

Identification of surface morphologic changes in the mitral valve leaflets and chordae tendineae of dogs with myxomatous degeneration

Brendan M. Corcoran, MVB, PhD; Alexander Black, BSc, MmedSc; Heather Anderson; Joanna Dukes McEwan, BVMS, MVM, PhD, DVC; Anne French, MVB, DVC; Paul Smith, BVetMed; Craig Devine, BVSc

Objective—To describe structural changes in the left atrioventricular (mitral) valve complex of dogs with endocardiosis by use of scanning electron microscopy.

Animals—5 clinically normal dogs and 4 dogs with mitral valve endocardiosis.

Procedure—The mitral valve complex from each dog was fixed and prepared for examination via scanning electron microscopy. Findings in valves from clinically normal and affected dogs were compared to identify surface changes associated with endocardiosis.

Results—Compared with findings in valves from clinically normal dogs, endocardiosis-affected mitral valve complexes had several morphologic abnormalities. Tissue swelling on the edge of valve leaflets, chordae tendineae, and the chordal-papillary muscle junction was evident. Damage to the valve complex endothelium was unevenly distributed; in some areas, denudation of endothelial cells had exposed the basement membrane or subendothelial valve collagen matrix. This damage was most noticeable on the leaflet edges and extended more to the ventricular aspect of the valve than the atrial side. Cell loss also extended to the chordae tendineae but was less apparent at the chordal-papillary muscle junction. The remaining endothelial cells on affected valves were arranged in less-ordered rows and had more plasmalemmal microappendages, compared with cells on unaffected valves.

Conclusions and Clinical Relevance—Morphologic changes associated with mitral valve endocardiosis in dogs were similar to those observed in humans with mitral valve prolapse. In dogs with mitral valve endocardiosis, gross changes in the valve complex may affect hemodynamics in the heart; alterations in the leaflet and chordal endothelium may contribute to pathogenesis of this disease. (*Am J Vet Res* 2004; 65:198–206)

lar myxomatous degeneration) is the most common acquired cardiac disease of dogs. It is mainly a disease of small or toy breeds but is particularly prevalent in Cavalier King Charles Spaniels worldwide¹⁻⁴ and is a major cause of illness and death in that breed in the United Kingdom, Europe, and North America.⁵ The severity of the disease and the extent of pathologic changes in the valve leaflets are age dependent.² Although much information is available on the incidence and prevalence, clinical signs, diagnosis, and management of this disease,^{2,3,5} data regarding the structural morphologic changes of diseased valve leaflets (in particular, the electron microscopic appearance of the affected valve) are limited. There are a few detailed descriptions of the electron microscopic appearance of mitral valves in clinically normal dogs^{6,7} but only occasional reference to changes in valves from dogs with endocardiosis.⁸

Mitral valve endocardiosis involves a progressive myxomatous degeneration of the valve complex (the valve annulus, leaflets, chordae tendineae, and papillary muscles), and the gross and histologic appearances of the affected valve leaflets have been described.^{1,9} This myxomatous degeneration results in disturbance of collagen deposition and organization and increased amounts of acid mucopolysaccharides in the valve. The disease is also recognized in humans and pigs, which show similar histologic changes^{10,11}; it may also occur in horses, although the pathologic features of the disease in that species are less well-characterized.¹² In humans, myxomatous degeneration is usually associated with **mitral valve prolapse (MVP)**, and this also appears to develop in dogs.^{8,13} To a certain extent, MVE and MVP can be used synonymously to describe the same disease process, although the former refers more specifically to valve-associated pathologic changes and the latter to abnormality of valve function. Furthermore, MVP is likely to be detected early and therefore might precede the overt development of MVE in dogs and humans.^{8,13}

Endocardiosis of the left atrioventricular (mitral) valve (**mitral valve endocardiosis [MVE]** or valvu-

Received April 21, 2003.

Accepted June 16, 2003.

From the Hospital for Small Animals, Division of Veterinary Clinical Studies, Royal (Dick) School of Veterinary Studies, College of Medicine and Veterinary Medicine, The University of Edinburgh, Easter Bush Veterinary Centre, Roslin, Mid Lothian, Scotland, UK EH25 9RG (Corcoran, McEwan, French, Smith, Devine); the Department of Anatomy, National University of Ireland, University College Galway, Galway, Ireland (Black); and the Division of Preclinical Veterinary Sciences, Royal (Dick) School of Veterinary Studies, College of Medicine and Veterinary Medicine, The University of Edinburgh, Summerhall, Edinburgh EH9 9RQ (Anderson). Dr. Dukes McEwan's present address is Department of Veterinary Clinical Studies, University of Glasgow Veterinary School, Glasgow, Scotland, UK G61 1QH.

Supported by the Kennel Club Charitable Trust and the Cavalier King Charles Spaniel Club of the United Kingdom.

Address correspondence to Dr. Corcoran.

However, although MVP is common in humans, it rarely (or at least not until an advanced age) results in clinically important disability.¹⁴

The etiology of MVE in all species is unknown, but the condition is believed by some to be a dystrophic process rather than a succession of repeated healing events.¹ This may be an inherited collagen defect, but in humans with MVP, no defects in genes encoding for collagen types I, III, and V (the major collagen constituents of the mitral valve) have been identified.^{14,15} Whether such gene defects are present in dogs with MVE remains to be elucidated. However, repeated trauma of the valve leaflets at the line of closure, resulting in alteration of endothelial cell function, might be implicated.¹⁶ Alteration in endothelial cell function as a consequence of trauma can result in proliferation and migration of the subendothelial **valvular interstitial cells (VICs)**.^{16,17} These VICs produce the contents of the valve matrix and are therefore important in the genesis of myxomatous degeneration. Furthermore, in affected valves, increased expression or activity of endothelin (acting as a growth promoter) might contribute to the myxomatous change; compared with normal tissue, expression of endothelin receptors on thickened areas of valve leaflets in dogs with MVE has been detected.¹⁶

In humans with MVP, damage and denudation of the valve leaflet endothelium have been detected by use of scanning electron microscopy.¹⁸ This suggests that endothelial loss is implicated in the disease process, but whether it is a consequence or cause of the disease process is unknown. The purpose of the study reported here was to describe and characterize morphologic changes in the mitral valve complex of dogs with MVE by use of scanning electron microscopy. Morphologic changes within the endothelium of the mitral valve leaflets were also assessed.

Materials and Methods

Entire valve complexes (including valve annulus, leaflets, chordae tendineae, and papillary muscles) were collected from 5 clinically normal dogs and 4 dogs with MVE. All dogs were client owned, and the clinically normal dogs were euthanized for reasons unrelated to cardiac disease. Euthanasia of affected dogs was requested by owners because of intractable congestive heart failure. The clinically normal dogs were 2 to 4 years old and of various mixed breeds; in all 5 dogs, there was no gross evidence of MVE on physical examination and no murmur was detected on auscultation of the thorax. The dogs with MVE were 9 to 15 years old and included 3 Cavalier King Charles Spaniels and 1 mixed-breed dog; the disease was confirmed on the basis of detection of a mitral valve murmur on auscultation of the thorax, echocardiographic evidence of disease, and findings of gross examination of the valve complex postmortem.

Dogs were euthanized by IV administration of pentobarbital sodium.⁹ All sample material was collected within 10 minutes of euthanasia and with informed client consent. Young dogs were used for the control group because it was believed they were unlikely to have any endocardiosis-associated valvular changes. Producing an age-matched group of clinically normal dogs would be difficult because all aged dogs are likely to have some evidence of endocardiosis. Although breed specificity has been associated with early-onset MVE, the disease is ubiquitous in old dogs and has an age-related prevalence.²

Hearts were removed from the dogs, and valve complexes were dissected with care to avoid handling damage of the endothelial surface; the excised tissues were gently washed with heparinized physiologic saline (0.9% NaCl; 5 U of heparin/mL) solution. The specimens were mounted on card to maintain normal anatomic alignment between the papillary muscles, chordae tendineae, and valve leaflets and immediately immersion fixed for 2 to 4 hours in a solution of 4% glutaraldehyde in 0.1M sodium cacodylate (pH, 7.4). The mitral valve complexes were rinsed for 30 minutes in 0.1M sodium cacodylate buffer, dehydrated through a series of graded ethanols, immersed for 15 minutes in the transitional solvent hexamethyldisilazane, and air-dried for 30 minutes.

After fixation, valve complexes were dissected into smaller segments of interest with the aid of a standard dissecting microscope. In all dogs, samples were collected from the septal (anterior) leaflet; however, in the affected group, samples were additionally collected from those areas of the mural (posterior) leaflet if marked changes were noted. All chordal samples were collected from chordae tendineae attached to the septal leaflet. No attempt was made to exactly match sampling sites between the 2 groups. For the purpose of scanning electron microscopic evaluation, specimens were mounted onto aluminium stubs and gold-coated by use of a sputter coater^b before being viewed in a scanning electron microscope^c at an accelerating voltage of 20 kV. The surface morphologic features of the valve leaflets, attached chordae tendineae, and papillary muscle-chorda junctions were examined via scanning electron microscopy at low (< 1,000X) and high (> 1,000X) magnification.

Results

Gross appearance of mitral valve complexes—There was no gross evidence of MVE in the valve complexes obtained from the clinically normal dogs. All these leaflets were thin with a translucent appearance and had no evidence of nodular thickening of the leaflet edges. The attached chordae tendineae were thread-like with no apparent thickening, and there was no evidence of chordal rupture. In the endocardiosis-affected group, all dogs had marked gross changes affecting both leaflets. The predominant feature was nodular thickening that primarily affected the free edge of the valve leaflet but also extended in certain regions to the mid and basal zones of the leaflets. In many chordae tendineae, distinctive thickening at the attachment to the leaflet was detected, and some ruptured chordae were noted in all affected valve complexes.

Scanning electron microscopic evaluation of valves from clinically normal dogs—The valve leaflet had multiple branching chordae tendineae attached to the ventricular surface of the valve. Viewed at low magnification, the surface of the valve leaflet had a crinkled appearance. Examination of the valve surface at high magnification revealed endothelial cells with prominent bulges (nuclei) that were ordered in distinct rows (Fig 1). The ordered arrangement and prominence of the nuclear bulges of cells were most pronounced at the distal (outer) zone of the leaflet close to the free edge. In proximity to the valve edge, the cells were oriented perpendicular to the valve annulus, whereas elsewhere, cells were oriented parallel to it (Fig 1). Compared with cells at the valve edge, cells in the midzone of the valve leaflet had a

more squamous appearance; they were less ordered in arrangement yet more densely grouped but also had prominent nuclear bulges and plasmalemmal microappendages. At the base of the valve (basal zone), cells were ordered in distinct cord-like rows and also had distinct nuclear bulges and microappendages. At all sites, but particularly in the midzone of the leaflet, clearly defined marginal folds that delineated the cell edges were observed (Fig 2). The nuclear bulges and microappendages appeared to be more pronounced in endothelial cells located on the ventricular aspect of the valve, compared with cells on the atrial aspect. Differences in arrangement of cells between the 2 sides of the valve were not consistently detected; in some areas of the atrial aspect of the valve, cells had a more cobblestone arrangement, compared with more orderly arrangement (ie, in distinct rows) of cells on the ventricular aspect. In the valves from the clinically normal dogs, no cell-free areas were observed. Microappendages were evident on endothelial cells in all zones of the valve; in a cell, the greatest density of microappendages was in the perinuclear area, rather than overlying the nuclear bulge (Fig 2). However, cells with less prominent

nuclear bulges usually had fewer microappendages, compared with cells with prominent bulges.

Similar cellular morphologic features were detected on the surface of chordae tendineae, which are also lined with endothelial cells (Fig 3). On the large chordae tendineae, the cells were well-delineated with prominent nuclear bulges; however, the nuclear bulges were not as prominent as those in endothelial cells at other valve sites and were similar to those in cells of the midzone of the valve leaflet. On the interchordal chordae tendineae, the cells had a flattened squamous appearance with distinct marginal folds. On the large chordae tendineae, endothelial cells were not ordered in straight rows but had a more helical appearance that corresponded with the orientation of collagen fibers in the chordae.

Scanning electron microscopic evaluation of valves from dogs with MVE—The striking difference between the valve leaflets from clinically normal and endocardiosis-affected dogs was the marked thickening and swelling of the free edge of the valve in the latter. The extent of this swelling included the chordae tendineae where they attach on the ventricular side of

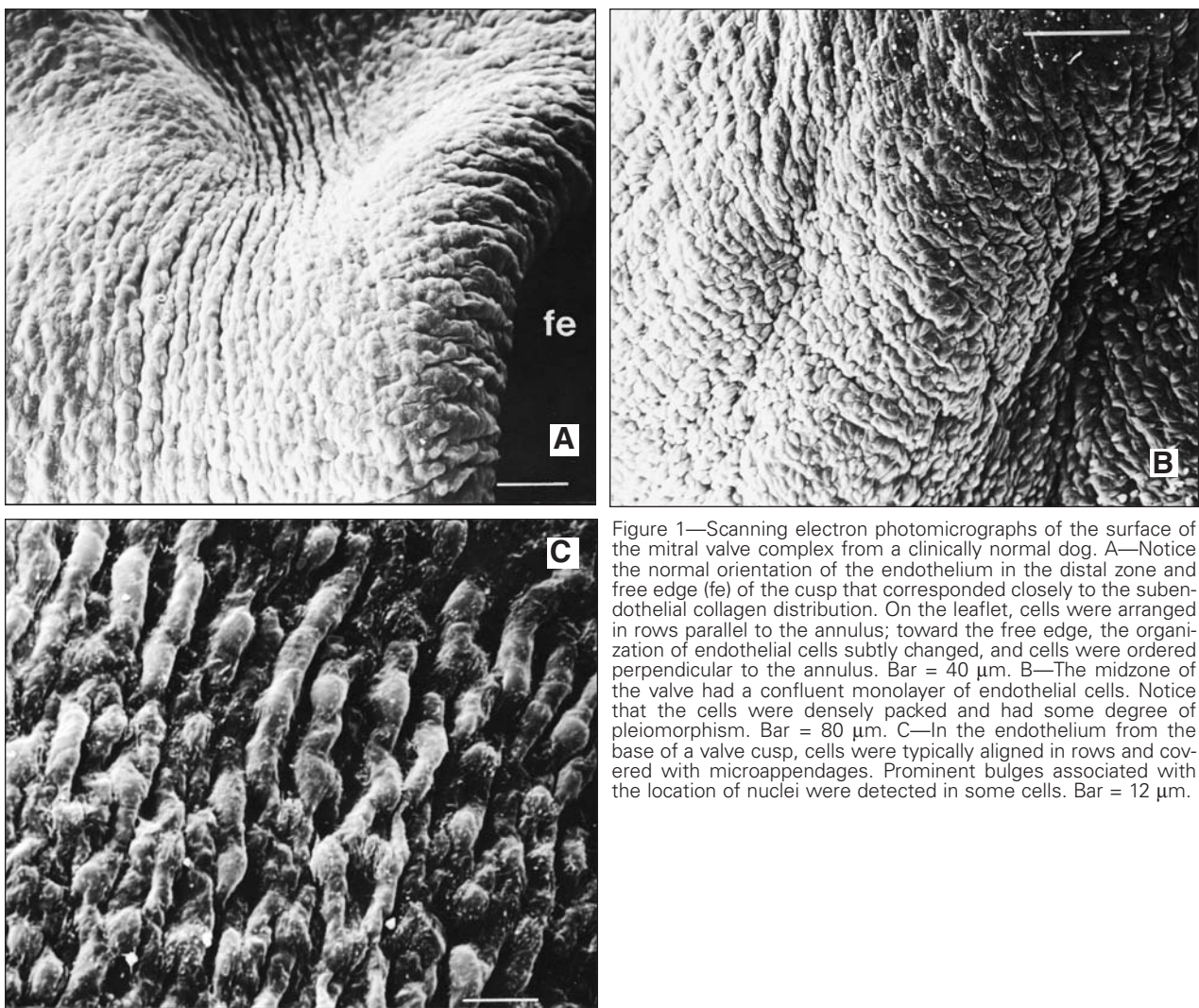


Figure 1—Scanning electron photomicrographs of the surface of the mitral valve complex from a clinically normal dog. A—Notice the normal orientation of the endothelium in the distal zone and free edge (fe) of the cusp that corresponded closely to the subendothelial collagen distribution. On the leaflet, cells were arranged in rows parallel to the annulus; toward the free edge, the organization of endothelial cells subtly changed, and cells were ordered perpendicular to the annulus. Bar = 40 μ m. B—The midzone of the valve had a confluent monolayer of endothelial cells. Notice that the cells were densely packed and had some degree of pleiomorphism. Bar = 80 μ m. C—In the endothelium from the base of a valve cusp, cells were typically aligned in rows and covered with microappendages. Prominent bulges associated with the location of nuclei were detected in some cells. Bar = 12 μ m.

the leaflet. Viewed at low magnification, no abnormalities on the valve surface were observed; however, several small tears were detected. Viewed at high magnification, marked damage to the endothelial surface was evident (Fig 4). This damage (detected primarily as cell-free areas of leaflet surface) had a patchy distribution but was most extensive at or close to the valve free edge. Similar patchy distribution of damage was also detected to a much lesser extent in the mid- and basal zones of the leaflet (Fig 5). The zones of endothelial cell denudation extended to the ventricular side and to the atrial side of the valve leaflet but did not extend far from the leaflet edge on the atrial aspect. In the damaged zones, there were areas of apparently normal endothelium with close cell apposition adjacent to areas of endothelial discontinuity in which there was an occasional absence of individual endothelial cells and areas of denudation. In the areas denuded of endothelium, the subendothelial basement membrane was visible or also

absent, thereby revealing the subendothelial valve structure (Fig 4). Cells were enmeshed in the subendothelial matrix that consisted of collagen, elastin, and VIC remnants; these cells included platelets, effete endocardial cells, and occasional erythrocytes and leukocytes (Fig 4–6). Large oval-shaped cells were also observed and assumed to be superficially adherent denuded endothelial cells. Endothelial cells adjacent to the denuded areas had many plasmalemmal microappendages, compared with cells in areas in which cell coverage appeared intact and cells on the surface of valves from clinically normal dogs. In endothelial cells adjacent to denuded areas, the most striking features of the microappendages were their number and location. The microappendages formed a uniform and dense cov-

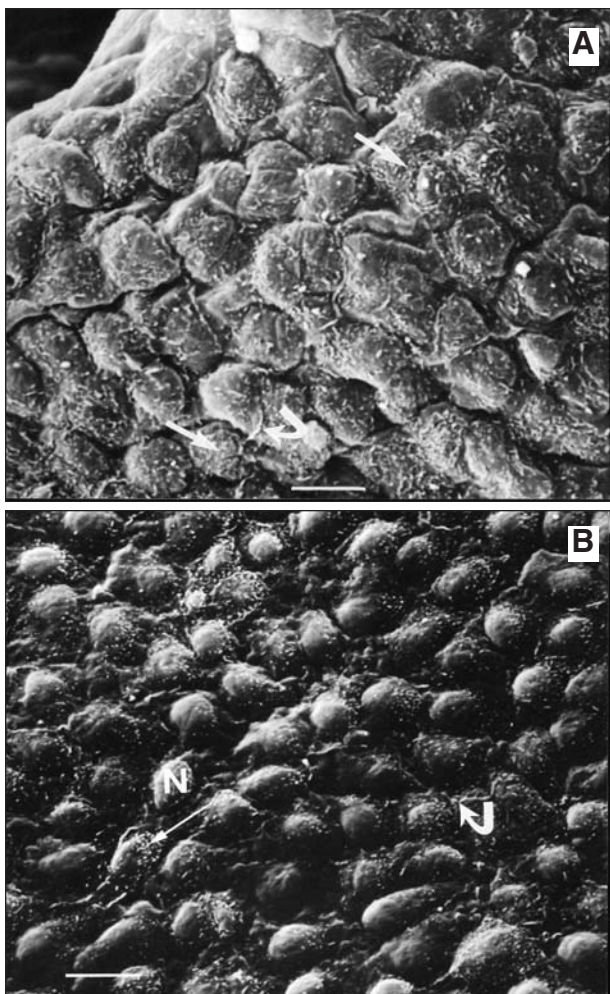


Figure 2—Scanning electron photomicrographs of the surface of the mitral valve complex from a clinically normal dog. A—The free edge of a mitral valve cusp typically contained plump endothelial cells; notice the scattered microappendages (straight white arrow) and distinct cell boundaries (marginal folds; curved white arrow). Bar = 10 μ m. B—The midzone of the valve leaflet was generally flat with squamous endothelium. Notice the cells had prominent nuclear bulges (N), microappendages (straight white arrow), and marginal folds (curved white arrow). Bar = 12 μ m.

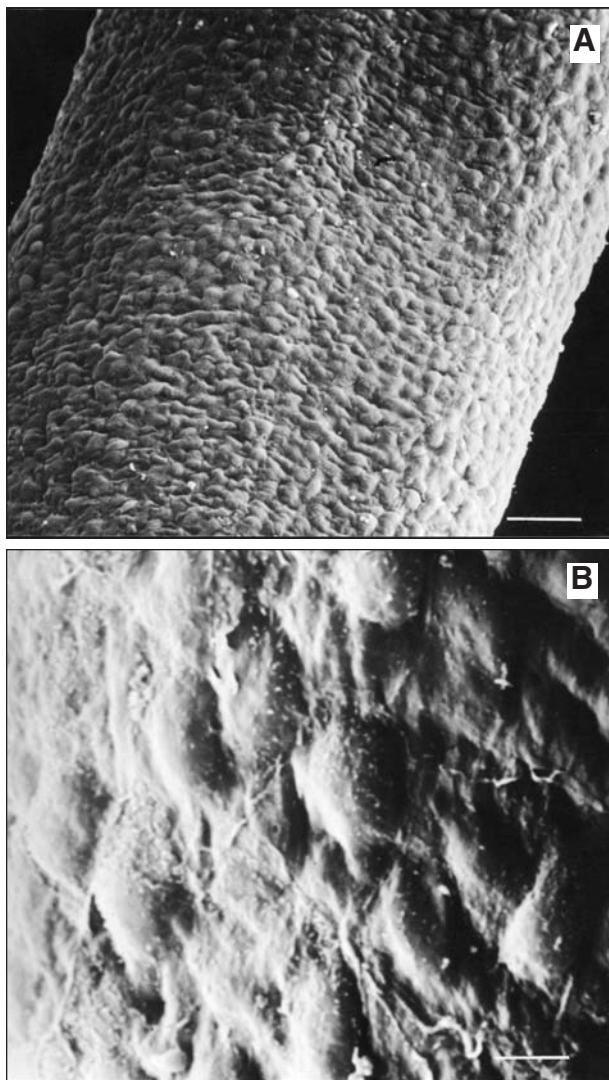


Figure 3—Scanning electron photomicrographs of the surface of chordae tendineae associated with the mitral valve complex from a clinically normal dog. A—The surface of each chorda was completely covered with endothelial cells that were arranged in a slightly helical fashion, which corresponded with the orientation of the underlying collagen bundles. Bar = 35 μ m. B—The surface of each large chorda comprised a squamous monolayer of endothelial cells. Notice the ridges (marginal folds) that represented locations where abutting endothelial cells overlapped. Scattered microappendages were detected on the surface of these cells. Bar = 6 μ m.

ering over the entire cell surface, including the nuclear bulges (Fig 4). Cells adjacent to the denuded areas also had wider marginal folds that delineated the cell boundaries, and at those same adjacent sites, large excrescences were detected, often near the valve edge or on the chordae tendineae; viewed at high magnification, these excrescences were comprised of denuded endothelium beneath which the basement membrane and collagen and elastin strands were exposed (Fig 6). However, endothelial damage was not consistently detected in regions of pronounced leaflet and chordal swelling; toward the mid- or basal zones of the valve leaflet, the endothelial cell covering usually appeared normal. In the basal zone of the valve, denuded areas of endothelium were less frequently observed; areas of damage had a pachydermatous appearance at low magnification, but denuded endothelium was revealed at higher viewing magnification (Fig 5).

At undamaged sites of the surface of the valve leaflet, the ordering into rows of the endothelial cells was less evident, compared with that observed in valves from clinically normal dogs; furthermore, the cell pleomorphism that was apparent in valves from clinically normal dogs was not as evident in valves from dogs with MVE. In the endothelial cells

at undamaged sites, prominent nuclear bulges were visible, but surface microappendages were not as evident, compared with cells in the valves from clinically normal dogs; however, prominent nuclear bulges were detected on the ventricular side of the leaflet. Overall, endothelial cells in the undamaged areas appeared to have a smoother surface profile, compared with cells in damaged areas of the valve.

Endothelial damage was also detected extending from the valve leaflet to the proximal portion of the chordae tendineae. The main findings were distinct swelling of the chordae and occasional bullous excrescences. Viewed at high magnification, denudation of the endothelium was evident, comparable to that detected on the valve leaflet; however, on the chordae tendineae, the endothelial cell loss was more patchy, and often single cells or small numbers of cells remained attached to them. At the chorda-papillary muscle junctions, the main finding was chordal swelling, and there was only minimal evidence of endothelial cell loss. The midsections of the chordae tendinae appeared to be least affected, with no apparent morphologic abnormalities, but there was still evidence of some loss of endothelial cells.

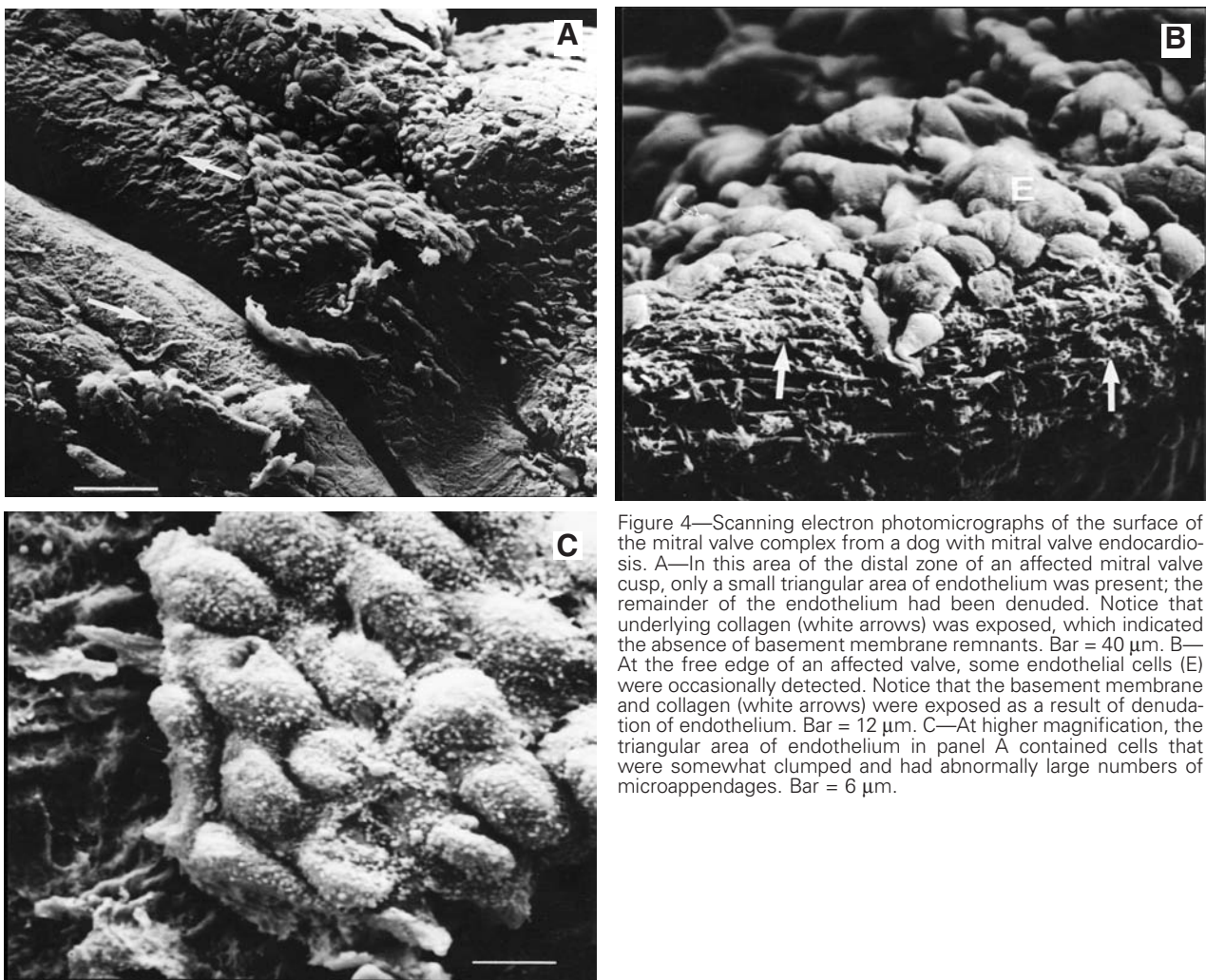


Figure 4—Scanning electron photomicrographs of the surface of the mitral valve complex from a dog with mitral valve endocardiosis. A—In this area of the distal zone of an affected mitral valve cusp, only a small triangular area of endothelium was present; the remainder of the endothelium had been denuded. Notice that underlying collagen (white arrows) was exposed, which indicated the absence of basement membrane remnants. Bar = 40 μ m. B—At the free edge of an affected valve, some endothelial cells (E) were occasionally detected. Notice that the basement membrane and collagen (white arrows) were exposed as a result of denudation of endothelium. Bar = 12 μ m. C—At higher magnification, the triangular area of endothelium in panel A contained cells that were somewhat clumped and had abnormally large numbers of microappendages. Bar = 6 μ m.

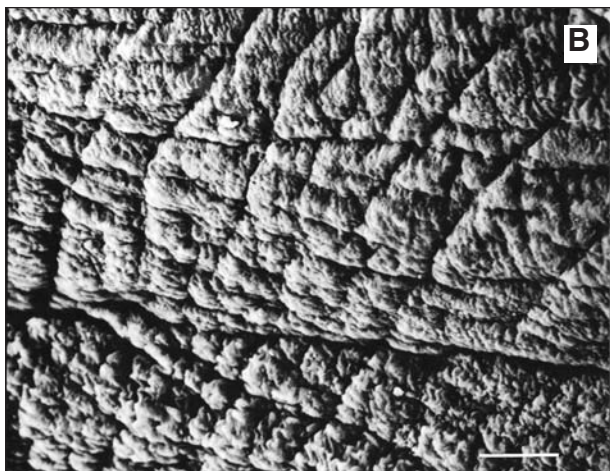


Figure 5—Scanning electron photomicrographs of the surface of the mitral valve complex from a dog with mitral valve endocardiosis. A—Some endothelium was retained in the midzone of this affected valve. Notice that most of the endothelium was denuded; some platelets (small white arrows) had adhered to the exposed basement membrane. Bar = 40 μ m. B—In the basal zone of an affected valve cusp, the damaged endothelium had a pachydermatous appearance. Notice that there was a complete lack of normal endothelium in this region. Bar = 35 μ m. C—At higher magnification, the basement membrane, collagen, and elastin underlying denuded endothelium in the basal zone were identified. Notice that some elements of endothelial cells (curved arrows) were visible, although these lacked normal morphologic features. Bar = 12 μ m.



Figure 6—Scanning electron photomicrographs of the surface of the mitral valve complex from a dog with mitral valve endocardiosis. A—Notice the large excrescences detected near the edge of an affected valve. Bar = 0.25 mm. B—No endothelium was observed in areas near the edge of an affected valve in which nodular changes were detected. Notice that the only structures observed were the basement membrane, collagen, and elastin strands (white arrows) with what appeared to be a few remnants of cytoplasmic extensions of valvular interstitial cells. Bar = 12 μ m.

Discussion

In the study of this report, the scanning electron microscopic appearance of the mitral valve from clinically normal dogs was similar to that reported previously in dogs.⁷ In our study and that of Sarpie,⁷ the dogs used to determine morphologic features of normal valves were of similar age. To the authors' knowledge, there are no reports in which the surface morphologic features of valves from MVE-affected dogs are described, although surface morphologic features of mitral valves have been described in humans with MVP.¹⁸ Viewed at low magnification, the thickening and distortion of the endocardiosis-affected valve leaflets were apparent, compared with valve leaflets from clinically normal dogs. These changes make the leaflets appear shrunken, but Kogure⁹ has demonstrat-

ed elongation of the chordae tendineae and enlargement of the leaflets in dogs with MVE; the distortion of the valve architecture contributes to prolapse of the mitral valve and results in the poorly coapted leaflet edges and mitral regurgitation that are typical of this disease.¹³ This alteration in valve configuration is a consequence of the myxomatous degeneration of the valve substance, which involves loss and destruction of the collagen matrix with concurrent increase in the quantity of ground substance (acid mucopolysaccharides).^{9,19}

At high magnification, the normal valve was lined by ordered rows of endothelial cells with prominent nuclear bulges and plasmalemmal microappendages. The cells were ordered in a set pattern that appeared to correspond with the orientation of the underlying collagen bundles. The endothelium formed a complete and intact covering over the chordae tendineae and leaflets. There was marked variation in the regional appearance of the endothelium, and this pleomorphism was a consistent finding. Regional endothelial cell pleomorphism and differences in cell function have previously been reported in the rabbit systemic vascular system,²⁰ and it is probable that the endothelial pleomorphism noted on valve leaflets in the study dogs is associated with functional differences at different sites or differences in the rate of proliferation or replacement of endothelial cells.^{7,21} In contrast with cells lining valves from clinically normal dogs, endothelial cells on the endocardiosis-affected valves that did not appear damaged *per se* were more uniform with less evidence of surface microappendages. Whether this implies a reduction in overall cell activity or cell replacement rates cannot be stated at this time.

In endocardiosis-affected valves, the most noticeable abnormalities were thickening of the leaflets and damage to the endothelial lining. The latter change was most evident near the edges of valve leaflets and extended to the chordae tendineae but only occasionally involved the base of the leaflets adjacent to the mitral valve annulus. It appeared that the orientation of the endothelial cells did not correspond with the orientation of the underlying collagen bundles in endocardiosis-affected valves, but this is a subjective assessment at present. Overall, the endothelial abnormalities that we identified in valves from dogs with MVE were similar to those reported for valves from humans with MVP.¹⁸ In endocardiosis-affected valves, the endothelial damage appeared to have a patchy distribution. In several areas of the valve surface, the loss of endothelial cells exposed the underlying basement membrane; in other areas, exposure of the underlying valve matrix was apparent. In the subendothelial matrix, single cells were observed that were effete endocardial cells, RBCs, or leukocytes. Platelets that were adherent to the subendothelial collagen were also detected. In humans with MVP, the patchy distribution of endothelial damage is also a consistent finding¹⁸; platelet adherence to exposed collagen and fibrin deposition in the valve matrix are also observed. These processes may contribute to thrombus formation and development of endocarditis, which are common problems in humans with MVP. However, in a scanning electron microscop-

ic investigation of mitral valves in humans,¹⁸ platelet attachment to the denuded surface of the valve was only reported in 1 patient, and that patient also had atrial fibrillation. In dogs with MVE, thrombus formation or endocarditis has not been reported, although it has been reported anecdotally that thromboembolic events may occur with this disease, particularly in Cavalier King Charles Spaniels.²² Although platelet adhesion to subendothelial collagen was evident in the study of this report, thrombus formation was not detected. Interestingly, Tanaka and Yamane²³ identified the reduced ability of platelets to aggregate in dogs with MVE and suggested that this was a consequence of exposure of platelets to chronic stimulation and stress injury as a result of mitral regurgitation, which thereby affected platelet function. In contrast, Olsen et al²² reported differences in the ability of platelets to aggregate (normal or increased), platelet numbers, and serum magnesium concentration between 2 subpopulations of Cavalier King Charles Spaniels with MVP. Data regarding the ability of platelets to aggregate in humans with MVP are similarly conflicting.²³ It remains to be determined if abnormalities of platelet function in dogs with MVE are important either in the development of this disease or in any potentially catastrophic consequences, such as cerebral or myocardial thromboembolism; at present, there is no explanation for why greater platelet adhesion in denuded areas of the endocardiosis-affected valves was not observed.

In our study, it is possible that the denudation of the endothelium in valves from MVE-affected dogs was a consequence of the tissue fixation and processing technique; if the excised tissue was swollen or damaged, the endothelium might have detached as a result of the processing conditions required for specimen preparation for scanning electron microscopy. However, detection of the consistently patchy variation in extent of endothelial cell loss and the presence of platelets lodged in the collagen fiber network would suggest that a biological effect was the likely explanation rather than fixation or processing artifact. Furthermore, endothelial cells adjacent to and within denuded areas had different phenotypic characteristics, which could only have developed *ante-mortem*.

Whether the patchy localized endothelial damage was associated with the regional pleomorphism detected among cells on the surface of valve leaflets from clinically normal dogs is unknown. The extensive damage along the free edge of the valve leaflets suggested that repeated trauma may be a cause of MVE,⁸ and the regional differences in morphologic features of cells may be a developmental process to protect the valve leaflets from trauma. In dogs predisposed to development of endocardiosis, abnormal function or replacement of these cells on valve leaflets may be important in the development of MVE. Alternatively, damage to the endothelium might occur secondary to damage within the valve structure. However, endothelial cell pleomorphism does appear to be associated with regional differences in cell proliferation and cell activity. In the study of this report, it was interesting that cells

located in endothelium-denuded areas or close to the margins of damaged areas had marked numbers of plasmalemmal microappendages, compared with cells in areas of intact endothelium. The exact function of the microappendages is not known, but such plasmalemmal projections increase the surface area of the cells; the structural appearance of the microappendages and their density on the endothelial cell surface are believed to indicate the degree of cell proliferation and level of overall cellular synthetic activity. In clinically normal dogs, the cells of the endocardium of the atrioventricular valves have the greatest density of microappendages.^{7,21} In valves from dogs with MVE, large numbers of microappendages per cell may reflect a protective and repair process (albeit probably unsuccessful) in response to severe endothelial loss. In a study⁷ by Sarphie of clinically normal dogs, endothelial cells lining mitral valves had more microappendages than did atrial endocardial cells (this phenomenon was most clearly visualized by use of transmission electron microscopy). Also, the endocardial cells on the ventricular aspect of the valve were reported by that investigator to be fewer and flatter than those on the atrial side, with fewer microappendages but a thicker acid mucopolysaccharide cell coating (the glycocalyx). Results of the study of this report appeared to contradict these findings, but transmission electron microscopy was not performed to support this contention. In the report⁷ by Sarphie, it is speculated that these pleomorphic differences between surfaces of the valve indicate differences in the rate of cell proliferation or differences in the hemodynamic forces to which each surface is exposed. Differences in the appearance of the endothelium and the density of the glycocalyx between the 2 sides of the aortic valve leaflets are also reported in rabbits.²⁰ The glycocalyx is a selectively permeable protective barrier, and damage to this barrier would appear to precede loss of endothelial integrity.^{24,25} Indeed, it is probable that changes in the composition of the glycocalyx contribute to general endothelial dysfunction in cardiovascular disease,²⁶ but whether alteration to this glycoprotein-polysaccharide coating of cells is present in mitral valves of dogs with endocardiosis remains to be elucidated.

Endothelial changes in valves of dogs with MVE have been previously reported.^{16,27,28} Compared with endothelial cells on normal mitral valves in dogs, Mow and Pedersen¹⁶ detected increased endothelin receptor density on endocardiosis-affected mitral valve leaflets, which correlated with severity of pathologic lesions and suggested that endothelin is important in the pathogenesis of MVE. As discussed, the damage to the valvular endothelium could be a consequence of endocardiosis-associated changes in the shape of the valve, resulting in mechanical damage from abnormal valve closure and shear stress caused by regurgitant blood flow.¹⁶ However, the endothelial loss also extends to the chordae tendineae and therefore is not restricted to the sites of maximal potential injury (ie, the free edges of the valve leaflets). Further indirect evidence of endothelial dysfunction in MVE includes recent

reports^{27,28} of reduced plasma nitric oxide activity and increased expression of NADPH-diaphorase in mitral valves of affected dogs, compared with that of valves in clinically normal dogs. Whether these changes are a cause or consequence of the disease process is not known. An alternative explanation is that subendothelial structures, such as the VICs, might be crucial to the maintenance of a healthy endothelial covering. Indeed, damage to endothelial cells does initiate VIC proliferation and migration to the valve surface,²⁹ and incorporation of VICs into the endothelium may be part of the normal repair process.¹⁷ However, these findings are only reported for experimentally cultured mitral valves from cattle, and it is not known if this occurs *in vivo*.

Valvular interstitial cells are fibroblast-like cells with additional contractile and pacemaker properties (myofibroblasts); they are involved in the maintenance of the valve structure and produce the matrix (collagen, elastin, and ground substance) in which they are embedded.³⁰⁻³² In our experience, abnormal VICs are present in the valves of MVE-affected dogs, and this suggests that such abnormalities are crucial to the development of MVE. Whether abnormalities of the VICs are solely the cause of the loss of collagen matrix and endothelium or endothelial damage and loss have a causal role in the development of myxomatous degeneration is not known. Endothelial damage does cause release of vasoactive peptides such as endothelin-1 that can interact with the subendothelial VICs.¹⁶ In cultured cardiac fibroblasts, endothelin-1 and -3 stimulate collagen production,³³ and endothelin-1 reduces collagenase activity. Because VICs function predominantly as myofibroblasts, they could be expected to respond to increased endothelin production in a similar manner.¹⁶ However, myxomatous degeneration appears to be a problem of dyscollagenesis in that there is a reduction in collagen production, and the collagen produced appears to be ultrastructurally abnormal.^{11,13,34} If expression of endothelin-1 in mitral valves is high as a consequence of endocardiosis-induced endothelial damage, then an increase in collagen production might be expected, rather than the loss of collagen that is observed in MVE. In the study of this report, the appearance of the endothelial cells in endocardiosis-affected valves suggested that they were responding to adverse circumstances, rather than being the progenitors of the pathologic change.

The possible role of collagen in maintaining a healthy endothelial covering should also be considered. The orientation of the endothelial cells of the mitral valve complex followed the ordered direction of collagen bundles. Consequently, for example, the cells on the chordae tendineae were orientated in a helical fashion. It is conceivable that damage to the collagen superstructure of the valve complex, such as that documented in published reports,^{9,19} could have affected endothelial cell alignment and therefore also cell function, health, and viability.

In conclusion, our data indicated that there is considerable endothelial damage in the mitral valve complex of dogs with MVE, which is similar to findings in mitral valves of humans with MVP. Whether this denudation of valvular endothelium is a cause or con-

sequence of mitral valve myxomatous degeneration in either species remains to be elucidated.

^aPentobject, Animalcare Ltd, York, England, UK.

^bEmscope SC500 sputter coater, Emscope Laboratories Ltd, Kent, England, UK.

^cHitachi 3-570 scanning electron microscope, Hitachi Ltd, Chiyodaku, Japan.

References

1. Buchanan JW. Chronic valvular disease (endocardiosis) in dogs. *Adv Vet Sci Comp Med* 1977;21:75–106.
2. Beardow AW, Buchanan JW. Chronic mitral valve disease in Cavalier King Charles Spaniels: 95 cases (1987–1991). *J Am Vet Med Assoc* 1993;203:1023–1029.
3. Darke PG. Valvular incompetence in Cavalier King Charles spaniels. *Vet Rec* 1987;120:365–366.
4. Haggstrom J, Hansson K, Kvarn C, et al. Chronic valvular disease in Cavalier King Charles Spaniel in Sweden. *Vet Rec* 1992;131:549–553.
5. Haggstrom J, Kvarn C. Update on mitral valve disease, in *Proceedings. 15th Am Coll Vet Intern Med Forum* 1997;202–204.
6. Fenoglio JJ, Pham TD, Wit AL, et al. Canine mitral complex. Ultrastructural and mechanical properties. *Circ Res* 1972;31:417–430.
7. Sarpie TG. Pleomorphic surface features of mammalian endocardium: fine structure of canine bicuspid valves. *J Mol Cell Cardiol* 1980;12:241–255.
8. Pedersen HD, Haggstrom J. Mitral valve prolapse in the dog: a model of mitral prolapse in man. *Cardiovasc Res* 2000;47:234–243.
9. Kogure K. Pathology of chronic mitral valve disease in the dog. *Jpn J Vet Sci* 1980;42:323–335.
10. Castagnaro M, Amedeo S, Bertolotto A, et al. Morphological and biochemical investigations of mitral valve endocardiosis in pigs. *Res Vet Sci* 1997;62:121–125.
11. King BD, Clarke MA, Baba N, et al. “Myxomatous” mitral valves: collagen dissolution as the primary defect. *Circulation* 1982;66:288–296.
12. Reef VB, Bain FT, Spencer PA. Severe mitral regurgitation in horses: clinical, echocardiographic and pathological findings. *Equine Vet J* 1998;30:18–27.
13. Pedersen HD, Kristensen BO, Norby B, et al. Echocardiographic study of mitral valve prolapse in Dachshunds. *Zentralbl Veterinarmed [A]* 1996;43:103–110.
14. Wordsworth P, Ogilvie D, Akhas F, et al. Genetic segregation analysis of familial mitral valve prolapse shows no linkage to fibrillar collagen genes. *Br Heart J* 1989;61:300–306.
15. Heeney AM, Sipouras PT, Schwartz RC, et al. Genetic evidence that mutations in the COL1A1, COL1A2, COL3A1 or COL5A2 collagen genes are not responsible for mitral valve prolapse. *Br Heart J* 1989;61:292–299.
16. Mow T, Pedersen HD. Increased endothelin receptor density in myxomatous canine mitral valve leaflets. *J Cardiovasc Pharmacol* 1999;34:254–260.
17. Lester W, Damji AA, Gedeon I, et al. Interstitial cells from the atrial and ventricular sides of the bovine mitral valve respond differently to denuding endocardial injury. *In Vitro Cell Dev Biol* 1993;29A:41–50.
18. Stein PD, Wang CH, Riddle JM, et al. Scanning electron microscopy of operatively excised severely regurgitant floppy mitral valves. *Am J Cardiol* 1989;64:392–394.
19. Pomerance A, Whitney JC. Heart valve changes common to man and dog: a comparative study. *Cardiovasc Res* 1970;4:61–66.
20. Sarpie TG. Anionic surface properties of aortic and mitral valve endothelium from New Zealand white rabbits. *Am J Anat* 1985;174:145–160.
21. Sarpie TG, Allen DJ. Scanning and transmission electron microscopy of normal and methotrexate-treated endocardial cell population in dogs. *J Submicrosc Cytol* 1978;10:15–25.
22. Olsen H, Kristensen A, Haggstrom J, et al. Increased platelet aggregation response in cavalier King Charles spaniels with mitral valve prolapse. *J Vet Intern Med* 2001;15:209–216.
23. Tanaka R, Yamane Y. Platelet aggregation in dogs with mitral valve regurgitation. *Am J Vet Res* 2000;61:1248–1251.
24. Sarpie TG. Surface topography of mitral valve endothelium from diet-induced, hypercholesterolemic rabbits. *Atherosclerosis* 1982;45:203–220.
25. Baldwin AL, Thurston G. Mechanics of endothelial cell architecture and vascular permeability. *Crit Rev Biomed Eng* 2001;29:247–278.
26. Beresewicz A, Czarnowska E, Maczewski M. Ischemic preconditioning and superoxide dismutase protect against endothelial dysfunction and endothelium glycocalyx disruption in the postischemic guinea-pig hearts. *Mol Cell Biochem* 1998;186:87–97.
27. Olsen LH, Mortensen K, Martinussen T, et al. Evidence of increased nitric oxide synthase activity in canine myxomatous mitral valve leaflets. *J Vet Intern Med* 2002;16:627–628.
28. Pedersen HD, Schutt T, Sondergaard R. Decreased nitric oxide metabolites (nitrate/nitrite) in plasma of dogs with mild mitral regurgitation. *J Vet Intern Med* 2002;16:628–629.
29. Lester W, Damji AA, Tanaka M, et al. Bovine mitral valve organ culture: role of interstitial cells in repair of valvular injury. *J Mol Cell Cardiol* 1992;24:43–53.
30. Filip DA, Radu A, Simionescu M. Interstitial cells of the heart valves possess characteristics similar to smooth muscle cells. *Circ Res* 1986;59:310–320.
31. Messier RH Jr, Bass BL, Aly HM, et al. Dual structural and functional phenotypes of the porcine aortic valve interstitial population: characteristics of the leaflet myofibroblast. *J Surg Res* 1994;57:1–21.
32. Mulholland DL, Gobleib AI. Cell biology of valvular interstitial cells. *Can J Cardiol* 1996;12:231–236.
33. Guarda E, Katawa LC, Myers PR, et al. Effects of endothelins on collagen turnover in cardiac fibroblasts. *Cardiovasc Res* 1993;27:2130–2134.
34. Tamura K, Fukada Y, Ishizaki M, et al. Abnormalities in elastic fibers and other connective-tissue components of floppy mitral valves. *Am Heart J* 1995;129:1149–1158.