REVIEWS

Energy, Structure, Conformation, and Heart Failure

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The problem of heart failure is analyzed from the point of view of conformation changes in submolecular cardiomyocyte structures. The development of heart failure as a consequence of abnormal function of contractile myocardial proteins is discussed. The properties of actin and the role of structural changes in actin molecules in the impairment of ATP energy utilization and generation of contractile force by actomyosin complexes are studied. Experiments on animal models and autopsy samples showed that conformation changes precede disturbances in energy utilization by myofibrils, abnormal functioning of the energy-producing and Ca^{2+}-transporting systems during the development of heart failure. It is proposed that rigid recombinations of submolecular structures in contracile proteins underlie impairment of myocardial contractility and resistance of the myocardium to regulatory factors and drugs (immobilization).

Key Words: *heart failure; conformation; energy; structure*

It is well known that severe heart failure can develop in the absence of macro- and microscopic pathognomic changes in the myocardium and vice versa patients with anatomically altered heart feel quite well, perform heavy physical work, live long, and die from noncardiac causes [29,101]. In the last case, morphological changes in the myocardium are completely compensated and the disease develops silently.

Clinical symptoms, the degree of disability, and systolic and diastolic function of the myocardium [45, 46,94] do not strictly correlate with structural and ultrastructural changes in cardiomyocytes [19,95] even in severe heart failure caused by congestive cardiomyopathy (CMP) and characterized by marked abnormalities in cardiomyocyte nucleus, T-tubular system, myofibrils, mitochondria (MC), and sarcoplasmic reticulum (SR) [67,118]. But all parameters are tended to decrease, which determine their coarse correlation [59, 94]. The absence of a strict correlation is probably due to nonspecific and nonpathognomic nature of morphological changes in CMP. These variances can be explained by the influence of compensatory mechanisms [34].

Thus, the absence of a strict correlation (or at least its long latency) between functional activity of the heart and morphological changes in the myocardium as well as between functional states of the myocardium and subcellular structures responsible for the contraction-relaxation cycle [9,12,17,38,39] suggests that the main causes of FH lie beyond the resolution (beyond the sight) of not only light microscopy but also ultrastructural methods.

It should be evaluated, what intracellular event(s) is(are) associated with reduced myocardial contractility.

Subcellular mechanisms of HF

It was established that severe HF induced by inflammatory damage to the myocardium, in particular by toxic-allergic myocarditis (TAM) [1] (this myocarditis causes death of 60% experimental animals within the first day), is characterized by almost simultaneous

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involvement of all subcellular cardiomyocyte structures responsible for the contraction-relaxation cycle [23]: systems of contractile myocardial proteins (SCMP), energy supply, and Ca^{2+}-transport through cardiomyocyte plasma membrane (i.e. sarcolemma, SR, and MC). The Ca^{2+}-transporting system determines the excitation-contraction coupling, regulates ionotropic response of the myocardium, enables cell relaxation upon Ca^{2+} sequestration from the cytosol, and participates in the regulation of cell metabolism [56,72].

The role of **contractile myocardial proteins** in adaptation to physical exercise in HF development is poorly understood. It was long accepted that the main contractile proteins (actin and myosin) do not play a role in the development of HF [5,80]. However, large-scale study conducted at the Center of Medical Biophysics (Ministry of Health of Georgia) on experimental animals [7,9,16-19,21] and autopsy material [6,8,10,87] demonstrated the important role of these proteins in the development of HF. In particular, force generation (Fig. 1, *a*, *b*) and energy transformation by SCMP in the left and right ventricles are considerably impaired in acute (myocardial infarction, sudden death) and congestive HF (valvular defects, essential hypertension, cardiosclerosis) [10].

Most impressive data were obtained on autopsy specimens taken during heart surgery (mitral stenosis and combined aortic and mitral valvular disease) (Fig. 1, *c*). It was shown that *in vivo* and *in vitro* contractility of SCMP sharply decreased in HF, especially combined with atrial fibrillation, but not in cardiosclerosis without HF (Fig. 1, *a*, *b*) (i.e. sclerosis not necessary induces changes in myofibrils). When cardiosclerosis is complicated by HF, changes in myocardial contractility are less pronounced than in valvular defects.

There are data that changes in SCMP precede disturbances in the energy supply system [9,17,19,23]. This is most pronounced in moderate pressure overload. For instance, in patients with 50% aortic stenosis adaptation to pressure overload, i.e. development of myocardial hypertrophy and chronic HF, lasts for year and longer. During the initial period of HF, no disturbances in the energy supply system appear until the impairment of SCMP contractility, while activity of the Ca^{2+}-transporting system even increases [9]. Cardiac glycosides are most effective during this period, which determines their beneficial use in HF.

Energy supply system plays a crucial role in the maintenance of structural organization [65] and functional activity [2,4,23,65,75,78,91,97]. Production of ATP in the myocardium is perfectly regulated and macroergic substances are utilized very economically.

The myocardium is enriched with MC; they occupy about 0.6-0.7 myofibril volume [43,119]. This promptly covers increased energy demands of the myocardium via stimulation of oxidative phosphorylation, the most efficient pathway of energy production. For instance, in the heart paced at 5 Hz the consumption of O_2 increases 5-fold over 10 min in comparison with the rest [133].

Resting myocardium is characterized by low number of open (functioning) blood capillaries [36] actomyosin complexes bound via strong bonds, and ATP-producing mitochondria. This is evidenced by the presence of myocardial zones characterized by different consumption of O_2 [132], content of ATP and total creatine, and considerable variability of succinate dehydrogenase (SDH) activity, the main enzyme of the Krebs cycle (from 0.16 to 1.97 U, i.e. 12-fold) [53]. This heterogeneity corresponds to metabolic demands in this particular zone and correlates with the intensity of blood flow [36,78], which provides for great reserve capacities of normal cardiomyocyte and makes it possible to recruit more MC in response to increased energy demands and activate more actomyosin complexes formed by strong bonds.

An important role in progression from latent to overt HF and then to severe HF refractory to usual therapy (cardiac glycosides, diuretics, and vasodilators, in particular, angiotensin-converting enzyme inhibitors) is played by disturbances in the energy supply system. For instance in TAM, the initial reduction of force developed by SCMP is potentiated by reduced availability of energy sources: the content of ATP and creatine phosphate (CP) decreases by 60 and 53%, respectively [23]. In experimental dilated cardiomyopathy (less severe than TAM), the content of ATP and CP decreases by 23 and 42%, creatine phosphokinase activity is inhibited by 34%, and the role of creatine phosphokinase reaction by 71%, which sharply restricts reserve capacities of the energy supply system [97]. Similar changes in the cardiomyocyte energy-generating system were found in human myocardium in dilated CMP [74,114]. R. Liao *et al.* assumed that the content of ATP in the myocardium decreases only in severe HF [97].

Thus, the initial drop in developed force caused by alterations of SCMP is than aggravated due to deficiency of available energy sources and their wasteful utilization. Impaired myocardial contractility in chronic HF is determined by a double mechanism: alterations of SCMP and energy deficiency.

These data attest to a relationship between generated force and the content of ATP and CP in TAM. However, neither in healthy heart nor in HF the parameters of cardiac systolic function correlate with the content of components of the adenylate or creatine phosphate systems in the myocardium. The same conclusions were previously made by K. Clarke *et al.*

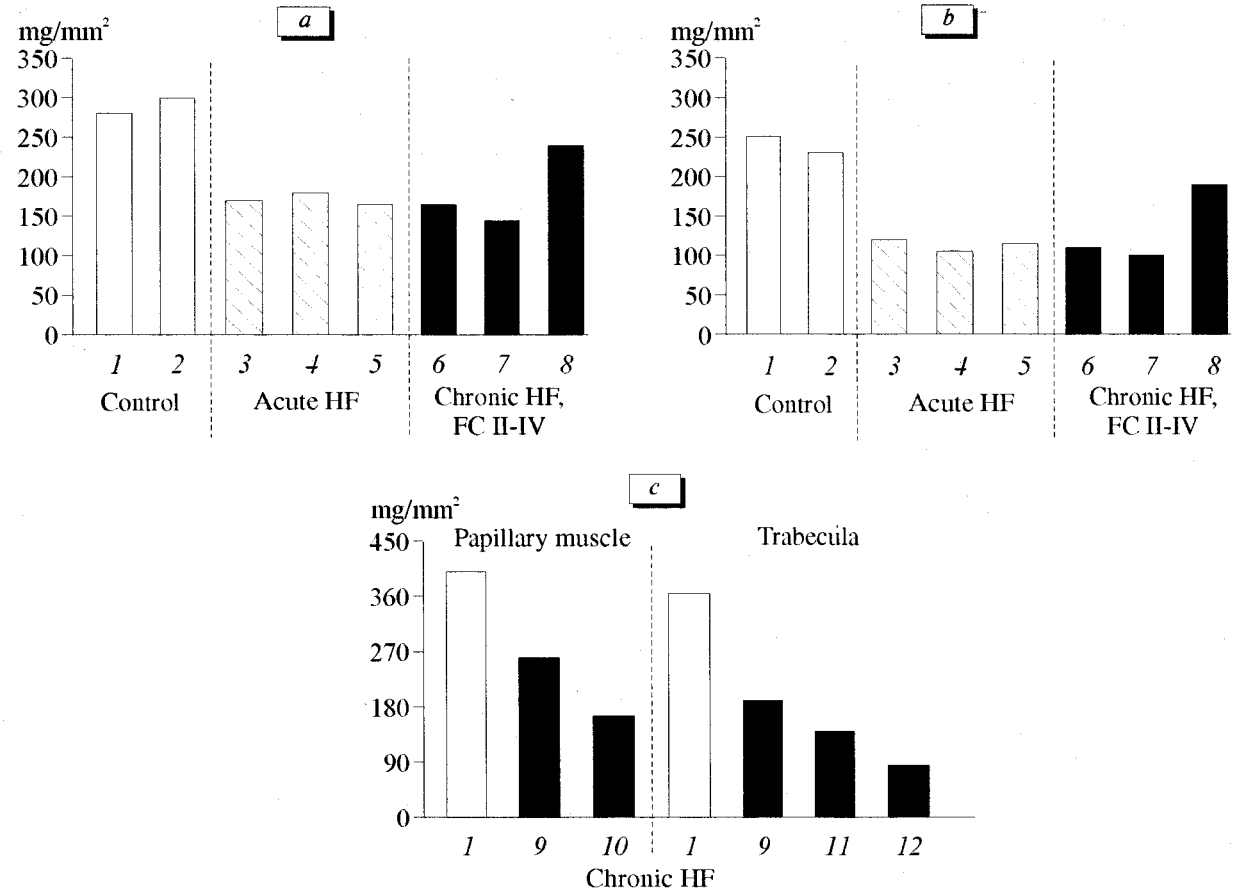

Fig. 1. Tension developed during auxotonic contraction by glycerinated muscle fibers from right (*a*) and left (*b*) ventricular trabeculae of failing heart (HF), normal human heart (autopsy material), and from left ventricular papillary muscles and trabeculae obtained during cardiac surgery. *1*) healthy subject; *2*) cardiosclerosis without HF; *3*) myocardial infarction; *4*) sudden death; *5*) stroke; *6*) heart defects; *7*) essential hypertension; *8*) cardiosclerosis with HF; mitral stenosis with HF, functional class II (FC II) without (*9*) and with (*10*) atrial fibrillation; mitral stenosis (*11*) and mitral-aortal defect (*12*) with HF (FC III) and atrial fibrillation. FC was determined according to NYDA.

[55]. This phenomenon can be attributed to the fact that ATP and CP do not represent free energy, which can be used for force generation, but only available source of this energy.

It should be noted that G. Schwartz *et al.* [122] observed a close correlation between the drop of segmentary contractility (by 24%) and the content of CP (r^2=0.97), inorganic phosphate (P_i, r^2=0.99), and CP/P_i ratio (r^2=0.98) during the first few minutes of coronary occlusion. However, myocardial contractility under these conditions only little depended on ATP content: ATP utilized during systole (only 15% [105]) was rapidly resynthesized.

Under normal conditions, myocardial contractility and diastolic function do not depend on ATP and CP, but on cytosolic phosphorylation potential (PP=ATP/ADP×P_i), which is equal to $\Delta G=dG/d\xi$ and determines the maximum heart work.

The important role of PP is confirmed by the fact that cytosolic PP in cardiomyocytes (~10^5/M) far surpasses that in other cells (1-5×10^3/M) [69]. In hamsters with CMP, ΔG of ATP hydrolysis during systole and diastole is far below normal [123]. It was also shown that the rate of relaxation closely correlates with $dG/d\xi$ in cardiomyocyte [60].

Myocardial contractility sharply decreased after attaining a critical value of ΔG of ATP hydrolysis (45-50 *vs.* 60 kJ/mol in normal myocardium [85]). As shown previously, such a decrease in ΔG of ATP hydrolysis occurred at coronary blood flow below 7.2 ml/min/g and Po_2=12 mm Hg [125]. It was also shown that blood flow rate depends on PP but not on the content of O_2 [105]. Disturbances in myocardial relaxation appear at the same ΔG of ATP hydrolysis (41 kJ/mol): SR losses its ability to accumulate Ca^{2+} [60,69].

In severe HF caused by TAM, the relationship between PP and parameters of systolic and diastolic cardiac functions is disturbed [14], while in dilated CMP complicated by coronary heart disease in humans, the correlation between PP, ATP/CP ratio, and ejection fraction disappears [74].

J. Giesen *et al.* [70] showed that mitochondrial respiration and the intensity of ATP formation in the reaction of oxidative phosphorylation in isolated MC

and in living heart are also regulated by PP. Published data suggest that changes in ΔG of ATP hydrolysis is the main mechanism of HF development [69].

Ca^{2+}-transporting system consisting of sarcolemma, SR, and MC also plays an important role in the regulation of contraction-relaxation cycle [5,40, 127]. Some investigators assume that impairment of Ca^{2+} homeostasis underlies the development of HF [72]. However, it should be noted that myocardial relaxation is a very energy-consuming process, since on the one hand, it involves many sarcolemmal ATPases, SR, and MC and, on the other hand, dissociation of strong bonds in the actomyosin complexes requires much ATP. That is why myocardial relaxation under normal conditions strictly depends on PP.

In HF caused by TAM [23], the ability of the Ca^{2+}-transporting system to release Ca^{2+} from SR cisterns decreased 2-fold, and its absorption and binding are reduced by 35 and 28%, respectively. Nevertheless, correlations normally observed between Ca^{2+} release from SR and heart rate (HR), functions of myocardial structures, and dP/dt_{max} and between Ca^{2+} absorption by SR and dP/dt_{max}, HR, and functions of heart structures are preserved in HF, while the correlation between the relaxation index and PP becomes more potent. At the same time, the correlations between other factors and PP weakens. This suggests that disturbances in the Ca^{2+}-transporting system play a less important role in the development of HF than abnormalities in SCMP and energy supply. There are data on enhanced activity of the Ca^{2+}-transporting system in the initial period of HF [9]. This can be explained by the fact that Ca^{2+}-transporting system is reliably protected from energy deficiency and its function is supplied by not only cellular (although compartmentalized [75]) ATP resources which are replenished predominantly via oxidative phosphorylation, but also autonomously [113,116] by its own energy-producing glycolytic system [116]. However, SCMP are also secured, since it contains all glycolytic enzymes.

SR couples and coordinates functional activity of myofibrils and MC. However, structural organization and local regulation of SR are characterized by high (at least functional) compartmentalization, since fine point depolarization can induce contraction of a half of particular sarcomere [82].

Inhibition of the Ca^{2+}-transporting system was observed only in terminal stages of HF [9,12,17,23, 72,106,127] caused by dilated CMP [111], TAM [23], hypertrophy [124], hereditary CMP in hamsters [130], alcoholic CMP [17,19,48]. Early disturbances in the Ca^{2+}-transporting system were revealed only in initially severe HF caused by TAM [23] or acute alcoholic intoxication [19,71] and were associated with early relaxation defects. This inhibition of Ca^{2+} transport is caused by considerable ATP depletion (activity of Ca^{2+}-ATPase, i.e. Ca^{2+} pump, is regulated allosterically by ATP and ADP [25,121]). Moreover, similarly to SCMP, Ca^{2+}-ATPase and Ca^{2+} transport are uncoupled under these conditions, slow channels in cardiomyocyte are closed and Ca^{2+} entry is impossible. The total content of Ca^{2+} in cardiomyocyte and in MC and SR decreased [24]. These processes lead to immobilization of cardiomyocyte aimed at preservation of ATP stores, prevention of Ca^{2+}-induced damage and cell death, but are destructive for the whole organism and result in myocardial hypofunction.

Thus, initial stages of HF inaccessible to light microscopy and ultrastructural analysis are characterized by primary disturbances in SCMP. These abnormalities are then aggravated by deficiency of free energy of ATP hydrolysis and later by disorganization of the Ca^{2+}-transporting system, which trigger a cascade of pathological events, cause metabolic and circulatory vicious circles underlying HF progression and the onset of HF refractory to usual therapy.

Role of structural and conformational alterations of sarcomere thin filament in the development of HF

Structure of contractile apparatus, a cardiomyocyte bioengine. Regulation of energy transformation. Sarcomere, an elementary contractile moiety of myofibrils, is an ultrastructure restricted by two neighbor Z lines with attached and oppositely directed thin actin filaments of two adjacent sarcomeres, which form I-disks of myofibrils. In the middle of the sarcomere, free ends of thin filaments enter into spaces between thick filaments arranged in a hexagonal lattice (A disk). Actomyosin complexes formed in the overlapping areas are responsible for generation of a drawing force and relative sliding of thin and thick filaments without changing their length, i.e. contraction.

In canine myocardium, the end-diastolic length of a sarcomere in the left ventricle varies from 2.07 [42] to 2.25 μ [20], while by the end of systole it decreased to 1.8-1.9 μ [20] (during normal contraction, shortening by 12%) or to 1.6 μ [42,89] (hypercontraction, shortening by 25%).

Thin filaments consist of actin, tropomyosin, and troponin (in a ratio of 7:1:1), while thick filaments consist primarily of myosin, large double-headed macromolecules with long superhelical tails; their aggregation yields a trunk of the thick filament. Myosin heads with ATP-hydrolysis site and site of interaction with thin actin filaments are located on the surface of these trunks in diastole.

During muscle excitation myosin heads are detached from the surface of thick filaments and get

intimately close to the thin actin filament forming a weak actomyosin bond (crossbridges). This is the first structural transformation accompanying excitation-contraction coupling. The weak bond becomes stronger; actomyosin complex formed by a strong bond represents a structure capable of generation of contraction force. Upon saturation of the thin filament regulatory proteins with Ca^{2+}, the actomyosin complex is transformed into a force-generating state [50,63], which lasts about 10 msec [66]. Summary contraction of solitary actomyosin complexes formed by strong bonds determined the total contractile response of the myocardium. Thus, structural rearrangements in SCMP occur long before force generation (changes in functional state). This clearly illustrates priority of structural changes over functional ones.

Although molecular mechanisms of force generation is poorly understood, the myosin hypothesis of biological mobility is most popular. According to this hypothesis the contraction force is generated due to changes in the position (slope) of myosin heads about thin filament or intramolecular conformational changes (recombinations) in myosin heads and changes in its shape [79]. However, the search for new evidence in favor of this theory yielded many contradictory data.

A method of reconstruction of actomyosin complexes by cross-hybridization [7,86,87] between actin (with or without native tropomyosin [26]) from myocardium of patients (or experimental animals) with HF and normal myosin or vice versa, as well as between natural ghost (actin) filaments from failing heart with or without minor proteins and normal myosin [7,18, 22] was developed at the Center of Medical Biophysics (Ministry of Health of Georgia). This method allowed us to prove that the force is generated and the energy is economically transformed in actin, whereas myosin heads provide support and, upon activation with actin, hydrolyze ATP, modulate free energy, and control the magnitude and rate of force generation [18,22,26,86,87].

In whole myofibrils and in the myocardium, the proportion between contractile response and generated force and the adequacy of this response to muscle load are regulated by pulses from mechanosensory structures [64] probably located in actin filament [31]. Under these conditions, the adequacy of contractile response is determined by the stretch of contractile structures, cardiomyocyte membrane, and probably by the amount of released integrin, as well as by synthesis and degradation of structures, in particular, myofibrils [64], the number of mobilized force-generating units, and quantitative changes in energy transformation.

Energy transformation in SCMP is regulated by a kind of internal combustion engine economizer [11], which couples ATP-hydrolysis (in myosin) and force-generation sites (in actin) and ensures economical energy transformation [10,11,15,16,21,22,89]. This economizer is located in thin actin filament of the sarcomere [22]. Myosin light chain 2 can also participate in this process [30,98].

Normally, ATP-hydrolysis and force-generation sites are tightly coupled through economizer and the actomyosin complex functions very economically (r= 0.87-0.93) [15,16,18,21,22]. Dysfunction of this regulator in chronic HF impairs coupling between these processes down to complete uncoupling and sharply disturbs economical energy transformation in actomyosin complex [10,11,21,22,89]. Thus, quantitative and qualitative disturbances in energy transformation in HF is a central process in HF development [10], whereas restoration of functional activity of this regulator with cardiac glycosides [10,11,15] normalizes economical energy transformation and determines positive clinical effect.

Actomyosin complex formed by strong bond as a force generator; regulation of force generation. The appearance of immediate (instant) Ca^{2+}-sensitive rigidity of muscle fibers during force generation [66] is a direct sign of formation of actomyosin complex, in which myosin head and thin filament is bound through a strong bond. This rigidity constitutes about 80% of its total rigidity [51]. It depends on the number of crossbridges [66] and closely correlates with developed tension [66,77] and ATPase activity of muscle fibers [77] in response to not only Ca^{2+}, but also inotropic agents (epinephrine, norepinephrine) [76].

Since in the rigor state (in the absence of ATP) all or almost all crossbridges are bound to thin filament by strong bonds, the instant rigidity in rigor state is taken as the maximum determined by 100% binding of myosin heads [51,66]. In relaxed state at ionic strength of 170 mM (physiological range), the number of crossbridges, actomyosin complexes formed by strong bonds, determined by the method of limited proteolysis [44], varies from 16% [89] to 25.5% [10,89], while in skinned myocardial fibers it constitutes 20% [20]. Small-angle x-ray diffraction analysis [102] showed that during *in vivo* relaxation the number of myosin heads located near the thin filament but not participating in force generation attains 30-40%. Matsubara *et al.* assumed that the great number of residual bridges ensures rapid response of the myocardium to stimulation.

In the initial stage of isotonic contraction, the number of crossbridges in the force-generating state little increases from 25.5 to 30.3% [89], while in the stage of maintenance of strained state it attains 76.7%. At the plateau of the isometric contraction, the number of actomyosin complexes formed by strong bonds in skinned myocardial fiber constitutes 63.7%, while in the rigor state it attains 83.5%. X-ray diffraction

analysis showed that the maximum force developed in isometric contraction corresponds to 86-96% cross-bridging [103,104], analogously, maximum Ca^{2+} activation (pCa=4.4) is characterized by 80-96% cross-bridging [102-104]. Normally, the lifetime of actomyosin complex formed by a strong bond increases from 5.8 sec during force generation to 44 sec during the stage of maintenance of strained state [15], when the force generated by single actomyosin complex and ΔG of ATP hydrolysis consumed for the maintenance of strained state are reduced 2.9- and 7.8-fold, respectively. From thermodynamic point of view, the processes of force generation and maintenance of strained state are quantitatively and qualitatively different: force generation is by one order of magnitude more energy-consuming process than its maintenance [15].

Thus, the integral value of myocardial strain under physiological conditions is controlled by the number of actomyosin complexes formed by strong bonds and the degree of coupling between force generation and ATP hydrolysis. In HF, mechanical effectiveness of contractile process is reduced due to impaired coupling between ATP hydrolysis and force generation down to their complete uncoupling [10,16-18,20-22, 89]; cardiac glycosides can restore this effectiveness to normal [11].

Structure of thin filament. In view of the key role of actin in force generation and impairment of SCMP contractility in HF [6,7,26,86-88], we studied the structure of natural thin filament and its protomers in HF caused by athyreotic cardiomyopathy (ATC) lasting for 2-3 months [13] and TAM [33]. Electron microscopy combined with optic and computer diffraction, as well as photographic and computer-assisted filtration of diffraction pictures and microphotographs were used; computer-assisted 3-D reconstruction was carried out using the method of modifying projecting functions.

Analysis of diffraction pictures of single natural thin filaments from normal myocardium (Fig. 2) revealed structural instability (multiple splitting of a meridional reflection at the 13th layer line) and abundance of reflections scattering over the whole diffraction picture and forming a large homogenous high-density spot in the central part. Both features attest to high flexibility of natural thin filaments from normal myocardium and contradict the classical concept of thin filament as a rigid stick.

Photographic filtration of diffraction pictures and removal of weak reflections untypical for actin helix, i.e. summation of subthreshold exposures (usually 9-10 negatives) to normal exposure, allowed us to identify reflections typical for genetic helix of myocardial thin filament. Normally (Fig. 2, *a*) they are seen on the 1st, 6th, 7th, and 13th layer lines and correspond to helix periodicities of 360 E (half-period of actin helix), 51 and 59 E (variable derivatives of 360-E periodicity), and 27.5 E (axial transfer constant; meridional reflection; spacing between projections of contralateral protomers on the helix axis). The latter reflection lies on the 13th (for 13/7 helix) or 28th layer lines (for 28/13 helix). The presence of a forbidden reflection 55 E can be interpreted as an artifact of negative contrasting caused by accumulation of a contrast substance in the cleft between protomer domains of normal actin filament and its coagulation under electron beam.

In experimental ATC, the diffraction patterns were sharply distorted: splitting of the meridional reflection on the 13th layer line drastically decreased or even disappeared, the number of scattered reflections and the central homogenous high-density area were reduced (Fig. 2, *b*); the reflection on the 6th layer line decayed, while the forbidden reflection disappeared (or considerably weakened). Simultaneously, all para-meridional reflections on layer lines characteristic of thin filament were sharply shifted to the meridian, so that the distance between contralateral reflections decreased, which reflects the increase in diameter of natural thin filament (1.4-fold) and F-actin filament copolymerized with tropomyosin-troponin complex [13].

Even more drastic changes were seen in diffraction pattern of single thin filament in TAM of 10-day duration (Fig. 2, *c*). It is characterized by higher stability of the transfer constant (absence of meridional reflection splitting, further shrinkage of the central homogenous spot in comparison with ATC). The decreased reflection intensity on the 6th layer line and the absence of scattered untypical (background) reflections attest to deep changes in protomer shape and a sharp decrease in their conformation mobility in TAM.

It should be noted that in severe HF in humans, the diameter of F-actin filament considerably increased in comparison with that in healthy individuals (our data).

Submolecular studies of actin protomer and monomer

Spatial reconstruction of thin filament (Fig. 3) showed that in HF caused by ATC [13] and TAM [33], actin protomer in rigor medium looks like an elongated ellipsoid arranged perpendicularly to the helix axis (in contrast to normal compact kidney-shaped protomers) and its size is 90×40×30 *vs.* 65×40×45 E in normal [33]. This results in smoothening of the interdomain cleft (confirmed by the disappearance of a forbidden reflection) and thickening of the thin filament in HF. The observed changes in the protomer size can result from its inability to take the compact kidney-shaped

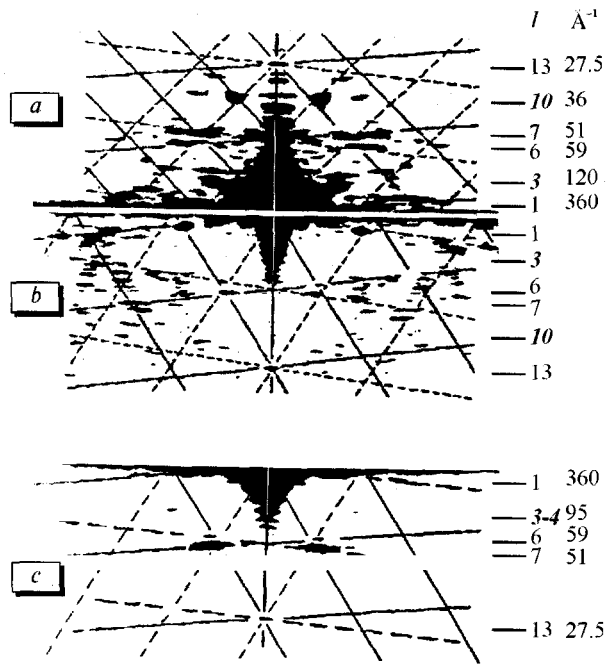

Fig. 2. Halves of photo-averaged diffraction pictures of natural myocardial thin filaments in normal (*a*, superposition of 28 pictures, upper part above the equator) and in heart failure (below the equator) caused by athyreotic cardiomyopathy (*b*, superposition of 24 pictures) or toxic-allergic myocarditis (*c*, superposition of 24 pictures). Solid and dotted lines shows front and hind reciprocal lattices. Layer lines and corresponding periodicities are shown on the right. Numbers of lines corresponding to forbidden reflexes are italicized.

conformation typical of normal actin protomer; in TAM and ATC, actin filament losses its conformation mobility. This phenomenon can be responsible for reduced force-generating capacity of SCMP in HF.

Electron microscopy and x-ray crystallography data are insufficient for construction of a realistic model of thin actin filament as a superhelical [73] single- [62] or double-strand [109] structure. Of crucial importance is the number of actin-actin interaction sites in the protomer and filament flexibility: 2 contacts → high flexibility → single-strand model; 3 or 4 contacts → relative rigidity → double-strand model [62, 81,107]. Although, the double-strand model proposed by K. Holmes *et al.* [81] is characterized by high flexibility. The actin:tropomyosin:troponin ratio is always 7:1:1.

Our models of thin filament from normal sarcomere and in TAM constructed using elementary density cross-sections at 9 E resolution are presented as single- and double-stranded filaments (Fig. 3).

The principal conclusion from this 3-D reconstruction of thin filament in HF caused by ATC or TAM is that protomer conformation in natural thin filament is altered: the protomer losses its conformational mobility and freezes as elongated structure arranged perpendicularly to the helix axis.

Pronounced structural and conformational disturbances in actin globule in HF caused by L-thyroxine cardiomyopathy (LTC) and especially by ATC were also detected by differential scanning microcalorimetry. These changes indicate considerable changes in the structure of outer functionally active actin domain in HF: loss of cooperativity and multiplet melting with a pronounced flat peak of excess heat capacity on the thermogram at 20-40°C. Considerable local intramolecular rearrangements in actin monomer in HF are probably responsible for *in vivo* denaturation of monomeric actin at physiological temperature. This destabilization of functionally active domain with low molecular weight considerably increases stabilization energy of monomer structure: standard enthalpy increased 4-fold, entropy and free energy increased 5- and 2.5-fold, respectively [27]. These findings indicate weakening of interdomain bonds and strengthening of intradomain hydrophobic bonds, which result in enlargement (smoothening) of the cleft between domains,* alteration in actin domain conformation, especially in the small domain, and enhance their rigidity [27].

In LTC of 2-3-month duration, monomer structure also undergoes considerable, though opposite changes: multiplicity of melting in the low-temperature region is reduced and protein stability increases above normal (judging from structure stabilization energy) [27]. Polymerization of actin in both pathologies stabilizes molecular structure, in particular, the outer domain. This stabilization is more pronounced in ATC and protects actin monomers from *in vivo* denaturation [28]. Despite structural stabilization and opposite deviations from normal, weakening of interdomain bonds is observed also in LTC. The mean length of F-actin filament (measured by double refraction in flow) in LTC and ATC decreases by 25 and 20%, respectively, in comparison with the normal level [88].

Both copolymerized filaments considerably differ from normal by melting parameters described by the all-or-nothing principle, despite the fact that the secondary structure and the content of α-helixes, β-structures, and random coil structures in HF caused by ATC remain unchanged [8]. Consequently, we deal with recombination rearrangements in actin protomer structure responsible for changes in melting parameters associated with weakening of bonds between domains and protomers [27,28].

Thus, in HF caused by ATC and LTC, thin filament becomes more rigid, while the bonds between domains and protomers weaken. These changes increase the distance between the centers of adjacent

*This confirms our interpretation of forbidden 55 E reflection in diffraction pictures of thin filament in ATC.

protomers, reduces actin polymerization capacity, and facilitate dissociation of thin filaments. That is why the most common length of reconstructed thin filament does not exceed 0.2-0.4 μ (*vs.* 0.8-1.2 in normal) or even 0.2 μ in chronic HF caused by pulmonary heart disease (Fig. 4). Under these conditions the nonenzymatic lysis of thin filaments, i.e. degradation of I-disks in sarcomeres (F-actin is resistant to proteolytic enzymes) against the background of preserved A-disks (myosin) is considered as an early phenomenon of myofibril lesion [17,18,38,39]. Changed actin properties and reduced conformation mobility of its outer domain decreases generated force, while degradation of thin filaments terminates contraction of affected sarcomere.

Of great importance is degradation of not only muscle, but also nonmuscle actin. It was shown that degradation of actin microfilaments with urea sulfate and blockade of microfilament synthesis with DNase I inhibit ATP-dependent K^+ channels, thus reducing its sensitivity to intracellular ATP [49]. Degradation of cytoskeleton microfilaments due to actin depolymerization results in the formation of apoptotic bodies and involvement of nonmuscle actin into aging [96].

The structure and conformation of actin protomer and monomer were directly studied by fluorescence resonance energy transfer using high-selective fluorescent probes. In HF caused by TAM or coronary occlusion (ischemic area), we observed changes in the orientation and microenvironment (polarization and anisotropy) of fluorescent probes bound to 4 amino acid residues of the outer actin domains: Cys374 (C-terminal amino acid residue is sensitive to polymerization and participates in binding with myosin), Cys10 (N-terminal region plays an essential role in force generation), two residues located near the isthmus, namely, Lys61 (participates in polymerization) and Tyr69 (participates in polymerization and interaction with nucleotides).

Structural changes in the outer domain, especially subdomain 2, were so pronounced that the distance between these amino acid residues measured by fluorescence resonance energy transfer after 1-h coronary occlusion and TAM increased by 10 and 14% and by

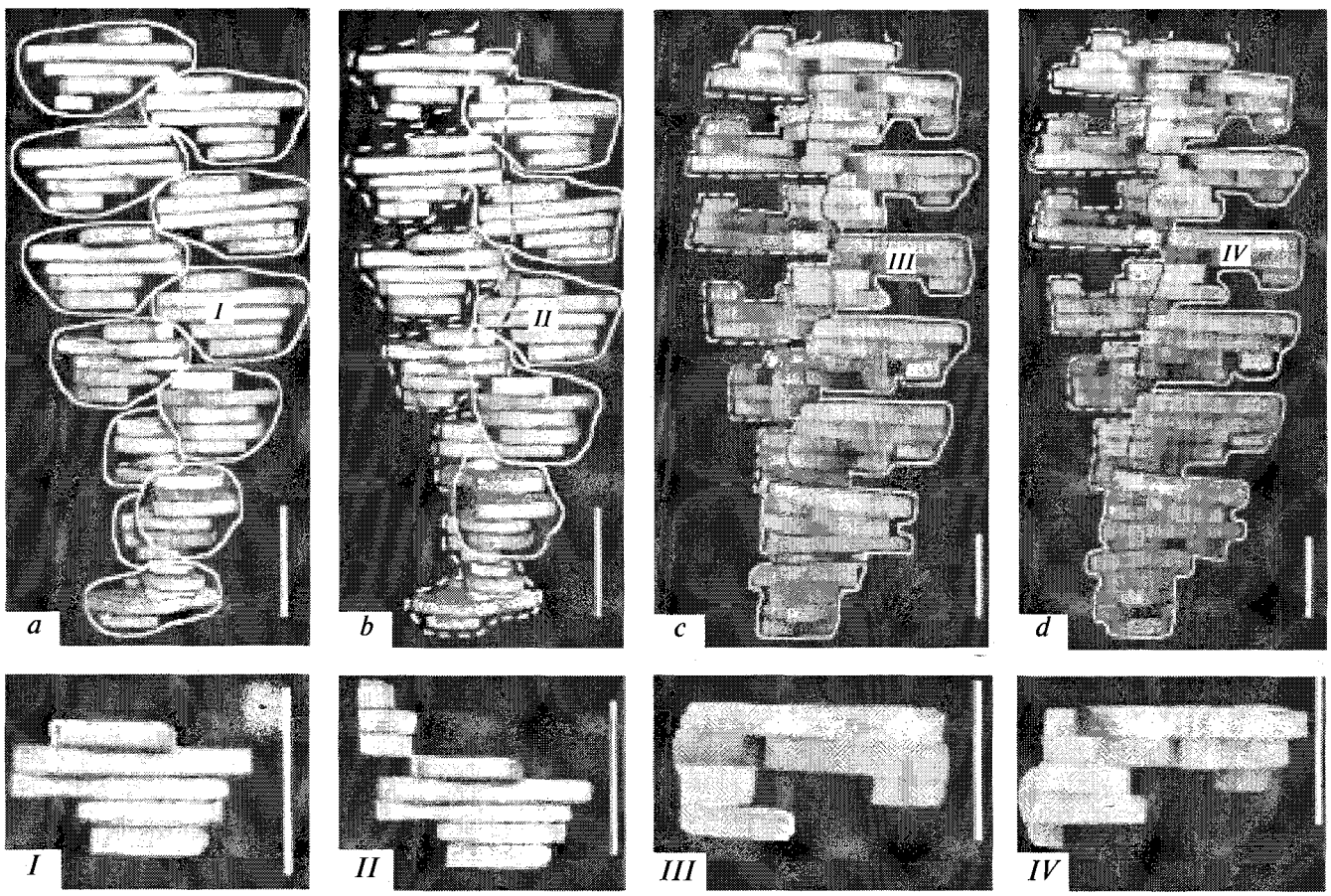

Fig. 3. 3-D reconstruction of a fragment of thin filament purified from tropomyosin with relative stable helix parameters (half-cyclefor 25/12 helix and complete cycle for 13/7 helix). Reconstruction of thin filaments from normal rabbit (*a, b*) and rabbit with toxic-allergic myocarditis (*c, d*). Protomer models (projections) *a* and *b*, as well as *c* and *d* differ in shape only (*I-IV*). Protomers in the filament can be only tentatively outlined and depending of chosen shape the model can be single- or double-stranded (*b* and *d*: T. G. Samsonidze and N. V. Karsanov, unpublished data).

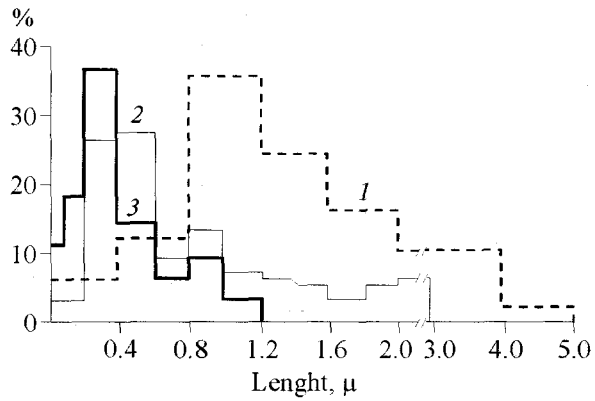

Fig. 4. Length distribution of F-actin myocardial filaments copolymerized with tropomyosin-troponin complex (reconstructed from thin filament) in healthy individuals (*1*, 1122 filaments) and in acute (*2*, myocardial infarction, 840 filaments) and chronic congestive heart failure (*3*, 1361 filaments).

24 and 33%, respectively [35]. Disturbances in actin polymerization suggest that both the outer domain and the isthmus are involved in recombinant rearrangements of actin protomer [8,88]. These rearrangements lead to radial elongation of the outer domain and smoothening of the interdomain cleft; the protomer freezes and, in contrast to normal protein, does not change this conformation in either contracting, or rigor medium [35], and undergoes no force-generating conformation rearrangements. Hence, in HF caused by TAM and coronary occlusion, the outer domain of actin protomer losses its conformation mobility.

Changes in the orientation and spatial localization of fluorescent probes in HF are accompanied by prolongation of the correlation time of conformation of the outer domain or its parts in nano- (for Cys374 from 18.6 to 29.5 nsec), micro-, and millisecond (comparable with force generation time) intervals. For instance, correlation time of slow component of tryptophan fluorescence in actin under conditions of TAM and acute myocardial ischemia increased 2-fold, while in LTC it increased 2.5-fold. Hence, actin in HF is characterized by reduced conformation mobility of local subdomain 1 regions and more exhensive subdomain 2 areas of the outer actin domain, including the isthmus.

Previously described [28,33,35] structural rearrangements confirm and define more concretely the location and nature of conformation changes in the tertiary structure of thin filament revealed by circular dichroism analysis. Interestingly, similar changes were observed in acute and congestive HF in humans caused by pulmonary heart disease, as well as in experimental HF caused by acute ischemia, pulmonary heart disease, TAM, ATC, and LTC (Fig. 5). These changes were associated with the development of HF irrespective of its mechanism and attested to decreased conformational mobility and reduced recombinant rearrangements in the main protein of actin filament, which interfere with proper folding of actin filament essential for its normal function in the force generation cycle [8].

Thus, ample clinical and experimental data suggest that actin protomer in HF losses its conformational mobility and the ability to transform from low-energy elongated linear state into high-energy compact kidney-shaped conformation normally corresponding to the force-generating state. This phenomenon is probably responsible for inability of pathological actin to transform into the force-generating state and inefficient energy transformation in the contraction-relaxation cycle.

Causes of changes in submolecular actomyosin structure in HF

Genetic apparatus plays an essential role in structural renewal, subcellular cell regeneration, and long-term adaptation of cardiomyocyte to pressure or volume overload, physical exercise, and long-lasting changes in neurohormonal environment. These factors modulate expression of not only heart-specific genes, but also genes in other organs and tissues [83].

In rodents characterized by high blood content of endogenous thyroid hormones, pressure overload and hypothyroidism induce a shift in the expression of myosin isoforms from V_1 (fast, skeletal, with high ATPase activity) to V_3 (slow, cardiac, with low ATPase activity). Some investigators attribute the decrease in ATPase activity in HF [41,106,126,128] to these shifts in myosin isoform spectrum. However, heart ventricles in humans and large animals contain primarily V_3 isozyme (96-100%), while the atria contain all three myosin isoforms [128]. Moreover, V_3 myosin isozyme expressed in response to pressure overload is characterized by high efficiency of the contractile process, while in HF it is characterized by low-efficient energy transformation [16,20-22]. Evidently, myosins possess different properties.

Comprehensive studies of the role of gene expression in the development of cardiac hypertrophy and HF led to a conclusion that expression of latent genes encoding all three cardiomyocyte systems responsible for the contraction-relaxation cycle is a physiological adaptive phenomenon, rather than the cause of HF development [93,106,128].

Mutation in cardiomyocyte genetic apparatus can underlie familial idiopathic hypertrophic CMP (mutations in chromosomes 1, 11, 14, and 15 [68,130]). This pathology manifests itself as concentric or asymmetric myocardial hypertrophy and is characterized by overexpression of mutant β-isoform of myosin heavy chain, where Asp232 in the nucleotide-binding center is substituted with Ser, while DNA nucleotide se-

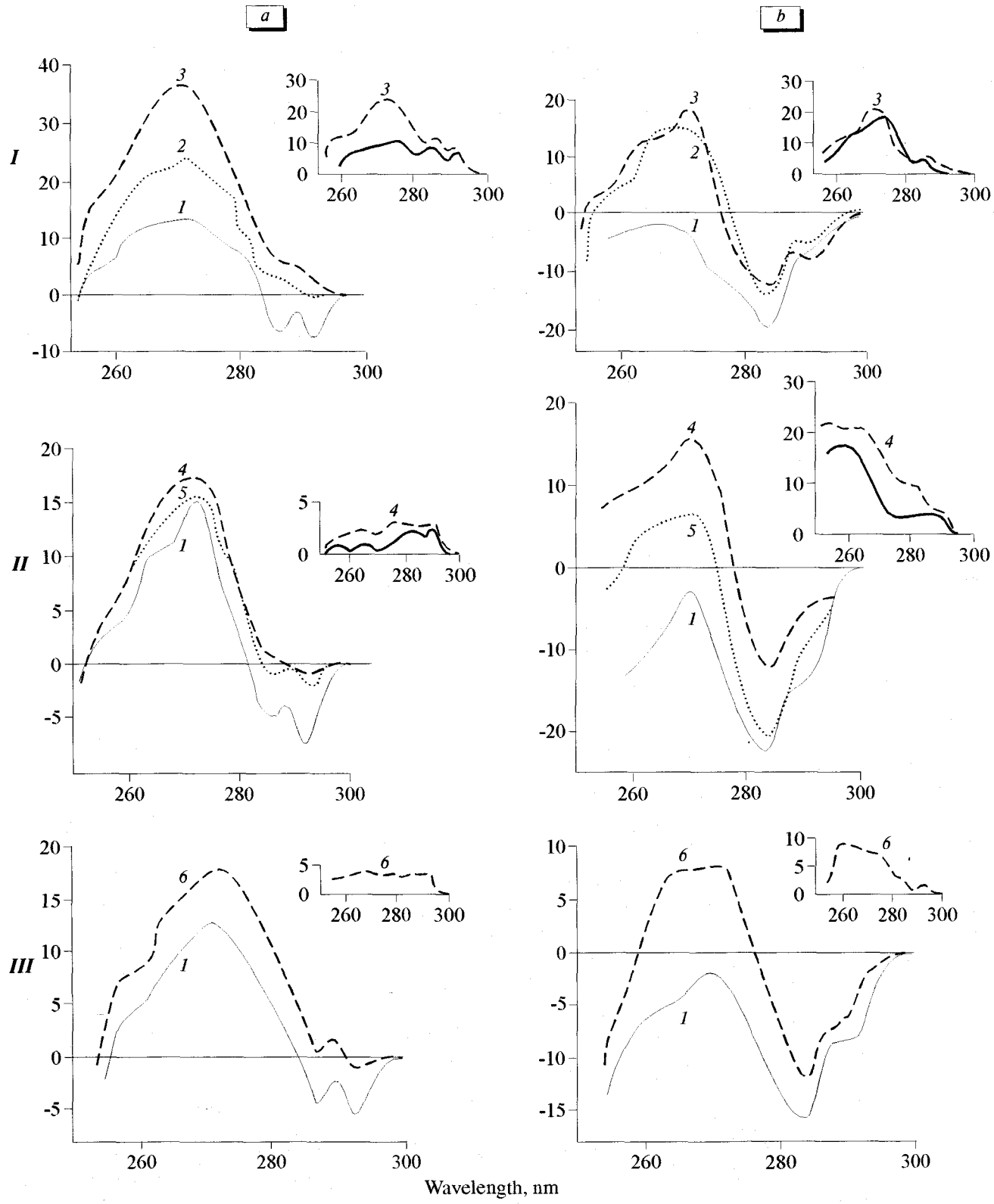

Fig. 5. Circular dichroism spectra of globular (*a*) and fibrillar (*b*) actin from normal myocardium (*1*) and in acute (*2*) and chronic congestive (*3*) heart failure in humans (*I*), experimental pulmonary heart disease (*4*) and athyreotic cardiomyopathy (*5*) in dogs (*II*), and experimental toxic-allergic myocarditis (*6*) in rabbits (*III*) [8]. Ordinate: [Δθ] ellipticity, (degree×cm²/mol)/10³. Little fragments: differential spectra; abscissa: wavelength, nm; ordinate: [Dq] ellipticity, (degree×cm²/mol)/10³.

quence remains unchanged [61]. Many mutations do not manifest clinically and have no pathological significance. They do not impair and sometimes even improve myocardial contractility [101-126], which implies that mutation, at least in hypertrophic CMP do not underlie the development of HF (but undoubtedly create specific condition for HF). However, crucial role of genetic abnormalities was demonstrated in hereditary CMP in hamsters [83,130].

The development of HF in normal and hypertrophied myocardium is probably determined by other factors. This conclusions drove us to an idea on posttranslational modifications of contractile proteins and other myocardial structures. For instance, acute myocardial ischemia culminating in acute HF induces rapid and pronounced damages to all three cardiomyocyte system responsible for the contraction-relaxation cycle: it decreases contractile activity of SCMP as soon as 5 min after onset (our observations) and impairs efficiency of the contractile process after 60 min [22]. Under these conditions no genetic abnormalities can play a role and we undoubtedly deal with posttranslational alterations of myocardial systems. This conclusion is also confirmed by circular dichroism data on unchanged secondary and, consequently, primary structure of actin in experimental coronary occlusion [8]. Finally, rapid recovery of cardiac function in severe HF during drug therapy also confirms the absence of mutations and the role of expression of actin isoforms [11].

S. Margossian *et al.* [99] assumed that the decrease in developed tension and actin-activated ATPase activity (by 25%) in dilated CMP is caused by degradation of myosin light chain 2 by specific protease. Indeed, elimination of this chain 10-fold reduces the rate of actin-myosin sliding *in vitro* (from 8.8 to 0.8 μ/sec) without considerable changes in ATPase activity, which significantly decreases efficiency of the contractile process. Incorporation of myosin light chain 2 or alkali myosin light chain 1 restores the rate of sliding by 50%, while incorporation of both chains completely normalized it [98]. It was established that heart dilatation in dilated CMP is caused by activation of enzymes catabolizing the connective tissue framework supporting myocardial muscle fibers [54].

Degradation of MC is of crucial importance for the pathogenesis of acute and chronic congestive HF and determines the development MC-forms of CMP [100]. However, all changes in MC are nonspecific and can be seen in other cardiac pathologies. Synthesis of ATP in MC is a complex process involving 56 various proteins; disturbances in some elements of this cascade can disorganize ATP synthesis [100,112,117,120].

Genetic apparatus plays a role in impairment of Ca^{2+} transport only at the final stages of HF, since no defects in Ca^{2+} transport were found at the initial stages of HF (our observations); gene expression plays physiological and adaptive, rather than pathogenetic role [128].

Posttranslational alterations in the myocardium can be induced by physical overload, drastic neurohormonal (humoral) shifts [4,9,29,32,92], repeated emotional stress [37,92], bacterial and viral infections, intoxication with diphtheria, pertussis, streptococcal, and staphylococcal toxins, fungal toxins (phalloidin), snake venoms (phosphodiesterase inhibitors), some drugs such as doxycycline, cyclophosphamide, furazolidone, doxorubicin (adriamycin), colchicine, cocaine, interleukin-2, prednisolone [90], alcohol and its metabolites (acetaldehyde), chronic poisoning with metals (cobalt, lithium, silicon, and barium), especially in combination with alcohol, and bromination of tryptophan residues [57]. Posttranslational modifications can result from disturbances of protein acetylation, methylation, ribosylation, palmitoylation, and phenylation (for instance, G-protein [110] coupling signal transduction) glycosylation [47], S-nitrosylation [68], and phosphorylation-dephosphorylation processes [24,47,111]. Muscle and cytoplasmic actins often serve as the target for various damaging factors (phalloidin, diphtheria toxin, dextrin, and actin-depolymerizing pancreatic DNase-I [134]).

This is only a brief survey of possible posttranslational alterations in the myocardium, cardiomyocyte, and various cells in the organism. This complex problem deserves special investigation. Posttranslational alterations modulate vital protein conformation and structure and, consequently, their functions.

Structure of proteins, in particular of contractile proteins, and especially their tertiary structure are in incessant motion. This motion is the measure of their nativity [3] and involves a wide frequency range from milli- to centimilliseconds (motion of some protein regions, is observed in actin and ATPase centers of myosin, SR, *etc.* [107,115,129,131]), as well as from micro- to nanoseconds (motion of individual amino acid residues, and further to pico- and femtoseconds [58]. These high-frequency motions have no force-generating effect, but they underlie milli- and subnanosecond force-generating conformation transformations: there are direct correlations between subnanosecon movements of thin filaments in glycerinated muscle fibers and rigor tension [52] and between nanosecond motions in myosin (probably around the ATPase center) and generated force [129].

High sensitivity of the above-mentioned motions to physiological fluctuations of Ca^{2+} concentration [108,131] and the fact that these low-frequency motions (segmentary, rotational, and vibrational [84,115]) with microsecond rates are detected in functionally active protein regions (in C-terminal and with a some-

what lower rate in N-terminal regions of actin polypeptide chain [52,131]) additionally confirm their role in force generation. Finally, the involvement of these motions in disturbances of myocardial contractility is confirmed by inhibition of nano-, micro-, and millisecond motions in actin protomer in HF caused by TAM, ATC, and LTC. These phenomena suggest that studies of the mechanism underlying the development of HF comes up to the submolecular level of knowledge.

Thus, all three principal systems of cardiomyocyte are involved in the development of HF: SCMP, energy supply system, and Ca^{2+}-transporting system. However, the impairment of contractile function in early HF is primarily associated with disturbances in SCMP, disorganization of energy supply appears later, and disturbances in Ca^{2+} transport join only in advanced stages of HF. Successive inactivation of these systems in HF is responsible for progressive immobilization of the myocardium, which is directed to its adaptation to novel conditions, but is harmful for the organism. Exhaustion of the reserve capacities of these myocardial systems, in particular of energy supply system, determines myocardial resistance to cardiotropic drugs.

Our data contradict the conventional myosin hypothesis of impaired myocardial contractility and prove the key role of actin, the second contractile protein, in force generation and efficient energy transformation by myocardial myofibrils during contraction. Disturbances in energy transformation due to structural and conformational changes in actin molecule and exhaustion of reserve capacities of the energy supply system are the central events in the formation of vicious circle determining the onset and progressive development of HF.

According to 3-D reconstruction data, changes in stabilization energy and enthalpy of actin monomer and actin filament in HF contribute to the loss of conformation mobility, determine the formation of a rigid structure of thin filament, and inhibit recombination rearrangements necessary for efficient energy transformation and force generation by actomyosin complexes. Posttranslational alterations of submolecular recombinant transformations in actin should be considered as a key mechanism underlying the impairment of contractile and relaxation functions of the myocardium in HF and low efficiency of drug therapy.

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