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Effects of Physical Training on Rat Myocardium. An Enzymatic and Ultrastructural Morphometric Study

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Rats subjected to physical training through swimming increased their weight at a slower rate than controls, which initialy had the same characteristics. The ratio heart weight/body weight was 23 % greater in the trained rats. However, the absolute weights of the hearts were only 7 % greater. The ultrastructural morphometric study, backed up by an analysis of the hierarchical variance, did not reveal significant changes neither in the myofibrillar and mitochondrial volume nor in the number of mitochondria per surface unit of myocardium.

Furthermore, no variations were recorded, due to training, in the amount of mitochondrial protein nor in the specific mitochondrial activities of malate dehydrogenase, cytochrome c oxidase and ATPase. It is therefore suggested that the increase in the measured parameters, due to training, is proportional to the increase in weight and size of the heart. On the other hand, the specific activity of LDH increased by 15 % after the first weeks of training.

The results of previous studies, concerning the influence of training on the muscular tissue of the rat, can be summed up by stating that, in the skeletal muscle, the authors coincide in asserting: the absence of muscular hypertrophy (19, 24); the existence of mitochondrial hypertrophy and hyperplasia (13, 19); and increase in the enzymatic activities of the mitochondria (15, 19) though LDH activity decrease (3, 12, 38).

Complete agreement does no exist with regard to the behavior of the cardiac muscle with physical training. Although an increase in the heart weight has been reported (2, 6, 26, 27), it is not known how this alteration takes place. At the

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ultrastructural level, an increase in the number of mitochondria has been described. Important changes in the structure and volume of the mitochondria were found after just one day of exercise (20, 28), but BANISTER *et al.* (4) and CVORKOV *et al.* (7) did not observe mitochondrial alterations, even after a prolonged period of training. The biochemical studies have show that no changes occur in Krebs's cycle (27) but glycolytic enzymes apparently increase (11, 12, 38).

In the present work, it has been analyzed the cardiac hypertrophy caused by training, mainly at the ultrastructural level, by means of a morphometric study, statistically assessed. This, up to now, had not been done. The ultrastructural observations were correlated with the enzymatic activity of trained and control cardiac muscle cells.

Materials and Methods

A total number of 82 male Wistar rats were used. At the beginning of the experiments, they were approximately two and a half months old. All animals had weekly weight control checks. After being sacrificed, the weight of the heart was recorded also. Physical training was carried out by letting the rats swim freely in water at 20° C until exhaustion, once per day. Exhaustion was reached after 30-40 minutes during the first week; after 1 to 2 hours during the third week and thereafter.

Two experiments were carried out for the ultrastructural study: 1) 4 rats were subjected to the exercise schedule described above over a period of six weeks (4 rats were used as controls). 2) In the second experiment 5 rats were trained for 12 weeks (5 rats served as controls). Twenty four hours after ending the last exercise, the animals were anaesthetized with ether, and fixed by perfusion with glutaraldehyde, according to HOMMES *et al.* (16). Small pieces of the left ventricular wall were removed and fixed further in 4 % cacodylate buffered glutaraldehyde, postfixed in 1 % veronal buffered osmium tetroxide (2 hours), dehydrated and embedded in Epon-812. Thin sections were studied with the light microscope in order to select the appropriate areas for electron microscopy. The ultrathin sections were double stained with uranyl acetate and lead hydroxide. The material was studied with a E M Siemens Elmiskop 1 A.

The surface of the transversal section of the fibers was measured by means of a planimeter in the thin sections.

For the ultrastructural morphometric study, 3 blocks from each rat were selected. From each block 14 electronmicrographs were selected at random (pictures showing nuclei, wide intercellular spaces or lacking myofibrillae were rejected). Mitochondrial and myofibrillar volume measurements were carried out on the micrographs (referred to as a percentage of the total cellular volume), and of the number of mitochondria (per 100 μm^2), according to the WEIBEL model (36), as has been applied to the myocardium by LAGUENS (21), with slight modifications. In this study it was used a 100 cm^2 grating (10 $\times$ 10 cm), formed by 100 squares with 1 cm sides. The micrographs were taken at 4,000 magnifications and they were photographically increased to 10.000 (one cm of the grating was the equivalent of one μm of the myocardium). An analysis of the hierarchical variance was applied to the results of the morphometric study (33). Significance of results was evaluated as well as the probable sources of variations (training, rats, sections and micrographs).

For enzymatic study 2 sets of animals (each 32 rats) were used. From each set 16 rats were subjected to the training described above; another 16 rats were used as controls. Each week (third to tenth of training), 2 trained rats and 2 controls were sacrificed and studied com-

paratively. Twenty four hours after ending the last exercise, the animals were decapitated. Heart mitochondria were isolated according to HOGEBOOM (14). Malate dehydrogenase activity was determined by the method of OCHOA (25); cytochrome c oxidase activity was determined according to the method of SOTTOCASA *et al.* (34); ATPase activity was determined according to the technique of PULLMAN *et al.* (31); LDH activity was determined following the method of KORNBERG (18). Proteins were determined by the method of LOWRY *et al.* (22).

Results

Body weight. The body weight increase in the trained rats was slower than in the controls. This effect took place mainly from the second week to the seventh of training. After the seventh week, the weight increase was equivalent in both groups of animals (table I).

Heart weight. The average of absolute heart weights of all trained rats, sacrificed throughout the experiments, was approximately 7 % higher than the average found for the controls.

Table I. *Effects of training on rat weights.* Mean values ± standard error of the mean of body weight and heart weight/body weight ratio throughout training.

	Body weights (g)		Heart weight/body weight	
Weeks	Controls	Trained	Controls	Trained
1	140±5	141±5	2.77±0.12	2.82±0.15
2	167±4	160±6	2.74±0.11	3.03±0.14
3	190±5	174±5	2.82±0.10	3.24±0.12
4	212±6	185±4	2.76±0.13	3.15±0.15
5	234±6	196±4	2.78±0.10	3.40±0.12
6	248±6	206±5	2.90±0.11	3.38±0.14
7	260±5	219±4	2.65±0.12	3.26±0.14
8	271±5	224±4	2.75±0.10	3.29±0.15
9	278±4	234±4	2.70±0.12	3.34±0.13
10	280±4	241±4	2.71±0.11	3.28±0.12
11	284±3	245±3	2.77±0.09	3.31±0.15
12	288±3	249±3	2.69±0.09	3.37±0.13

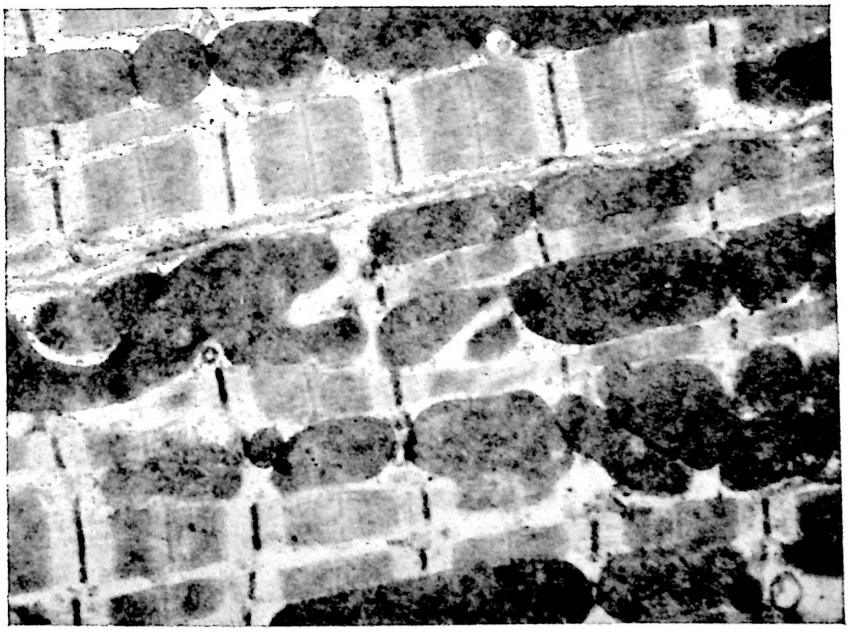

Fig. 1. *Myocardium of control rat* (× 10,000).

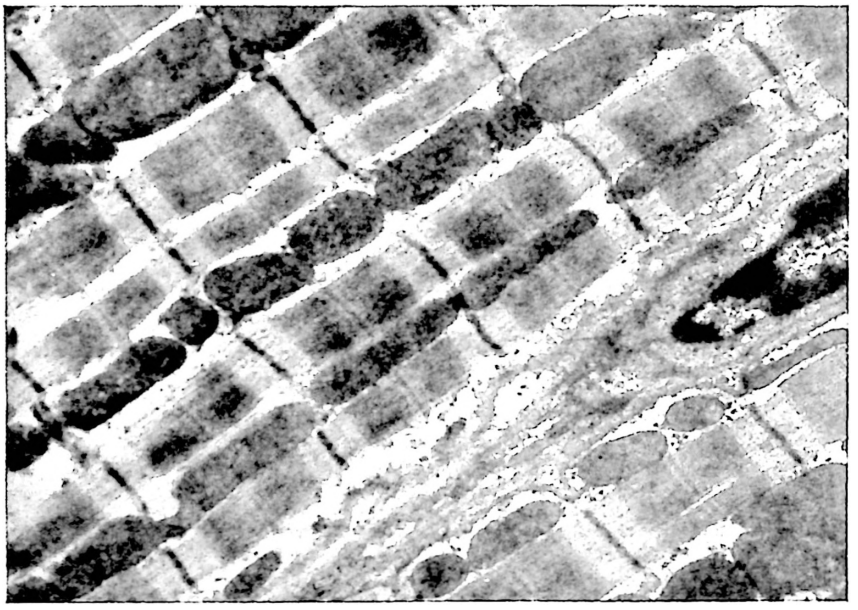

Fig. 2. *Myocardium of rat trained for 12 weeks showing a normal pattern* (× 10,000).

Fig. 3. *Myocardium of control rat showing mitochondrial swelling* (× 10,000).

The ratio heart weight/body weight, after 3 weeks of training, was approximately 23 % higher in the trained rats than in their controls. This ratio was 2.65 to 2.90 in the controls; while in the trained rats it was 3.15 to 3.40 (table I).

Light microscope. The histological pattern of the myocardium in the rats trained for 6 and 12 weeks was quite similar to that of the controls. The fiber width measurements in the transversal sections did not show any special difference between both groups of rats (the approximate average was 230 μm^2).

Electron microscope. The ultrastructure of the myocardium of trained rats over 6 and 12 weeks was also similar to that of the controls (figs. 1, 2). Moderate amount of swollen mitochondria were observed in experimentals and controls (fig. 3). On occasions fibers with contracted sarcomeres were observed, but these were found in the control hearts as well.

From ultrastructural observations no differences were appreciated in the proportion of the cellular organelles between the trained and the control speciments. In the morphometric study, the parameters measured were: *a*) volume occupied by the mitochondria; *b*) volume occupied by the myofibrils; and *c*) number of mitochondria per 100 μm^2 of myocardium. The results obtained from the rats trained for 12 weeks and their controls are given in table II. Identical results were obtained in the rats trained for 6 weeks.

Table II. *Morphometric ultrastructural measurements.*
Averages obtained of 5 rats trained for 12 weeks and 5 controls. Calculations were made from 3 sections from each rat. 14 electron-micrographs were taken from each section.

	Mitochondrial volume	Myofibrillar volume	Number of mitoch. per 100 μm^2
Controls	38.20	51.41	41.25
Trained	36.61	52.49	38.09

The results of the hierarchical variance analysis are shown in table III. Differences between experimental and control rats were not significant. Neither were the individual differences. Large differences were found, however, from one section to another.

Mitochondrial protein. The mitochondrial protein per wet heart weight did not vary troughout the period of physical training (table I).

Specific activities of the mitochondrial enzymes. In the third to tenth week experiment the specific activities of ma-

Table III. *Hierarchical variance analysis.*
Hierarchical variance analysis of averages obtained from 5 rats trained for 12 weeks and 5 controls. Calculations were made from 3 sections from each rat. 14 electron-micrographs were taken from each section. Fo_5 (1.8) = 5.32; Fo_1 (1.8) = 11.26; Fo_5 (8.20) = 2.45; Fo_1 (8.20) = 3.56; Fo_5 (20,390) = 1.57; Fo_1 (20,390) = 1.88. Only variations among sections (**) are significant. df among micrographs = 390.

Source of variation	df	Mitochondrial volume	Myofibrillar volume	Number of mit. per 100 μm^2
Between groups of rats	1	4.03	0.47	0.79
Among rats	8	0.30	0.67	1.63
Among sections	20	5.05 **	4.08 **	5.36 **

Table IV. *Mitochondrial protein and enzymatic actitivies.* Means ± standard error of the mean obtained from weekly measurements (third to tenth week) throughout the experiment.

	Controls	Trained
Mitochondrial protein (μg mit. prot./mg wet heart weight)	13.70±0.94	13.48±0.95
Malate dehydrogenase activity (μmol NADH ox./min/mg mit. prot.)	2.35±0.12	2.31±0.13
Cytochrome c oxidase activity (μmol cytochrome c red./min × mg mit. prot.)	0.334±0.030	0.345±0.030
Mitochondrial ATPase activity (μmol ATP hydrolyzed/min × mg mit. prot.)	4.10±0.22	4.13±0.22
LDH activity (μmol NADH ox./min × mg of supernatant prot.)	3.44±0.15	3.96±0.23

late dehydrogenase, cytochrome c oxidase and ATPase of the mitochondria did not show appreciable changes with training (table IV).

Specific LDH activity. From the third to the tenth week of physical training, the specific activity of LDH was approximately 15 % higher in the trained rats than in their controls. Such difference was maintained for the remaining weeks of the experiment, without appreciating any progressive increase or decrease during this time (table IV).

Discussion

The increase in body weight, slower in the trained rat than in the controls, confirms previous results (19, 24, 38). Such effect of the exercise was particulary evident in the first weeks of physical training.

The greatest heart weight/body weight ratio of the trained rats with respect to the controls (23 %) corresponded to that described in other studies (11, 26, 27). We have also analyzed the absolute weight values of the hypertrophic heart and control hearts in animals of the same initial age as well as body weight. The ratio between the averages obtained from each group of rats shows that the weigth of the hearts of trained rats was only slightly higher (7 %) than that of the untrained rats. Some authors have found a greater hearth weight in trained rats with respect to their control (2, 6, 39). In these experiments, however, body weight, at the end of the experiments, was similar in experimental and controls; the initial body weight was not taken into account. It appears that such results are not demonstrative of the heart weight increase due to exercise. As described above, at sacrificing moment, the control rats weight was higher than the weight of the trained ones, even if they started with the same age and weights. Similar body weigth in trained and untrained rats would indicate that the untrained ones are younger or were less corpulent at the beginning of the experiments. In either case, their heart would weigh less than that of the trained rats, even if these had not been trained.

The structural study of thin sections confirms the results on the absolute heart weights: the hypertrophy of the myocardia was not high enough to be detected with the measurements of muscle cells diameters.

An increase in the mitochondrial mass has been reported as a salient effect of exercise on cardiac muscle (2, 20, 28); although such changes have not been found by others (4, 7). However, a mor-

phometric study had not been performed. In the present work, the results of the hierarchical variance analysis, applied to the morphometric study, indicate that differences found between mitochondria and myofibrils of trained and control rats are not significant (table III). This applies also to individual differences. The difficulty found to contrast the differential effect between trained and control rats is due to the great differences among sections, even if a high number of micrographs were taken. Distribution of mitochondria in different myocardial fibers as well as in different areas of the same fiber was irregular. This distribution means that the data variance was great so that the power of the statistical test was affected. To detect significant differences it should be necessary to perform such a high number of observations that it would not be fulfilled. Consequently, without denying that some differences could arise from the physical training, it can be affirmed that they would be so small as to be masked by irregularity in the normal distribution of this organelle in the myocardial cells.

It was shown that the effect of exercise on the myocardium gives rise to large size mitochondria with a greater number of cristae than in the non trained rats (2, 20). However, in the present study these large mitochondria were also found in the control animals. Furthermore, they have been observed in the normal myocardium of rats and other mammals in previous studies (8, 9, 35). The same applies to the irregulary shaped and enlarged mitochondria of the myocardium, which have been attributed to the exercise (2, 20). These, nevertheless, appeared in the same proportion in trained rats and in the controls.

Another effect which have been attributed to physical training is a mitochondrial swelling in the myocardial cells (2). In the material used for this study, a mitochondrial swelling was observed in some sections of the trained as well as of the control rats. This change should be related to factors others than training. Probably the fixation plays an important role in its origin. In accordance with these results are those of BANISTER *et al.* (4), who sacrificed rats after different number of days of traning. Their findings indicate that: 1) A mitochondrial swelling occurs on the first day training. This is attributed to the hypoxia caused by the excesive exercise. 2) In the animals trained for a fews days the mitochondrial swelling diminished progressively, until it disappeared in the rats which underwent training for many days. On the other hand, these authors (4) and others (17) have shown that a resting period of twenty four hours, even after a single exercise, is enough for mitochondrial normalization. Therefore, the alterations, due to hypoxia, in the myocardium of the rats subjected to just one exercise until exhaustion seem to be reversible. As the days of training go by, adaptation would occur to hypoxia and its fine structural effects in mitochondria disapear.

The absence of variation in the mitochondrial protein in trained hearts (referring to the wet heart weight) is in agreement with the results of the morphometric study. No changes were detected. The increase in the overall mitochondrial mass, as a result of training, was proportional to the weight increase of the heart. With regard to the mitochondrial enzymes studied, their specific, activity, measured in relation to the mitochondrial protein, did not vary with training. This is in agreement with previous results (2, 26, 27, 37). Therefore, it can be stated that the increase of total enzyme activity is proportional to the increase of the mitochondrial mass, and to the increase in the mass of the cardiac muscle. All variations would be proportional to the heart hypertrophy.

In the present study, the specific activity of the extramitochondrial enzyme LDH showed a marked increase (15 %) in the

myocardium of the trained rats. Similar results have been referred to (11, 12). Recently, YORK *et al.* (39) have pointed out that this increase is due to an increase of the subunit M of the enzyme, and that pyruvate kinase activity also increases with exercise. An increase in the activity of LDH, in the subunit M, has also been observed in cases of cardiac hypertrophy, due to causes other than exercise, such as pulmonary stenosis (5, 10) or aortic (32); exposure to altitude (1, 23, 30); and anaemia (29). An increase in the pyruvate kinase activity has also been found to the later example.

Some authors (1, 5, 23) have assumed that the increase in the LDH activity, registered in the subunit M, is a heart response to low oxygen level. Cell adaptation to this condition would be connected to an increase of glycolytic enzyme activity.

Resumen

El peso corporal de las ratas sometidas a entrenamiento mediante natación aumenta más lentamente que el de los controles del mismo peso y edad iniciales. La relación peso corazón/peso cuerpo es un 23 % mayor en las ratas entrenadas; sin embargo, los pesos absolutos de los corazones son solamente un 7 % mayores. El estudio ultraestructural morfométrico, avalado con un análisis de la varianza jerárquica, no reveló cambios significativos en el volumen miofibrillar y mitocondrial ni en el número de mitocondrias por unidad de superficie del miocardio. Tampoco se han registrado variaciones con el entrenamiento en la proteína mitocondrial ni en las actividades específicas mitocondriales málico deshidrogenasa, citocromo c oxidasa y ATPasa. Se sugiere que el aumento que experimentan con el entrenamiento los parámetros medidos se mantiene proporcional al aumento de peso y tamaño del corazón. Por el contrario, la actividad específica láctico deshidrogenasa se incrementó un 15 % desde las primeras semanas de entrenamiento.

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