Review Article

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Connective tissue skeleton in the normal left ventricle and in hypertensive left ventricular hypertrophy and chronic chagasic myocarditis

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SUMMARY

Pictures certainly are worth a thousand words in the case of the structure of the connective tissue skeleton of normal and diseased myocardium. This report reviews the connective tissue matrix of the normal human myocardial tissue and the pathological myocardial fibrosis in left ventricular hypertrophy due to chronic arterial hypertension in humans and in human chronic chagasic myocarditis. The myocardial connective tissue matrix was studied employing a cell-maceration method that removes the myocardial tissue non-fibrous elements, and leaves behind a non-collapsed matrix, thus allowing a better three-dimensional view. Such information extends our knowledge of the expression of interstitial myocardial fibrous tissue in normal hearts and in hypertensive left ventricular hypertrophy and chronic chagasic myocarditis. The progressive accumulation of interstitial collagen fibers in both chronic cardiac diseases may be expected to decrease myocardial compliance and disrupt synchronous contractions of the ventricles during systole, contributing to a spectrum of ventricular dysfunction that involve either the diastolic or systolic phase of the cardiac cycle or both. In hypertensive heart disease myocardial fibrosis can be also implicated in the genesis of ventricular dysrhythmias, possible causes of sudden death among chronic hypertensive patients. Regarding chronic chagasic myocarditis, myocardial fibrosis is probably implicated in the genesis of malignant ventricular tachyarrhythmias (ventricular tachycardia and ventricular fibrillation), major causes of sudden death among patients with chronic Chagas' heart disease. The collagen distribution could interfere on the electrical properties of the myocardium. Fibrosis can block the cardiac impulse that may recycle (re-entry) through an alternative route and could slow conduction. In addition, the thick collagenous septa encompassing muscle fiber bundles could interfere with lateral impulse conduction, which would favor re-entry. Moreover, the methodology used is a useful tool to study the spatial organization of the collagen fibrils of the myocardium under normal and pathological conditions.

BACKGROUND

Pictures certainly are worth a thousand words in the case of the structure of the connective tissue skeleton of normal and diseased myocardium. Although useful biochemical and immunohistochemical investigation methods have been introduced, it is important to underline that morphology still has a central place in collagen network remodeling research. This report will review the connective tis-

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sue matrix of the normal human myocardial tissue and the pathological myocardial fibrosis in left ventricular hypertrophy due to chronic arterial hypertension in humans and in human chronic chagasic myocarditis. The myocardial connective tissue matrix was studied employing a cell-maceration method that removes the myocardial tissue non-fibrous elements, and leaves behind a non-collapsed matrix, thus allowing a better three-dimensional view [1–4].

THE NORMAL HEART

Control hearts, with an average weight of 280±40 g, from deceased individuals which cause of death was noncardiac were studied. The patients in the group had had no history of heart disease and the postmortem examination revealed their hearts to be without gross anatomic deformities of the valves, myocardium, and coronary vessels. The histologic examination of sections from the left ventricle free wall after staining with the acid dye Sirius red [5] showed thick (appearing yellow or yellow-red at polarization microscopy) and thin (appearing green at polarization microscopy) fibers of collagen, often colocalized, forming distinct perimysial sheaths which surrounded muscle fibers so as to group them together into bundles, and around intramyocardial coronary vessels. A fine endomysial sheath could be identified within the muscle bundles. The perimysial fibers were composed of strands orientated perpendicular to the long axis of the muscle (lateral connections), and of broad wavy longitudinal fibers (Fig. 1A-C). These findings were in keeping with those reported in the literature [6]. Moreover, the minor diameter of myocytes was measured in properly oriented cross-sections in each 200 fibers from each heart in hematoxylin-eosin-stained sections. The diameter of subendocardial fibers was not determinate since degenerative changes predominate in the subendocardial layers because oxygen demand and availability. Another method for the quantitative examination of the left ventricular myocardium was carried out at medium-power light microscopic fields (x250): a 100-point ocular Integration eyepiece II (Carl Zeiss) was used to estimate the volume fraction (%) of fibrosis in picrosirius-red-stained sections. Twenty (20) fields of subepicardial and midmyocardial zones were analyzed for each heart. The mean minor transverse diameter of the myocytes was 13.7±7.8 μm and the volume fraction fibrosis was 6.5±1.5% (mean(standard deviation).

The three-dimensional configuration of cardiac collagen has been determined by scanning electron microscopy [7–9]: the epimysium envelopes the entire cardiac muscle, the perimysium, which is an extension of the epimysium, serves to enwrap groups of myocytes, and the endomysium, as final arborization of the perimysium, supports and connects individual cells. The endomysial weave envelops each individual myocyte and is connected to adjacent myocytes by lateral struts.

Because this knowledge has been obtained through studies on whole fixed myocardial tissue without removal of its non-fibrous elements, a cell-maceration method was used to evaluate the myocardial connective matrix following removal of the myocardial tissue non-fibrous elements. This method allows a good demonstration of the three-dimensional architecture of the collagen fibers of the myocardium, and is not affected by the storage procedure [10]. Briefly, the fragments of myocardial tissue are thoroughly rinsed in distilled water, immersed in 2.5% phosphate buffered glutaraldehyde, rinsed in distilled water, and immersed in a 10% NaOH solution for maceration and removal of the cellular elements for 6–10 days at room temperature. After, they are rinsed in distilled water until they become transparent. Then, they are immersed in 1% tannic acid for 4 hours. Subsequently, the specimens are rinsed in distilled water overnight, post-fixed in 1% osmium tetroxide, rinsed, dehydrated in graded concentrations of ethanol, sectioned transversally in relation to the orientation of the myofibers with a very sharp and clean blade under dissecting microscope, and critical-point dried in liquid carbon dioxide. They are then glued to aluminum stubs, sputter-coated with gold and examined in a scanning electron microscope.

The organization of the connective tissue skeleton of the human control left ventricular myocardium sectioned transversally is quite similar to a honeycomb. The perimysium envelops groups of myocytes. The endomysium, as final arborization of the perimysium, supports and connects individual cells. The endomysial weave envelops each individual myofiber and is connected to adjacent myocytes by lateral struts presenting branches of variable size and extension. The range of the length and diameter of these struts is very wide. Collagen struts also connect myocytes to interstitial microvessels and perimysial collagen fibrils (Fig. 1D-G).

The stroma of the heart maintains the structure of the myocardium, determining tissue tensile, and

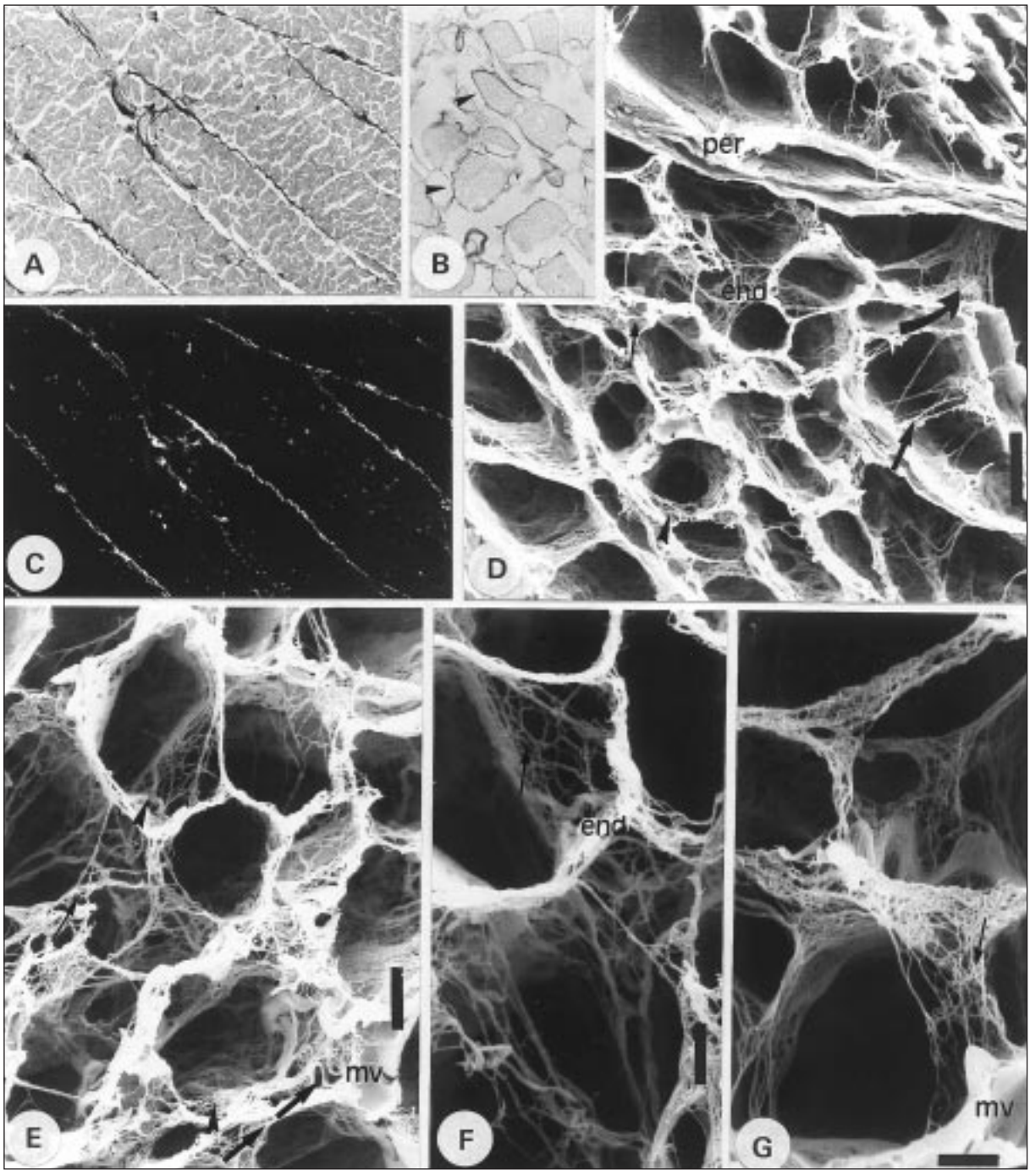

Figure 1. A, B, and C. Control myocardium weighing 280±40 g. Light microscopy: cross section. Collagen fibers are seen in a distinct perimysial sheath (thin arrows) surrounding muscle fibers grouped together into fiber bundles. A fine endomysial sheath (arrowheads) can be seen around individual fibers (C). Picrosirius red stain, A and C, direct light (collagen fibers appear black) and B, polarization microscopy (A and C, x58; B, x185). D, E, F and G. Scanning electron microscopy. The perimysium (per) envelops groups of myocytes. The endomysium (end, arrowheads) supports and connects individual cells. The endomysial weave is connected to adjacent myocytes by lateral struts (thin arrows) presenting branches of variable size and extension. Collagen struts connect myocytes to interstitial microvessels (thick arrows) and to the perimysial sheath (curved arrows) (D, bar = 20 μm; E, bar = 10 μm; F, bar = 10 μm; G, bar = 5 μm). (Panels C, D, E, and F are reprinted with permission from Rossi MA: Pathologic fibrosis and connective tissue matrix in left ventricular hypertrophy due to chronic arterial hypertension in humans, [3] Vol. 16, pp 1031-41. Lippincott Williams & Wilkins, 1998).

strength and stiffness [11]. Besides, it contributes to ventricular function through the transmission of myocyte-generated force to the atrial and ventricular chambers and to the relengthening of myocytes in diastole [12].

CONNECTIVE TISSUE MATRIX IN HYPERTROPHIED LEFT VENTRICLE

Hearts from deceased individuals with varying heart weights (hearts with mild hypertrophy, weighing 440±50 g, hearts with moderate hypertrophy, weighing 560±50 g, and hearts with severe heart hypertrophy, weighing 680±60 g) and a history of systemic hypertension were examined. None of the hearts had any evidence of cardiac disease, such as ischemia or valvular deformities. The myocytes and muscle fibers in hearts from all patients with a history of hypertension were significantly larger than those found in control patients with no history of hypertension. The minor transverse diameter of the myocytes affected by hypertension were 23.7±3.4 μm, 26.6±3.5 μm, and 32.8±5.8 μm in mild, moderate, and severely hypertrophied hearts, respectively, among patients with a history of hypertension. The volume fraction of fibrosis was respectively 15.4±3.1%, 22.9±6.9%, and 31.1±10.5% in hearts affected by hypertension as compared with 6.5% in normal hearts.

Interstitial and diffuse fibrosis could be observed in the hypertrophied hearts to a variable degree, but were present in all cases. Under polarization microscopy, picrosirius red-stained sections from mildly hypertrophied myocardium (Fig. 2A, B) showed a diffuse fibrosis characterized by thicker perimysial and endomysial collagen fibers as compared to controls. The moderately (Fig. 3A, B) and severely (Fig. 4A, B) hypertrophied hearts showed a diffuse marked increase in both endomysial and perimysial collagen fibers associated with scattered dense, sometimes stellate scars replacing small areas of myocyte dropout. These interstitial changes were more pronounced in the severely hypertrophied myocardium. An abnormal accumulation of collagen around intramyocardial coronary arteries was also evident. The muscle fibers appeared hypertrophic.

Under scanning electron microscopy, the most striking feature was the diffuse marked increase of pericellular collagen weave fibers (endomysial matrix), parallel to the increase of heart weight. The hypertrophied myocytes were encased in a dense weave of collagen fibrils continuous with those of adjacent myocytes. The lateral struts that usually tether one myocyte to others or the myocyte bundles to the perimysial collagen fibrils were obscured, immersed in the weave increase or, sometimes, markedly thick. The dimension of muscle fibers was markedly increased in hypertrophied hearts, although extremely variable from an area to another. Besides, a diffuse increase in the number of thick collagen fibers constituting broad bands and sheets of collagen surrounding disorganized muscle bundles (perimysial matrix) was observed. Scattered dense scar-like foci, apparently replacing areas of myocyte dropout, could be seen, mainly in the periphery of muscle bundles. This later finding was more commonly observed in moderately hypertrophied hearts, and, principally, in severely hypertrophied hearts. Importantly, a progressive disarray of the connective tissue skeleton of the myocardium could be seen in parallel to the progressive increase of cardiac hypertrophy (Figs. 2C-E, 3C-E, and 4C-E).

It appears obvious that as myocardial cells increase in size and mass, the structural integrity of the myocardium should be maintained by means of proportional increase in stromal connective tissue [13,14]. However, the balance between parenchyma and stroma depends on general factors such as blood flow and endocrine secretions [13]. The exact mechanism that promotes the accumulation of collagen in the interstitial compartment is still controversial. Two distinct phases have been distinguished in the development of myocardial fibrosis in experimental hypertensive heart disease [15]. First, there is a reactive accumulation of connective tissue in the interstitial space by de novo synthesis of collagen; the fibrogenesis response appears in the absence of parenchymal loss. Second, there is reparative (replacement) fibrosis or scarring which is an adaptation to the loss of parenchymal cells. The accumulation of collagen fibrils in the interstitial space has been attributed, in part, to growth factors produced and localized in response to mechanical load. Potential stimulators of collagen biosynthesis include locally produced agents, such as platelet-derived growth factor (PDGF) or transforming growth factor-β (TGF-β), or factors originated from the circulation or vascular wall, such as hormones of the renin-angiotensin-aldosterone system, angiotensin II and aldosterone, or endothelin [16]. The occurrence of myocardial cell necrosis in the hypertrophic heart was initially ascribed to decreased availability of oxygen in the center of muscle fibers with increased cross-sectional diameter [17]. Recent observations suggest that

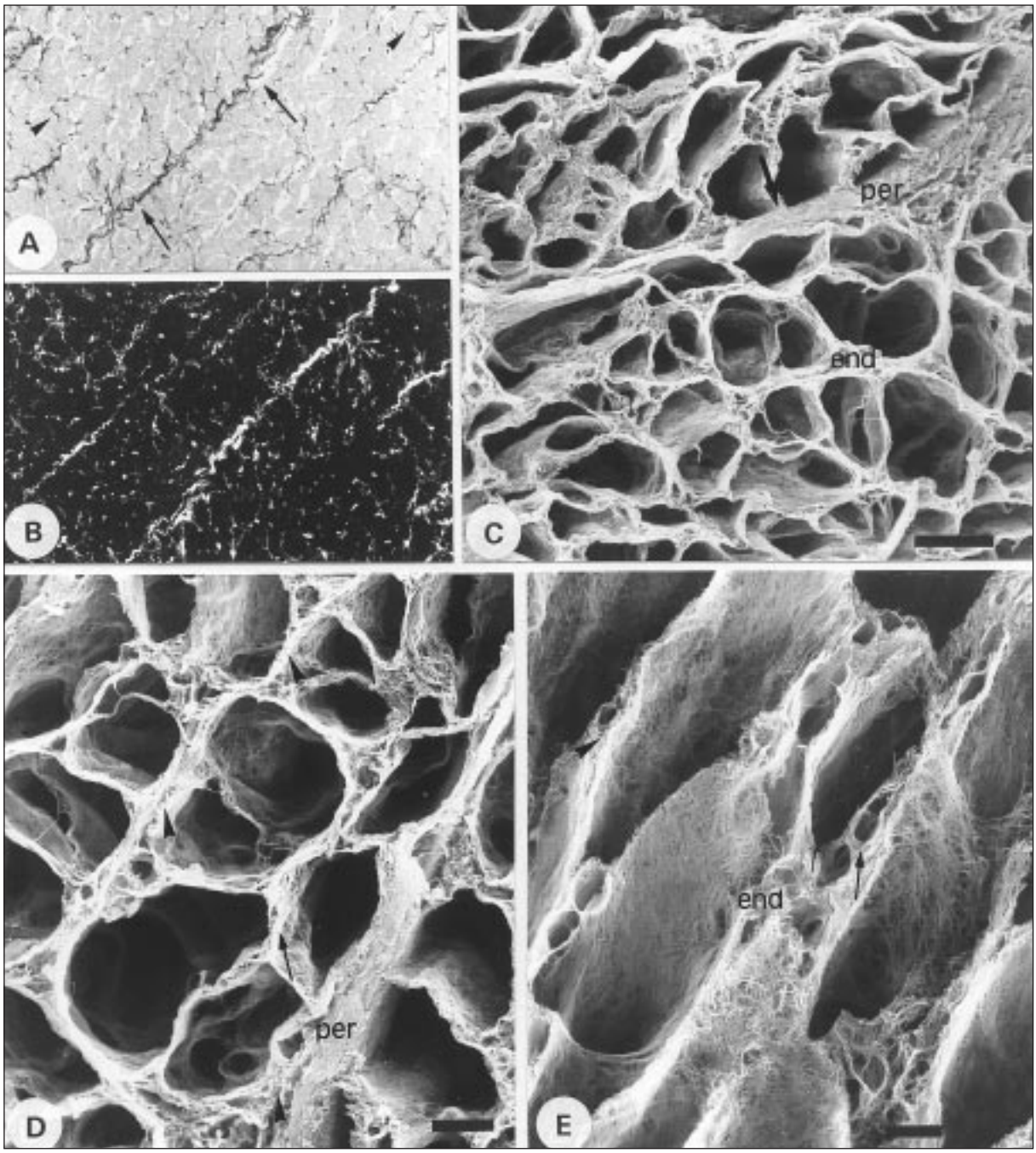

Figure 2. A, B. Hypertrophied myocardium weighing 440±50 g. Light microscopy: cross section. Diffuse fibrosis characterized by thicker perimysial (thin arrows) and endomysial (arrowheads) collagen fibers as compared to control can be clearly seen. Picrosirius red stain, direct light (collagen fibers appear black) and polarization microscopy (x58). C, D, and E. Scanning electron microscopy. A diffuse increase of pericellular collagen fibers (end, endomysial matrix) associated with broad bands of collagen fibers surrounding muscle fiber bundles (per, perimysial matrix) is present. The individual weave fibers are mostly directly continuous with those of adjacent cells (arrowheads). The lateral struts connecting one myocyte to another (thin arrows) or the myocyte bundles to the perimysial sheath (thick arrows) are obscured. These aspects can be clearly seen in obliquely sectioned myocardium (E). The cross diameters of the myocytes are increased (C, bar = 20 μm; D, bar = 20 μm; E, bar = 10 μm). (Panels C, D, and E are reprinted with permission from Rossi MA: Pathologic fibrosis and connective tissue matrix in left ventricular hypertrophy due to chronic arterial hypertension in humans, [3] Vol. 16, pp 1031-41. Lippincott Williams & Wilkins, 1998)

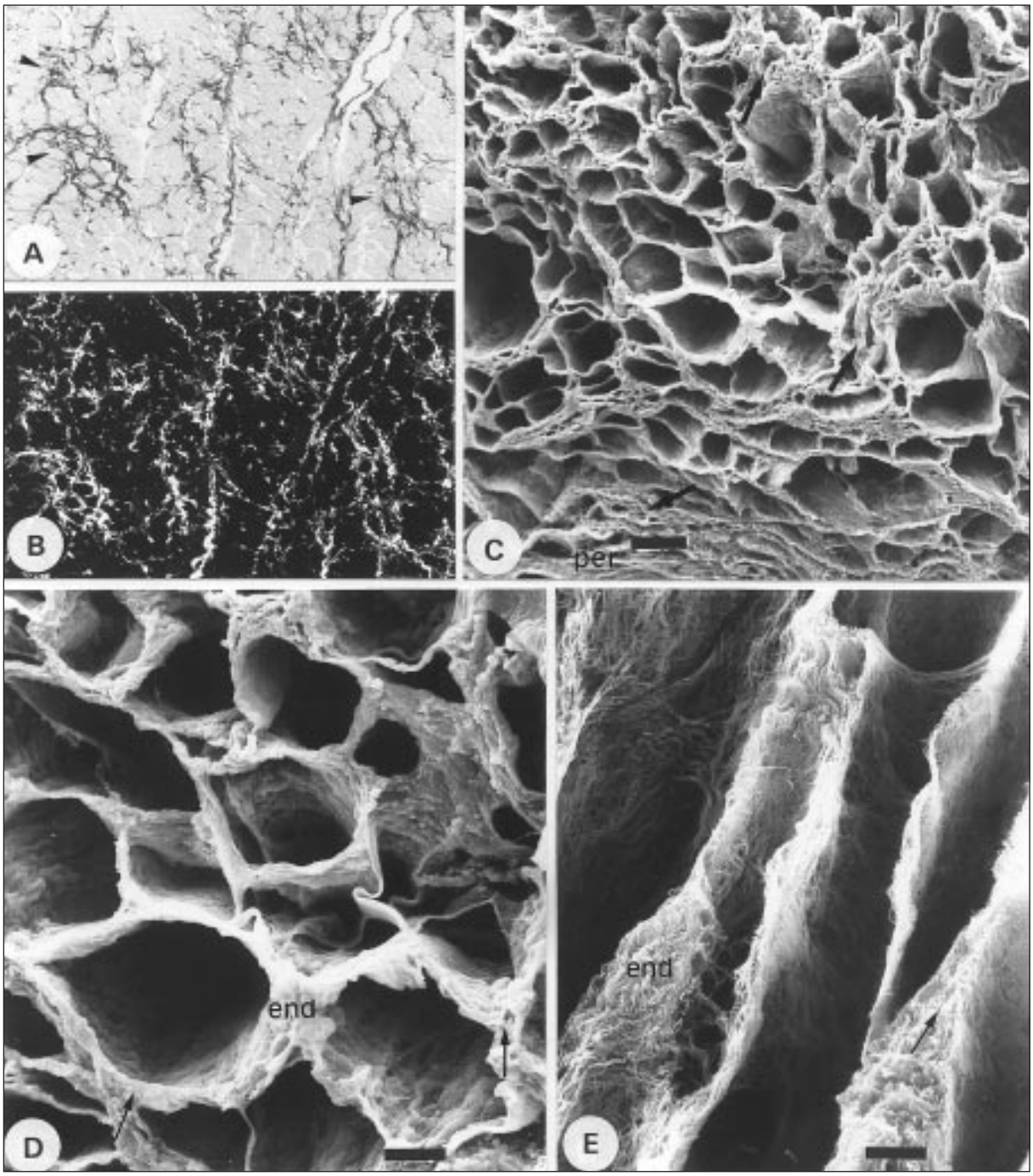

Figure 3. A, B. Hypertrophied myocardium weighing 560±50 g. Light microscopy: cross section. Diffuse increase in both endomysial and perimysial collagen fibers associated with scattered scars replacing areas of myocytes dropout (arrowheads) (x58). C, D, and E. Scanning electron microscopy. Hypertrophied myocytes with variable diameters are encased in a dense endomysial sheath (end) that completely obscures the lateral struts (thin arrows). Thick lateral connections tethering one myocyte to another can be seen in longitudinal sections (E, arrowheads). Scattered dense scar-like foci are present, mainly in the periphery of the muscle bundles (thick arrows) (C, bar = 20 μm; D, bar = 10 μm; E, bar = 10 μm). (Panels C, D, and E are reprinted with permission from Rossi MA: Pathologic fibrosis and connective tissue matrix in left ventricular hypertrophy due to chronic arterial hypertension in humans, [3] Vol. 16, pp 1031-41. Lippincott Williams & Wilkins, 1998).

left ventricle regions that manifest severe interstitial fibrosis may be subjected to chronic hypoxia due to reduced capillary density and increased oxygen diffusion distance [18,19]. The capillary-to-myocyte oxygen diffusion depends primarily on the expansion of the interstitial space due to deposi-

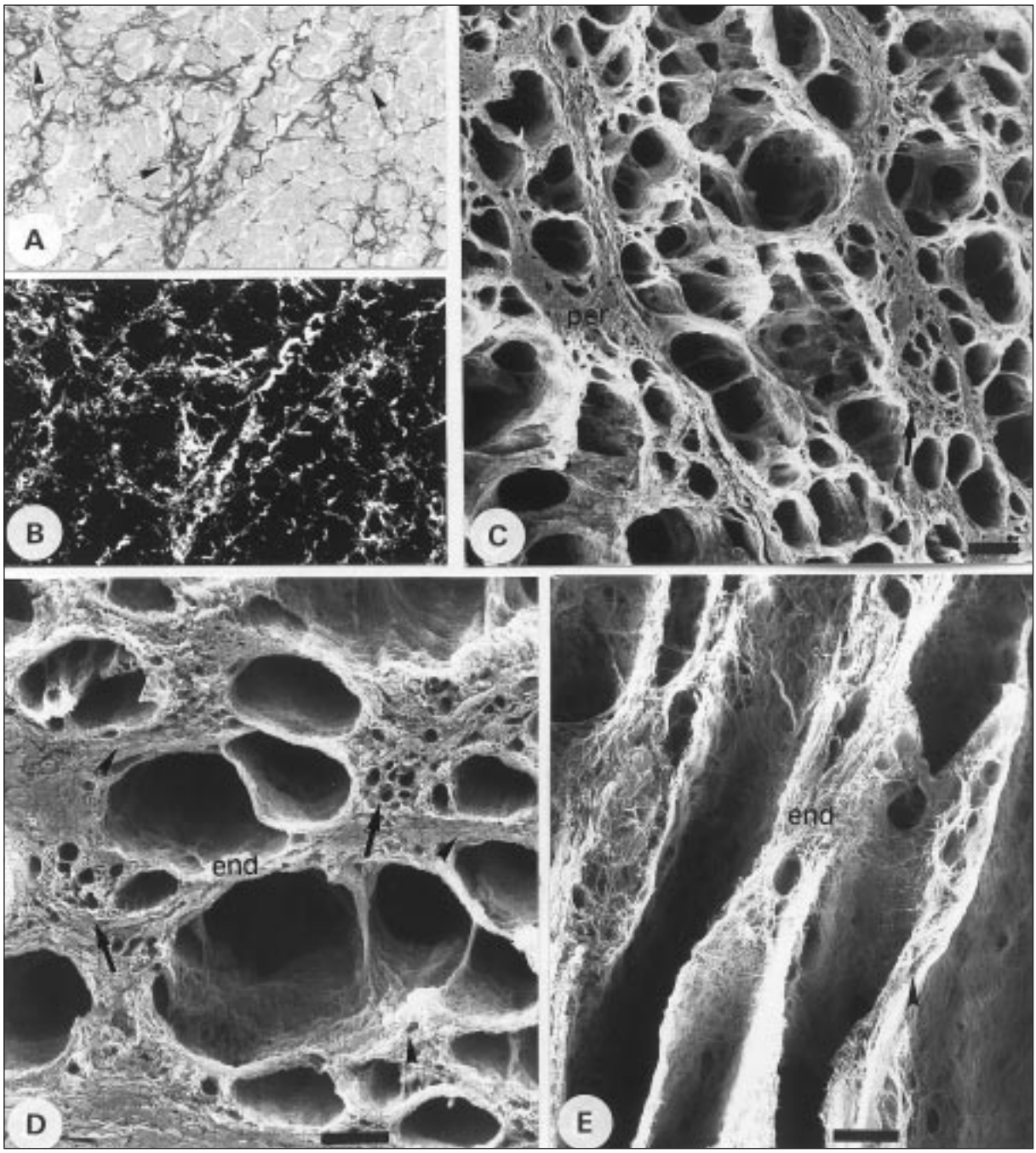

Figure 4. A, B. Hypertrophied myocardium weighing 680±60 g. Light microscopy: cross section. Diffuse marked increase in both endomysial and perimysial collagen fibers associated with dense scars replacing areas of myocytes dropout (arrowheads) (x58). C, D, and E. Scanning electron microscopy. The diameters of myocytes from severely hypertrophied myocardium are markedly variable. The myocytes are encased in a very dense sheath of endomysial collagen fibers (end, arrowheads) completely obscuring the lateral struts. Besides, a diffuse increase in the number of broad bands and struts of collagen surrounding disorganized muscle bundles (per, perimysial matrix) can be observed. Scattered dense scar-like foci replacing areas of myocytes dropout or extreme atrophy is seen (thick arrows). These aspects can be clearly seen in longitudinal section (E). (C, bar = 20 μm; D, bar = 20 μm; E, bar = 10 μm). (Panels C, D, and E are reprinted with permission from Rossi MA: Pathologic fibrosis and connective tissue matrix in left ventricular hypertrophy due to chronic arterial hypertension in humans, [3] Vol. 16, pp 1031-41. Lippincott Williams & Wilkins, 1998).

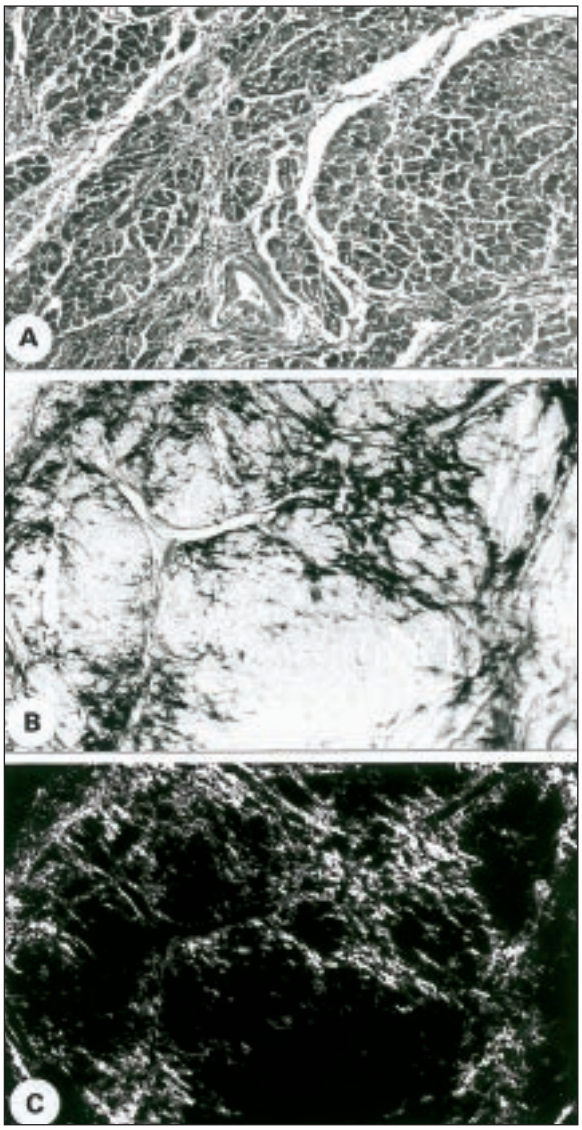

Figure 5. A. Chronic fibrosing myocarditis. Predominant perimysial and perivascular fibrosis combined with less pronounced endomysial fibrosis. (azan-Heidenhain, x80). B and C. Predominant perimysial fibrosis combined with less pronounced increase in the thickness of endomysial collagen fibers. (Picrosirius red stain, direct and polarization microscopy, x80).

tion of fibrillar collagen and secondarily on the increase in cross-sectional myocyte size. Thus, chronic hypoxia would adversely affect the function and viability of the collagen encircled myocytes. Our findings showing scar-like foci more frequently in association with moderate and severely hypertrophied hearts give support to the concept that interstitial fibrosis can lead to hypoxia of the collagen encircled myocytes. On the other hand, angiotensin II originating from exogenous or endogenous sources has been demonstrated to induce myocyte degeneration and necrosis and subsequent reparative fibrosis [20]. Recent investigation clearly demonstrated that the majority of angiotensin II-induced damage is caused by the local release of sympathetic stores of catecholamines [21]. Previous study from our laboratory had hypothesized that the degenerative and necrotic changes of myocytes in cardiac hypertrophy in iron deficiency anemia are caused by sympathetic overstimulation consequent to increased release of noradrenaline into the myocardium [14,22]. Besides, aldosterone has been shown to induce microscopic scarring secondary to myocytes necrosis [23]. Mineralocorticoid excess appears to induce myocyte injury through a decrease in intracellular potassium associated with urinary potassium excretion [24,25]. It has to be stressed, however, that the aldosterone-related myocardial damage has been demonstrated only in an animal model where one kidney was removed and the animal fed on a high sodium diet; it has not been shown that elevated levels of aldosterone by itself in animals with both kidneys functional and normal sodium intake can cause damage and fibrosis to the myocardium.

CONNECTIVE TISSUE MATRIX IN CHRONIC CHAGASIC MYOCARDITIS

Cases of Chagas' heart disease weighing between 420 and 655 g were selected. The cause of death was cardiac in all cases: sudden cardiac death, congestive heart failure or malignant arrhythmia. The diagnosis was based on previously established criteria: macroscopic features characterized by cardiomegaly with or without apical aneurysm, rosary-bead type epicarditis, dilatation of the subpulmonary infundibulum and microscopically by chronic fibrosing myocarditis and clinical and laboratory findings (positive complement-fixation and/or immunofluorescence tests for Chagas' disease done before death or in serous fluids at autopsy). Considering that the intensity of the inflammatory reaction varies from one case to another, we previously established to study exclusively cases with moderate to severe chronic myocarditis.

The histologic examination of the myocardium showed a chronic fibrosing myocarditis characterized by diffuse foci of myocardial cell loss and degeneration with an inflammatory response composed predominantly of mononuclear cells, and striking interstitial fibrosis. Myofibers containing parasites were very rarely detected. Diffuse interstitial fibrosis, as previously reported [26], was manifest by the finding of a diffuse increase in the thickness

of collagen fibers which surrounded the bundles of muscle fibers (the matrix of perymisial collagen), varying in intensity from one area to another, and around the intramural coronary vessels, combined with a less pronounced increase in the thickness of collagen fibers which surrounded individual myocytes (the matrix of endomysial collagen). Scattered dense stellate scars, replacing areas of 'dropout' of myocytes, could also be seen. The volume fraction of fibrosis of chagasic hearts (30.7±8.8%) was markedly increased in comparison to that found in control hearts (see above). The areas of fibrosis were associated with marked infiltrates of inflammatory cells consisting of lymphomononuclear cells (predominantly macrophages and a few lymphocytes), together with myocytes showing degenerative changes. Most of the cells within the bundles of muscle fibers appeared hypertrophied. The mean minor diameter of myocytes of the chagasic hearts was 23.25±6.28 μm as compared to controls (see above) (Fig. 5A-C).

The scanning electron microscopic study revealed that interstitial and diffuse fibrosis was present to variable but in all chagasic hearts. The most striking feature was a diffuse increase of thick collagen fibers constituting broad bands and sheaths of collagen surrounding disorganized muscle bundles (perimysial matrix). Tendon-like collagen fibers increased in number and became much thicker than those seen in normal hearts. The perimysial collagen bundles had a wavy appearance, most of them oriented parallel to the long axes of the myocytes. Thick collagen strands interconnected the endomysial collagen fibers in the perimysial collagen strands. This was associated with a less marked increase of the pericellular collagen meshwork (endomysial matrix), mainly in the periphery of the muscle bundles, adjacent to the perimysium and markedly variable from an area to another. The myocytes were encased in a dense weave of collagen fibrils continuous with those of adjacent myocytes. The thickness of the meshwork of collagen fibrils was extremely variable from one cell to another, but the thickest endomysial weaves were the nearest to the perimysial collagen strands. The arrangement of collagen fibrils of the endomysium had a complicated criss-cross pattern. The endomysial weave fibers are continuous with those of adjacent myocytes, obscuring the lateral connections. The dimension of the muscle fibers was markedly increased in chagasic heart, although extremely variable. Diffuse scar-like foci corresponding to areas of myocyte 'dropout', could be also seen. This disorganization of the connective tissue skeleton of the myocardium could be seen directly related to the intensity of the myocarditis and not in parallel to increase of heart weight. In addition, a remarkably developed perimysial and predominantly endomysial capillary network could be observed (Figs. 6 and 7).

The pattern of myocardial fibrosis in chronic Chagas' heart disease probably reflects the pathogenic mechanisms involved. Diffuse foci of myocardial myonecrosis may be the main etiology factor of the chronic expression in chronic chagasic myocarditis [27,28]. Many controversies characterize theories concerning the pathogenesis of chronic chagasic cardiomyopathy. One theory attributes an autoimmune basis to the chronic myocarditis of Chagas' disease [29–32], whereas another implicates a primary role for an impairment of the intrinsic and extrinsic cardiac autonomic nervous system [33,34]. A possible relationship between the presence of parasite antigens and the intensity of the inflammatory infiltrate has been observed during the chronic phase of the infection in both mice and man [35,36], suggesting therefore that the parasite may have a role in promoting a persistent antigenic stimulation throughout the chronic phase. However, more recently, clinical and experimental studies suggests that the consequences of infection converges on the microcirculation resulting in progressive and addictive focal cellular necrosis with associated inflammatory lymphomononuclear infiltrate, reactive and reparative fibrosis, and surrounding myocyte hypertrophy [32,37–42]. The present findings showing a well-developed capillary network in chagasic hearts is probable cause of slow blood capillary flux, which could contribute to the hypoxic changes in chronic chagasic myocarditis [43].

CONCLUDING REMARKS

Such information extends knowledge of the expression of interstitial myocardial fibrous tissue in normal hearts and in hypertensive left ventricular hypertrophy and chronic chagasic myocarditis.

The progressive accumulation of interstitial collagen fibers in both chronic cardiac diseases may be expected to decrease myocardial compliance and disrupt synchronous contractions of the ventricles during systole, contributing to a spectrum of ventricular dysfunction that involve either the diastolic or systolic phase of the cardiac cycle or both. In hypertensive heart disease myocardial fibrosis can be also implicated in the genesis of ventricular dysrhy-

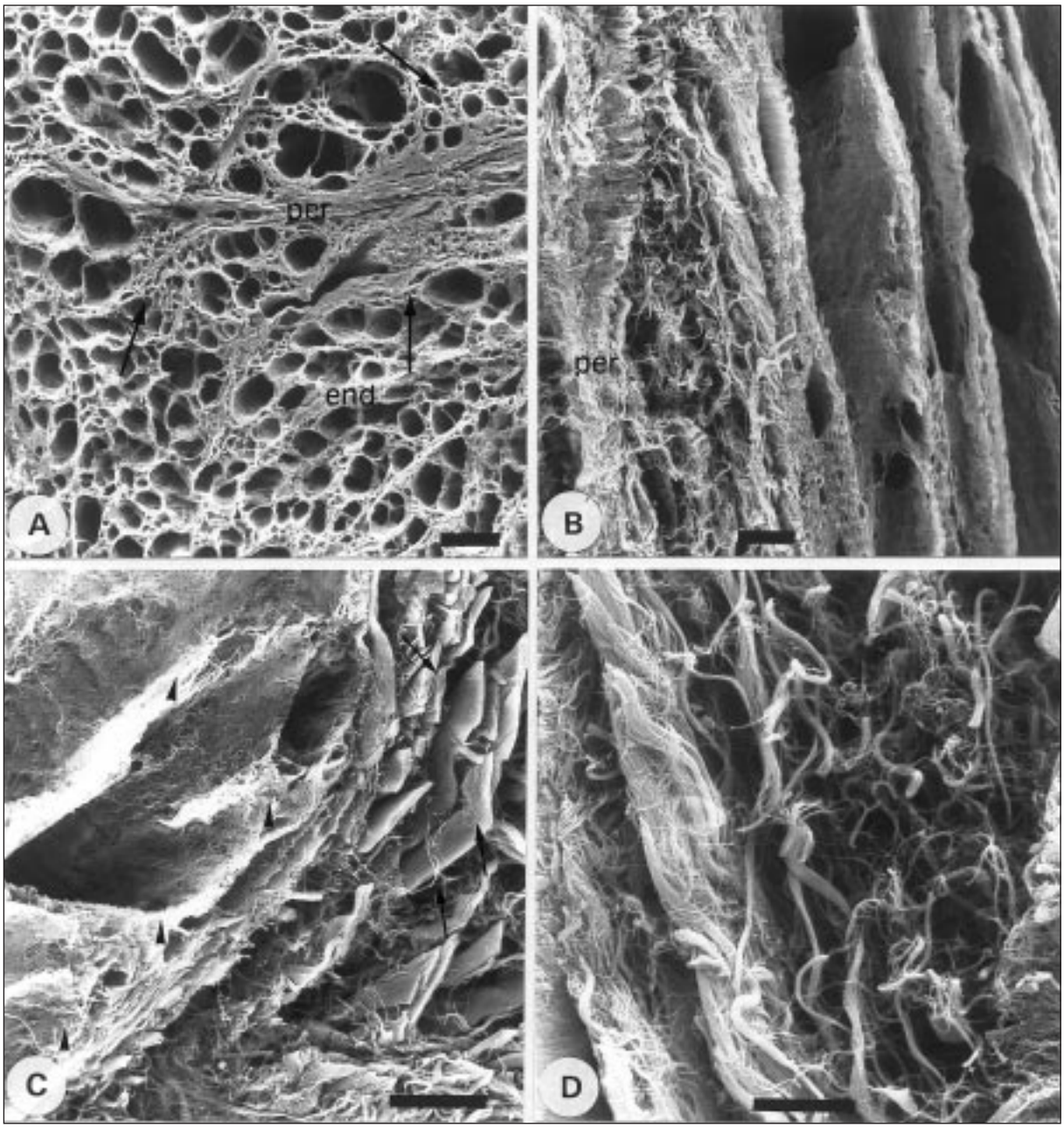

Figure 6. Scanning electron microscopy of chronic chagasic myocarditis. A. Broad bands of perimysial collagen fibers (p) surround muscle fiber bundles. The increase of pericellular collagen matrix (endomysial matrix, e) occurs mainly in the periphery of the muscle bundles, adjacent to the perimysium (arrows) (Bar = 50 μm); B. Longitudinally-oriented section showing thick perimysial collagen bundles (p) with a wavy appearance, most of them oriented parallel to the long axes of the myocytes (Bar = 20 μm); C. Obliquely-sectioned myocardium showing tendon-like perimysial collagen fibers (arrows) and dense pericellular collagen meshworks (arrow heads) (Bar = 10 μm); D. Very thick coiled perimysial fibers (Bar = 10 μm). (Reprinted with permission from Rossi MA: Fibrosis and inflammatory cells in human chronic chagasic myocarditis: scanning electron microscopy and immunohistochemical observations, [4] Vol. 66, pp 183-94. Elsevier Science, 1998.)

thmias, possible causes of sudden death among chronic hypertensive patients. Regarding chronic chagasic myocarditis, myocardial fibrosis is probably implicated in the genesis of malignant ventricular tachyarrhythmias (ventricular tachycardia and ventricular fibrillation), major causes of sudden death among patients with chronic Chagas' heart disease. The collagen distribution could interfere on the electrical properties of the myocardium. Fibrosis can block the cardiac impulse that may recycle (re-entry) through an alternative route and could slow conduction. In addition, the thick collagenous

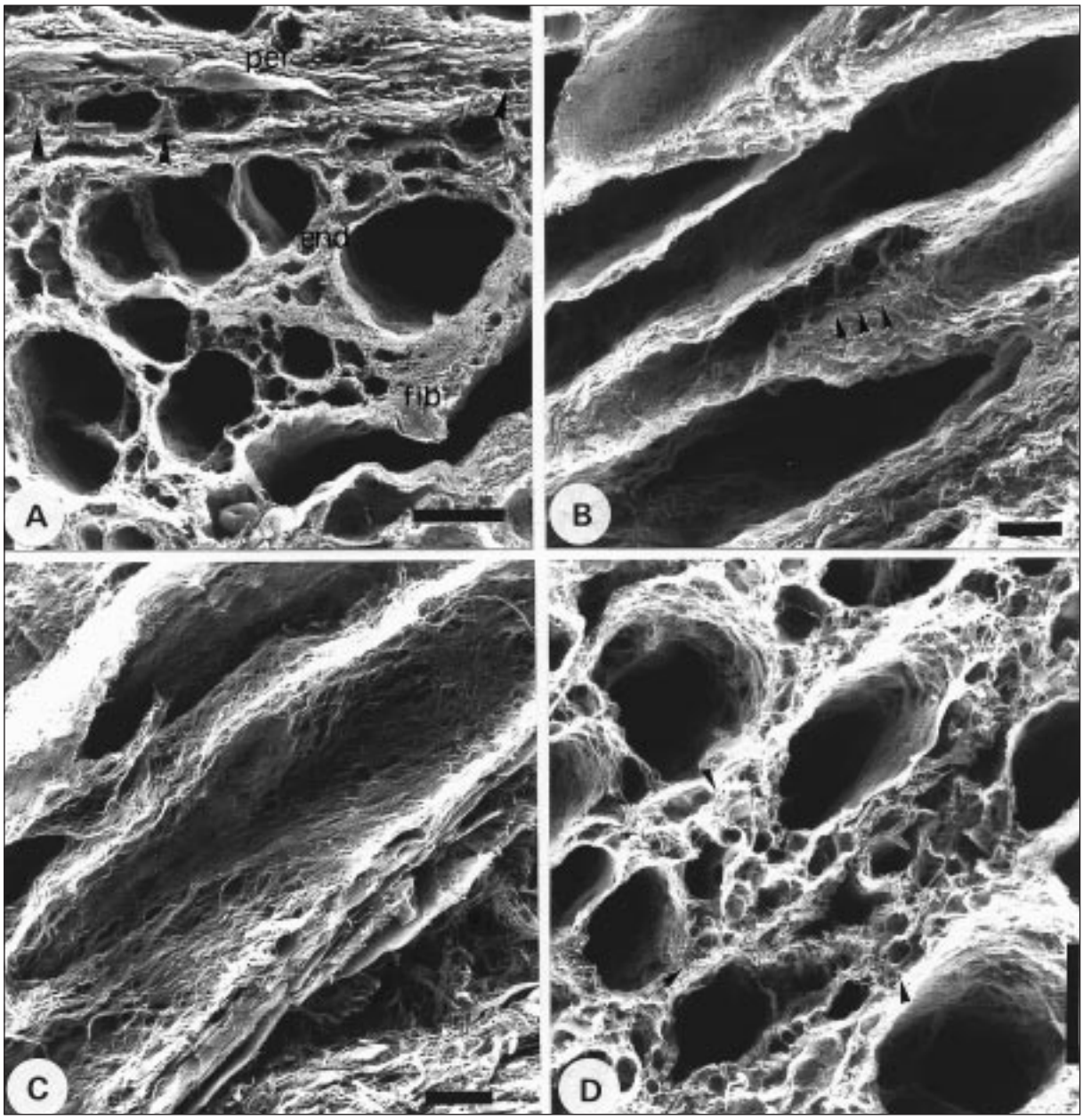

Figure 7. Scanning electron microscopy of chronic chagasic myocarditis. A. Thickened perimysial sheath (p). The adjacent myocytes are encased in a dense weave of collagen fibers (e). Perivascular fibrosis (v). The endomysial weave fibers are continuous with those of adjacent myocytes, obscuring the lateral struts. Broad bands and struts of collagen connect muscle bundles to the perimysial sheath (arrow heads) (Bar = 20 μm); B. Longitudinal section showing the thick lateral connections tethering one myocyte to another (arrow heads) (Bar = 10 μm); C. Arrangement of the collage fibrils showing a complicated criss-cross pattern (Bar = 10 μm); D. Thickened endomysial weaves continuous to those of adjacent myocytes. Remarkably developed endomysial capillary network can be seen (arrows) (Bar = 20 μm). (Reprinted with permission from Rossi MA: Fibrosis and inflammatory cells in human chronic chagasic myocarditis: scanning electron microscopy and immunohistochemical observations, [4] Vol. 66, pp 183-94. Elsevier Science, 1998.)

septa encompassing muscle fiber bundles could interfere with lateral impulse conduction, which would favor re-entry. This phenomenon was demonstrated in senescent hearts as compared to young hearts [44].

REFERENCES

1. *Ohtani O: Three-dimensional organization of the connective tissue fibers of the human pancreas: a scanning electron microscopic study of NaOH treated tissue. Arch Histol Jpn, 1987; 50: 557-566*

2. *Rossi MA, Abreu MA, Santoro LB: Connective tissue skeleton of the human heart: a demonstration by cell-maceration scanning electron microscope method. Circulation, 1998; 97: 934-935*
3. *Rossi MA: Pathologic fibrosis and connective tissue matrix in left ventricular hypertrophy due to chronic arterial hypertension in humans. J Hypertens, 1998; 16: 1031-1041*
4. *Rossi MA: Fibrosis and inflammatory cells in human chronic chagasic myocarditis: scanning electron microscopy and immunohistochemical observations. Int J Cardiol, 1998; 66: 183-194*
5. *Weber KT, Brilla CG, Janicki JS: Myocardial fibrosis: functional significance and regulatory factors. Cardiovasc Re, 1993; 27: 341-348*
6. *Robinson TF, Factor SM, Sonnenblick EH: The heart as a suction pump. Sci Am, 1986; 254: 84-91*
7. *Dolber PC, Spach MS: Picrosirius red staining of cardiac muscle following phosphomolybdic treatment. Stain Technol, 1987; 62: 23-26*
8. *Anderson KR, St John Sutton MG, Lie JT: Histopathological types of cardiac fibrosis in myocardial disease. J Pathol, 1979, 128: 79-85*
9. *Caulfield JB, Borg TK: The collagen network of the heart. Lab Inves, 1979; 40: 364-372*
10. *Robinson TF, Cohen-Gould L, Factor SM: Skeletal framework of mammalian heart muscle: arrangements of inter and peri-cellular connective tissue structure. Lab Invest, 1983; 49: 482-498*
11. *Robinson TF, Factor SM, Capasso JM et al: Morphology, composition, and function of struts between cardiac myocytes of rat and hamster. Cell Tiss Res, 1987; 249: 247-255*
12. *Yoshikane H, Honda M, Goto Y et al: Collagen in dilated cardiomyopathy. Scanning electron microscopic and immunohistochemical observations. Jpn Circ J, 1992; 56: 899-910*
13. *Monfort I, Perez-Tamayo R: The muscle-collagen ratio in normal and hypertrophic human hearts. Lab Invest 1962; 11: 463-470*
14. *Rossi MA, Carillo SV: Electron microscopic study of the cardiac hypertrophy induced by iron deficiency anaemia in the rat. Br. J Exp Pathol, 1983, 64: 373-387*
15. *Brilla CG, Weber KT: Reactive and reparative fibrosis in arterial hypertension in the rat. Cardiovasc Res, 1992; 26: 671-677*
16. *Bishop JE, Rhodes S, Laurent GJ et al: The regulation of collagen deposition in the hypertrophying heart. Ann N Y Acad Sci, 1995; 752: 230-239*
17. *Wearn JT: Morphological and functional alterations of coronary circulation. Harvey Lectures, 1941; 17: 754-777*
18. *Marcus ML, Harrison DG, Chillian WM et al: Alterations in the coronary circulation in hypertrophied ventricles. Circulation, 1987; 75(Suppl I): I-19-I-25*
19. *Sabbah HN, Sharov VG, Lesch M, Goldstein S: Progression of heart failure: a role for interstitial fibrosis. Mol Cell Biochem, 1995; 147: 29--34*
20. *Tan LB, Jalil JE, Pick R et al: Cardiac myocyte necrosis induced by angiotensin II. Circ Res, 1991; 69: 1185-1195*
21. *Henegar Jr, Blower BL, Kabour A, Janicki JS: Catecholamine response to chronic ANG II infusion and its role in myocytes and coronary vascular damage. Am J Physiol, 1995; 269: H1564-H1569*
22. *Rossi MA, Carillo SV, Oliveira JSM: The effect of iron deficiency anemia in the rat on the catecholamine levels and heart morphology. Cardiovasc Res, 1981; 15: 313-318*
23. *Campbell SE, Janicki JS, Matsubara BB, Weber KT: Myocardial fibrosis in the rat with mineralocorticoid excess. Prevention of scarring with amiloride. Am J Hypertens, 1993; 6: 487-495*
24. *Darrow DC: Effect of low potassium diet and desoxycorticosterone on the rat heart. Proc Soc Exp Biol Med, 1944; 55: 13-14*
25. *Darrow DC, Miller HC: The production of lesions by repeated injections of desoxycorticosterone acetate. J Clin Invest, 1942; 21: 601-611*
26. *Rossi MA: Patterns of myocardial fibrosis in idiopathic cardiomyopathies and chronic chagasic cardiopathy. Can J Cardiol, 1991; 7: 287--294*
27. *Rossi MA, Ramos SG: Pathogenesis of chronic Chagas' myocarditis: an overview. Cardiovasc Path, 1996; 5: 197-202*
28. *Rossi MA, Souza AC: Is apoptosis a mechanism of cell death of cardiomyocytes in chronic chagasic myocarditis? Int J Cardiol, 1999; 68: 325-331*
29. *Ribeiro-dos-Santos R, Rossi MA: Imunopatologia: In Cardiopatia chagásica, Cancado JR, Chuster M (eds). Fundacao Carlos Chagas: Belo Horizonte, 1985; 10-22*
30. *Ribeiro-dos-Santos R, Rossi MA, Laus JL et al: Anti-CD4 abrogates rejection and reestablishes long-term tolerance to syngeneic newborn hearts grafted in mice chronically infected with Trypanosoma cruzi. J Exp Med, 1992; 175: 29-39*
31. *Mengel J, Rossi MA: Chronic myocarditis pathogenesis: dependence on autoimmune and microvascular factors. Am Heart J, 1992; 124: 1052-1057*
32. *Kalil J, Cunha-Neto E: Autoimmunity in Chagas' disease cardiomyopathy: fulfilling the criteria at last? Parasitol Today, 1996; 12: 396--399*
33. *Oliveira JSM: A natural model of intrinsic heart system denervation: Chagas' cardiopathy. Am Heart J, 1985; 110: 1092-1098*
34. *Ramos SG, Matturri L, Rossi L, Rossi MA: Cardiac extrinsic neuropathy: lesions of mediastinal paraganglia in chronic chagasic cardiomyopathy. Cardiovasc Path, 1996; 5: 227-231*
35. *Ben Younās-Chennoufi A, Hontebeyrie-Joskowicz M, Tricottet V et al: Persistence of Trypanosoma cruzi antigens in the inflammatory lesion of chronically infected mice. Trans Roy Soc Trop Med Hyg, 1988; 82: 77-83*
36. *Higuchi ML, Brito T, Reis MM et al: Correlation between Trypanosoma cruzi parasitism and myocardial inflammatory infiltrate in human chronic chagasic myocarditis: light microscopy and immunohistochemical findings. Cardiovasc Path, 1993; 2: 101-106*
37. *Rossi MA, Goncalves S, Ribeiro-dos-Santos R: Experimental Trypanosoma cruzi myocarditis in BALB/c mice. The potential role of intravascular platelet aggregation in its genesis. Am J Pathol, 1984; 114: 209-216*
38. *Rossi MA: Microvascular changes as a cause of chronic cardiomyopathy in Chagas' disease. Am Heart J, 1990; 120: 233-236*
39. *Morris SA, Tanowitz HB, Witner M, Bilezikian JP: Pathophysiological insights into the cardiomyopathy of Chagas' disease. Circulation, 1990; 82: 1900-1909*
40. *Tanowitz HB, Morris SA, Factor SM et al: Parasitic diseases of the heart. I: Acute and chronic Chagas' disease. Cardiovasc Path, 1992; 1: 7-15*

41. *Marin-Neto JA, Marzullo P, Marcassa C et al: Myocardial perfusion abnormalities in chronic Chagas' disease as detected by thalium-201 scintigraphy. Am J Cardiol, 1992; 69: 780-784*
42. *Torres FV, Acquatella H, Condado A et al: Coronary vascular reactivity is abnormal in patients with Chagas' disease. Am Heart J, 1995; 129: 945-1001*
43. *Rossi MA, Carobrez SG: Experimental Trypanosoma cruzi cardiomyopathy in BALB/c mice: histochemical evidence of hypoxic changes in the myocardium. Br J Exp Path, 1985; 66: 155-166*
44. *Spach MS, Dolber PC: Relating extracellular potentials and their derivatives to anisotropic propagation at a microscopical level in human cardiac muscle. Evidence for electrical uncoupling side-to-side fiber connections with increasing age. Circ Res, 1986; 58: 356-371*

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