administered in culture. Success appears to depend chiefly upon satisfactory conditions for maintenance of the tissue. With *Locusta* fat body in M-20 medium[22], it was found that the rate of protein synthesis declined sharply during 1–2 days of culture, and the response to JH analog was quite variable. The response obtained with well-rinsed tissue in a defined medium demonstrates that VG synthesis can be induced in *Locusta* fat body by a JH analog in the absence of any other hormone, a conclusion difficult to establish by experiments in vivo[18,19], although the requirement for additional factors for full primary induction cannot be excluded, since a longer exposure and higher doses of hormone were necessary. The way is now open to several types of study that may throw light on the mechanism of stimulation of gene expression by JH.

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Muscle hexose monophosphate shunt activity following exercise[1]

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Summary. 1 day following prolonged treadmill walking untrained rats showed significant elevations in hexose monophosphate shunt reducing capacity in plantaris muscle. The increases were associated with accumulations of nuclei in the muscle interstitium, suggesting damage to the connective tissue element of the muscle.

Recent work from our laboratory[4] and from those of others[5,6] has demonstrated large increases in the activity of the hexose monophosphate shunt (HMS) in skeletal muscle following injury. We have concluded[4] the enhanced HMS activity that accompanies surgical trauma primarily results from accumulation of cells in the connective tissue, rather than from changes of activity within muscle fibers[6]. Boström et al.[7] also found large increases in the activities of several HMS enzymes in rat skeletal muscles following exhaustive swimming. These authors did not localize the response, and assumed the changes occurred in muscle fibers. Our purpose was to quantify and localize changes in HMS reducing capacity (HMSRC) in rat skeletal muscle following treadmill exercise.

Untrained male Sprague-Dawley rats weighing an average of 341 ± 3 g ($\bar{x} \pm SE$) were assigned to 2 groups: a non-exercise control group, and an exercise group. Animals in the exercise group were walked on a motordriven treadmill at a speed of 10 m $\times$ min^{-1}, without electrical stimulation, until they refused to maintain the speed of the treadmill. The animals walked 3.5–4 h for an average distance of 2.2 ± 0.08 ($\bar{x} \pm SE$) km. Rats from both the exercise and the non-exercise control groups were sacrificed by exsanguination following ether anesthesia at 0, 12, 24, 48 or 96 h post-exercise. HMSRC of the distal portion of 1 plantaris muscle from each rat was determined fluorometrically following the reduction of NADP using glucose-6-phosphate as substrate. Details of the assay were published earlier[4]. HMSRC

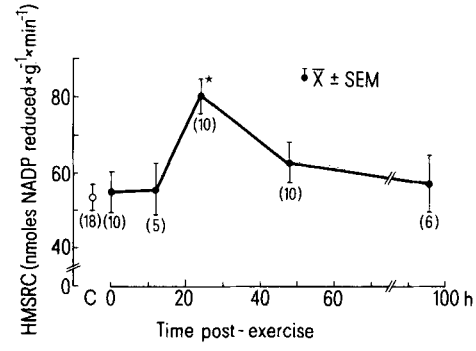

Figure 1. Hexose monophosphate shunt reducing capacity (HMSRC) of non-exercised control (C) and exercised rat plantaris muscles as a function of time following prolonged treadmill walking. Number of experiments in parantheses. * $p < 0.05$.

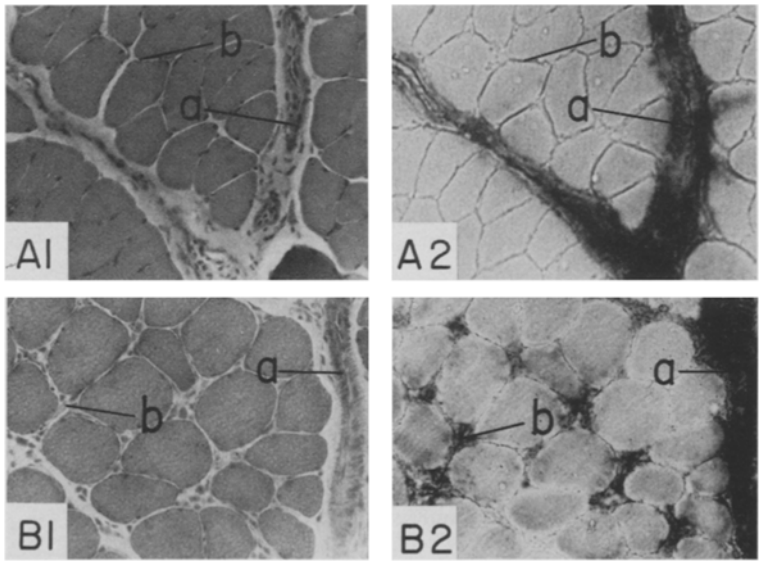

Figure 2. Serial sections of plantaris muscles from control (A) and exercised (B) rats taken 24 h after termination of exercise and stained for nuclei with hematoxylin and eosin (1) and activity of the hexose monophosphate shunt (2). The perimysium (a), which is the connective tissue element lying between adjacent fascicles, and the endomysium (b), which lies between muscle fibers, are indicated. ×215.

is reported in nmoles of NADP reduced ×g wet wt^{-1} ×min^{-1}. The distal half of the 2nd plantaris muscle was mounted on a specimen holder and frozen in 2-methyl butane cooled in liquid nitrogen. Cross-sections of these samples were cut (16 μm) and stained for nuclei with Harris' hematoxylin and eosin stain and for HMSRC using a modificiation[4] of the glucose-6-phosphate dehydrogenase procedure of Pearse[8]. A dialysis membrane technique[9] was used to reduce diffusion of stain from the site of enzyme activity. The experiment was repeated with a 2nd group of rats (n=4) to increase the number of samples analyzed for histochemistry and to determine the water content of the muscles during the post-exercise period. Since no differences were found among control values from different times, they were pooled for statistical treatment. Differences among the control and experimental means were tested using a one-way analysis of variance and Tukey's w. Treadmill walking resulted in a significant ($p < 0.05$) rise of about 50% in plantaris muscle HMSRC at 24 h after exercise over both control and 0 h post-exercise values (fig. 1). By 48 h following exercise HMSRC had declined to control levels. In both control and exercised rats HMSRC activity was primarily localized in the muscle interstitium in association with interstitial nuclei (fig. 2). In control animals the HMSRC stain was confined to perimysium, particularly in the regions of large blood vessels. However, in the exercised animals (24 h post-) HMSRC stain was also visible in the endomysium, again in association with nuclear accumulation. The nuclei belonged primarily to fibroblasts and macrophages, although some neutrophils and mast cells were in evidence. Stain in the endomysium of plantaris muscles from the exercised rats was confined to the deep portion of the muscle, which is primarily composed of high-oxidative-fast- and slow-twitch muscle fibers[10]. No changes in water content in the tissue were observed.

These results indicate that exercise-induced increases in HMS activity, like those observed following surgical trauma[4], are primarily localized in the muscle interstitium. The interstitial accumulation of cells presumably was associated with inflammation and degenerative/regenerative processes occurring from exercise trauma.

Exercise-induced injury to muscles has previously been identified from accumulations of macrophages and mononuclear cells in regions of necrotic muscle fibers[11], elevations in the histochemical staining intensities of acid hydrolases in interstitial fibroblasts and mononuclear cells[12], and fiber necrosis followed by regeneration[11,12]. Muscle fiber degeneration was not observed in the present study, which may be explained by the relatively low intensity[12] and/or duration[11] of the exercise. Also, we did not use electrical stimulation to encourage the animals to walk on the treadmill. Thus, the response was primarily confined to the interstitium of the muscle, suggesting the major damage was to the connective tissue. Connective tissue elements in the muscle transmit forces generated by muscle fibers to the bones, and absorb energy during eccentric contractions when work is done on the muscles. Since both concentric and eccentric contractions are performed by extensor muscles (e.g., plantaris muscle) during level walking, it seems reasonable to expect that in the untrained condition these forces may damage the connective tissue. Heikkinen and Vuori[13] demonstrated that alterations in connective tissue metabolism, as measured by hydroxyproline turnover, occur in mice during exercise training, and Tipton et al.[14] have reported increases in the strength of tendons and ligaments of chronically exercised rats. Experiments are in progress to study muscle injury following different types of exercise (concentric vs eccentric).

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