Myocardial Necrosis, Fibrosis, and DNA Synthesis in Experimental Cardiac Hypertrophy Induced by Sudden Pressure Overload

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SUMMARY The development of myocardial fibrosis as a result of cardiac hypertrophy was studied in 11 cats in which the pulmonary artery was banded, six rabbits in which the ascending aorta was banded, and eight cats with various congenital cardiac anomalies. Histological examination of the myocardium revealed multifocal areas of degeneration and necrosis with healing by fibrosis in the right ventricle of cats in which the pulmonary artery was banded and in the left ventricle of rabbits in which the aorta was banded. In five of eight cats with congenital anomalies, myocardial necrosis and fibrosis were not present in spite of heart weight to body weight ratios 2-4 times greater than in the experimental models. In the other three cats, fibrosis was subendocardial or diffuse rather than multifocal as in the banded animals. This suggests that the increased connective tissue found in animals with cardiac hypertrophy induced by banding the aorta or pulmonary artery is an artifact of the preparation. Autoradiographic studies of the myocardium of pulmonary artery-banded cats indicated that all newly synthesized DNA in this model is restricted to interstitial cell and endothelial cell proliferation.

Morphological alterations induced in an experimental model should be compared with those present in the naturally occurring condition in the same species and with those in man, to whom experimental results often are extrapolated.

The role of hyperplasia vs. hypertrophy of cardiac myocytes in the process of cardiac enlargement has been studied by several investigators. Determination of DNA content in experimental models of cardiac hypertrophy has generally shown that DNA concentration is the same as or increased compared to controls, indicating an increased total DNA content in the enlarged heart. However, it has been shown by autoradiographic localization of tritiated thymidine uptake in the rat heart following banding of the aorta, that the newly synthesized DNA is restricted almost completely to nonmuscular cellular elements in the myocardium.

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type of stimulus used to initiate the hypertrophy. Banding of the pulmonary artery in the cat is accompanied by an increase in hydroxyproline in the right ventricle, indicating increased connective tissue synthesis with this model. Reports of hydroxyproline and connective tissue content in hypertrophied human hearts, however, have indicated that in the absence of occlusive vascular disease, connective tissue content is not increased.

The purpose of the present investigation was to study the histological development of myocardial connective tissue formation in experimental animal models with sudden pressure overload on the myocardium, and to compare the histological features with those in naturally occurring cardiac hypertrophy in the same species. In addition, autoradiographic localization of tritiated thymidine was used to determine cellular localization of DNA synthesis in cats in which the pulmonary artery is banded.

Methods

EXPERIMENTAL ANIMALS AND SURGICAL PROCEDURE

Cats ranging in age from 8 weeks to 6 years were obtained either from a specific pathogen-free colony or from a conventional source of cats. Pulmonary artery stenosis was produced in 11 adult cats and aortic stenosis in six New Zealand White rabbits (weighing 2.5-3.5 kg) according to the method described by Spann et al. Anesthesia was induced with intravenous thiamylal sodium (25-30 mg/kg) and maintained with methoxyflurane administered with oxygen and positive-pressure respiration. Using sterile surgical procedure, the pulmonary artery or aorta was exposed via a left thoracotomy and a suture encased with polyethylene tubing and reinforced with stainless steel wire was placed around the vessel. Bands were 2.9 mm in diameter for cat pulmonary arteries and 3.5 mm in diameter for rabbit aortas. Bands with smaller diameters frequently produced acute congestive failure. The chest was closed by layers, the pneumothorax was evacuated, and the animal was allowed to recover from the anesthesia. Aspects, hydrothorax, hepatic congestion, or other signs of congestive heart failure did not occur in any animals included in the study. The control animals were 33 cats and 17 rabbits in which no surgical procedure was performed, and eight rabbits in which a thoracotomy and aortic dissection was performed but a band was not placed on the artery.

An additional eight cats with various forms of congenital heart disease were available for gross and histopathological examination. These cats weighed 1.15-3.5 kg and were 4 months to 1 year old. Congenital defects in this group of cats included ostium primum type atrial septal defects (five), patent ductus arteriosus (one), pulmonic valve stenosis (one), and ventricular septal defect combined with pulmonic valve stenosis (one).

GROSS PATHOLOGY AND HISTOPATHOLOGY

Cats were studied 3-32 days and rabbits 7-60 days after surgery. Animals were anesthetized with sodium pentobarbital (25 mg/kg of body weight) and the heart was fixed by retrograde perfusion through the aorta with phosphate-buffered saline followed by 2% phosphate-buffered glutaraldehyde. The atria were removed from the ventricles at the atrioventricular sulcus. The right ventricular free wall was dissected from the left ventricle and septum, and each portion was weighed and related to total body weight. Multiple sections of the heart from standard locations including both ventricles and the interventricular septum were embedded in paraffin, sectioned at 6 µm, and stained with hematoxylin and eosin and Gomori's aldehyde-fuchsin-trichrome stains.

AUTORADIOGRAPHY

Four of the 11 cats in which the pulmonary artery was banded and four cats (controls) that were not operated on were given an intravenous injection of tritiated thymidine (specific activity, 16 Ci/mmol), 0.5 µCi/g of body weight, and killed 3 hours later. The banded cats were studied at 3, 7, 10, and 14 days after surgery. Autoradiographs were prepared from 4-µm sections mounted on glass slides, deparaffinized, washed, and dip-coated with Kodak NTB2 liquid emulsion. Sections were air-dried, incubated in the dark for 1-3 weeks, developed with Kodak D-19 developer, and stained with hematoxylin and eosin. To quantify the number and distribution of tritium-labeled nuclei in the myocardium, full wall thickness sections of left and right ventricles and interventricular septum were examined by light microscopy. A calibrated eyepiece grid covering 0.25 mm² of tissue section at 100× magnification was used to count the number of labeled nuclei per grid. Adjacent tissue areas from endocardium to epicardium were counted for a minimum of 10 endocardial to epicardial columns and a minimum of 40 fields. The sections were divided into endocardial, midwall, and epicardial thirds (right, midwall, and left for interventricular septum) and the data were expressed as the number of labeled nuclei per square millimeter of tissue section.

Results

DEVELOPMENT OF HYPERTROPHY

During the study all animals remained in good condition and there was no significant change in body weight. In the cats in which the pulmonary artery was banded, right ventricular free wall weight to body weight ratios were moderately increased to a mean of 0.88 ± 0.66 (1 SEM) g/kg from a control mean of 0.67 ± 0.026 g/kg (P < 0.001), while the weight of the left ventricle plus septum decreased slightly from a control mean of 2.41 ± 0.066 g/kg to 2.08 ± 0.084 g/kg. The ratio of right ventricular free wall weight to left ventricle plus septum weight increased from 0.281 ± 0.007 in controls to 0.423 ± 0.026 (P < 0.001) in cats with the pulmonary artery banded. Right ventricular weight to body weight ratios in the four cats used for the tritiated thymidine autoradiographic studies were 0.75, 0.89, 0.95, and 1.03 g/kg.

Ventricular weight to body weight ratios in the cats with congenital heart disease were increased greatly from the normal animal values. Median right ventricular free wall weight to body weight was 2.55 g/kg (range = 2.08-7.13 g/kg), and median left ventricle plus septum to body weight was 4.20 g/kg (range = 3.55-10.65 g/kg). Although several of the cats with congenital heart disease had not grown well,
all appeared to be in good condition at the time of necropsy except one with emaciation (highest values given above). None had shown clinical signs of congestive heart failure.

Bands placed on the aortas of six rabbits for 7–60 days resulted in an increase in the mean left ventricle plus septum to body weight ratio, from 0.36 ± 0.036 g/kg in control rabbits to 1.59 ± 0.080 g/kg (P < 0.01) in the banded animals. There was no significant difference in right ventricular free wall weight to body weight ratios (controls, 0.39 ± 0.015 g/kg; banded, 0.42 ± 0.026 g/kg).

**HISTOPATHOLOGY**

Banding of the pulmonary artery in the cat resulted in multifocal areas of myofiber degeneration, necrosis, and fibrosis throughout the wall of the right ventricle, in the papillary muscles of the right ventricle, and in the right ventricular portion of the interventricular septum (Fig. 1). The size of these focal areas ranged from less than 30 μm up to 200 μm in diameter. Rarely, larger areas of fibrosis up to 1.0 mm in diameter were encountered. There was no predilection for subendocardial areas within the wall, all areas being equally affected. The left ventricle was normal.

By 3–5 days after banding of the pulmonary artery, the foci of myofibrillar degeneration consisted of necrotic myofibers characterized by acidophilic staining, loss of striations, a granular appearance of the cytoplasm, nuclear pyknosis, and karyorrhexis. A few cardiac histiocytes and fibroblasts were present. By 7–10 days, histiocytes and fibroblasts were numerous and collagen fibers were present. By 14 days and later, collagogenous scars with few fibroblasts or histiocytes were present. Neutrophilic or lymphocytic inflammatory cell infiltrates were not found at any stage of the lesion. There often was an increase in diffuse interstitial collagenous connective tissue as well as the multifocal areas of fibrosis.

Cats subjected to thoracotomy had mild to moderate fibroinflammatory lesions associated with the surgical procedure. The epicarditis was most severe over the atria, and less severe over the ventricles. Both ventricles were affected, but the inflammatory process was restricted to the epicardium and pericardium and did not appear to extend into the myocardium. The only other significant lesion was chronic passive congestion of the liver in some cats.

Banding of the rabbit aorta resulted in patchy focal myocardial necrosis with healing by fibrosis in the left ventricle similar to that seen in the right ventricles of cats in which the pulmonary artery was banded (Fig. 2). In the rabbit left ventricle, however, the distribution of the lesion was predominantly in the base of the free wall and in the subendocardial regions, particularly the papillary muscles. The left ventricular midwall and subepicardial regions were much less severely affected.

Cats with congenital heart disease had massive cardiac hypertrophy. Myocardial fibrosis detected by histological examination was much less extensive and of different distribution than that in animals with hypertrophy produced by banding of the artery. Five of eight cats examined had no histologically detectable fibrosis of the myocardium. One cat (patent ductus arteriosus) had fibrosis restricted to the tips of the left ventricular papillary muscles, one cat (atrial septal defect) had fibrosis of the right ventricular suben-

docardiand left ventricular midwall, and one cat (atrial septal defect) had diffuse fibrosis of both ventricles and septum, especially prominent near the base of the left ventricle.

**AUTORADIOGRAPHY**

Incorporation of tritiated thymidine at 3, 7, 10, and 14 days after pulmonary artery banding was restricted to endothelial and interstitial connective tissue cells in all four animals studied (Fig. 3). Labeled myocyte nuclei were not seen. The distribution of labeled nuclei is presented in Table 1. In the four normal control cats only occasional labeled endothelial cell nuclei were found. Banding of the pulmonary artery resulted in a marked increase in the number of labeled nuclei in the right ventricle, distributed evenly across the wall. In addition, there was a mild increase in labeled endothelial cell nuclei in the left ventricle of pulmonary artery banded cats compared to control animals. The increased number of labeled nuclei in the left ventricular epicardial region compared to other portions of the left ventricle is associated with a mild epicarditis due to the thoracotomy.

**Discussion**

Many experimental models of cardiac hypertrophy have been used in an attempt to understand the various functional, structural, and biochemical alterations which occur during the development of hypertrophy and failure in the human heart. Since an increase in outflow resistance from one or the other ventricle is a frequent cause of cardiac hypertrophy in man, surgical banding of the aorta or pulmonary artery in experimental animals frequently has been employed. Although the method produces a significant increase in ventricular mass, the sudden and severe strain on the myocardium does not mimic the course of events of slowly increasing outflow resistance during the development of cardiac hypertrophy in man. Therefore, alterations in the function or structure of the myocardium in animals subjected to acute banding of a major artery may not reflect those which occur as a result of disease in man. Nevertheless, such models have provided major contributions to the understanding of the mechanisms involved in cardiac hypertrophy and failure.

There was an average 40% increase in right ventricular mass produced in the present study by banding the pulmonary artery of the cat. This degree of hypertrophy is similar to or slightly less than that reported by other investigators, indicating that the degree of stress on the myocardium in the present study was similar to that induced by others. The mild decrease in left ventricular mass is similar to that reported by Buccino et al. and by Spann et al., although others have found no change in left ventricular mass following pulmonary artery banding in the cat.

Experimental cardiac hypertrophy seldom results in more than a 100% increase in right ventricular mass, and most studies report a 10–60% increase in weight. While some of these experimental models do develop signs of congestive heart failure, the degree of hypertrophy is much less than that which frequently occurs with spontaneous disease in man and animals. This degree of hypertrophy is most likely related to the long course of gradually increasing severity of
Figure 1. Right ventricular free wall of cats in which the pulmonary artery was banded. A: full wall thickness of right ventricle 5 days after banding the pulmonary artery; epicardium is at the top; focal areas of degeneration and necrosis of myofibers are indicated by arrows. B: higher magnification of a cross-sectional area of focal myocardial necrosis and early interstitial fibrosis in a cat 3 days after the pulmonary artery was banded. C: longitudinal section of a focal area of necrosis and interstitial fibrosis 5 days after banding the pulmonary artery of a cat. D: subendocardial focal scar formation in the right ventricle 14 days after banding the pulmonary artery of a cat. Gomori aldehyde-fuchsin-trichrome stain. 560x.
workload during the normal growth period. To produce an animal model with a more natural course and a degree of hypertrophy similar to that in man, it may be necessary to develop methods which produce a gradual degree of increase in the severity of the stress over a prolonged period. While this has been accomplished by banding the aorta of growing animals,\textsuperscript{14, 21, 22} use of ameroid clips,\textsuperscript{23} or by various methods of externally controlled progressive stenosis of the aorta or pulmonary artery,\textsuperscript{14-22} there is a need for better animal models which more closely reflect the spontaneous condition of cardiac hypertrophy in man.

Banding the pulmonary artery of the cat resulted in multifocal areas of myofiber degeneration and necrosis throughout the right ventricular free wall, right ventricular papillary muscles, and right ventricular portion of the interventricular septum. With time, the areas of necrosis were replaced with fibrous connective tissue scar formation. Banding of the aorta in the rabbit also produced multifocal areas of necrosis, but in contrast to the widespread distribution in the right ventricle, fibrosis in the left ventricle was more frequent in subendocardial regions. These areas of fibrosis in the cat and rabbit myocardium appear to be the morphological expression of increased hydroxyproline content previously reported in the myocardium of banded cats.\textsuperscript{1}

Although not determined in the present study, previous reports have demonstrated an increase in water content of the myocardium during the first 10 days after banding the pulmonary artery of the cat\textsuperscript{1} or rabbit.\textsuperscript{12} This apparently is inflammatory edema associated with the myocardial necrosis.

Histological examination of the myocardium from cats with congenital heart disease failed to reveal abnormal...
amounts of connective tissue in five of eight animals studied. In the other three, fibrosis involved confluent areas, especially in the subendocardial regions, and was not of a widespread multifocal nature as in the experimentally banded cats. This would suggest that the increased connective tissue in the experimental model is an artifact of the method of production of hypertrophy, and not a feature of the naturally occurring condition. Additional evidence that increased connective tissue in myocardial hypertrophy of cats with the pulmonary artery banded is an artifact may be found in previously reported studies of connective tissue content of hypertrophied human hearts. Blumgart et al. measured hydroxyproline content of human hearts and found that only nine of 23 hypertrophied human hearts had an increased level of collagen and that five of these nine had significant coronary artery disease. Oken and Boucek, in a study of 70 human hearts, found that only two of 14 hypertrophied hearts had an increase in concentration of hydroxyproline, whereas in most hypertrophied hearts the hydroxyproline concentration was the same as in normal tissue. Montfort and Perez-Tomayo also found that in the absence of ischemic heart disease there was no change in the concentration of collagen as determined by hydroxyproline measurement in hypertrophied human hearts. In a recent morphometric study of hypertrophied human hearts, Sasaki et al. found a correlation of the degree of fibrosis with increased weight of the heart. However, the most severe fibrosis was found in hearts from patients with vascular and

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<th>Table 1 Effect of Pulmonary Artery Banding in the Cat on Distribution of 3H-Thymidine Labeled Nuclei</th>
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<td><strong>Right ventricle</strong></td>
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renal disease. Therefore, it appears that both man and animals with naturally occurring cardiac hypertrophy in the absence of associated vascular diseases, an increase in collagenous connective tissue is unusual. Since the multifocal distribution of necrosis and fibrosis in cats in which the pulmonary artery was banded is different from the fibrosis found in three of eight cats with congenital heart disease in the present study, and is different from that commonly encountered in hypertrophied human hearts at autopsy, it appears that the lesion in the experimental animals is an artifact of the preparation.

Several mechanisms for development of focal areas of necrosis following banding of the aorta or pulmonary artery are possible. One explanation may be the development of a relative deficiency between the amount of blood flow to the myocardium and the increased oxygen demand produced by the sudden work overload. The model produces a sudden disparity in the normal relationship between the ventricular intraluminal systolic pressure and the coronary artery perfusion pressure, which, when coupled with the increased oxygen demand of the myocardium due to the increased workload, may result in zones of relative hypoxia in the heart. A similar type of myocardial degeneration and necrosis is produced by hemorrhagic shock in a variety of animal species, and by isoproterenol or norepinephrine. Whether circulating catecholamines, other hormones, or neurogenic factors associated with the surgery and work-load-induced stress may play a role in the pathogenesis of the focal degeneration and necrosis in this model has not been explored.

Further support for the hypothesis that cellular necrosis is due to relative oxygen deficiency during sudden work overload of the myocardium is found in the studies of Ratliff et al. Hemorrhagic necrosis of the myocardium produced by hypovolemic shock in the dog could be prevented by exposure to the animal to hyperbaric oxygen during the experimental shock period. Exposure of animals to hyperbaric oxygen increased delivery of oxygen to the heart with increased oxygen extraction from the blood. These authors concluded that shock-induced myocardial necrosis was due to a deficiency of oxygen delivery relative to oxygen demand.

The autoradiographic studies in the cats in which the pulmonary artery was banded indicated that "H-thymidine incorporation associated with DNA synthesis was localized principally in endothelial cell nuclei, with occasional interstitial fibroblasts or histiocytes also labeled. This suggests proliferation of the vascular bed to accommodate increased blood flow to the stressed myocardium. Previous studies have demonstrated that increased blood flow through vessels, such as occurs in collateral vessels to an area of experimental myocardial infarction, results in the proliferation of endothelial cells as demonstrated by "H-thymidine incorporation.

In considering the role of hyperplasia vs. hypertrophy of myocardial cells in the enlargement of the adult heart subjected to an increased workload, there appears to be a consensus of experimental evidence in favor of hypertrophy. However, Linzbach has provided morphological evidence that with extreme degrees of cardiac enlargement there is hyperplasia of myocardial muscle cells. Several previous studies also have provided perplexing results in which DNA concentrations in the experimentally enlarged heart either were not changed or were increased. An explanation for the increased DNA concentration without hyperplasia of the myocytes was provided by demonstrations in the rat with banded aorta, that all newly synthesized DNA was restricted to interstitial connective tissue and endothelial cells. The present autoradiographic study has extended this finding to the cat with banding of the pulmonary artery. The significance of this finding together with the fact that, in the rat, endothelial and fibroblast nuclei outnumber myocyte nuclei by approximately 4 to 1 should be kept in mind by investigators attempting to study nuclear-related functions of hypertrophied myocardial tissue. In myocardial homogenates, while the bulk of the cytoplasmic material is derived from muscle cells, the bulk of nuclear-related material is from non-muscle cells.

In conclusion, while experimental models are essential for gaining an understanding of underlying mechanisms of cardiac hypertrophy and failure, the search for a model which reflects the natural condition must continue, and each model should be thoroughly studied and compared to the natural disease. The limitations of any model must be thoroughly understood and taken into account when interpreting experimental results.

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Interaction of Capillary, Interstitial, and Lymphatic Forces in the Canine Hindpaw

HSING I CHEN, M.D., HARRIS J. GRANGER, PH.D., AND AUBREY E. TAYLOR, PH.D.

SUMMARY We used plethysmograph techniques to measure or calculate the tissue and capillary forces and flows (capillary pressure, tissue and plasma oncotic pressure, transcapillary pressure drop, lymph flow, and interstitial pressure) in a dog hindpaw preparation in situ at three different venous pressures (PV). Since lymph was flowing from the preparation, an isofiltration state represented an isofiltration state rather than the conventional isovolumetric or isogravitometric state. At an isofiltration capillary pressure of 12.8 mm Hg, lymph oncotic pressure averaged 3.8 mm Hg, plasma oncotic pressure averaged 20.9 mm Hg, and tissue pressure averaged -4.7 mm Hg (PV normal). The impedance in transcapillary forces averaged 0.5 mm Hg and represented the lymph flow contribution (lymph flow/filtration coefficient) to maintenance of the normal isofiltration state. As isofiltration capillary pressure increased to 24.9 mm Hg, interstitial fluid volume increased by 1.7 ml/100 g of tissue, tissue pressure rose by 4.6 mm Hg, lymph oncotic pressure fell by 2.2 mm Hg, and the transcapillary pressure drop increased to 5.6 mm Hg (PV = 20 mm Hg). At an isofiltration capillary pressure of 38.0 mm Hg, interstitial fluid volume increased by 17.5 ml/100 g, interstitial pressure rose to 10 mm Hg, lymph oncotic pressure fell to 0.5 mm Hg, and the transcapillary pressure drop increased to 6.3 mm Hg (PV = 30 mm Hg). At moderate levels of PV elevation, the transcapillary pressure drop and increased tissue pressure provided 80% of the counterbalancing tissue force, each contributing approximately 40%. At higher venous pressures, the only tissue force that opposed filtration was an increase in tissue pressure.

AS A RESULT of his study on the absorption of fluid from the connective tissue spaces in 1896, Starling proposed that the principal factors regulating fluid movement across the capillary wall are capillary hydrostatic pressure and the colloid osmotic pressure of plasma proteins. The Starling hypothesis later was extended to include interstitial hydrostatic and oncotic pressures, fluid flow measurements, and the dynamic theories of interstitial fluid flow.
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