

Electrophysiological estimation of the number of motor units within a human muscle

A. J. McCOMAS, P. R. W. FAWCETT, M. J. CAMPBELL, AND R. E. P. SICA

From the Muscular Dystrophy Research Laboratories, Newcastle General Hospital, Newcastle upon Tyne

SUMMARY An electrophysiological method is described for estimating the numbers of motor units in the extensor digitorum brevis muscle in man. The results obtained are compared with counts of axons in the nerve to the muscle. The significance of the sizes of the evoked motor unit potentials is discussed.

There is, at present, no satisfactory method for assessing the extent of denervation in human muscles during life. Thus, analysis of the density of the electromyographic interference pattern during maximum effort, although a valuable procedure in skilled hands, is not usually quantitative (see, however, Rose and Willison, 1967). In addition, it has the disadvantage of requiring the full cooperation of the patient and the maximum density depends on both the physical properties of the recording electrode and the muscle selected for study.

The purpose of the present paper is to describe a method which, although restricted to a particular muscle, nevertheless enables a numerical estimate of functional innervation to be made. It involves minimal cooperation by the patient and, indeed, has been employed successfully in subjects exhibiting confusional states. The principle of the method is a quantal one; thus, if it is possible to measure the amplitudes of the muscle action potentials generated, firstly, by a single motor unit of average size and, secondly, by the whole muscle, then the number of motor units within the muscle can be determined by division. In practice it is possible, by carefully grading the strength of an electrical stimulus applied to the appropriate motor nerve, to recruit successive motor units singly, and hence to calculate the mean motor unit potential amplitude; the response of the total population of units is then evoked by a maximal stimulus to the nerve. However, although the method appears simple in principle, a number of important underlying assumptions are involved. The nature of these assumptions and the techniques which have been devised for their verification are described in another section (see Results). In addition, the results achieved with this electrophysiological method are

compared with estimates of alpha motor fibres obtained by counting axons in specimens of muscle nerve.

It was anticipated that such a method for determining numbers of motor units might prove of value not only in clinical neurology, where the possibility of motor denervation must be considered frequently, but also as a research tool in the experimental investigation of various diseases of muscle and nerve. Both expectations have been realized. During the past two years the test has been used routinely in diagnosis and has proved capable of detecting degrees of denervation that could not be recognized by interference pattern electromyography. The application of the method to the experimental investigation of human diseases will be described fully in subsequent papers. Preliminary communication of some of this work has already been given (Campbell, McComas, and Sica, 1970; McComas, 1970; McComas and Sica, 1970; McComas, Sica, and Currie, 1970).

METHODS

SUBJECTS There were 41 subjects of both sexes aged between 4 and 58 years; they comprised technicians, medical staff, school children, and patients. In all subjects there was no evidence of neurological abnormality in the legs. The subjects rested comfortably in the supine position on a couch; the ambient temperature was regulated at 20-22°C and the investigated limbs were not warmed. Some of the subjects were examined on more than one occasion.

PREPARATION Experiments were performed on three muscles—the sartorius, brachioradialis, and extensor digitorum brevis (EDB). Of these only EDB was suitable

for the estimation of the number of motor units since it possessed the following characteristics:

1. The electrical activity evoked in EDB could be recorded without troublesome interference from other muscles.

2. Only one end-plate zone was present and hence all the evoked motor unit potentials could be made to summate by using appropriate electrode placements.

3. The overlying skin was thin, so that relatively large potentials could be recorded by surface electrodes.

4. The muscle belly itself was flattened, so that the component motor units were likely to be reasonably equidistant from the recording electrode.

The advantages of these four features are discussed in the two subsequent sections; the findings in the remainder of the paper refer exclusively to the extensor digitorum brevis (EDB).

STIMULATION The stimulating electrodes were a pair of chlorided silver screws which were covered by felt pads soaked in 10% sodium chloride solution. The electrodes had diameters of 10 mm and were spaced 2 cm apart in a Perspex holder; when placed in contact with the skin their combined d.c. resistance was 50-100 K Ω . They were positioned over the deep peroneal (anterior tibial) nerve at a site just above the ankle where the threshold of the nerve to electrical excitation was lowest; the cathode was distal to the anode. The electrode holder was then fixed in position with a rubber band. The stimuli were rectangular voltage pulses of 50 μ sec duration delivered from a Devices Ltd. stimulator, type 3072; the repetition rate was one per four seconds.

RECORDING The stigmatic, reference, and earth electrodes consisted of strips of silver foil, 8 cm long and 6 mm wide. They were coated with electrode jelly and held in position on the skin with insulating tape. The insulating tape was cut away at the sides of the stigmatic electrode to prevent the effective electrode area from being increased by electrode jelly spreading under the tape. The stigmatic electrode was placed so as to completely cover the end-plate zone of EDB (see Fig. 3) while the reference electrode was fastened over the sole; the earth electrode was situated approximately midway between the cathode and stigmatic electrodes. The combined d.c. resistance of the stigmatic and reference electrodes, when fixed in position, was approximately 10 K Ω . In children up to 16 years the stimulus artefact was frequently troublesome; for this reason smaller electrodes, 5 cm long, were used and the reference electrode was placed over the medial aspect of the foot rather than on the sole. This difference in electrode arrangement precluded meaningful comparisons of absolute amplitudes of evoked potentials in adults and children. However, it did not affect the estimation of the number of units or the sizes of motor unit potentials, when the latter were expressed as a percentage of the maximal response.

A second type of stigmatic electrode was used to map out the end-plate zones of individual units; it consisted of a chlorided silver ball electrode, approximately 2 mm in diameter, which was coated with electrode jelly.

The muscle potentials were fed through a source

follower, having an input impedance of 100 M Ω , to a parametric amplifier (Isleworth Electronics Ltd.) and thence to a cathode ray storage oscilloscope (Hewlett Packard Ltd., type 141A). The overall frequency response of the system was 3 dB down at 2 Hz. A 3 dB attenuation at 1 kHz reduced the noise level to 4 μ V (peak to peak) without noticeably affecting the amplitude of the muscle action potential. The evoked responses were superimposed on the storage oscilloscope and measured.

SKINFOLD THICKNESS At the end of an experiment the thickness of a fold of skin over EDB was measured, using a pair of Harpenden calipers. In 35 subjects aged 8 to 58 the mean thickness was 2.3 ± 0.34 mm and the range 1.6 to 3.2 mm.

NERVE FIBRE COUNTS Specimens of the lateral terminal branch of the deep peroneal nerve were obtained from a male aged 30 and a female aged 20 who had died within 24 hours of receiving severe head injuries. The nerves were removed within 24 hours of death, were mounted on cards, and were fixed in Fleming's fluid for 48 hours. Paraffin sections of nerve, 5 μ thick, were prepared and stained by the Weigert-Pal method. The sections were cut at a site approximately midway between the terminal bifurcation of the deep peroneal nerve and the medial border of EDB; it was proximal to the subdivision of the lateral terminal branch (see Fig. 3). Photographic enlargements (1,000 x) of the sections were made; the nerve fibres were then counted, and their myelinated diameters measured, using a protractor device similar to that described by Espir and Harding (1961).

STATISTICAL TREATMENT The significance of difference between mean values was calculated by the Student *t* test. Throughout the text means have been given with their standard deviations.

RESULTS

CALCULATION OF NUMBERS OF MOTOR UNITS The results of an experiment in a normal subject are shown in Fig. 1.

In this experiment the stimulus was gradually increased from a subthreshold value until 11 increments in the EDB muscle response had been obtained (Fig. 1a); the relatively low noise level in the recording system ensured that each increment could be readily distinguished.

These increments appeared to be quantal inasmuch as intermediate responses were never observed and therefore each was attributed to activation of an additional motor unit. In this example excitation of the 11 presumptive units evoked an action potential of approximately 440 μ V, corresponding to a mean motor unit potential of 40 μ V. The muscle response after supramaximal motor nerve stimulation was 8 mV (Fig. 1b) and hence the number of motor units was estimated to be 200.

The numbers of motor units in the EDB muscles

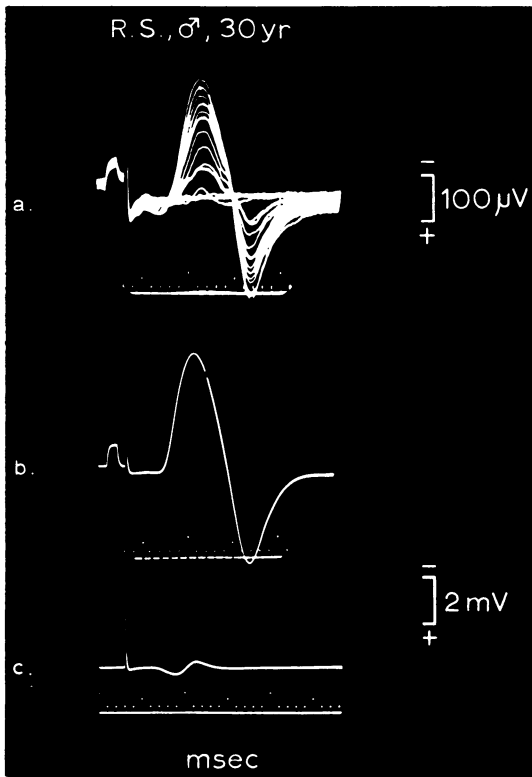


FIG. 1. Incremental responses of EDB following excitation of deep peroneal nerve with threshold and slightly suprathreshold stimuli (a) and maximal stimuli (b). In (a) several traces have been superimposed for each size of response. In the lowest trace (c) surface electrodes on the dorsum of the foot were used to excite selectively the first dorsal interosseus muscle and the electrotonically conducted potentials were recorded by the standard electrodes over EDB and the sole; the earth electrode was situated between the stimulating and recording electrodes.

of 41 healthy subjects aged between 4 and 58 years are shown in Fig. 2 where the estimates have been given with their standard deviations. It can be seen that, over the age spectrum examined, there was no correlation between the number of units and the age of a subject.

The reproducibility of the experimental method was tested by making 11 observations on the same EDB muscle of one subject; the successive experiments were separated by several days. In this subject the largest estimated number of units was 243 and the smallest 167, while the mean was 203 ± 28 . In 16 subjects in whom a second estimation was performed during the same recording session, the

larger value exceeded the smaller by an average of $11.4 \pm 7.6\%$. When the second examinations were carried out on separate days the discrepancy was greater and amounted to $33.7 \pm 31\%$ (six subjects).

VALIDITY OF EXPERIMENTAL METHOD As already stated, the method, although simple in principle, requires that a number of important underlying assumptions should be satisfied. These assumptions are enumerated below.

First assumption: electrical activity recorded is derived from single muscle If a single motor unit potential, used in the calculations, is actually generated by a muscle at a distance from the recording electrodes its amplitude will be misleadingly small and the computed number of motor units erroneously large. Similarly potentials from distant generators which summate with, or degrade, the maximum evoked potential will also affect the estimate. For these and other reasons (see above) the extensor digitorum brevis (EDB) was selected as the preparation of choice since this muscle is generally regarded as the only one innervated by the deep peroneal nerve below the ankle.

The arrangement of the innervation is shown in Fig. 3. It can be seen that the deep peroneal nerve divides on the dorsum of the foot into medial and lateral terminal branches. The lateral terminal nerve runs underneath EDB before splitting into branches which supply the four subdivisions of the muscle belly; the medial terminal branch runs distally to innervate the skin surrounding the cleft between the first and second toes. In addition, both the medial and lateral terminal branches may supply nerve twigs to the dorsal interossei (cf. *Gray's Anatomy*, ed. Davies, 1967). The exact arrangement of this accessory innervation was found to vary in the present study, since it sometimes involved only the first, or only the second, dorsal interosseus muscles. However, although potentials evoked in the interossei could be detected by the recording arrangement employed in the present study, they were inverted and very much smaller than the potentials in EDB (Fig. 1c); therefore they were unlikely to have influenced the present results to a significant extent.

The only other source of interfering muscle action potentials was the extensor hallucis longus. It appeared that this muscle could receive a motor twig from the deep peroneal nerve at the ankle. The contraction of the long extensor was detected by inspection and careful palpation of the muscle belly; when present, it was necessary to reposition the stimulating electrodes, usually by moving them distally.

One further check could be applied to ensure that

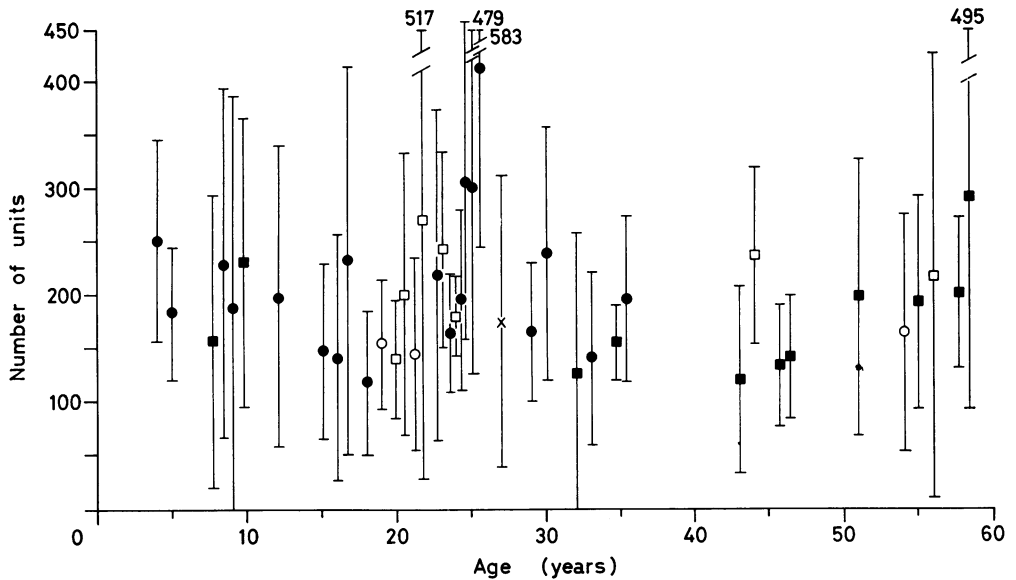


FIG. 2. Estimated numbers of units in EDB muscles of 41 control subjects aged 4 to 58 (mean number = 199 ± 60). For each subject the estimated value has been shown with ± 1 standard deviation (bars). No correlation was evident between the numbers of units and the ages of the subjects ($r = 0.03$). In 22 subjects (circles) the accessory peroneal nerve was sought and not found, in one it was present (cross), and in 18 it was not investigated (squares). Male and female subjects represented by filled and open symbols respectively.

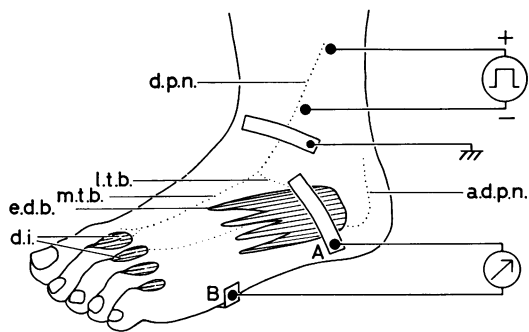


FIG. 3. Termination of the deep peroneal nerve on the dorsum of the foot and arrangements of stimulating and recording electrodes. A, stigmatic electrode over end-plate zone of EDB; B, reference electrode; a.d.p.n., accessory deep peroneal nerve; d.i., first and second dorsal interosseus muscles; d.p.n., deep peroneal nerve; e.d.b., extensor digitorum brevis; l.t.b. and m.t.b., lateral and medial terminal branches of deep peroneal nerve.

unitary activity was derived only from EDB. Thus it was possible with a small surface electrode to map out the end-plate zone of a stimulated motor unit

and to show that it lay within the confines of the muscle (see Fig. 6, right).

Before leaving the subject of muscle innervation, it is necessary to point out that, in approximately 20% of normal legs, the superficial peroneal nerve supplies a motor branch to the lateral part of EDB (Lambert, 1969; Infante and Kennedy, 1970). This branch, the accessory deep peroneal nerve, was detected in only one of 23 normal legs in the present study. In this subject stimulation of the accessory nerve evoked a potential in EDB which was 10% of that after excitation of the entire muscle; the total number of units in EDB was estimated to be 177, of which the deep peroneal nerve supplied 160. Accessory branches also occurred in two patients with chronic neuropathies and were responsible for 25 and 30% respectively of the potentials evoked from the whole muscles.

Second assumption: the incremental responses evoked by graded stimulation correspond to the activation of single motor units It is conceivable that two or more motor units might have had similar thresholds, causing a single increment in the evoked response. Although such a situation is quite feasible when a single stimulus is delivered, it becomes increasingly unlikely that the units will respond together on every

occasion as the number of trials increases. Furthermore, in isolated cases, additional evidence could be obtained to indicate that the increments corresponded to single units. For example, it could be shown that there was one and not two, localized end plate zones for the incremental response with the lowest threshold (cf. Fig. 6, right). Again, in each of three subjects with a chronic polyneuropathy, it was possible to demonstrate that there was a single all-or-nothing evoked response and, correspondingly that only a single motor unit could be recruited during a maximal voluntary contraction (McComas, Sica, Campbell, and Upton, 1971).

It was also theoretically possible that two motor nerve fibres having similar thresholds might have caused the number of units to be over- rather than under-estimated. This could have arisen if, on different occasions, one unit or the other responded or both fired together. In these circumstances the three types of response would have been erroneously interpreted in terms of three units, rather than two. There were two occasions when this alternating phenomenon could be recognized because of the distinctive potential configurations of the two units involved. However, more frequent occurrences could not be excluded, particularly as the stimulus strength was raised. The reason for the increasing likelihood of 'alternation' depended on the relationship between stimulus strength and the incidence of motor nerve fibres having given thresholds. Thus it can be seen from Fig. 4 that, in this experiment, the thresholds of the eight fibres with the lowest thresholds differed by a total of 10 V, whereas two further 10 V increases in strength evoked additional muscle responses corresponding to 54 and 104 units respectively.

As a result of this distribution of fibre thresholds it was not usually possible to distinguish with confidence the thresholds of more than the 10 most excitable fibres in control subjects.

One method of testing the accuracy of the experimental method, and the validity of the assumption at present under consideration, is to compare the calculated numbers of units with actual counts of fibres in the appropriate muscle nerve. Thus Cooper (1966) has reviewed published results and suggested that approximately half of the largest fibres in a muscle nerve are likely to be motor, while acknowledging that the proportions may vary in different muscles (cf. Boyd and Davey, 1968). In the present study fibres were counted in two specimens of the lateral terminal branch of the deep peroneal nerve (see Methods). In one of these nerves there was a clearly bimodal distribution of fibre diameters (Fig. 5). In this instance 50% of the population greater than $7\ \mu$ in diameter amounted to 438 fibres,

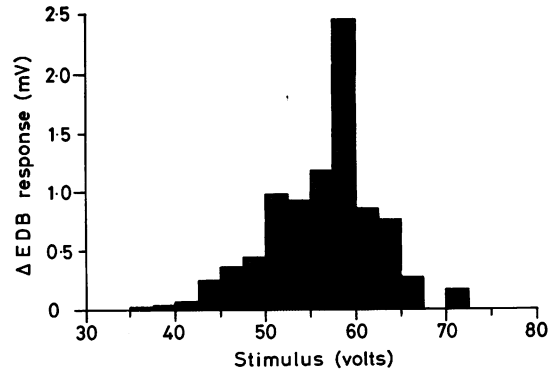


FIG. 4. Thresholds of motor nerve fibres to EDB on stimulating deep peroneal nerve at ankle. The size of the evoked muscle action potential is assumed to be proportional to the number of stimulated nerve fibres.

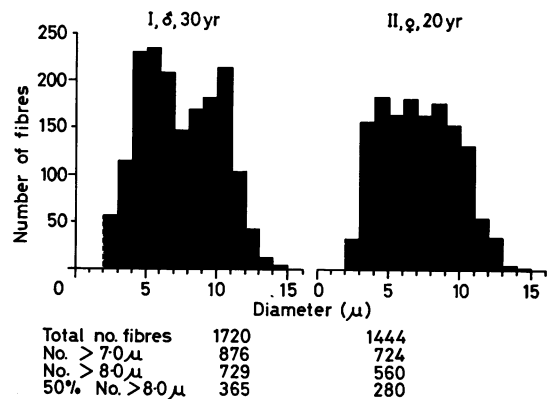


FIG. 5. Numbers of fibres with diameters of $2\ \mu$ or greater in the lateral terminal branch of the deep peroneal nerve in two young adults. In nerve I 1648 nerve fibres were counted at a second position 1 cm distally.

a number marginally in excess of the largest physiological determination, 414. On the other hand, if $8\ \mu$ were taken as the lower limit of the motor fibre range, then 50% estimates in the two nerves yielded values of 365 and 280 fibres which were both within the range of the physiological results. However, one of the factors which might have caused the 'anatomical' estimates to exceed the physiological ones is the existence of a nerve branch which does not terminate in EDB but which runs on to supply the dorsal interosseus muscles (see Fig. 3); small nerve twigs are also given to various tarsal joints (*Gray's Anatomy*, ed. Davies, 1967). Again, although the nerves were examined at some distance from the muscles (see Methods), it is known that branching

of nerve fibres can occur outside a muscle and this factor might also have affected the results. Discrepancies between the two types of estimate might also have arisen if some of the evoked motor unit potentials had been too small to be distinguished from background noise in the recording system. However, since the mean motor unit potential amplitude was $30\ \mu\text{V}$, and the noise level only $4\ \mu\text{V}$, peak-to-peak, it seemed very unlikely that any units had been missed in this way (see also Fig. 1a).

In conclusion, it would appear from the results of nerve fibre counting, that the electrophysiological estimates were likely to be within the correct range. As a consequence the assumption has been made in the remainder of the paper that the evoked increments corresponded to individual motor unit potentials.

Third assumption: evoked motor unit potentials summate algebraically The correct calculation of the mean motor unit potential amplitude requires that the action potentials generated by each of the motor units should interact algebraically in an additive and not a subtractive sense with respect to the recording electrodes. In order to achieve this condition, the stigmatic electrode was positioned so as to cover completely the end-plate zone of the muscle, while the reference electrode was situated on the sole. In these circumstances the excitation of each motor unit caused the stigmatic electrode to become initially negative, and then positive, in relation to the reference electrode; hence the evoked motor unit potentials were always additive as in Fig. 1a.

In the present study four different methods were employed to localize the end-plate zone in EDB: (1) evoked single unit method, (2) evoked multi-unit method, (3) volitional method, (4) end-plate 'noise' method.

In the *evoked single unit method* the stimulus was adjusted until a single all-or-nothing muscle response was obtained. A silver ball recording electrode was then moved over the skin until the point was found at which the initially negative response was largest and had the most rapid rate of rise (e, Fig. 6, right). The end-plate zone of the unit was considered to lie directly underneath this point. As the electrode was moved distally in the longitudinal axis of the muscle, the initial negative component of the evoked response declined until, at 1 cm distance, a positive-going wave was recorded instead. This initial positivity signalled initiation of impulses at distant end-plates, the muscle fibre membranes under the electrode activity acting as 'sources' of current, while the subsequent negative deflection marked the arrival of the propagated action potentials under

the electrode.

In the *evoked multi-unit method* of end-plate localization, the stimulus was made supramaximal and the silver ball recording electrode was moved to different sites in the long axis of the muscle. As in the previous method, the point corresponding to the largest initially negative response was found (e', Fig. 6, left); this point was judged to have the largest number of end-plates underneath it.

The *volitional multi-unit technique* was also employed to define the end-plate region of the muscle. A silver strip electrode was laid across the muscle to act as a stigmatic electrode with respect to a reference electrode on the sole. An incomplete volitional interference pattern was then produced when the subject attempted to extend the toes weakly. The configurations of the muscle action potentials were noted and the recording electrode was repositioned until all the volitional potentials became initially negative. In this position the electrode was judged to cover all the active end-plates and an example of this technique is given in Fig. 7a.

The final method was to search for *end-plate noise* in different parts of the muscle, using a coaxial needle electrode (Medelec Ltd.; see Fig. 7b and also Wiederholt, 1970).

The results of all four methods indicated that the end-plate zone of EDB lay along a line running across the muscle perpendicular to the extensor hallucis longus tendon and reaching the lateral border of the muscle approximately 1 cm from its posterior limit (see Fig. 3).

Fourth assumption: the motor unit potentials used in the calculation of the mean potential amplitude are representative of those generated by the total population of units The sizes of all the 380 motor unit potentials recorded in the 41 control subjects are shown in Fig. 8. Since the amplitudes of the evoked muscle potentials may have been influenced by factors such as skinfold thickness and effective electrode area (see Methods) to different extents in the various subjects, the motor unit potentials have been expressed as percentages of the corresponding maximal responses instead. It can be seen that, although the majority of units had potential amplitudes comprising between 0.2 and 0.6% of the maximal response, the results were skewed due to the presence of a small number of units with very much larger potential amplitudes. The significance of these findings and, in particular, the relationship between the amplitude of a motor unit potential and the 'anatomical' size of that unit are considered in a later section (see Discussion).

The problem of immediate relevance is whether the stimulating technique was likely to have preferen-

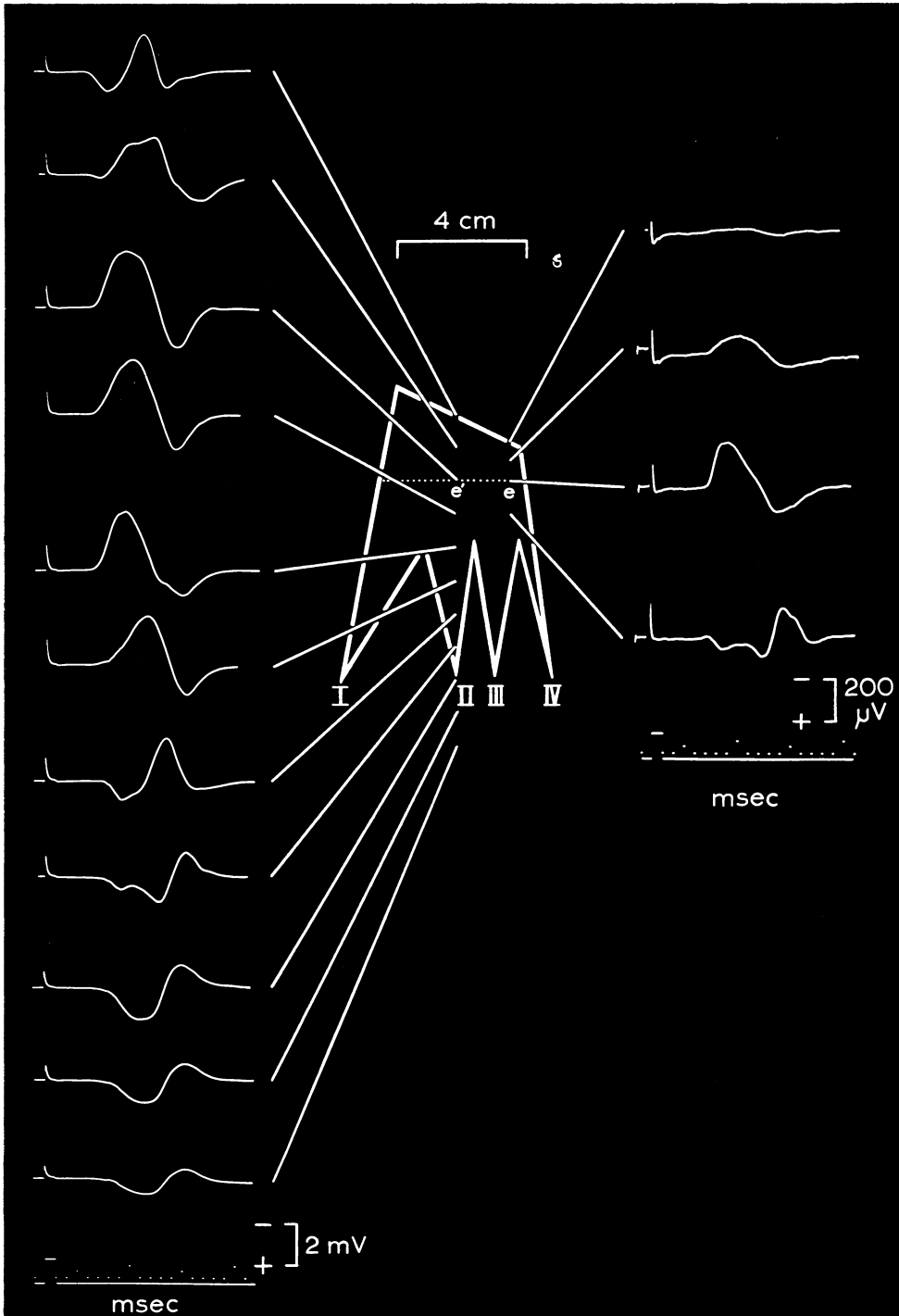


FIG. 6. Potentials evoked at different sites in long axis of EDB following stimulation of all motor units (left) and a single motor unit (right); dotted line across muscle indicates end-plate zone (see text).

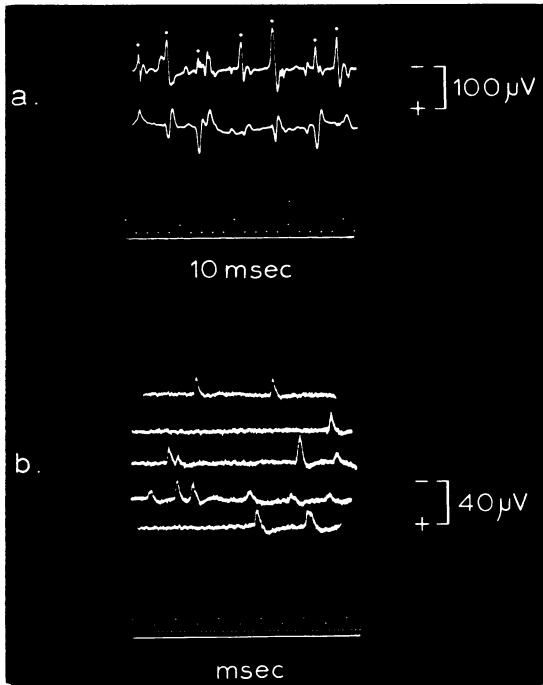


FIG. 7. (a) Volitional action potentials in EDB recorded simultaneously by one electrode over presumed end-plate zone (top trace) and one situated 1.0-1.5 cm posteriorly (lower trace). Dots indicate potentials in which the initial negativity was smaller or absent when recorded with the posterior electrode. (b) End-plate noise in EDB recorded with coaxial electrode.

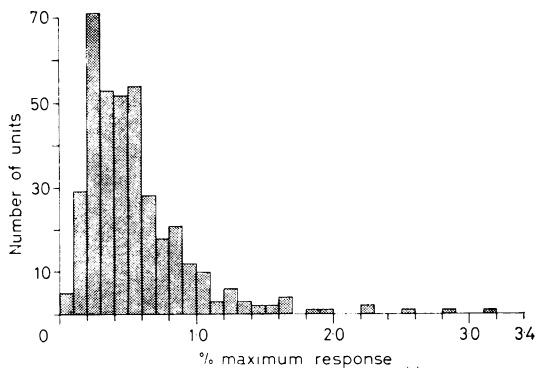


FIG. 8. Sizes of 380 motor unit potentials in 41 control subjects, expressed as percentages of maximum responses; mean size = $0.55 \pm 0.41\%$.

tially activated units generating potentials of a particular size. For example, it is well established from animal experiments (Erlanger and Gasser, 1937) that the largest nerve fibres have the highest conduction velocities and the lowest thresholds to electrical stimulation (for an analysis of the apparently conflicting observations in man, see the definitive study by Hausmanowa-Petrusewicz and Kopeć, 1967). Moreover, in the cat, McPhedran, Wuerker, and Henneman (1965) have obtained evidence that the fastest conducting motor nerve fibres to the soleus muscle innervate the largest numbers of muscle fibres; this correlation between conduction velocity and unit size, although present, was less marked in the gastrocnemius (Wuerker, McPhedran, and Henneman, 1965; see also Burke, 1967).

Probably the most direct test of selectivity in the present experiments is to compare the potential amplitudes of the first and last motor units activated by the graded stimulation technique in each subject. The results show that although the mean potential amplitude of the first singly excited motor units was greater than that of the last units (0.71 ± 0.62 and $0.56 \pm 0.38\%$ respectively), this difference was not significant ($P = > 0.1$). Furthermore, if the size of a nerve fibre had been the principal factor determining its threshold, then the same fibres should have had the lowest thresholds at different sites along the nerve; yet it could be shown by a simple experiment that this was not the case.

In this experiment a stimulus (S_2) was given to the common peroneal nerve at the head of the fibula sufficient to induce a potential (V_2) in EDB which had approximately 10% of the amplitude of the maximal evoked response (Fig. 9, upper). Another

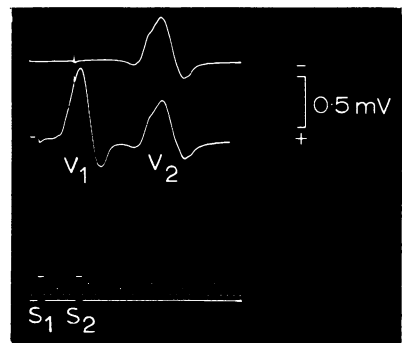


FIG. 9. Responses of EDB after submaximal stimulation of motor nerve fibres at two sites. V_1 , response following stimulus S_1 delivered to deep peroneal nerve at ankle; V_2 , response following stimulus S_2 delivered 6 msec later to common peroneal nerve at head of fibula. See text.

stimulator was then used to deliver a shock (S_1) to the deep peroneal nerve at the ankle 6 msec before S_2 and to evoke a response which was 15% maximal. Now, if the same fibres had the lowest thresholds at the knee and ankle then collision of nerve impulses should have occurred and caused V_2 to disappear. In fact, as can be seen from Fig. 9 (lower), V_2 was unchanged in size. This experiment demonstrated conclusively that the excitability of a nerve fibre was not influenced solely by its biophysical properties. However, it was equally apparent that the excitabilities of the nerve fibres could not have been determined randomly at the two sites. Thus, on a random basis, collision of impulses would have been expected to occur and to cause a 15% reduction in V_2 (since S_1 was 15% maximal). The absence of any decrement in V_2 suggested that the excitabilities of fibres were governed by some factor other than their biophysical properties; this factor was presumably the positions of the fibres in relation to the stimulating electrodes.

Even though the graded stimulation technique did not appear to select a biased population of units, it is nevertheless evident from Fig. 8 that the amplitudes of the unit potentials varied considerably. Consequently the estimates of mean motor unit potential amplitudes, and thence of the numbers of units, may have been correspondingly imprecise when based on the small samples of units employed in this study. It was for this reason that the calculated numbers of motor units were given with the standard deviations of the estimates for each subject in Fig. 2. However, it was possible to obtain a reasonably accurate estimate of the true number of motor units in a control subject by pooling the samples of motor units from all individuals. Thus the mean potential amplitude of the 380 units contained in Fig. 8 was $0.55 \pm 0.41\%$ of the total response and the reciprocal of this value, 183, corresponded to the idealized number of motor units in EDB.

DISCUSSION

In the preceding section an attempt was made to justify the various assumptions inherent in the method for determining the number of motor units. It was shown that it was possible to arrange for the recorded electrical activity to be derived from a single muscle and for the evoked potentials to summate algebraically. Furthermore, there was no evidence to indicate that the sample of units used in the calculation of the mean potential amplitude was not representative of the total population. Nevertheless, although the estimated numbers were of the same order as the values predicted from counts of nerve fibres, it was apparent that the results

ranged widely (cf. Fig. 2). One factor contributing to this large scatter was undoubtedly the error involved in assuming that individual increments in the evoked response always corresponded to the excitation of single motor units. Thus, although there could be little doubt that the smallest all-or-nothing evoked potential represented the excitation of a single motor unit, the possibility that 'alternation' of units occurred as the stimulus was increased could not be excluded (see Results). However, if only the smallest evoked response was used in the calculation of the number of units for each subject, then the mean number estimated from the pooled population of 41 units was 144. This value was reasonably close to the idealized number of 183 obtained by pooling all the 380 increments in the 41 subjects. Moreover, even though the tendency to 'alternation' might be expected to vary in different subjects, it was reassuring that repeated estimates for the same subject were in good agreement.

A second source of error in measurement arose from the possibility of including one of the few relatively large units within the sample. However, as stated previously, it is possible to indicate the homogeneity of a sample in terms of the standard deviation of the mean number of units.

Apart from errors in measurement, it was apparent that other factors might influence the results. In the first place Lambert (1969) and Infante and Kennedy (1970) have shown that the superficial peroneal nerve may send a motor branch to the extensor digitorum brevis muscle in approximately 20% of subjects. According to Lambert, this branch innervates the most lateral of the four subdivisions of the muscle belly. If each subdivision contained the same number of units, then the estimated value for the whole muscle in subjects with this accessory innervation would be reduced by 25%. However, it was apparent from Fig. 2 that the estimated numbers of units varied considerably even in subjects who did not possess the accessory branch.

The last possibility is that some of the variation between individuals was real, due to partial denervation of EDB in apparently healthy subjects, and this would appear to receive support from the studies of Swallow (1966) and Jennekens (1970). Thus, in subjects aged 17 to 40, Swallow found a greater than threefold difference in the numbers of nerve fibres in the medial terminal branch of the deep peroneal nerve proximal to its bifurcation at the base of the great toe (see Fig. 3; Swallow, 1966). However, it is recognized that variations in sensory innervation of the fingers can occur and it seems equally probable that similar anomalies may involve the toes. Indeed, Swallow himself described a communicating branch between the superficial and deep

peroneal nerves on the dorsum of the foot and pointed out that when this branch was large the medial terminal branch of the deep peroneal nerve was correspondingly small proximal to the confluence. A further consideration is that if denervation did occur in control subjects then a cumulative effect should be evident with respect to age. Both Swallow's data and our own (Campbell and McComas, 1970) indicate that quite severe denervation can occur in subjects *beyond the age of 60*. However, our present findings also suggest that, if denervation does take place in a healthy subject between the ages of 4 and 58, it is unlikely to involve a significant proportion of the nerve fibre population (Fig. 2). Similarly, in teased preparations of superficial peroneal nerves from control subjects, Arnold and Harriman (1970) have found evidence of active Wallerian degeneration in only 5 of 1,224 fibres. Fibres with short internodes, presumably resulting from degeneration and regeneration, were rather more common (58 of 1,224 fibres) but mainly affected subjects over the age of 60.

The findings of Jennekens (1970) are of considerable interest, since he has demonstrated grouping of histochemically similar muscle fibres in EDB and, to a lesser extent, in other muscles. Nevertheless, this grouping, although suggestive of denervation and reinnervation, cannot be regarded as definite proof since it may represent only the normal architecture in this muscle (see also Denny-Brown, 1929). Finally, it must be added that, even if some degree of denervation were shown to occur in EDB muscles of control subjects, it would in no way invalidate the diagnosis of 'pathological' denervation in a patient in whom the estimated number of units fell below the lower limit of the normal range.

If, in fact, the idealized number of 183 motor axons is close to the true value then interesting comparisons may be made with estimates derived for other human muscles. Thus, if it is assumed that 50% of the large fibres in a muscle nerve are motor (Cooper, 1966) then from the fibre counts of Feinstein, Lindegård, Nyman, and Wohlfart (1955) EDB would appear to have more axons than the first lumbrical (78) and first dorsal interosseus muscle of the hand (99) but fewer than the tibialis anterior (370).

Although the main purpose of this study was to devise a method for estimating the number of motor units in a human muscle, the sizes of the motor unit potential increments were also of considerable interest. It was found that, while the majority of the potentials had amplitudes close to the modal value, there was a small number of very much larger potentials. In assessing the significance of such potentials several factors were considered. First of

all, it was possible that the potentials were large because the active fibres were particularly close to the stigmatic electrode. However, the EDB muscle is relatively thin and within this flattened belly the motor units are presumably organized in overlapping cylinders of muscle fibres (cf. Buchthal, 1960; Edström and Kugelberg, 1968). Therefore it may be assumed that the stigmatic electrode was reasonably equidistant from the underlying motor units and that the recorded potentials reflected the sizes of the action currents generated within the muscle. On the other hand, the sizes of the motor unit potentials would certainly be affected, not only by the numbers of fibres within a unit, but also by the cross-sectional areas of individual fibres and the amplitudes of the active membrane potentials. In considering cross-sectional area, it is relevant that in various mammals fibres of histochemical type I are smaller than those of type II. However, although both types of fibre exist in the human EDB they were found to be similar in size (see also Brooke and Engel, 1969); thus in a total of 800 fibres from four muscles the mean diameters of type I and type II fibres were 45.4 and 49.6 μ respectively (Jennekens, 1970).

So far as the sizes of the active membrane potentials are concerned it is apparent from micro-electrode studies in control subjects that, although a considerable range exists, inadvertent trauma cannot be excluded as a cause of low values (McComas, Mrozek, Gardner-Medwin, and Stanton, 1968). If these various considerations are taken into account, it is probable that the size of an evoked motor unit potential is mainly related to the number of muscle fibres within the unit. In comparing the present results with those from animal experiments, it is of interest that both Wuerker, McPhedran and Henne-man (1965) and Burke (1967) have found evidence of a wide range in motor unit sizes in the cat gastrocnemius muscle. In both these studies the largest units had relatively fast twitches and were presumably composed of type II fibres.

In passing it should be noted that, by expressing the motor unit potential amplitudes as fractions of the maximal response rather than as voltages, such factors as skin thickness and subcutaneous fat do not affect comparisons of results between individuals. Nevertheless, comparison of absolute values can be justified insofar as the thickness of a fold of skin overlying EDB shows little difference between individuals (see Methods).

In conclusion it should be mentioned that the present method of estimating the number of motor units has been used in over 150 subjects during the past two years. It is sufficient, at the present time, to state that abnormally low values were always found in patients having clinical or electrophysio-

logical evidence of widespread denervation (McComas *et al.*, 1971) and that up to 70% denervation could be detected in other patients in whom the electromyographic interference pattern appeared normal.

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