ULTRASTRUCTURE OF THE MYOCARDIUM DURING PHYSICAL EXERTION, FATIGUE, AND REST

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Now that electron microscopy has provided much wider opportunities for the structural analysis of functional changes in organs, it is interesting to use this method to study the ultrastructure of the myocardium when it is working in different conditions—during increased functional activity, fatigue, and rest. The results of such a study could be useful, first, to provide a deeper insight into the pathogenesis and mechanisms of heart failure, and second, to develop ways of preventing this condition. They could also have a bearing on athletic medicine. No investigations of this nature could be found in the literature.

EXPERIMENTAL METHOD

Albino rats (20 animals) were divided into four groups: 5 animals swam in warm water (32-35°) for a single stretch of 4 h (group 1—physical exertion once); 5 animals carried out this procedure for 2 h twice a week for 6-10 months (group 2—training for physical exertion); another 5 animals swam for 6 h daily for 1-2 months (group 3—chronic overloading); and finally, 5 animals were controls (group 4). The animals were decapitated immediately after a routine exercise. Pieces of brain were fixed in Carnoy's fluid and embedded in paraffin wax. Sections cut to a thickness of 5 μ were stained by Nissl's method. Neurons of the cutaneo-motor area of the cerebral cortex, Area PA^m, layer 5, were studied. The cross-section of the neurons and of their nuclei in this zone was determined. This was done by obtaining the product of two mutually perpendicular diameters (major and minor) of the neuron and of its nucleus. In the cutaneo-motor area of each experimental animal, an average of 100 neurons and nuclei was measured (about 500 measurements to a group). The statistical analysis was carried out with the aid of a "Ural-2" computer. Pieces of myocardium were fixed in 1% OsO_4 solution and embedded in a mixture of butyl- and methylmethacrylates. Sections were cut on a type LKB 4801A ultratome and investigated with a type ÉM-7 electron microscope.

EXPERIMENTAL RESULTS

In the animals subjected to physical exertion once, moderate changes in the ultrastructure of the myocardium were observed. They took the form of swelling of the mitochondria, the formation of granules in them and a punctate translucency of their matrix, disorganization of the cristae to a certain degree, and clearing of the sarcoplasm. In the same cell, along with mitochondria showing these changes, many others had a normal structure (Fig. 1). When the animals were overfatigued as a result of prolonged daily exertion, these changes were much more marked. Most of the mitochondria were greatly swollen, their matrix was translucent, and the cristae were partly or completely destroyed. The sarcoplasm was translucent. The tubules of the sarcoplasmic reticulum were greatly dilated, and empty areas were seen between the myofibrils (Fig. 2). These changes were particularly well defined in a rat dying after carrying out these exercises for a month. In animals subjected to systematic moderate exercises (group 2), the ultrastructure of the muscle cells was only slightly different from that observed in the control (Fig. 3). In some sections from these rats particularly large groups of mitochondria were found, on the basis of which it could be assumed that their number had increased.

Examination of layer 5 of Area PA^m of the cortex of the control animals showed that hypochromic cells with signs of chromatolysis of peripheral type were present among the nerve cells of normochromic type. The nuclei of the neurons, like the neurons themselves, were of average size (mean cross sectional area of neuron 94 μ^2, of nucleus 45 μ^2) and rich in chromatin. The nucleoli were large, regular and circular in shape, and located centrally. The cutaneo-motor area of the cortex of the animals fatigued by swimming

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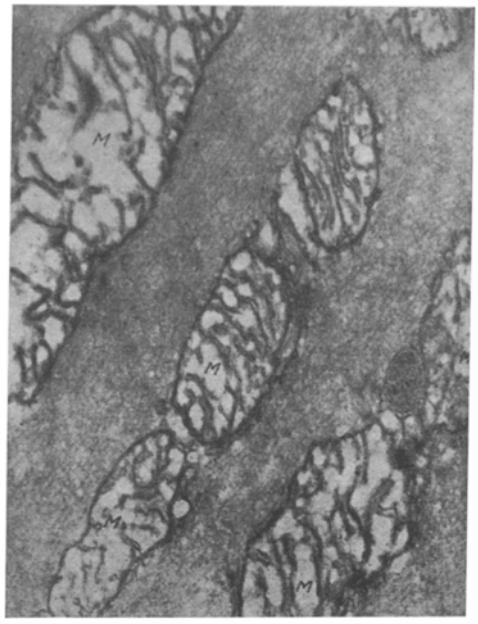

Fig. 1. Swelling of mitochondria (M), translucency of matrix, and disorganization of their internal septa in myocardium of a rat after physical exertion once. 30,000 ×.

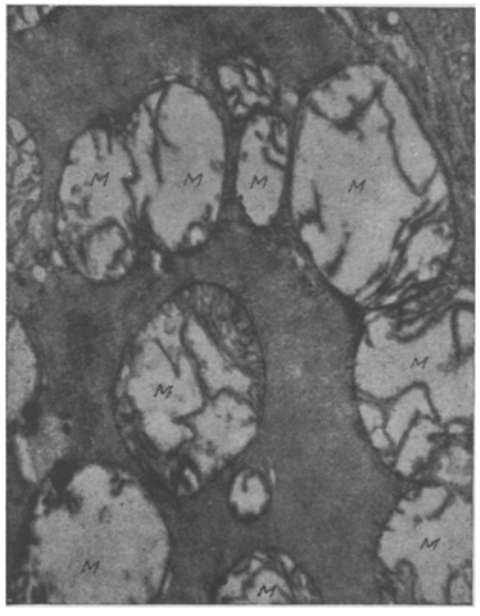

Fig. 2. Changes in the mitochondria (M) in the myocardium of a rat subjected to daily and prolonged physical exertion. These changes are more marked than those in Fig. 1. 30,000 ×.

contained large, clear, hypochromic cells. The Nissl substance of these cells was in a state of peripheral chromatolysis, the nucleus was lighter, and the nucleoli were in various states (ectopia, hypertrophy, disintegration, and atrophy). The mean cross sectional area of the neuron was 118 μ^2, and of the nucleus 59 μ^2. Investigation of the cerebral cortex of the animals receiving systematic, moderate exercises revealed definite preservation of the cytoarchitechtonic picture. Layer 5 of the cortex contained neurons of normochromic type. The nerve cells were rich in Nissl substance and sometimes peripheral chromatolysis was present. The nuclei were regular and circular in shape, with a centrally situated nucleolus and a well marked granular structure*.

The results of statistical analysis of the measurements of the neurons and their nuclei in Area PA^m of the cortex thus demonstrated that their values were raised in the animals exercised once only. Meanwhile, the amount of Nissl substance in the cytoplasm was reduced. In the animals receiving training exercises of moderate severity these changes were much less marked and the neurons of these animals were practically indistinguishable from the corresponding cells of the control rats.

The facts described above may be understood on the basis of modern concepts of the principles governing intracellular regenerative processes. Data reported in the literature, together with the results of the authors' experimental investigations, indicate that under the influence of various pathogenic stimuli the ultrastructures of cells suffer damage; but if the stimulation stops in good time, the fine structure of the cells is gradually restored and the injured organoids are repaired or renewed. It has also been discovered that intensive functional activity of cells is made possible and also accompanied by an increase in the number of their ultrastructures. The chief importance of these observations, made by means of the electron microscope, is the discovery of the regenerative and hyperplastic processes taking place continuously within the cell. The term "intracellular" can be suggested for this form of regenerative reaction and it has been identified as the most universal type of such reactions – found in all organs without exception. Finally, taking into account the data described above, corrections have been introduced into modern views of the principles governing regeneration in mammals and man [2, 3, 4, 5]. Similar statements have been made recently by other authors [7].

*The consultant for this part of the investigation was G. N. Krivitskaya, senior research worker at the Institute of the Brain, Academy of Medical Sciences of the USSR.

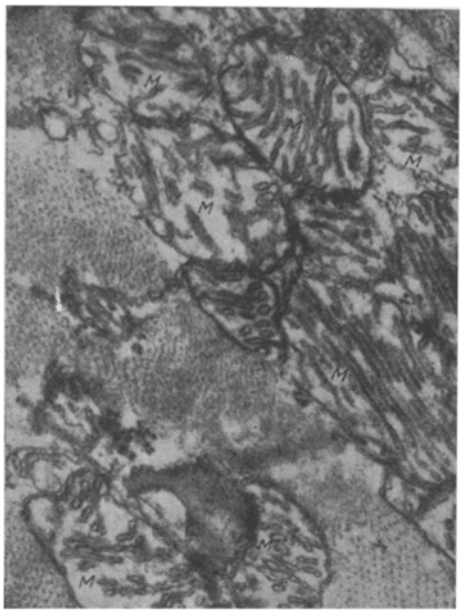

Fig. 3. Slight changes in mitochondria (M) in the myocardium of a rat subjected to physical exertion after preliminary training. 30,000 ×.

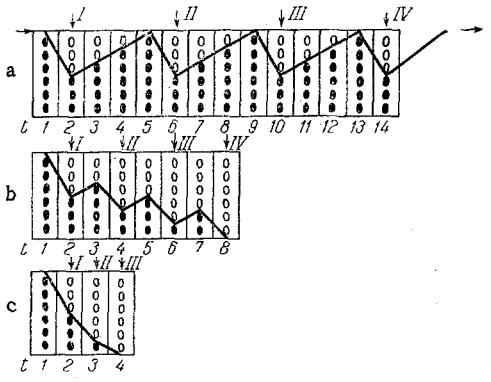

Fig. 4. Scheme representing relationship between frequency of exposure to fatigue and intracellular regenerative reaction. Black circles) unchanged mitochondria; unshaded circles) changed mitochondria; ↓) moment of action of fatigue.

The physiological prototype of intracellular reparative regeneration taking place after injury to organs is the continuous process of cyclic renewal of intracellular structures. It has been shown, for example, that the mean life span of the liver mitochondria is 10-12 days [6], of the myofibrils 30 days [8], and so on.

The fact that functional loads, if applied to animals periodically at sufficiently long intervals (2nd group of experiments) do not give rise to progressive changes in the ultrastructures of the myocardium can be explained in the light of the foregoing account by assuming that in the interval between the action of these forces the architectonics of the cells in fully restored. In other words, the rhythm of action of the disturbing forces coincides with the cycle of physiological renewal of the structures. In contrast to this state of affairs, in animals exposed more frequently (daily) to intense fatigue (3rd group of experiments), complete restoration of the ultrastructures had not yet taken place before each new exposure to fatigue and the material resources of the cells gradually became exhausted. This is shown schematically in Fig. 4, from which it will be seen that the faster the rhythm of action of fatigue on the cell, the less the cell was able to repair the damage to its ultrastructures before the next exposure to fatigue (Fig. 4, b, c). If complete recovery took place, however, exposure to fatigue could be repeated indefinitely without any after effects (Fig. 4, a).

The rate of renewal of the ultrastructures at which the regenerative cycle was able to reach completion in the intervals between exposures to fatigue can render the cell practically invulnerable. Hence, it follows that systematic and moderate functional loads can activate the rate of the intracellular repair process and maintain it continuously at a high level, while if these training exercises continue for a long time they lead to moderate hypertrophy of the cells, i. e., to an increase in the number of their ultrastructural components. This creates a reserve of structural and functional cell units and ensures a high degree of renewal; as we have seen, this may minimize the extent of injury to the organ caused by the action of a strong stimulus. One of the more important consequences of systematic functional loads on organs is thus what might be called the "training" of the intracellular physiological regenerative process. In contrast to this, absence of systematic exercises or a lowering of functional demands on an organ and on the cells composing it, may retard this process, leading to inhibition of regeneration of the ultrastructures and then to a decrease in their number. This is clearly seen in electron-microscopic investigations of the organs of animals subjected to starvation or to atrophy as a result of lowered function [1, 9]. With a sudden increase in the functional load, such cells will naturally be injured quickly. This may perhaps account for the greater sensitivity of the aging organism to functional loads: the rhythm of renewal of the cell ultrastructures is slower and protracted in these circumstances, and the total number of ultrastructures is fewer, so that the time interval between

successive actions of the disturbing forces, although adequate for regeneration of the organoids of a young organism, is not long enough in the old.

It must be considered that these remarks apply not only to physical loads, but also to the action of other factors on the organism. Under the influence of certain chemical substances, for example, changes take place in the ultrastructure of the cells, which is fully restored before the next exposure to the stimulus if the rate of application is not too rapid; but, these changes progress and terminate in necrosis when the interval between exposures to the chemical factor is too short for complete regeneration. The possibility is not therefore ruled out that this interrelationship between intracellular regenerative processes and various factors acting on the cell is a general biological principle.

The morphological results described above showed that during exposure to physical loads, definite changes in intracellular structures take place not only in the working organ (heart muscle), but also in the central nervous system and, in particular, in the cells of the cutaneo-motor area of the cerebral cortex. Uncontrollable and gradually increasing loads may lead to considerable structural changes in the cells of the myocardium and central nervous system. Conversely, loads alternating with periods of rest adequate to allow completion of the repair process gradually begin to be accompanied by progressively less marked changes in the cells of the central nervous system.

By regulating the work of the internal organs, in the course of this regulation, the central nervous system itself undergoes changes analogous to those taking place in the cells of the working tissues. The more intensive the functional activity of an organ, the more clearly defined the intracellular changes, not only in the organ itself, but also in the central nervous system. Hence it follows that the recovery period is not limited to intracellular reparative processes in the working organ, but it is occupied by such processes in the corresponding parts of the nerve tissues as well. An important condition of complete restoration of the working capacity of the organism is synchrony of the repair processes in the two systems – peripheral (working) and central (regulatory). Disturbance of this synchrony of these repair processes may be a cause of the onset of pathological states. In fact, complete restoration of the ultrastructure of the heart muscle cells after physical loading is not in itself evidence of adequacy of rest and a state of preparedness for work, without knowledge that the fine structure of the cells in nervous centers responsible for the control of the work of the heart and changing in the course of its work has likewise been restored completely to normal. If this has not happened – even if the normal structure of the muscle cells has been fully restored – their activity will be imperfect. In such cases it remains unexplained, as a rule, why the apparently normal myocardium has failed.

The cycle of physiological regeneration of intracellular structures is not stable. It is constantly changing, ensuring adaptation of the cell to differences in the working conditions of the organ. This regulation of the intensity of intracellular physiological regeneration and this continuous adaptation of its course to the requirements of the organism may be among the structural bases of the trophic function of the nervous system. Acceleration of the process of renewal of the ultrastructures and an increase in their number, accompanied by hypertrophy of the cells, are responsible for adaptation of an organ to external influences, i.e., for its state of training.

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