REVIEW

Transformations of Mitochondria in Damaged Cardiomyocytes

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Time course of intermitochondrial contacts in rat cardiomyocytes is studied in myocardial hypertrophy followed by its regression, alcoholic cardiomyopathy, acute pancreatitis, and acute diffuse purulent peritonitis. Contacts between mitochondria do not depend on the type of disease. Subacute and chronic myocardial hyperfuntion is characterized by hyperplasia of contacts involving an increase in the number and extent of mitochondrial associations resulting from these contacts during exposure to a damaging factor and a decrease in the number of contacts and mitochondrial associations after liquidation of the cause of pathological process. In a severe disease (acute diffuse purulent peritonitis) the number of contacts between mitochondria drops and associations of damaged mitochondria disintegrate, causing exhaustion of cardiac muscle energy. The results confirm Sarkisov's recombination theory that rearrangements of elements in biological system involve alterations of the quantity and quality of its function. This helps the heart overcome the critical stage of disease and later ensure hyperplasia of mitochondria and other structures in cardiomyocytes. This phenomenon possesses the characteristics of a biological regularity and is one of the earliest compensatory adaptive reactions of injured heart.

Key Words: *cardiomyocytes; intermitochondrial contacts; recombination transformations*

Development of heart diseases of any etiology is governed by common regularities playing the leading role in the pathogenesis and outcome of disease [9]. One of the most important of these regularities is the rate of involvement in pathological process of a complex of compensatory adaptive reactions at all levels of a live system, particularly at the ultrastructural level, among the "elemental components" of biological processes [13]. These reactions permit the heart to adapt rapidly to changed conditions of functioning. Any exposure of the heart leads to its hyperfunction associated with intense degradation of hyperfunctioning structures [9,10]. Therefore, resynthesis of intracellular organelles is a factor determining the duration of heart function in disease.

The concept of intracellular regeneration was formulated in the 1950s by D. S. Sarkisov. According to this concept, the pattern of all compensatory-adaptive reactions is universal and is based on increase in the number of actively functioning intracellular organelles [11,12,15]. Therefore, heart hyperfunction during its long overexercise is caused by hyperplasia of mitochondria (MC) and other intracellular structures in the cardiomyocyte (CM), leading to myocardial hypertrophy. The intracellular regeneration theory explains the development of compensatory adaptive reactions aimed at increasing the number of functioning organelles during pathological process. It is unclear what ensures the necessary level of heart contractions during acute period of disease,

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for example, in myocardial infarction, when myocardial hyperfuntion leads to death of many MC and eventually to energy deficit [9]. Formation of new intracellular structures takes a rather long time. Recently it was suggested that adaptation and compensation processes at all levels of live matter organization should be regarded as qualitative changes in the system based on the mechanism of recombination (rearrangement) of its elements[14]. This mechanism requires no novel structures and permits a live system to realize a variety of adaptation reactions before triggering synthetic and resynthetic processes underlying hyperplasia of cellular organelles and increase of the system's volume.

There is evidence that compensatory adaptive processes in injured myocardium represent a set of stereotypical changes in CM ultrastructure and normal heart reactions to damaging factors [9,10,17]. Mitochondria undergo the greatest spectrum of changes, which is explained by the level of their function, conditions of this function, and cell requirement in ATP energy [1]. The number of MC in a cell, number and pattern of their cristae packing, and frequency and intensity of heart beats and level of its metabolism are directly related [6,9,10]. Therefore, MC forming energy potential of the cell and thus ensuring the function of any live system remain an important object of research carried out in health and disease.

It was shown that in murine heart cells MC are bound by special structures: intermitochondrial contacts (IMC), representing zones of increased electron density at sites where the external membranes of neighboring MC are drawn together [3] (Fig. 1, *a-c*). IMC are very stable structures [16], and MC groups united by them (clusters) synchronously loose their energy potential if one organelle is pierced with a laser beam [2]. The major part of studies in this problem are carried out by biologists and are devoted mainly to normal distribution and structure of IMC [5]. On the other hand, it is obvious that if these structures really unite MC in a common system, they play an essential role in mechanisms of CM energy supply, particularly in heart diseases of different origin. However, only few scientists discuss this problem [4,7,8].

From this viewpoint, it is very important to evaluate IMC as a probable compensatory adaptive mechanism which was not studied during and after heart diseases. Our aim was to follow up the CM IMC reaction during a slowly progressing disease characterized by relatively stable compensation and a favorable course, acute poisoning rapidly eventuating in death from heart failure, and during a subacute process when animals survive and compensatory adaptive reactions start to develop in the heart. In addition, in order to understand the role of IMC in adaptation mechanisms, we followed up the changes in these structures after liquidation of pathological factor.

Experiments were carried out on 135 adult male white rats weighing 230-250 g. Chronic processes were induced as follows: myocardial hypertrophy by stenosis of abdominal aorta (days 3, 15, and 30 of experiment) followed by its regression (days 15 and 30 after elimination of aortic stenosis) and chronic alcoholic intoxication (inhalation of ethanol vapors for 98 days); acute heart involvement in purulent diffuse peritonitis (intraperitoneal injection of 10% fecal suspension) from which all animals died, and a subacute process induced by ligation of pancreatic duct and splenic vein, running a relatively benign course and eventuating in recovery. These models represent a wide spectrum of diseases occurring in practical medicine. Left-ventricular myocardium was examined by morphological and morphometric methods. Mitochondrial population in CM is heterogeneous in structure and function [18], and therefore, the number of IMC was analyzed for 3 cell zones: interfibrillar, subsarcolemmal, and perinuclear.

In control samples many organelles were bound by IMC in all MC subpopulations. The number of IMC in the interfibrillar zone was 36.3±2.4, in subsarcolemmal 44.3±4.0, and in perinuclear zone 44.6±3.8 per 100 MC. The mean number of MC cristae was 28.4±2.1.

Histological study of cardiac muscle on day 3 after induction of myocardial hypertrophy showed plethoric capillaries, erythrocyte sludging in capillaries and venules, and contractures of some CM. Electron microscopy showed damage to individual CM with sarcoplasmic edema. The signs of hyperfunction were observed in the majority of CM: the contours of nuclei were irregular and secondary lysosomes accumulated near the nuclei. Accumulations of MC in all 3 zones of CM were larger than normally, organelles were swollen, their cristae fragmented and homogenized, and matrix homogenized. The number of IMC decreased only in the interfibrillar zone of CM (to 46.7±3.7), in two other zones it decreased to 34.4±2.9. Volume density of myofibrils decreased significantly, as did the number of cristae in MC (to 19.6±0.7), which may result from myocardial hyperfunction and a predominance of destructive processes in the cell over synthetic during this period.

After 15 days, the number of CM changed by contraction and their morphological heterogeneity increased; there were cells with pronounced sarcoplasmic edema, large foci of myofibril lysis, myofilament fragmentation, and karyopyknosis. Polymorphism and swelling of MC progressed. In all CM

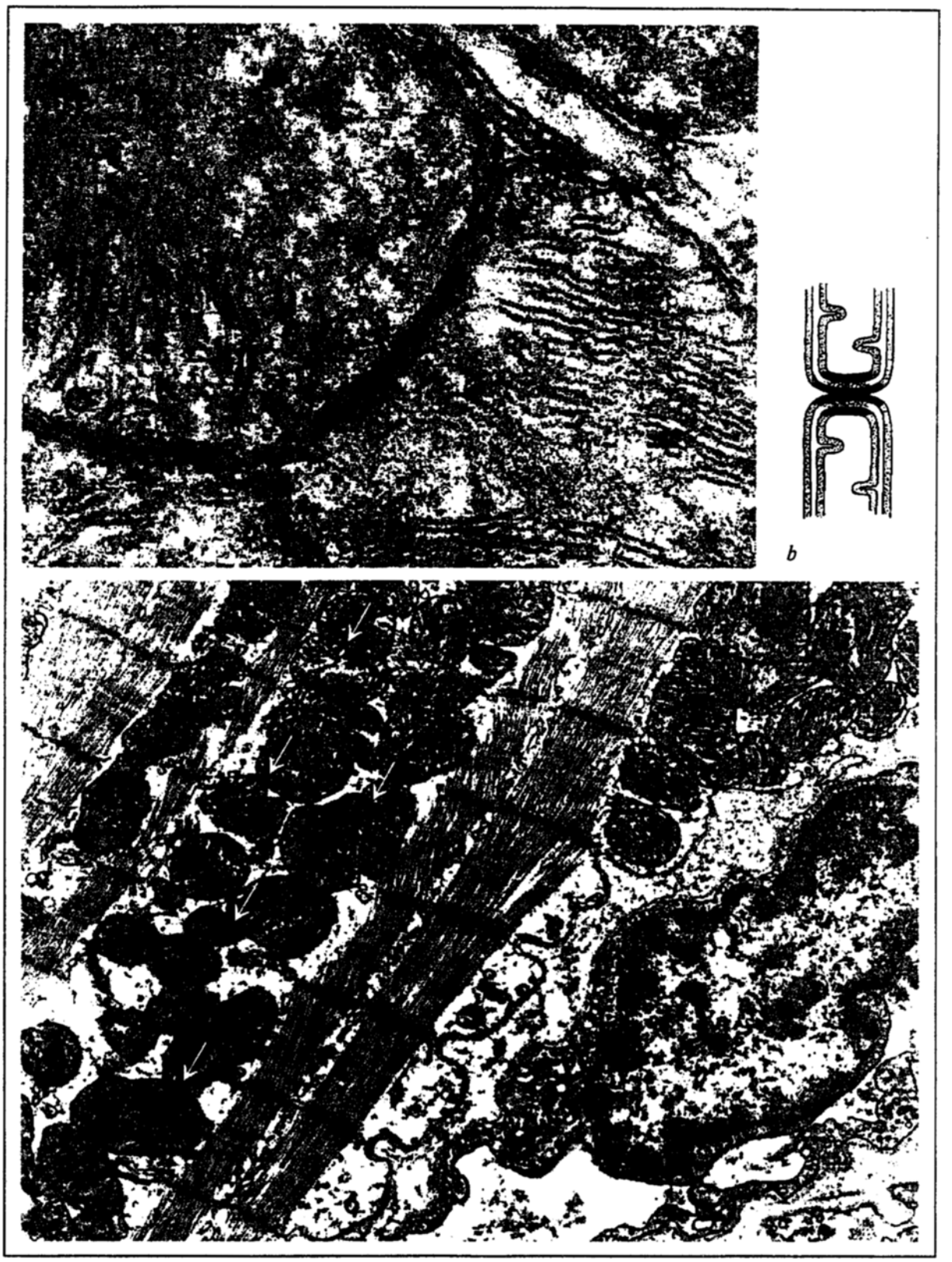

Fig. 1. Intermitochondrial contact in cardiomyocytes (*a*), its structure (*b*) [3]; *c*) intermitochondrial contacts in cardiomyocytes are shown with arrows. *a*) ×50,000; *c*) ×15,000.

zones these organelles formed large accumulations in which the number of IMC almost doubled (to 73.1±4.3). The number of cristae in MC increased to 24.9±1.9. Under sarcolemma, MC hyperplasia was most expressed in cell sites closest to capillaries.

Thirty days after stenosis of the abdominal aorta, the heart weight increased by 43.6%. Histological examination showed CM hypertrophy and moderate growth of perivascular and interstitial stroma. The absolute area of transverse section of CM was 577.23 μm^2, which is twice as large as in the controls. The parenchyma/stroma ratio changed: the relative volume of stroma increased 1.3 times and the relative volume of parenchyma decreased 1.1 times in comparison with the control. The overwhelming majority of MC formed clusters united by very dense and long IMC. Moreover, these contacts were detected between organelles with destroyed cristae and matrix and intact membrane. The number of IMC increased 1.85 times in comparison with the control, but the number of cristae in MC dropped in comparison with the previous check-up. This may be indicative of exhaustion of energy and plastic reserve of hypertrophic myocardium.

Microcirculatory disorders and moderate sclerosis of perivascular and interstitial stroma observed after abdominal aorta stenosis had been eliminated and cardiac hypertrophy regressed for 15 days. Damaged CM with decreased number of MC were seen. Organelles were irregularly oriented and there were destructive changes in many of them. In relatively intact CM, MC formed accumulations in which the number of IMC was almost one-third less: 44.9±3.3, i. e., virtually normal. The number of cristae in MC increased, remaining smaller than in the control.

Thirty days after elimination of aortic stenosis, heart weight decreased significantly, being only 15.8% higher than in control. There were small foci of sclerosis in the myocardium, and CM remained slightly enlarged in comparison with the control. The absolute area of a CM transverse section was 413.07 μm^2, which is significantly less than on day 30 of myocardial hypertrophy (577.23 μm^2). The parenchyma/stroma ratio remained the same as at the height of myocardial hypertrophy. The size of CM nuclei and myofibril thickness notably decreased. MC decreased in size and continued to form accumulations, but did not join each other so tightly as in previous experiments. The number, length, and electron density of IMC markedly decreased and sometimes they were absent even between associated MC. The number of IMC was lower than in the control in different zones of a cell (33.3±2.2).

Plethoric capillaries and moderate sclerosis of perivascular and interstitial myocardial stroma were found in animals subjected to chronic alcohol intoxication. Many CM were hypertrophied, although atrophic cells with foci of myolysis were also noted. Electron microscopy of hypertrophic CM showed hyperplasia and swelling of MC containing numerous cristae varying in shape; many organelles had the signs of destruction. MC formed large accumulations, particularly in the subsarcolemmal zone of CM. Virtually all of them were united in clusters by long IMC with high electron density. The number of IMC was as high as 99.2±3.7, which is 2.4 times higher than in the control.

Microcirculatory disorders, stromal edema, and pronounced contractures of individual CM and of CM groups with myofibril fragmentation were found in the myocardium of animals which died from acute diffuse peritonitis. Sarcoplasmic edema and numerous foci of myofilament lysis were seen in the majority of CM. Mitochondria were swollen, there were marked destructive changes and even ruptures of external membranes in the majority of organelles. The number of cristae dropped, particularly in organelles of CM interfibrillar zone. Sometimes there were small accumulations of MC without IMC. Relative volume and surface density of MC dropped, which objectively confirmed their destruction.

By the end of the first day of acute pancreatitis the condition of rats deteriorated considerably; after 3 days their motor activity increased, and by day 7 they looked normal. Sludges and microthrombi in some capillaries and venules and empty microvessels, numerous mosaic lesions such as CM contractures and sarcoplasmic edema in some cells were revealed in the myocardium of rats sacrificed on day 1 of experiment. Electron microscopy showed small foci of myofilament lysis in some CM. Mitochondria formed small groups, in many of them matrix vacuolization and fragmentation of cristae were seen. Electron density and mean number of IMC decreased to 31.2±0.9 *vs.* 41.7±2.5 in the control. In parallel with this, volume and surface density of MC and the number of their cristae decreased, probably due to progressing destruction of organelles. On day 3 electron microscopy showed accumulations of MC, the majority of which were united in clusters or associations by IMC. The number of IMC increased to 46.6±1.0, i. e., it was slightly higher than in the control. On day 7, moderate hypertrophy of CM was observed. The overwhelming majority of MC were swollen, their number in accumulations increased considerably, the majority of organelles were united in clusters by long IMC with high electron density. The number of IMC increased in all cell zones to 76.7±3.2.

Thus, myocardial exertion in all experiments led to CM hyperfunction requiring an adequate energy

supply to hyperfunctioning myofibrils [9]. The necessary energy can be supplied only at the expense of hyperfunction of MC resulting in their destruction. The total number of MC and IMC decreases. Energy deficiency develops, which signals triggering of compensatory processes. Under such conditions, a decrease in the number of MC and their cristae may be compensated by MC clusters with IMC, which maintains heart rate in acute pancreatitis and aortic stenosis. In extreme exertion of the myocardium, when destructive changes surpass a certain threshold, MC are destroyed and IMC disappear. This is a morphological indicator of energy depletion causing cardiac failure. Such a picture is observed in acute diffuse purulent peritonitis. Therefore, formation of associations from damaged MC by means of IMC provides a necessary level of energy and permits the organism survive in moderate heart exertion. Every pathological process is characterized by specific morphological features due to its etiology.

If animals do not die during the first days of disease, compensatory adaptive processes develop, aimed primarily at increasing the ATP energy production. In order to ensure cardiac function, this level of energy should be sufficient for maintaining myofibril hyperfunction and reproduction of destroyed organelles. It can be achieved by hypertrophy and hyperplasia of intracellular structures, primarily MC and their cristae. Such processes start on day 3 of acute pancreatitis. However, during this period myocardial injuries are still evident, the number of cristae and hyperfunctioning MC is decreased, and energy deficit persists. The number of IMC rapidly grows, leading to formation of still larger MC associations in CM after 7 days of experiment. This compensatory reaction may improve energy supply to the heart and involves no pronounced MC hyperplasia and hypertrophy.

In chronic diseases — myocardial hypertrophy and alcoholic cardiomyopathy — the above-mentioned compensatory reactions are observed, including hypertrophy and hyperplasia of organelles. The number of MC cristae does not reach the control level. This can be explained by the fact that even in a hypertrophied myocardium, CM hyperfunction persists and progresses if the cause of heart exertion is not liquidated [9]. In addition, the myocardial stroma/parenchyma ratio shifts toward predominance of the stroma. Therefore, the major part of energy is spent on maintaining cardiac contractile function and the lesser part for biosynthesis and repair of subcellular structures. As a result, energy deficit still persists in a certain measure, causing MC hyperfunction and destruction, and the loadover of retained or reproduced cristae progressively increases. A vicious circle is formed, and this imbalance programs the "delayed" cardiac failure. Part of CM die in chronic alcoholic poisoning. Surviving cells continue functioning, which results in their hyperfunction, hyperplasia of intracellular structures, and hypertrophy of CM. The number of cristae in MC is decreased. In both experiments the number of IMC sharply increased under such conditions, as did associative activity of MC. This probably largely balanced the impaired energy homeostasis, although did not liquidate energy deficit completely.

When a harmful exposure ceases (abdominal aortic stenosis is liquidated), CM hyperfunction is needed no longer, there is no need in increased energy supply to these cells, and hence, no cause of MC hyperfunction. Disappearance of energy deficit as the basic cause of compensatory reactions in a damaged heart brings about a decrease in the content, length, and electron density of IMC, and in MC associative activity, which in this case is a purely adaptive reaction.

Thus, reaction of MC associations formed by means of IMC is one of the first reactions in a complex of structural and functional manifestations of compensatory adaptive mechanisms in the heart. It is probably the key compensatory adaptive reaction providing CM function at the initial stages of pathological processes in the heart. The recombination theory [14] about two variants of changes in substance qualities in the course of adaptation to changed conditions of functioning helps understand the essence of this reaction: the quality of a substance changes 1) due to quantitative changes in constituent structural elements and 2) due to structural recombinations, i. e., alteration of mutual disposition of the system's elements with their quantity unchanged. Structural recombination of a substance results in appearance of its new quality capable of providing the necessary quantity of the structure's function as well. This variant of adaptation may require the minimum time and is therefore of the greatest importance under conditions of heart disease. Its efficacy largely determines the probability of overcoming the emergency stage of disease by the heart and of further development of MC hyperplasia and hypertrophy, requiring a longer time. Thus, the first and the second variants of recombination theory take place during heart adaptation to new conditions of functioning. However, the reaction of MC association by means of IMC is no less important during the stage of relatively stable compensation, because it is probably due to this reaction that the necessary level of energy supply to hyperfunctioning CM is maintained under conditions of incomplete resynthesis of MC cristae. The last but not the least, in

adaptive processes such as myocardial hypertrophy regression after its overexertion is liquidated, a decrease in the number and size of MC associations due to a decrease in the number of IMC contacts is apparently one of the first reactions reducing energy production. The amount of energy providing for reproduction of intracellular structures is thus reduced, and, as a result, the volume of CM and myocardium decreases.

The formation of MC associations by means of IMC is a stereotypical reaction. It is a biological regularity which does not depend on the etiology of heart involvement and largely influences the disease pathogenesis, duration, and outcome.

Thus, we can assert that the IMC are labile structures increasing the number and size of cardiomiocyts MC associations in hyperfunction of contractile myocardium under conditions of energy deficit, thus stimulating energy production and transportation. This intracellular reaction is stereotypical and does not depend on the cause and type of disease. It possesses the properties of a biological regularity and is characterized by a drop in the IMC number at the beginning of pathological process in the heart, IMC hyperplasia with increase in the number and size of MC associations during relatively stable compensation, and, finally, by decrease in IMC number and MC associations after liquidation of the cause of pathological process. If a pathological exposure of the heart is extremely grave, destructive changes in MC predominate over resynthesis processes, and the number of IMC drops, involving disintegration of MC associations; this causes energy depletion of the myocardium and heart arrest. After cessation of heart exhaustion which caused myocardial hyperfunction, the number of IMC and MC associations in CM decreases, reflecting heart adaptation to new conditions of functioning, involving no MC hyperfunction or increased energy supply to the cells. Thus, the detected regularity in formation and disintegration of MC associations by means of IMC hyperplasia or destruction during heart exercise under conditions of disease or during heart adaptation to decrease of myocardial exercise indicates that these processes are aimed at regulation of energy production and transportation in contractile myocardium. Therefore, they should be regarded as one of the most important compensatory adaptive reactions of an injured heart supplying energy to hyperfunctioning CM before MC hyperplasia and hypertrophy.

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