left ventricles, 78 days after the fistulas had been closed, were not different ultrastructurally or grossly from those in dogs with patent fistulas. After 178 days of closure, ventricular mass and volume had decreased; mass was still significantly greater than that in normal dogs, but cavity volume was not. Ultrastructural abnormalities in dogs after 178 days of closure were much less marked than those in dogs with patent fistulas, and ultrastructure was hardly distinguishable from normal. The findings in these experiments indicate that gross and ultrastructural abnormalities produced in the left ventricle by chronic volume overload are largely, if not completely, reversible.

KEY WORDS canine myocardial hypertrophy aortocaval fistula distorted intercalated disks

Morphological abnormalities and ultrastructural changes associated with cardiac hypertrophy and dilation have been investigated extensively. However, the regression of these changes has received relatively little attention despite its great clinical relevance to the correct timing of cardiac surgery. Early indirect evidence of regression of chronic changes that accompany cardiac pressure or volume overload included the diminution of electrocardiographic voltage following the correction of pulmonary stenosis (1) and the radiological demonstration of a decrease in the size of the cardiac silhouette or in the chamber volume following aortic valve replacement (2). Recent studies in man have emphasized that the left ventricular dilation and hypertrophy resulting from the volume load imposed by mitral (3) or aortic (3, 14) regurgitation may often be slow to regress or fail to regress following valve replacement with a prosthesis.

Although clinical studies of this type are very important, methodological limitations make the complete documentation of the regression of hypertrophy difficult or impossible in man. Also, studies in animals have been limited to observations of the reduction in heart weight in rats following the removal of a stimulus to hypertrophy such as hypertension (5, 6), hyperthyroidism (6), anemia (6), hypoxia (7), or exercise (7). Therefore, the present study was performed to determine structural and ultrastructural alterations in response to regression. The model selected for study was the moderate cardiac dilation and hypertrophy resulting from the large circulatory volume overload induced by an aortocaval fistula in the dog (8, 9). The chronic compensatory response to a pressure load is hypertrophy without cavity dilation (10), but the response to a volume load consists of dilation with approximately commensurate hypertrophy (8, 9, 11). The details of the process of regression depend on the nature of the initial stimulus as well as other factors, and observations in any one specific situation are not necessarily
generally applicable. Although there were disadvantages inherent in the present model, it was useful because lesions induced by cardiac volume overload are common in man, a fairly uniform degree of moderate cardiac dilation and hypertrophy could be produced (8, 9), and the stimulus could be removed without great trauma and risk of cardiac damage. Hemodynamic parameters and gross and ultrastructural changes in the left ventricle were documented 4–6 weeks after an infrarenal aortocaval fistula was established. After a similar interval in other dogs, the fistulas were closed; these dogs were then studied again after 3 or 6 months. Partial reversion of dilation and hypertrophy was established, and some gross and ultrastructural characteristics were documented.

Methods

Under sterile conditions, side-to-side infrarenal aortocaval fistulas (14–16 mm in length) were created in mongrel dogs (17.4–25.9 kg). The dogs were anesthetized with intravenous infusions of thiopental and pentobarbital; anesthesia was maintained with halothane, nitrous oxide, and oxygen.

After definite limb edema, ascites, or both and dyspnea, had developed (28–43 days), the dogs were randomly studied while the fistula was patent (7 dogs), or the fistula was closed at that stage. Dogs with closed fistulas were studied 3 months (5 dogs) or 6 months (6 dogs) later. To ensure that the dogs would be closely matched, 7 dogs that did not develop clear signs of clinical cardiac failure were discarded from the protocol. Also, because of severe heart failure before 28 days had elapsed (28–43 days), the dogs were randomly allocated to the control group.

TABLE 1

<table>
<thead>
<tr>
<th>Dog no.</th>
<th>Fistula</th>
<th>Body weight (kg)</th>
<th>Heart rate (beats/min)</th>
<th>Femoral arterial pressure (mm Hg)</th>
<th>Aortic arch pressure (mm Hg)</th>
<th>Left ventricular end-diastolic pressure (mm Hg)</th>
<th>Cardiac index (ml/min kg⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Present</td>
<td>20.0</td>
<td>125</td>
<td>90/37</td>
<td>130/65</td>
<td>38</td>
<td>389</td>
</tr>
<tr>
<td>2</td>
<td>Closed</td>
<td>22.0</td>
<td>60</td>
<td>120/60</td>
<td>120/65</td>
<td>7.5</td>
<td>109</td>
</tr>
<tr>
<td>3</td>
<td>Present</td>
<td>20.0</td>
<td>140</td>
<td>160/42</td>
<td>150/70</td>
<td>42</td>
<td>241</td>
</tr>
<tr>
<td></td>
<td>Closed</td>
<td>21.5</td>
<td>59</td>
<td>120/57</td>
<td>105/60</td>
<td>7</td>
<td>100</td>
</tr>
<tr>
<td>3</td>
<td>Present</td>
<td>19.1</td>
<td>120</td>
<td>125/40</td>
<td>100/42</td>
<td>27</td>
<td>434</td>
</tr>
<tr>
<td></td>
<td>Closed</td>
<td>18.2</td>
<td>73</td>
<td>105/55</td>
<td>98/62</td>
<td>3</td>
<td>140</td>
</tr>
</tbody>
</table>

Anesthesia was induced with halothane, and maintenance with halothane, nitrous oxide, and oxygen using positive-pressure ventilation. After the posterior abdominal wall had been exposed through a lower anterior midline incision, the small, colored plastic markers previously sewn to each end of the aortocaval fistulas were located. The aorta and the vena cava in the region of the fistula were isolated from other structures but were not separated from each other. Clamps were placed around both vessels above and below the fistula, the anastomosis was dissected, and the defect in each vessel was closed with a continuous suture of 5/0 cardiovascular silk. Furosemide was given again at the completion of surgery; penicillin and streptomycin were given daily for 1 week. Freshly obtained blood from one donor was usually infused during the last part of the operation, since bleeding from dilated veins on the posterior abdominal wall often occurred. Care was taken not to unnecessarily aggravate the existing circulatory overload in the absence of blood loss. On the other hand, it was undesirable for the dogs to be anemic postoperatively, because anemia can perpetuate cardiac changes. The hemoglobin level 1–3 weeks later was 13.7 ± 2.2 (SD) g/100 ml and did not differ from that in dogs in the control group.

Dogs with fistulas closed for 3 or 6 months, dogs with patent fistulas, and control dogs were studied using the same protocol. Also, similar hemodynamic studies using sterile techniques were performed on three dogs before their fistulas were closed, and the complete protocol was followed at death 3 months later. The dogs were sedated with morphine (2 mg/kg, im), promazine (1.5 mg/kg, im), and promethazine (1.5 mg/kg, im). Local analgesia with lidocaine was used for catheter insertions. The aorta and left ventricle were catheterized by the retrograde route through a right femoral arteriotomy using a no. 5 Courand catheter attached to a Statham P23Db pressure transducer. Pressures, referred to midchest level without correction for intrapleural pressure, were recorded with the electrocardiogram on a multichannel direct-writing recorder (Hewlett-Packard, model 7718A). Cardiac output was measured in duplicate by left ventricular injection and left femoral arterial sampling of indocyanine green dye (Gilford densitometer model 103IR). The dog was then anesthetized with an intravenous injection of pentobarbital, and the chest was quickly opened. A midwall sample of muscle (0.5 g) was taken from the anterolateral section of the left ventricle.
The right ventricle was vented; a clamp was placed across the atrioventricular groove, and the pressure-volume relation of the left ventricle was determined in duplicate (12). At a filling pressure of 20 mm Hg the two pressure-volume curves differed by 2.4 ± 0.8 (80) ml in normal dogs, by 2.1 ± 2.5 ml in dogs with patent fistulas, and by 3.0 ± 2.8 ml in dogs with closed fistulas. There was no systematic difference between the first and second curves. The right ventricle and the atria were resected from the left ventricle including the septum; the left ventricle and septum were weighed.

Seven dogs with patent fistulas were studied after 37 ± 4 days. Five dogs were studied 78 ± 17 days after closure of a fistula that had been present for 37 ± 6 days, and six other dogs were studied 178 ± 4 days after closure of a fistula that had been present for 34 ± 6 days. Inspection at the end of each procedure confirmed that the fistulas were closed and that the suture lines were free of obvious infection.

The significance of the difference between group means was evaluated by Student's t-test.

**ELECTRON MICROSCOPY**

Small specimens of left ventricular myocardium measuring approximately 1 mm² were immersed in cold 3% glutaraldehyde in 0.1M cacodylate buffer (pH 7.4). After 16 hours the tissues were washed in buffer and postfixed for 1 hour in 1% osmium tetroxide in 0.1M cacodylate buffer (pH 7.4). Specimens were dehydrated in graded solutions of ethanol and embedded in Araldite. Blocks were oriented under a dissecting microscope prior to sectioning on a LKB ultramicrotome. Sections were stained with lead hydroxide and examined under an electron microscope (JEM T6 or Philips 300).

**INTERCALATED DISK WIDTH**

The thickness of the intercalated disks was measured using a modification of the method suggested by Laks et al. (13).

**QUANTITATIVE ANALYSIS OF THE MITOCHONDRIAL POPULATION**

From each specimen electron micrographs of 10-15 separate fibers were taken. The perinuclear mitochondrial zone was not included in the micrographs used for histometric analysis, and only well-fixed fibers were selected for study. The methods of Bishop and Cole (14) were used to assess quantitatively the characteristics of the mitochondria. The parameters estimated were (1) the number of mitochondria per 100 μm², (2) the percent of fiber volume occupied by mitochondria, and (3) the ratio of surface area to volume of the mitochondria. Weibel (15) has presented mathematical justifications for three-dimensional interpretations of two-dimensional ultrastructural samples. The formulas suggested by Loud et al. (16) were used in the calculations.

**Results**

Individual hemodynamic measurements made in three dogs with patent fistulas that were subsequently closed and measurements repeated 3 months later are shown in Table 1. Cardiac index, left ventricular end-diastolic pressure, arterial pulse pressure, and heart rate were grossly elevated when the fistula was patent; these parameters returned to normal 3 months after the fistula was closed. Table 2 presents mean values of hemodynamic parameters, left ventricular mass, and left ventricular volume at the time of final study in all of the dogs. Left ventricular mass was 27% greater in the dogs with patent fistulas than it was in the normal dogs (Table 2). Although there was some evidence of regression of hypertrophy, the ventricular mass after 6 months of closure was still significantly greater than normal (P < 0.05) (Table 2). On the other hand, ventricular volume at a given filling pressure appeared to have regressed almost half the way to normal after 3 months; however, it was still significantly different from that in control dogs but not different from that in dogs with patent fistulas. Six months after closure, ventricular volume was significantly different from that in dogs with patent fistulas but not from that in control dogs (Table 2).

**MYOCARDIUM WITH PATENT FISTULA**

The hypertrophied fibers exhibited both qualitative and quantitative differences from normal fibers. Nuclei were elongated with much euchromatin and large nucleoli. Sarcomeres in hypertrophied myocardium were more frequently malaligned than were those in normal myocardium. This condition resulted, for example, in the apposition of the Z lines of the sarcomeres of one fibril with the central areas of adjacent sarcomeres (Figs. 1 and 2). Not all adjacent sarcomeres showed such extreme malalignment; some showed good alignment (Fig. 3B) similar to that in normal fibers (Fig. 2A) or various degrees of less severe malalignment (Fig. 2B). Therefore, quantification of this abnormality did not seem feasible.

Many intercalated disks were irregular and hyperconvoluted (Fig. 3B); the wavy contours of the normal intercalated disk that are shown in Figure 3A were lost. The irregular folding led to a significant increase in the average width of the disks (Table 3).

Mitochondria varied in size and distribution. Mitochondria in normal muscle were frequently large and several sarcomeres in length (Fig. 2A), but in hypertrophied muscle they were smaller (Fig. 2B). There was a significant increase in the number of mitochondria in 100 μm² of hypertrophied muscle fiber when compared with normal muscle fiber but, because of their smaller size, there was a significant decrease in the ratio of mitochondrial volume to fiber volume (Table 3).

**MYOCARDIUM AFTER CLOSURE OF FISTULA**

Three months after closure of the fistulas, intercalated disk width and mitochondria were not
<table>
<thead>
<tr>
<th>Measurements in Normal Dogs and Dogs with Patent Fistulas and Closed Fistulas</th>
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<tr>
<td><strong>Table 2</strong></td>
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<tr>
<td>------------------------------------------------</td>
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<tr>
<td><strong>Body weight (kg)</strong></td>
</tr>
<tr>
<td>------------------------------------------------</td>
</tr>
<tr>
<td>Normal (8)</td>
</tr>
<tr>
<td>Patent fistula (7)</td>
</tr>
<tr>
<td>Fistula closed 3 months (5)</td>
</tr>
<tr>
<td>Fistula closed 6 months (6)</td>
</tr>
</tbody>
</table>

All values are means ± sd. Number of dogs studied is given in parentheses. NS = not significant.

* P value compared with normal dogs.

† P value compared with dogs with patent fistulas.
FIGURE 1

Myocardium from a dog with a patent aortocaval fistula showing loss of alignment of corresponding components of adjacent sarcomeres. The solid arrows to the left of the cross indicate Z lines, and the open arrows indicate the center or M bands of sarcomeres. The Z lines of the sarcomeres of one myofibril are opposite the M bands of its neighboring myofibril. Bar = 0.5 μm.

significantly different from those in dogs with patent fistulas, but they were significantly different from those in normal dogs (Table 3).

Six months after closure of the fistulas, intercalated disks and mitochondria were significantly different from those in dogs with patent fistulas (Table 3). Mitochondria had increased in size (Fig. 2C), and the number and the volume of mitochondria relative to fiber volume were not different from those in normal dogs (Table 3). Although the average width of intercalated disks was significantly less than that in dogs with patent fistulas (Table 3), there was still some irregularity and hyperconvolution of the disks (Fig. 3C); the average disk width was just midway between that in normal myocardium and that in myocardium from dogs with patent fistulas (Table 3). Malalignment of adjacent sarcomeres was definitely less frequent than that in dogs with patent fistulas but possibly more frequent than that in normal myocardium.

Discussion

The present experiments attempted to document whether cardiac dilation and hypertrophy in response to a chronic volume overload regress, and, if they do, to ascertain the rate of this regression and characterize some of the accompanying ultrastructural changes. In our model, chronic dilation with approximately commensurate hypertrophy was induced in response to volume overload (8, 9, 11); this hypertrophy is distinct from that initially unaccompanied by cavity dilation which is induced by a chronic pressure overload (10). The details of hypertrophy and its regression depend on individual circumstances; the present observations in a relatively simple model represent only one approach to a complicated question.

This study also attempted to document the ultrastructural changes that occur in response to volume overload. The most obvious measurable changes in the left ventricle were the thickening and the increased folding of the intercalated disks and the decrease in size and relative volume of mitochondria. The loss of the usual apposition of Z lines and other sarcomere components of adjacent myofibrils, previously reported in this preparation (9), was observed but was not amenable to quantification. Similar intercalated disk changes have been described in the pressure-overloaded hypertrophied right ventricle of the dog with pulmonary stenosis, both with (14) and without (13) cardiac failure, but they have not been described in the dilation and hypertrophy associated with volume-overload lesions. A decrease in the size of mitochondria of the right ventricle has also been described in the dog with pulmonary stenosis (14); however, in the presence of various cardiac pressure- and volume-overload lesions most other authors have described mitochondria as large (17-19) or unchanged (7, 20). The reason for these differences is unclear. Perhaps mitochondrial size varies with the nature, duration, and severity of the overload lesion since mitochondria can, apparently, change their size rapidly; it has been reported that mitochondria increase their relative surface area by nearly half in rats exercised for several hours by swimming (21). Meerson (19) has reported an increase in the proportion of fiber occupied by mitochondria within the first week of imposition of left ventricular pressure overload by
aortic stenosis in rabbits followed by subsequent normalization and, finally, a decrease in the proportion of cell occupied by mitochondria. In this respect our results are consistent with the latter phase, but Meerson (19) has reported a persistent increase in the size of individual mitochondria rather than the decrease that we found.

Most previous information about the regression of hypertrophy and dilation has been indirect or limited to observations on heart weight. Heart weight has been shown to decrease in rats following removal of a stimulus consisting of hypertension (5, 6), hyperthyroidism (6), anemia (6), hypoxia (7), or exercise (7). We are not aware of previous observations on structural and ultrastructural changes accompanying reversion of cardiac dilation and

**FIGURE 2**

A: Myocardium from a normal dog. Large mitochondria (M, and M₂) measure more than two sarcomeres in length. Components of sarcomeres of adjacent myofibrils were aligned. Bar = 0.5 μm. B: Myocardium from a dog with a patent aortocaval fistula. Mitochondria are small and measure about one sarcomere in length. Toward the right of the figure the components of adjacent sarcomeres become malaligned. Bar = 0.5 μm. C: Myocardium from a dog 6 months after closure of the aortocaval fistula. Although the mitochondria is larger than that in the normal dog (A), the average mitochondrial characteristics were not different from normal. Bar = 0.5 μm.

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hypertrophy. In our experimental preparation, regression of both dilation and hypertrophy of the left ventricle was considerably slower than was their appearance. None of the parameters associated with dilation and hypertrophy examined 3 months after the fistulas had been closed were significantly different from those in dogs with patent fistulas. After 6 months, cavity size and characteristics of mitochondria were not significantly different from those in normal dogs, but left ventricular weight and width of the intercalated disks were still greater than those in normal dogs. There is no a priori reason why regression of the various changes should occur together; relatively rapid changes in size and volume of mitochondria might be expected on the basis of previous observations (19, 21), but it is more difficult to conceive of the regression in the changes in the intercalated disks, although this regression did occur.

The question might be raised concerning the assessment of alignment of corresponding components of adjacent sarcomeres. Unfortunately, there
was no satisfactory method to quantify this observation, but loss of alignment did occur, as previously seen in the left ventricle of dogs with aortocaval fistulas (9) and in the right ventricle of dogs with pulmonary stenosis (14); in addition, the realignment occurred 6 months after removal of the overload. Although the alignment of sarcomeres may depend on the degree of stretch at which the muscle is fixed, similar malalignment was found in dogs with patent aortocaval fistulas when their ventricles were fixed by coronary perfusion at high ventricular distending pressure (9). Furthermore, the same method of immersion fixation was used throughout the study so that the findings in the several groups should be comparable. The realignment of sarcomeres implies that myofibrils which had malaligned were pulled back into alignment by other intact cell components.

Obviously, the present results cannot be extrapolated to all types of cardiac hypertrophy and dilation. The lesion studied produces volume overload with dilation and hypertrophy; but the chronic adaptive process depends on the nature of the overload lesion so the process of regression will vary. Furthermore, the overload lesion was suddenly imposed and its duration was short compared with the usual clinical situation. The type and duration of lesion studied has previously been found to be unassociated with measurable depression of left ventricular contractility (8), although severe volume overload of longer duration, or at least that due to aortic regurgitation, leads to depression of left ventricular function (4, 11). When aortic regurgitation is associated with left ventricular failure in human subjects, Gault et al. (4) have found no improvement in chronic ventricular dilation or in the tension-velocity relation after aortic valve replacement. Similarly, Baxley and Dodge (3) have found very little, if any, decrease in ventricular mass or volume 4–14 months after mitral or aortic valve replacement (3). Chronic fibrotic or other changes occurring in the presence of lesions of long duration and the additional presence of rheumatic, ischemic, or other myocardial disease in human subjects may impair or prevent regression of hypertrophy or dilation. However, it has been shown that cardiac dilation and hypertrophy and some of their ultrastructural accompaniments are capable of at least partial regression.

Acknowledgment

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