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EFFECT OF STRENUOUS PHYSICAL EXERCISE ON DESTRUCTIVE AND REPARATIVE PROCESSES IN THE RAT LIVER

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Most physiological, biochemical, and morphological investigations have been devoted to the study of the response of skeletal muscle to physical exercise. However, for a correct training program to be formulated it is necessary not only to study the state of the muscles but also to have some idea on how other vitally important organs and, in particular, the liver, respond to physical exercise. An important role in the solution of this problem must be played by the method of electron microscopy, for the limited light-optical evidence of the state of the liver during physical exercise demonstrates only an increase in the number of binuclear cells and differences in RNA and glycogen levels [2, 5, 6, 10].

EXPERIMENTAL METHOD

Experiments were carried out on 19 male Wistar rats weighing 200-300 g. The rats were trained to run on a treadmill at a speed of 35 m/min, which is regarded as work of high intensity [9]. One group of rats ran on the treadmill 5 times a week for 1 month; the other group for 1.5 months. Animals of the same age and sex, which were untrained, served as the control. All animals received food [9] and water ad lib. Pieces of liver were fixed in 2.5% glutaraldehyde solution in S-colloidal buffer at pH 7.2-7.4, and postfixed with osmium tetroxide. After training for a month material was taken immediately after the last training session (four rats) and 24 h later (five rats); two rats served as the control. In group 2 material was taken (four rats) immediately after the last training session, and four rats served as the control. The material was embedded in Epon and sections were stained by Reynolds' method and examined in the JEM-100C electron microscope. Relative volumes of mitochondria, rough endoplasmic reticulum, peroxisomes, and glycogen were calculated by the use of a random step grid [8].

EXPERIMENTAL RESULTS

A study of the ultrastructure of the liver cells of animals trained for 1 month and killed immediately after the last training session revealed no changes in the nuclei compared with the control. They contained small nucleoli, the heterochromatin was uniformly distributed throughout the nucleus, and some binuclear hepatocytes were seen. Mitochondria were more numerous in all hepatocytes than in the control (Table 1) and the principal changes took place in them and in the rough endoplasmic reticulum (RER). The degree of these changes differed not only in different hepatocytes of the same animal, but also in the cytoplasm of the same hepatocyte, suggesting individual sensitivity at both cell and organelle level.

The mitochondria showed a more or less palely stained matrix with the appearance of myelin figures (Fig. 1). Different parts of the same mitochondrion could react differently. Some mitochondria with swollen matrix and whose cristae

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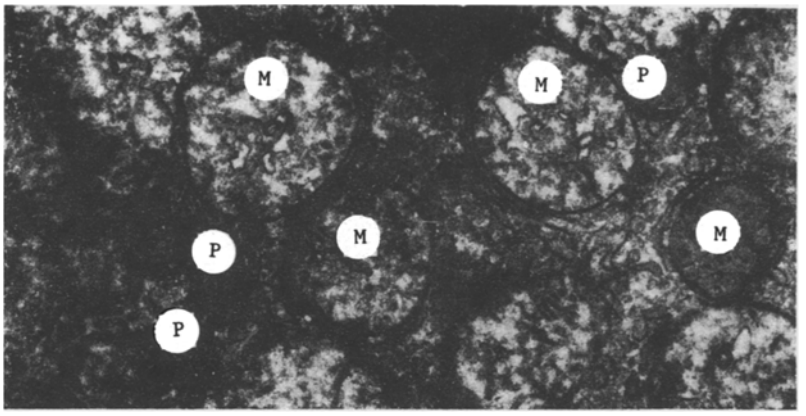

Fig. 1. Changed mitochondria in hepatocytes of rats trained for 1 month. 17,430×. Here and in Figs. 2 and 3: N) nucleus, M) mitochondrion, P) peroxisome, FD) focal degeneration.

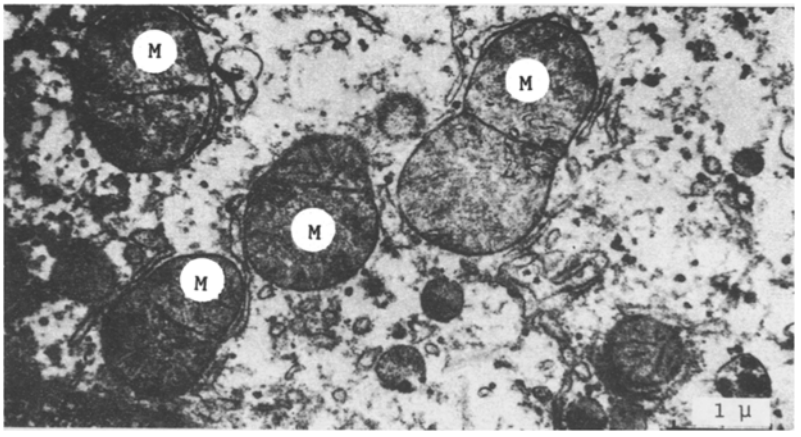

Fig. 2. Mitochondria dividing along cristae in hepatocytes of rats trained for 1 month (fixation 24 h after last training session). 13,860×.

had disappeared were completely separated by a crista from the unchanged part of the organelle. Even mitochondria with a swollen matrix were entwined by cisterns of the RER. Changes in the RER were characterized by the fact that the ribosomes on the cisterns were irregularly arranged and in some hepatocytes lengthened cisterns of the RER were preserved only around the mitochondria (which were normal in structure or swollen with a pale matrix). In some cells the whole cytoplasm, free from other organelles, was filled with vesicles of the smooth endoplasmic reticulum (SER) and with autophagic vacuoles. The Golgi complex was small in size but the ends of its cisterns were widened and contained floccular material. The Golgi complex was localized near the biliary capillaries, which were almost completely closed, unlike in the control.

Peroxisomes were found more often in the cytoplasm of all the cells than in the control (Fig. 1). They were uniformly distributed throughout the cell and varied in size from 0.5 to 0.9 μm. Fat and glycogen were absent in all hepatocytes. The Kupffer cells were large and phagosomes were found in their cytoplasm. Many sinusoids were dilated.

"Dark" hepatocytes were found in the liver in this series of experiments; their nucleus contained a loosely looped nucleolus and their cytoplasm contained many mitochondria and cisterns of the RER. Investigation of liver cells from rats killed 24 h after the last training session, after training for 1 month, showed that as before the hepatocyte contained changed mitochondria with a pale matrix (Table 1). Many hepatocytes contained autophagic vacuoles, evidence of death of some of their organelles. However, reparative processes predominated in the hepatocytes: many mitochondria with a very dense matrix and dividing along their cristae appeared (Fig. 2), the membranes of the RER became more uniformly covered with ribosomes, glycogen appeared, and "dark" hepatocytes also were present. Many peroxisomes were seen in the cytoplasm as before.

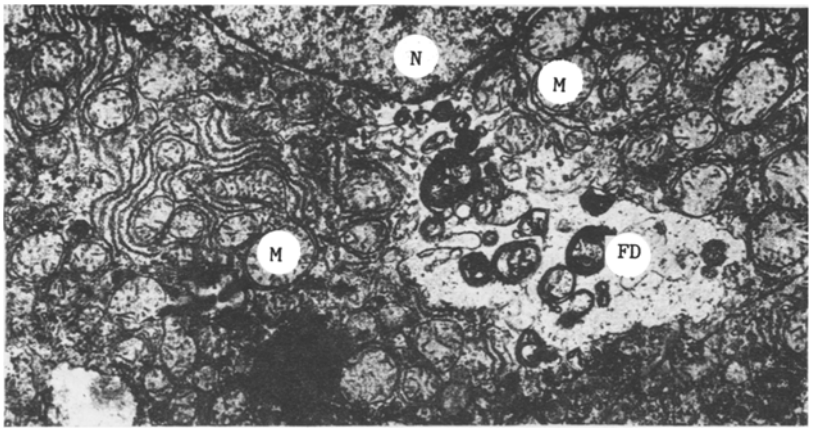

Fig. 3. Focal degeneration in hepatocyte of rat trained for 1.5 months. 7900×.

TABLE 1. Relative Volumes of Mitochondria (M), Rough Endoplasmic Reticulum (RER), Peroxisomes (P), and Glycogen in Hepatocytes of Rats Trained for 1 and 1.5 Months Compared with Control

Character of experiment	M	Swollen mitochondria	RER	P	Glycogen
Control	22,1±1,9	—	16,3±1,63	1,45±0,29	20,4±1,63
Training for 1 month, fixation immediately	24,25±1,69	9,5±1,08	14,7±1,47	3,05±0,21	—
Training for 1 month, fixation after 24 h	25±1,75	6,35±0,9	20±0,16	2,2±0,55	7,1±1,07
Training for 1.5 months	27,35±1,9	—	21,1±1,6	2,7±0,67	18,35±1,65

The study of the ultrastructure of the hepatocytes after running at a speed of 35 m/min for 1.5 months showed that a considerable degree of adaptation of the liver to strenuous physical exercise had taken place. The nuclei contained large, loosely looped nucleoli, there were some binuclear cells, and the nuclear membrane contained numerous large pores. All the mitochondria differed in their ultrastructure from the control. They contained a matrix of normal density and quite a large number of cristae. Calculations showed that the relative volumes of mitochondria, RER, and peroxisomes were increased compared with the control (Table 1). All mitochondria were entwined with long cisterns of the RER, parallel rows of which could be seen around the nuclei also. Among the profiles of the SER there were concentrations of glycogen granules. The Golgi complex showed a marked increase in size, mainly due to vacuoles with contents of varied density and to small vesicles. The Golgi complex was located around the closed biliary capillaries. The large Kupffer cells contained phagosomes. Compared with the control animals, the hepatocytes of rats of this experimental group contained less fat and a somewhat smaller Golgi complex, and nearly all their biliary capillaries were closed. In addition, unlike in the control, the cytoplasm of the experimental hepatocytes contained large foci of focal degeneration (Fig. 3). These consisted of areas of cytoplasm containing myelin figures and fragments of organelles (mitochondria) and membranes of the reticulum, surrounded by a membrane. These zones of focal degeneration were located in the hepatocytes close to a biliary capillary or sinusoid, evidently because the residual bodies are eliminated together with biliary products into the biliary capillary or into the Disse's space.

The investigation shows that during a month of training marked degenerative and reparative processes are observed in the hepatocytes. The degenerative changes includes swelling of the mitochondria, disappearance of the cristae, slipping of the ribosomes from membranes of the RER; reparative changes include the appearance of binuclear cells and of "dark" hepatocytes, division of mitochondria along the cristae, the appearance of "dark" mitochondria, and an increase in the number of peroxisomes. The individual sensitivity at cell and organelle level has already been emphasized. This can be explained on the grounds that mitochondria, even in the same cell, are in different phases of functional activity, as a result of which they differ in their degree of damage. The fact that hepatocytes after 1 month of training contain many changed mitochondria and foci of focal degeneration indicates that the rhythm of repair of the mitochondria and ribosomes on membranes of the RER does not coincide with the rhythm of training, and as a result, some of the organelles begin to "wear out" [7]. It must be noted, however, that the rate of physiological regeneration in the liver after training for 1.5 months was certainly increased, for the ultrastructure of the mitochondria and cisterns of the RER was the same as in the control, but their number was increased

(Table 1). It can be concluded from the morphology of the nucleus, mitochondria, and cisterns of the RER that the protein-synthesizing system of the cell was adapted to this particular load. The ultrastructure of the Golgi complex and biliary capillaries is evidence that hepatocytes elaborate bile products, but their elimination is inhibited because nearly all the biliary capillaries are closed [4]. Strenuous physical exercise evidently inhibits the process of bile excretion, and this is bound to have some effect on the state of the body as a whole.

The discovery that the number of peroxisomes was increased at all times of the investigation is an interesting fact. Because these organelles contain catalase, D-amino-acid oxidase, and urate oxidase [1, 3], peroxisomes evidently are involved in the detoxication of breakdown products of the organelles and of certain metabolic products and they contribute to an increase in the nonspecific resistance of the organ [11, 12]. The results of the present investigation thus demonstrate the great importance of physical training as a means of promoting more rapid physiological regeneration of the hepatic organelles and increasing the general resistance of the body to damaging agents.

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