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The Wilmer Institute, Johns Hopkins Hospital and University Baltimore, Maryland.

The two Skeletal Nerve-Muscle Systems in Frog* **.

By

STEPHEN W. KUFFLER.

With 9 figures in the text.

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OTTO LOEWI has profoundly influenced, directly and indirectly, many fields of physiology. The force and stimulus of his original concepts and investigations has not diminished through the years. The vitality of his ideas is most clearly shown by their wide continued discussion and especially by the fact that they are still put to repeated new experimental tests. This is particularly true for the studies of nerve-muscle transmission processes. The present author had the good fortune of frequent meetings with Professor LOEWI during many pleasant summers at the Woods Hole Marine Biological Laboratories. There, among a new generation of rising scientists LOEWI is at his best spreading his own youthful enthusiasm for the scientific approach to all problems of life. This paper is presented to him, coming from one of many grateful friends.

Only a small aspect of nerve-muscle problems will be discussed here. The main emphasis will be placed on a series of recent investigations which have demonstrated the existence of two functionally distinct systems of nerve-muscle connections. (I) The "Large-Nerve System" or "Twitch System". The first designation emphasizes the neural elements, the second the effector elements. The system consists of nerve fibers of relatively large diameter, conducting at about 8 to 40 m/sec., and of the muscle elements which are innervated by these nerve fibers. This nerve-muscle complex sets up in the normal living animal the well-known muscle twitches which lead to phasic movement. (II) The "Small-Nerve System" or "Slow Muscle Fiber System". This consists of nerve fibers of relatively small diameter, conducting at about 2 to 8 m/sec., and of the muscle fibers in connection with them. This second system leads to relatively slow maintained contractile action, never to muscle twitches or conducted muscle impulses. The "slow" muscle fibers, although skeletal and striated, possess quite distinct properties from the twitch muscle fibers.

* In honor of Prof. O. LOEWI on his 80th birthday.

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The differences in innervation patterns, the electrical, pharmacological, and functional properties of the two types of nerve-muscle systems will be discussed. The full evidence for all findings cannot be presented and is found elsewhere. At the outset it is emphasized that strict analogies between frogs and mammals do not seem to exist. A different pattern of organization and of mechanisms has been clearly demonstrated. A recent series of papers has dealt with these questions (HUNT 1; KUFFLER and HUNT; KUFFLER & VAUGHAN WILLIAMS 1, 2).

A. Differentiation between the Small-Nerve and Large-Nerve Systems.

Differential block of nerve fibers: TASAKI and his collaborators were the first investigators to give clear evidence that stimulation of isolated small diameter motor fibers caused contractile responses which differed from those set up by excitation of the larger nerve fibers. Preferential elimination of fast conducting (and therefore large) nerve fibers can also be obtained by passing an appropriate constant current through the nerve (KUFFLER & GERARD). A still simpler and more reliable technique has recently been developed by KUFFLER & VAUGHAN WILLIAMS 1. In ventral roots a controlled selective block of different nerve elements was obtained by the use of square pulses. Provided the correct combination of pulse duration (about 0.6—1.0 msec), intensity and inter-electrode distance is selected, the faster fibers are blocked at the anode, while the slower ones pass through. In this way practically all the small-nerve fibers to a chosen muscle can be stimulated simultaneously. The present method permits separate and repeated stimulation of different groups of nerve fibers and of their muscle elements.

Contractile responses: Whenever all the motor nerve fibers above a conduction velocity of 8 m/sec. (at about 20—22° C) are blocked, stimulation of the remaining slower fibers causes a typical contractile response of slow time course. An example is shown in Fig. 1. Single stimuli to the small-nerve group innervating the iliofibularis caused only very little tension. At a frequency of 4/sec, however, a slow tension rise, developing over many seconds, can be seen. At higher frequencies the rate of tension rise, recorded isometrically, increases progressively. Almost the same tension peak is reached at all frequencies above 40/sec. The gradual tension build-up is a characteristic phenomenon when small-nerve fibers, conducting at about 2—8 m/sec, are stimulated. Complete relaxation (not shown in Fig. 1) may take seconds or minutes, depending on recording conditions.

In contrast, a single stimulus to the large motor nerve fibers, conducting at 8—40 m/sec, causes the well known twitch response which in a sartorius at 20—24° C may rise to a peak in 20—30 msec. and relax within 0.1 seconds. Twitches also sum, but having a relatively short

contraction time, high frequencies are needed to produce a smooth, fused tetanus.

The mechanical response differences alone are so striking that a ready distinction can be made between the two nerve-muscle systems. The separate function of certain motor nerve fibers is clearly indicated by these experiments (see also below).

Differentiation of individual slow muscle fibers and twitch fibers: It became important to dertermine whether distinct groups of muscle fibers responded to stimulation of small and large-nerve fibers. Although indirect evidence for two different striated muscle fiber groups was

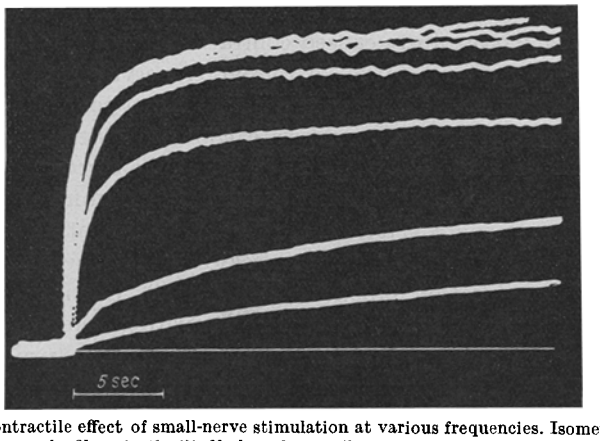

Fig. 1. The contractile effect of small-nerve stimulation at various frequencies. Isometric tensions, set up in slow muscle fibers in the iliofibularis by small-nerve stimulation at frequencies of 4, 10, 20, 30, 40, 50 and 75 per second, maintained for the duration of the sweep. No twitch motor unit activity present. All seven exposures superimposed on one record. At the three highest frequencies the final tension was similar but the rate of rise differed. Maximum tension 2 g. Initial tension 0.5 g. Tension recorded by a transducer coupled to a D.C. amplifier, base line drawn in. (from KUFFLER & VAUGHAN WILLIAMS[2]).

available from several sources, it remained possible that individual muscle fibers had a dual role, namely of slow or fast contractile action, depending on the nerve fiber type through which they were excited. In fact, that was the prevalent explanation for the well known "tonic" phenomena which have been seen by many workers (see reviews by BRECHT, and KRÜGER). Experiments with intracellular recording electrodes have now clearly established that different muscle fibers are involved in the small-nerve and large-nerve response. Electrodes placed within a cell record only the activity of that cell, and are hardly affected by simultaneous activity of surrounding tissues.

Fig. 2 illustrates the two main response types which can be obtained from numerous frog muscles. In Fig. 2a the microelectrode was inserted into a muscle fiber which had a membrane potential of 90 mV. When

the nerve to the iliofibularis was excited a large 120 mV potential resulted. This is the action potential associated with muscle twitches, as already shown by NASTUK & HODGKIN. It is analogous in many respects to the nerve impulse and shows the "overshoot" or potential reversal phenomenon, i. e. the spike exceeds the membrane potential. Furthermore, the action potential propagates along the whole length of a muscle fiber and apparently activates the contractile elements as it sweeps along the fiber surface. Fig. 2b shows the second principal response type which is always small (8—15 mV) and has a slower and more complex time course (note the slow sweep speed) than the propagated muscle impulse. Its characteristics will be discussed in detail below. It has been named the "Small-Nerve Junctional Potential" or s. j. p. in former studies (KUFFLER & GERARD).

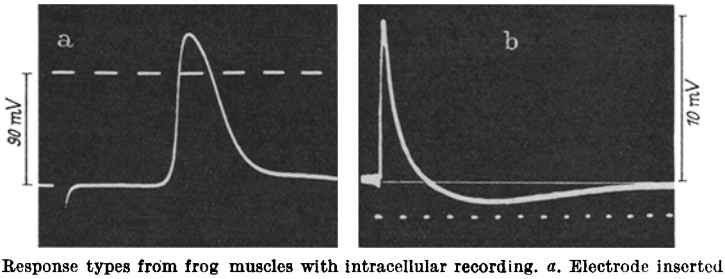

Fig. 2. Response types from frog muscles with intracellular recording. *a.* Electrode inserted into a twitch muscle fiber of iliofibularis. Resting potential difference between inside and outside 90 mV. Zero membrane potential indicated by upper sweep interrupted at 1000 c.p.s. Maximal nerve stimulation causes a propagating muscle impulse of 120 mV, i.e. 30 mV in excess of membrane potential. *b.* Electrode in slow muscle fiber, resting potential 52 mV, zero potential not shown. Typical response following small-nerve stimulation, called small-nerve junctional potential (s.j.p.). Potential peak is only 10 mV. Note the large hyperpolarization or "positive afterpotential" and slow time course. For details of rising phase of s.j.p. see Fig. 3. Time marker 50 c.p.s., base line added (modified from KUFFLER & VAUGHAN WILLIAMS[1]).

Individual muscle fibers gave either s. j. p.'s or conducted impulses, never both. Further, it was found that the propagated impulse type was always associated with stimulation of large diameter nerve fibers, while small-nerve stimulation alone caused the distinctive s. j. p.'s of Fig. 2b. *Therefore it seems established that no individual muscle fiber receives both kinds of innervation or gives both types of response.* There exist then two different systems of nerve muscle connections involving different groups of muscle fibers, namely the twitch fibers and "slow" muscle fibers.

B. Innervation Pattern of Frog Muscles.

Multiple innervation of individual slow muscle fibers: By stimulating one or a few small-nerve fibers it was shown by KUFFLER & GERARD that the potentials which set up s. j. p.'s were confined to the nerve-muscle junctions and their spread was electrotonic over several millimeters around the endplate regions. In recent studies, when the majority of small-

nerve fibers to muscles could be excited, it was found that s. j. p.'s were recorded anywhere along the course of slow muscle fibers if microelectrodes were suitably inserted. This fact alone led to the conclusion that slow muscle fibers are densely innervated.

A close examination of s. j. p.'s, of the type seen in Fig. 2b, gave more details about the innervation density and furnished good evidence that these potentials are complex in nature and at any point are caused by the activity of several small-nerve fibers. Fig. 3, taken from a recent

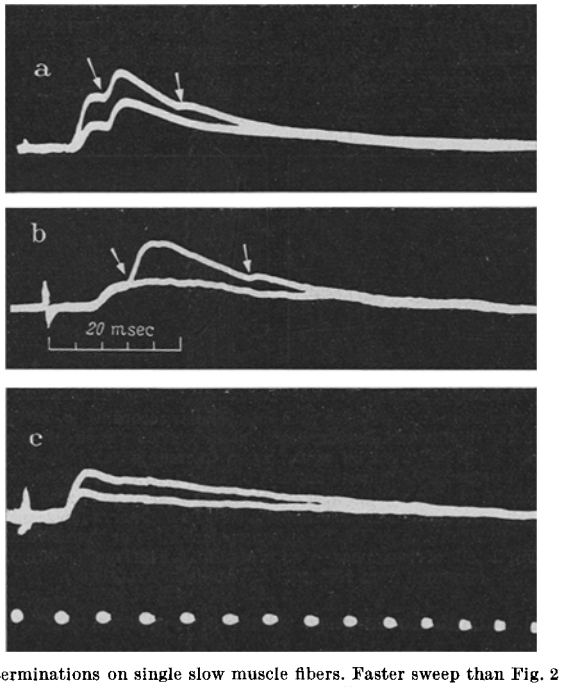

Fig. 3. Multiple terminations on single slow muscle fibers. Faster sweep than Fig. 2b; early phase only of s.j.p. is shown. Intracellular records from three different slow muscle fibers in iliofibularis. Graded stimulus strength to small-nerve fibers in ventral root brings in additional "steps". In each record two sweeps superimposed, s.j.p. components (arrows) are set up by different small-nerve axons with junctions in vicinity of electrode tip. Time marker in *c* 100 c.p.s.; s.j.p. peaks 8 to 11 mV. Hyperpolarization phase of s.j.p.'s seen on slower sweep only (from KUFFLER & VAUGHAN WILLIAMS[1]).

study, shows the early phase of s. j. p.'s when the stimulus to small-nerve fibers to the iliofibularis was graded. Gradual increments of stimulus strength excited more and more small-nerve fibers and at the same time new discrete components were added to the s. j. p.'s. In Fig. 3 two sweeps were superimposed in each record at two different strengths of a stimulus to ventral root 10. The simplest and smallest potential is obtained at threshold intensity (e. g. lower tracing in Fig. 3b and c), set up by a small-nerve axon(s) of relatively low threshold. As many as

seven distinct steps have occassionally been seen in one s. j. p. with finely graded stimulation. In Fig. 3 some of the s. j. p. steps marked by arrows are set up by small-nerve fibers of different conduction velocities. The later steps are generally brought in by stronger stimuli and are therefore caused by relatively high threshold fibers of slow conduction speed. Since s. j. p.'s spread only electrotonically and are not propagated and since the recording range of an intracellular electrode under present conditions seems limited to a few millimeters, it is concluded that many small-nerve junctions exist along a short stretch of slow muscle fibers. Further, the finding that s. j. p.'s are recorded along the whole course of slow muscle fibers indicates that the dense innervation covers the entire fiber length.

Twitch muscle fiber innervation: As early as 1885 SANDMANN presented histological evidence that individual muscle fibers in the sartorius can have several endplates along their course. Physiological evidence for multiple terminations on individual muscle fibers was given by KATZ & KUFFLER for the sartorius, which is practically a pure twitch muscle with a predominant large-nerve innervation. Recently multiple innervation of individual twitch muscle fibers was also demonstrated in several other frog muscles and in cat (HUNT & KUFFLER). With intracellular electrodes inserted into single muscle fibers portions of the motor nerve supply to the muscles were stimulated. It was shown that one muscle fiber can carry propagated impulses arising at different junctions along its course. In the experiment of Fig. 4 the divided pelvic and tibial branches of the sartorius were excited separately, each setting up a propagated impulse (and twitch) in the same fiber. The latency of the impulses is different, mainly due to the varying conduction distance between the endplates and the intracellular electrode. When both branches were excited simultaneously, only the muscle impulse with the shorter latent period, from the nearest endplate, reached the electrode. The other impulse was extinguished by "collision".

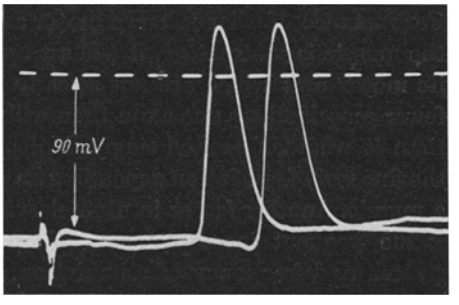

Fig. 4. Multiple innervation of twitch fibers. Intracellular recording from a muscle fiber in the sartorius. Two exposures superimposed with separate stimulation of the tibial and pelvic branch of the nerve to the muscle. Both branches set up a propagated impulse in the same fiber. Note different latencies due to difference in conduction distance between electrode and the nearest endplate of the tibial and pelvic branch. With simultaneous stimulation of both branches only the impulse of shorter latency is seen. Resting potential 90 mV, upper sweep indicates zero potential modulated at 1000 c.p.s. (from HUNT & KUFFLER).

These studies show once more the multiple innervation of individual twitch fibers in many muscles. In these fibers, however, there certainly

exist relatively long nerve free stretches, in contrast to the innervation of slow muscle fibers.

The motor unit: A motor horn cell with its nerve fiber and all the muscle elements which it innervates is the motor unit according to a purely structural definition. Gradation of muscle movement and of tension is brought about principally by motor unit recruitment. Functionally, as judged by contractile performance, the individual motor unit is not always a well defined entity. Not all the muscle fibers "belonging" to a motor unit need contract with each motor nerve impulse, and not all muscle fibers contract fully. The following phenomena should be considered in this context. If one muscle fiber is innervated by more than one nerve fiber it will be shared by more than one motor unit. This is true for many twitch fibers and for all slow muscle fibers. A motor unit overlap can therefore occur when many units contract simultaneously. For instance, if a large-nerve motor axon is excited while a great part of the muscle is contracting, it will set up activity only in those portions of its motor unit which are not already being stimulated. By contracting fewer muscle fibers one axon then will cause the addition of less tension than it would if excited singly. Multiple innervation will thereby affect the fineness of gradation depending on contractile background activity in a muscle. Another factor in normal twitch-motor unit activity of the frog is the frequent incompleteness of transmission. Depending on muscle stretch and frequency of stimulation, fewer or more muscle fibers take part in the activity of one motor unit (KUFFLER 2; LIBET & WRIGHT). No local graded contractions are, however, known to occur normally in twitch fibers on nerve stimulation.

The effect of multiple innervation of slow muscle fibers on their contractile output is more complex than in twitch fibers. In the latter the whole muscle fiber always gives a propagated impulse and a maximal twitch, whether excited through one or more endplates. The slow muscle fibers, however, are excited merely around the small-nerve junctions, giving local electrical and contractile changes. The more local areas, distributed along the length of a slow muscle fiber, become involved, the stronger it will contract. Each slow muscle element, therefore, having a multiaxonal innervation, belongs to numerous motor units. Furthermore, the extent of local changes also depends on the frequency of stimulation which contributes to the very fine gradation of which a slow muscle fiber is capable (see later).

C. Specific Properties of Slow and Twitch Fibers.

Membrane potentials: Under favourable experimental conditions, for instance in a sartorius muscle, a resting potential of over 90 mV is measured between the inside and outside of twitch muscle fibers (LING

& GERARD). In slow muscle fibers the membrane potential has always been found to be lower, usually around 60 mV in selected measurements (solid squares Fig. 5 A). The fact that resting potentials fall into two groups emerges clearly from this diagram. While there are certain difficulties in measuring intracellular potentials in slow muscle fibers the results were consistent in all experiments (for details see original paper).

Junctional potentials: There exist striking differences between endplate potentials (e. p. p.'s) at the junctions of twitch fibers and large-nerve fibers, and the s. j. p.'s at small-nerve junctions. E. p. p.'s usually rise to 30—40 mV before setting up a propagating muscle impulse and their subsequent time course is largely obscured by the spike. In curarized,

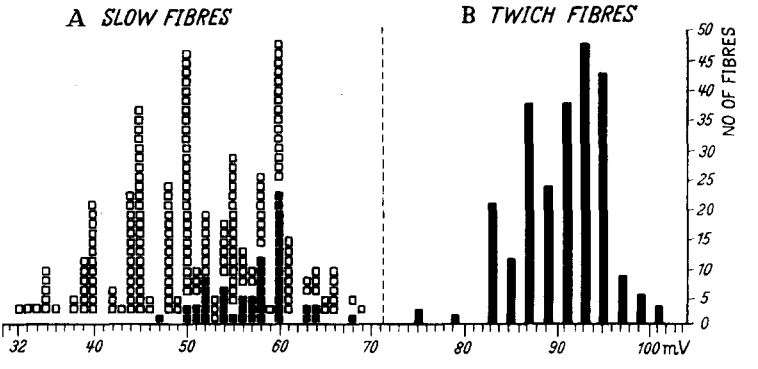

Fig. 5. *A* Resting potentials of 244 slow muscle fibers of iliofibularis. Open squares represent unselected potentials, solid squares are measurements selected according to certain rigid criteria. Note absence of potentials above 70 mV. *B* Resting potentials from 239 twitch fibers in the sartorius. Distribution peak over 90 mV. (from KUFFLER & VAUGHAN WILLIAMS[1]).

fatigued or even normal junctions (KUFFLER 2) e. p. p.'s can be studied uncomplicated by spikes. The most recent and accurate study of the e. p. p.'s and of junction processes was made by FATT & KATZ. In short, the e. p. p. may be regarded as a depolarization which rises to a peak in 1—1.5 msec. and declines to the base line in about 30 msec. along an exponential time course. In contrast, the s. j. p. does not exceed 15 mV, does not set up propagating impulses and its declining or restorative phase is complex and slow. The s. j. p. consists of: (I) a rapid depolarization, made up of several steps (Fig. 3) which in a synchronous s. j. p. may have a rise time of 2—3 msec. (II) a repolarization phase which is approximately exponential but always swings over into a hyperpolarization. The latter may be 20—40% of the initial depolarization. (III) a final restoration, or return to the original membrane potential. The whole s. j. p. complex may last for 0.4 seconds.

Some additional characteristics of s. j. p.'s and of slow muscle fibers become apparent during repeated stimulation. Even at high frequencies

the slow muscle fibers cannot be depolarized much over 30—35 mV. An equilibrium between the depolarizing and restorative processes is soon reached. The contribution of individual s. j. p.'s during trains of small-nerve stimuli has recently been analyzed. Fig. 6a is an intracellular record from a slow muscle fiber made up of three separate but superimposed exposures of one, two and three nerve volleys at 15 msec. intervals. The height and time course of the second s. j. p. was derived by subtracting the first potential from the combined potential set up by two nerve volleys, while the third potential is given by the difference between two and three s. j. p.'s. The individual potentials are then plotted separately

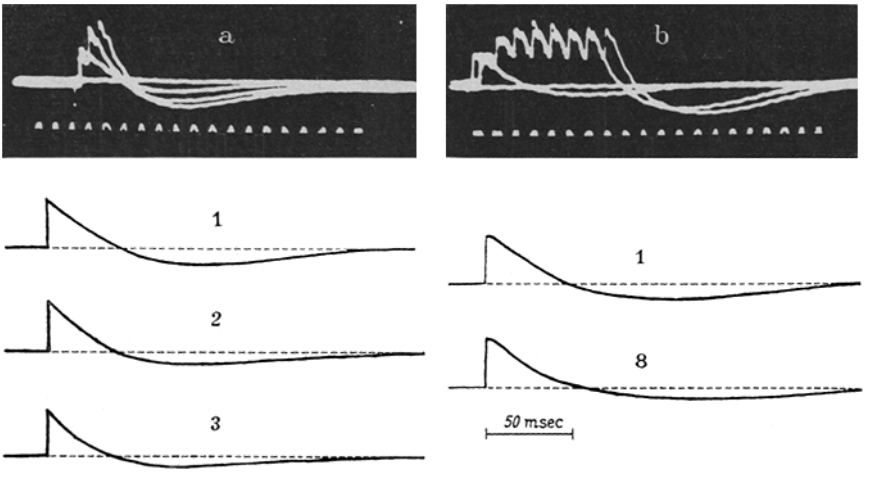

Fig. 6. Intracellular recording from slow muscle fibers in the iliofibularis during repeated stimulation of small-nerve fibers within the 10th ventral root. The membrane potential was 50 and 53 mV in the two fibers, the single s.j.p. 9 mV in both. Time base in upper records 50 c.p.s. *a* Three sweeps superimposed giving one, two and three nerve stimuli; intervals between shocks are 15 msec. Below are plotted in sequence from above the single s.j.p., and the contributions of the second and third nerve volleys, the latter two derived by subtraction (see text). – *b* Three superimposed sweeps with one, seven and eight stimuli. Intervals 20 msec. The single s.j.p. starts earlier and does not coincide with the first s.j.p.'s of the two series at 50/sec. Below are plotted the single s.j.p. and the contribution of the 8th nerve volley, which is the difference between the potential set up by 7 and 8 stimuli. Note the accurate superposition of the two tetani.
(from KUFFLER & VAUGHANWILLIAMS[1]).

below, for better comparison. In Fig. 6b a tetanus of 7 and 8 stimuli at 50/sec. has been superimposed and the difference between these constitutes the contribution of the 8th small-nerve volley. This again is plotted below for comparison with a single s. j. p. It appears that even during relatively intense activity, as during a depolarization plateau, the successive s. j. p.'s are not greatly changed. It was concluded from such results that the initial event in slow muscle fibers is a short depolarization which in turn initiates a series of processes which are determined by the slow fiber characteristics. The stability of the restorative processes (re-

polarization and hyperpolarization) even during continued activity, indicates that the electrical characteristics of the muscle fiber are not greatly changed by stimulation.

"Electrical" and "chemical" properties: Several muscles, such as the sartorius or adductor longus, do not contain a significant number of slow fibers. In others the slow muscle fibers (and small-nerve innervation) are confined to distinct regions, for instance in the iliofibularis or semitendinosus. Such muscles can be divided into "twitch portions" and "slow portions". The latter, however, always contain a mixed population of slow and twitch fibers. Either whole twitch muscles or twitch portions can be taken for experimentation and their

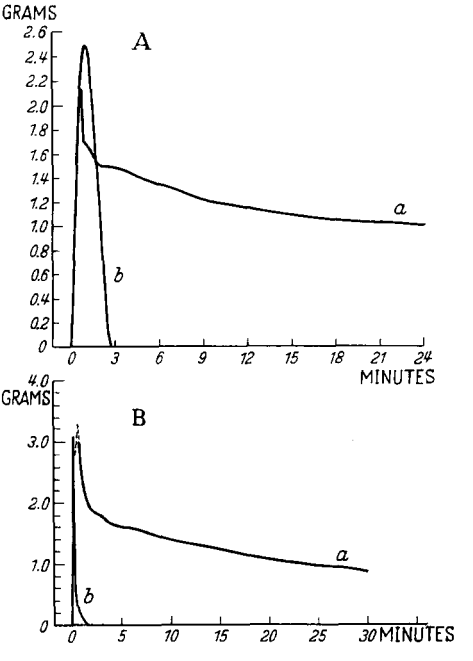

Fig. 7. Isometrically recorded tensions during chemical and electrical excitation of slow and twitch fibers. *Aa* Slow portion of the iliofibularis immersed into 10^{-5} ACh. Within the first minute there occured noticeable transient propagated twitch fiber activity, followed by a gradual tension decline. Initial tension 0.5 g. *Ab* Adductor longus in isotonic KCl develops transient tension rise only. Initial tension 1.0 g. *Ba* Slow iliofibularis portion during constant current flow of 50 μA maintained some tension for 30 minutes. Initial tension 0.5 g. *Bb* Current of 180 μA passed through adductor longus sets up transient tension rise only. Initial tension 1.0 g. Twitch portion of iliofibularis gave similar tension curves as the adductor longus. Large tensions which occur during the first 30 seconds of drug action or of current flow, are not plotted (from KUFFLER & VAUGHAN WILLIAMS[2]).

behaviour compared with parts containing slow muscle elements. The following results have regularly been obtained (I) Strong electric shocks of short duration applied "directly" to large-nerve innervated muscles cause twitches only, while similar stimuli to small-nerve innervated muscles are followed by long maintained shortening or tension changes. (II) Constant current passed through twitch muscle fibers will produce a tetanus, followed by a transient cathodal contracture, lasting 2 minutes or less although current flow is maintained. The same current causes prolonged (up to 60 minutes) contractile changes in slow muscle fibers. (III) Depolarizing agents, e. g. KCl or Acetylcholine (ACh) act on twitch fibers and on slow fibers in a similar manner to constant current.

Two examples are shown in Fig. 7. The slow portion of the iliofibularis was immersed in 10^{-5} ACh and the tension development was

recorded isometrically (Fig. 7 Aa). The quick initial tension development is due to asynchronous twitches and declines rapidly while the remaining tension has a slow time course and is still appreciably present after 24 minutes. If either the twitch portion of the iliofibularis or a whole twitch muscle is immersed into ACh the tension change is transient, resembling that in Fig. 7 Ab. This record was in fact obtained from an adductor muscle immersed in isotonic KCl solution, but a strong ACh solution would have caused a similar effect. Although such solutions depolarize the muscle rapidly, they do not cause a maintained tension and in spite of the continued presence of KCl, relaxation has taken place within 3 minutes. In Fig. 7 Ba a constant current of 50 μ A was passed through the slow portion of an iliofibularis for 30 minutes, causing maintained tension. 180 μ A passed through the adductor longus in Fig. 7 Bb, however, ceased to be effective after less than 2 minutes, although current flow was kept constant.

Experiments of this kind have led to the conclusion that important differences exist between twitch and slow muscle fibers in their contractile response to long lasting stimuli which may be either constant currents or depolarizing drugs. These phenomena are well known and have been established especially by SOMMERKAMP, WACHHOLDER and VON LEDEBUR, WACHHOLDER and NOTHMANN. The present series of investigations merely correlate them with the small-nerve innervation and slow muscle fibers (see General COMMENT).

D. Reflex Activation of the Small-Nerve System: Interaction of Twitch and Slow Fibers.

In a decapitated frog "at rest", without obvious visible contractile activity, one can usually record s. j. p.'s from the surface of many muscles. These potentials are set up reflexly and can be enhanced or inhibited by afferent nerve impulses arising in the periphery. Whenever the frog executes a visible movement there appear large propagating impulses in twitch muscle fibers which to a large extent obscure the smaller s. j. p.'s in the slow fibers.

A certain amount of separation in the reflex activation of the two systems can be shown simply by touching the frog's skin while recording from some muscles which receive small-nerve innervation. It is thus frequently seen that small-nerve discharges have a relatively low reflex threshold (not electrical threshold), i. e. they are activated in great numbers by "gentle" touch, while stronger pressure is needed to cause twitch reflexes. While such stimulation presumably sets up an afferent discharge pattern which resembles that during normal activity, electrical stimulation of afferent pathways in skin and muscle nerves leads to a more artificial but better controlled reflex activation. By electrically exciting

the central ends of cut nerves one can set up reflex contractions of appreciable magnitude in slow muscle elements, without bringing in twitch discharges. The latter appear, however, when the afferent volleys reach a certain strength or frequency.

An example is shown in Fig. 8 giving the tension development and also the electrical discharge pattern during such a reflex. In this experiment stimulation at 20/sec. to the central end of the cut tibial nerve caused s. j. p. reflexes (b) and the associated slow muscle tension (a) seen

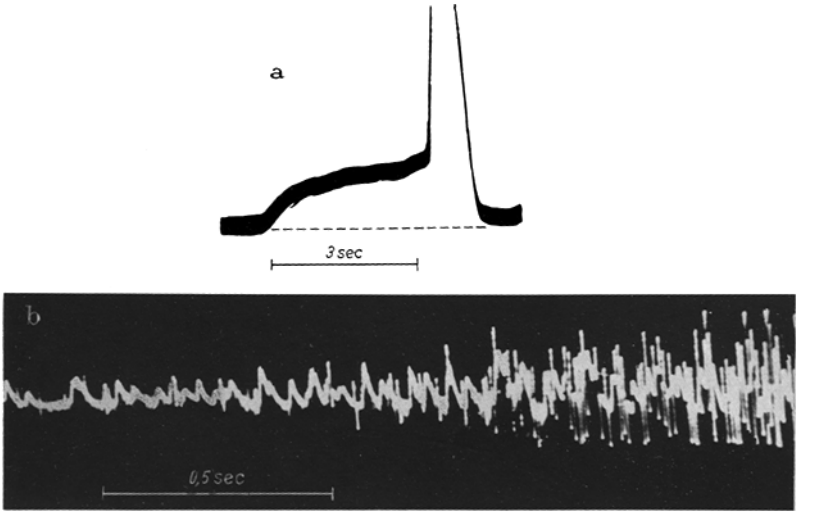

Fig. 8. Reflex excitation of the small-nerve and large-nerve systems in a spinal frog. Tension and electrical discharges recorded from partially isolated ventral head of the semitendinosus. The central end of the cut tibial nerve was stimulated submaximally at 20/sec. *a* For about 3 seconds the reflex activity was exclusively in small-nerve fibers causing a slow tension rise. A slight increase in the stimulus strength set up a sudden burst of twitch reflexes, accompanied by a quick tension rise (tension peak off the record). Reflex stimulation discontinued shortly after twitches started. *b* Electrical activity which accompanies slow tension shows s.j.p.'s only, while during the twitch reflex propagating muscle impulses are seen. Note faster time scale in *b* which illustrates early phase only of twitch reflexes and the s.j.p.'s immediately preceding them (from KUFFLER, LAPORTE & RANSMEIER).

in the first portion of the records. An increase in the strength of the afferent stimuli set up a sudden burst of twitch reflexes in addition to the s. j. p.'s.

Although the above experiments do not furnish detailed information of spinal organization, they indicate that small-nerve reflexes utilize some mechanism which are distinct from the twitch reflexes. On the whole the discharges seem to be distinguished by their more continuous and maintained nature, besides being set up more readily by a variety of stimuli. They are, however, subject to excitation and inhibition in a similar manner to twitch reflexes and are seen to be coupled with them during light twitch contractions. During more synchronous twitches

they cannot be detected, and are probably obscured by the larger propagated impulses.

In isolated nerve-muscle preparations some aspects of the mechanism of interaction between the two fiber types could be tested more satisfactorily. If twitches are added to slow muscle fiber contractions, the tensions sum, as expected from the existence of two separate systems. There do occur, however, some other interactions of possible interest. In Fig. 9a a series of twitches (tension peak off the record) were superimposed on a small-nerve tetanus and at the end of the twitches the

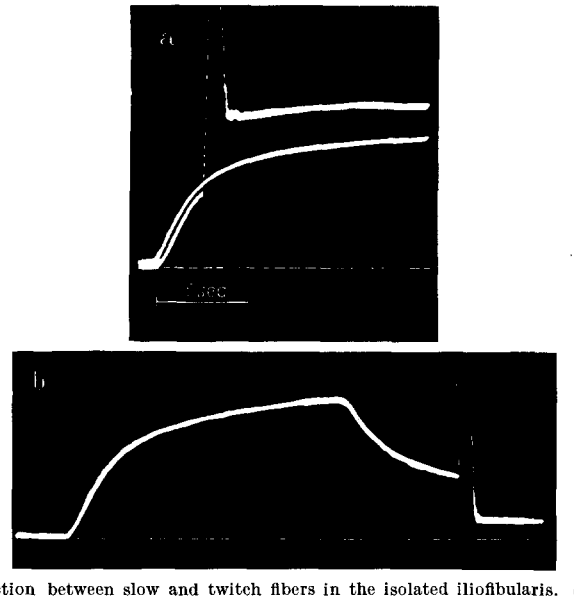

Fig. 9. Interaction between slow and twitch fibers in the isolated iliofibularis. *a* Two tension records superimposed. Small-nerve stimulation at 30/sec. causes a slow smooth tension rise reaching peak value of 0.6 g. In the second superimposed exposure a short burst of twitch fiber activity is added (tension off the screen) during continued small-nerve stimulation. After the tetanus the slow muscle fiber activity maintains a higher final tension value (0.75 g.) than it would have reached without the interposed twitch tetanus. *b* Small-nerve stimulation at 30/sec. sets up tension of 0.8 g. in 20 seconds. During slow relaxation following cessation of small-nerve excitation a short tetanus in twitch fibers causes a collapse of most of the residual tension. Initial tension 0.5 g in *a* and *b*. (from KUFFLER & VAUGHAN WILLIAMS[2]).

slow muscle fibers maintained a greater tension than they could have developed alone. While such tension synergism is seen when both nerve-muscle systems are simultaneously active, the opposite effect can also be demonstrated in Fig. 9b, namely twitch activity accelerating the tension decline *after* slow muscle fiber activity. A tension residue, subsiding very slowly, is usually seen after cessation of small-nerve activity. Such a tension can be partially or completely collapsed by interposition of twitches (Fig. 9b). Since residual slow muscle tension is also abolished by stretch, this interaction phenomenon seems purely "mechanical" and

like all other tension effects it is greatly influenced by initial muscle tension. It is not known whether these types of interaction play a significant role in the intact animal.

General Comment.

A great part of the experimental evidence presented here has been known for a long time, stemming from many sources. In the first place the important early studies of SOMMERKAMP on "tonic" muscles should be mentioned. He established clearly that portions of the iliofibularis showed distinct contractile behaviour in response to "chemical", "electrical" and other forms of stimuli. On the whole his views have been strikingly confirmed, but he thought that "tonic" muscle fibers could give twitch responses in addition to the slow maintained contractions. This was, and still is, a widely held view. SOMMERKAMP's "Tonusbündel" is the region in the iliofibularis where the slow muscle fibers are located and there seems little doubt that his "tonic" elements have now been identified with the intracellular recording method. These studies have also shown physiologically that small-nerve fibers supply only the "Tonusbündel" but not the twitch portion of the iliofibularis, a finding which has histological support (GÜNTHER). The numerous studies of WACHHOLDER and his coworkers on the "tonus" problem appear also to have been done on the slow muscle fiber system. More recently BRECHT and FENEIS have come to doubt the validity of available evidence for special contractile properties of different muscle fibers, as a result of experiments with ACh immersed preparations. In a later study BRECHT and EPPLE, however, postulate the existence of different fiber types, those where ACh depolarizes the endplates only and sets up twitches (e.g. sartorius fibers), and fibers where the depolarization spreads over the whole surface and causes slow contractile changes (some fibers in the rectus abdominis). The present findings are in essential agreement with these conclusions. If one assumes that ACh acts at small-nerve junctions, practically the whole fiber surface would be involved by the depolarization.

Detection of slow muscle fibers by their reaction to ACh alone may not always be sufficient since the sensitivity even of twitch muscles is variable (WACHHOLDER and NOTHMANN). This, in fact, led to the belief that "non-tonic" muscles may be changed into "tonic" ones according to the season or the animal's metabolic state. For a definitive identification of slow muscle fibers the innervation must also be established. Since the innervation pattern is fixed, as seen in experiments with intracellular electrodes on frogs at all seasons, the slow muscle fiber identity must also be fixed.

Many observations classed as "contracture", or residual shortening, and sometimes unselectively called Tiegels contractures, may have been

due to slow muscle fiber stimulation. The well-known contracture of BREMER, however, obtained most favourably by direct or indirect double stimulation has recently been shown (BREMER and DESMEDT) to be accompanied by propagating muscle impulses in twitch fibers. As stated above, the differences between contractures (non-propagated reversible contractions) in twitch fibers and slow fibers are great, but merely quantitative (Fig. 7). Other differences, like the absence of propagating impulses and different membrane properties may well be called qualitative since they set the fiber types completely apart. It is clear now that the well-known assay for ACh on the rectus muscle works only on slow muscle fibers.

The extensive histological studies of KRÜGER and his collaborators over the past years, summarized in a recent book, have to be noted. On muscle crossections they find fibers with "Felderstruktur" in locations which contain slow muscle elements, e. g. in the slow portion "Tonusbündel" of the iliofibularis, while they identify muscle fibers with "Fibrillenstruktur" in the twitch portion or in muscles like the sartorius. On this basis a correlation of "Felderstruktur" fibers with slow muscle fibers is indicated. In our own studies on the ordinary mammalian skeletal muscle system, however, we have not been able to identify muscle fibers which correspond to the frog's slow elements. They all were found to conduct muscle impulses and give twitches. This physiological test by itself does not exclude differences within the mammalian skeletal system (see below). The *functional* differentiation, however, of mammalian structures in which KRÜGER finds "Felderstruktur" does not seem clear to us.

The present picture of slow muscle fiber activation as contrasted to twitch fiber excitation appears to be as follows. In twitch fibers motor nerve impulses set up junctional potentials which in turn depolarize the surrounding muscle membrane and start selfpropagating muscle impulses. These sweep along the whole muscle fiber length and activate in an unknown manner the underlying contractile system. There seems little scope left for gradation in single twitch fibers under normal conditions. In contrast, no propagated impulses occur in slow fibers, the small-nerve motor fibers set up local potentials at the junctions which in turn cause local contraction. Since slow muscle fibers possess numerous junctions along their whole course, a nearly simultaneous activation of many parts of such a muscle fiber can take place. Very fine grading of the contractile action in individual slow fibers is brought about (I) by varying the number of active nerve fibers reaching a muscle fiber, and thereby creating more or fewer distinct spots of contraction and (II) by changing the frequency of small-nerve impulses, leading to different intensities of junctional depolarization and thereby to graded contractile

action. The mechanisms underlying the facilitation processes are apparently multiple and presumably occur in nerve terminals, within the junctions themselves, and in the contractile system. Gradation based on local contractions in fibers is well known in many crustaceans muscles (see reviews by KATZ 2, WIERSMA).

In both twitch and slow fibers the membrane change is the precursor of contractile change, but there seems to be a striking difference between the fiber types in the speed of "accomodation" to depolarization. In one case a persistent loss of membrane charge leads to a prolonged, in the other to a transient contraction, i. e. the coupling process between surface and contractile elements is broken slowly or more quickly (Fig. 7). Apparently the method of depolarization—electrical, chemical, mechanical—is not the important factor. Thus there is no evidence which indicates that current flow as such, accompanying the muscle impulse or applied through electrodes, excites the contractile elements directly, nor does KCl or ACh do so. Contractions can be set up by simultaneous depolarization of the whole muscle fiber, for instance by "massive" direct transverse stimulation (SANDOW), without longitudinal current flow or propagating muscle impulses, or by immersion in drugs. In the latter case no significant current flow develops at all if the whole length of a fiber is affected simultaneously. Drug contractures can, however, be abolished or partially reversed if the surface polarization is restored by a current even in the continued presence of the depolarizing agent (KUFFLER 1; FLECKENSTEIN, HILLE and ADAM). In the case of ACh, curare can prevent contracture development or reverse the ACh effect, presumably by its action on the surface and not on the contractile elements. Therefore it seems to be the reduction of membrane potential which is responsible for the initiation of the contractile event. The linkage between surface and fiber interior is not known (see KATZ 3).

Function of small-nerve system: By their constitution the slow muscle fibers appear to be suited to relatively slow contractions only. They can maintain tensions, without neuromuscular fatigue or block, much longer than twitch fibers. Their contractile state is regulated in very fine steps and, in contrast to twitch fibers, is more sensitive to changes of the ionic composition of the environment. Apart from these peripheral properties there exists a specialized central nervous organization controlling the slow muscle fibers through the small-nerve fibers. This is shown by the different reflex excitability of the small-nerve system and especially by the tendency for a continued discharge "at rest", in the absence of obvious movement. Stepped up small-nerve activity, frequently precedes and then accompanies twitch action (Fig. 8), a sign that the two systems also work in unison. This observation agrees with KOBAYASHI, OSHIMA, TASAKI, who recorded discharges in nerve.

The foregoing considerations alone suggest that the small-nerve system may serve as a supplementary organization to the twitch system. It was suggested already many years ago that the "tonic" frog muscles of SOMMERKAMP and WACHHOLDER are largely concerned with the clasp reflex. Such an exclusive functional specialization is not likely since slow muscle fibers are widely distributed in the body, are not confined to particular functional units like flexors or extensors and are found in males and females at all seasons. Further, they are also present in eye muscles (for details see KUFFLER and VAUGHAN WILLIAMS 2). It seems reasonable, therefore, to assign to the small-nerve system a role in general postural activity. In the sense that we are dealing here with an organization which tends to be active for long periods, does not effectively participate in phasic movement, and is relatively resistant to fatigue, one may well call the small-nerve system "tonic". In this context the slow muscle fibers appear more economical for gradual tension development, since their energy can be expended in fine steps according to need, while in the twitch fiber energy is liberated in a sudden maximal burst (HILL).

The actual tension produced by slow muscle fibers in different muscles varies, since their density fluctuates greatly in different regions of the body. The ratios of slow and twitch fibers are not known, but slow fibers seem to be greatly outnumbered wherever tests with intracellular electrodes have been made. More histological counts using KRÜGER's method, correlated with physiological tests, would be of interest. In some muscles slow fibers can produce 10—15% of the maximal single twitch tension. However, the optimal working condition for slow fibers are low initial tensions, while twitch fibers operate most effectively under higher tensions. Furthermore, isotonic recording conditions, as used in most of the studies by earlier workers, will tend to exaggerate the slow muscle fiber contribution.

Comparative aspects: A system similar to that of frog has not been found in mammals (cat) in spite of prolonged search. The cat's lumbosacral ventral root outflow contains a well defined grouping into large and small-nerve fibers with diameter peaks around 4—6 μ and 12—15 μ (ECCLES and SHERRINGTON). LEKSELL found that the small diameter fibers produced little or no muscle tension, but enhanced the afferent discharge from muscle spindles. In later investigations of the role of these fibers it was possible to isolate large numbers to one muscle and test for contractile effects. It was concluded that the whole group comprising about $^1/_3$ of the ventral root fibers to hind limbs has no effect on the ordinary contractile system but innervates exclusively the intrafusal muscle fibers within spindles (KUFFLER, HUNT and QULLIAM). By causing minute contractile effects in the muscular elements of the spindles, they

excite the sensory elements by stretch and cause afferent discharges. KOBAYASHI, OSHIMA and TASAKI, in agreement with HUNT and KUFFLER'S general results, report that small-nerve excitation in their studies has not caused detectable tension, but speculating from indirect evidence they link small-nerve activity with decerebrate rigidity. A similar view has been held by HÄGGQUIST for some time. HÄGGQUIST'S findings on animals rendered spastic as a result of cord hypoxia have been repeated and essentially confirmed in our laboratory (unpublished). In each case, however, it is thought that undegenerated large nerve fibers remained in sufficient numbers to account for the spastic state. The intrafusal muscle elements in mammalian spindles seem to us to possess many similarities with slow muscle fibers of frog. Besides being densely innervated by a small diameter nerve spectrum, their activation involves facilitation, they probably give graded contractions, and they also exhibit a similar pharmacological behaviour under the influence of ACh. This drug apparently does not excite the sensory nerve terminals but only the intrafusal muscle fibers and the ACh effect is blocked by curarine without appreciably changing the threshold of the afferent discharge to stretch (HUNT 2).

It has now been well established, especially by the later studies of HUNT, that the mammalian small-nerve system plays a role in reflex activity by regulating the afferent flow of discharges from muscle spindles (see HUNT'S review 1). Although small-nerve fibers cause no direct muscle tension in the ordinary striated muscle fibers, they exert an *indirect* effect on posture. In contrast, the frog's small-nerve system produces principally a direct postural effect besides innervating spindles (KATZ 1). While the frog's mechanism of regulating postural activity seems relatively simple, slow and economical, the cat's organization seems more complex but the additional complexities allow much greater precision (MERTON; GRANIT & STRÖM).

Summary.

1. The results of recent investigations on two distinct nerve-muscle systems in the frog are presented. Each system consists of a special group of nerve fibers which innervate a separate set of striated muscle fibers. (I) The "Twitch System" or "Large-Nerve System" is composed of large diameter ventral root fibers which conduct at 8 to 40 m/sec. and innervate muscle fibers which give the well known propagating muscle impulses and twitch contractions. (II) The "Slow Muscle System" or "Small-Nerve System", consisting of small diameter fibers of 2 to 8 m/sec. conduction velocity which innervate the "slow" muscle fibers. Various properties of the two systems were studied, largely with the use of intracellular electrodes.

2. The small-nerve fibers cause slow contractile changes, resembling smooth muscle activity. Repetitive stimulation is needed for a significant effect. The rate of rise of tension and the amount finally developed, depend on the frequency of nerve stimulation.

3. Each slow muscle fiber is densely innervated by numerous small-nerve fibers over its entire length. Twitch fibers of several muscles have also been shown to receive more than one large-nerve termination, but these are relatively widely spaced. No innervation overlap occurs between the two systems. The motor unit response and its role in grading contraction is discussed in relationship to multiple innervation of slow and of twitch muscle fibers.

4. Slow muscle fibers have membrane potentials of about 60 mV as compared with over 90 mV in twitch fibers. Slow fibers do not give propagating muscle impulses on nerve stimulation but are activated around their numerous junctional regions where local electrical and contractile changes occur.

5. The small-nerve junctional potential (s. j. p.) differs greatly from the larger and faster endplate potential (e. p. p.) which is found at twitch muscle fiber junctions. S. j. p.'s, as recorded with intracellular electrodes inserted anywhere in slow muscle fibers, are composite potentials, set up by the activity of several small axons at their nerve endings. S. j. p.'s rise in 2—3 msec. to a peak of 8—15 mV which is followed by a slow membrane restitution process leading into a phase of hyperpolarization with a gradual return to the resting level. The entire s. j. p. complex may last about 300 to 400 msec. as compared with approximately 30 msec. for the e. p. p.

6. It is concluded that the slow muscle fibers are identical with some of the muscular elements contained in SOMMERKAMP's "Tonusbündel". The slow fibers also seem responsible for most of the "tonic" phenomena which were extensively studied by WACHHOLDER and other workers. Slow muscle fibers show a specific "electrical" and "chemical" excitability. If depolarized by currents or by drugs they maintain a tension or shortening for prolonged periods, while twitch fibers relax quickly. Assays for Acetylcholine are always done on slow muscle fibers. The relationship between surface charge and contraction is discussed.

7. The small-nerve and large-nerve systems can be activated separately or together by reflex excitation and appear to act synergistically in the maintenance of posture.

8. The differences between the frog's and cat's small-nerve systems are discussed. The small-nerve fibers in mammals innervate muscle spindles exclusively and do not cause muscle tension directly. They exert, however, an important indirect effect on the spinal reflex mechanisms.

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Dr. STEPHEN W. KUFFLER, Wilmer Institute, John Hopkins Hospital and University Baltimore/Maryland USA.