Effects of exercise and training on serum enzyme and tissue changes in rats

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HIGHMAN, BENJAMIN, AND PAUL D. ALTLAND. Effects of exercise and training on serum enzyme and tissue changes in rats. Am. J. Physiol. 205(1): 162-166. 1963.—Young adult male rats received 1-20 successively daily 6-hr exercise tests in a rotating cage. Serum glutamic oxalacetic transaminase and aldolase and blood urea nitrogen values rose sharply and serum alkaline phosphatase fell immediately after each of the first four tests with only partial recovery after an overnight rest. Subsequently, the serum alkaline phosphatase and blood urea nitrogen values returned to normal, but the transaminase and aldolase values were slightly elevated even after 17-20 tests. Weight loss was 15% in 3-6 days and 7% after 17-20 tests. Transient fatty changes were noted in the thigh muscles after the first test. Necrotic muscle lesions, most pronounced after three tests, regressed after the 1st week. Rats given 17-20 successive daily 6-hr exercise tests, contrary to untrained rats, showed no apparent fatigue, no muscle lesions, and no significant changes in serum enzymes immediately after a 16-hr exercise test. These findings emphasize the importance of properly graded training exercises.

The importance of exercise in maintaining a good physical condition is well recognized, but there is little experimental work in animals to indicate what serum enzyme and tissue changes take place during training. In a recent study (1), we have shown that forced exercise of rats in a rotating cage for a 16-hr period, with 5-min rest periods at 30-min intervals, produced a marked rise in certain serum enzyme levels, fatty changes in the viscera, and necrosis of scattered muscle fibers. The present study was undertaken to determine the effects of shorter periods of exercise and of training or conditioning on serum enzyme and tissue changes. Evidence is presented showing that adequate training not only improves performance during a prolonged period of exercise but also greatly reduces serum enzyme and pathologic changes. Alterations in serum enzymes have been reported after strenuous exercise in man (2-5) and have been attributed to alterations in cells short of necrosis (4). However, further clarification is needed since no pathologic studies of the muscles or other tissues have been reported in these investigations on man.

MATERIALS AND METHODS

Several lots of Sprague-Dawley male rats were exercised in groups, as previously described (1), in a cage rotated to require the rats to walk 6.9 m/min. The rats in the youngest lot were about 80 days old and weighed 240-280 g at the beginning of the experiment; the rats in the oldest lot were about 140 days old and weighed 250-350 g. All rats were housed and exercised in an air-conditioned room maintained at 23-25 C. Food and water were inaccessible during exercise periods. The exercising rats were rested 5 min in each 30-min period. Twenty rats were sacrificed immediately after a 16-hr exercise test, 6 after a 12-hr test, and 15 after a 6-hr test (Table I). Most of the other rats were exercised daily. Groups were sacrificed immediately or 18-24 hr after the last of a series of 1-20 successive daily 6-hr exercise tests (Fig. 1, Table I). To test the value of training, some rats were exercised 6 hr on each of 17-20 successive days. After a 24-hr rest period, these trained rats, along with untrained controls, were sacrificed immediately after subjection to a 16-hr exercise test. Forty-five unexercised control rats, including 23 fasted and deprived of water for 16 hr, were sacrificed in groups at intervals along with exercised rats from the same lots. The other 22 unexercised controls were not fasted since preliminary studies indicated no significant differences in the parameters tested between such animals and those deprived of food and water for 6 hr. No significant diurnal variations in blood values were detected in unexercised controls.

Heart blood for serum enzyme studies was obtained during Nembutal anesthesia immediately preceding sacrifice. Serum values of glutamic oxalacetic transaminase (SGOT), glutamic pyruvic transaminase (SGPT), lactic dehydrogenase (SLD), alkaline phosphatase (SAlP), and aldolase (SAld) and values of blood urea nitrogen (BUN) were determined by methods previously described (1).

The heart, liver, kidney, adrenal, and thigh muscles...
TABLE 1. Effect of various periods of exercise and previous training on serum enzyme and BUN values in rats

<table>
<thead>
<tr>
<th>Exercise Periods</th>
<th>No. of Rats</th>
<th>SGOT</th>
<th>SGPT</th>
<th>SLD</th>
<th>SAkP</th>
<th>SAld</th>
<th>BUN</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 X 6 (Fed)</td>
<td>22</td>
<td>275±11</td>
<td>64±10</td>
<td>2.43±0.40</td>
<td>1,647±92</td>
<td>79±4</td>
<td>25±4</td>
</tr>
<tr>
<td>0 X 6 (Fasted)*</td>
<td>23</td>
<td>305±16</td>
<td>64±10</td>
<td>2.11±0.87</td>
<td>1,540±139</td>
<td>49±4</td>
<td>87±11</td>
</tr>
<tr>
<td>3 X 6 (UT)</td>
<td>15</td>
<td>520±39</td>
<td>88±4</td>
<td>2.43±0.49</td>
<td>1,647±92</td>
<td>79±4</td>
<td>25±4</td>
</tr>
<tr>
<td>1 X 12 (UT)</td>
<td>6</td>
<td>952±18</td>
<td>93±8</td>
<td>3.29±0.87</td>
<td>774±24</td>
<td>94±5</td>
<td>31±5</td>
</tr>
<tr>
<td>1 X 16 (UT)</td>
<td>20</td>
<td>938±28</td>
<td>108±6</td>
<td>2.89±0.60</td>
<td>108±6</td>
<td>314±19</td>
<td>47±4</td>
</tr>
<tr>
<td>1 X 16 (T)</td>
<td>10</td>
<td>428±20</td>
<td>61±4</td>
<td>2.61±0.35</td>
<td>42±6</td>
<td>108±12</td>
<td>27±2</td>
</tr>
<tr>
<td>17 X 20 X 6 (T)</td>
<td>13</td>
<td>522±45</td>
<td>80±4</td>
<td>2.39±0.78</td>
<td>109±7</td>
<td>102±13</td>
<td>24±4</td>
</tr>
</tbody>
</table>

* This group of controls was fasted 16 hr along with the groups exercised 16 hr. † Untrained rats (UT) not exercised previously. ‡ The groups (T) on the last 2 lines were trained by 17-20 successive daily 6-hr exercise tests. After a rest period of 24 hr, the last (T) group was bled while the preceding (T) group was exercised for 16 hr and then bled for samples. Blood samples from the other four exercise groups were also obtained immediately after exercise.

of most of the rats were fixed in buffered (pH 7.0) formalin. Routine paraffin sections of these organs were stained with hematoxylin and eosin, and frozen sections were stained for fat with oil red O.

FIG. 1. Changes in values of serum alkaline phosphatase (SAkP), serum glutamic oxalacetic transaminase (SGOT), serum aldolase (SAld) and blood urea nitrogen (BUN) in rats immediately and 18 hr after successive daily 6-hr exercise tests in a rotating cage. On the extreme left are the four mean composite values of 22 unexercised control rats sacrificed in groups at intervals along with the groups from the same lots of exercised rats.

RESULTS

In a typical group of ten rats exercised 6 hr daily, the mean loss in body weight was 7.9% immediately after the first 6-hr exercise period and 4.6% the next morning following an overnight rest period with access to food and water. The weight loss reached a low of 7.5% after four periods and a diminution to 1.2% the next morning. Thereafter, the animals gained slowly, but their mean weight at the end of the seventeenth exercise period was still about 5% below their initial weight. Ten control rats were placed in the exerciser, but not exercised, and were deprived of food and water for 6 hr daily. Their mean body weight dropped about 3% during each fasting period, but increased by the next morning to about 1% above their prefasting weight; their net weight gain in 6 days was comparable to that of nonfasting controls.

Ten rats were trained by exercising 6 hr on 17 successive days. After a 24-hr rest period, they were exercised for 16 hr along with eight untrained rats from the same lot. Ten other untrained controls from the same lot were deprived of food and water but not exercised during the same 16-hr period. The mean loss in body weight during the 17-hr period was 13% for the trained and 16% for the untrained exercising rats and 7% for the unexercised controls. None of the exercising or control animals died.

The rats exercised 6 hr daily showed a sharp rise in SGOT, SAld, and BUN and a fall in SAkP values (Fig. 1) after each of the first four exercise tests with a partial restoration to normal values each following morning after an overnight 18-hr rest period. Subsequently, despite the continuation of daily exercise tests, the deviation from normal values diminished and the effect of an overnight rest period became less pronounced. The SAkP and BUN values returned to normal, but the SGOT and SAld values were slightly above control values even after 17-20 exercise tests (Table 1). The SGPT and SLD showed a similar but proportionally lesser rise and subsequent fall in values than those of SGOT and SAld (Table 1), because of marked variations in values in individual animals, the significance of the changes in SLD values is uncertain.
The changes in blood values after a 12-hr exercise test were intermediate between those found after a 6-hr and 16-hr test (Table 1).

Untrained rats exercised 16 hr (Table 1) showed a sharp drop in mean SAkP values and a sharp rise in values of BUN and of the other enzymes in the serum. After 9-12 hr, a number of these untrained rats appeared fatigued and discontinued exercising by sliding during all or part of the 25-min interval between rest periods. In contrast, the trained rats continued to exercise (walk) throughout the 16-hr test period and showed no significant changes in blood values after the test except for a fall in SAkP values comparable to that seen in fasting controls (Table 1).

HISTOPATHOLOGIC CHANGES

As described previously (1), pathologic study of untrained rats sacrificed after a 16-hr exercise test often revealed significant fatty changes in the myocardium, liver, kidney, and muscle and depletion of lipid in the adrenal cortex. Similar fatty changes were seen but rarely after a 6-hr test except in the muscles. Moderately severe fatty changes appeared transient, since they were not noted in rats given repeated tests. In rats given three or more exercise tests, the incidence of slight fatty changes in the thigh muscles was even less than in the unexercised controls (Table 1).

Pathologic study of untrained rats sacrificed after a 16-hr exercise test often revealed scattered necrotic or partially necrotic muscle fibers or segments; some necrotic fibers or portions thereof were infiltrated by macrophages or large mononuclear cells, as previously illustrated (1). The severity of the necrosis was graded by counting the number of necrotic muscle fibers seen in a stained paraffin section about 1 cm² in area. The finding of 1 or 2 necrotic fibers was not considered significant since these were also seen in some unexercised control rats. The lesions were graded mild if 3-15 necrotic fibers were noted in a section, moderate if 16-35 necrotic fibers were found, and severe if 36-150 fibers were necrotic. It should be noted that even in severe lesions the necrotic fibers constituted only a minute proportion of all the muscle fibers. As shown in Table 2, significant necrosis was found in about 27% of rats given a single 6-hr exercise test, reached a peak of 63% after three exercise tests, and then declined rapidly and was rarely seen after eight or more exercise tests.

Regression of lesions was evidenced most frequently by infiltration and margination of the necrotic muscle fiber or its necrotic portion by mononuclear cells (1) and its apparent eventual replacement by a dense cluster of mononuclear cells. The grading of these regressing or postnecrotic lesions was similar to that of the necrotic lesions and based on the number of clusters of mononuclear cells seen in the same sections. Sections containing less than three clusters were considered negative since one or two such clusters were seen in about 25% of the unexercised controls. Postnecrotic lesions were most pronounced after two to
SERUM ENZYMES AND LESIONS IN RATS AFTER EXERCISE

Our studies emphasize the importance of training. In this and the preceding study (1), untrained rats exercised for 16 hr became fatigued and showed a sharp rise in SGOT, SGPT, SLD, SAld, and BUN values and a fall in SAKP, and many showed necrosis of scattered muscle fibers and fatty changes in the liver, kidney, heart, adrenal, and striated muscle. In contrast, trained rats, given 17-20 successive daily 6-hr exercise tests and then exercised for 16 hr (Tables 1 and 2), showed no apparent fatigue, no significant histopathologic changes, and no significant changes in blood values, except a fall in SAKP values. These findings support Selye's views on a general adaptation syndrome (6).

In an untrained rat, even a 6-hr test, which appeared to be well tolerated, can be damaging and produce changes in the blood values and tissues similar to but less severe than the changes after a 16-hr exercise test. After three or four daily 6-hr exercise tests, the effects appeared to be cumulative and the changes were comparable to those found after a 16-hr test (Tables 1 and 2); the mean body weight fell about 17%. However, when the daily exercises were continued beyond 4 days, conditioning became evident by a return to near normal blood values, resolution of the lesions, and partial restoration of the weight loss. The SGOT, SLD, and SAld values after 17-20 daily exercise tests were above control values (Table 1). These findings are consistent with those of Cantone and Cerretelli (3) who reported that the serum aldolase activity in rest, in subjects undergoing a 50 days training and in athletes in a period of activity, was two to four times higher than in sedentary subjects. These findings are also of importance in the differential diagnosis of myocardial infarction (7).

We have reported somewhat similar changes in blood values in dogs after administration of large doses of catecholamines (8) or after exposure to simulated high altitude (9, 10), and in rats after exposure to cold (11). In the dog, however, there was a rise in SAKP. These findings suggest that the serum enzyme changes are in part nonspecific and that the specific muscle lesions found after prolonged exercise in the rat may not be the major factor contributing to the changes in blood values. Alterations in permeability of mitochondria or the cellular membrane have been considered to be important factors in the increase in serum enzymes (1, 3, 4, 8-11); this would permit certain cellular enzymes to escape and accumulate in the blood. This hypothesis would explain the elevations in blood values in animals recovering or showing no demonstrable lesions.

Except for a marked fall in SAKP values in unexercised control rats fasted and deprived of water for 16 hr, there was little or no difference between the serum values in five exercise tests (Table 1); significant lesions, rarely severe, were seen in 23% of the rats sacrificed after one or two exercise tests and in 61% after eight or 12 tests. Mild postnecrotic lesions were seen in only 20% of rats sacrificed after 17-20 exercise tests. These findings indicate that the postnecrotic lesions are transient and resolve rapidly even in animals undergoing training.

The degree of mononuclear cell infiltration varied in different necrotic fibers or portions of the same fiber indicating that the onset of necrosis was not uniform in time. Other muscle changes noted after exercise in some instances included fragmentation and atrophy of scattered muscle fibers, indistinct striation, cytoplasmic basophilia, marked proliferation of muscle nuclei, formation of multinucleated giant cells, and a focal interstitial fibroblastic proliferation and infiltration by various types of inflammatory cells (Fig. 3). These findings suggest that some fibers may recover after undergoing changes short of necrosis.

In trained rats given a 16-hr exercise test, significant fatty changes were seen only occasionally in the liver, kidney, or myocardium; the incidence was not significantly different statistically from that found in the unexercised controls. Similarly, in sharp contrast to the findings in untrained rats after a 16-hr test, the trained rats showed no significant fatty changes or necrosis in the muscle.

There was no close correlation between the severity of the muscle lesions and the degree of enzyme elevations in the serum. Most of the rats with severe lesions had marked elevations of enzymes in the serum. However, there were some rats with mild or insignificant lesions that had higher elevations of enzymes in the serum than other rats with severe lesions.
such animals and those of unexercised controls fed ad libitum (Table 1). The drop in $SAkP$ values in trained rats (T) exercised 16 hr was comparable to that in fasted controls and, therefore, is attributable to the fasting and water deprivation during the exercise period. In untrained rats (UT), the $SAkP$ values were lower than in corresponding unexercised controls. This indicates that exercise contributed to the fall in $SAkP$ values in such rats.

The regulating mechanism for $SAkP$ differs in the rat from that in man or dog. In the rat, ligation of the bile duct causes a fall in alkaline phosphatase (12) instead of a rise as in man and dog. Accordingly, it is unlikely that $SAkP$ values would fall in man after strenuous exercise.

Species differences in the behavior of other serum enzymes may also be anticipated.

Although there was no close correlation between the degree of elevation of the blood values and the severity of demonstrable lesions, most of the animals with severe lesions showed marked serum enzyme elevations. Accordingly, determination of serum enzyme elevations may be helpful in evaluating individual or group exercise training programs. A program causing marked serum elevations or weight loss would suggest the development of tissue damage and the need for caution and a less strenuous and more gradual initial training program.

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REFERENCES
