RELATIONS BETWEEN STRUCTURE AND FUNCTION IN THE DESIGN OF SKELETAL MUSCLES

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INTRODUCTION

In two recently published papers (11, 18) a detailed study was made of the properties of motor units in the soleus and medial gastrocnemius muscles of the cat. In particular, it was shown that there were great variations in the size of individual motor units and, paralleling them, important differences in their contractile characteristics. The full significance of these findings did not become apparent, however, until the observations reported in the preceding paper (7) had been made, indicating that the excitability of motor neurons is an inverse function of their size. From this it follows that the participation of a motor unit in graded motor activity is dictated by the size of its neuron. A corollary of this conclusion is that the total amount of contractile activity of a unit decreases as its size increases. These new findings on motor units have prompted a histochemical study of the soleus and m. gastrocnemius (MG), using recently developed methods (14) of staining muscle fibers for adenosine triphosphatase (ATPase). The results of this study are presented here. They provide a background for a functional analysis of the data on motor units. The hypotheses emerging from this analysis, in turn, offer a rationale for the histological findings. The conjunction of these different findings, all concerned with the same muscles, permits us to point out some previously unrecognized principles which govern the design of skeletal muscles.

METHODS

Strips from 12 soleus and 15 medial gastrocnemius muscles, each approximately 2 cm. x 5 mm. x 5 mm., were obtained from anesthetized cats and were tied to a splint, which was held parallel to the direction of the fibers. The splinted muscle was frozen by plunging it into a mixture of dry ice and 95% alcohol at -70° C. Cross sections 10 μ in thickness were cut in a cryostat maintained at -18° C. Sections were mounted on chilled glass slides, thawed and dried before a fan at room temperature for 30 min. Mounted sections were fixed for 10 min. in Novikoff's (13) calcium formol. Mitochondrial ATPase at pH 7.2 was demonstrated by the lead method (17). The medium contained adenosine triphosphate (ATP), lead nitrate, magnesium sulfate and tris maleate buffer. Dinitrophenol, an activator of mitochondrial ATPase, was added to the medium to counteract the inhibition resulting from fixation (14). The sections were incubated at 37° C. for 1–2 hours, rinsed, and immersed in ammonium sulfide. They were mounted in glycerogel without dehydration.

1 This study was supported by a research grant from the National Science Foundation.
2 Predoctoral Fellow of the National Institutes of Health.
Demonstration of mitochondrial ATPase depends on a combination of the released orthophosphate with lead to form lead phosphate. Subsequent treatment with ammonium sulfide results in a dark brown precipitate of lead sulfide. This ATPase activity requires the presence of magnesium ions (10) and is enhanced by dinitrophenol (14).

Fibers were classified on the basis of the over-all degree of ATPase activity and the size and distribution of their mitochondria. In the medial gastrocnemius, the large pale fibers with relatively few mitochondria were designated A fibers. These fibers represent the classical "white" fiber. The two other fiber types are probably variants of the classical "red" fiber. Those with moderate enzymic activity and pronounced peripheral aggregates of mitochondria are designated B fibers. The smallest fibers which show intense ATPase activity and are filled with small mitochondria are designated C fibers (cf. 12, 14, 16).

Low-power photomicrographs of representative fields were taken and each fiber in the photograph was labeled A, B or C according to its ATPase activity. All of the fibers were then cut out individually or in groups and sorted into the three types. From the weight of each group of cutouts, the number of fibers in it and the weight per unit area of the paper, the average diameter and cross-sectional area of each type of fiber was computed. For the purpose of the calculations it was assumed that the fibers were round. With this procedure the average dimensions were obtained, but not the standard deviations.

RESULTS

Medial gastrocnemius

Although the ATPase technique used in this study of cat muscle differed from that employed by Stein and Padykula (16) in their investigation of rat muscles, the histological appearance of the MG was essentially alike in the two species. In sections of cat MG prepared as described above three types of muscle fibers can be distinguished. Their appearance and distribution under low magnification are illustrated in Fig. 1. Large, pale fibers, which closely resemble the A fibers described by Stein and Padykula in the rat gastrocnemius, predominate in all fields. Scattered among them are small, dark cells which look similar to the C fibers of rat muscle. A third type, intermediate in size and in the intensity of its ATPase reaction, corresponds closely to the B fibers in rat muscle. Examples of these three types of fibers are shown at higher magnification in Fig. 2.

The appearance of the A fibers in Fig. 1 is not exactly the same everywhere. Some cells are definitely lighter and contain fewer mitochondria than others. In order to ascertain whether differences in enzymatic activity at different levels of the sarcomere might account for these minor variations, longitudinal sections of muscle were prepared. It was found that each of the three types of fibers has a characteristic appearance which does not vary but is uniform throughout its observable length.

There is a tendency for A fibers which are assembled together in a group to resemble each other very closely, which suggests that they may belong to the same motor unit. The size, background, and mitochondrial content of B fibers also vary from cell to cell, but it is difficult to judge whether fibers of similar appearance are grouped together. C fibers vary slightly in their appearance, but appear to be more uniform than A and B fibers. Large groups of B or C fibers are never seen in solid assemblies, which suggests that the fibers of type-B or type-C motor units are spread out diffusely or in chains among A fibers.
The data in Table 1 indicate that the predominance of A fibers seen in Fig. 1 is found in the proximal, middle, and distal portions of the muscle. In number, A fibers constitute about 50% of the fiber population. Since A fibers are the largest of the three types, their total cross-sectional area on a percentage basis is even larger, sometimes exceeding 70%.

There are some regional variations in the B- and C-fiber population. The proximal part of the muscle contains 20–35% B fibers by number or area. The same region contains 10–25% C fibers by number, but 10% or less by area. In the middle and distal portions of the muscle the B and C fibers are more evenly divided in number and area.

The percentages of A, B, and C fibers in the proximal parts of three different specimens are given in Table 1. The means of these values are 54.5% A fibers, 29.9% B fibers, and 15.7% C fibers. The difference in the mean per cent of A fibers relative to B fibers and of A fibers relative to C fibers is very significant: $0.01 > P > .001$ (Student’s t test); for B fibers relative to C fibers $0.2 > P > 0.1$.

In the middle and distal portions of the muscle there are 48.3% A fibers, 26.2% B fibers, and 25.6% C fibers (means of values in Table 1). The differ-
ence in the mean per cent of A fibers relative to B fibers and A fibers relative to C fibers is very significant (P < .001), but the difference in the mean per cent of B fibers relative to C fibers is insignificant.

The mean diameters and mean areas ($\mu^2$) of A, B, and C fibers in different parts of the MG and in different specimens are also given in Table 1. The size of the fibers varied considerably from animal to animal, therefore over-all averages were not computed. It is clear that A fibers were consistently greater in cross-sectional area than B fibers, which were consistently larger than C fibers. The ratios of these areas to each other, however, varied with the particular sample and the region from which it came.

An advantage of the ATPase method of studying muscle is that it permits a striking visualization of the smallest blood vessels. A close examination of Figs. 1 and 2 will reveal the presence of darkly stained capillaries located at the interstitial angles between fibers. The density of capillaries is greatest around C fibers, somewhat less around B fibers, and least around A fibers. Unless they are adjacent to B or C fibers, A fibers may have no associated capillaries at all. Occasionally a capillary can be seen partially or almost entirely encircling a B or C fiber, but never an A fiber. The density of capillaries around soleus fibers (Fig. 3) is

<table>
<thead>
<tr>
<th>Muscle</th>
<th>Mean Cross-Sectional Area, $\mu^2$</th>
<th>Mean Diameter, $\mu$</th>
</tr>
</thead>
<tbody>
<tr>
<td>MG prox.</td>
<td>58.4 31.3 25.5</td>
<td>72.0 66.5 61.2</td>
</tr>
<tr>
<td>MG dist.</td>
<td>46.4 23.8 19.8</td>
<td>49.0 22.0 19.1</td>
</tr>
<tr>
<td>Sol. lat.</td>
<td>64.0 30.0 26.0</td>
<td>64.0 30.0 26.0</td>
</tr>
<tr>
<td>Sol. mid.</td>
<td>41.0 21.0 18.0</td>
<td>41.0 21.0 18.0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table 1. Numbers, areas, and sizes of muscle fibers in soleus and m. gastrocnemius muscles</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number, %</td>
</tr>
<tr>
<td>Muscle</td>
</tr>
<tr>
<td>Total Cross-Sectional Area, %</td>
</tr>
<tr>
<td>MG prox.</td>
</tr>
<tr>
<td>MG dist.</td>
</tr>
<tr>
<td>Sol. lat.</td>
</tr>
<tr>
<td>Sol. mid.</td>
</tr>
<tr>
<td>Sol. sup.</td>
</tr>
</tbody>
</table>
FIG. 2. Characteristic features of A, B, and C fibers in m. gastrocnemius (middle portion) at high power: type A, large, with few mitochondria; type B, intermediate in size and enzymatic activity, with subsarcolemmal distribution of large mitochondria; type C, small, with marked background activity and numerous small mitochondria. 10μ. ATPase section; pH = 7.21. X800.

apparently greater than it is around B fibers in MG. This may be due to the fact that B fibers in MG usually share their capillaries with adjacent A fibers instead of other B fibers. As will be pointed out in the discussion, the vascularity of muscle fibers varies not only with the type of the fibers, but also, in an indirect way, with the size and "usage" of their motor units.

Soleus

The characteristic appearance of the cat soleus, as revealed by the mitochondrial ATPase method, is illustrated in Figs. 3 and 4. Samples taken from the medial, intermediate, and lateral parts of the muscle showed no regional difference in the mitochondrial content of the fibers. In all respects, they resemble most closely the type-B fiber of heterogeneous muscles. The over-all intensity of the reaction is intermediate between that of A and C fibers. The background activity of soleus fibers appears slightly darker and more uniform than in gastrocnemius B fibers. Soleus fibers have numerous small mitochondria, some of which are filamentous in appearance. Although the density of mitochondria is somewhat greater at the periphery of the cells and near capillaries, the subsarcolemmal aggregates are less pronounced
than in the B fibers of heterogeneous muscles. There are slight variations in
the intensity of the reaction from fiber to fiber, but these are minimal in
comparison with the extremes produced by the same methods in MG. Oc-
casionally a few lighter fibers have been observed in a cluster and even more
rarely a large, lone, darker fiber is seen. The evidence, however, leaves no
doubt that the soleus of the cat is a very homogeneous muscle with respect
to fiber type.

In general the fibers of soleus varied less in diameter from part to part
and animal to animal than in any other muscles which were studied. The
mean fiber diameters of the lateral, intermediate, and medial portions of this
muscle in one sample were 60.3, 59.7, and 63.9 μ. respectively. As Table 1
indicates, the diameters of B fibers in MG were in this same range or slightly
below it. The cross-sectional area of soleus fibers ranged from 2,800 to
3,700 μ².

Around every soleus fiber there are four to eight capillaries, which also
show intense ATPase activity. They occur with great regularity at the inter-
stitial angles where one capillary can be shared by three to four muscle

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Fig. 3. Mitochondrial ATPase activity of soleus, showing uniformity of ATPase
activity and fiber size. Note intense ATPase activity of capillaries around each fiber. 10μ,
section; pH = 7.2. X220.
fibers and they are scattered elsewhere around the periphery where they can be shared by two fibers. It is obvious that every soleus fiber has an ample blood supply, as do the B and C fibers of heterogeneous muscles. This observation is consistent with all that is known about the functional properties of red fibers.

Intrafusal muscle fibers

In the course of these studies observations were also made on the intrafusal fibers inside muscle spindles. Boyd (1) has described two distinct types: 1) “nuclear bag” fibers with a concentration of nuclei in their central portion, which are longer, larger in diameter (20–30μ.), and with greater myofibrillar density; 2) “nuclear chain” fibers with a single row of nuclei running through the equatorial region, which are shorter, smaller in diameter (10–20μ.), and lower in myofibrillar density. When examined in our ATPase material, spindles clearly had two types of fibers which could be distinguished by their size and enzymatic activity. Examples are shown in Fig. 5, A and B, which are from a soleus and a flexor digitorum longus, respectively. The small size of intrafusal fibers is clearly evident, seven to eight of them occupying less total area than one large extrafusal fiber. The spindle in Fig. 5A has one large fiber containing a moderate number of mitochondria and six
Fig. 5. Mitochondrial ATPase activity of muscle spindles in soleus (A) and flexor digitorum longus (B). A: note one large, nuclear bag fiber with scattered mitochondria and seven small nuclear chain fibers with many mitochondria. Nerves and vessels also present. B: note two nuclear bag fibers with moderate ATPase activity and six nuclear chain fibers, densely packed with mitochondria. 10μ, section; pH = 7.2. ×500.
small fibers with a greater density of mitochondria. The spindle in Fig. 5B has two larger fibers which are somewhat darker than the single one in Fig. 5A and a group of smaller fibers which are extremely dark. It is worth noting that even a very homogeneous muscle such as the soleus contains two types of intrafusal fibers. Spindles are supplied by blood vessels which are surprisingly large for the size of the structures they supply.

**DISCUSSION**

The foregoing observations indicate that there are three types of muscle fibers in pale muscles such as the m. gastrocnemius of the cat, but only one type of fiber in red muscles such as the soleus. Physiological studies (18) strongly suggest that individual motor units are uniform in their fiber composition, from which we may conclude that there are three types of motor units in MG, but only one type in soleus. In the discussion which follows we shall attempt to show how these different types of motor units contribute to the over-all performance of the triceps group. For this purpose we shall draw upon data obtained in our prior studies on these muscles.

*Significance of axonal conduction velocity of motor units*

The conduction velocities of axons may have special significance in the timing of events in the nervous system as many authorities believe (4). We are less concerned with that possibility, however, than with some other implications of conduction velocity suggested by the data in Tables 2 and 3. Line 3 of Table 2 contains a listing of the mean tensions of all soleus units grouped according to their conduction velocities and line 4 contains the contraction times of the same groups of units. The corresponding data for MG are found in Table 3. The significance of conduction velocity for this study is that it is a measure of axonal diameter (9). The data in Tables 2 and 3, therefore, indicate that the maximal tensions or "sizes" of motor units bear a direct relationship to the diameter of their axons as well as to their conduction velocity. Small fibers innervate small units. As line 4 in both tables indicates, these tend to be slowly contracting. Large fibers supply large units which contract rapidly. In the case of soleus, a homogeneous muscle, the mean tensions increase quite uniformly from the slowest to the fastest group of fibers. The correlation between conduction velocity and tension is equally impressive for MG up to about 100 m/sec., but the last two groups, with six and two units each, are slightly below the peak of mean tension.

Since the soleus is a homogeneous muscle with fibers which are relatively uniform in size in a given animal (Table 1), we may conclude that variations in the diameter of its axons are related chiefly to the number of muscle fibers innervated by each axon. In the case of MG, however, the diameter of each axon may be related to the type and size of the muscle fibers it supplies as well as their number.
### Table 2. Mean tensions and contraction times of soleus motor units grouped according to their conduction velocities

<table>
<thead>
<tr>
<th>Conduction Velocity, m/sec.</th>
<th>50.0–54.9</th>
<th>55.0–59.9</th>
<th>60.0–64.9</th>
<th>65.0–69.9</th>
<th>70.0–74.9</th>
<th>75.0–79.9</th>
<th>80.0–84.9</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. No. of units</td>
<td>1</td>
<td>14</td>
<td>12</td>
<td>24</td>
<td>21</td>
<td>9</td>
<td>1</td>
</tr>
<tr>
<td>2. Contraction times</td>
<td>177</td>
<td>100</td>
<td>115</td>
<td>104</td>
<td>101</td>
<td>85</td>
<td>90</td>
</tr>
<tr>
<td>3. Mean tensions, g.</td>
<td>4.7</td>
<td>9.5</td>
<td>14.9</td>
<td>16.2</td>
<td>15.0</td>
<td>17.0</td>
<td>20.6</td>
</tr>
<tr>
<td>4. Conduction Velocity</td>
<td>50.0–54.9</td>
<td>55.0–59.9</td>
<td>60.0–64.9</td>
<td>65.0–69.9</td>
<td>70.0–74.9</td>
<td>75.0–79.9</td>
<td>80.0–84.9</td>
</tr>
</tbody>
</table>

Numbers in parentheses in line 4 indicate number of units on which contraction times were obtained.

### Table 3. Mean tensions and contraction times of m. gastrocnemius motor units grouped according to their conduction velocities

<table>
<thead>
<tr>
<th>Conduction Velocity, m/sec.</th>
<th>55.0–59.9</th>
<th>60.0–64.9</th>
<th>65.0–69.9</th>
<th>70.0–74.9</th>
<th>75.0–79.9</th>
<th>80.0–84.9</th>
<th>85.0–89.9</th>
<th>90.0–94.9</th>
<th>95.0–99.9</th>
<th>100.0–104.9</th>
<th>105.0–109.9</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. No. of units</td>
<td>1</td>
<td>1</td>
<td>4</td>
<td>3</td>
<td>15</td>
<td>17</td>
<td>17</td>
<td>21</td>
<td>15</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>2. Contraction times</td>
<td>177</td>
<td>100</td>
<td>115</td>
<td>104</td>
<td>101</td>
<td>85</td>
<td>90</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3. Mean tensions, g.</td>
<td>4.7</td>
<td>9.5</td>
<td>14.9</td>
<td>16.2</td>
<td>15.0</td>
<td>17.0</td>
<td>20.6</td>
<td>72.1</td>
<td>64.8</td>
<td>49.0</td>
<td>40.2</td>
</tr>
<tr>
<td>4. Conduction Velocity</td>
<td>55.0–59.9</td>
<td>60.0–64.9</td>
<td>65.0–69.9</td>
<td>70.0–74.9</td>
<td>75.0–79.9</td>
<td>80.0–84.9</td>
<td>85.0–89.9</td>
<td>90.0–94.9</td>
<td>95.0–99.9</td>
<td>100.0–104.9</td>
<td>105.0–109.9</td>
</tr>
</tbody>
</table>
Significance of tetanic tensions of motor units

As Eccles and Sherrington (3) showed, the contractile properties of soleus and MG differ considerably. MG weighs 2 1/2-3 times as much as soleus, but it develops about 6 times as much tetanic tension (e.g., 15,000 vs. 2,500 g.). This disparity in tension per unit of weight may be due in part to differences in the arrangement of the muscle fibers in the two muscles. In general, muscles with obliquely running fibers such as MG have more but shorter fibers for their weight than muscles like soleus with nearly longitudinal fibers of greater length. As a consequence, they can develop more tension, but cannot shorten as much. To a considerable extent, however, the greater power of MG is also due to the predominance in it of large, pale (type-A) fibers, which are lacking in the soleus (Table 1).

Not only is the power of MG greater, but its "dynamic range" greatly exceeds that of soleus. The largest MG unit (120 g.) developed about three times as much tension as the largest unit in soleus (40.4 g.). The smallest unit encountered (0.5 g.) developed about one-sixth as much tension as the smallest unit (3.2 g.) in soleus. The three types of muscle fibers in MG probably account for its greater dynamic range. Many other muscles, however, which develop less total tension and presumably less tension per motor unit, such as the extraocular muscles, also contain three types of fibers (12). This is not as surprising as it may first appear. The demands for delicacy, power, and speed from one small, specialized muscle may well be more stringent than for larger muscles.

The distributions of unit tensions in soleus and MG depart strikingly from a "normal" distribution but resemble each other closely (cf. 11, 18). In both muscles there is a predominance of units which develop small tensions and a progressively decreasing percentage of larger units. A similarly skewed distribution has been found by Olson and Swett in their studies on flexor digitorum longus and flexor hallucis longus (unpublished observations). The functional significance of this distribution pattern was not apparent until it was found (6, 15) that the excitability of motoneurons is related to the diameter of their axons. The relation is inverse, the cells with the thinnest axons being the most excitable and those with the thickest axons being least excitable. As Tables 2 and 3 indicate, the tetanic tensions or sizes of motor units are also related to the diameter of their axons, but in a direct way. It follows that as the size of motor units increases their susceptibility to discharge decreases.

This relationship between the size of a motor unit and the excitability of its motoneurons is all that is required to interpret the puzzling distribution of unit sizes. Clearly, the order of recruitment of motor units is the same as the left-to-right order of unit sizes (Fig. 6, ref. 11; Fig. 4, ref. 18) of the studies on motor units. Small tensions are produced and precisely controlled by selective mobilization of varying numbers of small motor units, of which many are available. With the demand for further tension larger increments
are added through the contributions of progressively larger motor units. The same principle applies to both soleus and MG although the range of unit sizes available in MG is much greater.

It is of interest to inquire into the fineness of control afforded by this arrangement throughout the whole range of unit sizes. It is obvious that the large number of small motor units varying slightly in their sizes could endow the system with extremely fine control at the low or vernier end of the scale. What happens at the other end of the scale? Is there loss of fine control when the units which are recruited may contribute increments of tension of up to 120 g.? In answering this question we must consider the relation of the various possible increments of tension to the total muscle tensions already developed prior to each new addition. Since recruitment of a new unit of any size occurs after all of the smaller units in that muscle are already active, it is easy to estimate what per cent of the total tension is contributed by the last addition to it. To do this all the unit tensions were grouped in decades. Line 1 of Table 4 gives the sums of the unit tensions in each decade. Line 2 contains the total cumulative tensions, i.e., the sum of all unit tensions in each column plus the totals of all the columns to the left. The per cent of tension contributed by new units to the tensions already developed by all units of smaller size is shown in line 3. For example, the figure 6.4 in the left-hand column was obtained by dividing 10 g. (the next largest unit) by 156.3 g. (the cumulative total of all units below 10 g.). If all increments of tension are viewed as percentages of the pre-existing muscle tension in this manner, it is clear that there is no loss of fine control as the total tension approaches a maximum.

This treatment of the data is intended only as an illustration of a basic principle in the control of muscle tension. A number of factors combine to make the actual control finer and more precise than the scheme outlined above would achieve. There are about three times as many motor units in an average MG as there were in our experimental sample of it, hence each addition to the total tension is an even smaller percentage of the total, as shown in line 4 of Table 4. With more units, the spectrum of sizes is more nearly continuous. Further, the relative thresholds of motoneurons at the low end of the scale are much closer together than at the high end. Finally, in the case of slowly increasing tensions, it is clear that units are recruited into the total discharge in overlapping fashion. New units, that is, are added to the discharge well before the last recruits reach their maximal tension levels. The result is a smooth crescendo of total tension.

**Significance of speed of contraction of motor units**

The intrinsic speed of different muscles varies widely as Hill (8) has pointed out in discussing the significance of this property. Although speed is vital in certain kinds of muscles, it is more costly in terms of energy than slow contraction. In the design of a muscle nature must, therefore, make a compromise between the advantages of speed and the necessity for economy; it must decide, as Hill says, "... whether to save time or energy."

It is now apparent that this compromise usually takes the form of having within a muscle a wide range of contraction speeds, represented in its various motor units. Even in a red muscle such as the soleus, which consists exclusively of type-B fibers in the cat, there is a more than threefold range of contraction speeds (58–193 msec.). In a pale muscle such as m. gastrocnemius, which is composed of three types of units, the full range of contraction speeds is, curiously, somewhat less than in the soleus (17.8–129 msec.). It includes, however, units which contract far more rapidly than the fastest soleus unit. As its histological appearance suggests, MG is composed chiefly of rapidly contracting units. Although a few of its units are slow and some are extremely small, the muscle is designed principally for rapid, powerful contractions rather than for economy. The opposite is true of soleus.
Table 4. Per cent contribution of single motor units to the tension developed by all smaller units of m. gastrocnemius

<table>
<thead>
<tr>
<th>Grouping of Units</th>
<th>&lt;10 g.</th>
<th>10.0-19.9</th>
<th>20.0-29.9</th>
<th>30.0-39.9</th>
<th>40.0-49.9</th>
<th>50.0-59.9</th>
<th>60.0-69.9</th>
<th>70.0-79.9</th>
<th>80.0-89.9</th>
<th>90.0-99.9</th>
<th>100.0-109.9</th>
<th>110.0-120.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Total tensions, g.</td>
<td>156</td>
<td>152</td>
<td>387</td>
<td>252</td>
<td>358</td>
<td>323</td>
<td>443</td>
<td>519</td>
<td>587</td>
<td>278</td>
<td>107</td>
<td>345</td>
</tr>
<tr>
<td>2. Cumulative totals, g.</td>
<td>156</td>
<td>308</td>
<td>691</td>
<td>947</td>
<td>1304</td>
<td>1628</td>
<td>2072</td>
<td>2590</td>
<td>3178</td>
<td>3456</td>
<td>3563</td>
<td>3908</td>
</tr>
<tr>
<td>3. Contribution of 1 unit divided by cumulative totals, %</td>
<td>6.4</td>
<td>6.5</td>
<td>4.3</td>
<td>4.2</td>
<td>3.8</td>
<td>3.7</td>
<td>3.4</td>
<td>3.1</td>
<td>2.8</td>
<td>2.9</td>
<td>3.1</td>
<td>3.1</td>
</tr>
<tr>
<td>4. Contribution of 1 unit divided by cumulative totals (est. for whole muscle)</td>
<td>2.1</td>
<td>2.2</td>
<td>1.4</td>
<td>1.4</td>
<td>1.3</td>
<td>1.2</td>
<td>1.1</td>
<td>1.0</td>
<td>0.9</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
</tbody>
</table>

Table 5. Tetanic tensions of motor units of m. gastrocnemius grouped according to their speed of contraction

<table>
<thead>
<tr>
<th>Contraction times msec.</th>
<th>&gt;100</th>
<th>100-90</th>
<th>89.9-80</th>
<th>79.9-70</th>
<th>69.9-60</th>
<th>59.9-50</th>
<th>49.9-40</th>
<th>39.9-30</th>
<th>29.9-20</th>
<th>&lt;20</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of units</td>
<td>3</td>
<td>1</td>
<td>4</td>
<td>2</td>
<td>3</td>
<td>9</td>
<td>18</td>
<td>24</td>
<td>15</td>
<td>3</td>
</tr>
<tr>
<td>Mean max. tension, g.</td>
<td>4.1</td>
<td>5.1</td>
<td>8.8</td>
<td>9.3</td>
<td>14.5</td>
<td>33.4</td>
<td>45.6</td>
<td>59.8</td>
<td>44.6</td>
<td>47.5</td>
</tr>
<tr>
<td>Range of tensions, g.</td>
<td>3.1-5.9</td>
<td>5.1</td>
<td>8.2-12.5</td>
<td>8.1-10.4</td>
<td>5.0-26.6</td>
<td>4.3-107</td>
<td>8.0-84.3</td>
<td>13.0-120</td>
<td>5.8-114</td>
<td>44-54</td>
</tr>
</tbody>
</table>
Tables 2 and 3 show that the mean tension and contraction time of a motor unit are both correlated with the conduction velocity of the nerve fiber which innervates the unit. The units of lowest threshold (i.e., most slowly conducting) are, in general, the smallest and the most slowly contracting. The units with high thresholds are, in general, large and rapidly contracting. To bring out this association between unit size and speed of contraction in a more direct way, all of the MG units were arranged in decades according to their contraction times (Table 5). It is evident that there is a striking increase in unit tensions as the contraction times become shorter. Only one group of units, those in the decade from 39.9–30.0 msec., was “out of order.” An explanation for the relation between size and contraction speed is proposed in the following section.

"Usage," a physiological basis for the interrelations between size, contraction speed, and type of motor units

In the preceding paper it was pointed out that motoneurons which are small are more readily discharged synaptically than large motoneurons. As a result of their greater susceptibility to discharge, small cells are fired much more often than large ones. In consequence, the small motor units with which they are connected are heavily “used” in comparison with large units. The “usage” of any motor unit, in fact, is probably in inverse ratio to its size.

Usage is the link which connects the apparently unrelated properties of size and speed in a meaningful way. Since units of small size are often used intensely and for prolonged periods, they must of necessity consist of muscle fibers which function economically and are not subject to fatigue. They must always be ready to respond despite a preceding period of prolonged activity. Rapidly contracting pale fibers cannot meet these requirements, as we have shown (18). Slowly contracting red fibers have the appropriate resistance to fatigue. Hence, the small size of a unit, which implies heavy usage, necessarily specifies red fibers and these, in turn, are slowly contracting.

The recent observations of Vrbová (19) on muscles whose tendons had been cut are in harmony with these views. After tenotomy the soleus altered from a slow to a fast muscle. Concurrently there was a significant slowing in the contraction speed of the contralateral soleus which was used more intensively due to ipsilateral weakness. The author believes that these changes in contraction speed were due to the decrease in ipsilateral motoneuron activity and the increase in contralateral activity which accompanied tenotomy.

The collective properties of the soleus also constitute an argument for the usage theory which has been advanced. The range of conduction velocities is from a very slow 50.7 m/sec. to a moderately slow 81.3 m/sec., indicating that its motoneurons are small, low-threshold cells which are used heavily. It is a red muscle and its motor units range from very slow to moderately slow. The low threshold of the whole soleus in stretch reflexes, as compared with MG, has been pointed out by Denny-Brown (2). The muscle
is relatively resistant to fatigue, as Ranvier first reported. All of these facts are consistent with the hypothesis that the size of motoneurons determines their threshold and their threshold determines their usage.

It seems likely that the great variations in mitochondrial ATPase seen in Figs. 1–5 are related to equally great variations in the usage of different types of muscle fibers. The average level of contractile activity of a muscle fiber presumably dictates its need for mitochondrial ATPase. Large, pale fibers, which are innervated by large motoneurons (18), contract infrequently and therefore require little ATPase. Small, dark fibers, which are innervated by smaller neurons, contract more frequently and require ample supplies of ATPase.

The size-principle applied to gamma units

A gamma nerve fiber with the intrafusal muscle fibers it innervates may be regarded as a special kind of motor unit on a small scale. If the hypotheses we have advanced are valid generally, they should apply to these diminutive units. In the following respects the anatomical and physiological properties of gamma units seem consistent with the size principle and its corollaries.

1) There is an obvious relation between the diameters of gamma fibers as a group and the size and number of the intrafusal fibers they innervate. Gamma fibers are thinner than the axons passing to the smallest alpha motor units and, correspondingly, they supply fewer and smaller muscle fibers (Fig. 5). The larger, or $\gamma_1$, axons supply the larger, or nuclear bag fibers; the smaller, or $\gamma_2$, axons innervate the smaller, or nuclear chain, fibers. These observations, which are well established (1), suggest a correspondence between the size of the gamma fiber and either the size of the intrafusal fibers it innervates or their total cross-sectional area, which is similar to the relation between alpha fiber and the muscle fibers they innervate.

2) The tensions developed by contracting gamma units cannot be detected. This is consistent with the relationship between axonal diameter and maximal tetanic tension for alpha units. An examination of the graphs of maximal tension versus conduction velocity for soleus and MG (11, 18) reveals that extrapolation of this relationship down into the range of gamma fibers would bring the tensions to levels below the sensitivity of the tension-recording equipment (0.2 g.).

3) The threshold of gamma motoneurons is apparently as low as the size of their axons indicates that it should be. All investigators who have recorded from ventral root filaments or small muscle nerves have noted a fluctuating but more or less continuous discharge of gamma motoneurons. The nature of the afferent impulses required to maintain gamma activity is not known. Evidently, the threshold of gamma cells is so low that nothing short of eliminating all afferent flow will abolish it.

4) The contraction speeds of intrafusal muscle fibers are believed to be slow, as the small size of their motoneurons would demand and as the heavy usage of gamma units would require. There is not a great deal of information
available on intrafusal muscle fibers. Boyd (1) states that both types of fibers in spindles are "slower" than extrafusal fibers. The nuclear chain fibers are probably slower than the nuclear bag fibers. The appearance of the intrafusal fibers in Fig. 5 is consistent with this statement. The larger nuclear bag fibers have a lower density of mitochondria than the small nuclear chain fibers, just as B fibers have a lower density than C fibers. If increasing mitochondrial density indicates decreasing speed of contraction as it does in the case of extrafusal fibers, the smaller nuclear chain fibers should be considerably slower than the nuclear bag fibers. The large size of the blood vessels supplying spindles is probably related to the greater metabolic requirements of their smaller, but more active muscle fibers as much as to the presence of sensory endings on them.

In summary, as far as one can ascertain at present, the basic size principle which dictates the properties of alpha motor units also governs the properties of gamma motor units. The latter, in fact, appear to represent an extension of the principle into a smaller and more specialized system of motor units.

A final synthesis

Let us now see whether a single, internally consistent explanation for the available information can be formulated, which will account for the histochemical and physiological differences between soleus and MG in terms of their precise functions. If they were unrelated muscles serving completely different ends, their distinctive properties would probably be attributed to their specific roles in the body. The fact that they are two heads of the same muscle complex, functioning in parallel and inserting on the same tendon, limits the possible causes for their differences and helps to focus attention on the significant factors.

Since both muscles cause extension of the foot, it may be assumed that each contributes in a different way to this action. That is to say, their contributions might be expected to differ with respect to maximal tension, speed of contraction, amount of shortening, economy of action, and degree of usage. Which of these aspects of contraction are best served by segregating them in different heads?

Consider first MG. This muscle develops about twice as much tension on a weight basis as soleus, and the range of its unit tensions reflects this fact. It is, in general, rapidly contracting and fatigues quickly. Its most useful qualities, speed and power, result from the nature and disposition of its fibers, which are largely pale and run obliquely to the long axis of the muscle. This arrangement accommodates more fibers which can therefore develop more tension. The fibers, however, are necessarily shorter. Their shortness and oblique course both limit the contractile range of the muscle. Clearly MG has serious shortcomings in over-all performance. It cannot shorten very much, it fatigues rapidly and, accordingly, is not suited for prolonged use.
The soleus seems to be designed to provide all the properties which MG does not possess. It has longer fibers which are arranged almost parallel with the long axis of the leg. Thus, more shortening is possible, though at the expense of power. Redness of muscle fibers is associated with economy and resistance to fatigue. These characteristics are necessary in soleus because the small size of its motoneurons results in heavy use of the muscle.

It must be assumed that the segregation of different properties in soleus and MG came about because of demands which could not be met by a single muscle. The power and speed necessary for running and jumping required one arrangement of fibers; the capacity to shorten enough to produce a full range of ankle extension called for another. In response to the need separate heads evolved, each designed for a special purpose, each complementing the other mechanically. In short, nature discovered long ago that two heads are better than one!

**Summary**

1. Relations between the functional properties of motor units and the mitochondrial adenosine triphosphatase (ATPase) content of their muscle fibers were investigated in a heterogeneous and a homogeneous muscle which insert on the same tendon.

2. Three types of fibers could be distinguished histochemically in m. gastrocnemius (MG) of the cat (Figs. 1 and 2): 1) large, pale A fibers with few mitochondria predominate in all fields; 2) scattered among them are small, dark C fibers which show intense ATPase activity and are filled with small mitochondria; and 3) intermediate in size and ATPase activity are B fibers with peripheral accumulations of mitochondria. In contrast, the soleus muscle is very homogeneous in ATPase sections, containing only B fibers (Figs. 3 and 4). The percentages, mean diameters and mean cross-sectional areas of each type of fiber, as well as their total cross-sectional area, were determined (Table 1). There are four to eight capillaries around each B or C fiber, but usually none around A fibers unless they are adjacent to a B or C fiber.

3. Two types of intrafusal fibers were distinguished in muscle spindles (Fig. 5): 1) larger fibers with a moderate number of mitochondria (presumably ‘‘nuclear bag’’) and 2) smaller, more numerous fibers with a greater density of mitochondria (presumably ‘‘nuclear chain’’).

4. Data obtained in prior studies on motor units in MG and soleus were assembled in four tables showing the relations between axonal conduction velocity, maximal tetanic tension, and contraction speed. The functional significance of these properties and their relation to the histochemical findings are discussed.

5. A hypothesis is advanced that the interrelationships between the functional properties of motor units and the mitochondrial ATPase content of their fibers depend upon the size of the motoneurons which innervate
them: the size of the cell dictates its excitability, its excitability determines
the degree of use of the motor unit, and its "usage" in turn, specifies or in-
fluences the type of muscle fiber required. It is suggested that this size prin-
ciple also governs the properties of gamma motor units.

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