mortality[16]. Prostaglandins are known modulators of hematopoiesis[17] and elevated levels are seen after both trauma[18] and radiation[19]. Thus, increased $PGE_2$ levels induced by trauma shortly before or shortly after radiation may enhance hematopoiesis and therefore survival, while increased amounts induced both by radiation and subsequent late trauma may result in death associated with sepsis[20]. We are currently directing our research efforts toward delineating the role of these biological modifiers in trauma-enhanced survival/mortality with radiation exposure.

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2 Research was conducted according to the principles enunciated in the 'Guide for the Care and Use of Laboratory Animals' prepared by the Institute of Laboratory Research, National Research Council.
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## The susceptibility to exercise-induced muscle damage increases as rats grow larger

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*Summary.* Glucose-6-phosphate dehydrogenase and N-acetyl-β-glucosaminidase activities were both elevated after eccentric exercise indicating that this type of exercise causes muscle damage. Muscle damage as measured by glucose-6-phosphate dehydrogenase activity in the vastus intermedius was greater and occurred later in larger rats indicating that the susceptibility to muscle damage is increased and the repair process delayed in older and larger animals.
*Key words.* Rat muscle; eccentric exercise; delayed muscle soreness; glucose-6-phosphate dehydrogenase; N-acetyl-β-glucosaminidase.

Heavy physical exercise performed by one unaccustomed to exercise generally results in muscular pain and stiffness. This 'delayed muscle soreness' has been mainly attributed to eccentric work[1-3]. Eccentric work, which is a component of most normal exercise, results from lengthening a muscle against a force. Although the prime physiological cause of this soreness is not known, Hough's suggestion[4] that this soreness was due to muscle damage has been substantiated. Muscle exhibited Z-band disorganization two days after eccentric exercise (running down stairs) in human subjects[5], and I-band widening was observed immediately after eccentric exercise in rats[3]. The extent of exercise-induced muscle damage has been assessed biochemically by measuring the activity of either lysosomal enzymes[6], or glucose 6-phosphate dehydrogenase (G6PDH)[3], the first enzyme in the pentose phosphate pathway. The purpose of this study was to determine which biochemical marker of muscle damage was the most sensitive and then to use this marker to determine the effect of animal size on the extent of muscle damage.

*Experimental.* Male Sprague-Dawley rats obtained from the East Carolina University School of Medicine animal facilities, were used in these experiments. The rats were individually caged in a temperature-controlled room (20–23 °C) with lights on from 07.00 to 19.00 h, and were allowed free access to food and water. They were exercised by running down an 18° grade on a motor driven treadmill. Running downhill biases the work towards the eccentric type thus causing increased muscle damage. The time and intensity of exercise was adjusted for each group such that about 90% of the animals could complete the exercise protocol. The large rats (411 ± 6 g) rat at 16 m/min for 90 min, the 269 ± 5 g rats ran at 25 m/min for 200 min, and the small rats (79 ± 2 g) rats at 16 m/min for 120 min. The rats in each size group were randomly subdivided into groups that were unexercised (control) or exercised. The control group (0) remained sedentary in their cages until they were sacrificed. The rats in the exercise groups were sacrificed 1 day after exercise (group 1), 2 days after exercise (groups 2), etc. Immediately after sacrifice the muscles were removed, frozen between liquid nitrogen cooled aluminum blocks, and stored for enzyme analysis. In the first experiment both G6PDH and N-acetyl-β-glucosaminidase (NAG) were assayed in the soleus and vastus intermedius. In subsequent experiments G6PDH was assayed in the vastus intermedius. The frozen muscles were homogenized (1/10, w/v) using a Polytron at a setting of 5 for 5 s in 50 mM Tris/HCl buffer, pH 7.6, containing 0.3% triton X-100. After centrifugation for 2 min in an Eppendorf model 5212 centrifuge, the supernatant was assayed for enzymatic activity. The activity of G6PDH was measured by following the reduction of $NADP^+$ at 340 nm in a 50 mM glycine assay buffer, pH 9.2, initially containing 0.4% bovine

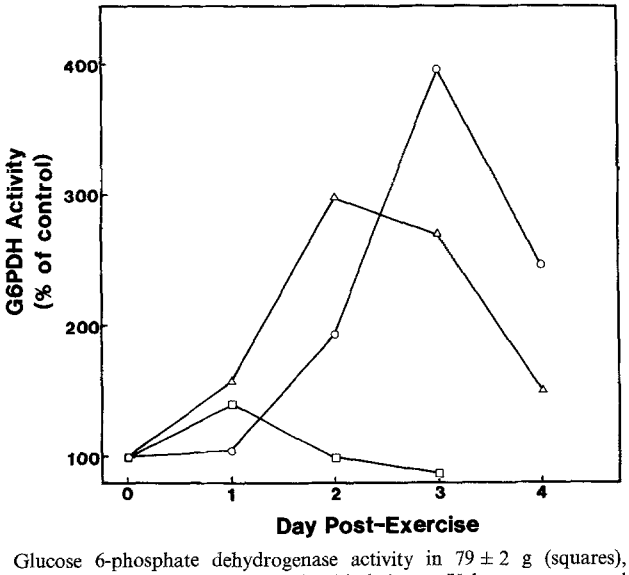
serum albumin and 1.0 mM $NADP^{+}$[3]. The enzyme activity is expressed as nmoles $NADP^{+}$ reduced/min/mg protein. NAG activity was measured using 4-methylumbelliferyl-N-acetyl-β-glucosaminide as substrate[7]. Protein concentration was determined using the biuret reaction[8] with bovine serum albumin as standard.

*Results and discussion.* The activities of G6PDH and the lysosomal enzyme NAG were measured in the soleus and vastus intermedius both before and for 4 days after eccentric exercise (table). In agreement with Armstrong et al.[3] the activity of G6PDH increased in both muscles after exercise. This increase in G6PDH activity is likely associated with the degeneration-regeneration process[9]. The activity of NAG was also increased in the same muscles following exercise indicating that lysosomal enzymes are also involved in the process of muscle damage and repair. Our observation of increased lysosomal enzyme activity after eccentric exercise substantiates Friden's et al. finding of increased lipofuscin granules in human subjects after eccentric exercise[10]. The maximum increase in G6PDH activity was 296 and 47% in the vastus intermedius and soleus respectively while the increase in NAG activity was only 65 and 30% in the same muscles. Thus while both of these enzymes are good biochemical markers of muscle damage, G6PDH was used in subsequent studies because of its greater responsiveness.

Glucose 6-phosphate dehydrogenase (G6PDH) and N-acetyl-β-glucosaminidase (NAG) activity in muscles of 411 ± 6 g rats

| Hours post exercise | Enzyme activity (nmoles/min/mg protein) Vastus intermedius | | Soleus | |
|---|---|---|---|---|
| | G6PDH | NAG | G6PDH | NAG |
| Control | 1.65 ± 0.12 | 1.96 ± 0.09 | 4.55 ± 0.24 | 1.59 ± 0.04 |
| 24 | 1.74 ± 0.11 | 1.65 ± 0.07 | 4.85 ± 0.55 | 1.89 ± 0.09 |
| 48 | 3.18 ± 0.61 | 2.08 ± 0.15 | 6.71 ± 0.50* | 1.95 ± 0.32* |
| 72 | 6.54 ± 1.14* | 3.23 ± 0.59* | 5.48 ± 0.52 | 2.06 ± 0.11* |
| 96 | 4.06 ± 0.62 | 2.28 ± 0.16 | 4.12 ± 0.19 | 1.85 ± 0.07 |

Values are means ± SE for 10 observations. Asterisks indicate the value is statistically different ($p < 0.05$) from the control value.




Glucose 6-phosphate dehydrogenase activity in 79 ± 2 g (squares), 269 ± 5 g (triangles), and 411 ± 6 g (circles) rats. Values are expressed as percent of control. The control values were 3.80 ± 0.16, 1.95 ± 0.19, and 1.65 ± 0.12 nmoles/min/mg protein for the 79, 269, and 411 g rats respectively.

The effect of rat size (age) on exercise-induced muscle damage was assessed by measuring the activity of G6PDH in small and medium sized rats as a function of time after exercise and comparing these data to the data from the large rats obtained in our first experiment. The G6PDH activity increased with increasing rat size (fig.). The small rats (79 ± 2 g) showed only a 40% increase while the large rats (411 ± 6 g) which ran at the same intensity for slightly less time had a 296% increase. The medium sized rats (269 ± 5 g) showed an intermediate 197% increase even though they had to be run both longer and faster to achieve the same degree of 'tiredness' as the other two groups. It thus appears that exercise-induced muscle damage increases as the animal gets older and larger.

The time course of the G6PDH activity shows that the maximal increase occurs 1, 2, and 3 days post exercise in the small, medium, and large rats respectively. Since G6PDH is associated with this repair process, it appears that this process is delayed in the larger animals. Thus, it seems that older and larger animals are not only more susceptible to exercise-induced muscle damage, but recover from this damage more slowly than younger and smaller animals.

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## Supramarginal cells in the rat pituitary cleft revealed by scanning electron microscopy

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*Summary.* An unusual cell type consisting of free elements widely scattered over the marginal epithelium of the rat pituitary cleft is revealed by SEM. Most of these supramarginal cells characteristically have irregularly shaped cell bodies from which thin branched processes extend. Supramarginal cells bear resemblances to Kolmer (epiplexus) cells and to supraependymal cells of the brain ventricles. Their ultrastructural features make it probable that supramarginal cells are phagocytes, and can be regarded as scavengers of the cleft. Considering the close topographical association between the hypophysial cleft and the floor or the 3rd ventricle, supramarginal cells may be members of the motile macrophagic Kolmer cells populating the ventricular surfaces of the brain.
*Key words.* Rat pituitary cleft; scanning electron microscopy; supramarginal cells.