A STUDY OF "SIMPLE DISUSE ATROPHY" IN THE MONKEY

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Other than in immobilization as occurs, for example, in the treatment of fractures, there are very few conditions which cause simple disuse atrophy of skeletal muscles. Immobilization experiments have been carried out by Froboese (1), Legg (2), Thompson (3) and Lippman and Selig (4), but their results are not in agreement. Differences in technique are, no doubt, responsible for many of the discrepancies. The present study was undertaken in an attempt to clarify this problem.

METHOD. Six young macacus rhesus monkeys were employed in the experiment. By applying a body and leg cast, an attempt was made to reduce the activity of the gastrocnemius-soleus muscles to a minimum, although it was realized, of course, that muscles so immobilized are still subject to "static" activity due to "stretch" and other tonic reflexes, which influences can only be abolished by nerve section.

Before applying the plaster, the extremity was first placed in a stock-inette sleeve extending distally beyond the toes and proximally high up over the abdomen. The extremity was then wrapped very thoroughly with cotton batting to prevent pressure sequelae. Preliminary experiments showed that ischemic paralysis and pressure sores would be produced very easily by improper applications of the cast, and that the success of the experiment depended primarily upon the avoidance of such complications.

In applying the plaster bandages, the limb was held in a position of slight flexion at the knee, in order to obtain maximum relaxation of the gastrocnemius-soleus muscles and to avoid stretching. At designated periods of one, two, three, four, six, and ten weeks, respectively, the casts were removed. Using aseptic technique, the gastrocnemius-soleus muscles were dissected from their proximal and distal attachments.

Immediately following the excision, the muscle group was weighed and then bisected longitudinally. One portion was used for histologic study and the other for chemical analyses. For histologic studies, the sections

1 This work was carried on with the assistance of a grant from the Council on Physical Therapy of the American Medical Association.
were stained by the hematoxylin and eosin, Van Gieson, and a modified
Ranson pyridine silver-methods.

In chemical analyses, water content was determined by desiccation of
the muscle over sulphuric acid under reduced vapor pressure. Total nitrogen
was determined by the Kjeldahl method and the protein content calculated.

In several preliminary experiments, the atrophied muscles were tested
for threshold responses to faradic and galvanic stimulation and compared
with the controls.

**Results.** Within one week after immobilization there was gross evi-
dence of a decrease in muscle bulk. This was verified by weighing, which
revealed a loss of 4.9 per cent, as compared to the control side. In the

<table>
<thead>
<tr>
<th>TABLE 1</th>
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<tbody>
<tr>
<td><strong>Atrophy of simple disuse</strong></td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Wt. specimen on removal</th>
<th>ONE WEEK</th>
<th>TWO WEEKS</th>
<th>THREE WEEKS</th>
<th>FOUR WEEKS</th>
<th>SIX WEEKS</th>
<th>TEN WEEKS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wt. left</td>
<td>12.28</td>
<td>12.19</td>
<td>11.45</td>
<td>10.62</td>
<td>10.30</td>
<td>9.77</td>
</tr>
<tr>
<td>Wt. right</td>
<td>11.67</td>
<td>11.50</td>
<td>10.96</td>
<td>10.60</td>
<td>10.92</td>
<td>10.22</td>
</tr>
<tr>
<td>Weight difference</td>
<td>0.61</td>
<td>1.57</td>
<td>2.14</td>
<td>5.83</td>
<td>5.51</td>
<td>4.71</td>
</tr>
<tr>
<td>Per cent wt. loss</td>
<td>4.9%</td>
<td>12.8%</td>
<td>15.7%</td>
<td>27.1%</td>
<td>29.8%</td>
<td>32.5%</td>
</tr>
<tr>
<td>Wt. 1/2 to be desiccated</td>
<td>5.63</td>
<td>5.27</td>
<td>5.15</td>
<td>5.12</td>
<td>5.11</td>
<td>5.15</td>
</tr>
<tr>
<td>Constant wt. after prolonged desiccation</td>
<td>1.46</td>
<td>1.28</td>
<td>1.57</td>
<td>1.25</td>
<td>1.88</td>
<td>1.49</td>
</tr>
<tr>
<td>Water content in gms.</td>
<td>4.06</td>
<td>3.99</td>
<td>4.67</td>
<td>3.79</td>
<td>4.88</td>
<td>5.17</td>
</tr>
<tr>
<td>Per cent water</td>
<td>73.5%</td>
<td>74.9%</td>
<td>74.8%</td>
<td>74.3%</td>
<td>74.8%</td>
<td>74.3%</td>
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<tr>
<td>Total nitrogen</td>
<td>0.196</td>
<td>0.195</td>
<td>0.216</td>
<td>0.200</td>
<td>0.278</td>
<td>0.246</td>
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<tr>
<td>Protein N x 6.25</td>
<td>1.225</td>
<td>1.217</td>
<td>1.351</td>
<td>1.156</td>
<td>1.500</td>
<td>1.446</td>
</tr>
<tr>
<td>Per cent protein</td>
<td>22.6%</td>
<td>22.5%</td>
<td>21.6%</td>
<td>21.6%</td>
<td>21.1%</td>
<td>21.9%</td>
</tr>
<tr>
<td>Specimen number</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
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</tbody>
</table>

other specimens subjected to longer periods of inactivity (2, 3, 4, 6 and 10
weeks, respectively), the atrophy was found to be increased accordingly,
as indicated in table 1.

Grossly, the atrophied muscles appeared somewhat paler than those of
the control side. Microscopically, the muscle bundles were definitely
smaller in diameter. The individual muscle fibers were narrower in cross-
section and in longitudinal section showed prominent cross-striations, the
Q bands appearing denser than normal. In cross-section, the fibers
appeared more homogeneous, and Cohnheim's zones were less prominent,
implying a decrease in the amount of sarcoplasm. Longitudinal striations
were not observed. The sarcolemmal and muscle nuclei were not obviously
increased in number nor altered in size, and there was no evidence of mito-
The connective tissue elements were not increased. There was no change in the intra-muscular blood vessels. The intramuscular nerves and nerve endings were intact and normal in appearance, as demonstrated by the silver stain.

Chemical studies showed that the proportions of water and protein remained essentially the same as in normal muscle. For example, in the ten-week specimens, despite a 32.5 per cent weight difference between the atrophied and the control muscles, the variation in protein content between the two was but 0.30 per cent, and the difference in water content was too slight to have any significance.

Electrical stimulation of the atrophied muscles with faradic and galvanic current gave prompt responses similar to those obtained in normal skeletal muscle.

Discussion. Formerly, the atrophy resulting from lesions of the nervous system, as well as in the arthritides in which activity is limited, has been attributed to disuse. This concept of disuse atrophy, however, is not strictly correct and needs to be clarified.

By simple atrophy of disuse is meant that wasting of muscle tissue which results solely from a curtailment of its specific function, namely, contraction, and without any accompanying disturbance of its nerve or blood supply. The atrophy resulting from disuse was found to be a very slow process. As such, it differs greatly from muscle atrophy resulting from neuron lesions. Computation of weight loss by comparing the inactive muscles with those of the opposite extremity, is subject to error. It may be pointed out that because of the limitation of activity of the one extremity, there was an increased use imposed upon the opposite one, which might result in some hypertrophy. Such a criticism seems quite justified. On the other hand, when one compares the general behavior of the animals used with that of the untreated animals, it is quite evident that the former group are less active than normally. Another fact to be considered is the normal variation in weight of the same muscle groups of opposite extremities. Studies by Lipschutz and Audova (5) on normal rabbits indicate that this variation is slight.

Histologic studies established the fact that this type of atrophy is associated with very simple structural changes. The loss in muscle bulk can apparently be accounted for on the basis of a diminished quantity of sarcoplasm in the individual muscle fibers. It is quite likely that slight changes also occur in the myofibrillar elements. If so, the changes are not revealed by the usual staining methods. The atrophied muscle fibers appeared to be packed more closely together, so that in a microscopic field of a definite area, a larger number of fibers was seen than in a corresponding area of normal muscle. There was no evidence of degeneration of the
muscle fibers, nor were there any of the usual features indicating an attempt at regeneration so characteristic of neurogenic atrophies.

Chemical analyses for water and nitrogen showed no significant change in water content between the atrophic and the normal muscle. The differences were small and occurred in both directions. This makes it highly improbable that there was any change in the degree of hydration of the protein. The quantitative decrease in protein content of this type of atrophy is associated with a decrease in the weight of the total muscle bulk, so that the relative proportion remains the same as that of normal muscle.

Physiologic tests likewise gave evidence of the absence of any degenerative process. Faradic and galvanic stimulation produced responses similar to those obtained in normal muscle.

Thompson, using rabbits, reported degeneration of muscle fibers with fibrosis, after six weeks' immobilization. He commented, however, upon the occurrence of circulatory and pressure complications in his experiment. This, no doubt, also accounted for the very rapid wasting which occurred. Froboese, likewise, obtained degeneration of muscle fibers and replacement with fibrous tissue. Edema and pressure from the cast, no doubt, were responsible for these changes. It has been demonstrated by the present study that when such complications are avoided, the inactivity of muscle, such as is obtained by immobilization, does not give rise to any degeneration of muscle tissue.

Davenport and Ranson (6) studied the changes in skeletal muscle following tenotomy. After a period of from five to eight days, contracture occurred with a 20 per cent loss of weight. There was no degeneration of muscle fibers, nor increase in nuclei. They found an increase in the diameter of the fibers, more pronounced longitudinal striations, and blurred and wavy cross striations, which they considered characteristic of myostatic contracture. Such muscles, however, have been deprived of their "stretch reflex," and, hence, an additional factor has been added to that of simple inactivity. This, no doubt, accounts for the very rapid rate of atrophy resembling that which results following nerve section.

Lippman and Selig (4) found only a slight amount of muscle atrophy following fixation of the limb of the rabbit. They stated that such atrophy is not appreciable before the lapse of at least a month. Our results, however, imply a more rapid rate of atrophy, which corresponds to that commonly observed clinically.

In order to ascertain the status of the anterior horn cells which subserve the skeletal musculature subjected to inactivity, one monkey was treated as follows:

The left upper extremity was immobilized in a plaster of Paris cast for a period of one year. During this period the cast was removed at three-
month intervals to determine the state of the immobilized extremity, particularly to insure the avoidance of ischemic and pressure complications. Histologic examination of the atrophied muscles showed simple changes similar to those reported above. The cervical enlargement of the spinal cord and the peripheral nerves derived therefrom were examined. Employing hematoxylin and eosin and the Davenport silver stain, no difference could be demonstrated between cells of the right and left anterior cornu of the spinal cord. The peripheral nerves were likewise normal in appearance.

The factor of disuse has been mentioned by some in explanation of the changes observed in the anterior horn cells subserving extremities which have been amputated (Spatz, 7). These changes, designated as "axonal chromatolysis," have been considered retrograde and consist of a displacement of the nucleus to the axon hillock and a clumping of the Nissl bodies in that locality with a paling of the remaining cytoplasm. Other authors attribute these changes to injury to the axons.

The results of our experiment indicate that such muscular inactivity as is obtained by immobilization does not lead to demonstrable changes in the anterior horn cells of origin of the respective nerve supply. This is further supported by the lack of any changes in the intramuscular motor nerve endings.

CONCLUSIONS

1. Disuse atrophy is a distinct entity and is simple in character, as revealed by histologic findings.

2. Disuse atrophy consists primarily of a uniform reduction of the bulk of each muscle cell, especially of the sarcoplasm. It is not attended by any evidence of degeneration or attempts at regeneration. Irritability to electrical stimuli remains unaffected.

3. There is no alteration in the proportions of water and nitrogen content.

4. Simple disuse is not associated with any demonstrable changes in the anterior horn cells of origin of the respective nerve supply, and the atrophy of the peripheral musculature due to disuse does not result in such changes.

REFERENCES

(3) Thompson, T. C. J. Bone and Joint Surg. 16: 569, 1934.