INTERSTITIAL MICROFIBRILS IN THE MYOCARDIUM OF PATIENTS WITH RHEUMATIC AND CONGENITAL CARDIAC DEFECTS

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Electron-microscopic investigation of biopsy specimens of heart tissue from patients with rheumatic and congenital cardiac defects revealed aperiodic microfibrils, the number of which was proportional to the fibrosis of the myocardium, on the basal membranes of the capillaries and muscle fibers and also in the lumen of the T-tubules and their vacuolar expansions. If signs of rheumatic carditis are present the myofibrils are less regular and their number somewhat greater. Microfibrils are rutheniophilic and argyrophilic and consist of elementary fibrils of reticular fibers. Their hyperplasia is the ultrastructural equivalent of the reticular skeleton of the hypertrophied myocardium in patients with cardiac defects.

KEY WORDS: *Reticular microfibrils; hypertrophy of the myocardium; rheumatic heart defects; congenital heart defects.*

Microfibrillary material is frequently observed [3, 4, 7, 13] on the basal membranes of the capillaries and of muscle and nerve fibers of the myocardium of patients with rheumatic (RHD) and congenital heart defects (CHD). This paper describes the results of an electron-histochemical study of the nature of the microfibrils.

EXPERIMENTAL METHOD

Biopsy material from the left and right auricles and left ventricle of the heart from 114 patients with RHD and 11 patients with CHD and from the right auricle of one patient with a pericardial cyst, was studied in the light and electron microscopes. Pieces of tissue for electron microscopy were fixed with glutaraldehyde and osmium and then embedded in methacrylates or Araldite. Ultrathin sections were stained with lead citrate and uranyl acetate. In four cases some of the pieces were fixed in fixatives with the addition of 0.001% ruthenium red. In seven cases the ultrathin sections were stained with ammoniacal silver; a freshly prepared solution of Gomori's ammoniacal silver [5] was poured into the bath of the ultratome blade; after 2-3 min of cutting the strip of sections was drawn out on grids with Parlodion-carbon film, dried by touching with filter paper, and examined unstained in the electron microscope.

EXPERIMENTAL RESULTS AND DISCUSSION

Focal or diffuse sclerosis of the myocardium was found in histological sections from most patients stained with azan and picrofuchsin. In those areas bundles or sheets of parallel, unblanced, aperiodic microfibrils, 7-18 nm in thickness, were seen in the electron microscope on the basal membranes of muscle fibers, capillaries, and nerve fibers, and other bundles were seen lying freely in the interstitial tissues. The quantity of microfibrillary material was proportional to the degree of fibrosis and sclerosis. In the normal myocardium of a patient with a pericardial cyst and in areas of myocardium free from fibrosis in patients with RHD and CHD there were no or very few microfibrils. In CHD they were usually

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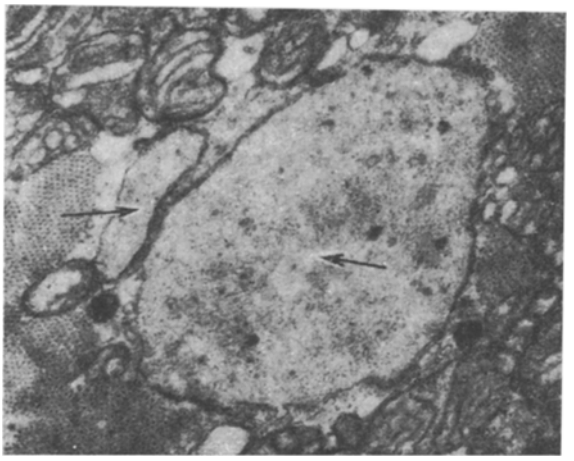

Fig. 1. Microfibrils in lumen of vacuolar expansion of T-tubules in muscle cell of myocardium of left auricle (arrows), 26,250×.

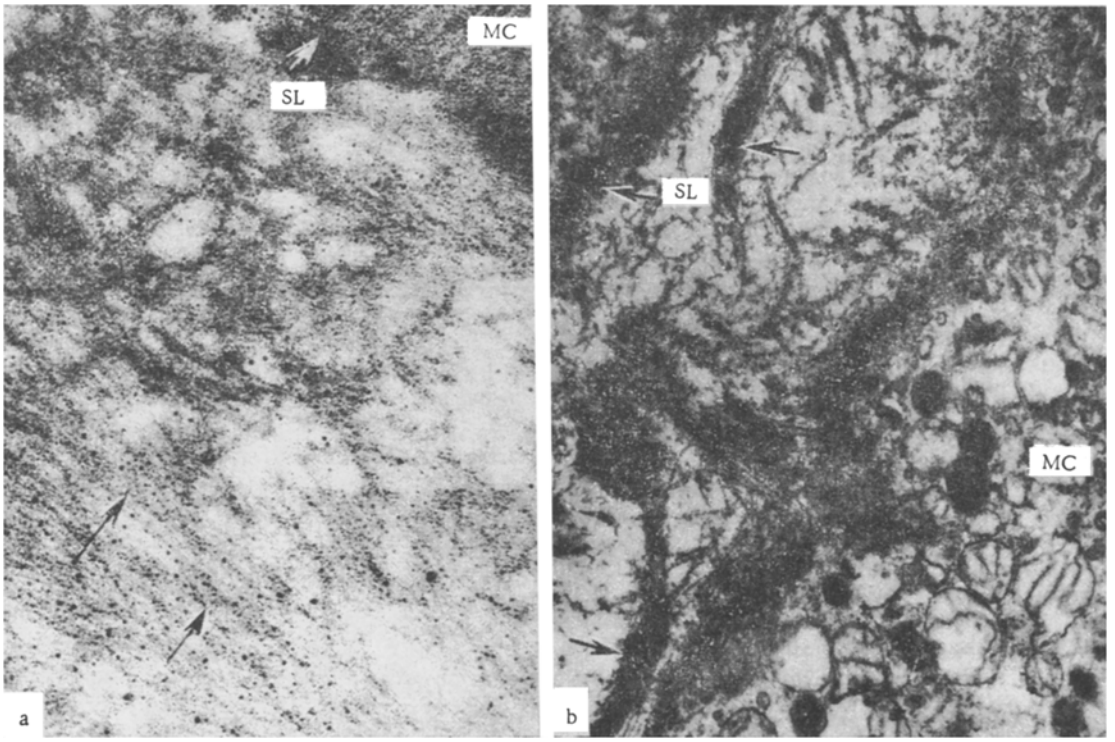

Fig. 2. Microfibrils (arrows) on surface of muscle cells after staining ultrathin section with ammoniacal silver (a) and after fixation of tissue with addition of ruthenium red to fixative (b). MC) muscle cell; SL) sarcolemma. Magnification: a) 30,000×, b) 25,700×.

arranged in a relatively thin, loose layer, but in RHD, by contrast, they formed a thick, dense layer. Under high power, a paler center could be seen in their transverse sections.

The bundles of microfibrils were in close contact with the basal membrane and penetrated along with it into depressions in the sarcolemma and into the T-tubules, and sometimes they completely filled the lumen of the T-tubules and their vacuolar expansions (Fig. 1). Relatively fewer microfibrils were seen on capillaries and nerve fibers. No differences were observed between the quantity of microfibrillary material on the surface of muscle fibers of different thickness (hypertrophied, normal, or atrophied).

If morphological signs of rheumatic carditis were present in the auricle (mucoid and fibrinoid swelling, rheumatic granulomas, microscopic foci of nonspecific inflammation) the microfibrils were more frequently arranged not strictly parallel, or even irregularly; loosening of the bundles and sheets on the surface of the muscle fibers was accompanied by an increase in the quantity of microfibrillary material unconnected with the basal membranes.

Veil-like processes often covered the layer of microfibrils on the surface of the muscle cells or, on the other hand, they appeared to separate the cells from the bundle of microfibrils lying freely in the interstitial tissues. If signs of rheumatic carditis were present in the auricle, patterns of unwinding of the collagen fibrils [10], i.e., their breaking up into microfibrils, also were observed. In these places the number of collagen fibrils was reduced.

Ruthenium red considerably increased the electron density of the basal membranes and microfibrils. After treatment of the ultrathin sections with ammoniacal silver, chains of silver grains, sometimes alternating with a definite period of about 15 nm, were discovered along all the microfibrils. Silver impregnation gave better results in methacrylate ultrathin sections (Fig. 2).

Gamma-globulin and complement were determined (I. S. Kazakova) by an immunofluorescence method in cryostat sections from 29 patients with RHD and four patients with CHD. None were found in the patients with CHD. Deposits of γ-globulin on the sarcolemma of the muscle fibers were found in nine patients with RHD, in conjunction with complement in four of them. Microfibrils were present on the surface of the muscle fibers in 24 of these 29 patients with RHD. The degree of the ultrastructural changes in the muscle cells was independent of the thickness of the microfibrillary layer on their surface. However, when deposits of both γ-globulin and complement were found on the sarcolemmas of the muscle fibers, many microfibrils were found in the electron microscope on the surface of the muscle cells with considerable destruction of their organelles. A corresponding degree of changes in the muscle cells was found in only 11 (44%) of the remaining 25 patients.

Hyperplasia of the interstitial microfibrils was characteristic of areas of fibrosis and sclerosis of the myocardium in both RHD and CHD [9]. Its greater severity in RHD reflects the greater degree of myocardial sclerosis.

Electron-histochemical studies showed rutheniophilia and argyrophilia of the microfibrils, including microfibrils in the vacuolar expansions of the T-tubules. Vacuoles with argyrophilic contents also were visible in the light microscope [16]. These results indicate that the microfibrils are identical with the elementary fibrils of the reticular fibers. The reticular skeleton of the hypertrophied myocardium in RHD and CHD is known to be composed of interfascicular and perimysial reticular fibers [6]. In the electron microscope they corresponded to bundles of microfibrils in the interstitial tissue and on the surface of the muscle fibers. Cuffs of microfibrils also were present around the nerve fibers, capillaries, and blood vessels [7].

During recurrence of rheumatic carditis the microfibrils in the bundles lost their strictly parallel arrangement; they were loosely arranged, and their number was increased. Evidently not only hypertrophy of the myocardium [6], but also inflammatory changes with proliferation of connective-tissue cells [8] play a role in the coarsening of the reticular skeleton of the myocardium.

The close topographic connection between the microfibrils and the veil-like processes of the fibroblasts suggests that the latter participate in the synthesis of the materials from which the microfibrils are formed. On the other hand, the even closer spatial connection with the basal membranes justifies the hypothesis that muscle cells participate in microfibrillogenesis [1, 9]. It is in harmony with the idea of synthesis of the basal membrane proper by the cells (epithelial, endothelial, muscle) [11, 14, 15]. The basal membranes consist of collagen and noncollagen proteins rich in carbohydrates [12]. Their carbohydrate components are evidently responsible for the rutheniophilia of the basal membranes and microfibrils. Muscle, endothelial, and connective-tissue cells can all participate in the synthesis of the components of the microfibrils. Loosening and thickening of the basal membranes of the muscle fibers in rheumatic carditis [3, 4], in particular, may be related to the accelerated formation of new microfibrils. The results of the present experiments also indicate the possibility of reconstruction of collagen fibrils into thin argyrophilic microfibrils during recurrences of rheumatic carditis. Under these circumstances, exhausted fi-

brils are evidently split into thin aperiodic fibrils, for the number of collagen fibrils with the characteristic period is correspondingly reduced.

The microfibrillary material is identical with the "homogeneous and micellar masses" in which Mitin et al. [2] saw deposits of immunoglobulins. Parallel immunofluorescence and electron-microscopic investigations showed the absence of correlation between hyperplasia of the microfibrils and the deposition of immune complexes in the myocardium in RHD. Destructive changes in the muscle cells were not found to depend on the number of microfibrils on their surface, in agreement with observations by other workers [9, 10]. In four cases when immune complexes were discovered on the sarcolemma and in blood vessel walls, the degree of the changes in the muscle cell organelles was considerable, but no preferential destruction of the subsarcolemmal organelles [2] was observed.

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