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PROPERTIES OF MOTOR UNITS IN FAST AND SLOW SKELETAL MUSCLES OF THE RAT

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SUMMARY

1. Three kinds of motor unit have been distinguished in soleus (SOL) and extensor digitorum longus (EDL) muscles of adult rats on the basis of differences in isometric twitch contraction times measured at 35° C.

2. SOL contains about thirty motor units of which about twenty-seven are slow and three are intermediate with average contraction times of 38 and 18 msec respectively.

3. EDL contains about forty fast motor units with an average contraction time of 11 msec.

4. Average values for maximum isometric tetanic tension were 4·7 g for fast and slow units and 5·85 g for intermediate units.

5. Average values for twitch-tetanus ratios ranged from 0·2 to 0·227 for EDL muscles, SOL muscles and the three kinds of motor units.

INTRODUCTION

The results of earlier work on the dynamic properties of whole muscles of the rat (Close, 1964, 1965) were interpreted on the assumption that the muscle is made up of a fairly uniform population of fibres having similar mechanical properties. It has now been observed that the soleus muscle contains some motor units which differ considerably from whole muscle in the time course of twitch responses. Some of the properties of extensor digitorum longus and soleus muscles have been re-examined in the present work together with the properties of motor units in these muscles. Three kinds of motor units have been distinguished on the basis of differences in time course of the isometric twitch and these are referred to as fast, intermediate and slow units. Intermediate and slow motor units have been found in soleus muscles whereas extensor digitorum longus is virtually homogeneous and composed almost entirely of fast motor units. It will be shown that only about one in ten soleus motor units is an intermediate unit and that the characteristics determined from contractions of whole soleus muscles are very nearly the same as the average values obtained for slow units.

METHODS

Dissections were performed on 5-week-old and 12-week-old female rats (Wistar strain) which were anaesthetized with 50 mg sodium pentobarbital/kg body wt administered intraperitoneally, followed by about one quarter of the initial dose every 1–2 hr. The right hind limb was denervated by transecting the nerves to all muscles with the exception of either soleus muscle (SOL) or extensor digitorum longus muscle (EDL). SOL or EDL was exposed and prepared for recording isometric contractions as described previously (Close, 1964); the skin overlying the lower part of the leg was removed completely. In most instances the L_4 and L_5 ventral roots were exposed by laminectomy and transected proximally near the spinal cord.

In setting up the preparation the animal was placed in the prone position and the dissected limb was put into a Perspex bath through a hole in one wall. The skin of the thigh was stretched over a flange surrounding the hole and tied in that position, thereby making the bath leak-proof. The bath contained about 130 ml. of Ringer solution (NaCl, 137 mM; KCl, 5 mM; $CaCl_2$, 2 mM; $MgCl_2$, 1 mM; NaH_2PO_4, 1 mM; $NaHCO_3$, 2 g/l.; glucose, 2 g/l.). This fluid was bubbled continuously with a gas mixture containing 95 % O_2 and 5 % CO_2, maintained at 35–36° C and replaced at the rate of about 2–3 ml./min. The leg was held in the horizontal position by forceps.

Isometric contractions of the muscle were recorded with the proximal tendon clamped securely to a rigid frame and the distal tendon attached directly to a strain gauge (Statham, Gl-4-250). The total compliance of the recording system was $2{\cdot}5 \times 10^{-4}$ cm/g. A Tektronix preamplifier (Type Q) was used in conjunction with the transducer; signals were displayed on a Tektronix 502 oscilloscope and photographed. All contractions were recorded with the muscle set at the optimal length determined from twitch contractions of the whole muscle (Close, 1964).

Two methods of stimulation were used to elicit contractions of single motor units. The twitch responses of low-threshold motor units were obtained in early experiments by stimulation of the whole sciatic nerve. Here the nerve was stimulated repetitively at 1 or 2 c/s with very small electrical pulses just sufficient to excite one or two axons. In later experiments on 12-week-old animals motor units were stimulated indirectly by way of ventral root filaments which were subdivided progressively until they contained only one axon running to the muscle. In these preparations the skin around the medial incision over the vertebral column was drawn up to form a pool for oxygenated mineral oil which bathed the ventral roots. Axons of motor units in EDL muscles are distributed equally in L_4 and L_5 ventral roots whereas those of units in SOL muscle occur predominantly in L_5. The isometric twitch, latent period and isometric responses to repetitive stimulation at various frequencies were recorded for stimuli applied first to the sciatic nerve, then to L_4 and L_5 ventral roots and ventral-root filaments. These filaments were dissected under a microscope and care was taken to ensure that each contained only one motor axon supplying the muscle under investigation and that the stimulus did not escape to the remainder of the root.

The maximum isometric tensions developed during twitch and tetanic contractions of the whole muscle were determined at the beginning and end of each series of recordings. The mean value for the ratios of total tetanic tension at the end of the experiment to the initial value (i.e. P_0final/P_0initial) was 1·0 (0·96–1·09) for SOL muscles and 0·985 (0·97–1·02) for EDL muscles. Average values for similar ratios of twitch tensions (i.e. P_t final/P_t initial) were 0·955 (0·86–1·005) for SOL and 1·04 (0·97–1·13) for EDL. These results showed that the muscles, and hence the motor units, underwent little or no change during the course of experiments which usually lasted about 6 hr.

The definitions of contraction time (T_c), maximum isometric twitch (P_t) and tetanic (P_0) tensions, intrinsic speed of shortening, and the optimal length are the same as those given in earlier papers (Close, 1964, 1965).

RESULTS

Sciatic nerve–muscle preparations. Figure 1 shows some records of twitch contractions of whole soleus muscles and soleus motor units from animals of different ages. The records of contractions of motor units comprise

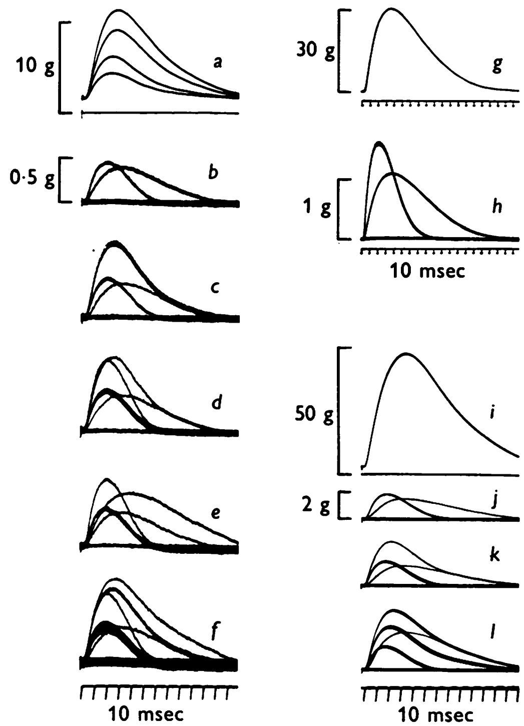

Fig. 1. Records of isometric twitch contractions of whole soleus muscle (*a*, *g*, *i*) and low-threshold motor units (*b–f*, *h*, *j–l*). The muscles were obtained from 5-week-old (*a–f*), 12-week-old (*g*, *h*) and 14-week-old (*i–l*) rats. The uppermost record in *a* is the maximal twitch of a whole muscle, the other records are for submaximal twitches obtained by graded stimulation of the sciatic nerve. The muscle weights were: *a* = 29 mg, *g* = 86 mg and *i* = 130 mg.

many superimposed traces obtained during threshold stimulation of the sciatic nerve. With an appropriate stimulus repeated once every second the 'low-threshold' motor units either contracted or failed to contract depending, presumably, on fluctuations in the threshold of the motor axons concerned. Invariably two kinds of responses were obtained when the nerve was stimulated in this way. In one T_c was about 17–19 msec whereas for the slower response T_c was 28–35 msec depending on the age of the animal (Fig. 1*b*, *h*, *j*). These are referred to as responses of intermediate and slow motor units respectively, to distinguish them from the more rapid response of fast motor units present in muscles such as EDL (below).

The muscle fibres of SOL are arranged in parallel (Close, 1964). Consequently the motor units act in parallel and their isometric responses summate. Examples of this summation are shown in Fig. 1*c* and *k* which are made up of records of the twitch responses of a SOL intermediate unit and a SOL slow unit and, above these, the summed response of the two units contracting simultaneously. The other records shown in Fig. 1*d*, *e*, *f* and *l* show different combinations of responses of additional intermediate and slow units brought into play by small increases in stimulus amplitude. Further recruitment of units produces submaximal twitch responses for which the contraction time increases with an increase in twitch size (e.g. Fig. 1*a*). This effect is probably due to a progressive increase in the proportion of slow motor units contracting and not to re-excitation of muscle fibres through a 'back-response' of the kind described by Brown & Matthews (1960).

The isometric response to two maximal stimuli applied to the nerve at different intervals has been determined for EDL and SOL muscles *in situ* in 4-week-old rats and adult rats. In these muscles the maximum twitch tension evoked by the first stimulus was not reduced by a second stimulus applied to the nerve in the absolute refractory period of the muscle fibres. Rat EDL and SOL muscles differ in this respect from some cat muscles in which the twitch response to indirect stimulation is reduced by a second nerve volley which abolishes a 'back-response' in the nerve and thereby prevents re-excitation of muscle fibres (Brown & Matthews, 1960, Fig. 8; Buller & Lewis, 1962, Fig. 2). This difference between cat and rat muscles may be due partly to differences in muscle size and the magnitude of the electrical change resulting from the summed effects of the muscle fibre action potentials.

TABLE 1. Mean values ($\pm$standard deviation) for the isometric twitch contraction time (T_c), half-relaxation time ($T_{\frac{1}{2}R}$) and maximum isometric twitch tension (P_t) of 'low-threshold' motor units of soleus muscles

	T_c (msec)	$T_{\frac{1}{2}R}$ (msec)	P_t (g)
	5-week-old animals		
Whole muscle (n = 5)	26·5 ± 1·75	34·0 ± 2·4	9·25 ± 1·3
Slow motor units (n = 7)	28·0 ± 2·45	34·5 ± 2·25	0·38 ± 0·07
Intermediate motor units (n = 8)	17·0 ± 1·3	20·0 ± 1·6	0·44 ± 0·08
	12-week-old animals		
Whole muscle (n = 8)	35·5 ± 1·8	51·0 ± 3·7	29·0 ± 5·8
Slow motor units (n = 14)	35·0 ± 4·3	51·0 ± 8·8	1·0 ± 0·27
Intermediate motor units (n = 11)	19·0 ± 2·1	23·5 ± 3·6	1·14 ± 0·22

Some characteristics of contractions of whole SOL muscles are listed in Table 1 together with the characteristics for low-threshold intermediate and slow motor units of the same muscles.

Ventral root filament–muscle preparations. Figure 2 shows representative records of contractions of an EDL muscle and fast motor unit, and Fig. 3 shows similar records obtained for a SOL muscle, a slow motor unit and an intermediate unit. The records for the fast unit of EDL (Fig. 2*B*) and the slow unit of SOL (Fig. 3*B*) resemble those for the whole muscles. In contrast the intermediate unit of soleus (Fig. 3*C*) differs from both the slow and fast units, not only in twitch time course, but also in the summation of contractions at low repetition rates and the rates of rise and fall of tension during tetanic contractions.

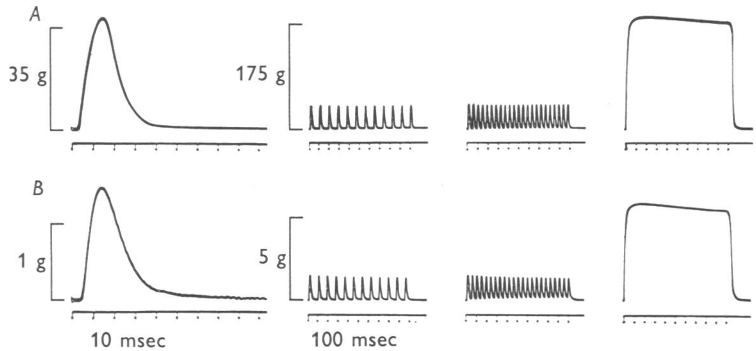

Fig. 2. Records of the isometric twitch and isometric responses to repetitive stimulation at 10, 20 and 250 c/s for a whole EDL muscle (*A*) (muscle weight = 73 mg) and a fast motor unit (*B*) from a 12-week-old rat.

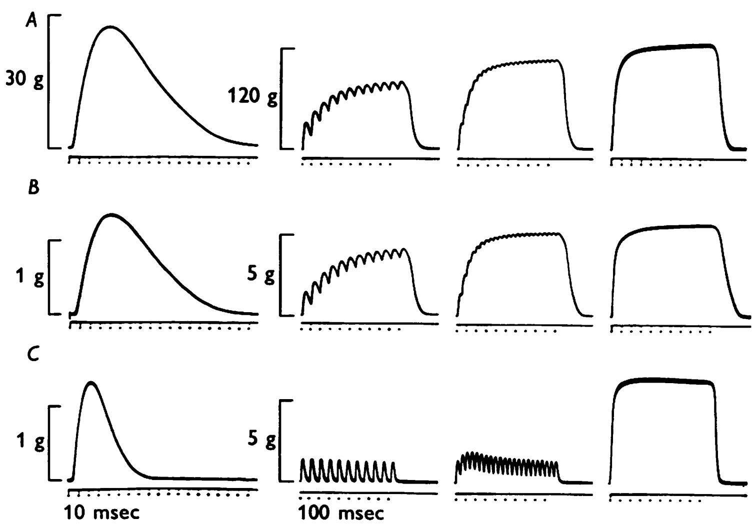

Fig. 3. Records of isometric contractions of a whole soleus muscle (*A*) (muscle weight = 64 mg), a soleus slow motor unit (*B*) and a soleus intermediate motor unit (*C*) from a 12-week-old rat. Reading from the left the records are the isometric twitch and the responses to repetitive stimulation at 10, 20 and 200 c/s. Contractions of whole muscle were elicited by indirect stimulation of the sciatic nerve trunk in the leg whereas the motor units were stimulated through ventral root filaments in the vertebral column.

Figure 4A shows the distribution of contraction times for forty-nine units from six EDL muscles; all these units were stimulated indirectly through ventral root filaments. One unit having a contraction time of

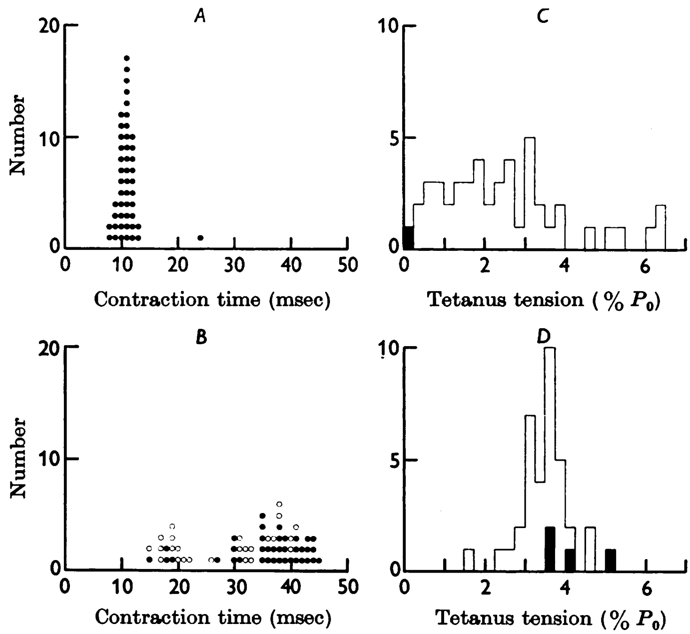

Fig. 4. The diagram *A* shows the distribution of isometric twitch contraction times for EDL motor units stimulated through ventral root filaments. The distribution of the maximum isometric tetanic tension of the same units is shown in *C* plotted as percentage of P_0 for the whole muscles. The black block in *C* represents the tetanic tension for the EDL unit with contraction time of 23·5 msec. Diagrams *B* and *D* show the distributions of contraction times and tetanic tensions for SOL motor units. The filled circles in *B* are for units stimulated through ventral root filaments and the open circles are for units isolated by threshold stimulation of the sciatic nerve. Units with contraction times less than 25 msec are intermediate ones and the remainder are slow motor units. The tetanic tensions for SOL muscles stimulated through ventral root filaments are shown in *D* as percentage of P_0 of the whole muscle; the black sections are for intermediate units and the unfilled sections are for slow units. The data plotted in these diagrams are summarized in Table 2.

23·5 msec has been classified as an intermediate motor unit and the remainder are fast motor units. The maximum isometric tetanic tension was determined for forty-seven of the forty-nine EDL units and the distribution of these values is shown in Fig. 4*C*. The intermediate unit found in one EDL muscle was very small (black block in Fig. 4*C*) and contributed only 0·24% of the tetanic tension of the whole muscle. The

TABLE 2. Mean values ($\pm$standard deviation) for isometric twitch contraction time (T_c), half-relaxation time ($T_{\frac{1}{2}R}$), maximum isometric tetanic tension (P_0), and the twitch:tetanus ratio (P_t/P_0) for extensor digitorum longus (EDL) muscles and motor units, and soleus (SOL) muscles and motor units of 12-week-old rats. The average muscle weight was 79 (64–90) mg for six SOL muscles and 77 (72–85) mg for six EDL muscles

	T_c (msec)	$T_{\frac{1}{2}R}$ (msec)	P_0 (g)	P_t/P_0
	EDL muscles			
Whole muscles (n = 6)	11·75 + 1·3	7·7 ± 1·2	186·0 + 11·0	0·2 ± 0·018
Fast units (n = 48)	10·8 ± 1·07	10·5 ± 1·9	4·7 ± 2·9	0·211 ± 0·06
Intermediate unit (n = 1)	23·5	28·0	0·41	0·17
	SOL muscles			
Whole muscles (n = 6)	36·0 ± 1·95	51·0 ± 4·3	145·0 ± 21·0	0·21 ± 0·022
Slow units (n = 34)	38·0 ± 3·7	57·0 ± 8·3	4·74 ± 0·81	0·227 ± 0·0315
Intermediate units (n = 4)	17·4 ± 1·7	22·0 ± 2·45	5·85 ± 0·84	0·20 ± 0·01

characteristic properties of six EDL muscles and forty-nine EDL units are listed in Table 2.

The contraction times of thirty-eight SOL motor units stimulated through ventral root filaments are plotted as filled circles in Fig. 4*B*. These contraction times are distributed in two groups with mean values of 17·4 msec for the four units with T_c < 25 msec and 38 msec for the thirty-four units with T_c > 25 msec. The mean and median values for T_c are the same for the second group (T_c > 25 msec) and the distribution appears to be normal. The difference between the means for the two groups is 20·6 msec and the standard error is 4·7 msec; t = 4·4 and for 38 degrees of freedom P is less than 0·01. Consequently the null hypothesis may be rejected and the difference between the mean values for T_c may be considered real. The values for T_c of soleus units (Table 1*B*) obtained by threshold stimulation of the sciatic nerve are plotted for comparison as open circles in Fig. 4*B*. The half-relaxation times ($T_{\frac{1}{2}R}$) also formed two distinct groups and for individual motor units there is direct correspondence between distributions of T_c and $T_{\frac{1}{2}R}$ such that units with short contraction times between 15 and 22 msec have half-relaxation times between 17 and 29 msec. Soleus motor units with T_c < 25 msec have been classified as intermediate ones and those with T_c > 25 msec are slow units. Among the thirty-eight soleus units isolated by subdivision of ventral roots only four were intermediate and the remainder were slow. The maximum isometric tetanic tension was determined for thirty-six of the thirty-eight soleus units. The values showed a normal distribution for P_0 about a mean value of 4·86 (2·25–6·6) g. In Fig. 4*D* the distributions of tetanic tensions for intermediate and slow motor units are shown by the filled and open blocks respectively. The range of values of P_0 for SOL units is less than the range for EDL units but the mean values are about the same for the two groups.

A summary of the characteristics of the thirty-eight soleus motor units and the six soleus muscles is given in Table 2. These results show that slow motor units predominate in contractions of soleus muscles and that temporal characteristics determined from contractions of the whole muscle are very nearly the same as the average values obtained for slow motor units. The twitch:tetanus ratio (P_t/P_0) is virtually the same not only for whole SOL muscles and intermediate and slow motor units, but also for EDL muscles and fast motor units.

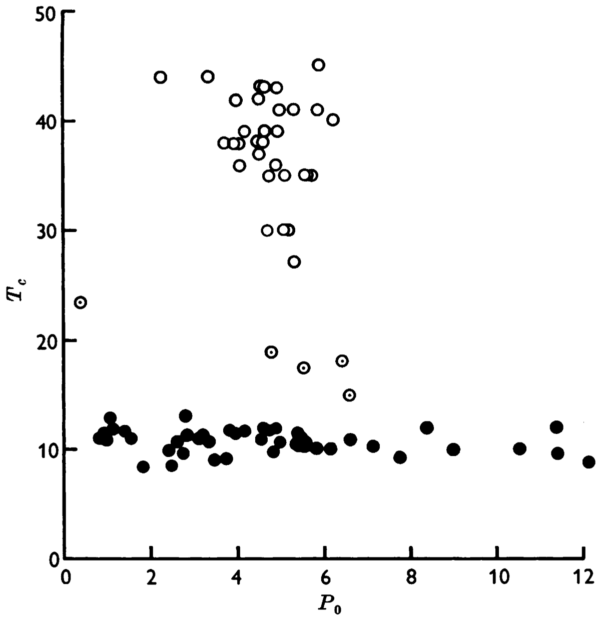

Fig. 5. The relation between isometric twitch contraction time (T_c) in milliseconds and maximum isometric tetanic tension (P_0) in grams (Table 2) for thirty-two slow motor units from SOL (○), five intermediate motor units from SOL and EDL (⊙) and forty-six fast motor units from EDL (●).

Henneman & Olson (1965) found large motor units to be generally faster than small units in some cat muscles and they suggested a functional basis for this relationship. In contrast, there was no correlation between size (P_0) and contraction time for any of the rat motor units described above (Fig. 4, Table 2). These results have been re-plotted in Fig. 5 to show the relation between T_c and P_0 for thirty-two slow units from SOL, five intermediate units from EDL and SOL and forty-six fast units from EDL. The average value for P_0 was 4·74 g for the slow units, 4·76 g for the intermediate units and 4·7 g for the fast units. Furthermore P_t and P_0 are directly proportional and there is no correlation between T_c and P_t/P_0 for the three kinds of motor unit. This is consistent with the view that the principal difference in the contraction times for fast and slow muscles is due to difference in intrinsic speed of shortening (Close, 1964, 1965).

DISCUSSION

The number of motor units present in individual EDL and SOL muscles may be estimated approximately by dividing the average P_0 for whole muscles by the average P_0 for motor units. In this instance it is necessary to assume that (1) there is no branching of axons in ventral roots and the whole motor unit responds to stimulation of its axon in that region, (2) there is exact summation of the responses of all motor units in isometric contractions of the whole muscle, and (3) the sample of motor units obtained by subdivision of ventral roots is representative of the whole population. Using the mean values for P_0 given in Table 2, the estimate of the average number of motor units is 186/4·6, i.e. 40 in EDL muscles and 145/4·86, i.e. 30 in SOL muscles. The estimate for SOL is to be compared with the finding of Gutmann & Hanzlikeva (1966) that thirty-two *a* motor axons enter this muscle. Among the thirty-eight soleus motor units isolated by subdivision of ventral roots only four, or about 10 % of the total number, were intermediate units and the remainder were slow. The chances of obtaining by dissection a ventral root filament with a single axon of either an intermediate or a slow unit are probably proportional to the numbers of each of these present in the muscle. On this basis it is estimated there may be an average of three intermediate and twenty-seven slow motor units in soleus muscles. The number of slow motor units may be estimated in another way by comparing the tensions in whole muscles and slow units during the relaxation phase of the twitch. The twitch of an intermediate unit usually ends within 90 msec after the onset of contraction and at that time the tension in the whole muscle is borne, presumably, by the slow motor unit component. The average twitch tension at 90 msec was 14·9 g (12·7–20·6 g) in six whole soleus muscles and 0·56 g (0·125–1·08 g) in thirty-four SOL motor units. The average number of slow motor units in a soleus muscle estimated by dividing the mean tension in the whole muscle at 90 msec by the mean tension for the slow units is twenty-seven.

The tension developed by the intermediate and slow motor unit components during isometric tetanic contractions of soleus muscles may be estimated from the product of the number of units present and the average maximum tetanic tension for each kind of unit. Thus twenty-seven slow motor units having an average P_0 equal to 4·75 g would develop about 128 g or 88 % of the average P_0 for the whole muscles (Table 2). Similarly three intermediate motor units with an average P_0 equal to

5·85 g would contribute about 12 % of the tension developed by a muscle during an isometric tetanus (Table 2).

Stein & Padykula (1962) determined the distribution of fibres with different histochemical properties in rat fast and slow muscles and suggested that type A fibres are the classical 'white' muscle fibres and that B and C fibres are two kinds of 'red' muscle fibre. This view is supported by the work of Henneman & Olson (1965) which showed that A fibres predominate in cat gastrocnemius muscle and most of the motor units are fast (Wuerker, McPhedran & Henneman, 1965) whereas cat soleus muscle is composed entirely of B fibres and all the motor units are slow (McPhedran, Wuerker & Henneman, 1965). The existence of three kinds of motor unit and three kinds of muscle fibre in rat limb muscles points to the possibility that each motor unit is made up of one kind of muscle fibre. Moreover, the estimated sizes of the intermediate and slow motor unit components given above correspond approximately with the sizes of C and B fibre components shown in Fig. 13 of Stein & Padykula (1962). Their photomicrograph shows that 30 % of the muscle fibres present in the section were C fibres and that these constituted 20 % of the total cellular cross-sectional area. Furthermore, Stein & Padykula (1962) found higher sarcoplasm/fibril ratios for C fibres than for B fibres and this probably means that the tetanic tension developed per unit cross-sectional area is less for C fibres than for B fibres. In other words the C-fibre component probably contributes less than 20 % and the B-fibre component more than 80 % of the maximum isometric tetanic tension of the soleus muscle. These estimates of the tension developed by the C and B fibre components are approximately the same as those given above for the tension developed by the intermediate and slow motor unit components respectively. Nevertheless, this correspondence may be fortuitous and more work should be carried out on these and other muscles to determine whether there is a correlation between histochemical properties and speed of contraction.

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