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SYSTEMIC MORPHOFUNCTIONAL ASSESSMENT OF THE STATE OF CARDIOMYOCYTES OF EXPERIMENTAL ANIMALS AFTER PROLONGED PHYSICAL LOAD WITH SUBSEQUENT REST

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Research relevance. Modern elite sport needs continuous improvement of training methods and technologies in the training process, taking into account the individual characteristics of the athletes' body.

In accordance with these requirements, coaches choose, in their opinion, the most optimal training regimens for athletes long before the competition and immediately before the competition, often paying tribute to not entirely justified ideas that the greater the training load, the higher the sports results shown by the athlete should be. In this process, breakdowns sometimes occur when athletes are not able to show seemingly objectively expected results, and in rare cases it ends tragically, even with the sudden death of athletes [3, 5, 6]. To clarify many issues related to the regimen, duration, level of training loads [2], it is advisable to reproduce models on experimental animals [4, 8, 9, 10, 11]. In many sports medicine studies, when studying the state of the cardiovascular system, even under experimental conditions, the emphasis was on organometric or electrocardiographic moments [7, 8,]. And what happens inside the heart muscle cell itself remains a little consecrated question.

Our morphofunctional studies of the cardiac muscle of experimental animals at the cellular and ultrastructural levels yielded unexpected results. The latter could shed some light on the above problems and curiosities. The problem was that in experimental

animals that were trained through swimming, the magnitude of the force of contraction of the myocardial strip sample varied over a very wide range, despite the fact that the conditions for keeping and training were the same for all animals. Later it became obvious that the observed unexpected result are related to the problem of physical overload, fatigue of athletes during exhausting training or competition, to the problem of restoring the body of athletes after large long-term limit loads. It also became clear that both the observed phenomenon and its analysis are of significant practical importance.

Research aim: to find out the nature of the impact of a combination of extreme physical activity and different duration of post-exercise rest on the function of myocardial contractility in experimental animals.

-To achieve this goal, the following tasks were set:

1. Carry out a comparative analysis of the patterns of ultrastructural organization of cardiac muscle cells in two groups of animals: a) normal healthy animals living with normal daily physical activity, and b) animals adapted to prolonged maximum physical activity.

2. Assess the strength of myocardial contractility using a multivariate systemic quantitative morphological analysis based on the methodology of Quantitative Functional Morphology (QFM).

3. Check the adequacy and evaluate the accuracy of the QFM method for measuring cardiomyocyte myocardial contractility by conducting simultaneous and parallel measurements of the force of myocardial contractility using a direct measurement method by micromechanography.

4. Carry out a comparative correlation analysis of the factors that may cause the detected scatter in the data on myocardial contractility in swimming rats.

Research methods and organization. The study was conducted on 17 practically healthy outbred male white rats, weighing 230-280 grams. Of these, 5 animals served as controls with normal physical activity in cage, the remaining 12 rats were subjected to forced daily swimming. The swimming course was successfully completed by 5 animals, which, along with control rats, underwent further research. The study used 6 main methods: 1. Modeling physical activity in the form of daily one-hour swimming for 55 days with rest every 7th day. The duration of swimming at the beginning was 15-30 minutes, increasing to 1 hour by the end of the first week. Animals swam in a group, in a bath indoors, with water at room temperature, in July-August-September. The water level in the bath was higher than the

standing height of the rats, so they could not reach the bottom with their feet to rest.

2. From five control rats and from five swum rats, after slaughter in compliance with the rules of euthanasia, the heart, several skeletal muscles from the hind limb, and lungs were taken, which were subjected to histological and electron microscopic studies.

3. A longitudinal piece of the heart muscle measuring 6x2x2mm, cut from the intramural section of the middle third of the left ventricle lateral wall, was handed over to an employee of the Laboratory of Molecular Cardiology of the Institute of Cardiology of the Ministry of Health of the Republic of Armenia. The mechanical activity of the strip was recorded using a 6Mx2B mechanotron force sensor in the isometric mode. Pieces for electron microscopic examination of the myocardium were also cut out from the immediate adjacent section of the indicated zone. A number of indicators of myocardial contractile function were measured on a micromechanograph.

The recorded curves were used to determine the contraction parameters: Pmax-maximum contraction amplitude, P/S-maximum contraction amplitude relative to the strip cross section, dP/dt-stress development rate, relaxation rate.

4. On electron microscopic images of transverse and longitudinal sections of cardiomyocytes, morphometric and stereological studies of mitochondria, mitochondrial cristae, myofibrils, myofilaments, T-system, sarcoplasmic reticulum and sarcoplasm were carried out. Their specific numerical, area and volume parameters were determined (Svcrist, Svmit, Svmfb, Vvmfb, Vvspl.).

5. According to the morphometric and stereological data of the ultrastructures of cardiomyocytes, a virtual integrative systemic indicator of the contractile potential of cardiomyocytes was measured, calculated according to the mathematical model developed by us and the methodological approach, which we called the Methodology of Quantitative Functional Morphology (Fig. 1). The method is recognized as an invention and patented in the Russian Federation [1].

6. Digital data were processed by the methods of variation statistics. Correlation analysis was carried out using the computer program Excel.

$$f_{cdct} = K \bullet Vv_{2_{mfbr}} \bullet N_{ATP} \bullet \varphi =$$

$$= K \bullet Vv_{2_{mfbr}} \bullet \left(\frac{1}{\frac{1}{Sv_{crst}} + \frac{1}{Sv_{mit.fr} \bullet 4{,}49} + \frac{Vv_{spl}}{452{,}11} + \frac{1}{a \bullet Sv_{mfb} \bullet 4{,}49} + \frac{Vv_{mfb}}{3067{,}21}} \right) \bullet v_1 \bullet \varphi.$$

Figure 1. Mathematical model for measuring the virtual maximum contractile potential of a unit volume of cardiomyocytes.

The studies were carried out in the period from 1985 to 2022, at the Central Scientific Research Laboratory of the Yerevan Institute for Advanced Medical Training of the USSR Ministry of Health, later in the National Institute of Health of the Health Ministry of the Republic of Armenia, at the Laboratory of Molecular Cardiology of the Yerevan Research Institute of Cardiology of the Ministry of Health of the Arm. SSR, at the Virchow Institute of Pathology, Charite Medical Faculty of the Humboldt University, Berlin, in the German Heart Center Berlin, in the after Orbeli Institute of Physiology NAS RA. The following devices were used: an LKB-III ultramicrotome, an EMV 100L transmission electron microscope, a micro-mechanograph with a 6Mx2B mechanatron force sensor, a CARL-ZEIS Amplival light microscope, computers with Word, Excel, Picture Manager, and Photoshop programs, Internet.

Analysis of research results. In order to maintain the objectivity and independence of the studies by two different methods, we did not discuss the results of the study until the final data of the micromechanographic and QFM studies were ready. When we began to compare the data obtained, the following picture caught my eye. In swum rats, the QFM contractility index was on average 26.5% higher than the QFM indicator of the myocardial contractility force of healthy control rats, that is, prolonged swimming caused adaptive changes and the myocardium became almost a third more enduring than the myocardium of control animals. At the same time, while in healthy control QFM rats, the cardiomyocyte contractility index of 5mm3 varied from the average value within 16%, then in all five swum rats, the spread in the myocardial contractility index was greater (27%). When we began to compare the values of the contractility forces obtained by

the two methods, we revealed a certain parallelism between the curves. Correlation analysis showed a high direct correlation between the contractility data of both methods (correlation coefficient r=0.862).

It became noticeable that the strength of contractility in swum rats generally tends to increase from the first animal to the last (Table 1). However, a very significant spread in the values of micromechanographic indicators of contractility was a real surprise for us. This scatter between the largest and smallest values of the contractility index in swimming rats was almost 7 times greater (240%) than that of the contractility index determined for the same animals by the QFM method (27%). At the beginning, such a large scatter in swum rats suggested the inaccuracy of the experiment, the non-standard conditions of the micromechanography process for different animals. However, being confident in the high professionalism and conscientiousness of the employee of the Institute of Cardiology, then after discovering the parallelism between the contractility data curves obtained by the two methods, we saw in this spread a challenge, that requires study and explanation.

We were looking for an explanation for this phenomenon, we began to analyze whether there were differences between animals. We found out that due to the fact that one micromechanographic study could be carried out in a day, this process dragged on for five days. The main difference between the animals was that some of them were at rest after a 55-day swim, that is, they did not swim for a different number of days. The first rat (37u) was decapitated the day after the last day of swimming, and the rest of the rats rested from one day (39u) to four days (42u). And the idea arose that the value of the duration of rest played a positive role in showing higher values of contractile force. The correlation analysis between the contraction force and the number of days of rest was high both for the QFM indicator (r=0.633) and for the micromechanographic indicator (r=0.767). This concept or pattern is somewhat "violated" by the first animal. We tend to attribute this to the individual characteristics of the animal. Without taking into account the first animal, the correlation indicator (r) increases significantly: for the QFM indicator: from r=0.633 to r=0.787, and for the micromechanographic indicator: from r=0.778 to r=0.920.

There was a need to look for ultrastructural equivalents of the functional inequality observed in swum rats. In general, in control, normal rats no significant qualitative difference was observed in the ultrastructural picture. Naturally, the morphometric and stereological parameters differed, as a result of which different values

of the QFM contractile force were obtained. But in swum rats, a noticeable enlargement of mitochondria, an increase in the density of cristae, and thickening of myofibril bundles were observed. Along with these changes, which were common to varying degrees for all swum rats, there were also some qualitative differences in the pattern of ultrastructural organization within the group of swum rats, especially between swum rats with relatively low and high contractility indices. These changes were taken into account by morphometric and stereological parameters, and they were reflected both in the value of the integrative indicator of QFM contractility (fcdmc) and in the micromechanographic indicators of contraction force Pmax.

We found it expedient to briefly compare the features of the ultrastructural patterns of only those animals whose heart muscle showed the highest and lowest values of the contractility index both among five swimming rats and among five control rats (Tables 1 and 2).

Table 1.

Cardiomyocyte contractility data from swum rats obtained by two methods. Micromechanographic data were obtained at an impulse frequency of 0.3 Hz, (M±m).

Parameters	Swum animals				
	Rat 37i	Rat 39i	Rat 40i	Rat 41i	Rat 42i
Pmax. (g)	0,0703± ±0,0006	0,0384± ±0,0003	0,0322± ±0,0001	0,1204± ±0,0007	0,2492± ±0,0008
fcdm. (5mm3)	2880±135	2786±141	2522±135	3236±180	3307±154

These animals are, from swum: No. 42i (highest score) and No. 40i (lowest), and from control animals Rat 2 control (highest), and Rat 1 control (lowest). This numbering corresponds to the marking of animals shown in Figure 1 and in Tables 1 and 2. We note right away that in all ten animals, no pathological structural changes were found in cardiomyocytes at the ultrastructural level. Changes relate to adaptive processes.

Table 2.

Data on indicators of contractility of cardiomyocytes (fcdm. 5mm3) of control, practically healthy rats (M±m).

Parameters	Control animals				
	Rat1	Rat 2	Rat 3	Rat 4	Rat 5
fcdm. (5mm3)	2096±75	2467±101	2353±101	2328±98	2400±110

But there are similarities between animals 42i and Rat1 control. This means not only that they have the highest rates, each in their group, but there is also a similarity in the ultrastructural organization. Both animals have active mitochondria, rich in cristae, with an enlightened matrix. The shape of mitochondria approaches round or oval. Mitochondria are arranged in columns with a width of one or one and a half mitochondrion. The bundles of myofibrils are relatively thickened, with a more direct course. This once again emphasizes the importance of individual differences for the contractile function of the heart. That is, not only the duration of post-exercise rest could play a favorable role.

In this aspect, it should be pointed out that there is a certain similarity between the ultrastructural patterns of cardiomyocytes in rats 42i and control Rat 1 with the ultrastructural pattern of myocytes of the flying muscles of insects, in particular wasps. In both cases, there is a very rational and economical use of space, the maximum reduction in the time of transfer of macroergs from mitochondria to myofibrils. The shape of myofibril bundles provides them with a

minimum expenditure of forces to overcome friction against the walls of “hollow cylinders” formed from mitochondria. Most mitochondria have the appearance of uniform triangles with concave sides, which provides the maximum contact surface for exchange with myofibril bundles. The shape of the latter, in turn, provides a reduction in friction of the outer surface, fast and uniform delivery of macroergs to all sections of the myofibril bundle, including its central section. Similar shapes and sizes of myofibril bundles provide maximum synchrony and maximum total force of contraction, as occurs in the myocytes of flying muscles of insects (several hundred times per second).

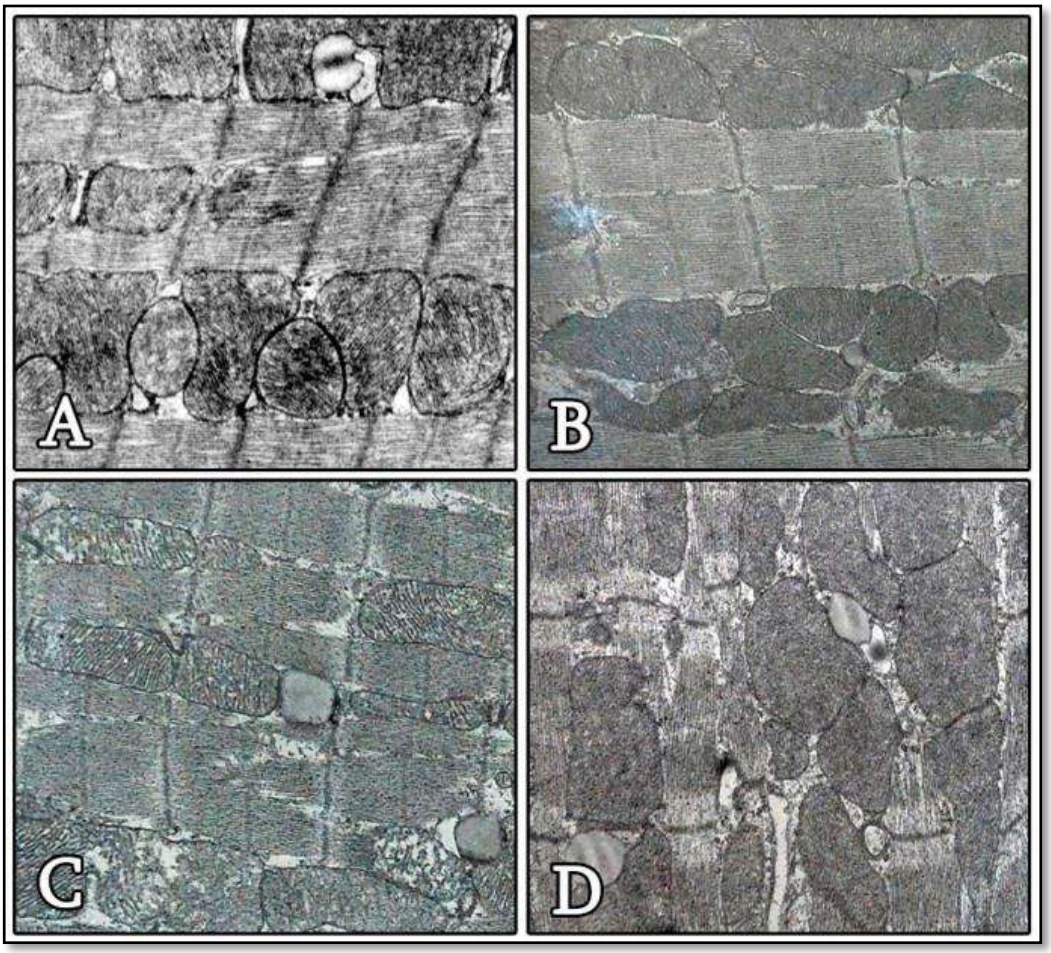

Figure 2. Ultrastructural changes in cardiomyocytes of swum (A-Rat 42i, B-Rat 40i) and control rats (C-Rat 2 control, D-Rat 1 control). Magnification: 26400x There is a markedly more ordered arrangement of mitochondria and myofibril bundles in cardiomyocytes A, B, and C.

Conclusions. 1. In swum rats, the contractile force potential was significantly higher than in control rats.

2. In both control-normal and swum rats, there was a significant scatter in the values of the contractile potential index.

3. The scatter in the values of the contractile force of the heart muscle is especially pronounced among the micromechanography data.

4. Individual, congenital features of the ultrastructural cytoarchitectonics of

cardiomyocytes also play a role in the ability of the myocardium to show high contractility data.

5. Rest after prolonged physical activity had a positive effect on the value of cardiomyocyte contractility, when post-exercise rest was at least three days.

6. The QFM method can be repeatedly reproduced on the same material, since the material embedded in resins remains for years.

7. The QFM method makes it possible to calculate the absolute margin of safety, the functional reserve of tissue biological systems, without its destruction. With micromechanography, the examined tissue sample is no longer suitable for research.

8. It is desirable to conduct experiments with a combination of intravital non-invasive functional studies with subsequent systemic morphological determination of the strength margin of cardiomyocytes, myocardium based on the QFM methodology.

9. The detected features of ultrastructures, their spatial organization, in control animals are individual innate, and changes in ultrastructures, restructuring of their spatial organization in swum animals are adaptive-compensatory in nature.

11. The latter are superimposed on the already existing congenital type of ultrastructural cytoarchitectonics. In animals with an innate optimal spatial organization of the cardiomyocyte, such layering leads to optimal structural remodeling and, accordingly, special positive results.

12. Taking into account the characteristics of the body of athletes, it is advisable to choose for each athlete an individual mode of the duration and strength of loads, as well as the duration of post-load rest based on objective data, especially before the upcoming competitions.

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ՓՈՐՁԱՐԱՐԱԿԱՆ ԿԵՆԴԱՆԻՆԵՐԻ ԿԱՐԴԻՈՄԻՈՑԻՏՆԵՐԻ ՎԻՃԱԿԻ ՀԱՄԱԿԱՐԳԱՅԻՆ ՄՈՐՖՈՖՈՒՆԿՑԻՈՆԱԼ ԳՆԱՀԱՏՈՒՄ ՏԵՎԱԿԱՆ ՖԻԶԻԿԱԿԱՆ ԾԱՆՐԱԲԵՌՆՈՒՄԻՑ ԵՎ ՆՐԱՆ ՀԱՋՈՐԴԱԾ ՀԱՆԳՍՏԻՑ ՀԵՏՈ

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ՀՀ ԳԱԱ Օրբելու անվան ֆիզիոլոգիայի ինստիտուտ, Երևան, Հայաստան,
ՀՀ ԱՆ Սրտաբանության գիտահետազոտական ինստիտուտ, Երևան, Հայաստան

ԱՄՓՈՓԱԳԻՐ

Առանցքային բառեր: Մարզիկների ֆիզիկական վերականգնում, կարդիոմիոցիտ, միկրոմեխանոգրաֆիա, քանակական ֆունկցիոնալ մորֆոլոգիա:

Հետազոտության նպատակը: Պարզել էքստրեմալ ֆիզիկական ակտիվության և հետմարզական հանգստի տարբեր տևողության համակցության ազդեցության բնույթը փորձարարական կենդանիների սրտամկանի կծկողականության ֆունկցիայի վրա: **Հետազոտության մեթոդները և կազմակերպումը:** Հետազոտությունն անցկացվել է 10 սպիտակ առնետների վրա, որոնցից 5-ը գտնվել են նորալ, սովորական պայմաններում, իսկ մնացած հինգը ենթարկվել են ակտիվ ֆիզիկական ներգործության՝ երկարատև լողի միջոցով: Լողացած առնետների սրտամկանի շերտերի կծկման ուժը չափվել է միկրոմեխանոգրաֆիայի եղանակով: Էլեկտրոնային մանրադիտակի միջոցով ուսումնասիրվել են սրտամկանի կտորները՝ և՛ լողացած, և՛ նորմալ առնետներից: Օգտագործելով կարդիոմիոցիտների ուլտրակառույցների ստերեոլոգիական պարամետրերը և հատուկ մշակված մաթեմատիկական մոդելը, որը կոչվում է QFM, և որը ճանաչված է որպես գյուտ, հաշվարկվել է կարդիոմիոցիտների կծկման ներուժի ինտեգրատիվ ցուցիչ:

Ստացված արդյունքների վերլուծություն: Կծկման պոտենցիալի QFM ցուցիչի արժեքները լողացած առնետների մոտ միջինը 26%-ով ավելի բարձր են, քան նորմալ կենդանիների մոտ: Պոտենցիալ կծկման ցուցիչի արժեքների տատանումներ են հայտնաբերվել և լողացած (27%), և նորմալ առնետների (16%) մոտ: Կծկողականության միկրոմեխանոգրաֆիկ ցուցիչի արժեքը մեծապես տատանվել է (240%): Լողացած առնետների խմբում դրական հարաբերակցություն (կոռելյացիա) է հայտնաբերվել

կծկման ցուցիչի արժեքի և լողի ընթացքին հաջորդած հանգստի օրերի թվի միջև: Կարդիոմիոցիտների ներբջջային կառուցվածքային տարածային տատանումները հայտնաբերվել են ինչպես նորմալ, այնպես էլ լողացած առնետների մոտ:

Համառոտ եզրակացություն: 1. Առնետների երկարատև ֆիզիկական ակտիվությունից հետո հանգիստը դրական ազդեցություն է ունեցել կարդիոմիոցիտների կծկման վրա:

2. Նորմալ, չվարժեցված կենդանիների կարդիոմիոցիտների ուլտրակառույցների տարածական կազմակերպման անհատական առանձնահատկությունները բնածին են:

3. Լողացած առնետների ուլտրակառույցների տարածական վերակազմավորումները կրում են հարմարվողական-փոխհատուցողական բնույթ: Տարբեր կենդանիներ, անհատական առանձնահատկություններով պայմանավորված, ունեն բնածին տարբեր կարողություններ՝ ծանրաբեռնվածություններին լավագույն ուլտրակառուցվածքային հարմարվողականություն ապահովելու համար:

4. Ներբջջային վերակազմավորումները վերադրվում են արդեն գոյություն ունեցող ուլտրակառուցվածքային ցիտոարխիտեկտոնիկայի բնածին տեսակի վրա: Դեռևս մարզումներից առաջ կարդիոմիոցիտների օպտիմալ տարածական կազմակերպում ունեցող կենդանիների մոտ նման լրադրումը հանգեցնում է կարդիոմիոցիտների ավելի լավ կծկման:

5. QFM-ի եղանակով կծկման ներուժի չափումը համարժեք (ադեկվատ), միշտ վերարտադրվող մեթոդ է սրտամկանի բիոպտատի փոքրածավալ նմուշով, որը պարփակված է խեժի բլոկի մեջ:

СИСТЕМНАЯ МОРФОФУНКЦИОНАЛЬНАЯ ОЦЕНКА СОСТОЯНИЯ КАРДИОМИОЦИТОВ ЭКСПЕРИМЕНТАЛЬНЫХ ЖИВОТНЫХ ПРИ ПРОДОЛЖИТЕЛЬНОЙ ФИЗИЧЕСКОЙ НАГРУЗКЕ С ПОСЛЕДУЮЩИМ ОТДЫХОМ

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АННОТАЦИЯ

Ключевые слова: физическое восстановление спортсменов, кардиомиоцит, микромеханография, количественная функциональная морфология.

Цель исследования. Выяснение характера воздействия сочетания предельных физических нагрузок и разной продолжительности постнагрузочного отдыха на функцию сократимости миокарда у экспериментальных животных.

Методы и организация исследования. Исследование проведено на 10 беспородных белых крыс, из коих 5 служили контролем (норма), а остальные пять подверглись физической нагрузке в виде продолжительного плавания. На полосках миокарда плававших крыс измерили силу сокращения методом микромеханографии. Кусочки миокарда, как плававших, так и контрольных крыс изучали методом электронной микроскопии. По стереологическим параметрам ультраструктур кардиомиоцитов и специально разработанной математической моделью КФМ, признанной изобретением, вычислен интегративный показатель потенциала сократительной способности.

Анализ полученных результатов. Величины показателя сократительного потенциала у плававших крыс в среднем на 26 %-ов выше, чем у контрольных животных. Обнаружена вариация величин показателя сократительного потенциала среди плававших (27%), и контрольных крыс (16%). Величина микромеханографического показателя сократимости варьировала в больших пределах (240%). В группе плававших крыс обнаружена положительная корреляция между величиной показателя сократимости и количеством дней отдыха последовавших за курсом плавания. Обнаружены структурные вариации внутриклеточной архитектоники кардиомиоцитов как контрольных, так и плававших крыс.

Краткие выводы.

1. Отдых после продолжительной физической нагрузки положительно отразился на сократимости кардиомиоцитов.

2. Индивидуальные особенности пространственной организации ультраструктур кардиомиоцитов животных без тренировки имеют врожденный характер.

3. Перестройки пространственной организации ультраструктур у плававших крыс носят адаптационно-компенсаторный характер. Разные животные, в силу индивидуальных особенностей, обладают разной способностью обеспечить оптимальную ультраструктурную адаптацию к нагрузкам.

4. Перестройки наслаиваются на уже существующий врожденный тип ультраструктурной цитоархитектоники. У животных, обладающих до тренировок оптимальной пространственной организацией кардиомиоцитов такое наслоение приводит к лучшим показателям сократимости.

5. Метод измерения сократительного потенциала по КФМ является адекватным, всегда воспроизводимым методом на заключенном в смолы мизерным биоптате миокарда.

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