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Functional differentiation of skeletal muscles

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Abstract

According to BUCHTHAL the histogram of duration and voltage from the motor unit of biceps brachii muscles gives only one peak respectively as can be seen in Fig. 14 in his paper which reports the data covering the experiment on 1,268 motor units. However, his histogram seems to be made of the spikes led off from several motor units because the histogram shows no much fluctuations in voltage as 50μV to 1,000μV and in duration as 1 msec to 20 msec. Therefore, if observations are actually based on a single motor unit, two peaks may reasonably be expected on the histogram, because two kinds of the motor units, kinetic (phasic) and tonic, have respective individual characteristics of their own shikes The histological observation shows that many white and red muscle fibers are intermingled with each other even in one single fasciculus, and it is supposed that the fasciculus does not correspond to a single motor unit. Moreover, the shape of the spikes, which was formerly considered as a motor unit, is not a pure diphasic form, but irregular and polyphasic ones, and also electromyographically a single motor unit controls the area of more than 10mm in width (BUCHTHAL). From these facts, it is probable that the histogram by BUCHTHAL was made of the spikes composed of muscle fibers belonging to several different motor units. Our observations done by the above stated method showed clearly the pure diphasic spikes. Therefore, we are of the opinion that these spikes obtained by our method are led off from only one or from a few muscle fibers belonging to the same motor unit. These spikes are lower than 550μV in voltage and shorter than 5.5 msec in duration and every individual spikes show uniform diphasic pattern. There exist two kinds of spike groups, in the histogram one which is composed of high voltage with short duration (1.0-1.5 msec.), and the other of low voltage with long duration (2.0-4.0msec.). The former may be of kinetic (phasic) motor unit and the latter is of tonic motor unit, because the white muscle fibers with a larger diameter may have a higher voltage than the red and the white fibers that perform rapid contraction may show shorter duration in wave form. In the two cases having spinal cord tumors, two kinds of spikes with respective and individual characteristics were observed in the same EMG. These will be two different kinds, kinetic (phasic) and tonic motor units. In the case of amyotrophic lateral sclerosis, two kinds of spikes appeared, but since both of them were of short duration, they might be considered to be of kinetic (phasic) motor unit (or its intermediate motor unit). Furthermore, since its histological findings revealed that the red muscle fibers were all atrophied and degenerated and showd only white muscle fibers to be normal, it is obvious that the kinetic (phasic) motor unit with a shorter duration is derived from white muscle fibers. Therefore, in our opinion the widely accepted concept that spikes including even irregular wave forms all belong to the motor unit seems not to be true, but these spikes seem to represent a combination of several pure spikes though not so many, and those muscle fibers belonging to the same motor unit appear to be intermingling themselves in a relatively wide area.

The reason for this contention may be explained as follows. If the muscle fibers belonging to the same motor unit were agglomerated, clearcut diphasic spikes should appear even with a fairly big electrode, and if the accepted concept be true, these spikes can never be picked up at the distance so far apart as 2.0cm. or 2.5cm. (0.5-1.2cm. by BUCHTHAL) as has been poible in our experiments. Furthermore, the histogram composed only of these pure spikes reveals two peaks, and therefore, we believe it is reasonable to say that these two peaks indicate the existence of kinetic (phasic) and tonic motor units. As a small number of motor units located in between these two peaks can be recognized, these are believed to be the muscle fibers poeing an intermediate stainability as revealed in the histological examination. However, further studies are required before giving any definitive conclusion on this point.

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FUNCTIONAL DIFFERENTIATION OF SKELETAL MUSCLES

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Since RANVIER'S study[1] (1874) it has been well known that there are red and white muscles in the skeletal muscles. The former performs slow contraction and the latter does rapid one. Many investigators (GRÜTZNER[2], BELL[3], BULLARD[4], DENNY-BROWN[5] and others) revealed the histological and physiological differences between these two kinds of muscle fibers. DENNY-BROWN and others[6] (1929) showed that the red muscle is concerned in tonic postural adjustment and the white in phasic rapid contraction.

In the physiological field there are some reports dealing with the functional differentiation of the skeletal muscles. RIJLANT[7] (1933) reported that the motor units may be divided into two groups by the frequency of spike discharge, one group having large potential, the frequency of 40 per second corresponding to contraction, and the other having small potential, and the frequency of 15 per second and corresponding to tonic activity. In this field of studies there are investigations by NUMOTO[8] and TOKIZANE[9], which indicate that the kinetic motor unit is innervated by the pyramidal system, while the tonic motor unit is controlled by the extrapyramidal system. For the purpose of establishing morphological and functional differences between these two components of skeletal muscle, histochemical, morphological and physiological studies have been carried out with a special consideration for the relation between the function and the structure of individual muscle fibers. The experimental data of our studies are briefly reviewed in the present report.

Morphological and histochemical observations of the white and red muscle fibers

Histological observations of the femoral biceps muscle of a cat revealed that there are three sorts of muscle fibers, i. e. the faintly stained large white muscle fibers, the deeply stained small red muscle fibers and the moderately stained muscle fibers intermingled in a fasciculus (Fig. 1).

In a muscle spindle, the red and white muscle fibers can also be observed (Fig. 2). The trunk muscle involved in the postural adjustment and M. soleus having tonic function are consisted of a decidedly greater

Fig. 1. Microgram of the cross-section of the femoral biceps of a normal cat. The fresh tissue was fixed with Baker's solution, frozen, sectioned, and stained with Sudan black B at room temperature. Picture shows clearly that the muscle is composed of three sorts of muscle fibers (× 150).

Fig. 2. Photograph shows the muscle spindle of M. gastrocnemius of a cat. Six red muscle fibers, appearing dark and small in diameter, and two large white muscle fibers stained faintly can be seen in a muscle spindle (central upper part, Sudan black B staining, × 600).

number of the red muscle fibers, while M. gastrocnemius and other peripheral muscles of the extremities having phasic function tend to contain more of the white muscle fibers. The white muscle fibers tend to gather mostly in the external part, while the red muscle fibers are mostly in the internal part and near the tendon.

The difference in stainability of the two muscle fibers, red and white, will depend upon the quantitative difference in the lipid and fat contents of their mitochondria in sarcoplasma. Therefore, further histochemical studies were conducted in order to detect succinic dehydrogenase by using neotetrazolium as the hydrogen acceptor and phosphorylase by the method of Takeuchi under the suspicion that there must be differences between the two kinds of muscle fibers in their energy metabolism. The results demonstrated that the large muscle fibers are stained light and small muscle fibers dark with succinic dehydrogenase, while on the contrary, under the reaction of phosphorylase of the adjacent section from the same tissue block, the large muscle fibers are stained dark and small muscle fibers faintly. They look like positive and negative films of the same picture. It is noteworthy that succinic dehydrogenase, which is localized in mitochondria and takes a part in the respiration as a member of TCA cycle, is found abundantly in red muscle fibers undergoing persistently tonic contraction and also the phosphorylase, which is contained in myogen of sarcoplasma and mediates glycogenolysis, is found in a greater quantity in the white muscle fibers, which contract rapidly and energetically.

Under electron-microscope, it has been established that the white muscle myofibrils have little fine mitochondria sparsely scattered and have well-developed sarcoplasmic reticulum, while the red myofibrils have round mitochondria in sarcoplasma arranged densely on both sides of Z-band of each fibril. Recently HUXLEY[10] stated that the sarcoplasmic reticulum connects each myofibril (inocomma) on both sides of Z-bands and transmits the impulse of muscle contractions from myofibril to myofibril. The muscle fibers moderately stained by Sudan black B as revealed by light microscope, which seem to belong to the category of intermediate group between the red and white muscle fibers, were also recognized by electron-microscope.

Concerning the innervation of muscle fibers, since Sherrington's observation many reports appeared claiming that there are large and small motor nerve fibers in the muscle, which divide into numerous small branches reaching white or red muscle fiber, but it is still obscure how these nerve fibers communicate with each red or white muscle fiber. It may be supposed that the large nerve fibers would emanate from large nerve cells in the anterior column and terminate in white muscle fibers and the small fibers from the small nerve cells reach the red muscle fibers. In order to prove this assumption the nerve branchcs were traced histologically up to the endplates. However, it was not possible to clarify this point because both of these two nerve fibers become about the same size (5-6 microns in diameter) in the vicinity of the endplate, though it was revealed that those branches coming from one nerve terminate selectively in the red

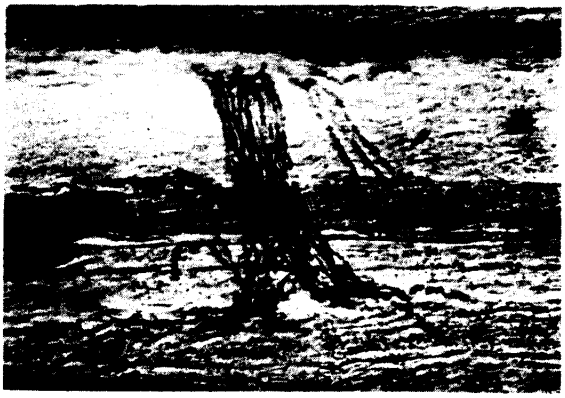

Fig. 3. Microgram of preterminal nerve fibers (Sudan black B staining, ×600). Branches of one nerve fiber seem to be terminating selectively in the red muscle fibers.

muscle fibers as illustrated in Fig. 3.

Next, cat's peroneal nerve was exposed and stimulated with alternating electric current at the frequency of 60 per sec., and immediately after the stimulation 10% formalin was injected into the artery distributed to gastrocnemius muscle, and the muscles were fixed in a state of contraction. The contraction knots were found only in red muscle fibers on the slice specimens. (Fig. 4), while the white muscle fibers did not show any contraction knots. In the control slices without stimulation no knots were observed. This fact suggests that one nerve fiber innervates the same kind of muscle fibers exclusively, and these results seem to be of great importance in substantiating the meaning of spikes of electromyograms in our experiments.

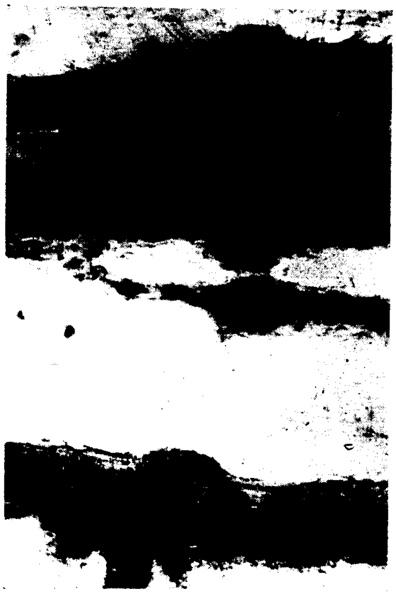

Fig. 4. Contraction knots can be observed only in red muscle fiber when the motoneurone is stimulated.
(Sudan black B staining, ×600)

Electromyographic study of human muscles

There are two hypotheses on the arrangement of the motor unit, a muscle fiber group which gets a common impulse from one anterior horn cell. (1) A motor neurone innervates a muscle fiber group which forms a fasciculus and each motor unit is arranged in a mosaic form (Fig. 5-A). (2) Muscle fibers supplied by several motoneurones are intermingled in a fasciculus (Fig. 5-B). In the latter case a motor unit is distributed in a rather wide area. If the former is true, a potential picked up with the electrode should be supplied by a single motoneurone, but if the latter is correct, it should be supplied by several different motoneurones.

In order to clarify the above questions on the motor unit and also to find out the arrangement of a motor unit the experiments were conducted on human muscle. An effort was made to pick up as much as possible a single motor unit to clarify the nerve fiber distribution.

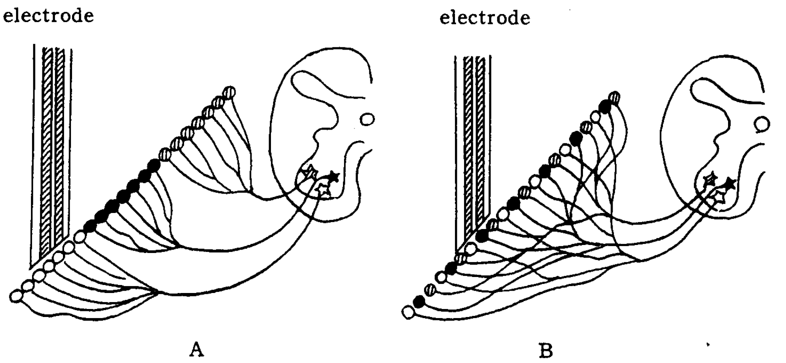

Fig. 5. Two schematic drawings showing the arrangement of motor unit in muscle according to the hypotheses described in the text.

The unit activity of deep flexor digitorum muscles of normal person was recorded with concentric needle electrode during very weak voluntary contraction against the weight of 5 to 10 grams to the middle finger (Fig. 6). The electode used was bipolar, consisting of two insulated 60 μ enameled wires in a bare ♯23 needle shaft. For the selection of the position of needle tip, a loud speaker was used for detecting stronger and more uniform sounds.

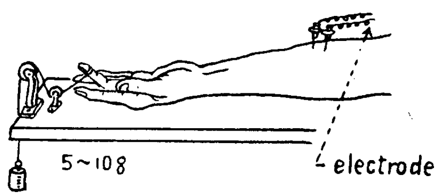

Fig. 6. Schematic drawing showing the way to induce a weak contraction of M. flex. dig. profundus.

Observations on normal persons :

Case 1 : Diphasic spikes of 350 μV and 1.0 msec were seen as in Fig. 7. Each spike had the same amplitude of negative and positive phase and each spike shape was very similar. Records led off simultaneously from this point and other point in the muscle 2 cm. away from it rectangular to the long axis of the muscle with double beam oscillograph proved to be identically the same in voltage and duration and synchronized exactly. The potentials led off from the other part of the same muscle showed a little smaller and longer spikes of 100 μV and 3.0 msec (Fig. 8).

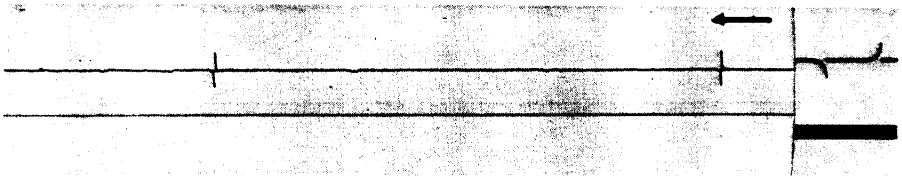

Fig. 7. Electromyogram of M. flex. dig. profundus of normal person, showing the spike of 350 μV and 1.0 msec in duration (calibration 200 μV, time mark 1 msec).

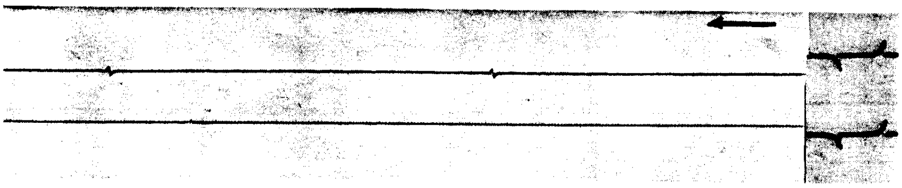

Fig. 8. Electromyogram of M. flex. dig. profundus of normal person, recorded at a point other than the one given in Fig. 7, showing the spike of 100 μV and 3.0 msec. (calibration 200 μV, time mark 1 msec).

Case 2: In this case pure diphasic spikes with short duration of 1.5 msec and 300 μV were observed. Spikes from two different points in a muscle 2.5 cm. apart from each other paralleling to the long axis were synchronized and had the same voltage and duration. Another type of spikes was also observed, that had 4.0 msec and 200 μV from other point of the same muscle.

Observations on the patient of flaccid paralysis due to spinal cord diseases :

Spikes from muscle of several patients having lesions in anterior column of the spinal cord were examined. These flaccid muscles have little active muscle fibers so that rather pure spikes of a single motor unit can easily be obtained even under a voluntary contraction.

Case 1: The patient had a spinal cord tumor at the level of L_{1-2}. Myographic observations revealed rather regularly discharged spikes with short duration of 1.0 msec and 100 μV in the rectus femoris muscle, which showed paresis clinically.

Case 2: The patient had a spinal cord tumor at the level of C_4. In this case two kinds of spikes were observed in the paralyzed deltoid muscles. One was large spikes with short duration of 1.0 msec and 120 μV and another was small spikes with long duration of 2.5 msec and 80 μV (Fig. 9). In these diseases such as two cases described above lower voltage seems to be given when compared with normal person.

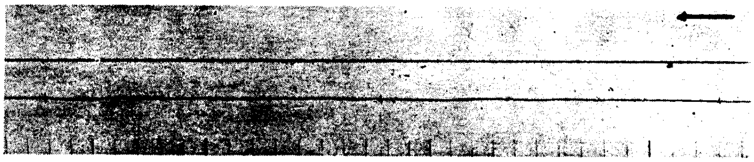

Fig. 9. Electromyogram of the paralyzed deltoid muscle of the patient with spinal cord tumor (C_4). It shows two spikes, one of 120 μV, 1 msec. and the other of 80 μV, 2.5 msec. (calibration 100 μV, time mark 8.3 msec).

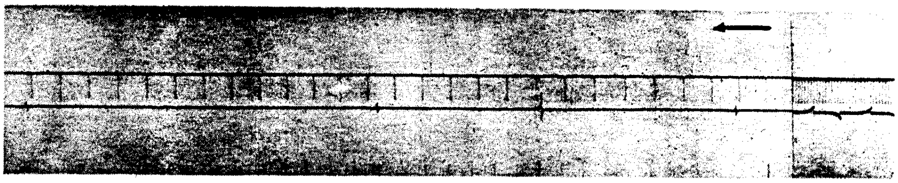

Fig. 10. Electromyogram of M. flex. dig. profundus of the patient with amyotrophic lateral sclerosis, showing spikes both assumed to belong to kinetic motor unit: one of 400 μV, 2.0 msec and the other of 100 μV, 1.5 msec (calibration 100 μV, time mark 8.3 msec).

Case 3: The patient was suffering from amyotrophic lateral sclerosis. The electromyogram showed two kinds of spikes on flexor digitorum profundus muscle. Larger ones were of 400 μV and 1.5 msec and smaller ones were of 100 μV and 1.5 msec. Both were of a shorter duration (Fig. 10). Histological examination on biopsy material of the muscle taken from the area where the spikes had been led off, proved that red muscle fibers were atrophied or disappeared, while white muscle fibers remain almost normal (Fig. 11).

Histogram of the voltage and duration made up of pure motor units:

Figures 12 and 13 show the histograms drawn with the voltage and the duration of 200 motor units having pure diphasic spikes, which are taken by bipolar concentric needle electrode from the flexor digitorum profundus muscle of 19-year-old female. The voltage is distributed between 50 μV and 550 μV, and has two distinct peaks at 100-200 μV and 300-450 μV. The histogram of duration has also two peaks at 1.0-1.5 msec and 2.0-4.0 msec, but these two peaks are not so distinct as that of the voltage. When the voltages and the durations of the motor units are plotted on ordinate and abscissa

Fig. 11. Microgram of M. flex. dig. profundus from the patient with amyotrophic lateral sclerosis (Sudan black B staining, × 300). Red muscle fibers are atrophied or disappeared while white muscle fibers remain almost normal.

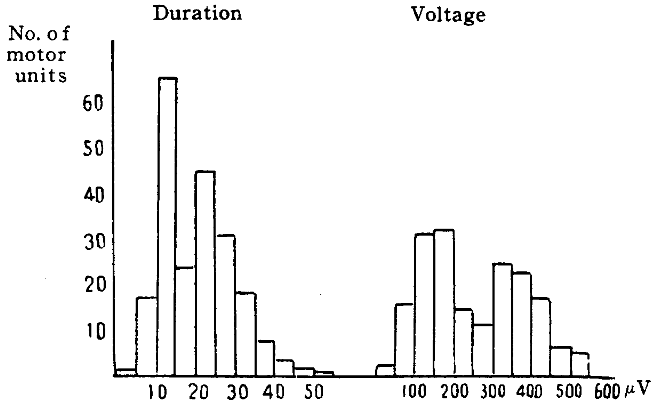

Fig. 12. Histogram recorded from M. flex. dig. profundus of a normal person, showing the duration and voltage of 200 motor units.

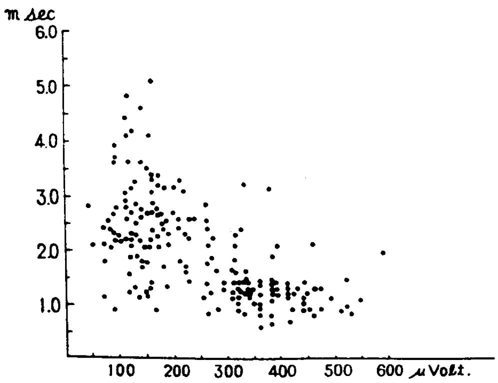

Fig. 13. Spots in the histogram showing voltage and duration of 200 motor units led off from normal M. flexor digitorum profundus.

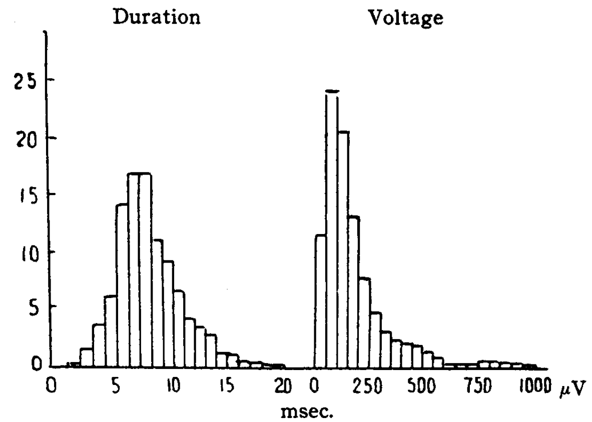

Fig. 14. Histogram of normal human biceps brachii muscle, showing the duration and voltage of motor unit activity. (by Buchthal)

respectively, they can be practically divided into two groups (Fig. 13): one group with high voltage and short duration and another group with low voltage and long duration. However, there can be observed, although a few in number, some intermediate ones.

Discussion and Summary

According to BUCHTHAL[11] the histogram of duration and voltage from the motor unit of biceps brachii muscles gives only one peak respectively as can be seen in Fig. 14 in his paper which reports the data covering the experiment on 1,268 motor units. However, his histogram seems to be made of the spikes led off from several motor units because the histogram shows no much fluctuations in voltage as 50 μV to 1,000 μV and in duration as 1 msec to 20 msec. Therefore, if observations are actually based on a single motor unit, two peaks may reasonably be expected on the histogram, because two kinds of the motor units, kinetic (phasic) and tonic, have respective individual characteristics of their own shikes The histological observation shows that many white and red muscle fibers are intermingled with each other even in one single fasciculus, and it is supposed that the fasciculus does not correspond to a single motor unit. Moreover, the shape of the spikes, which was formerly considered as a motor unit, is not a pure diphasic form, but irregular and polyphasic ones, and also electromyographically a single motor unit controls the area of more than 10 mm in width (BUCHTHAL[11]). From these facts, it is probable that the histogram by BUCHTHAL was made of the spikes composed of muscle fibers belonging to several different motor units.

Our observations done by the above stated method showed clearly the pure diphasic spikes. Therefore, we are of the opinion that these spikes obtained by our method are led off from only one or from a few muscle fibers belonging to the same motor unit. These spikes are lower than 550 μV in voltage and shorter than 5.5 msec in duration and every individual spikes show uniform diphasic pattern. There exist two kinds of spike groups, in the histogram one which is composed of high voltage with short duration (1.0-1.5 msec.), and the other of low voltage with long duration (2.0-4.0 msec.). The former may be of kinetic (phasic) motor unit and the latter is of tonic motor unit, because the white muscle fibers with a larger diameter may have a higher voltage than the red and the white fibers that perform rapid contraction may show shorter duration in wave form.

In the two cases having spinal cord tumors, two kinds of spikes with respective and individual characteristics were observed in the same EMG. These will be two different kinds, kinetic (phasic) and tonic motor units. In the case of amyotrophic lateral sclerosis, two kinds of spikes appeared, but since both of them were of short duration, they might be considered to be of kinetic (phasic) motor unit (or its intermediate motor unit). Furthermore, since its histological findings revealed that the red muscle fibers were all atrophied and degenerated and showd only white muscle fibers to be normal, it is obvious that the kinetic (phasic) motor unit with a shorter duration is derived from white muscle fibers.

Therefore, in our opinion the widely accepted concept that spikes including even irregular wave forms all belong to the motor unit seems not to be true, but these spikes seem to represent a combination of several pure spikes though not so many, and those muscle fibers belonging to the same motor unit appear to be intermingling themselves in a relatively wide area. The reason for this contention may be explained as follows. If the muscle fibers belonging to the same motor unit were agglomerated, clear-cut diphasic spikes should appear even with a fairly big electrode, and if the accepted concept be true, these spikes can never be picked up at the distance so far apart as 2.0 cm. or 2.5 cm. (0.5—1.2 cm. by BUCHTHAL[11]) as has been possible in our experiments. Furthermore, the histogram composed only of these pure spikes reveals two peaks, and therefore, we believe it is reasonable to say that these two peaks indicate the existence of kinetic (phasic) and tonic motor units. As a small number of motor units located in between these two peaks can be recognized, these are believed to be the muscle fibers possessing an intermediate stainability as revealed in the histological examination. However, further studies are required before giving any definitive conclusion on this point.

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