
BASIC CONCEPTS IN CARDIOLOGY

Arnold M. Katz, MD, FACC, *Guest Editor*

Cardiac Interstitium in Health and Disease: The Fibrillar Collagen Network

KARL T. WEBER, MD, FACC

Chicago, Illinois

Composed of type I and III collagens, the valve leaflets, chordae tendineae and collagen matrix of the myocardium form a structural continuum. Synthesized by cardiac fibroblasts, these fibrillar collagens support and tether myocytes to maintain their alignment, whereas their respective tensile strength and resilience resist the deformation, maintain the shape and thickness, prevent the rupture and contribute to the passive and active stiffness of the myocardium. An acquired or congenital defect in this collagen network can lead to abnormalities in myocardial architecture, mechanics or valve function. In the hypertrophic process that accompanies a pressure overload, for example, increased collagen synthesis, fibroblast proliferation and a structural and biochemical remodeling of the matrix are seen. This includes distinctive patterns of reparative and reactive myocardial fibrosis, each of which alters diastolic and systolic myocardial stiffness and may lead to pathologic

hypertrophy. Alternatively, a loss of collagen tethers or decline in matrix tensile strength can be responsible for regional or global transformations in myocardial architecture and function seen in the reperfused ("stunned") myocardium and in dilated (idiopathic) cardiopathy. Inherited disorders in the transcriptional and posttranslational processing of collagen can also alter the biophysical properties of the network.

Future studies into collagen gene regulation, gene switching events and the control of collagen synthesis and degradation are needed to develop a more complete understanding of the relation between the collagen network and acquired and inherited forms of heart disease and to utilize therapeutics that will prevent, retard or regress abnormal collagen matrix remodeling.

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This article is dedicated to Thomas F. Robinson, PhD (1944-1988)—dedicated and creative scientist, respected colleague and friend.

K.T.W.

The heart is a muscular pump composed of cardiac myocytes (1). In recent years, however, it has been recognized that, whereas cardiac myocytes and the coronary vasculature are central to the contractile function and viability of the myocardium, so too is the extracellular matrix, or cardiac interstitium, and its type I and III fibrillar collagen matrix in

particular. Collagen fibers, the major structural protein of the interstitium, serve several functions: 1) they provide a scaffolding that supports muscle cells and blood vessels (2); 2) they act as lateral connections between cells and muscle bundles to govern architecture (2,3) while coordinating the delivery of force, generated by myocytes, to the ventricular chamber (4); and 3) their respective tensile strength and resilience (5) are important determinants of diastolic and

This article is part of a series of informal teaching reviews devoted to subjects in basic cardiology that are of particular interest because of their high potential for clinical application. The intent of the series is to help the clinician keep abreast of important advances in our understanding of the basic mechanisms underlying normal and abnormal cardiac function.

From the Cardiovascular Institute, Michael Reese Hospital, University of Chicago Pritzker School of Medicine, Chicago, Illinois. This study was supported in part by Grant No. R01-HL-31701 from the National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, Maryland.

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Address for reprints: Karl T. Weber, MD, Michael Reese Hospital, Lake Shore Drive at 31st Street, Chicago, Illinois 60616.

systolic myocardial stiffness (6,7) and serve to resist myocardial deformation, maintain shape and wall thickness and prevent ventricular aneurysm and rupture (8,9). Less is known about other connective tissue elements of the heart and a discussion of the cytoskeleton and glycosaminoglycans, for example, is beyond the intent of this review.

The fibrillar collagen matrix of the heart is an active participant in the hypertrophy that accompanies various forms of chronic pressure overload (i.e., aortic stenosis and renovascular and essential hypertension). Like the hypertrophic remodeling seen in skeletal muscle (10), where both extracellular matrix and muscle cells increase in dimension, an accumulation and enhanced dimension of collagen fibers, together with their realignment relative to muscle, have been observed in left ventricular pressure overload hypertrophy (see Ref. 6 for review). Initially, the physical transformation of collagen fibers is an adaptive process that facilitates the concentric growth of the myocardium and augments its generation of force while increasing diastolic stiffness. Subsequent alterations in the concentration of collagen, the proportion of type I and III collagens, or the alignment of collagen and muscle fibers, or both, will determine whether this adaptation becomes pathologic (6,7). In other circumstances, such as the "stunned" myocardium (11) or dilated primary myocardial heart disease (12), a physical or biochemical abnormality of collagen tethers has been observed, each of which may be responsible for the respective regional or global transformation in myocardial architecture, including its thinning, impaired contractility and enlargement of the ventricular chamber.

Continued investigation into the remodeling of the extracellular matrix as well as the control and regulation of collagen synthesis and degradation is needed to fully elucidate the role of the collagen network in health and disease. Nevertheless, it would appear timely, at this juncture, to review our current understanding of the collagen network to heighten general awareness of the important potential contributions of a disorder of fibrillar collagen in various expressions of cardiovascular disease. This review also suggests that such a broader perspective of the myocardium, which includes connective tissue, is necessary to develop our understanding of the various entities that will come to be recognized as interstitial heart disease. Such a perspective mandates that in our consideration of the origins of heart disease, including the appearance of heart failure and ventricular arrhythmias, we include acquired and inherited disorders of intracellular and extracellular events fundamental to synthesis, fiber formation and degradation of collagen.

Historical Perspective

In 1907, Holmgren (13) described the extracellular matrix and fibrillar collagen that connected skeletal muscle fibers. The terms epi-, peri- and endomysium were used by Nagle

(14) to characterize the various structural components of the extracellular matrix in muscle. In 1960, Hort (15) suggested that the collagen matrix of the myocardium prevented its excess dilation. He further observed that the configuration of perimysial fibers was related to the state of ventricular distension (i.e., wavy in contraction and stretched with dilation) and proposed that the recoil of the network of thin collagen fibers that enveloped muscle contributed to diastolic filling. About the same time, muscle physiologists had become interested in the contribution of elastic elements to muscle shortening. Modeled as springs that were arranged in series or in parallel with contractile element (16,17), these elastic elements had no clear anatomic counterpart, although they were presumed to be connective tissue. The advent of the scanning electron microscope brought the ultrastructural nature of the collagen matrix, the major elastic element of the myocardium, into perspective (see later). Immunohistochemical studies and biochemical analysis identified the fibrillar collagen matrix as consisting primarily of type I and III collagens.

The fractional volume of collagen in the myocardium is small. Its high tensile strength, however, influences myocardial stiffness, as is the case for any composite material or alloy that is composed of various elements, each having different elastic constants. The collagen concentration of the right ventricle is approximately 30% greater than that of the left ventricle (18) because its myocytes are smaller.

Structure, Function and Metabolism of the Normal Collagen Matrix

The Cardiac Interstitium

The interstitium of the myocardium includes fibrillar connective tissue; various cells, such as fibroblasts and plasma cells, and a gel-like ground substance composed of glycosaminoglycans and glycoproteins. Cardiac nerves and the coronary vasculature also reside in the interstitium. Myocytes represent only one-third of the number of cells; however, their volume accounts for over two-thirds of the myocardium (19). Fibroblasts may represent as much as two-thirds of the cells. Type I and III collagens are the dominant components of fibrillar connective tissue. Elastin is also seen, but to a lesser extent (20). In arteries, on the other hand, elastin is present in larger quantities providing a resilience to vessels necessary for their cyclical distension.

The interstitium serves several functions. These include 1) providing a structure that supports and tethers cardiac myocytes, intramyocardial coronary arteries, arterioles, capillaries and veins; 2) maintaining a defense mechanism against invasion by foreign protein, bacteria or viruses; 3) aiding in the nutrition of myocytes, by facilitating the exchange of substrates between myocytes and their capillaries; and 4) providing a lubricant for contracting myocytes. In keeping with the heart's known autocrine and paracrine

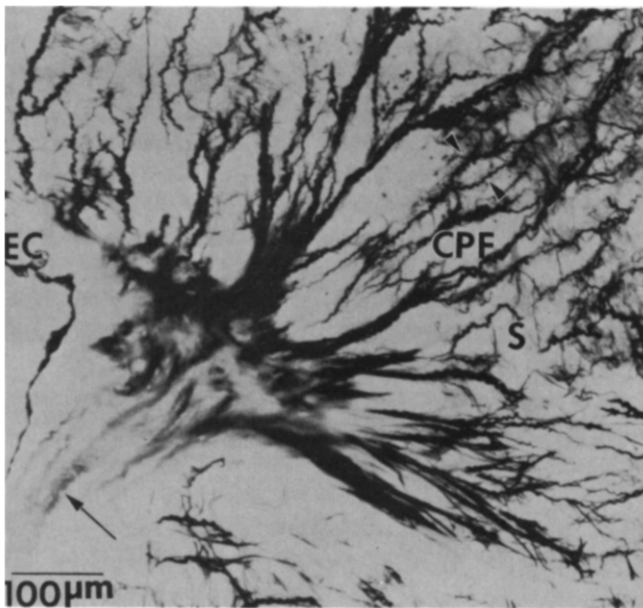


Figure 1. Insertion of the chordae tendineae into the papillary muscle (arrow) is shown to arborize into a network of coiled perimysial fibers (CPF) that are interconnected by strands (S) of collagen. EC = epimysial collagen fibers. (Reproduced with permission from Robinson et al. [21].)

properties, the interstitium will likely be shown to contain putative mitogens that mediate cell growth. These mitogens will normally reside in or gain entry to the interstitium. In this review, we will focus solely on the structural protein, collagen and its contribution to the myocardium in health and disease. A broader perspective of the cardiac interstitium is beyond the scope of this review.

The Collagen Network of the Heart

The leaflets of the semilunar and atrioventricular (AV) valves, together with chordae tendineae and the intramyocardial collagen matrix, form a structural continuum (Fig. 1) that represents the collagen network of the heart (21). A biochemical defect (22) within this collagenous network or its physical interruption (23) has the potential to adversely alter its tensile strength and tethering-support function and thereby alter the architecture and mechanical properties of the myocardium. The floppy mitral valve, for example, reflects an abnormality in the type I/III collagen ratio that may also be present in the matrix as well. At present, such a defect is thought to be confined to the valve leaflets, or the exteriorized portion of the network. This may not be the case. The involvement of the matrix could explain why some patients with degenerative mitral valve disease do not have a satisfactory response to valve replacement. The importance of preserving the structural integrity of the mitral valve apparatus-matrix network during surgical repair of the diseased mitral valve has recently been emphasized (24,25).

The valves, chordae tendineae and collagen matrix of the myocardium consist primarily of fibrillar type I and III collagens (22,26,27). The extracellular matrix of muscle is subdivided into epi-, peri- and endomysial components (28,29). Recent studies by Eghbali et al. (30,31), using cDNA probes specific for these collagens and in situ hybridization, indicate that in the myocardium cardiac fibroblasts, *not* myocytes, are responsible for the production of type I and III collagens. Cardiac fibroblasts are also likely to produce the proteolytic enzyme collagenase that is responsible for collagen degradation (32). Therefore, it must be recognized that disorders of cardiac fibroblasts, the cells involved in collagen synthesis and degradation, may result in interstitial heart disease (see later). Hence, and despite the importance of the contractile element, or cardiac myocyte, cardiac fibroblasts will undoubtedly come to be recognized as playing an important role in the appearance and progression of cardiovascular disease as well as in the regression of myocardial fibrosis.

Components of the collagen matrix. The *epimysium* is the collagenous matrix that lies below the endothelium of the epicardium and endocardium; it surrounds the myocardium. Figure 2 depicts the various components of the matrix including the epimysium. The epimysium forms a complex array of collagen fibers that covers muscle (left panel, Fig. 3). Robinson et al. (20) found that once this array of epimysial fibers is aligned parallel to muscle with stretch (right panel, Fig. 3), an exponential rise in passive muscle tension occurs with additional increments in muscle length. Hence, the epimysium resembles a parallel elastic element that resists and modulates compressive forces and that preserves the sarcomeric unit from excessive and disruptive degrees of stretch. The epimysium is an important determinant of the maximal length of papillary muscle.

The *perimysium* consists of tendon-like extensions of the epimysium (Fig. 2) that arborize into a weave to aggregate myocytes into myofibers. Perimysial strands of collagen, located in natural spaces between muscle bundles, join adjacent weaves (Fig. 3). Shearing forces, present between contracting muscle bundles, are borne by these strands. Given that the tensile strength of type I collagen exceeds that of steel (33), perimysial strands are well suited to accommodate these forces and to minimize the dissipation of force generated by myocytes. Collagen fiber tensile strength is proportional to fiber thickness (5). It therefore follows that thicker collagen strands would permit a more powerful contraction of the myocardium. Furthermore, and because perimysial strands provide lateral connections between myocyte bundles, they prevent the malalignment or slippage of cardiac muscle. An absence of disruption of these strands would permit slippage that is necessary for the concentric or eccentric remodeling of the myocardium. Weaving through perimysial strands, in a direction parallel to muscle, are

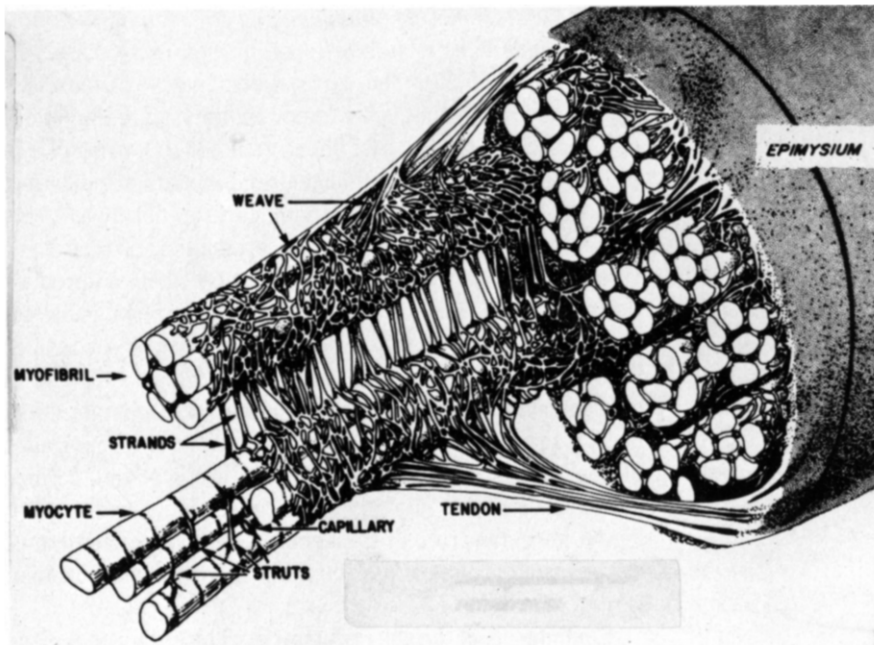


Figure 2. Schematic representation of the collagen matrix of the myocardium and its epimysial, perimysial and endomysial components (see text). (Reproduced with permission from Weber et al. [6].)

broad collagen fibers or tendons (Fig. 4). Coiled perimysial fibers also run parallel to muscle fibers. Robinson et al. (21) have shown that these coiled perimysial fibers are continuous with chordae tendineae.

Endomysial collagen fibers consist of fibrils that connect adjoining myocytes to one another and to their neighboring capillaries (left panel, Fig. 5), and to a meshwork of fibrils that surround individual myocytes. The endomysial fibers connecting cells have been termed struts by Borg and Caulfield (2). Struts insert into the basal lamina lateral to the Z band of the sarcomeric unit (34) (right panel, Fig. 4). Inside the cell they may communicate with the cytoskeleton that supports the actin-myosin contractile protein apparatus (35) and that also may influence myocyte stiffness. Thus, one can envisage that these struts join together the contractile apparatus of adjacent cells. These lateral cell to cell connections further serve to coordinate the transduction of force to the ventricular chamber and to prevent cell slippage during the cardiac cycle. Winegrad and Robinson (36) showed that, even in the absence of the intercalated disc, cardiac muscle fibers contract in a coordinated manner because of these collagen tethers. Factor and Robinson (37) suggested that the forceful contraction and relengthening of the myocardium are related to the presence of intercellular tethers and the endomysial weave. In this connection, Iwazumi and ter Keurs (38) noted that the restoring force seen in cardiac tissue was diminished in isolated cardiac myocytes prepared with collagenase.

Fibrillar type I and III collagens. On the basis of electrophoretic analysis, the myocardium of the rat (26) and of the nonhuman primate (27) contains type I and III collagens. The collagen matrix consists primarily of type I collagen

Figure 3. Silver-stained epimysial collagen fibers in the normal rat papillary muscle. **A**, Unstretched muscle, where collagen fibers have a complex arrangement. **B**, Stretched muscle and epimysial fibers now arranged parallel to muscle fibers (arrow). Additional stretch is now associated with an exponential increase in muscle tension. (Reproduced with permission from Robinson et al. [20].)

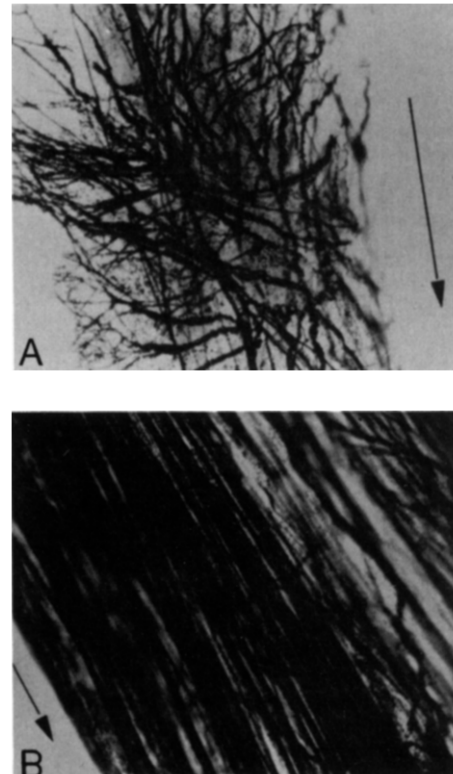
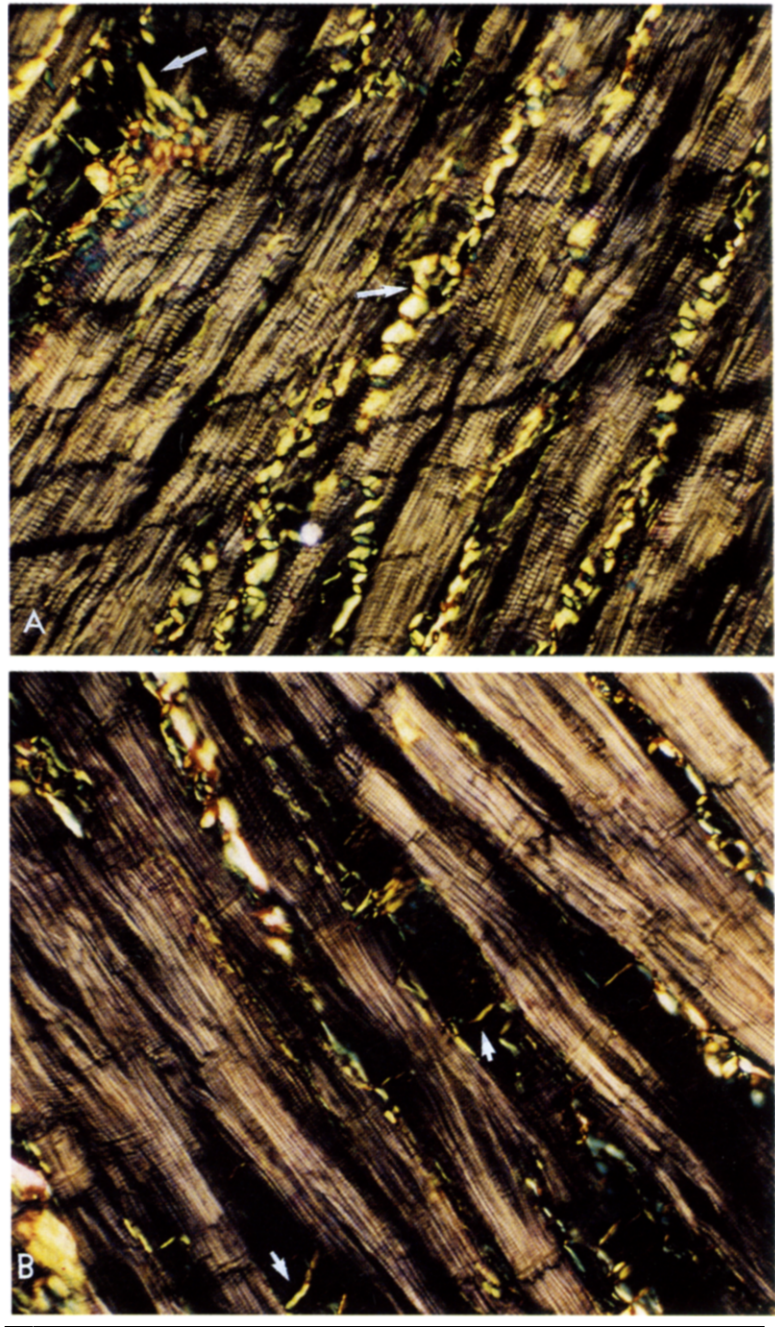


Figure 4. Normal human myocardium stained with Sirius Red and viewed by polarization microscopy. **A**, Coiled thick perimysial fibers (yellow or yellow-red) coursing between rows of organized muscle are seen. Collagen strands (**arrows**) connecting adjacent muscle fibers are quite evident. **B**, The nature of these strands is further identified (**arrows**). Note that the majority of collagen fibers are thick and appear yellow or yellow-red in color. The proportion of thin, green colored collagen fibers is quite small. (Reproduced with permission from Weber et al. [12].)



(85%); type III represents 11% of the total collagen protein. Valve leaflets contain 20% type III collagen (22). Type I collagen fibers have a substantial tensile strength (5,33), whereas type III collagen fibers possess a resilience that is ideal for maintaining structural integrity and distensibility of the network. Type I and III collagens are each thought to be present in most collagen fibers (39).

Fibrillar type I collagen normally aggregates into predominantly thick fibers, whereas type III collagen typically forms thin fibers (40). With use of the collagen specific stain,

Sirius Red F3BA in a supersaturated picric acid solution, together with polarized light, an enhanced birefringence and differential coloration of thick and thin collagen fibers can be found (41,42). This histochemical technique has been used to distinguish the fibrillar nature of the collagen network (11,43-45) in normal and diseased myocardium. Immunohistochemical methods (34,46) using monospecific antibodies to type I and III collagens have indicated that endomysial tethers are composed of both type I and type III collagen fibers.

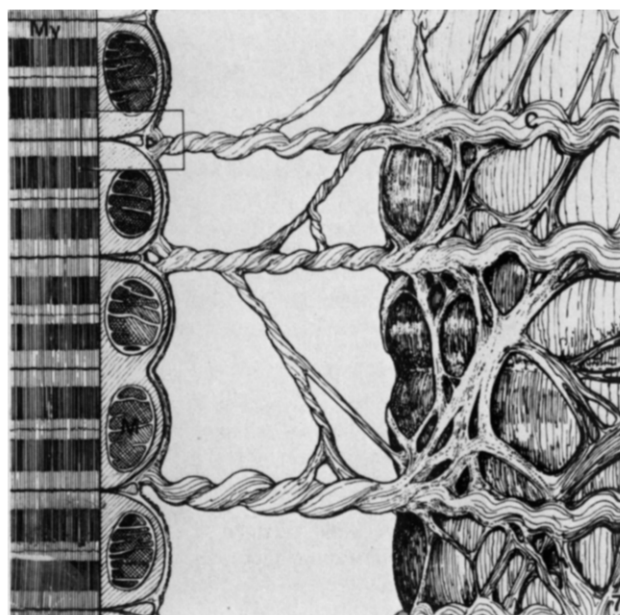
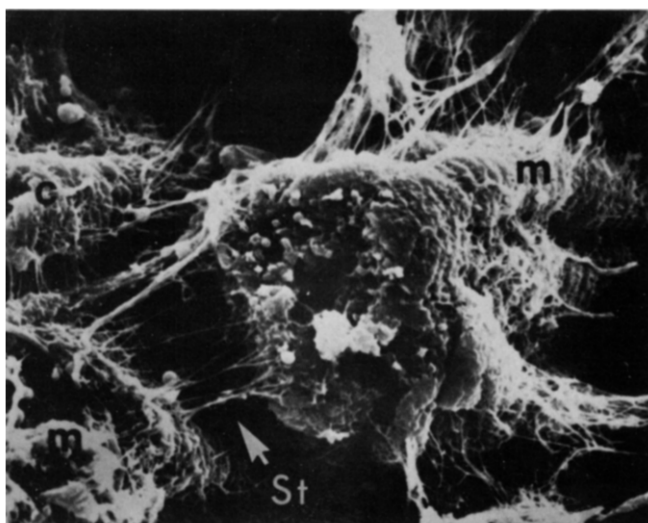


Figure 5. Endomysial collagen fibers between myocytes. **Left panel,** Scanning electron microscopy image of endomysial fibers or struts (St) seen in the nonhuman primate myocardium supporting myocytes (m) to one another and their capillaries (c). (Reproduced with permission from Weber et al. [3].) **Right panel,** Diagrammatic representation of endomysial fibrils (C) inserting near Z band of myocytes and communicating with intermediate filaments inside the cell. M = mitochondrion; My = myofilament. (Reproduced with permission from Robinson et al. [34].)

Collagen synthesis and degradation. In studying mRNA to type I and III collagens in isolated cardiac cells and using the *in situ* hybridization technique in cultured cardiac cells and whole heart tissue, Eghbali et al. (30,31) found that fibroblasts, not myocytes, contain the message for these fibrillar collagens. Myocytes and fibroblasts contain the mRNA to type IV collagen. A remarkably ordered series of transcriptional, posttranscriptional and posttranslational events mark the biosynthesis of collagen and the formation of collagen fibrils. These events are reviewed elsewhere (47). Controversy exists as to whether fibrillogenesis actually occurs in fibroblasts, in the extracellular matrix, or in both. In the embryonic chick cornea, Birk and Trelstad (48) present evidence that fibrils of varying diameter are formed inside fibroblasts before they are deposited in the interstitium. They further demonstrate that fibroblasts control the direction in which fibrils are oriented. Once collagen fibrils are deposited in the extracellular matrix, they are cross-linked to create a very stable protein. Whether these findings apply to the heart remains to be elucidated.

The daily fractional synthesis rate of collagen is 0.56% for the right and left ventricles of the dog (49) and 0.88% for the cockerel heart (50). Collagen half-life is estimated to be

80 to 120 days. Therefore, myocardial collagen, whose synthesis rate is low relative to that of noncollagen protein, has a half-life 10 times longer. Moreover, and even though fibroblasts represent 70% of the heart's total cell population (51), these cells are normally quiescent.

Fibroblasts remain quiescent out of the cell cycle, or Go stage, until recruited back into the cell cycle when they can be stimulated to proliferate. The interaction of certain mitogenic agents or growth factors with cell surface receptors is fundamental to the regulation of fibroblast proliferation. In some cases a complex interaction of several growth factors is required to induce quiescent fibroblasts to proliferate (52). The addition of platelet-derived growth factor to quiescent cells, for example, initiates proliferation by making fibroblasts "competent" to respond to the addition of other growth factors, termed "progression" factors. In keeping with this competence-progression model of fibroblast growth (53), cardiac fibroblasts residing in the interstitium would normally be exposed only to progression factors and therefore are quiescent. It is not until competence factors are made available that these cells are able to proliferate. It remains to be determined whether cardiac fibroblasts strictly adhere to the competence-progression model. The various growth factors that contribute to this paradigm also remain to be elucidated.

Collagen degradation in the myocardium has not been measured, but it would appear to be equivalent to synthesis, given the stability of its collagen concentration. Cardiac fibroblasts are again likely to be the cells responsible for degradation. Collagen fiber breakdown is mediated by collagenase. Montfort and Perez-Tamayo (54) identified collagenase as residing in the perimysium and endomysium by

immunohistochemical labeling. Given that fibroblasts do not store this enzyme after it is formed, these findings suggest that the myocardium has a latent collagenase system. Whether this latent collagenase exists in either of its two known forms, as a zymogen or a tissue-inhibitor complex, remains to be elucidated (for review see Ref. 32).

At present, the control and regulation of collagen genes and collagenase gene expression are unknown. These are particularly relevant topics if we are to prevent, retard or correct various forms of interstitial heart disease.

Collagen Matrix Remodeling

Pressure Overload Hypertrophy

In ventricular hypertrophy that accompanies a volume overload state, such as an arteriovenous fistula or anemia, or after thyroxine administration, collagen concentration remains unchanged. This is not the case in various states of left ventricular pressure overload hypertrophy, where an increase in collagen concentration and a structural and biochemical remodeling of the collagenous matrix of the left ventricular myocardium are seen (for review see Ref. 3 and 6). Thus it would appear that myocyte and collagen compartments are under separate as well as common controls. In both large and small mammalian hearts, banding of the ascending or abdominal aorta, coarctation of the renal artery, perinephritis and genetic hypertension are each associated with collagen accumulation in the myocardium. This accumulation of collagen, which could also be termed *fibrosis*, should not be considered synonymous with myocyte necrosis in that cell loss need not be present. Furthermore, although an elevation in left ventricular pressure work is common to each model, this elevation does not guarantee that systolic pressure itself is the stimulus to collagen synthesis and degradation. A number of putative mitogens may be operative depending on the etiologic basis of the pressure overload.

In humans with aortic valve stenosis and symptomatic heart failure (55-59), myocardial collagen concentration is increased three- to sixfold. Collagen volume fraction is also increased in patients with systemic hypertension; the greater the collagen accumulation, the more likely are patients to have symptomatic heart failure (60).

Collagen synthesis and degradation. As already indicated, collagen synthesis is quite low (0.6%/day) relative to synthesis of noncollagenous protein (7.2%/day). After the induction of a pressure overload, noncollagenous protein synthesis increases promptly in the affected ventricular myocardium. By day 5, it is 75% greater than baseline (49,50), returning to control levels at the end of 2 weeks. The increase in collagen synthesis is slower, but more persistent. Protocollagen proline hydroxylase activity increases within days and is followed by enhanced radioactive proline incorporation into hydroxyproline (61,62). A six- to eightfold increase in colla-

gen synthesis (4%/day) is seen. Collagen synthesis remains threefold greater than normal at 2 and 4 weeks (49). Autoradiographic evidence of fibroblast proliferation reaches its peak several days after the rise in collagen synthesis (63,64). The relevance of these findings to the competence-progression model of fibroblast growth remains to be determined. It can be said, however, that the rise in collagen synthesis is seen first in existing fibroblasts and is soon thereafter followed by fibroblast proliferation. At present, it is unclear if the population of fibroblasts responsible for collagen synthesis also participates in cell division. Moreover, the signal and transducer that link the pressure overload state to fibroblast proliferation remain to be determined.

A rise in collagen synthesis is not seen in the unaffected ventricle (49). This finding suggests that the release of local mitogens within the pressure-overloaded myocardium, the entry of putative mitogens into the interstitium of the involved myocardium or differences between ventricles are responsible for collagen accumulation. Circulating growth factors would be expected to alter collagen synthesis in both ventricles. Myocyte necrosis is not a requisite element for the appearance of *reactive fibrosis*.

Biochemical studies suggest that collagen degradation also increases with the pressure overload, but returns to baseline more quickly than does the elevation in collagen synthesis (50). We found (27,43,44) morphologic evidence (see later) of collagenase activity, including disrupted collagen fibers, during the evolutionary phase of hypertrophy. The presence of collagen degradation would lower the absolute elevation in collagen concentration that is predicted solely on the basis of an elevation in collagen synthesis.

Because the increase in myosin synthesis is more rapid than collagen synthesis, myocyte hypertrophy and a decline in both relative collagen content and concentration are seen early after pressure overload (49). Once myocyte growth stabilizes, although enhanced collagen synthesis persists and exceeds collagen degradation, collagen concentration and content will each increase to reach normal values within several weeks and greater than normal values at 4 weeks or more.

New collagen formation and structural remodeling of existing collagen. Caulfield (65) reported that with systemic hypertension the diameter of perimysial tendons and the density of the perimysial weave are increased in the hypertrophied human myocardium. Studies conducted in our laboratory in the nonhuman primate with perinephritic hypertension (27,66) and in the rat with suprarenal abdominal aorta banding or perinephritis (44,45) revealed a similar morphologic pattern of remodeling. These studies also extended our knowledge of the evolutionary and progressive nature of this remodeling and its functional consequences. In established hypertrophy, existing perimysial tendons, weaves and strands were greater in dimension (Fig. 6). A greater number of intermuscular spaces were now also

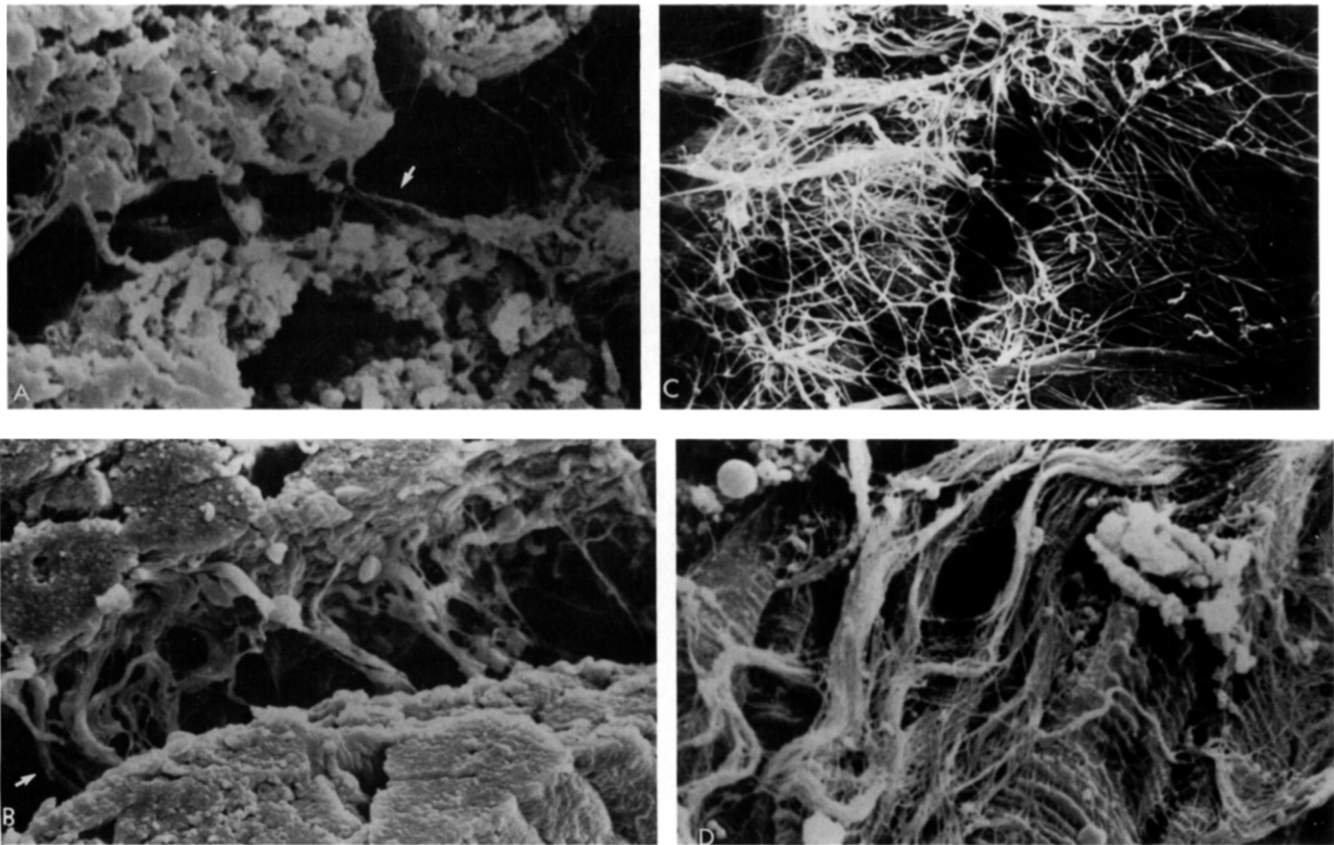


Figure 6. Scanning electron microscopy demonstrates the progressive increase in the dimension of the collagen matrix that is seen in the nonhuman primate myocardium with left ventricular pressure overload due to perinephritis. **A**, Normal myocardium and perimysial strands (**arrow**) between myocyte bundles. **B**, Thickened strands (**arrow**) are evident in hypertrophy. **C**, Normal myocardium with perimysial weave and tendons. **D**, Thickened tendons and dense weave seen with hypertrophy.

occupied by newly formed perimysial fibers (Fig. 7). In addition to this interstitial fibrosis, a perivascular fibrosis was evident involving intramyocardial coronary arteries (67). From their location around arteries collagenous septa radiated outward into the intermuscular space between organized myofibers. This pattern of interstitial and perivascular fibrosis, also termed streaky fibrosis (68), represents one aspect of the structural remodeling of the interstitium in chronic pressure overload hypertrophy.

Our studies also identified the evolution of the remodeling process. It began with evidence of collagen fiber degradation, edematous-appearing intermuscular spaces and an increased formation of type III collagen. These findings had disappeared with the established phase of hypertrophy. This sequence, which resembles the early and late stages of dermal wound healing (69), was observed in both rat and

primate myocardium. The mechanism responsible for the interstitial edema is not clear. It could be a response to an increased production of hydrophilic glycosaminoglycans (70), such as hyaluronic acid. In hypothyroidism, hyaluronic acid is increased in the myocardium and edema is frequently seen (71). In the case of myocyte injury, Judd and Wexler (72,73) found interstitial edema to accompany increased hexosamine formation. Alternatively, Laine (74) and others (75,76) have found coronary microvascular permeability to increase in response to either systemic hypertension or angiotensin infusion. Such an increase in permeability would cause interstitial edema. This issue is deserving of study because of its potential importance in developing preventive pharmacologic therapy.

Additional patterns of structural remodeling. In established hypertrophy, we observed other structurally distinct patterns of myocardial fibrosis. One pattern was a *reactive* process and included a unique arrangement of type I and III collagen fibers that began as thin collagen fibers extending outward across muscle fibers, perpendicular to their long axis. Thick collagen fibers, running in a direction parallel to muscle and perpendicular to the thin collagen fibers, subsequently became entwined in an ever increasing amount to form an orthogonal grid of thin and thick collagens fibers.

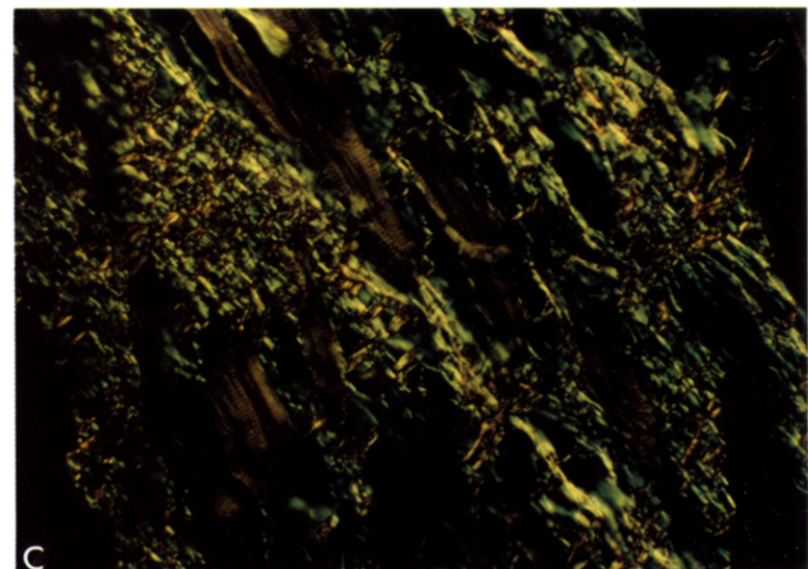
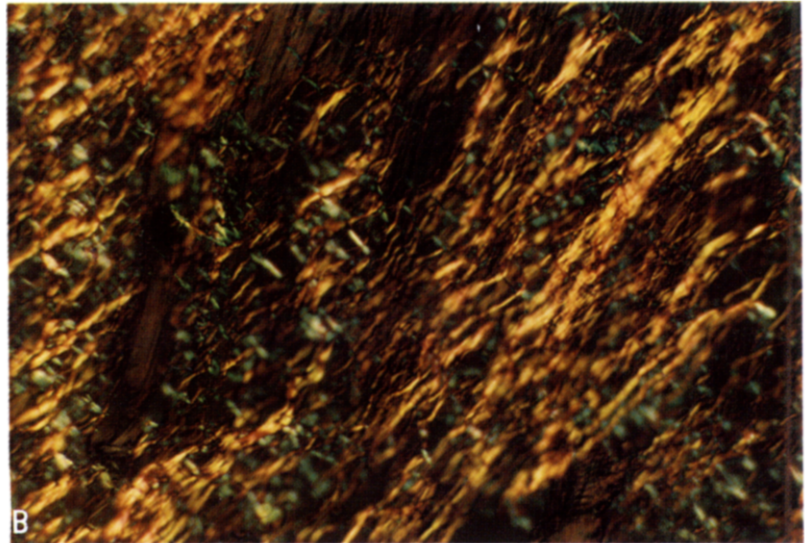
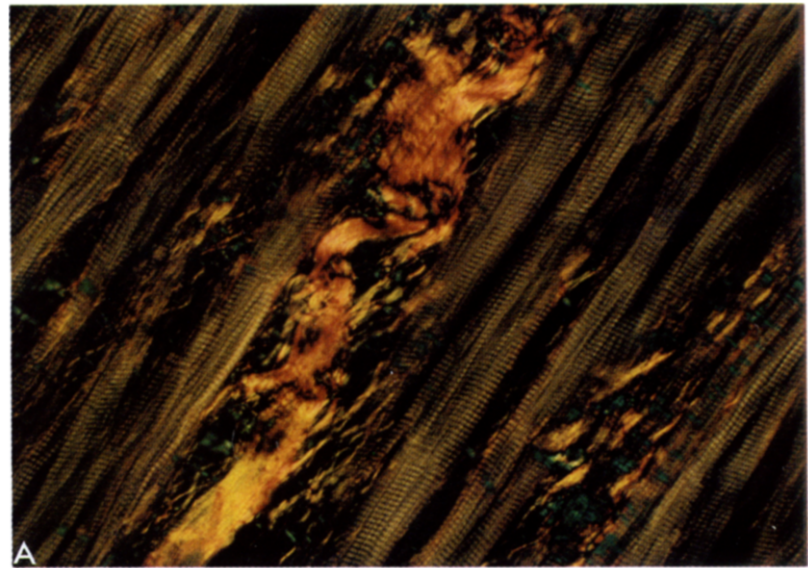


Figure 7. Picrosirius-polarization technique in the hypertrophied nonhuman primate myocardium. **Top panel,** Heavy perimysial tendons are found in a greater number of intermuscular spaces. Original magnification $\times 100$, reduced by 24%. **Middle panel,** A second pattern of fibrosis consists of an orthogonal grid of thick and thin collagen fibers that envelop muscle fibers. Note that thin (green) collagen fibers cross over muscle at nearly right angles and are entwined by thick (yellow) collagen fibers at right angles. Original magnification $\times 100$, reduced by 26%. **Lower panel,** Another orthogonal grid, 90° different from that seen in the middle panel, is seen in response to cell loss. Note that the thick (yellow) collagen fibers cross between muscle fibers at nearly right angles and are entwined by thin (green) collagen fibers at right angles. Original magnification $\times 100$, reduced by 25%.

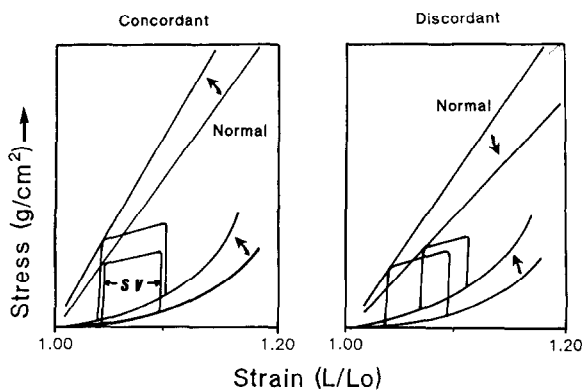


Figure 8. Systolic and diastolic stress-strain relations are shown for the normal myocardium and under conditions where systolic and diastolic stiffness are altered concordantly (left) or discordantly (right) in the hypertrophied ventricle as collagen concentration increases and its alignment with cardiac muscle becomes abnormal; see text for discussion. L/LO = end-diastolic fiber length normalized to its unstretched length at zero filling pressure; SV = stroke volume.

This grid (Fig. 7) begins to surround muscle fibers and will eventually envelop them.

We have been able to evoke this pattern of perimysial fibrosis in the rat using isoproterenol in small doses and have monitored the sequence of its formation over 8 days (43). The resultant orthogonal grid of collagen fibers resembles that seen with long-standing hypertension.

Late in established hypertrophy, cell loss is evident and accompanied by a reparative fibrosis. Here the fibrous tissue formation also resembled an orthogonal grid of thick and thin collagen fibers. In this case, however, the grid is 90° different from the reactive fibrosis described before (Fig. 7). The replacement scar consists of thick collagen fibers that run perpendicular to muscle fibers now adjacent to one another and into which thin collagen fibers are entwined, followed subsequently by thicker collagen fibers. In studying discrete regions of replacement fibrosis that accompanies coronary microsphere embolization, we (77) found that short, thick, taut collagen fibers, bridging the void left by cell loss, were arranged in series with viable muscle.

Functional consequences of collagen remodeling. In considering the impact of collagen accumulation and remodeling on myocardial stiffness, the systolic and diastolic stress-strain relations of the intact ventricle were examined (27,44,45,77). In the established phase of hypertrophy in the rat, when collagen volume fraction had risen significantly from normal (3% to 5%) to occupy 8% to 12% of the myocardium, systolic stiffness and diastolic stiffness at larger strains were each increased (Fig. 8). This concordant increase in stiffness could not be attributed to a conversion

of myosin isoforms (67). On the other hand, the increase in force generation, a useful adaptation in the pressure-overloaded myocardium, could be related to the increased dimension and number of interfascicular perimysial strands. These strands served to reduce the dissipation of myocyte-generated force and to preserve stroke volume. The increased dimension of the parallel elastic-like elements, represented by the perimysial weave and tendons, served to increase diastolic stiffness. Thus, with interstitial fibrosis, the increment in systolic and diastolic stress-strain relations would be expressed clinically as diastolic left ventricular dysfunction with a preservation of systolic function. This represents one form of pathologic hypertrophy.

For greater increments in collagen volume fraction, representing up to 20% or more of the hypertrophied myocardium, an increment in diastolic stiffness at all strains was evident. For this degree of fibrosis, invoked experimentally by isoproterenol, systolic stiffness declined (Fig. 8). This discordant response between diastolic and systolic stiffness was related to the appearance of a fibrous meshwork that surrounded myocytes and that served to retard length-dependent myocyte force generation. Thus, when interstitial fibrosis is combined with perimysial fibrosis, both diastolic and systolic left ventricular dysfunction will be evident. This is another form of pathologic hypertrophy.

The reparative fibrosis accompanying coronary microsphere embolization, characterized by the in series addition of collagen to muscle, led to an increment in diastolic stiffness at larger strains. This has been termed the fibrosis type of decreased myocardial distensibility (78). It represents yet another expression of pathologic hypertrophy. For the degree of cell loss obtained in this experimental model, systolic stiffness was sustained and even increased. We would expect, however, that when a large (e.g., 40%) portion of myocardium was lost, active stiffness would decline.

Collectively, these studies of collagen structure and myocardial stiffness emphasize that it is not solely the amount of myocardium occupied by collagen, but the structural characteristics and arrangement of collagen fibers with respect to myocytes that influence the mechanical behavior of the myocardium. Moreover, the structural remodeling of collagen can account for the appearance of pathologic hypertrophy with various expressions of diastolic and systolic left ventricular dysfunction.

Further evidence in support of the role of collagen in altering myocardial stiffness has been obtained from other studies. In the hypertrophy that accompanies thyroxine administration (79), myocardial collagen concentration and cardiac muscle stiffness are each unchanged from normal. The increase in myocardial collagen concentration and the expected increase in diastolic stiffness seen in aorta-banded

animals are not seen when rats are pretreated with a lathyrogen (80).

Ischemic Heart Disease

Cell loss. Reparative or replacement scarring of necrotic myocardium is a well recognized, long-term adaptation that maintains the structural integrity of the ventricle in the absence of myocytes, especially because muscle cells cannot regenerate. The early aspects of collagen matrix remodeling in response to cell loss was examined by Sato et al. (81), who followed the structural remodeling of the collagen matrix after 20, 40 and 120 min of coronary occlusion in the pig. Using scanning electron microscopy, they found a progressive disruption of collagen fibers appearing by 20 min in the subendocardium. Glycoproteins began to disappear by 40 min, whereas epimysial fibers and endomysial struts had disappeared at 2 h. In the absence of this restraint, sarcomere lengths in these regions of the myocardium increased to 3.6 μm , which is likely the result of dyskinetic wall motion. These findings again underscore the protective role of epimysial fibers in resisting deformation and sarcomeric overdistension. They also suggest the presence of collagenase activation with prolonged ischemia. The early disruption of collagen fibers in the infarct zone was not seen in irradiated animals made leukopenic before coronary artery ligation (82), suggesting that either inflammatory cell-released collagenase or mononuclear cell-fibroblast interaction (32) to produce collagenase may be contributory to collagen degradation in this setting.

The early inflammatory response and alterations in collagen metabolism to myocardial cell loss were monitored by Judd and Wexler (72,73) in the dog. Myocardial edema and enhanced glycosaminoglycan formation was observed for the first 5 days after cell loss and resolved thereafter. Cellular infiltration including fibroblasts was at its peak on day 3. Protocollagen proline hydroxylase activity increased on day 1, reaching a peak by day 3, whereas hydroxyproline concentration had increased significantly in necrotic regions by day 5 and continued to increase until day 12, after which it remained stable. The early increase in collagen synthesis before fibroblast proliferation suggests that collagen synthesis in existing fibroblasts was first to increase. Subsequently, a greater number of metabolically active fibroblasts contribute to enhanced collagen formation.

Over a 6 week period after myocardial infarction, the collagen concentration in the infarcted segment rises progressively to 5 times its normal value (83). In noninfarcted regions, collagen concentration is unchanged. The accumulation of collagen leads to a progressive contraction of the infarct zone. The ventricular chamber, on the other hand, can increase in size over this interval, particularly if the infarction is large. Pfeffer et al. (84) found a progressive enlargement in left ventricular chamber size and filling

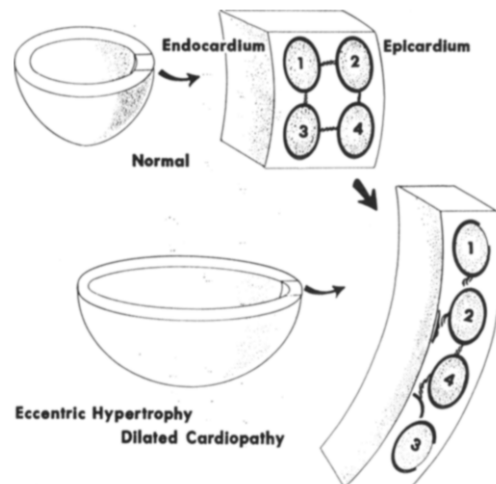


Figure 9. Negative muscle fiber slippage and wall thinning in dilated cardiopathy requires that collagen tethers be disrupted.

volume 1 year after an anterior myocardial infarction. The mechanism or mechanisms responsible for this progressive dilation remain speculative.

Stunned myocardium. "Stunned" myocardium is the term used to describe viable myocardium that has been salvaged by reperfusion, but in which there is transient, postischemic contractile dysfunction. Implicit in the term is the recovery of myocytes that are not irreversibly injured. Zhao et al. (11), using scanning and high voltage transmission electron microscopy, have examined the structural integrity of fibrillar collagen in a coronary artery occlusion-reperfusion model of stunned myocardium. Implantable sonomicrometer crystals were used to assess regional myocardial architecture and shortening. In this canine model of ischemia and reperfusion, the paradoxical expansion and thinning of the involved myocardium were accompanied by the degradation of collagen fibers and the loss of their structural integrity. Oxygen metabolites or free radicals, acting like a disulfide agent, a known activator of the latent collagenase-tissue inhibitor complex (32), may have caused collagen fiber breakdown. Caulfield et al. (85) recently showed that an infusion of the disulfide agent oxidized glutathione can cause collagen fiber disruption in the myocardium. Collagenase deactivation, by free radical scavengers having sulfhydryl groups, might accelerate the return in contractile function of the stunned myocardium. This area is deserving of study given that it could extend our pharmacologic concepts of cardioprotection and interventional cardiology. It is of further interest to note the occurrence of ventricular rupture soon after reperfusion with streptokinase (86) and that this may be prevented with beta-adrenergic blockade (87). Even though the current dictum "time is muscle" is pervasive, the salvage of myocytes with reperfusion techniques must be carried out within the context of protecting the entire myocardium, including its extracellular matrix.

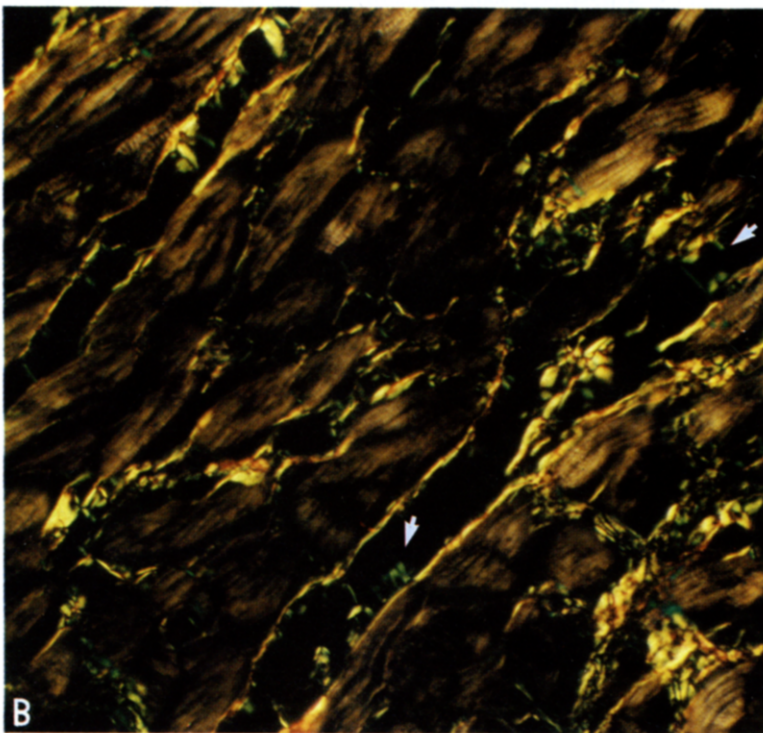
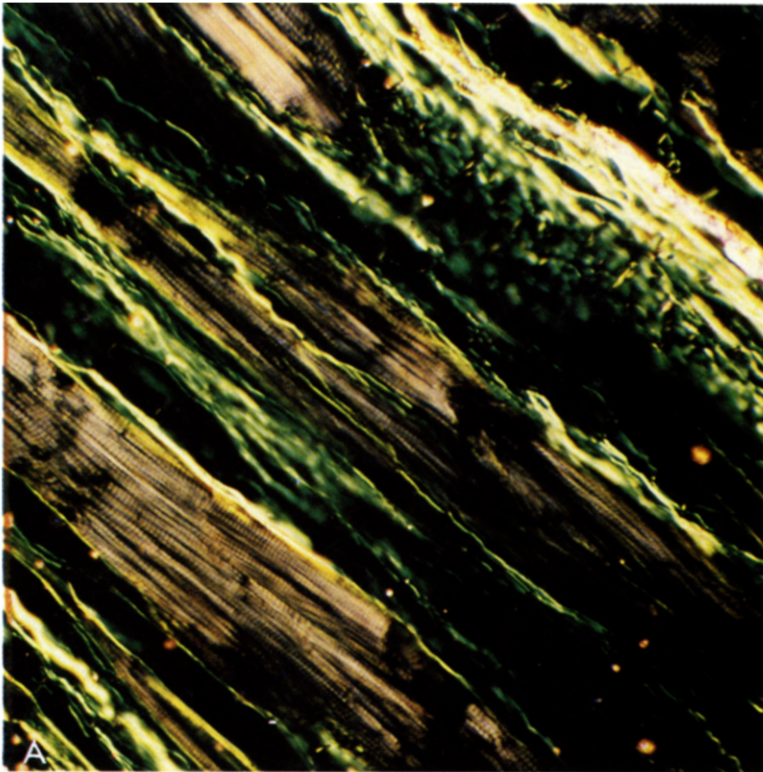


Figure 10. Human myocardium in dilated cardiopathy (stained with the picosirius red-polarization technique). **A**, An excess proportion of thin collagen fibers (green) is seen as well as fewer perimysial strands between muscle fibers. (Reproduced with permission from Weber et al. [12].) **B**, Perimysial strands are markedly reduced in number and disrupted (arrows), whereas intermuscular spaces are expanded as if edematous. Perimuscular collagen sheath is also disrupted. Compare with normal myocardium in Figure 4. Original magnification $\times 100$, reduced by 19%.

Heritable Diseases

Given the importance of collagen and elastin in maintaining structural integrity and resilience of the heart and vasculature, it is not unexpected that genetic disturbances in type I or type III collagen formation or elastin would be associated with structural defects of heart valves and blood vessels, respectively. Less well appreciated are the potential abnormalities of the collagen matrix that may involve the myocardium in these disorders. In this connection, the utility of pharmacologic agents that may prevent or retard blood vessel rupture or heart valve deformation have not been systemically examined.

Ehlers-Danlos and Marfan syndromes. Various subclasses of the Ehlers-Danlos syndrome, having autosomal dominant or recessive patterns of expression (88), have enhanced blood vessel fragility that may eventuate in rupture. The responsible biochemical defect is either unknown or proposed to be reduced synthesis of type III collagen that may compromise blood vessel resilience.

In Marfan's syndrome, aortic aneurysm and dissection and mitral valve prolapse are found. The nature of altered collagen biosynthesis or fiber formation is not entirely clear. Defects in cross-linking, the quality or quantity of the alpha chains in type I collagen, or elastin fibrillogenesis have each been proposed (see Ref. 22 and 88 for review).

Mitral valve prolapse. The results of studies that examined the relative proportions and synthesis of type I and III collagens have yielded conflicting results (for review see Ref. 22). An increase in type III collagen synthesis has been associated with redundant mitral valves, and may account for their poor tensile strength and increased resilience. In degenerative valve disease, a complete absence or decreased amount of type III collagen has been observed as is the case in patients with mitral valve prolapse and cerebral aneurysm. Given the prevalence of mitral valve prolapse, acquired and congenital defects in tissue collagen are likely to be found.

Idiopathic (dilated) Cardiopathy

The normal perimysial strands of the human myocardium are shown in Figure 4. It has been our contention that an absence or disruption of these muscle bundle to muscle bundle tethers would permit cardiac muscle fiber slippage, fiber realignment and wall thinning (Fig. 9). In addition, because its tensile strength would be reduced, the myocardium cannot resist deformation. As a result, the ventricles dilate and the left ventricular chamber becomes spherical as intracavitary stress becomes equal in all directions. In post-mortem human hearts, each of which had previously been shown (89) to have abnormal thinning and spherical configuration of the ventricle, we observed (12) the following (Fig. 10): 1) collagen tethers were markedly reduced in number;

perimysial strands either were present but disrupted, or were absent; and 2) an excess proportion of thin collagen fibers, perhaps representing type III collagen, was observed by the polarized light and picrosirius red technique. These findings have led us (12) to propose the term *cardiopathy*, and to reasonably expect that a spectrum of interstitial heart disease of diverse etiology will be found to account for some cases of primary myocardial disease.

Senescent Myocardium

Intermolecular cross-linking of collagen increases with age. This occurs in heart valves (90) and myocardium (91) and serves to retard collagen degradation by collagenase. Hence, there is an increase in the concentration of insoluble collagen. The collagen concentration of the senescent myocardium has been reported to be either increased (92-94), particularly in the subendocardium, or unchanged (95,96). In the senescent Fischer 344 rat (97) and Wistar rat (98), the accumulation of collagen within the subendocardium increases muscle stiffness. On the basis of the alignment of collagen and muscle, the accumulation of collagen can alter myocyte loading, as noted earlier for chronic left ventricular pressure overload. Encircled by fibrillar collagen, muscle may not be stretched and, therefore, myocyte work is reduced (45). Unloaded, myocytes become atrophic (99). Elsewhere in the myocardium and near the periphery of fibrous tissue, loading of myocytes may be increased, leading to their hypertrophy. This hypothesis remains to be rigorously examined, but may provide one explanation for the prevalence of left ventricular hypertrophy in the elderly (99). The mechanism responsible for endomyocardial fibrosis in the senescent myocardium is unclear. Specific abnormalities in fibroblast-mediated collagen synthesis or degradation, subendocardial perfusion or permeability of the endothelial surface of the endomyocardium need to be examined.

Future Directions

It is now appreciated that the myocardium contains a collagen matrix that is a major determinant of its architecture, structural integrity and mechanical properties. This fibrillar matrix consists primarily of type I and III collagens having epimysial, perimysial and endomysial components. The interrelations among the morphologic features of these components, their biochemical characteristics and biophysical properties and the function they serve, need to be further refined. The same holds true for the various patterns of myocardial fibrosis that have been observed in pressure overload hypertrophy. These structure-function correlations will serve to decipher abnormalities in diastolic and systolic stiffness, target collagenolytic therapy for a reactive fibrosis associated with impaired ventricular function and aid in our

understanding of disorders of collagen synthesis, type I or III collagen formation, fibrillogenesis and defective or excessive collagen degradation.

Future studies of collagen and collagenase gene regulation and collagen biosynthesis and degradation in the heart, together with the mechanisms operative in the regulation of cardiac fibroblast proliferation, are necessary elements to fully elucidate the role of the cardiac interstitium in health and disease. Interstitial heart disease is an entity that embraces primary or secondary abnormalities of the cardiac interstitium and its structural, immunologic and nutritional functions. For example, excess, abnormal or inadequate fibrillar collagen represent structurally based expressions of this hitherto unrecognized entity. The etiologic factors that lead to disorders of the collagen matrix, and which, in turn, lead to a compromise in the mechanical properties of the myocardium, require recognition and systematic investigation. These will be accomplished through the coordinated efforts of basic and applied scientists and the integration of advances from the molecular and cell biology laboratory into pathophysiologic and clinical expressions of cardiovascular disease. In so doing, it will be possible to enter the 21st century armed with corrective forms of therapy, targeted at the remodeled interstitium.

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