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The Effects of an Exercise Training upon Subsequently Induced Cardiac Enlargement[1] (41363)

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Abstract. One group of rats was trained in a 10-week exercise program and a comparable group left in their cages. The animals of both groups were then subjected to abdominal aortic constriction and injection with [^{3}H]thymidine 3 days later. After one more day the hearts were removed and perfused with degradative enzymes. Following separation of cell types, greater levels of [^{3}H]thymidine incorporation were found in muscle and nonmuscle cells of trained rats.

Cardiac enlargement, generally regarded as a pathological condition in humans, may result from increased cardiac work requirements. Enlarged hearts have been observed clinically and produced experimentally by volume or pressure overload. At least in early stages of work overload the increase in cardiac mass appears to be a compensatory adaptation (1–4). Such enlargement may theoretically occur by an increase in cell number (hyperplasia) or in cell size (hypertrophy). Cardiac enlargement induced in adult animals results from muscle cell hypertrophy rather than hyperplasia even though nonmuscle cell division does occur (5, 6). If rats 21 days of age are subjected to aortic constriction the subsequent cardiac enlargement due to pressure overload proceeds with division of muscle as well as nonmuscle cells (7, 8). This ability of rat myocytes to undergo cell division may relate to the observation that the incorporation of tritiated thymidine into myocyte DNA largely ceases at from 17 to 120 days of age (8–10).

The hearts of adult and neonatal rats do not differ only in their ability to enlarge with myocyte hyperplasia in response to overload challenge. The cardiac performance of the adult hypertrophied heart appears, at least according to most investigators, to be compromised. In contrast, the enlarged neonatal heart retains normal function when subjected to hemodynamic stress resulting from either volume infusion or catecholamine challenge (7).

While it is generally agreed that repetitive exercise has a beneficial effect upon the heart, the mechanism and quality of this effect are incompletely understood. It has been shown that when adult rats are first subjected to a program of swimming (11) or treadmill (12, 13) exercise, their hearts have an increased capacity to retain contractility after an acute increase in afterload or hypoxia. This study was initiated to determine whether an exercise regimen induced differences in the biochemical responses of hearts to aortic constriction.

Materials and Methods. Twenty female Sprague–Dawley rats obtained from Timco Breeding Laboratories were divided randomly into two groups of 10. One group (112 ± 3.1 g) were left in their cages except for occasional weighings (sedentary, Sed). The other group (111.5 ± 5.1 g) was subjected to a program of treadmill running lasting for 10 weeks. The training program involved the use of a motor-driven Quinton rodent treadmill set at 15° angle with 10 rats running simultaneously. The training program began with two 10-min runs daily at 19

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m/min the first week and ended with daily 1-hr runs at 25 m/min. The gradually increasing program was similar to that of Cutilletta *et al.* (13) except that the 1-hr, 25 m/min runs were extended through the 9th and 10th weeks. After the training program the Sed group weighed 153.7 ± 3.9 g and the exercised group 159.8 ± 6.2 g.

During the week following the exercise regimen each rat was subjected to subdiaphragmatic aortic constriction by a modification of the method of Morkin and Ashford (14). The rats were anesthetized with ether, their abdomens opened and the stomachs and intestines retracted. The abdominal aorta was isolated above the renal vessels and a silk ligature passed around it. The ligature was then tied around both the aorta and a 22-gauge needle, followed by removal of the needle. This constriction, approximating the size of the needle, resulted in pressure elevation above this segment. The peritoneum was sutured together and the skin closed with wound clips.

On the third day after the aortic constriction the rats were intraperitoneally injected with [*methyl*-^{3}H]thymidine by the schedule of Neffgen and Korecky (15). At 2-hr intervals beginning at 9 AM each rat received three ip injections of 1 μCi [^{3}H]thymidine/g body wt (69.4 Ci/mmole in sterile aqueous solution).

On the following day the muscle and nonmuscle cells of the heart were separated by a modification of the method of Cutilletta *et al.* (16). Each rat was injected ip with 500 units of sodium heparin and killed by cervical dislocation 30 min later. The heart, along with 5–10 mm of aorta was rapidly removed, and placed in 50 ml of cold Glick's phosphate buffer (116 m*M* NaCl; 5.5 m*M* KCl; 5.5 m*M* glucose; and 2 m*M* sodium phosphate, pH 7.2) containing 2 m*M* EGTA and 10 units/ml sodium heparin. The hearts were then trimmed, weighed, attached by the aorta to a recirculating perfusion apparatus and washed with 5–10 ml of the Glick's, EGTA, heparin solution at 37°. The hearts were then flushed with 5 ml Glick's solution and perfused for 1 hr with a 37° Glick's solution containing 0.05% collagenase (Sigma, Type 1); 0.1% hyalurondase (Sigma, type 1-S) and 1% bovine serum albumin (Sigma, fraction V). The perfused hearts, now gelatinous sacs, were finally flushed with 10 ml cold Glick's solution. After removal of the aorta and other vascular tissue the hearts were minced with iris scissors and transferred to a loose-fitting Dounce homogenizer with 10 ml cold Glick's solution. This and all subsequent steps in the cell preparation were at 0–5°. After five gentle strokes of the pestle the homogenate was filtered through 100-mesh silk bolting cloth. The filtered cell suspension was then centrifuged at 50*g* for 3 min. The supernatant solution was removed and set aside as the nonmuscle cell fraction. The pellet was twice resuspended in 10 ml Glick's solution and recentrifuged for 3 min at 50*g*. The pellet was then resuspended in 5 ml Glick's solution and carefully layered over 5 ml of 3% Ficoll (Sigma, Type 70) in Glick's solution and centrifuged for 3 min at 60*g*. This step was repeated once and the pelletted muscle cells finally washed twice with 10 ml Glick's solution and centrifuged for 3 min at 50*g*. The nonmuscle cells were also washed twice with 10 ml Glick's solution and centrifuged at 800*g* for 5 min. The pellets of washed muscle and nonmuscle cells were resuspended with 1 ml Glick's buffer and samples were taken for light microscopy. The cells were stained with Schiff's reagent and counterstained with fast green. At least 100 cells per sample were counted to determine cell purity. The muscle cells were readily distinguished by their large cytoplasmic contractile protein content (16).

DNA extraction and measurement was initiated by suspending the cells in 2 ml of 0.5 *M* NaOH and heating at 70° for 5 min. After adding 1.74 ml H_2O and chilling on ice, 0.26 ml of concentrated perchloric acid was added making the solution approximately 0.5 *M*.

After a 30-min incubation on ice the mixture was centrifuged at 2000*g* for 10 min. The pellet was resuspended with 2 ml 0.5 *M* perchloric acid and incubated again for 30 min on ice. After another centrifugation the supernatant solutions were combined and the pellets resuspended with 1 ml

TABLE I. DNA AND PROTEIN CONTENT OF PURIFIED HEART CELLS

	Sedentary (9)[a]		Exercised (7)	
	Muscle cells	Nonmuscle cells	Muscle cells	Nonmuscle cells
μg DNA	102.6 ± 30[b]	230.1 ± 54.3	112.9 ± 23.8	231.9 ± 41.2
mg Protein	18.6 ± 6.2	2.44 ± 1.1	22.2 ± 5.2	2.36 ± 0.8
Prot(mg)/DNA(μg)	0.182 ± .041	0.011 ± .008	0.197 ± .037	0.012 ± .007

[a] The number in parentheses is the number of animals in each group for this and subsequent tables.
[b] The mean ± the standard deviation for this and subsequent tables.

of 0.5 *M* perchloric acid. After a 30-min incubation at 70° the mixture was similarly centrifuged. The pellet was again suspended with 1 ml 0.5 *M* perchloric acid and heated at 70° for 30 min and recentrifuged. The latter two supernatant solutions were pooled for DNA and tritium analyses. The pellets were finally suspended with 2 ml of 0.5 *M* NaOH and heated at 70° until dissolved. The method of Burton, with calf thymus DNA as a standard, was utilized to determine the DNA content of the perchloric acid extractions (17, 18). The protein content of the residue dissolved in 0.5 *M* NaOH was determined by the modified Lowry assay of Zak and Cohen (19). While no measurable DNA was found in the cold acid extracts the 70° extracts, as seen in Table I, were rich in DNA. While the muscle cells of the exercised rats appeared slightly higher in DNA content than those of sedentary rats there were no significant differences. The nonmuscle cell DNA content from both groups was even more similar.

The tritium content of the acid extracts was measured by liquid scintillation spectrometry both before and after the addition of activated charcoal (50 mg Norit A per 2 ml) to remove aromatic [^{3}H]thymidine (10).

Results and Discussion. The muscle cell preparations from all animals appeared to be 97 ± 3% pure. The nonmuscle cell preparations were 97.5 ± 1.9% pure, i.e., free from muscle cells to this extent.

As seen in Table I there were no significant differences in cell size as exhibited by the similar ratios of protein to DNA content from exercised and sedentary animals. At this point in time (4 days after aortic constriction) no differential degree of cell hypertrophy is observed in this manner. There were also no significant differences noted between the total heart weights of each group (sedentary 917 ± 22 mg vs exercised 920 ± 31 mg).

Table II indicates that both the radioactivity appearing in the cold perchloric acid extracts and that left unabsorbed by charcoal are similar in both groups of animals.

TABLE II. TOTAL RADIOACTIVITY OF PERCHLORIC ACID EXTRACTIONS

	Sedentary (9)		Exercised (7)	
Extract	Muscle cells	Nonmuscle cells	Muscle cells	Nonmuscle cells
Cold $HClO_4$ (dpm)[a]	3659 ± 856	1095 ± 256	4191 ± 720	1239 ± 503
70° $HClO_4$ (dpm)	3515 ± 963	9720 ± 2630	6436 ± 1007	17971 ± 2902
70° $HClO_4$ after charcoal (dpm)	115 ± 56	232 ± 71	220 ± 69	373 ± 42

[a] The total radioactivity of each extract in disintegrations per minute.

TABLE III. SPECIFIC RADIOACTIVITY OF DNA IN THE PERCHLORIC ACID EXTRACTS

Sedentary (9)		Exercised (7)	
Muscle cells	Nonmuscle cells	Muscle cells	Nonmuscle cells
36.2 ± 4.7[a,b]	53.1 ± 11[b]	58.0 ± 14	81.9 ± 13

[a] Results are expressed as dpm/μg DNA.
[b] The cells from sedentary group incorporated significantly less [^{3}H]thymidine than those from the exercised group ($P \leq 0.02$ from Student's t test).

This result is consistent with there being no significant differences in thymidine uptake or metabolism as a function of the exercise program.

The specific radioactivity of DNA in the hot perchloric extracts is seen in Table III. This was calculated from the radioactivity absorbed by charcoal and the DNA content before charcoal absorption.

Both muscle and nonmuscle cardiac cells of exercised rats exhibited a greater degree of [^{3}H]thymidine incorporation after aortic constriction than that found with sedentary rats. This difference in DNA associated [^{3}H]thymidine was found to be statistically significant with both cell types (Student's t test, $P < 0.02$). An analogous exercise program has been found to render rat hearts better able to tolerate increases in afterload and hypoxia and also to more rapidly respond with compensatory hypertrophy after aortic constriction (13). Preexercised animals have also been shown to retain more cardiac contractility than sedentary animals after aortic constriction (12).

Although the increased incorporation of [^{3}H]thymidine by muscle cells appears to correlate with the differences in cardiac behavior of exercised and sedentary rats noted above, the precise mechanism of this increase is not known. It is generally accepted that, after about 21 days of age, rat hearts do not respond to pressure overload with muscle cell mitosis. Whether an exercise program prior to pressure overload alters this situation appears unlikely.

A possible explanation for the exercise-induced increased [^{3}H]thymidine incorporation could be an increase in muscle cell polyploidy. Aortic constriction has been shown to result in an increased heart muscle cell polyploidy within 10 days after aortic constriction (21). Other, perhaps less likely but possible, explanations might include polynucleation, alterations in DNA turnover, enhanced thymidine uptake, or even increased mitochondrial DNA synthesis.

In any case, the exercise program has induced an observable difference in the response to aortic constriction as reflected in the increased amount of [^{3}H]thymidine incorporation in muscle and nonmuscle cell DNA. This difference was not accompanied by significant alterations in the ratio of cellular protein to DNA as seen in Table I.

It has been observed that when rats younger than 22 days of age are subjected to aortic constriction their hearts exhibit some muscle cell division as part of the compensatory enlargement (7, 8). Also previously shown is that, when these young rats are subjected to aortic constriction, their subsequently enlarged hearts retain a normal aortic peak flow velocity, cardiac index, and stroke index. These cardiac performance indicators were markedly reduced with adult rats subjected to aortic constriction (7).

The possible existence of exercise-induced muscle cell hyperplasia in response to aortic constriction with adult rats is one rational interpretation of the increased [^{3}H]thymidine incorporation. With rats less than 22 days of age subject to aortic constriction, cardiac muscle cell hyperplasia correlates with greater retention of cardiac function. Exercised adult rats retain more of their normal cardiac function after overload induced cardiac enlargement. While there may be several explanations of the increased [^{3}H]thymidine incorporation in

the cardiac muscle cells of aortic constricted preexercised adult rats, the one explanation perhaps least illogical at this point is at least a degree of muscle cell hyperplasia. The significance of exercise-induced increases in cardiac nonmuscle cells after aortic constriction is even less explicable at this point in time.

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