J. Physiol. (1953) 121, 289–317

SMALL-NERVE JUNCTIONAL POTENTIALS. THE DISTRIBUTION OF SMALL MOTOR NERVES TO FROG SKELETAL MUSCLE, AND THE MEMBRANE CHARACTERISTICS OF THE FIBRES THEY INNERVATE*

BY STEPHEN W. KUFFLER AND E. M. VAUGHAN WILLIAMS†

From the Wilmer Institute, The Johns Hopkins Hospital and University, Baltimore 5, Maryland, U.S.A.

(*Received* 19 *January* 1953)

The ventral roots in the frog contain two distinct groups of motor nerve fibres, which subserve two different functions in their innervation of skeletal muscles. Repeated stimulation of nerve fibres of small diameter, about 5μ, causes slow, graded muscular contractions which are accompanied by non-propagated muscle potentials of small amplitude and relatively long duration (Tasaki & Mizutani, 1944; Tasaki & Tsukagoshi, 1944). The larger nerve fibres, in the region of 12μ diameter, set up the familiar motor unit twitches accompanied by fast propagated muscle action potentials. Kuffler & Gerard (1947) demonstrated that when one or a few small-diameter motor nerve fibres were isolated by dissection and stimulated, the resulting muscle potentials were confined to the regions of the neuromuscular junctions; they were accordingly named 'Small-nerve junctional potentials' (s.j.p.'s). The mechanical effects of stimulation of the small motor nerves were studied in detail by Kuffler, Laporte & Ransmeier (1947) who found that quite large tensions could be developed in numerous muscles widely distributed over the body. Differences in the organization of the reflex responses of small and large-diameter motor fibres were also demonstrated. It was concluded that the small motor nerves are part of a specialized neuromuscular system with a specific 'holding' or 'tonic' function in the maintenance of the frog's posture.

In the latter two studies it could not be decided whether the two groups of nerves went to different types of fibres within the muscle or whether both innervated the same single muscle fibres. The possibility existed that a single muscle fibre could give two different types of response, according to the type

* This investigation was supported by a research grant from the National Institutes of Health, Public Health Service.

† Fellow of the Rockefeller Foundation; from the Department of Pharmacology, Oxford, England.

of nerve fibre through which it was stimulated. A duality of response has been observed in crustacean muscles (Katz & Kuffler, 1946).

The first purpose of the present investigation was to determine whether small-nerve fibres innervate a distinct set of muscle fibres. Secondly, it was proposed to study the characteristics of the small-nerve junctional potentials by newer methods. It will be shown that the small-nerve fibres supply quite distinct muscle elements, whose behaviour in many respects bears little resemblance to that of the twitch fibres. These special muscle fibres possess some properties not hitherto seen in skeletal muscle, and will be referred to as 'slow' muscle fibres. They receive innervation from numerous nerves, all of small diameter, whose terminal contacts are distributed along the whole fibre length. In contrast to the 'fast' twitch-producing muscle fibres they have a smaller membrane potential, never give propagated muscle action potentials on nerve stimulation and do not show a reversal of membrane potential ('overshoot') during activity.

In the second paper some special properties of these 'slow' striated muscle fibres will be examined. It will be shown that the present results furnish an adequate explanation of the earlier findings of Sommerkamp (1928), Wachholder & Ledebur (1930) and others concerning 'tonic' muscles. Also some questions which have been raised concerning the nature of 'contractures' in muscle (Gasser, 1930; Bremer, 1932) may find at least a partial answer in these studies.

It is appropriate at this point to consider further the term 'small-nerve fibres'. In these papers the expression is used to describe the nerve fibres in the ventral roots which conduct at 2–8 m/sec (see below), and which innervate the slow muscle fibres. There clearly exist still slower (and smaller) ventral root fibres of different function (e.g. sympathetic), and in some parts of the body even the same diameter fibres may not play the same role. It is not known whether there is any difference (e.g. biochemical) other than the obvious one of size (of perhaps secondary importance) associated with nerve fibres subserving distinct functions. It is possible that nerve fibres causing diverse effects are themselves practically identical along their course, and that the difference in their function is determined by properties not of their own, but of the effector organs upon which they terminate. Since the association of the 'small' and 'large' nerve fibres with distinct groups of muscle fibres has now been established, it may perhaps be more appropriate to classify the two systems according to the effectors, i.e. 'slow skeletal muscle system' and 'twitch system'. For the sake of conformity with previous work, however, the established term 'small-nerve fibres' will be retained, but it is emphasized that it refers only to nerves supplying slow muscle fibres in frogs. The term 'small-nerve system' will be more inclusive and be applied to the slow muscle fibres together with all nervous structures which activate them. This will be in

contrast to the twitch fibres and their nervous connexions which will be called the 'twitch system'. These definitions should not be extended to mammals, in which many mechanisms and their organization are different (Kuffler & Hunt, 1952).

METHODS

Muscles, together with their nerves and appropriate spinal roots, were dissected from summer and winter frogs of the species *Rana pipiens* and *R. catesbiana*. Records from single muscle fibres were obtained with glass capillary electrodes (Ling & Gerard, 1949) with tips certainly well under 1·0 μ, and smaller than could be measured accurately with a light microscope (Zeiss water-immersion objective, overall magnification $\times 900$). They were filled with approximately 3 M-KCl and had a d.c. resistance of 10–40 MΩ. The input circuit was similar to that described by Nastuk & Hodgkin (1950).

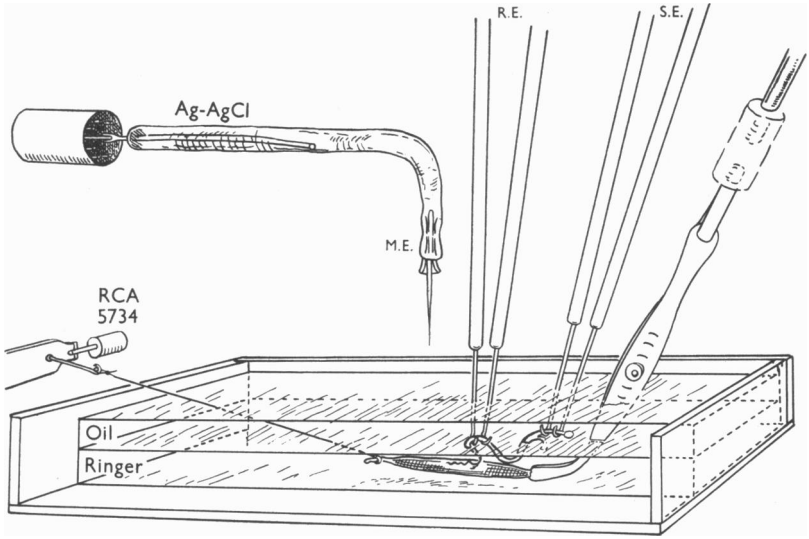

Fig. 1. Scheme of experimental set-up. For intracellular recording the muscle was usually gripped at both ends and stretched over a curved lucite block to minimize movement during stimulation. M.E., microelectrode; R.E., recording electrodes; S.E., stimulating electrodes. The KCl-filled microelectrode makes a liquid junction with KCl-agar. The Ag-AgCl electrode is soldered to a clip which fits directly to the grid of a cathode follower.

Intracellular electrodes are easily dislodged from muscle fibres by contractions, especially during twitches. This problem is less serious during the relatively slow movement set up by small-nerve stimulation, although occasionally it was necessary to stretch the muscle to a suitable extent by trial and error and select recording electrodes (usually long tapered, easily bent) which after impalement moved freely with the muscle. The muscle rested against a transparent base-plate, except when tensions were recorded (Fig. 1). In many experiments it proved possible to record from slow or twitch fibres, even during prolonged tetanic stimulation, without serious movement artifacts, the electrode tip staying inside muscle cells (Figs. 9, 11). To reduce muscle movement contributed by the twitch fibres the twitch portion of the iliofibularis was frequently dissected away.

Tension records were obtained with an RCA 5734 transducer, fitted to a smaller version of the apparatus described by Talbot, Lilienthal, Beser & Reynolds (1951). Some modifications were made to render the valve itself less vulnerable to accidental damage.

RESULTS

Differential block of nerve fibres

Tasaki and his co-workers had distinguished between the muscle responses to small and large motor nerves by stimulating dissected nerve fibres. This technique, though providing unequivocal evidence concerning the types of response to nerves of different diameters, did not permit identification of the muscle fibres which were responding. Kuffler & Gerard (1947) also used the single fibre method, but in addition they differentiated between nerve fibre groups by blocking the faster fibres by pressure and by constant current. The latter method was quickly reversible and permitted satisfactory differentiation for a few minutes at a time, but the preparations were not very stable, and it was certain that a fair proportion of small-nerve fibres was blocked along with the large. A stable and more complete differentiation was, however, achieved in the following way.

Appropriate motor roots were dissected out together with the required muscle and its nerve. The preparation was then set up as shown in Fig. 1. The motor root and the nerve to the muscle were lifted from the Ringer solution into paraffin oil upon electrodes for stimulating and recording respectively. When muscle potentials were to be recorded with external electrodes, the muscle also was lifted into oil, but when individual fibres were to be pierced with the microelectrode, the muscle was left in the Ringer. The motor roots were then stimulated and the nerve impulses reaching the preparation were recorded from the nerve just before its entry into the muscle. The stimulating cathode was placed near the origin of the motor root with the anode at variable distances away (Fig. 2). A square current pulse was then passed through platinum electrodes. If the stimulus was short (0·1–0·2 msec) and sufficiently strong, all the nerves in the root were stimulated and impulses passed down to the muscle. With longer stimuli, however, some or all the nerve fibres could be blocked at the anode. Apparently impulses from fast and slow fibres started off at the same moment in the vicinity of the cathode (Rushton, 1949), as seen in Fig. 2*B*, but the faster fibres reached the second electrode while the anodal effect still persisted (Fig. 2*D*). By adjusting the duration and strength of the pulse correctly in relation to the interelectrode distance it could be arranged that the anodal polarization persisted sufficiently long for the faster fibres alone to be blocked, while the slower ones passed through. The example presented in Fig. 2 shows how, with an effective interelectrode distance of 4 mm and a pulse of 0·55 msec, fibres travelling at 9 m/sec (or faster) would be blocked because they would arrive at the anode while the pulse still persisted, but those travelling at 6 m/sec (or more slowly) would be let through.

Another factor besides conduction velocity seems to play a role, namely, that small-nerve fibres are more resistant to anodal block even if placed in a

uniform field with large fibres. Presumably less current actually penetrates the small fibres than the larger ones, since the space between fibres serves as a relatively more effective shunt to the small-diameter fibres. This seems to be the basis for the constant current blocking method used in our previous studies.

The present method never failed, although occasionally it required prolonged trials to obtain the correct combination of pulse and interelectrode distance.

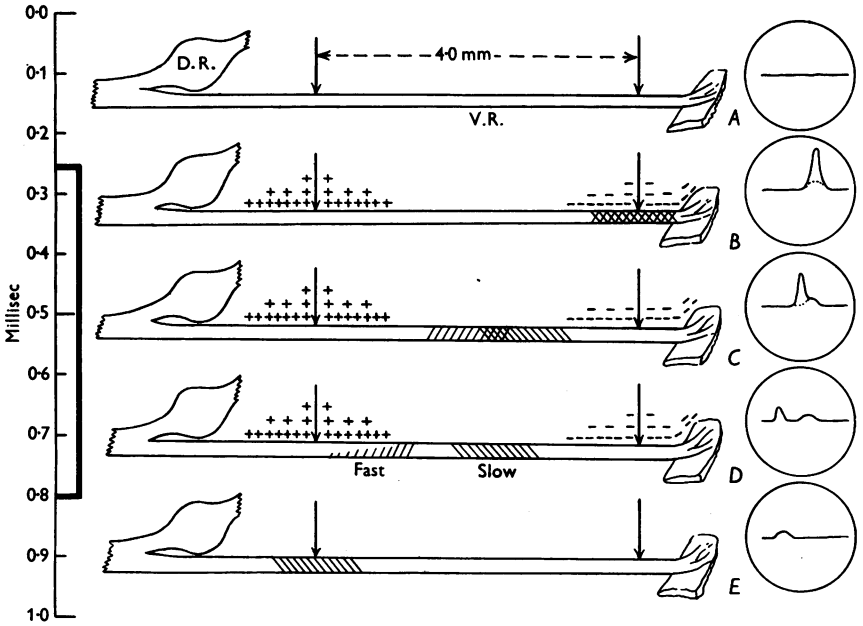

Fig. 2. Scheme of blocking procedure and suggested mechanism of large nerve block. Electrodes on the ventral root (V.R.) lifted into paraffin oil. The ventral root is depicted as attached to a piece of spinal cord on the right. D.R., dorsal root ganglion. *A*, before excitation; *B*, nerve impulses started at cathode after the beginning of a square pulse; *C*, 0·25 msec after start of impulses some separation of slow and fast fibres occurs; *D*, most of the faster nerve impulses have reached the anode and are blocked; *E*, after cessation of square pulse only the slower impulses pass through. The events depicted in each picture take place at times corresponding to the picture's position on the scale at left. The thick black line represents the duration of the current pulse. Right: expected potentials in ventral root at the times depicted. Shaded areas represent the active regions. (See text for additional factors in blocking.)

In this fashion desired groups of fibres could be selected at will as is shown in Fig. 3. The preparations remained stable for hours, i.e. the same select group of nerves could repeatedly be stimulated. A further advantage was that nearly all the small-nerve fibres to a given muscle could be stimulated simultaneously. Whenever both fast and slow fibres in a root were to be excited either the polarity of the current was reversed so that the anode was at the origin of the root, or a second pair of electrodes was placed lower down on the nerve and

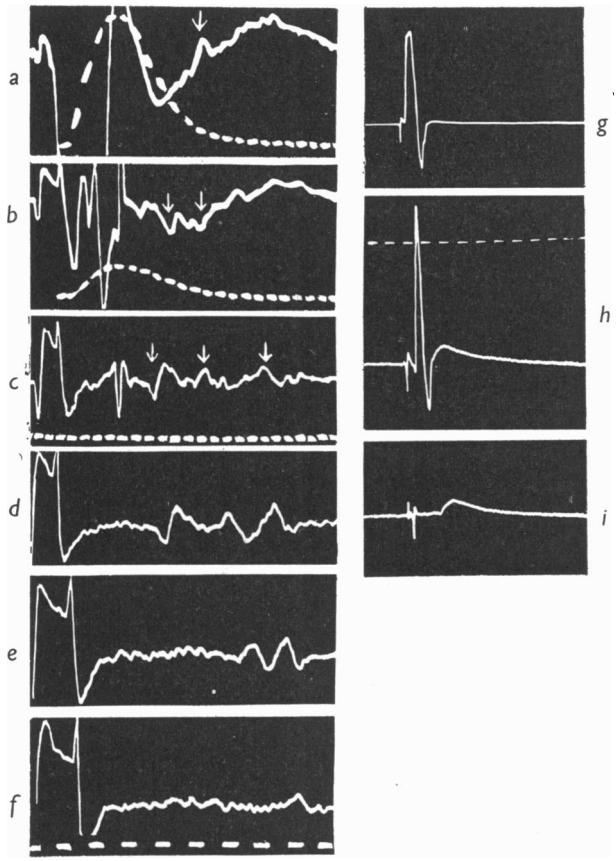

Fig. 3. Progressive block in root, recorded from peripheral nerve and from muscle. Stimulating electrodes arranged as in Fig. 2. Left: leading from nerve before its entry into iliofibularis. *a*, stimulus of 0·6 msec duration to V.R. 10 causes maximal twitch tension recorded by transducer on lower beam, modulated at 100 c/s. Large nerve potentials on upper record cause early deflexions (off the screen); arrow marks potentials of some small-nerve fibres. Note fast time-base on this beam (calibration in *f*). *b*, stronger stimulus blocks many large fibres, causes smaller twitch, fewer early potentials and excites more small-nerve fibres (arrows). *c*, stimulus increased, blocks all but one large-nerve fibre, causing no measurable twitch tension at this sensitivity; three distinct small-nerve groups seen (arrow). *d*, optimal stimulus for small nerves alone, no twitch contraction. *e–f*, duration of stimulus increased progressively, also blocks small-nerve impulses. Sweep speed for all nerve potential traces is given by 1000 c/s modulation in *f*. *Records from muscle surface.* *g*, stimulus as in *a*, large potential of 20 mV recorded. *h*, amplification increased 10 ×, most large-nerve fibres blocked. Diphasic potentials of few twitch units are followed by monophasic small-nerve junctional potentials (s.j.p.'s). *i*, as in *d*, all fast fibres blocked; small-nerve fibres set up s.j.p.'s only. Time base 200 c/s.

the fast fibres stimulated alone or in combination with the slower (and higher threshold) small-nerve fibres.

It was not possible to apply this blocking technique of the roots satisfactorily to the whole nerve. Large nerve trunks are surrounded by a connective tissue sheath and contain sensory fibres also. It is likely that in such a situation the entry and exit of current is less sharply localized in relation to the elements which it is desired to block.

Fig. 3 illustrates the selective blocking of nerve fibres of different conduction velocities within ventral root 10. Recording was done from the nerve to the iliofibularis about 33 mm from the proximal stimulating electrode. A non-blocking maximal stimulus (Fig. 3*a*) sets up impulses in the relatively fast motor fibres (action potential peak off the screen) and in a few slower fibres (arrow) which from the subsequent records can be identified as small-nerve fibres. Simultaneously a record of the twitch tension was superimposed, taken on the second beam at a slower speed. In Fig. 3*b* a stronger stimulus blocked at the anode most of the larger fibres, and also stimulated a greater number of small fibres (arrows). In Fig. 3*c* all the large-nerve fibres but one were blocked; no tension was recorded at this sensitivity and the slow fibres can be clearly distinguished. In Fig. 3*d* further increase in the stimulus strength blocked all fibres except three groups conducting at 6, 4·8 and 3·6 m/sec. This represents maximal small-nerve stimulation. In Fig. 3*e–f* the duration of the stimulus was increased (without further change in strength) and the remaining small-nerve groups were successively blocked in the order of their conduction velocities. Fig. 3*g* presents the same situation as Fig. 3*a* but with records taken from the muscle surface (iliofibularis). Only a large muscle action potential is seen. In Fig. 3*h* (amplification $\times 10$) most of the large nerves were blocked, the remaining ones setting up propagated impulses, while the small nerves set up the subsequent slow potential, made up of s.j.p.'s. In Fig. 3*i*, corresponding to Fig. 3*d*, all but the small-nerve fibres were blocked, leaving the s.j.p.'s alone. By this method the previous findings correlating nerve fibres and their conduction velocities (Kuffler & Gerard, 1947) with their effects on muscle were confirmed in a more precise manner. Particularly useful in the present experiments was the availability of a 'pure' preparation of virtually all the small-nerve motor fibres.

Distinct innervation of muscle fibres giving fast and slow contraction

Many hundred muscle fibres were entered with microelectrodes and their nerve supply was stimulated. Two principal types of response were observed. One was the large (110–130 mV) propagated muscle action potential of Fig. 4*a* associated with a muscle twitch (Nastuk & Hodgkin, 1950). In its electrical properties it resembles the nerve impulse, and it seems clear that its function is to activate locally the contractile mechanism as it sweeps along the muscle

fibre. The other type of response, seen in Fig. 4*b* (note time scale ten times slower), was a slow, relatively small potential which could be identified as the s.j.p. obtained in earlier studies by external electrodes.

In addition, there is shown in Fig. 4*c* an end-plate potential (e.p.p.) obtained in the same position as Fig. 4*a*, and with the same amplification and time scale, but after transmission was blocked by a previous high-frequency tetanus. In some respects it resembles the s.j.p. and represents an intermediary process between the nerve impulse and the propagated muscle impulse; it is confined to the junctional region, and is usually seen only in fatigued or otherwise blocked junctions. Under special conditions, however, the e.p.p. is also found

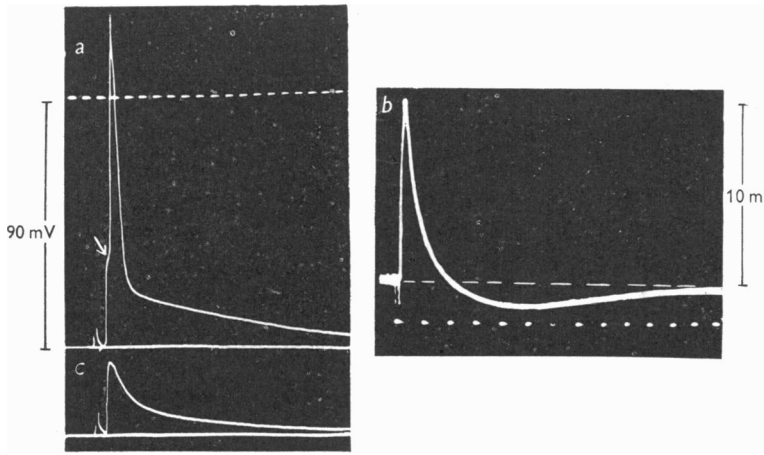

Fig. 4. Types of response of striated muscles to nerve stimulation, recorded with intracellular electrodes from iliofibularis. *a*, twitch fibre with propagated muscle impulse, recorded at an end-plate. Electrode inside muscle fibre was 90 mV negative relative to outside. Zero membrane potential indicated by upper sweep with intensity modulation of 500 c/s. Total action potential 120 mV, i.e. 30 mV 'overshoot'. Arrow marks end-plate potential component. If recorded some distance from junction, the end-plate component is absent. *c*, same electrode position as in *a* after transmission was blocked by previous high frequency stimulation. Resting potential 90 mV. *b*, electrode in slow muscle fibre. Typical s.j.p. response following small-nerve stimulation. Note sweep speed reduced (50 c/s modulation). Potential peak only 10 mV, resting potential 52 mV, zero potential not shown. Large hyperpolarization or 'positive afterpotential' (see also Fig. 9).

in normal muscles (Kuffler, 1952). Its main role is not to activate the contractile system but to set up the propagated muscle impulse. The slower s.j.p., on the other hand, is associated with the development of tension (see later).

No single muscle fibre gave the two main response types. The propagated action potential (with overshoot) or the e.p.p. was only seen with stimulation of the large-nerve group and a fibre giving this response never exhibited any potential change on stimulation of small-nerve fibres only. Conversely,

muscle fibres which gave the slow s.j.p. response did so exclusively on stimulation of the small-nerve fibres but gave no potential with large-nerve excitation. In view of the large number of fibres entered, and of the fact that all the large nerves, and most, if not all, the small nerves, were separately stimulated in each test, the results are regarded as adequate proof that no individual muscle fibre can receive both kinds of innervation and give both types of response. Evidence that hardly any nerve fibres escaped stimulation is provided by the fact that only rarely did a muscle fibre fail to respond at all in these tests. Additional evidence of two distinct types of muscle fibre is derived from the finding of a large difference in the membrane potential of the muscle fibres innervated by small and large nerves (see below).

There are thus two quite different systems of nerve-muscle connexions in frog skeletal muscle involving two separate groups of muscle fibres. The one is the twitch system associated with large-nerve innervation, the other the slow system with small-nerve connexions.

Multiple innervation of individual slow muscle elements by small-nerve fibres

If one or a few small-nerve fibres are stimulated in a whole muscle or particularly in a muscle 'strip' preparation, one can record s.j.p.'s with external electrodes which may be sharply localized to a small junctional region. The potentials decrease with distance from the junction, and disappear at a few millimetres (Kuffler & Gerard, 1947, fig. 1). The spatial decrement is similar to that of the end-plate potential (e.p.p.) of curarized muscle originally observed with external leads and recently confirmed by Fatt & Katz (1951) with internal electrodes. In the studies of s.j.p.'s with external electrodes it was reported that a single small-nerve fibre might innervate several regions of a muscle, causing distinct local contractions at each area where the junctional potentials were recorded. Whenever a large number of small-nerve fibres was excited, however, it was difficult to localize distinct junctional foci and therefore the pattern of the distribution of small-nerve fibres on individual muscle fibres could not be determined.

In the present experiments iliofibularis muscles with their ventral roots were set up and the small nerves exclusively stimulated by the technique already described. The location of the slow contraction was then looked for under the microscope. It was found to be confined to a region extending for about 15 mm on the side of the muscle on which the nerve entered, and coincided with the 'tonus bundle' described by Sommerkamp (1928). Microelectrodes were inserted into this area, mainly into muscle fibres running on the muscle surface, but occasionally the electrode was also thrust through the surface layer into the fibres lying more deeply. The small-nerve fibres were always excited first and if a 'slow' fibre had been entered, an s.j.p. was recorded. Whenever no potential change occurred, the whole nerve to the muscle was maximally

stimulated, and as a rule a propagated impulse was set up. Whenever one obtained s.j.p.'s after 'properly' (see later) entering a slow muscle fibre, the potentials varied little in size (8–15 mV) or time course, provided the small-nerve stimulus was maximal. This relative lack of variation is significant, when considered in relation to the local nature of s.j.p.'s which has already been established by their spatial decrement over a few millimetres observed in muscle-strip preparations by Kuffler & Gerard (1947). If long stretches of slow muscle fibres existed without innervation it would follow from the localization of the potentials to the nerve junctions that sometimes no potentials at all, or potentials of all transitional sizes should have been recorded, since several hundreds of such fibres were penetrated at random and some intermediate regions without innervation would certainly have been entered. Such extreme variations in potentials were actually observed if, for example, microelectrodes

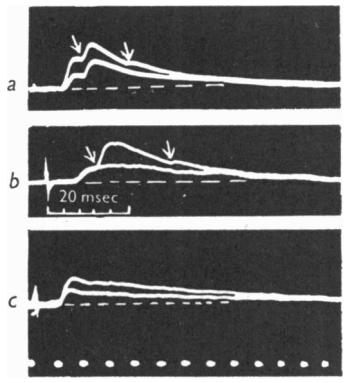

Fig. 5. Multiple terminations on single slow muscle fibres. Faster sweep than Fig. 4*b*; early phase only of s.j.p. shown. Intracellular record from three different slow muscle fibres in iliofibularis. Graded stimulus strength to small-nerve fibres in ventral root brings in additional 'steps'. In each record two sweeps superimposed. S.j.p. components (arrows) are set up by different nerve axons with junctions in vicinity of electrode tip. Sweep speed in *c* 100 c/s; s.j.p.peaks 8–11 mV. Hyperpolarization phase of s.j.p.'s seen on slower sweep only (see Fig. 9).

were inserted at random into curarized or fatigued twitch fibres, and the end-plate potentials recorded. Since there are only a few such end-plates in each twitch fibre, there are considerable stretches from which no potentials, or only small potentials, are obtained. One may therefore tentatively conclude from the absence of such blank areas in slow muscle fibres that no region is far from a nerve ending. Each fibre must, therefore, receive numerous nerve endings, and it is probable that adjacent endings are from different axons.

Evidence that different axons do supply the same fibre is presented in Fig. 5. Unlike propagated muscle impulses, which are all-or-nothing, s.j.p.'s recorded from the interior of a slow muscle fibre could be graded by altering the stimulus strength. Thus in Fig. 5, with all small-nerve fibres excited, the s.j.p.'s showed

several inflexions or 'steps' during the early part of their time course. By gradually weakening the stimulus strength, thereby exciting fewer small-nerve fibres, the potentials became less complex. Further variation of stimulus strength caused each potential step to fall out or come in at a definite threshold. At a certain stimulus strength the steps disappeared, leaving an s.j.p. of simple time course, but smaller amplitude (Fig. 5*b*, *c*, lower trace). Only the following interpretation seems compatible with the observations: each step in the s.j.p. complex represents a depolarization by a different small-nerve fibre; several fibres innervate the selected muscle fibre in the vicinity of the microelectrode and add their contributions in discrete quanta to the total potential recorded, which is the resultant of them all (see also Fig. 9). The potential component with the shortest latent period has normally the lowest threshold and represents junctional excitation by the fastest small-nerve fibre. The potentials which appear later as steplike additions are set up by nerve fibres of higher threshold and slower conduction velocity. It is also clear that the recorded amplitude of the contributions of different small-nerve fibres to the s.j.p. complex, are not equal. A small addition to an s.j.p., e.g. at the second arrow in Fig. 5*a*, most likely represents the contribution of a small-nerve junction some distance from the microelectrode tip set up by a more slowly conducting nerve fibre. In Fig. 5*b* the potential was obtained in two large steps and one small. The largest component (second step) was presumably set up nearest to the electrode by a nerve fibre conducting more slowly than that which gave rise to the first but smaller step (from a more distant junction). The third step (second arrow) arose from the most distant junction excited by an even slower fibre. In Fig. 5*c* the s.j.p. showed two steps only, apparently set up by two axons of similar conduction velocity.

From the present results, therefore, it may be concluded that individual slow muscle fibres are densely innervated over their whole length by a great number of small-nerve fibres.

The resting potential of fast and slow muscle fibres

In measuring the resting potentials of cells somewhat arbitrary criteria have to be adopted in estimating the validity of the results obtained. For instance, if a region on the inside surface of the sartorius containing little connective tissue was selected for making the measurements, and if small electrode tips (about 0·5 μ) and a 'good' micrometer advance was used, it was found that the uniform results of Ling & Gerard (1949) were readily confirmed, and the resting potentials were remarkably constant, within a few mV, even if a large series of fibres was measured. For example, one such series, of twenty-one fibres penetrated in succession, and without the rejection of any results, gave a mean resting potential of 95·6 mV (S.D. about mean, 2·4 mV); and another, of forty-six successive fibres without rejections, gave a mean of 90 mV (S.D. 2·6 mV).

A third series of 113 fibres gave a mean of 93·4 mV. When such fibres were entered the full membrane potential developed within 1 msec (Nastuk & Hodgkin, 1950). In other regions of the same muscle, however, even when the same electrode was used and with conditions apparently unchanged, the resting potential values often showed appreciable scatter. Frequently these variable results were associated with the presence of visible connective tissue strands. In still other instances high membrane potentials (90–95 mV) in individual fibres were observed, but the final value was reached in a gradual fashion as the electrode advanced. In these cases some dimpling of the muscle fibre surface could often be seen under the microscope during manipulation. In other fibres the electrode might 'snap in', yet give a low resting potential reading. Occasionally obvious fibre damage was seen in such instances.

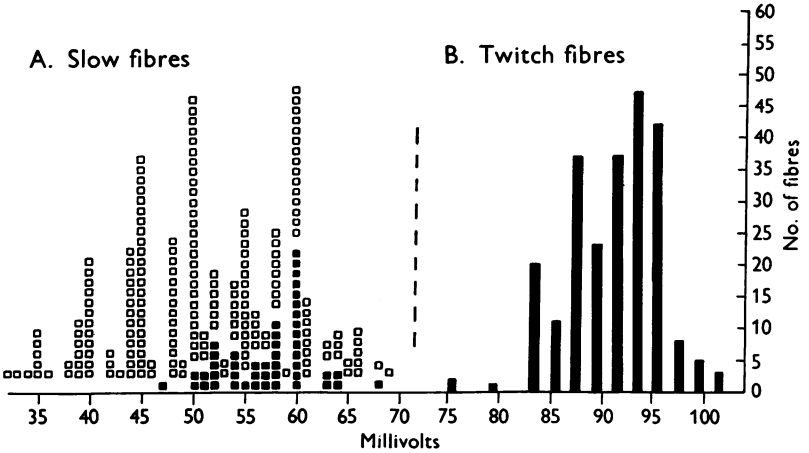

Fig. 6. *A*, resting potentials of 244 slow muscle fibres of iliofibularis. Open squares represent unselected potentials with distribution peak of 60 mV. Note absence of potentials above 70 mV (see text). *B*, resting potentials from 239 twitch fibres in the sartorius. Distribution peak about 95 mV.

With due regard to such difficulties significant differences have nevertheless been observed between the resting potentials of the slow fibres and the twitch fibres in all the muscles studied. The slow fibres showed consistently a lower resting potential than the twitch fibres. The membrane potential measurements of 244 fibres which gave s.j.p.'s are presented in Fig. 6*A* in two groups. The open squares represent individual unselected measurements and only fibres with obvious damage were not included. The filled-in squares represent the potentials of slow fibres selected according to the following criteria: (i) the electrode 'snapped' into the fibre, (ii) the resting potential did not fall off, (iii) the potential did not change with slight movement of the electrode. The membrane potential readings were made visually on the face of a cathode-ray tube. The observer's bias in selecting certain values is obvious. Only few

readings of 49 or 59 mV were made, the natural tendency, since the accuracy was only to within 1–2 mV, being to select the round numbers 50 or 60. The distribution peak of the selected measurements was 60 mV. The population had a skew distribution, the majority being below the peak. This was to be expected since damage or imperfections in method could only serve to reduce the resting potential. The absence of potentials above 70 mV is highly significant in view of the measurements of twitch fibre potentials. As mentioned above, in several large series of measurements (Fig. 6*B*) on the deep sartorius surface, averages of 90–96 mV were obtained from fibres giving twitch responses to nerve stimulation. While the 'twitch portion' of the iliofibularis was not as favourable to consistent results as the sartorius, or some other muscles, and the scatter of the measurements was greater, the peak resting potential values obtained from twitch fibres in this muscle also were always at least 20 to 30 mV higher than those from the slow fibres. This estimate is derived from more than 500 twitch fibres in the semitendinosus and iliofibularis.

In a small series of measurements the tendency would be to attribute differences in observed membrane potentials to such accidents as the presence of tough connective tissue or damage to the fibres. We feel that such an explanation of the difference between slow and twitch fibre resting potentials cannot be accepted. Very large numbers of fibres of both kinds were entered and the membrane potentials distributed themselves into two populations of the same shape, mounting to peaks at 60 and 95 mV respectively. Since both populations included many fibres in which confidence was felt (by the criteria mentioned) that the 'true' values had been obtained, the conclusion seems justified that the slow fibres regularly have a resting potential about 2/3 that of the twitch fibres.

Characteristics of the s.j.p.'s

Extracellular electrodes

The approximate time course of s.j.p.'s was determined on small muscle-strip preparations by Kuffler & Gerard (1947) and by Katz (1949) on the thin extensor digiti IV muscle. The s.j.p.'s rose to a peak in about 2 msec and decayed in about 50–60 msec. Fig. 7 shows some of the difficulties which arise if recording is done from a whole muscle (see also Tasaki & Tsukagoshi, 1944) like the iliofibularis. In Fig. 7*a–c* one electrode was placed at the distal end of the muscle while the other was moved to different positions on its surface. The small nerves exclusively within the 10th motor root were excited. Since in this case the active elements were surrounded by a mass of inactive tissue (twitch fibres) some of the conditions of volume conductor recording apply. The largest concentration (and size) of s.j.p.'s was obtained when the roving electrode was in the central part of the slow muscle fibre region (Fig. 7*c*). When both electrodes were close together on that region, a diphasic potential (Fig. 7*d*) was recorded, apparently the resultant of potential swings in the

same direction, but of different time course. Under some conditions even a spike-like potential was obtained. It is thus apparent that external records may not always give reliable information about the true course of s.j.p.'s.

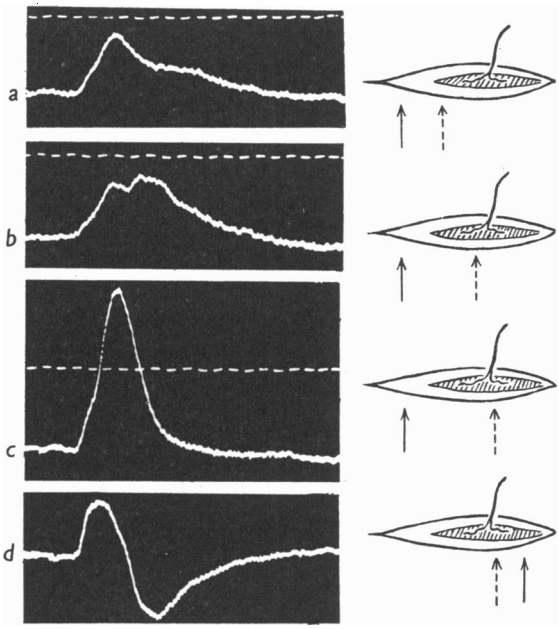

Fig. 7. Recording of s.j.p.'s from muscle surface of iliofibularis after block of all twitch-producing nerve fibres. Right: electrode positions in relation to slow muscle fibre portion (shaded area). *a–c*, one electrode stationary. Largest potential (i.e. highest density of small-nerve junctions) at position shown in *c*. In *d*, s.j.p.'s arise asynchronously under both electrodes causing diphasic potential. Time base, 500 c/s.

Intracellular recording. S.j.p. time course with single stimuli

As stated above, s.j.p.'s obtained with internal electrodes are remarkably constant in size and time course wherever they are recorded. This is only true if the majority of the small-nerve fibres with endings in the vicinity of the electrode are stimulated, since the recorded s.j.p.'s represent a composite potential derived from neighbouring junctional regions. Individual s.j.p.'s, however, even if obtained with certainly maximal stimulation of all nerve elements, i.e. without anodal block as in Fig. 9, do show fluctuations in size (about 10–15%) when excitation is repeated. The factors leading to this variability have not been specially studied, but might be due to small irregularities in conduction in the small-nerve terminals. The s.j.p. itself has the following three easily distinguishable phases.

(i) *Depolarization.* The first phase consists of a rapid depolarization (i.e. the interior becomes less negative relative to the exterior) made up of several steps,

which can be seen on a fast time-base as distinct inflexions when small-nerve impulses do not arrive simultaneously (Fig. 5). On occasions a single s.j.p. has been found to be made up of as many as seven separate component steps. The total depolarization in response to a single small-nerve volley has never been more than 16 mV (25% of the membrane potential), the most frequent value being 8–10 mV. The time taken to reach the peak depolarization depends upon how nearly synchronous are the different steps, but for one step or for a single synchronous s.j.p. the rise-time is 2–3 msec.

(ii) *Repolarization and hyperpolarization.* The second phase is a slow repolarization which always swings below the original resting potential into a hyperpolarization. The latter might be called a 'positive afterpotential' in conventional terminology, since it would be recorded as a positive potential with external leads. It is, in fact, an increase in negativity of the interior relative to the exterior. The fall of the potential from the peak of the depolarization to the peak of the hyperpolarization or positive after-potential is approximately exponential. Many potentials have been plotted semi-logarithmically, and the simple exponential sought which can be most accurately superimposed upon them. Fig. 8 shows such a line superimposed upon the falling phase of an s.j.p. which is also illustrated in Fig. 10. These plots regularly showed that the s.j.p. deviated slightly from a simple exponential. The simple exponential curves, fitted as closely as possible to a number of s.j.p.'s, were found most frequently to have a half time of 23–39 msec, i.e. the time constants (fall to $1/e$) were 33–56 msec.

The voltage of the hyperpolarization is from 20 to 40% of the initial depolarization, the most frequent values being about 30%. Here again the size and shape of this phase is constant only if all of the fibre endings in the vicinity of the electrode are stimulated. One of the criteria of 'adequate' small-nerve stimulation is seen in Fig. 9, which shows the effect of altering the stimulus strength on the records obtained from a single fibre. Just as the initial depolarization increased or decreased in steps in Fig. 5, the phase of hyperpolarization also exhibited gradations of response. If the stimulus is reduced until only a single small step remains, the phase of hyperpolarization may not be perceptible. In this connexion it should be mentioned that whenever there was doubt whether some of the small fibres had been blocked along with the large, an unequivocal maximal small-nerve volley could easily be obtained by giving a non-blocking stimulus with the cathode placed peripheral to the anode. The records shown in Fig. 9 were, in fact, made with such non-blocking stimuli of different strengths. It should be noted that in Fig. 9*a* and *b* the large potentials are obviously not identical, although nerve stimulation was maximal and therefore the same small-nerve fibres were excited.

(iii) *Final restoration of membrane potential.* The third phase consists of a return to the original resting potential after the hyperpolarization phase.

This is very slow and the total duration of the hyperpolarization is usually 200–240 msec, the longest observed being near 0·4 sec.

In Table 1 are presented the extreme and most frequent values of several parameters of s.j.p.'s, taken from measurements of numerous records. Since some, though very little, movement is associated with each small-nerve volley,

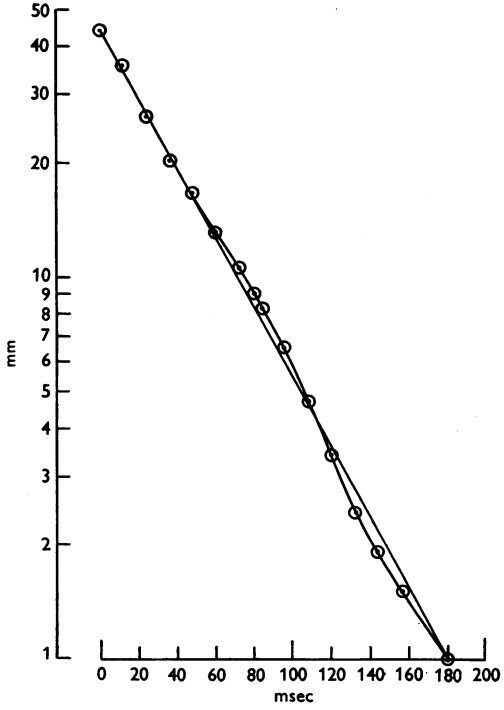

Fig. 8. Time course of s.j.p., depolarization peak to hyperpolarization peak recorded with intracellular electrode plotted on semi-logarithmic scale. Note deviation from exponential, indicated by straight line. This was a regular finding. Half-time of decay 32·4 msec. Ordinate: 1 mV = 5 mm.

the possibility of artifacts in these measurements has to be considered. The regularity with which very similar records were obtained, and the fact that variations in initial tension, which greatly affect movement, did not affect the s.j.p.'s, provide strong evidence that the potentials observed were genuine membrane effects. In practice, movement artifacts (caused, for example, by simultaneous twitch fibre stimulation) were easily recognized, mainly by their irregularity. In the series of stimuli of increasing strength from which Fig. 9 was obtained, for instance, the muscle twitched even before the first sign of an s.j.p. appeared, since the large-nerve fibres have a lower threshold. The twitches did not dislodge the electrode or introduce visible artifacts into the record and caused no appreciable potential change in this slow fibre. The movement which

was added on top of the twitches by inclusion of small-nerve fibres must have been relatively small. Still more convincing evidence is presented below that even in the presence of such movement as is associated with a tetanus, successive records could be identical (Fig. 11).

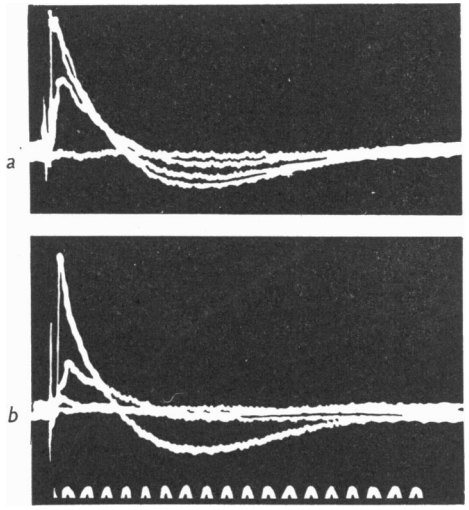

Fig. 9. Intracellular records from a slow muscle fibre of iliofibularis showing composite nature of s.j.p.'s. No block, entire ventral root 10 is stimulated with graded shocks (most of twitch portion cut, to reduce movement). *a*, three stimuli set up s.j.p.'s of different sizes. Two of the shocks produce nearly identical potentials and the large s.j.p.'s differ mainly in the 'positive' after-potential size. *b*, same electrode position, three different intensities of stimuli. Near threshold the s.j.p. component is quite small. Note that during all records the muscle twitched without dislodging electrode. Largest potential 9 mV, time base 50 c/s.

TABLE 1. Parameters of s.j.p.'s

	Amplitude	
Single s.j.p.	Extreme	Most frequent
Peak depolarization (mV)	16	8–10
% of resting potential	27	12–18
Hyperpolarization (mV)	4·0	2·5–3·5
% depolarization	20–46	25–35

Response to a tetanus

Frequency	Depolarization value (mV)	% of s.j.p.	Hyperpolarization value (mV)	% of depolarization
40/sec	13–23	180–240	5·0– 7·1	37–55
100/sec	16–28	200–270	5·6–13·2	35–55
150/sec	30–33	200–220	12–15	50

Effects of two successive stimuli

If two or more small-nerve stimuli are given at close intervals the potentials sum. The question naturally arises whether the first potential in any way affects a subsequent one. If the interval between volleys is long (Fig. 10*a*), the

measurement of the potential changes contributed by the second impulse presents no difficulty, because the first s.j.p. has already returned close to the original base-line before the arrival of the second. At shorter intervals, however (Fig. 10*b*) in order to calculate the contribution of the second volley alone, a single volley was superimposed upon the same record. It was then possible by subtracting this addition from the total potential change to obtain the parameters of the second s.j.p. (shaded area). It should be emphasized that

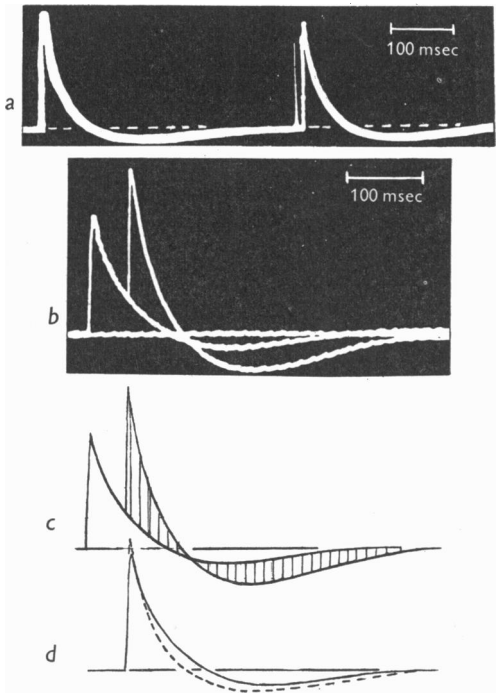

Fig. 10. Intracellular records showing the effect of two successive stimuli. *a*, 400 msec interval, the second s.j.p. is 10% smaller. *b*, 50 msec interval, exposure of single stimulus and double stimulus superimposed. Early phase of s.j.p.'s coincides accurately. *c*, tracing of (*b*), contribution of second s.j.p. indicated by shaded area. *d*, tracing of first s.j.p. (solid line) plotted together for comparison with second s.j.p. (dotted line) derived by subtraction. The latter is about 10% larger and has a faster hyperpolarization.

this procedure involves some important assumptions. It is implied that the processes which are initiated by the first volley, including the hyperpolarization and restoration of the resting potential, may continue unchanged to the end of their normal course and form the base-line for the second membrane change which is superimposed. The response to the double volley is thus the resultant of two discrete processes, which, once triggered, continue in a stable manner,

and the total potential is simply the algebraic sum of the potentials which each would have produced separately. In Fig. 10*b* the total potential after two nerve volleys seems to swing more steeply toward the phase of hyperpolarization than it does after one volley alone. The subtraction method, however, reveals that the second s.j.p. did not differ greatly from the first. It is plotted in Fig. 10*d*, together with a tracing of the s.j.p. resulting from a single volley, so that the contributions of the first and second volleys may be compared. This illustration is representative of many measurements. Since, however, individual s.j.p.'s show appreciable fluctuations in size (up to 15%), small differences between successive s.j.p.'s as seen in Fig. 10 cannot be taken as significant.

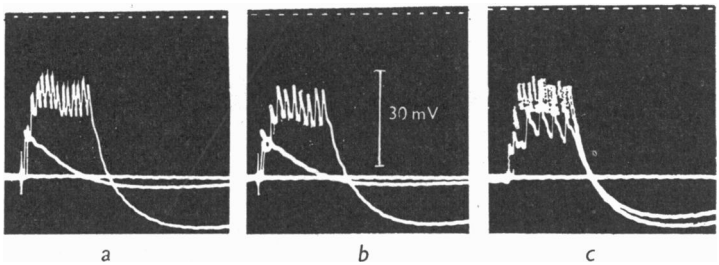

Fig. 11. Intracellular records from one slow muscle fibre. Maximal nerve stimulation, no block. *a*, separate exposures of a single s.j.p. and of a tetanus at 150/sec superimposed. *b*, as *a* but tetanus at 100/sec. *c*, 50/sec and 150/sec superimposed. Resting potential 52 mV, zero line modulated at 50 c/s. Note that ceiling depolarization is reached with 100/sec stimulation. Records at 150/sec in *a* and *c* nearly identical.

Effect of a tetanus

If several shocks are given at regular intervals, the successive depolarizations build up to a plateau, whose average height depends upon the frequency of stimulation. In Fig. 11 are presented the results of stimulation of the same single muscle fibre by exciting the whole of ventral root 10 at frequencies of 50, 100 and 150 shocks/sec. No anodal block was used and muscle movement was reduced by removing most of the twitch portion of the iliofibularis. It is clear that beyond 100/sec (Fig. 11*b*) relatively little further depolarization is added by increased stimulation frequencies. (The maximal tension development at frequencies around 50/sec (Kuffler & Vaughan Williams, 1953, fig. 2) should be noted in this context.) In Fig. 11*c*, the depolarizations produced by 50/sec and 150/sec stimulus series have been superimposed for comparison.

Fig. 12*a* is an intracellular record of the responses of a single slow fibre to first one, then two, then three shocks at 10 msec intervals, each consecutive sweep being superimposed. The height and time course of each successive s.j.p. can be derived in the manner already described, by subtracting the other potential changes. The course of the derived second and third s.j.p. of Fig. 12*a*

is plotted below for comparison with the first. Fig. 12*b* is a record of the effects of one, of seven and of eight shocks to a single fibre (a different one from that of 12*a*), all superimposed. The accuracy with which these successive records coincide provides convincing evidence that no serious artifacts have been introduced by movement. This figure also shows that the decay and recovery of the potential following seven or eight stimuli is almost identical since the tracing of one can be superimposed upon the other. This implies that once a plateau has been reached, previous activity does not further modify the course of succeeding additions of potentials. The last s.j.p. of a series as in Fig. 12*b* may be almost the same as the first, as is shown in Fig. 12b_8, where

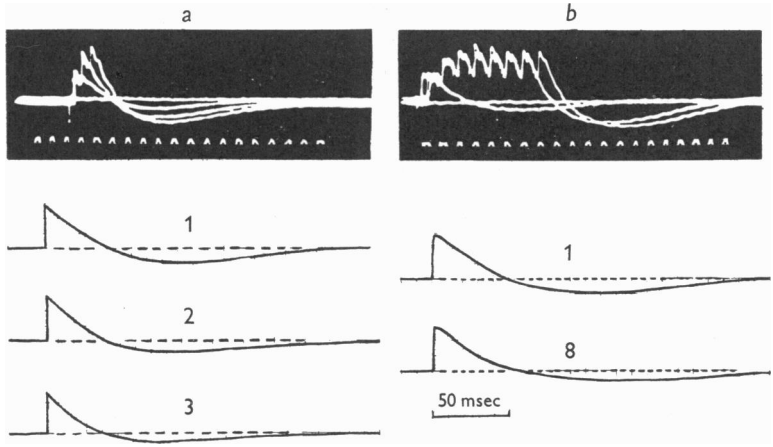

Fig. 12. Intracellular recording from slow muscle fibres in the iliofibularis during repeated stimulation of small-nerve fibres within the 10th ventral root. The membrane potential was 50 and 53 mV in the two fibres, the single s.j.p. 9 mV in both. Time base in upper records 50 c/s. *a*, three sweeps 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 the subtraction method (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 subtracted contribution of the 8th nerve volley, being the difference between the potentials set up by seven and eight stimuli. Note the accurate superposition of the two tetani, making movement artifacts improbable.

the s.j.p. at the end of a tetanus of eight stimuli has been derived by subtraction of the superimposed tetanus of seven stimuli. It is compared with a tracing of the single s.j.p. which is also seen in Fig. 12b_1. Their conformity confirms the supposition that, although individual components may be slightly affected by their predecessors, the plateau seen during a tetanus is built up by the simple addition to one another of basically similar units.

Limitation of depolarization plateau. At any given frequency of stimulation a 'plateau' is reached, because the membrane repolarizes during the interval

between stimuli to the extent by which it is depolarized by each stimulus. Even at 150/sec the membrane was never completely depolarized. The plateau is built up of individual s.j.p.'s of similar characteristics. The initial event of each s.j.p. is a brief (2–3 msec) phase of depolarization which may represent a transfer of charge by ionic flux. This is followed by restorative processes (repolarization, hyperpolarization) of relative stability which continue, as revealed by the subtraction method, even if further impulses are added. The stability implies a membrane structure whose electrical characteristics vary little during the restoration process.

It was thought of interest, therefore, to construct a simple electrical model by which the observed behaviour of the membrane could be reproduced. In Fig. 13, circuit 1 contains a voltage source E, representing the membrane e.m.f., the positive pole being the outside of the fibre at ground potential. It is assumed that the source is of high impedance, i.e. that if current is drawn, the voltage will drop; R_1 (50 kΩ) is, therefore, introduced to represent the internal resistance of the membrane battery. R_2 (100 kΩ) represents the transverse leak resistance of the membrane; the voltage across R_2 (=resting potential) is 60 mV. C_1 (1 μF) represents the membrane capacity. Recording leads are placed across the membrane resistance, R_2. (R_4 of 2·2 MΩ, the input impedance of the D.C. amplifier, has to be included in any circuit analysis.)

If now the membrane resistance is temporarily reduced by closing a switch S (a high-speed relay) for a definite length of time (3–5 msec), thereby including a smaller resistance R_3 in parallel with R_2, the condenser C_1 discharges during the closure of the switch; then slowly recharges when the switch opens again, with a time constant determined by C_1, R_1 and R_2. The smaller R_3, the more rapidly C_1 will discharge, and if R_3 is zero then C_1 will remain totally discharged throughout the closure of the relay. Fig. 13*a* shows the course of discharge and recharging of C_1 for four different values of R_3 from zero to 10 kΩ.

Fig. 13*b* illustrates how a 'plateau' is reached in this circuit if R_3 is 10kΩ (which reduces the 'membrane leak resistance' to one-eleventh of its former value during the closure of S) and the relay is closed (for 3 msec on each occasion) at 50 closures/sec. In Fig. 13*c* the plateau reached at 50/sec is compared with that reached at 30/sec. There is an obvious similarity between the behaviour of this model and that of the slow fibre membrane, but there is absent the phase of hyperpolarization. This can easily be supplied by the addition of a further R-C circuit, as shown in circuit 2 (C_2=0·3 μF and R_5=1 MΩ). One end of R_5 is returned to the potentiometer terminal (P) of R_1. Fig. 13*d* is a record from circuit 2 of exactly the same operation as Fig. 13*c*, with P at position C. In order to increase the 'positive afterpotential', P is turned toward position B. In this way the circuit can be made to provide good imitations of any of the records obtained from inside slow fibres. Fig. 13*e* and *g*

are recorded from circuit 2, while *f* and *h* are from microelectrodes within single slow muscle fibres.

The interest of this model is in the fact that it demonstrates how the type of complex potential response observed in slow muscle fibres could, from a purely electrical point of view, be explained on the hypothesis that a nerve

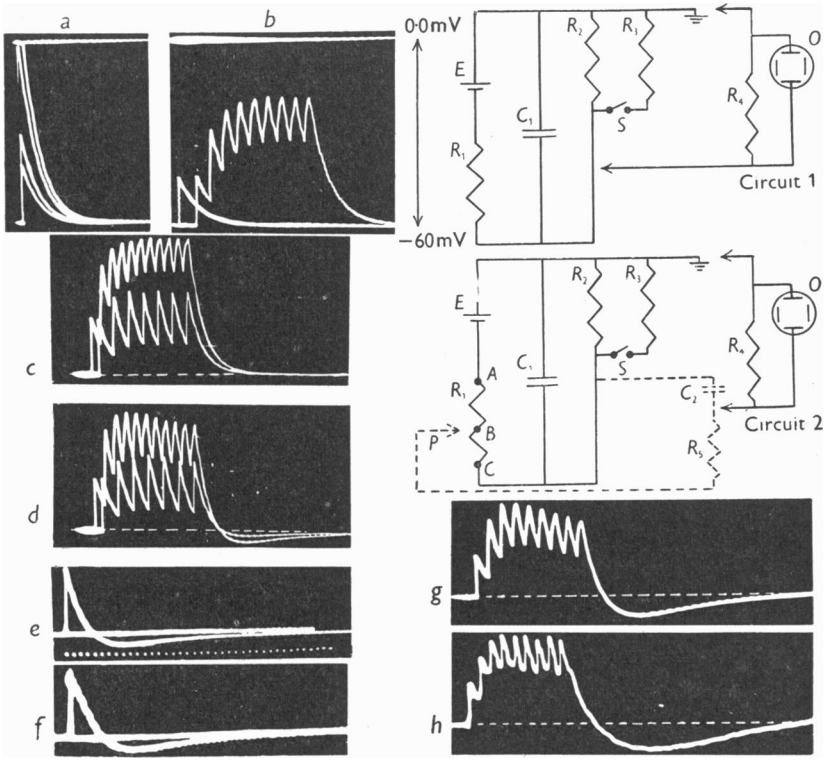

Fig. 13. Potential changes in model circuits compared with s.j.p.'s obtained by intracellular recording. *a*, key *S* of circuit 1, operated by a high speed relay, closed for a few msec. Record obtained when R_3 is at four different values from zero to 10 kΩ. When R_3 is zero, C_1 is discharged throughout closure of relay. *b*, record obtained from circuit 1 when switch *S* is closed for 3 msec ($R_3 = 10$ kΩ), at 50 closures per second. *c*, as *b*; comparison of records when relay frequency is 30/sec and 50/sec. *d*, record from circuit 2; conditions otherwise as in *c*. Position of potentiometer arm *P*, at *C*. *e* and *g*, 'artificial s.j.p.'s'. Position of *P* between *B* and *C*. *f* and *h*, actual s.j.p.'s obtained with microelectrode inside a slow muscle fibre after nerve stimulation. R_1, 50 kΩ; R_2, 100 kΩ; R_3, 0–10 kΩ; R_4, 2·2 MΩ; R_5, 1 MΩ; C_1, 1 μF; C_2, 0·3 μF; *E*, 90 mV. Closure of the relay switch *S* short-circuits the 'membrane leak resistance', R_2, with the smaller resistance R_3.

volley did no more than reduce the membrane leak resistance by a factor of about ten. The remainder of the potential course is the result of current flow through a very simple circuit. The similarity between the records from circuit

and muscle provides no evidence, of course, that the electrical characteristics of the membrane are, in fact, similar to those of the model.

Neuromuscular delays and conduction in terminal regions

The usual method of obtaining conduction velocities was by recording the small-nerve potentials near their entry into the muscles at a known distance from the motor roots (Fig. 3). The slowest small-nerve conduction velocity observed was 1·8 m/sec, the fastest 7·5 m/sec at about 22° C. Another method was to take the rise of the junctional potential as an index of arrival of the impulses at the small-nerve 'end-plates'. Neuromuscular delay times at the junctions, i.e. between arrival of the nerve impulse at the terminal region and the setting up of the s.j.p., were included in the 'conduction' time measured by this method. In many instances these delays could not have been longer than 2 msec, when the actual conduction time down the nerve (assuming uniform conduction velocities between root and muscle terminals) was subtracted from the total time between stimulus and commencement of the s.j.p. On the other hand, as seen for example in Fig. 5*b*, total conduction times as long as 29 msec occur for distances of 29 mm at a temperature of 22° C, implying a conduction velocity of 1 m/sec. The discrepancy between calculations by the two methods may be due to: (i) nerve impulses from the slowest conducting nerves were too small to be seen when the leads were on the nerve near its entry into the muscle, although the s.j.p.'s they produced were readily recorded from inside a single muscle fibre; (ii) there occurs a tapering, and consequent slowing of conduction, in the intramuscular region due to extensive branching of nerve terminals; (iii) neuromuscular delays may be long in some of the fibres. It is suspected that slowing due to extensive branching and consequent tapering is the principal factor in producing the apparent slower rates of conduction. The conduction velocities for small-nerve fibres of 4–8 m/sec, obtained by Kuffler & Gerard (1947), should now be revised to include the slower conducting elements of 2 m/sec or less. No data are at present available to convert conduction velocities into diameter spectra, as can be done for cats.

DISCUSSION

Once small-nerve stimulation was found to cause local contractile effects, two possibilities were considered: (i) the slow contractions could occur in muscle fibres which also gave propagated impulses and twitches. In that case the small-nerve action could 'set' the initial tension on which twitches were superimposed. (ii) The existence of two separate nerve-muscle systems could be postulated. The second alternative has now clearly been demonstrated. In the absence of propagated membrane changes or of some other mechanism by which the activation within the contractile substance could spread, efficient tension development could hardly be expected in muscle elements of some

length since much of the work would be expended in stretching the inactive elastic tissue (see Kuffler & Vaughan Williams, 1953). The innervation pattern which enables the individual slow muscle fibres to shorten at multiple points along their course seems to overcome the shortcomings created by the absence of propagated impulses. In fact, with a synchronous small-nerve volley the activation will be as simultaneous as the dispersion of the impulses due to differences in conduction velocity permits.

Knowledge of the precise innervation density by separate axons will have to await histological determination. Such investigations have been made in crustaceans by Harreveld (1939) and Harreveld & Wiersma (1939), where local contractile activation also occurs. It is likely that the small-nerve junctional contacts differ from those of the larger nerve fibres which show an appreciable terminal ramification in frogs (Couteaux, 1947). In a study of the innervation of the sartorius and of the iliofibularis muscle Günther (1949) found fine nerve fibres concentrated in the 'tonus bundle' region. Although the terminal regions could not be resolved, the small fibres showed a pattern which distinguished them from the better known 'end-plates'. The internal electrode probably records s.j.p. components over a distance of several millimetres, assuming a spatial spread similar to that found in twitch fibres by Fatt & Katz (1951). Since as many as seven separate components of one s.j.p. were seen, it is likely that the density of contacts is great. This arrangement creates a mechanism by which activity in individual fibres can be graded in very fine steps. Not only can the foci of contractile activation along the fibre course be made more or less dense, but the area of each active region and the intensity of its activity can be graded either by adding new nerve elements or by increasing the frequency of impulses. The tensions developed by appropriate reflex activation of small-nerve fibres can approach those observed with electrical stimulation (at 20/sec) of the majority of the small-nerve fibres reaching an individual muscle (Kuffler *et al.* 1947, fig. 3). A spinal mechanism for a combination of synchronous and high frequency discharge seems to exist.

Multiple innervation, i.e. more than one nerve fibre ending on one muscle fibre, is not confined to slow vertebrate muscle fibres. Although less dense, it is present in the frog sartorius (Katz & Kuffler, 1941), and it has lately been demonstrated also for other twitch muscles of the frog and shown to be common in the cat. The cricothyroideus is particularly interesting. Feindel, Hinshaw & Weddell (1952) have demonstrated histologically the existence in this muscle of adjoining end-plates on one muscle fibre, and Hunt & Kuffler (1953, in preparation), by the use of internal electrodes, have shown recently in the same muscle that the individual end-plates on single muscle fibres can be supplied by separate axons from different nerves. Isolated instances of multiple end-plates have been shown histologically by Cole (1946) and by Jarcho, Eyzaguirre, Berman & Lilienthal (1952) in rats, and physiologically in

the adductor longus of the frog by Kuffler (1942, fig. 1). Evidence for a dense innervation of muscle elements within mammalian spindles was more recently obtained by Hunt & Kuffler (1951).

The potentials from the junctions of small nerves to slow fibres have been revealed in these studies as strikingly different from those of large-nerve twitch-fibre junctions, as seen both by external and intracellular recording methods. In the latter studies, however, Fatt & Katz (1951) did come to the conclusion that the end-plate potential itself (distinct from the propagated muscle impulse which can be recorded by a microelectrode at an end-plate) did not exceed the resting potential, i.e. it showed no 'overshoot'. In this respect a similarity exists. In the twitch fibre junctions the relatively short intense transmitter effect causes a localized depolarization which usually leads to the setting up of a propagated muscle impulse. Unless such propagation is caused, the depolarized region (the e.p.p.) recovers in the same manner as it does after an applied subthreshold current pulse. At the small-nerve junctions, the transmitter effect also appears to last only a few milliseconds, but the subsequent course of the membrane change is quite different, being about ten times slower, and including a phase of hyperpolarization. The exact time course of a 'simple' s.j.p. is not known since it would be necessary to excite one small-nerve junction alone and find its centre with an internal electrode; this has not so far been found practicable. There is no reason, however, to assume that it differs from the usual composite s.j.p. which always has a phase of hyperpolarization (positive after-potential). The latter becomes increasingly evident with repeated stimulation and is present at all ranges of the resting potential at which s.j.p.'s were obtained.

It should be mentioned that the individual s.j.p.'s remained similar in size and shape even though the membrane potential occasionally fell by 20–30% between records. It was observed, however, that when such a fall of resting potential had occurred, the original value could occasionally be restored by a mechanical 'adjustment' of the micromanipulator, without pulling the electrode out of the fibre. For such reasons caution has been exercised in estimating resting potential values (see pp. 299–301).

It is not known whether the observed behaviour of the slow fibres occurs only in response to nerve stimulation. It would be of interest, for example, to see if another type of stimulus, such as a subthreshold current pulse, would evoke the same sequence of repolarization and hyperpolarization; or if a stronger pulse could give rise to propagation in a slow fibre. It has not been possible to insert two microelectrodes into a single slow fibre, however, and so this information is not yet available.

Potential sequences in some respects analogous to s.j.p.'s were recorded by Laporte & Lorente de Nó (1950) in the postsynaptic region of the superior cervical ganglion of the turtle under the influence of tubocurarine. Both

positive and negative phases do occur in this preparation and show a relationship similar to that of the corresponding phases of s.j.p.'s after single or repeated stimuli. Positive components, set up by presynaptic impulses were seen by Brock, Coombs & Eccles (1952) in motor-horn cells with intracellular electrodes. An attempt to account for the potential changes of slow muscle fibres on the basis of ionic fluxes (see Hodgkin's review, 1951), seems premature in the absence of further experimental evidence. The electrolyte content of the fibres is not yet known.

The apparent inability of slow muscle fibres to conduct propagated impulses on nerve stimulation can probably not be explained on the basis of a lower resting potential alone. Propagated impulses have been seen in twitch fibres when the resting potential has fallen below 60 mV after injury or 'ageing'. It should be noted that in slow fibres after maximal small-nerve stimulation the potential of the whole membrane changes more or less simultaneously at all points along the fibre. Longitudinal currents, such as those which flow between active and inactive regions during the conduction of a propagated impulse and which account for the propagation, are probably of little importance in slow fibres, except perhaps during the initial phase when an asynchronism in the time of arrival of impulses at adjacent junctions would permit a potential difference to develop between them. Throughout the depolarization plateau, during tetanic stimulation, current spread along the axis cylinder should certainly be absent; nor can there be longitudinal currents during chemical depolarization when the whole length of the fibre is immersed into a depolarizing solution (Kuffler & Vaughan Williams, 1953).

The innervation and junctional potentials of slow fibres have been established as being in marked contrast to those of the much studied twitch muscle fibres. The lower resting potential is another property which sets these muscle fibres apart. The possibility of some unknown factor causing the quite consistent lower membrane potential reading cannot be excluded. In spite of several hundred measurements, even *one* verified potential of 90 mV would necessitate a revised interpretation of the resting potential data of slow muscle fibres. As pointed out above, however, the evidence for two distinct nerve-muscle systems is not based on membrane potential measurements.

SUMMARY

1. In the organization of the frog's skeletal musculature there exist two distinct systems of nerve-muscle connexions. One is associated with 'large-nerve' fibres of conduction velocities about 8–40 m/sec which lead to propagated muscle impulses and quick contractile responses. This is called the twitch system. The second is the 'small-nerve' system, associated with nerve fibres which conduct at 2–8 m/sec and lead to localized electrical responses in the muscle and slow contractions. The behaviour of single elements in the latter

system was studied in this investigation by recording potentials with intracellular electrodes.

2. A method is described which permits convenient exclusive stimulation of fibres of different diameters within the ventral roots. By choosing an appropriate interelectrode distance and a rectangular electric pulse of requisite duration and intensity, nerve impulses can be selectively blocked at the anode, according to their speed of conduction and diameter.

3. If microelectrodes are inserted into muscle fibres two different types of electrical response can be recorded: (i) a non-propagated relatively slow change which is set up by small-nerve fibres and can be identified as the small-nerve junctional potential (s.j.p.); (ii) the well-known propagated muscle action potential, caused by large-nerve fibres in the familiar twitch fibres. The small-nerve innervated elements have been named 'slow' muscle fibres; they give local contractions only and have other special properties which set them apart from other skeletal muscle fibres. No overlap of innervation between the distinct muscle fibre groups occurs, and accordingly no muscle fibre has been found to give both s.j.p.'s and propagated muscle impulses.

4. The resting potentials of several hundred muscle fibres were measured. The slow fibres show consistently lower potentials, with a peak distribution at 60 mV, while the twitch fibres exhibited resting potentials of 90–95 mV, in confirmation of previous work. Some technical difficulties associated with these measurements are discussed.

5. Each s.j.p. obtained with maximal nerve stimulation is a composite potential and is set up by the junctional activity of numerous small-nerve fibres (Figs. 5 and 9). Since the local nature of s.j.p.'s is known and since they are of remarkably constant size when recorded anywhere along the slow muscle fibres, it is concluded that small-nerve fibres innervate individual muscle elements densely along their whole course. The numerous junctional contact areas, by locally activating the contractile system over a large stretch, apparently compensate for the absence of propagated muscle impulses.

6. The characteristics of single s.j.p.'s were analysed and their principal features are the following: the composite s.j.p.'s rise in a few msec to a peak depolarization of 7–15 mV, and the restitution takes place along an approximately exponential time course with a half time of 23–39 msec and leads into a phase of hyperpolarization (positive after-potential). The latter phase reaches 20–50 % of the depolarization peak and is followed by a gradual return to the original resting potential level. The whole potential complex may last up to 0·4 sec at 20–24° C and differs from the end-plate potential found at twitch fibre junctions.

7. With repetitive small-nerve stimulation the s.j.p.'s sum and after an initial rising phase a depolarization plateau is established depending on the frequency of stimulation. The component parts of the membrane change during

a tetanus were analysed, and it is concluded that the summed potential results from the addition of basically similar units. Each individual potential component appears to follow a stable time course which is unaffected by preceding or subsequent membrane activity.

8. A simple electrical model which reproduces many features of the s.j.p.'s is presented.

REFERENCES

BREMER, F. (1932). Researches on the contracture of skeletal muscle. *J. Physiol.* **76**, 65–94.

BROCK, L. G., COOMBS, J. S. & ECCLES, J. C. (1952). The recording of potentials from motoneurones with an intracellular electrode. *J. Physiol.* **117**, 431–460.

COLE, W. V. (1946). A gold chloride method for motor end-plates. *Stain Tech.* **21**, 23.

COUTEAUX, R. (1947). Contribution à l'étude de la synapse myoneurale. *Rev. canad. Biol.* **6**, 563–711.

FATT, P. & KATZ, B. (1951). An analysis of the end-plate potential recorded with an intra-cellular electrode. *J. Physiol.* **115**, 320–370.

FEINDEL, W., HINSHAW, J. R. & WEDDELL, G. (1952). The pattern of motor innervation in mammalian striated muscle. *J. Anat., Lond.*, **86**, 32–48.

GASSER, H. S. (1930). Contractures of skeletal muscle. *Physiol. Rev.* **10**, 35–109.

GÜNTHER, P. G. (1949). Die Innervation des M. sartorius und des M. ileofibularis des Frosches. *Anat. Anz.* **97**, 175–191.

HARREVELD, A. VAN (1939). The nerve supply of doubly and triply innervated crayfish muscles related to their function. *J. comp. Neurol.* **70**, 267–284.

HARREVELD, A. VAN & WIERSMA, C. A. G. (1939). The function of the quintuple innervation of a crustacean muscle. *J. exp. Biol.* **16**, 121–133.

HODGKIN, A. L. (1951). The ionic basis of electrical activity in nerve and muscle. *Biol. Rev.* **26**, 339–409.

HUNT, C. C. & KUFFLER, S. W. (1951). Further study of efferent small-nerve fibres to mammalian muscle spindles. Multiple spindle innervation and activity during contraction. *J. Physiol.* **113**, 287–297.

JARCHO, L. W., EYZAGUIRRE, C., BERMAN, B., & LILIENTHAL, J. L., JR. (1952). The spread of electrical excitation in skeletal muscle. Some factors contributing to the form of the electromyogram. *Amer. J. Physiol.* **168**, 446–457.

KATZ, B. (1949). The efferent regulation of the muscle spindle in the frog. *J. exp. Biol.* **26**, 201–217.

KATZ, B. & KUFFLER, S. W. (1941). Multiple motor innervation of the frog's sartorius muscle. *J. Neurophysiol.* **4**, 209–223.

KATZ, B. & KUFFLER, S. W. (1946). Excitation of the nerve-muscle system in crustacea. *Proc. Roy. Soc.* B, **133**, 374–389.

KUFFLER, S. W. (1942). Further study on transmission in an isolated nerve-muscle fibre preparation. *J. Neurophysiol.* **5**, 309–322.

KUFFLER, S. W. (1952). Incomplete neuromuscular transmission in twitch system of frog's skeletal muscles. *Fed. Proc.* **11**, 87.

KUFFLER, S. W. & GERARD, R. W. (1947). The small-nerve motor system to skeletal muscle. *J. Neurophysiol.* **10**, 383–394.

KUFFLER, S. W. & HUNT, C. C. (1952). The mammalian small-nerve fibres: A system for efferent nervous regulation of muscle spindle discharge. *Res. Publ. Ass. nerv. ment. Dis.* **30**, 24–47.

KUFFLER, S. W., LAPORTE, Y. & RANSMEIER, R. E. (1947). The function of the frog's small-nerve motor system. *J. Neurophysiol.* **10**, 395–408.

KUFFLER, S. W. & VAUGHAN WILLIAMS, E. M. (1953). Properties of the 'slow' skeletal muscle fibres of the frog. *J. Physiol.* **121**, 318–340.

LAPORTE, Y. & LORENTE DE NÓ, R. (1950). Potential changes evoked in a curarized sympathetic ganglion by presynaptic volleys of impulses. *J. cell. comp. Physiol.* **35**, 61–106.

LING, G. & GERARD, R. W. (1949). The normal membrane potential of frog sartorius fibres. *J. cell. comp. Physiol.* **34**, 383–396.

NASTUK, W. L. & HODGKIN, A. L. (1950). The electrical activity of single muscle fibres. *J. cell. comp. Physiol.* **35**, 39–73.

RUSHTON, W. A. H. (1949). The site of excitation in the nerve trunk of the frog. *J. Physiol.* **109**, 314–326.

SOMMERKAMP, H. (1928). Das Substrat der Dauerverkürzung am Froschmuskel. *Arch. exp. Path. Pharmak.* **128**, 99–115.

TALBOT, S. A., LILIENTHAL, J. L. JR., BESER, J. & REYNOLDS, L. W. (1951). A wide range mechano-electronic transducer for physiological applications. *Rev. sci. Instrum.* **22**, 233–236.

TASAKI, I. & MIZUTANI, K. (1944). Comparative studies on the activities of the muscle evoked by two kinds of motor nerve fibres. *Jap. J. med. Sci.* **10**, 237–244.

TASAKI, I. & TSUKAGOSHI, M. (1944). Comparative studies on the activities of the muscle evoked by two kinds of motor nerve fibres. Part II. *Jap. J. med. Sci.* **10**, 245–251.

WACHHOLDER, K. & VON LEDEBUR, J. (1930). Untersuchungen über 'tonische' und 'nicht tonische' Wirbeltiermuskeln. *Pflüg. Arch. ges. Physiol.* **225**, 627–642.