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EXPERIMENTAL INDUCTION OF INTRINSIC VALVE STENOSIS

S. RAJU, M.D., A. CIBULSKI, M.D., M. B. HENDRIX, M.D.

From the Department of Surgery, Veterans Administration Hospital, and the University of Mississippi Medical Center, Jackson, Mississippi

True pulmonic and aortic valvular stenoses were produced in dogs by chemical cautery of valve cusps during cardiopulmonary bypass. The intrinsic valve stenoses were confirmed by phonocardiography, gradient measurements, angiography, and pathologic examination. The lesions were similar to clinical valvular stenosis in several respects, including the presence of dystrophic calcification. Unlike the hitherto available models of supravalvular stenosis, the present preparation is amenable to a greater variety and spectrum of experimental studies.

UNLIKE regurgitant valve lesions, for which several different experimental models are known,[7,12,15] stenotic valve lesions have been extremely difficult to produce experimentally.[16,18] Heretofore, there has not been a satisfactory experimental model that has successfully created intrinsic valvular stenosis at any of the locations in the heart. Due to the lack of suitable experimental models, workers have resorted to other techniques that tend to create lesions in the arterial wall itself, rather than in the valve.[4,5,16,18] Most of these simulations have used supravalvular locations and thus have been of limited value, since clinically occurring valve stenosis is predominantly valvular. This deficiency has been felt most acutely in the aorta, because extrinsic supravalvular stenosis is supracoronary in position. It is obvious that in the latter instance, in which the coronaries are below the stenotic valve lesion, the coronary flow, myocardial function, and other related parameters can be quite different from those obtained in the clinical setting. For this reason, we initiated experiments directed toward the possible production of true intrinsic valve stenosis in experimental animals. Such a model would be far superior to the presently available extrinsic models for several other basic studies, such as the study of dystrophic calcification in the valve leaflets, flow turbulence distal to the valve lesions, susceptibility to endocarditis, chronology and extent of ventricular hypertrophy, regional myocardial flow study in the hypertrophied myocardium, and others. After unsuccessful trials with several methods, including electrocautery of the leaflets, a suitable model of intrinsic valve stenosis was created using chemical cautery of the valve leaflets. The method is reported in detail below.

Materials and Methods

Pulmonic Valve Stenosis

Animals. Mongrel dogs (20–25 kg each), treated and checked for chronic parasites, were used for the experiment.

Production of Valve Stenosis. Under phenobarbital anesthesia, the dogs were cannulated via femoral vein, jugular vein, and the femoral artery for placement of cardiopulmonary bypass. EKG and arterial pressure, as well as the central venous pressure (CVP), were monitored continuously. A left thoracotomy was performed, and the animal was placed on cardiopulmonary bypass. Normothermia was used in all the dogs studied. When the flow rates had stabilized to optimal levels (75–100 ml/kg), electrical ventricular fibrillation was induced, and a left ventricular sump

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Reprint requests to Seshadri Raju, M.D., Department of Surgery, Veterans Administration Center, Jackson, Mississippi 39216.

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was inserted through the left atrial appendage. The pulmonary artery was then opened above the valve through an incision 1.5 cm in length. Both the cephalad and caudal surfaces of the pulmonary valve cusps were cauterized with silver nitrate sticks, with care taken to keep the chemical outside the ventricular cavity. After cauterization, which took only a few seconds to perform, the valve cusps were carefully irrigated with saline and the irrigant was aspirated immediately. The arteriotomy was then closed in the usual manner and the animal gradually weaned off cardiopulmonary bypass. The pericardiotomy was partially closed to prevent herniation of the heart, and the chest was closed, leaving a tube drain connected to an underwater seal. The drain was removed in 3 to 4 hours if hemostasis proved satisfactory. Animals were kept overnight in a heated cage, and potassium and fluid supplements given intravenously.

Aortic Valve Stenosis

A technique similar to that described above was used in adult mongrel dogs in an attempt to produce aortic valve stenosis. The results were inconsistent, with a high operative mortality. More recently, cautery of valve leaflets in small puppies has been carried out for the production of lesions in a more predictable fashion. Even a mild stenosis is expected to progress with growth of the animal. Silver nitrate cautery of aortic valves was performed in puppies weighing no more than 5–7 kg each. Inflow occlusion with mild hypothermia was used. A left thoracotomy was performed with the puppy in an ice bath with core temperature down to 30 C. At this point the vena cavae were snared and the aorta was clamped and opened below to expose the aortic valve cusps. The valve cusps were cauterized with silver nitrate sticks as before. Following closure of the centimeter-long arteriotomy with 5-0 prolene continuous sutures, the inflow occlusion snares and the aortic clamps were removed; perfusion was maintained by manual massage of the heart after removal of air from the left ventricle by aspiration through the apex. With rewarming, and defibrillation when necessary, an autogenous rhythm was established with good perfusion pressure, and the chest was closed as before.

Results

Pulmonic Stenosis

In eight attempts to create valve stenosis, there was one operative death. Seven dogs survived the cardiopulmonary bypass procedure for creation of pulmonic stenosis. Two of these were sacrificed subsequent to the procedure at two weeks and four weeks, respectively, with gross pulmonic stenosis identified at necropsy. The necropsy findings are provided in detail elsewhere in this article. All seven animals developed pulmonic murmurs typical of stenotic lesions as early as a week after surgery. The phonocardiogram was characteristic (Fig. 1). Chest x-ray revealed a prominent pulmonary knob in all five long-term survivors. Clear-cut right ventricular enlargement[8] was present in three dogs; in two others, borderline right ventricular enlargement was detected. In one dog, the cardiac silhouette was normal. Electrocardiograms revealed obvious right ventricular enlargement in only one of the five long-term survivors, but canine electrocardiograms are frequently unreliable.[9] The right heart catheterization revealed gradients (Fig. 2) ranging up to 125 mmHg in the long-term survivors, and valve areas ranging from 0.24 sq cm to 0.42 sq cm (mean = 0.35 sq cm) (Table 1). There was no reduction in the pulmonic valve area as calculated, signifying progression of the lesion, in any of the long-term survivors during the two-year period of the study (Table 1). Increasing the cardiac output by Isuprel® (Breon) administration increased the gradient across the valve (Table 1). Angiocardiography done at the time of right heart catheterization revealed characteristic pulmonary valvular stenosis in all the animals studied, and marked infundibular hypertrophy was evident on angiography.

In two dogs, this secondary hypertrophy was evidently obstructive; Isuprel administration resulted in a disproportionate increase in gradient that could not be attributed solely to an increase in cardiac output.

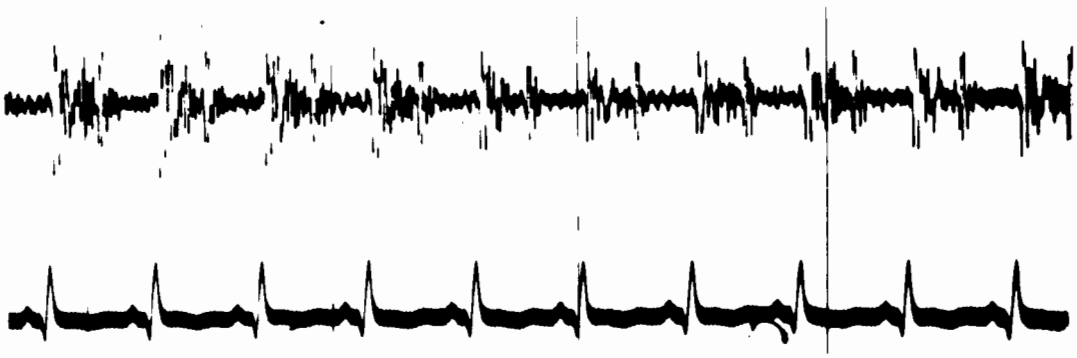

FIG. 1. Phonocardiogram in a dog with pulmonic lesion.

Pathology. At the time of death or deliberate sacrifice, the valve was studied grossly. In the two dogs that were sacrificed a few weeks after surgery, the valve cusps were grossly deformed and covered with thick granulation tissue. However, no thrombus adherent to the valve was noticed. Due to injury, the valve was enormously thickened and obviously immobile. There was obvious gross valve stenosis (Fig. 3), due almost entirely to valve thickening and immobility. There was some commissural shortening that became somewhat more pronounced later, but commissural fusion was not a major feature of this experimentally induced valve stenosis. Microscopically, the chemically burned valves showed destruction of endothelium, growth of granulation tissue, and hyaline degeneration of the collagenous matrix. An inflammatory cell infiltrate was in evidence at this time, presumably due to the chemical burn.

Abnormal Vascularity. A vascular granulation tissue was present even in distal parts of the valve cusp, whereas in the normal canine valve cusp blood vessels, if present, are confined to the base.

Late Changes. At the end of two years, the chemically burned valve cusps remained stenotic due to thickening and immobility. The edges were rolled and

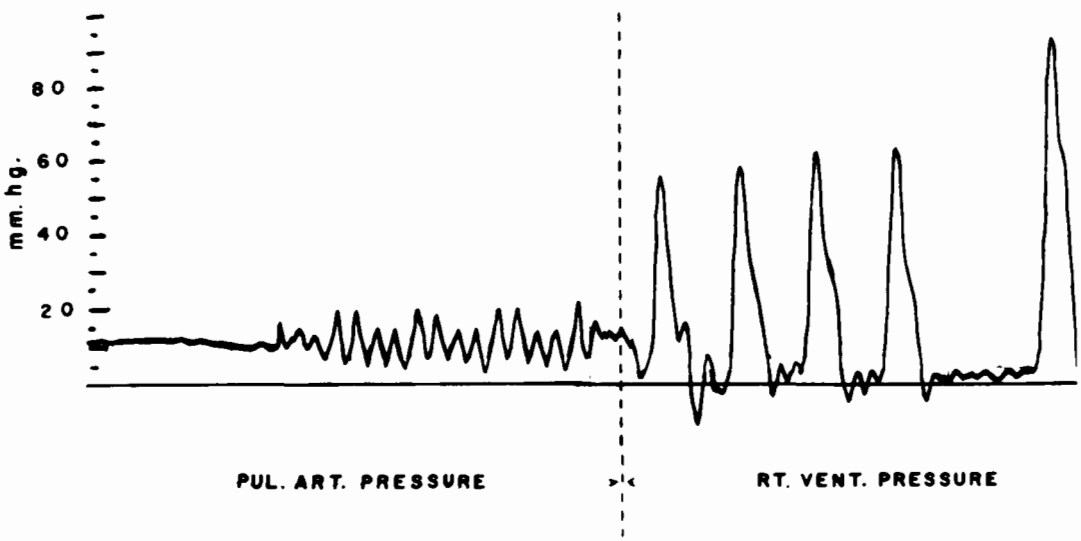

FIG. 2. Pullback tracing across pulmonic valve showing gradient. Notice a small infundibular gradient.

TABLE 1. *Experimentally Induced Pulmonic and Aortic Stenosis*

Animal	Age of Valve Lesion	Valve Gradients and Areas: Cardiac Output (l/min)	Valve Gradient (mmHg)	Valve Area (sq cm)
Pulmonic stenosis				
Dog 1	2 wk	NM	40	
	1 mo	NM	25	
Dog 2	1½ mo	NM	62	
	4 mo	NM	45	
	11 mo	3.1	33	0.42
	11 mo	5.2*	46	
	2 yr	2.6	28	0.41
	2 yr	4.5*	50	
	2 yr	5.6*	66	
	2 yr	8.6†	125	
Dog 3	4 wk	NM	17	
	10 mo	2.0	30	0.33
	10 mo	4.0*	46	
	2 yr	1.9	20	0.30
	2 yr	3.2*	39	
	2 yr	3.6†	58	
	2 yr	3.8†	67	
	2 yr	4.5*	72	
Dog 4	1 wk	NM	15	
	10 mo	1.5	10	0.32
	10 mo	2.5*	46	
	2 yr	2.1*	35	0.24
	2 yr	6.5*	78	
Dog 5	3 wk	NM	23	
	1 yr	1.9	17	0.4
	1 yr	2.0*	33	
	1 yr	2.7*	38	
Dog 6	1 wk	1.3	8	0.39
Aortic stenosis				
Dog 7	1 mo	NM	10	
	4 mo	2.0	8	0.63
	4 mo	2.6*	44	0.63
	14 mo	3.6*	70	0.69
	14 mo	6.0*	148	
Dog 8	2 wk	NM	10	

NM = not measured.
* With Isuprel.
† With Levophed® (Breon).

frequently measured 2–4 mm in thickness, while normal valve cusps are only a fraction of this thickness. There was no commissural fusion. The valve cusps showed abnormal thickening, fragmentation of collagen and elastic fibers, and extensive fibrosis (Fig. 4). The granulation tissue was now largely replaced by fibrosis, and endothelial ulceration was no longer in evidence. Even though the hypervascularity of the early stages had largely diminished, abnormal vessels were still present in the valve cusps (Fig. 4). Areas of dystrophic calcification were in evidence (Fig. 4).

At the time of death, five hearts were bisected by the method of Herrmann,[11,12] and right ventricle/body weight ratio was calculated (Table 2). All five exhibited right ventricular hypertrophy according to this criterion.

Aortic Valve Stenosis

Creation of aortic stenosis was successful in four animals. Of these four dogs, two were doing well when they suddenly died at eight days and 24 days, respectively, while being exercised, reminiscent of the sudden death following aortic valve stenosis. A third animal died during anesthesia for catheterization at four weeks. However, the animals had developed systolic ejection murmurs in the aortic valve area characteristic of aortic stenosis, and gross aortic stenosis was confirmed at necropsy in all three premature deaths. Left heart catheterization revealed valve gradients up to 148 mmHg in the remaining long-term animal (Table 1). Cineangiography demonstrated features characteristic of aortic stenosis described above. The heart exhibited left ventricular hypertrophy as measured by the method of Herrmann (Table 2).

One puppy recently underwent successful silver nitrate cautery of the aortic valve cusp under moderate hypothermia and inflow occlusion as described. Three months after surgery it developed irreversible pulmonary edema and died. At necropsy an extremely tight valve stenosis with an effective orifice of not more than a few millimeters across was found (Fig. 5). The left ventricle was bisected and showed intrinsic hypertrophy when measured by the method of Herrmann (Table 2).

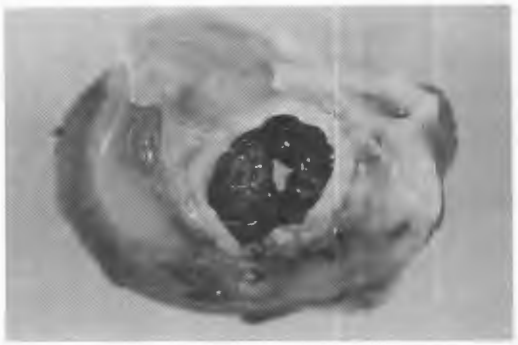

FIG. 3. A stenosed pulmonic valve showing the superior surface.

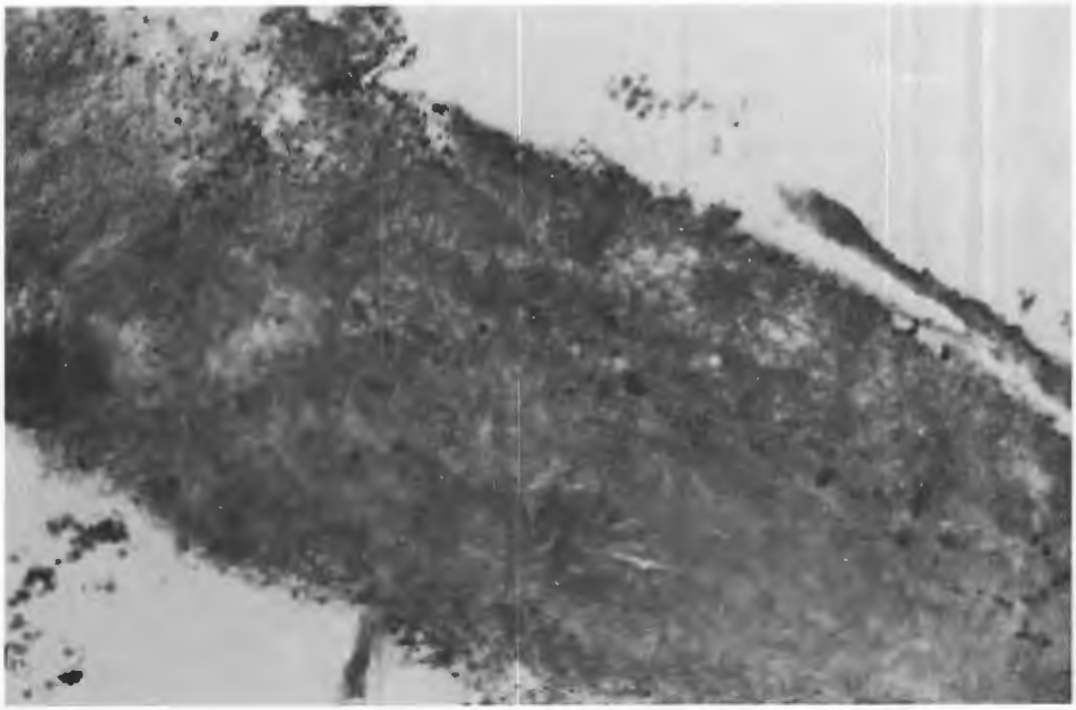

FIG. 4A. Stages in experimental valve stenosis. Necrosis of collagen.

TABLE 2. *Ventricular Hypertrophy in Experimental Valvular Stenosis*

	No. of Animals	Ventricle wt/Body wt Ratio			
		Mean Right Ventricle/ Body Wt	Range	Mean Left Ventricle/ Body Wt	Range
Pulmonic stenosis	6	0.045	0.0366–0.0054		
Normal animals*	200	0.00265	0.00178–0.00400	0.00369	0.00268–0.00472
Aortic stenosis	2			0.00685	

* Data from Herrmann.[11]

Discussion

Over the years there have been several attempts to produce valve stenosis at various cardiac positions,[1,3,4,7,10,12,15] but to date, except for the present method, these have not been successful. There have been several successful simulations,[5,15,18] the most notable being the creation of pulmonic artery stenosis close to the valve ring by placing a constricting band around the artery. This model has been used for several experimental studies,[5,7] where the preparation has been used as a hemodynamic simulation of pulmonic valvular stenosis. Attempts to create mitral stenosis have been more ingenious.[1,3,10,13] These have included tightening the mitral valve ring, invagination of atrial tissue into the valve ring, use of corrosive substances injected intramurally around the valve ring, and suture of a stenotic prosthetic device in the valve annulus. Attempts to produce aortic valvular stenosis have been least successful, probably because production of a significant aortic valve stenosis

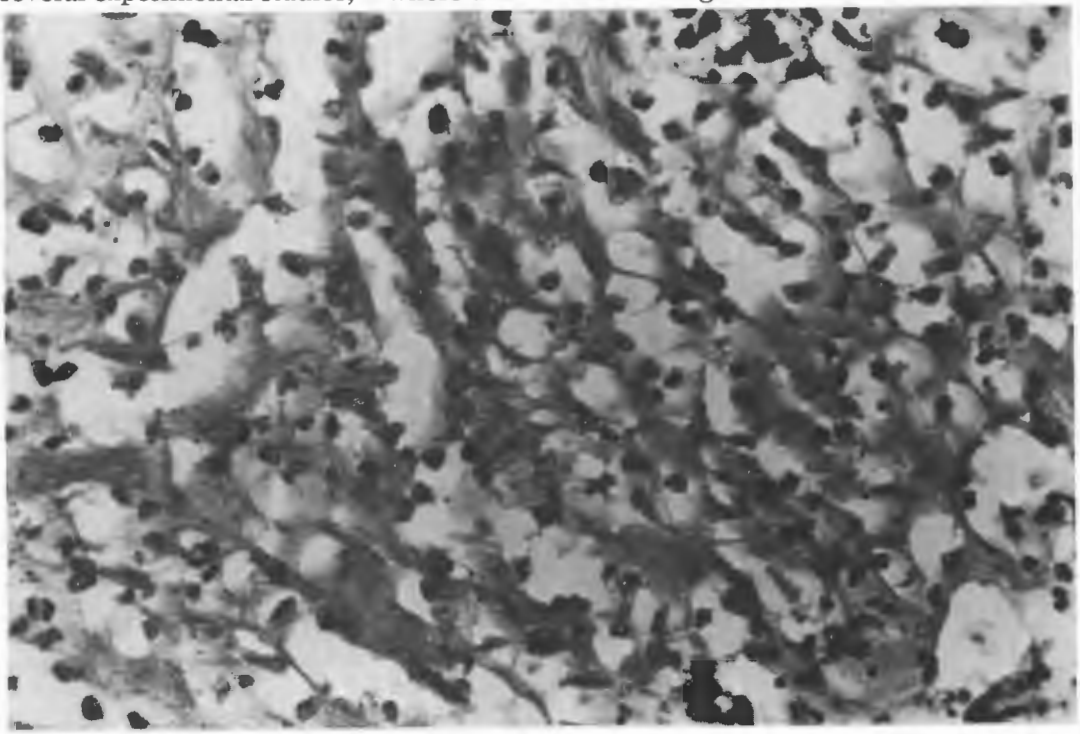

FIG. 4B. Stages in experimental valve stenosis. Vascular granulation tissue.

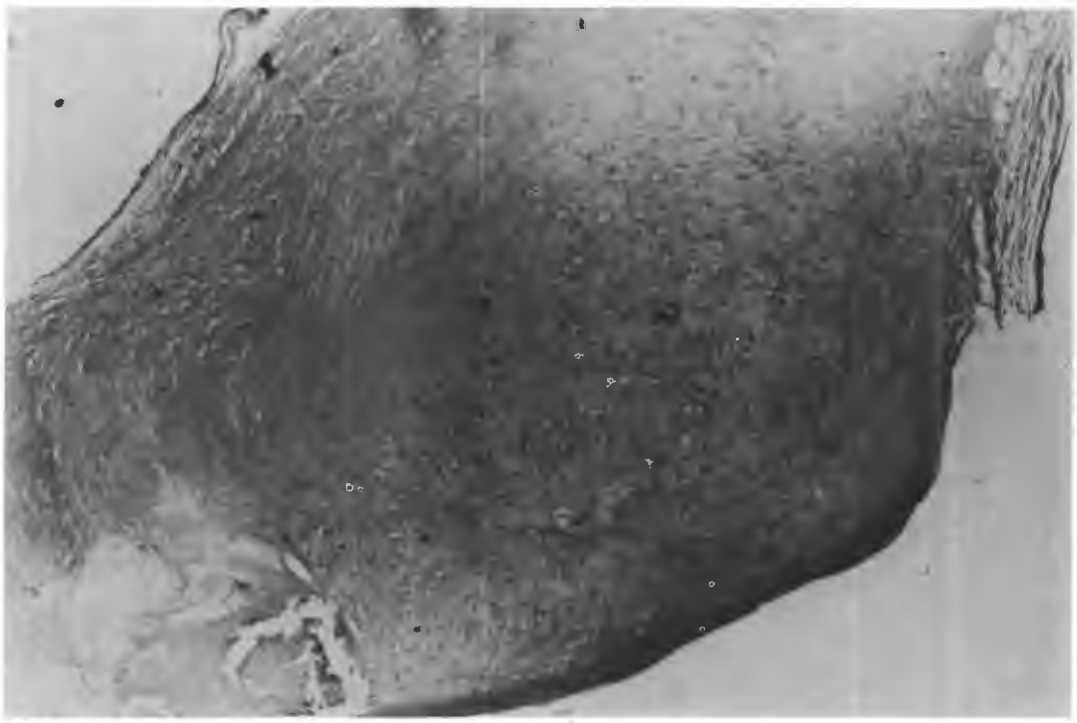

FIG. 4C. Stages in experimental valve stenosis. Thickening and fibrosis of valve cusp.

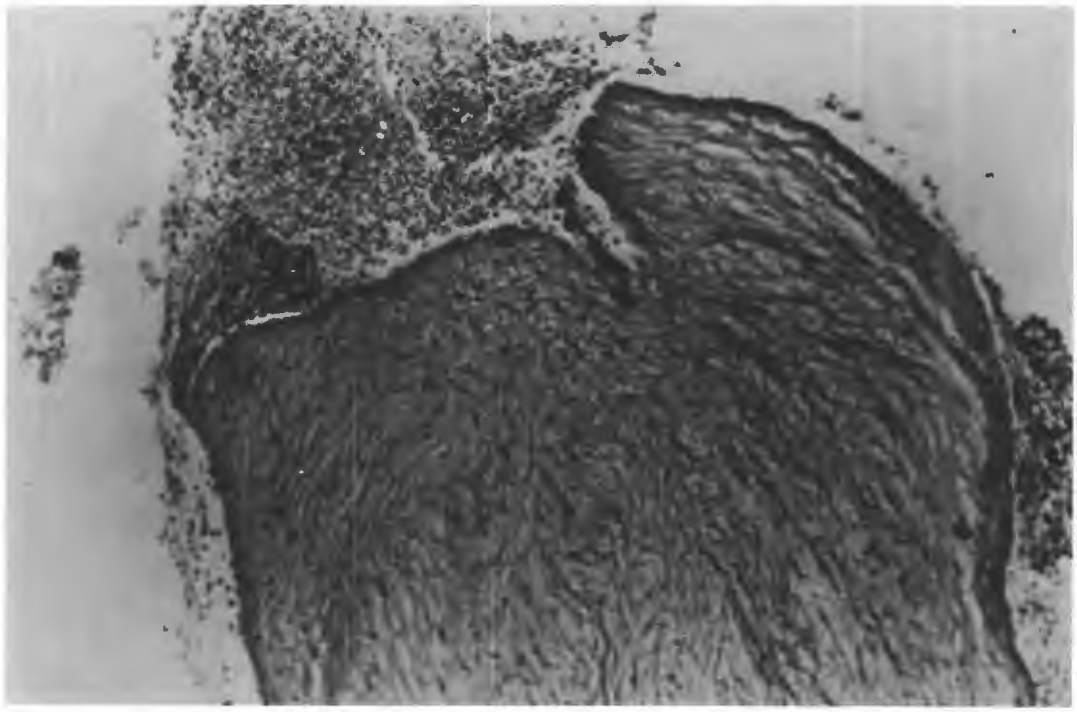

FIG. 4D. Stages in experimental valve stenosis. Organizing thrombus on valve surface.

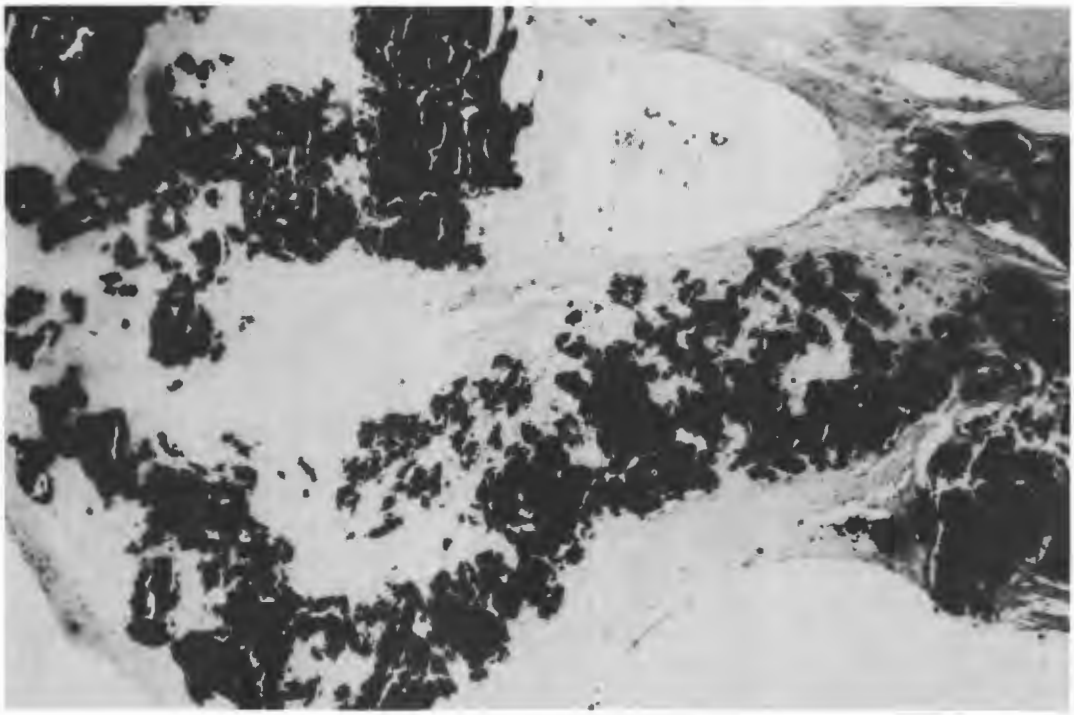

FIG. 4E. Stages in experimental valve stenosis. Dystrophic calcification.

results in a high degree of intraoperative mortality.[16,18] A high gradient supracoronary aortic stenosis in adult mongrel dogs has been described in which the lesions had been created when the animals were puppies[16,18]; mild aortic stenosis created at an early age developed into severe stenotic lesions in adult animals. However, this experimental model was not an intrinsic aortic valvular stenosis; an extrinsic stenosis was created by placing a constricting band around the aorta in the supravalvular position, similar to the model of pulmonary artery stenosis described above. It is clear that such extrinsic aortic stenotic models, while being of unquestionable value in some areas of investigation, are limited in versatility, as they do not incorporate modification of valve tissue itself and consequently do not duplicate the features of clinical valve stenosis completely. This drawback is particularly obvious in the aortic area, where the existing extrinsic stenotic model is a supravalvular stenosis above the level of take-off of coronary arteries. The supravalvular type of aortic stenosis does indeed occur clinically but is comparatively rare. The most common valvular stenosis is subcoronary in position, involving disease of the valve cusps and commissures. This feature results in significant changes in coronary flow patterns, which may further aggravate the severe manifestation of the disease by limiting blood supply to the myocardium under stress and thereby adversely affect muscle function. An intrinsic valvular stenosis as an experi-

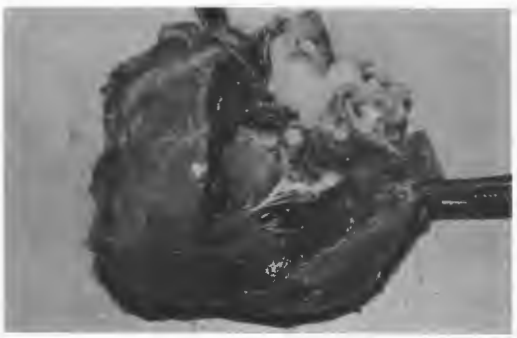

FIG. 5. A stenosed aortic valve; the annulus has been divided for better display.

mental model will be more useful in other ways than those presently available, such as for studies involving dystrophic calcification, subacute bacterial endocarditis, and turbulence patterns.

Using silver nitrate cautery of the valve cusps, we have successfully produced intrinsic valve stenosis in several dogs, both in the pulmonic and aortic positions. The valve lesions resemble clinical valve stenosis in several ways: characteristic murmurs are present and radiographic changes are consistent, as is the ventricular hypertrophy following the creation of stenotic valve lesions. Catheterization pressure studies and angiography are similar to the clinical variety of valve stenosis. Even under microscopy, features of the experimental model are not dissimilar to the clinical material, including the presence of dystrophic calcification. The sudden death of two dogs who were doing very well following the creation of aortic valve stenosis is of particular interest; these deaths were reminiscent of the sudden death sometimes seen in patients with aortic valve stenosis. At the time of sacrifice, differential measurement of ventricular thickness revealed right ventricular hypertrophy in all five dogs with pulmonic stenosis and left ventricular hypertrophy of significant proportions in long-term dogs with aortic valve stenosis. In the former instance, evidence of infundibular hypertrophic stenosis was sought during angiography. While hypertrophy was evident in all the five dogs so studied, in two animals the hypertrophy was obstructive, as demonstrated by the Isuprel test. Others have demonstrated similar obstructive muscular hypertrophy[2,6,14,17] secondary to valve stenosis.

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