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HISTOCHEMICAL AND PHYSIOLOGICAL PROPERTIES OF CAT MOTOR UNITS AFTER SELF- AND CROSS-REINNERVATION

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SUMMARY

1. This report describes selected histochemical and physiological properties of the motor units of adult cat soleus muscle approximately one year after self- and cross-reinnervation with the nerve of the heterogenous flexor hallucis longus (f.h.l.). Self-reinnervated f.h.l. motor units are also considered. Whole muscles were tested for fibre reaction to alkaline pre-incubated ATPase, α-glycerophosphate dehydrogenase (α-GPD) and reduced nicotinamide adenine dinucleotide diaphorase (NADH-D). Motor units were isolated and studied by splitting the ventral root in acute preparations.

2. The histochemical fibre type profile in the self-reinnervated muscle was comparable to normal muscle as was mean twitch contraction time, twitch–tetanus ratio and fatigue index. The mean tetanic tension of the soleus self- and cross-reinnervated motor units appeared close to a normal soleus whereas the mean tetanic tension of the f.h.l. self-reinnervated units was significantly less than a normal f.h.l.

3. An average of 14% of the fibres of the soleus cross-reinnervated muscles had high ATPase and a α-GPD staining intensity in contrast to normal and self-reinnervated soleus in which such fibres are absent. Thus alkaline lability of myofibrillar ATPase increased in some fibres of what was originally a homogeneous population. The small increase in the number of densely staining fibres for ATPase at an alkaline pH (14%) was associated with a 73% decrease in (mean) contraction time (41 ± 11 ms) of the thirty-three cross-reinnervated muscle units studied, with no unit's contraction time greater than 60 ms. Mean contraction times for the self-reinnervated soleus and f.h.l. muscles were 78 ± 31 ms and 27 ± 8 ms respectively.

4. All fibres of the soleus cross-reinnervated muscles showed intense reaction to NADH-D, as was true of self-reinnervated soleus. This staining pattern is typical of normal soleus. In concordance, these motor units consistently demonstrated a high resistance to fatigue when stimulated for a four-minute period.

5. These results suggest that in the adult self- and cross-reinnervated soleus muscle,

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there is some active mechanism which regulates the eventual size of motor units as reflected by tetanic tension.

6. Change in contraction time from that typical for a soleus unit to that similar to an f.h.l. unit remains incomplete one year after cross-reinnervation. Within this time this partial change in single motor units reflects incomplete neural control of this property rather than a mixture of self- and foreign-innervation.

7. A greater degree of independence from neural control to conversion of the histochemically demonstrated myofibrillar ATPase activity exists than is the case for contraction time.

INTRODUCTION

The degree of control of the phenotypic characteristics of muscle fibres exerted by the motoneurone remains undetermined to a large extent. One model frequently used to investigate this problem has been the cross-reuniting of transected nerves which normally innervate a predominantly slow or fast muscle (Buller, Eccles & Eccles, 1960). Most investigators using this model have studied physiological and/or biochemical properties of whole muscles. This approach does not allow one to differentiate the exactness of the reinnervation process from the completeness of the control that the motoneurone has on the phenotypic characteristics of the muscle fibres.

By determining the contractile characteristics of single motor units in self- and cross-reinnervated muscles the completeness of conversion of each contractile parameter in each unit can be determined more precisely. Also, by combining investigation of the physiological properties with investigation of the histochemical profile, it can be determined whether the motor unit's normally comparable physiological and histochemical properties (Burke, Levine, Tsairis & Zajac, 1973; Burke, Levine, Salcman & Tsairis, 1974) are subject to similar conversions. In this study these relationships were investigated using a model of self- and cross-reinnervation. The cat hind limb muscles chosen for investigation were the homogeneous soleus and the heterogeneous (mixed) flexor hallucis longus (f.h.l.). F.h.l. is the largest and the most lateral head of the digit flexor pair (see Goslow, Stauffer, Nemeth & Stuart (1972) for complete discussion). Some similar whole muscle and motor unit data for self- and cross-reinnervated cat soleus and the medial digit flexor, flexor digitorum longus (f.d.l.), have been reported by Burke and his colleagues (Burke, Dum, O'Donovan, Toop & Tsairis, 1979; Dum, Burke, O'Donovan & Toop, 1979) and Bagust, Lewis & Westerman (1981). In addition to the f.d.l. data, the latter study included an analysis of forty-four soleus motor units cross-reinnervated by the f.h.l. nerve.

METHODS

Surgical reinnervation methods

Pentobarbitone sodium (30 mg/kg body weight, I.P.) was used to anaesthetize six adult cats for surgical cross- and self-reinnervation. The soleus and f.h.l. of one leg were always surgically self-reinnervated while in the opposite leg cross-reinnervation was performed. The side that was self-reinnervated or cross-reinervated was alternated from cat to cat. The surgical procedures consisted of a mid-sagittal skin incision over the calf of the leg followed by a partial separation

of the medial from the lateral head of the gastrocnemius and the plantaris muscles. The two heads of the gastrocnemius were separated proximally for about two-thirds of the muscle's length. The nerves to the soleus and f.h.l. were exposed for about 10 mm so that a 6-0 silk suture could be secured through the epineurium at points on the nerves such that when the sutures were tightened after complete nerve section, the two appropriate sectioned ends of the nerve stumps were drawn together. Then the two heads of the gastrocnemius muscles were sutured together with gut while the skin was closed with silk. All cats were maintained post-surgically for 12–14 months before physiological measurements were performed and muscles removed for histochemical processing.

Physiological methods

To study the mechanical properties of motor units, the animal was anaesthetized with pentobarbitone sodium (20 mg/kg body weight, I.V.) after an initial injection of acepromazine (1 mg/kg). As described in detail elsewhere (Goslow *et al.* 1972; Reinking, Stephens & Stuart, 1975; Stuart, Mosher, Gerlach & Reinking, 1972) functional isolation of single alpha axons was performed by ventral root dissection following laminectomy from L_3 to S_2. A muscle oil pool was maintained at 37 ± 1 °C with a DC regulated heating pad. Each muscle was tied by a 4 cm length of nylon line (compliance 0·45 μm/kg) to a strain ring (compliance 30 μm/kg) attached to a rack and pinion.

An electrode for ventral root filament stimulation (0·1 ms pulses) and for antidromic recording of action potentials was placed in a spinal oil pool. A second stimulating electrode was attached to the muscle nerve near the nerve-muscle junction. Recordings of whole muscle and single motor unit electromyography (e.m.g.) were obtained with bipolar insulated copper wires (0·1 mm o.d.).

Confirmation of functional isolation of a single alpha axon was provided by: (1) ventral root filament stimulation at a threshold voltage that gave an all-or-none twitch and/or tetanic response about 50% of the time; (2) a corresponding all-or-none e.m.g. record from the muscle which maintained a consistent wave form and (3) an all-or-none action potential from the spinal nerve filament following antidromic stimulation of the muscle nerve distally. Avoidance of subsequent recruitment of other axons in the experimental paradigm was provided by increasing the threshold voltage from two to five-fold. If a second axon was not recruited in this range, the voltage was set at just above threshold where it remained for all physiological tests. For those muscles (five soleus and two f.h.l. muscles) in which both the foreign and parent nerves were present, the distal muscle nerves were carefully separated and treated individually. In these instances, the confirmation test as outlined above indicated whether a cross- or self-reinnervated unit had been isolated prior to the motor unit tests.

An active tension–length curve was generated for the whole muscle preceding motor unit isolation. For five soleus muscles this included both a 1500 ms tetanus at 100 Hz as well as a twitch at each length. For all other whole muscles only twitch active tension–length curves were generated following a supramaximal shock of the muscle nerve. The muscle was lengthened in 2 mm increments. Active tension–length curves were similarly generated for each isolated motor unit. Fast twitch units (contraction times $\leqslant 45$ ms) were stimulated at 200 Hz for 600 ms and slow twitch units (contraction times > 45 ms) were stimulated at 100 Hz for 1500 ms. Potentiated twitches (see below) were generated at the shortest length corresponding to maximum active twitch and all other motor unit tests were performed at the shortest length corresponding to maximum tetanic tension. In addition to obtaining the active tension–length curve for each motor unit the following were measured.

1. *Axonal conduction velocity.* At the end of each experiment an *in situ* measurement of the length of the entire muscle nerve was made from the spinal cathode to muscle nerve cathode. This length divided by antidromic conduction time provided a measure of axonal conduction velocity.

2. *Contraction time of the potentiated twitch.* Motor units were stimulated at 100 Hz for 1500 ms or 200 Hz for 600 ms (see above). Two seconds later a maximal twitch was generated. This procedure was repeated at 5 s intervals until the twitch had grown to a peak amplitude and twitch time had increased to its maximum. Twitch contraction time was measured from the onset of the e.m.g. to the peak of twitch tension.

3. *Response to repetitive stimulation.* Motor units were stimulated at 5, 10, 15, 20, 30, 40, 50, 100 and 200 Hz for 600 or 1500 ms each. A 10 s interval existed between each tetanic train.

4. *Response to unfused tetani (sag test).* Motor units were stimulated repetitively for 600 ms with interstimulus interval at 125% of potentiated twitch contraction time.

5. *Fatigue test.* Motor units were stimulated at 40 Hz for 330 ms at 1 s intervals. This procedure

was continued for 4 min. The fatigue index chosen was the ratio, expressed as a percentage, of the accumulated peak tension during the first 2 min divided by the accumulative peak tension during the entire 4 min test (Reinking *et al.* 1975).

Histochemical methods

The soleus and f.h.l. muscles of each cat were removed and a complete cross-section about 5 mm thick was taken from the muscle belly. It was then frozen in isopentane at −160 °C. Cryostat cross-sections of 10 μm thickness were cut at −20 °C. The histochemical techniques used were those for demonstrating myofibrillar adenosine triphosphates (ATPase) with acid or alkaline pre-incubation (Guth & Samaha, 1970), reduced nicotinamide adenine dinucleotide diaphorase (NADH-D) (Novikoff, Shin & Druker, 1961), and α-glycerophosphate dehydrogenase activity (α-GPD) (Wattenberg & Leong, 1960). A trichrome stain was used to identify morphological detail (Engel & Cunningham, 1963).

Fibre types were identified from photographic prints of ATPase, NADH-D and α-GPD. Criteria for identifying fibre types as fast twitch glycolytic (f.g.), fast twitch oxidative glycolytic (f.o.g.) and slow twitch oxidative (s.o.) have been described (Peter, Barnard, Edgerton, Gilespie & Stempel, 1972).

Statistical treatment

When three groups were to be compared, a one-way analysis of variance was used. This was followed by the Duncan Multiple Range test to determine the difference between any two groups when a significant ($P < 0.05$) probability was obtained in the analysis of variance test.

RESULTS

In the nerve cross-united muscles studied (five soleus, two f.h.l.), functional nerve anastomoses formed such that both self- and cross-reinnervation resulted. The degree of cross- and self-reinnervation was estimated by comparing the twitch tensions elicited by stimulation of both the original and foreign nerve. Twitch tensions, percentage reinnervation and motor unit sample sizes are given in Table 1 for the three self-reinnervated and seven cross-reinnervated muscles studied.

Stimulation of the self-reinnervating nerve produced the greatest tension in two nerve cross experiments (Table 1, cats 1 and 6), whereas in two preparations stimulation of the cross-reinnervating nerve resulted in tensions greater than three times the force produced by the self-reinnervating nerve (Table 1, cats 2 and 4). In two nerve crosses, tensions produced from each nerve were similar (Table 1, cats 3 and 5).

The motor unit properties for the f.h.l. self-reinnervated (f.h.l.-S), soleus self-reinnervated (soleus-S) and soleus cross-reinnervated (soleus-X) groups are illustrated in Table 2. The f.h.l.-S data were generated from four muscles, two of which were partially innervated by the soleus nerve (motor unit samples: 10, 7, 5, 3), the soleus-S data from four muscles, two of which had some cross-reinnervation by the f.h.l. nerve (samples: 8, 5, 3, 1) and the soleus-X data from five muscles, all of which had some soleus reinnervation (samples: 9, 6, 5, 3,10). The extent of cross-reinnervation to the f.h.l. by the soleus nerve was small. Therefore, no data on cross-reunited f.h.l. motor units is presented. Further, some motor units thought to be the result of the cross-reinnervation could not be proven by our criteria and hence are not included here. Axonal conduction velocities were successfully measured for 40 % of the f.h.l.-S population, 76 % of the soleus-S sample and 56 % of the soleus-X sample. Technical limitations prevented this measurement for 100 % of the samples.

The mean twitch contraction time for the soleus-X population was 41 ms (S.D. ± 11), which was significantly shorter ($P < 0.05$) than the 78 ms (S.D. ± 31) for the soleus-S units. Ranges for the two samples were 18–60 ms and 40–150 ms, respectively. However, the mean for soleus-X units was not significantly different ($P > 0.05$) from the f.h.l.-S population mean of 27 ms (S.D. ± 8), the range being

TABLE 1. Whole muscle data

Cat No.	Muscle	Surgical preparation*	Twitch tension (N)† Self	Foreign	Sample size‡
1	Soleus	Cross- 40%	1·76	—	10
		(Self- 60%)	—	1·18	8
2	Soleus	Cross- 77%	—	4·51	3
	Soleus	Self- 100%	3·14	—	5
	F.h.l.	Self- 100%	7·84	—	7
3	Soleus	Cross- 56%	—	0·98	9
	F.h.l.	Self- 100%	1·18	—	3
	F.h.l.	Cross- 0%	—	—	—
		(Self- 100%)	5·88		5
4	Soleus	Cross- 95%	—	1·37	6
5	Soleus	Cross- 57%	—	0·27	5
		(Self- 43%)	0·20	—	1
6	F.h.l.	Cross- 46%	0·64	—	—
		(Self- 54%	—	—	10
7	Soleus	Self- 100%	4·51	—	3

Animals were adult cats weighing between 2·5 and 3·5 kg.

* Cross-, cross-reinnervation; self-, self-reinnervation. Bracketed nerve manipulation unintended. Percentage of cross- or self-reinnervation determined by the percentage of twitch tension produced by stimulation of the self and foreign nerves.

† Measured via self-reinnervating nerve or cross-reinnervating nerve.

‡ Motor units sampled through the respective self or foreign nerve reinnervation.

18–24 ms. The frequency distribution of contraction time of muscle units from each of the three experimental conditions is illustrated in Fig. 1.

The most meaningful way of expressing muscle unit tension is as a percentage of whole muscle tension. Such a procedure normalizes for animals and/or muscles of different size. Whole muscles were not tetanized in this study, however, as we feared whole muscle nerve damage. Also in some cases, glycogen depletion procedures to tag individual muscle unit fibres were done and we did not want to deplete glycogen prematurely by tetanizing the whole muscle. Thus muscle unit tensions are expressed here in absolute force and compared to similar data where available.

Mean (± S.D.) values for absolute twitch and tetanic tensions are presented in Table 2. All but two f.h.l.-S muscle units produced measurable twitch tensions. The distribution of peak tetanic tensions for the three experimental populations is shown in Fig. 2 together with each population's response to unfused tetanus. Those units that showed a 'sag' or level response are shaded; those that showed summation are open (see below). The f.h.l.-S mean tetanic tension was 0·15 N (range 0·015–0·59 N), the soleus-S units averaged approximately 0·22 N (range 0·025–0·51 N), and the mean

TABLE 2. Comparison of motor unit properties

Experimental treatment	A.c.v. (m/s)	Contractile responses					
		Twitch c.t. (ms)	Twitch tension (N)	Tetanic tension (N)	Twitch/tetanus	Potentiation (%)	Fatigue index (%)
F.h.l.-S ($n = 25$)	82±20	27±8	0·04±0·05	0·15±0·14	0·25±0·14	102±114	77±16
Soleus-S ($n = 14$)	76±15	78±31	0·04±0·02	0·22±0·12	0·20±0·12	12±31	53±3
Soleus-X ($n = 33$)	81±10	41±11	0·02±0·02	0·20±0·14	0·12±0·11	31±54	54±10
Significance	n.s.	$27 \leqq 78$	n.s.	n.s.	n.s.	$102 \geqq 12 = 31$	$77 \geqq 53 = 54$

Values are means±S.D. A.c.v., axonal conduction velocity; twitch c.t., contraction time, measured from onset of e.m.g. to peak tension; twitch/tetanus, twitch to tetanus tension ratio; potentiation, percentage twitch potentiation following a repeated tetanus twitch procedure; fatigue index, expressed as a percentage of the cumulative force developed over a 4 min fatigue test that occurs during the first 2 min of the test; n.s., not significant ($P < 0·05$).

for the soleus-X units was 0·20 N (range 0·015–0·57 N). While the mean tetanic tension of soleus-S muscle units was similar to the published values for samples of normal soleus units of 0·25, 0·11 and 0·11 N (Bagust, 1974; Burke *et al.* 1974; Mosher, Gerlach & Stuart, 1972), the mean tension of the f.h.l.-S units was less than one-half the 0·31 N mean found for a normal sample of muscle units from this muscle (Goslow *et al.* 1972).

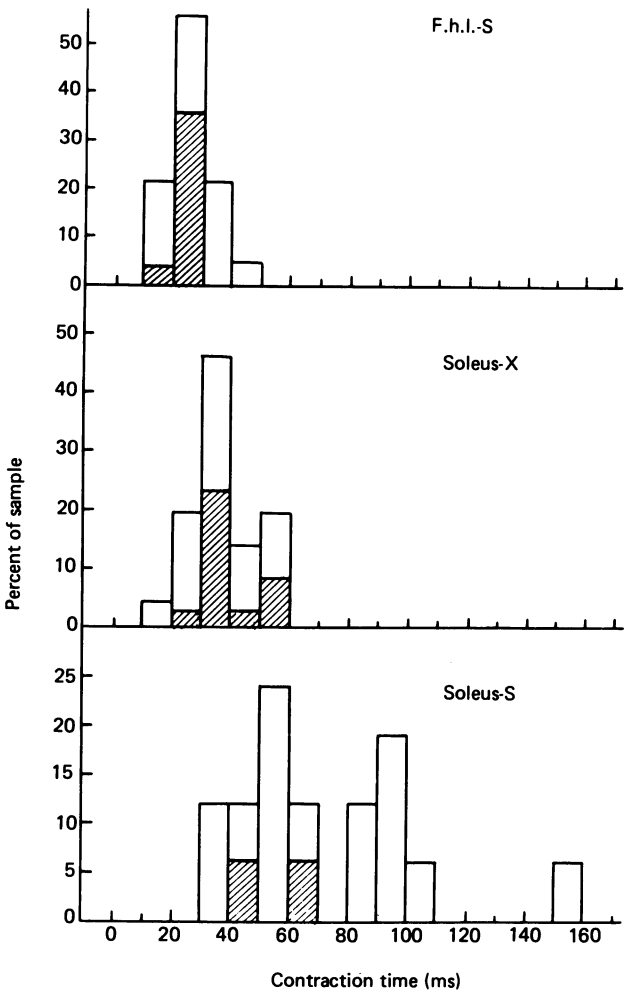


Fig. 1. Population distribution of twitch contraction times of the f.h.l.-S, soleus-X and soleus-S motor units. Those units that showed a 'sag' or level response to an unfused tetanus are shaded; those that showed a conventional summation are open.

Burke and his colleagues have suggested that a 'sag' test is useful in differentiating fast from slow twitch motor units (Burke, Levine, Zajac, Tsairis & Engel, 1971; Burke *et al.* 1973). In their studies of the heterogeneous medial gastrocnemius of the cat, Burke *et al.* (1971, 1973) found that type f.f. (fast twitch, fatigable) and f.r. (fast twitch, fatigue resistant) muscle units demonstrated a 'sag' in the tension profile

during unfused tetani. Type S (slow twitch, fatigue resistant) units, in contrast, showed a 'no sag' response in that the tension profile exhibited conventional summation. In subsequent studies of cat medial gastrocnemius a small number of units with a 'flat' tension profile intermediate between the f.f., f.r. and s muscle unit types have been found (Proske & Waite, 1974; Reinking *et al.* 1975).

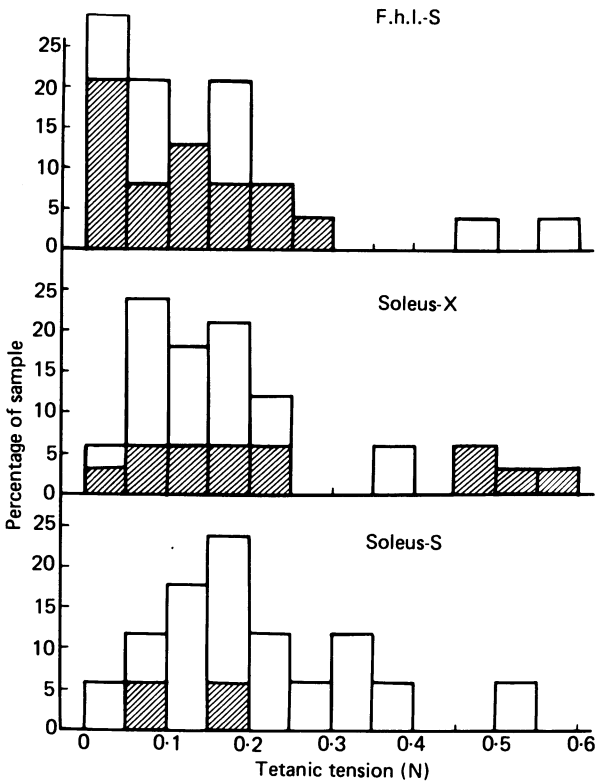


Fig. 2. Distribution of motor unit peak tetanic tensions for the f.h.l.-S, soleus-S and soleus-X populations. Peak tetanic tensions are expressed in absolute N. The mean size of the soleus-S units is comparable to normal units, although f.h.l.-S unit means are smaller than means from their respective normal populations. Though the three means are not significantly different, the soleus-X population appears to be intermediate between the f.h.l.-S and soleus-S populations. Those units that showed a 'sag' or level response to an unfused tetanus are shaded; those that showed a conventional summation are open.

Inspection of Fig. 2 reveals that 63% of the f.h.l.-S and only 12% (2 of 17 units) of the soleus-S population showed a 'sag' or level response to an unfused tetanus. These values are as expected for normal f.h.l. and soleus muscle. In contrast to the soleus-S data, 39% (13 of 33 units) of the soleus-X units showed a 'sag' or level response. Thus, based on this test, 61% of the population of soleus-X units could be considered type s., and 39% type f.r. units. The 'sag' response did not seem to be associated with a particular muscle unit size in either the f.h.l.-S or soleus-X populations.

The twitch–tetanus ratios for the three populations are given in Table 2. A mean

of 0·25 (range 0·02–0·53) was found for the f.h.l.-S, and a mean of 0·20 (range 0·02–0·39) for the soleus-S units. These means and ranges are comparable to the published values for normal units of these two muscles (see Discussion). While the mean twitch–tetanus ratio of 0·12 (range 0·03–0·54) found for the soleus-X units was similar to that of the soleus-S sample ($P > 0·05$) this ratio was significantly lower ($P < 0·05$) than that of the f.h.l.-S group.

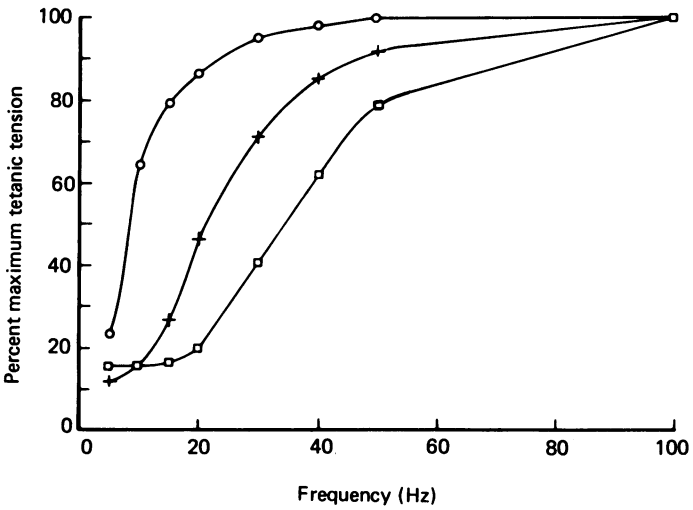


Fig. 3. Average effect of stimulation frequency on tension developed by motor units of f.h.l.-S (□), soleus-S (○) and soleus-X (+). Tension at a given frequency for each motor unit is expressed relative to the maximum tetanic-tension developed by that unit. A total of 25, 14 and 33 units is represented for the f.h.l.-S, soleus-S and soleus-X.

The degree of twitch potentiation following the repeated tetanus–twitch procedure used in this study is indicated in Table 2. Though all three experimental groups demonstrated an over-all twitch potentiation following tetani, they responded to different degrees. The f.h.l.-S population showed a mean twitch potentiation of 102 %, although one unit of the twenty-three studied was depressed following tetanus. The soleus-S units had a mean of 12 % potentiation with four units of seventeen being depressed. Finally, the soleus-X units showed 31 % potentiation with one unit out of thirty-two showing a depressed response following tetanus. Comparisons of twitch contraction times and peak tetanic tensions to post-tetanic response showed no statistically significant patterns.

The mean percentage of maximum tension developed by each of the three muscle unit populations upon stimulation delivered at varying frequencies from 5 to100 Hz is shown in Fig. 3. At 20 Hz the percent of maximum tension produced for the three experimental populations was markedly different ($P < 0·05$): 18, 86 and 46 % for the f.h.l.-S units, soleus-S and soleus-X units, respectively. The relationship between the percentage maximum tension generated at 20 Hz stimulation and the twitch contraction time for the muscle units within the three experimental groups is shown in Fig. 4. The mean contraction time and percentage of maximum tension at 20 Hz

(±S.D.) for the f.h.l.-S, soleus-S and soleus-X populations, respectively, were 27±8 ms and 22±12%; 78±31 ms and 84±20%; and 41±11 ms and 51±24%. From the data in Fig. 4 one could predict that a motor unit with a contraction time of 25 ms would produce 20% of its maximum tension and one with a contraction time of 50 ms would produce 80% of its maximum tension when stimulated at 20 Hz. A

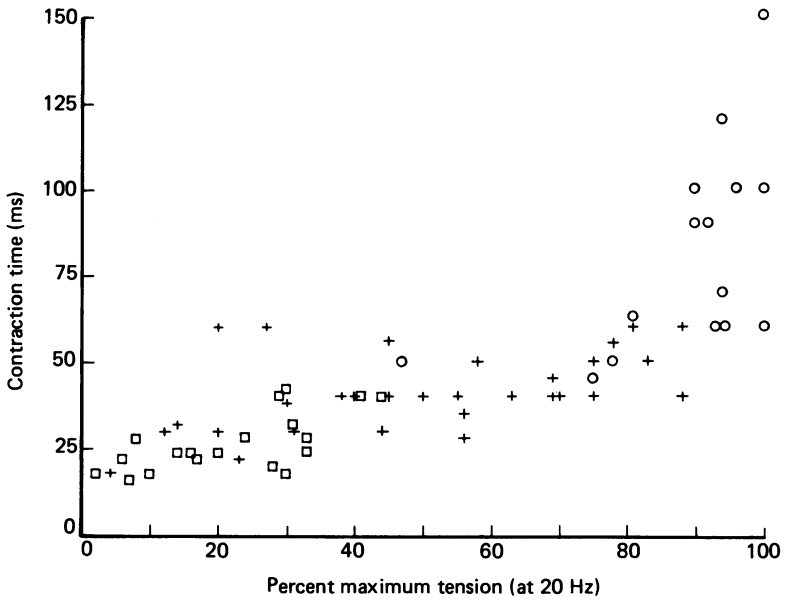


Fig. 4. The relationship between the percent maximum tension generated at 20 Hz stimulation and the twitch contraction time for the motor units of the f.h.l.-X (□), soleus-S (○) and soleus-X (+) populations.

rather linear relationship seems apparent between contraction times of 20 and 40 ms, with percent of maximum tensions ranging from 0 to 60% when stimulated at 20 Hz. The curvilinearity of this relationship becomes apparent at contraction times greater than 40 ms and tensions above 60%. There does not appear to be any qualitative difference in the relationship between contraction times and motor unit tension when stimulated at 20 Hz among and the three experimental situations studied. There is considerable overlap in the plot in Fig. 4 when comparing f.h.l.-S, soleus-S and soleus-X motor units, but all follow the same basic relationship with contraction time and tension produced at 20 Hz.

The histochemical fibre type profile of the normal cat soleus is exclusively that of slow oxidative (s.o.) fibres (Ariano, Armstrong & Edgerton, 1973), with rare exceptions. Multiple cross-sections of a soleus-X muscle stained for myofibrillar ATPase (pH 10·4 and 4·2), α-GPD and reduced NADH-D were prepared. A mean of 14% (S.D. = ±9%; range 4–37%) of soleus-X fibres produced a dark reaction with alkaline pre-incubation ATPase. In almost every fibre that stained intensely with the alkaline myofibrillar ATPase reaction, an intense reaction was also observed for α-GPD. The completeness of the conversion was also illustrated by the reversal of the ATPase staining intensity when pre-incubated at pH 4·35. All fibres had an intense

activity for NADH-D, and consequently those staining intensely for the ATPase reaction satisfied the criteria of being classified as f.o.g. fibres. The maintenance of a high oxidative capacity of all the soleus fibres, including those cross-reinnervated, is consistent with the observed fatigue indices of these units (54 ± 10 %), demonstrating physiologically the resistance to fatigue of the units of normal (Mosher *et al.* 1972) or self-reinnervated (Table 1) soleus motor units.

Cross-sections of a reinnervated f.h.l. muscle stained for alkaline and acid pre-incubated ATPase and NADH-D were also prepared. Most fibres showed intense alkaline pre-incubated ATPase reaction but few stained intensely for NADH-D. As in normal f.h.l. muscle, most fibres were fast glycolytic (f.g.) type.

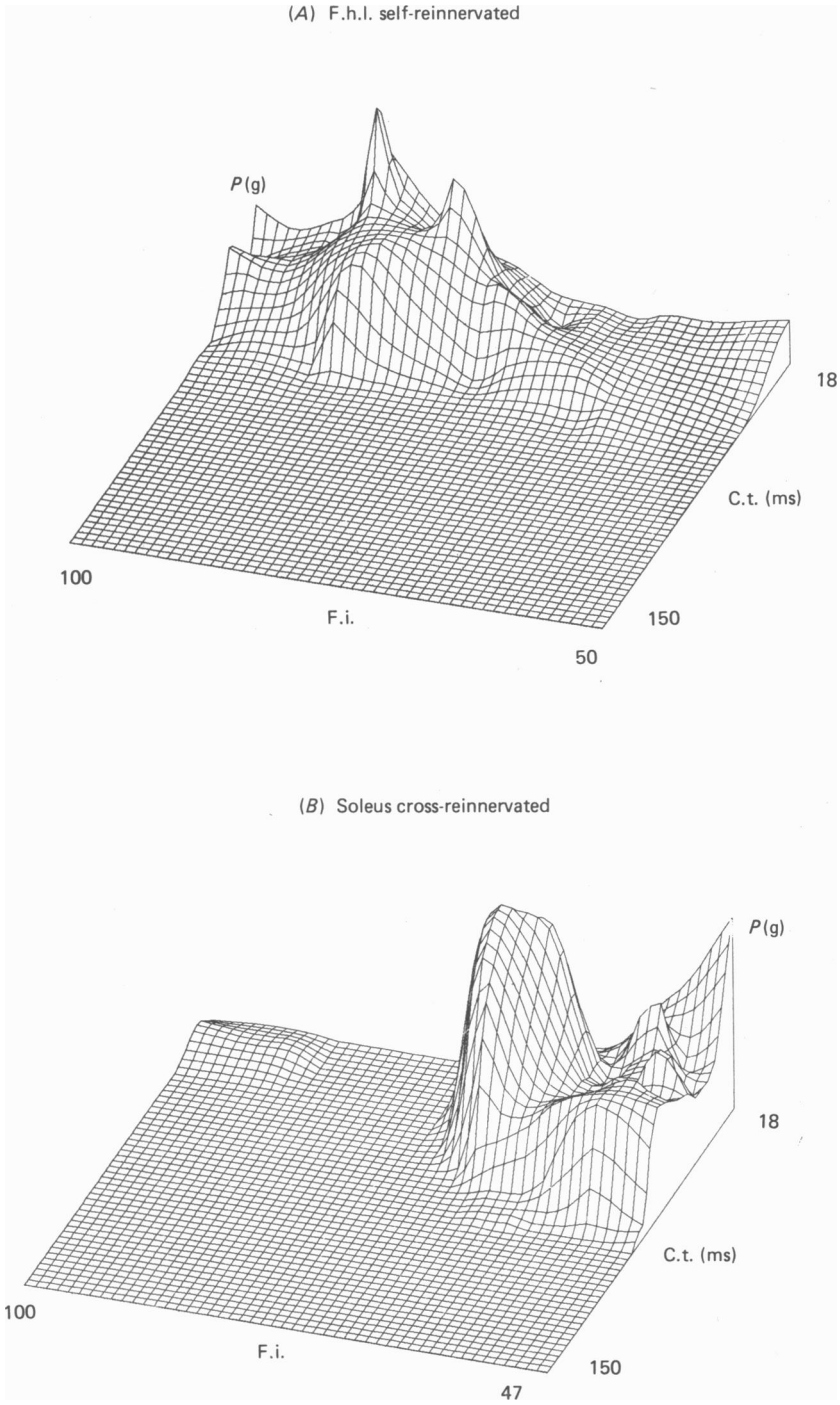
The percentage of fibres which converted histochemically in the soleus-X preparations appeared unrelated to the extent of foreign *vs.* original nerve reinnervation, being similar for all soleus muscles (14 ± 9 %; range 4–37 %) whereas the 'degree of crossing' as determined by the percentage tension of each of the innervating nerves varied from 40 to 95 %. In the two 'purest' crosses, for example, 77 and 95 % of the twitch tension was produced through the foreign nerve (Table 1) and the percentage of converted fibres was 14 % in each cross. A cross-reinnervation that resulted in only 4 % fibre type conversion had a 56 % conversion in terms of tension. Also while a high alkaline ATPase activity was seen in a mean of 14 % of soleus-X fibres, contraction times were reduced by about 73 % relative to what would be expected on the basis of self-reinnervated f.h.l. muscle (Table 2).

Summaries of the contractile properties of single motor units for the f.h.l.-S, soleus-S and the soleus-X preparations are represented in Fig. 5 *A–C* (see Figure legend). A composite of all three muscle conditions is shown in Fig. 5*D*. In the composite the prominent peaks in Fig. 5*A B* and *C* are labelled as *A*, *B* and *C* in Fig. 5*D*. Note particularly that although the contraction time of the soleus-X motor units decreased, no change in the fatigue index of these units was found.

DISCUSSION

The present data support the concept that the contraction time of a muscle fibre is controlled by the motoneurone which innervates it. However, it remains uncertain and rather unlikely that contraction time is exclusively regulated by the motoneurone. Observations on 'pure' cross-reinnervation of a slow muscle with axons that normally innervate a predominantly fast muscle show that a slow muscle's contraction time is not completely converted to that of a fast muscle (Buller *et al.* 1960; Buller, Mommaerts & Seraydarian, 1969). There is always the chance that some 'slow motoneurone' influence in a predominantly fast nerve can be exerted in a pure cross-reunion of nerves when whole muscle properties are studied. This problem is partially overcome by studying single motor units as in the present study. The contraction time was about 75 % converted from that typical of a self-reinnervated soleus to a self-reinnervated f.h.l. muscle. Statistical analysis of contraction time for each of the experimental conditions studied showed that the mean contraction time for the f.h.l.-S (27 ± 8 ms) did not differ ($P > 0{\cdot}05$) from that of the soleus-X (41 ± 11 ms). However, one cannot state that the values for the two groups are the same based on the power of the statistical test.

It is apparent from the data presented in Fig. 4 for the soleus-X units that


(A) F.h.l. self-reinnervated
P(g)
18
C.t. (ms)
100
F.i.
150
50
(B) Soleus cross-reinnervated
P(g)
18
C.t. (ms)
100
F.i.
150
47

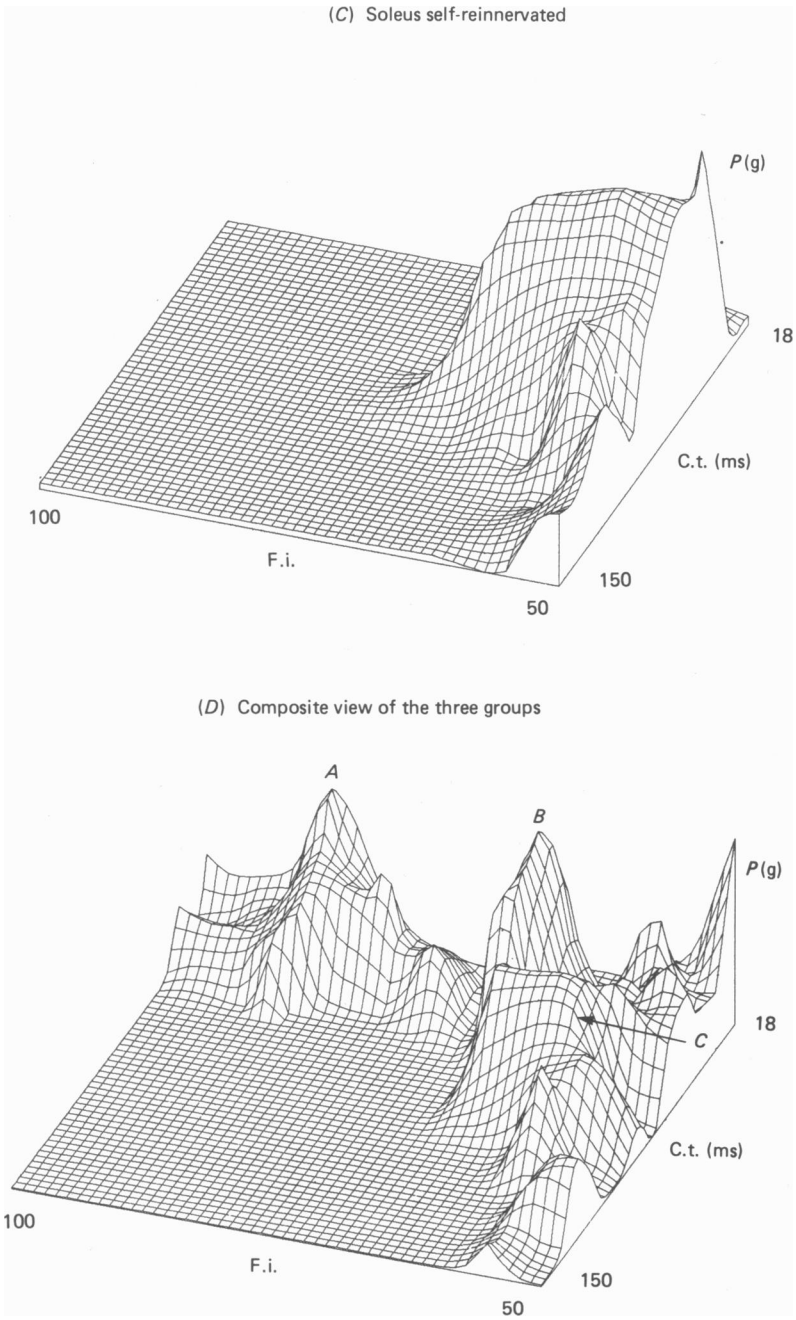


Fig. 5. Three-dimensional summarizations of motor unit populations using tetanic tension (*P*), fatigue index (f.i.) and contraction time (c.t.) (see text). The graphs were drawn by using all of the motor units reported in Figs. 1 and 2. The graphics program was informed of the absence of data beyond the ranges at which data was obtained in order to avoid extrapolation of data beyond the appropriate points. The topographic configuration is a function of the density of muscle units as well as their tension levels. The smoothed curves are a result of the predicted values based on these variables (Burt, 1978).

contraction time varies in a predictable way with the percentage of maximum tension produced when stimulated at a given frequency. If it is assumed that 'fast' and 'slow' motoneurones maintain their usual firing patterns after being severed and self- or cross-united, then not only is the cross-reinnervated motor unit subjected to a new impulse pattern but it is also exposed to a marked change in its usual active tension developed relative to maximum tetanic tension. If a slow motor unit was activated typically at 5–10 Hz it would produce about 20–40% of maximum tetanic tension (Fig. 3). But if it was then exposed to 20 Hz, it would produce more than 80% of maximum tetanic tension. Consequently, crossed nerve effects may be induced by modulating tension (compare Buller *et al.* 1960) as well as the impulse pattern or some neurotrophic factor.

The present data demonstrate that the mean twitch contraction times of the f.h.l.-S (27 ms) and soleus-S (78 ms) populations are similar to the means reported for normal muscle unit samples (Burke *et al.* 1974; Goslow *et al.* 1972; McPhedran, Weurker & Henneman, 1965; Mosher *et al.* 1972). Bagust & Lewis (1974) reported a similar mean twitch contraction time for a population of soleus-S muscle units sampled after six months post-operatively, but found statistically significantly less scatter than for the normal animal. The range of contraction times they reported for the soleus-S population (54–119 ms) was narrower than that found in the present study (40–150 ms). We lack a normal population for statistical comparison, but visual inspection of the contraction time distribution of our soleus-S population (Fig. 1) and that of Bagust & Lewis (1974; see Fig. 1, p. 94) suggests that the range of contraction times of soleus-S units may increase from 6 months to 1 year post-operatively. In contrast, however, Bagust *et al.* (1981) reported mean and range values for soleus-X (f.h.l. nerve; 6 months post-opertively) which were almost identical to the findings reported here after 1 year of reinnervation. These data suggest that motoneurone influence on contraction time in the soleus-X population is complete after 6 months, but incomplete in the soleus-S population. This difference may reflect sample bias or the more subtle mechanical and neural influences which act upon a motor unit to control its twitch time.

Mean twitch–tetanus ratio for the soleus-S population is comparable to published values for normal soleus units (Bagust, 1974; Burke *et al.* 1974), as well as soleus-S units 6 months after reinnervation (Bagust *et al.* 1981). The ranges of twitch–tetanus ratios in soleus-S units in the latter study (0·17–0·37) are reported to be 1·5 times smaller than the control muscle, but again, in the present study one year after surgery the ranges are broader (0·02–0·39) and more approximate to published values for normal soleus (Bagust, 1974; Burke *et al.* 1974). These findings, as well as the shorter contraction times for the soleus-X units discussed above, are consistent with the idea that there may have been an actual increase in the maximal rate at which the muscle units can shorten. However, Close & Luff (1974) have pointed out that a dissociation of intrinsic speed of shortening and duration of the active state can occur for mammalian fast twitch muscle so that caution is warranted in inferring the results of one to the other. The partial conversion of soleus-X units toward that of normal f.h.l. units is also shown by the intermediate range of data points for soleus-X units when percent of peak tetanic tension at 20 Hz stimulation is plotted against twitch contraction time for the three experimental preparations (Fig. 3).

In their studies of both soleus-S and f.d.l.-S muscle units, Bagust& Lewis (1974) reported a variance of tetanic tensions significantly broader than their control muscles. Populations were primarily skewed toward the large units, i.e. the mode was shifted to low tension producing units. Similar findings were reported by Bagust *et al.* (1981) for soleus-X (f.d.l. and f.h.l. nerve) and f.d.l.-X (soleus nerve). These variances were most significant when muscle unit tensions were expressed as a percentage of whole muscle tetanic tension, but were also evident when expressed in absolute force. The present absolute data can be compared to reports of normal populations from animals of about the same size. Our sample of f.h.l.-S muscle units are skewed toward the right demonstrating a population of smaller than normal units (Goslow *et al.* 1972). The distribution of soleus-S units, however, matches closely the population of normal units reported by Mosher *et al.* (1972). Though the mean sizes of the three populations are not significantly different, the distribution of the soleus-X population appears intermediate between the f.h.l.-S and soleus-S populations. It remains to be seen whether the tetanic tension of soleus-X units is a function of the f.h.l. motoneurone or the soleus muscle.

Thompson & Jansen (1977) demonstrated extensive sprouting of intact motoneurones to result in much larger than normal muscle units in partially denervated adult rat soleus. Furthermore, 9–19 weeks later the units were still large suggesting they would remain so. In some contrast, however, are the experiments of Westerman (1978). Using a partial denervation preparation via ventral root section kitten and young adult cats, it was shown that the range of sizes of f.h.l. muscle units 10–21 weeks after surgery was also much larger (2–3 ×) than normal, but after 46–51 weeks the range of sizes was normal. Though the sprouting phenomenon has its distinct time course (see Thompson & Jansen, 1977), Westerman's data as well as the results of this study support the suggestion made by Bagust & Lewis (1974) that there are a finite number of muscle fibres that a motoneurone will eventually support. This implies that some of the same factors controlling muscle fibre motoneuronal innervation in the neonate (for review see Fambrough, 1976) also exist in the adult.

Though the contraction time of soleus motor units is substantially reduced (73%) under the influence of the f.h.l. nerve, the histochemical profile for alkaline preincubation ATPase reaction shows a much smaller conversion (14%) toward a 'fast-twitch' profile. Romanul & Van Der Meulen (1967) reported in adult rat and cat soleus cross-reinnervated for four months with f.h.l. or f.d.l. nerves a relationship between whole muscle contraction time and percent of glycolytic fibres similar to that seen in the present study when relating contraction time with alkaline ATPase dark fibres. Estimations from Fig. 9 in Romanul & Van Der Meulen (1967) show a 75% conversion relative to a normal fast muscle contraction time with about a 30% conversion to high α-GPD activity. (If a fibre in the cross-reinnervated soleus muscle had a dark alkaline ATPase staining characteristic it also stained darkly with α-GPD. Also, some fibres not stained darkly for ATPase did stain darkly for α-GPD.) This marked change in contraction time with only a small change in histochemically demonstrated ATPase of α-GPD is a consistent observation. Crockett & Edgerton (1975) found about a 90% conversion in contraction time with about a 20% conversion in ATPase in the guinea-pig soleus after cross-reinnervation. Burke *et al.* (1979) reported a 100% conversion physiologically with $< 10\%$ histochemical change

in ATPase. A similar response has been observed in the soleus of cats three to four months after low thoracic complete spinal transection where a 90% change in contraction time toward that of a normal fast muscle occurs while only an 18% increase in dark alkaline ATPase fibres was found (Edgerton, Smith, Eldred, Cope & Mendell, 1980*b*). Similar observations have been made on the rat and guinea-pig soleus after limb immobilization (Booth & Kelso, 1973; Maier, Crockett, Simpson & Edgerton, 1976). These results demonstrate that the contraction time can change markedly without a concomitant change in the histochemically demonstrated myofibrillar ATPase and that this change can be induced using a variety of manipulative techniques on the neuromuscular system. This is consistent with the statement made by Buller *et al.* (1969) that 'the change in ATPase activity in the cross-innervated cat soleus muscle is therefore small even when the motor innervation is completely changed'.

The absence of a proportionate change in the histochemically demonstrated ATPase and contraction time could be interpreted in several ways, one of which is that the ATPase procedure is neither quantitative nor specific. Although this is true to some degree, there is a very predictable relationship between the proportion of dark alkaline ATPase fibres in a normal muscle and its contraction time. This relationship also exists for motor units. Kugelberg (1973) has reported an accuracy of ± 2 ms in the rat soleus when testing for single motor units and predicting contraction time on the basis of the pH sensitive ATPase reaction. However, when a fibre's contraction time is modified these normally occurring relationships with ATPase may become disrupted.

Burke *et al.* (1979) studied the histochemical and physiological properites in adult cats of seventeen soleus motor units cross-reinnervated by the nerve of f.d.l. Observed for the soleus-X units was a mean twitch contraction time of 53 ms (37–80), twitch–tetanus ratio of 0·18 (0·05–0·44), and a 6% incidence of post-tetanic depression. All other physiological criteria were characteristic of type s. units and it was concluded that f.d.l. motoneurones reinnervate soleus muscle to produce typical s. muscle units with faster contraction times and less post-tetanic depression than normal soleus units. A greater diversity of cross-reinnervated muscle units is seen in the present study. Twenty of the thirty-three units showed summation during the unfused tetanus and thirteen units showed a 'sag' or a level response. Based on the 'sag' test, then, though it is not always easy to interpret, 61% of the population could be considered type s. units. Of this population, all had contraction times of < 55 ms and all but one were highly resistant to fatigue. One unit showed post-tetanic depression. These units appear to be similar to the soleus cross-reinnervated units reported by Burke *et al.* (1979). In addition, however, 39% of our population did not show summation to unfused tetanus, had contraction times of $\leqslant 60$ ms, and were highly resistant to fatigue. These units are regarded as type fast twitch, fatigue resistant (f.r.) units. These data suggest, then, that the axons of slow twitch, fatigue resistant (s.) and f.r. motoneurones are selectively favoured by the soleus muscle in the process of reinnervation or that the particular phenotypic expression of fatigue resistance, which is related to mitochondrial content, is controlled primarily within the muscle and not by the motoneurone (Edgerton, Goslow, Rasmussen & Spector, 1980*a*).

An additional observation on the histochemical enzyme profile was that if a muscle fibre stained darkly with alkaline ATPase and was light with an acid ATPase, it also stained darkly for a α-GPD and remained darkly stained with NADH-D. This is typical of the f.o.g. profile (Peter *et al.* 1972). Also, it was observed that some of the fibres stained darkly with α-GPD but not with alkaline ATPase. No fibres were observed which stained darkly for alkaline ATPase but not for α-GPD. The same finding has been reported in the soleus of the unilaterally immobilized hind limb of guinea-pigs (Maier *et al.* 1976) and in the soleus of cats chronically (3 months) spinalized at approximately the level of the twelfth thoracic vertebra (Edgerton *et al.* 1980*b*). These observations are also consistent with a report in which a close relationship of phosphofructokinase activity assayed biochemically and myofibrillar ATPase activity was shown (Baldwin, Winder & Holloszy, 1975). A close relationship between contraction time and phosphofructokinase (Prewitt & Salafasky, 1967) and contraction time and α-GPD (Romanul & Van Der Meulen, 1967) has been shown in cross-reinnervated cat soleus, as well. Also the cross-reinnervated soleus muscle showed no change in its malate dehydrogenase activity even though phosphofructokinase increased as contraction time decreased (Prewitt & Salafasky, 1967). Chronically stimulated muscle also follows this pattern (Pette, Ramirez, Muller, Simon, Exner & Hildebrand, 1975). These data suggest a rather strong gene co-ordination between glycolytic enzyme activity and myofibrillar ATPase activity, as suggested by Golisch, Pette & Pichlaier (1970).

In general these data suggest that following reinnervation of adult skeletal muscle fibres by a foreign motor neurone there is only partial control of several of the more commonly tested physiological properties. The small percentage of conversion of histochemical alkaline ATPase to dark staining fibres, the generally longer contraction time and the maintenance of fatigue resistance of soleus-X motor units innervated by a pool of motor neurones that normally innervate predominantly fatiguable muscle fibres with shorter contraction times is consistent with the concept of incomplete neural control. The data further demonstrate a dissociation of the close relationship between the standard histochemical ATPase staining and the contraction time of muscle fibres that exists in normally innervated adult muscle.

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