

**EFFECTS OF  
NERVE CROSS-UNION ON FAST-TWITCH AND SLOW-GRADED  
MUSCLE FIBRES IN THE TOAD**

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**SUMMARY**

1. A method is described for resolving isometric tetanic tension developed by fast-twitch and slow-graded components of heterogeneous toad muscles. This makes use of the difference in threshold for excitation of low threshold nerve fibres which normally innervate the fast-twitch muscle fibres and high threshold nerve fibres which innervate slow-graded muscle fibres.

2. The sartorius muscle contains only fast-twitch muscle fibres whereas the posterior semitendinosus (PST) contains both fast-twitch and slow-graded muscle fibres, the latter contributing 10–15% of the maximum isometric tetanic tension.

3. Following surgical cross-union of nerve to sartorius and PST muscles, both the fast-twitch and slow-graded muscle fibre components of the PST are reinnervated by low threshold nerves originally innervating sartorius fast-twitch fibres, and sartorius fast-twitch muscle fibres are reinnervated by both low threshold and high threshold nerves formerly supplying the fast-twitch and slow-graded muscle fibre components of the PST.

4. The characteristic mechanical responses of fast-twitch muscle fibres and slow-graded muscle fibres were not transformed up to 134 and 200 days respectively following nerve cross-union.

5. PST nerve partially innervated the sartorius muscle whereas sartorius nerves completely innervated the PST muscle. Isometric tetanic tension declined markedly during repetitive indirect stimulation of cross-innervated sartorius muscles, whereas the tetanic contractions of cross-innervated PST showed a plateau of tension and resembled the response of normal muscles.

6. Normal, cross-innervated and self-innervated PST muscles gave sustained contractures in the presence of acetylcholine whereas PST muscles denervated for 120 days gave phasic contractures similar to those of normal, cross-innervated and self-innervated sartorius muscles.

## INTRODUCTION

The demonstration of neural control of the speed of mammalian muscles (Buller, Eccles & Eccles, 1960; Buller & Lewis, 1965; Close, 1965) has raised the question whether similar influences are exerted through motoneurons of other kinds of fast and slow muscles in other animals. In this connexion Feng, Wu & Yang (1965), Zelená, Vyklický & Jirmanová (1967) and Hník, Jirmanová, Vyklický & Zelená (1967) found little or no change in the speed of response of avian muscles following cross-union of the motor nerves to focally innervated fast muscle fibres and multiply innervated slow muscle fibres. Miledi & Orkand (1966) examined some of the properties of frog iliofibularis muscle innervated by the nerve which normally innervates sartorius muscle. They reported a change in the time course of acetylcholine contractures of the cross-innervated iliofibularis muscle from the prolonged contracture which is characteristic of normal slow-graded muscle fibres to a phasic response which resembles that of fast-twitch muscle fibres, but they did not show whether this change was brought about through innervation by alien nerves or denervation.

In the present work an attempt has been made to determine whether functional neuromuscular connexions develop following cross-union of the nerves to fast-twitch muscle fibres and slow-graded muscle fibres of toad skeletal muscles, and whether the characteristic all-or-nothing twitch and graded contractions of these two kinds of muscle fibres are altered by nerve cross-union. Some of the results have been reported briefly elsewhere (Hoh & Close, 1967).

## METHODS

The experiments were carried out on sartorius (SART) and posterior semitendinosus (PST) muscles of adult toads (*Bufo marinus*) of body weight ranging from 60 to 171 g obtained from Queensland, kept alive in the laboratory at room temperature, and force-fed with minced liver every 1-2 weeks.

*Operations.* Cross-union, self-union or denervation operations were performed on nerves to sartorius and PST muscles in one leg and the contralateral nerves and muscles were kept intact as controls. Ether was used as anaesthetic and all operations were performed in aseptic conditions.

In cross-union operations the nerves to sartorius and PST muscles were transected and cross-sutured with fine silk. The proximal part of the PST nerve was tied to the distal stump of the sartorius nerve deep in the thigh along the normal course of the sartorius nerve but the proximal stump of the sartorius nerve was passed between the anterior and posterior semitendinosus muscles and tied to the distal stump of the PST nerve on the superficial surface of the PST muscle. In this way the sites of nerve union were kept some distance apart, thereby reducing the chances of a nerve growing back into its original muscle. The chances of this occurring were virtually excluded in a few preparations in which one muscle was excised and its nerve was allowed to innervate an alien muscle transplanted from the contralateral limb. Innervation of transplanted PST muscle by sartorius nerve was carried out in one animal. In five other animals the contralateral sartorius muscle was detached from

all connexions except its origin on the pubis and transposed to lie 'back to back' along the ipsilateral sartorius muscle; the tendons of insertion of the sartorius muscles were tied together, the PST nerve was tied to the stump of the sartorius nerve and the PST muscle was excised.

Self-union operations were performed on fourteen animals by transecting and reuniting the nerve to each muscle in turn.

Denervation of sartorius and PST muscles was carried out in nine animals. This was done at first by excising most of the sciatic nerve but in later operations the only part removed was the branch of the sciatic nerve which leaves the main trunk under the pyramidalis muscle to supply sartorius, PST, anterior semitendinosus and the rectus internus major and minor muscles. During dissections of denervated muscles for experiments, the spinal nerves of the sciatic nerve were stimulated electrically to ascertain whether reinnervation had occurred in the experimental muscle.

Operations were performed on seventy-nine animals; the mortality rate was 81% in the first series of operations due to wound infection but this was reduced to 31% in the last batch of operated animals with topical application of antibiotic powder ('Cicatrin'-Calmic) containing neomycin sulphate, zinc bacitracin, cysteine, threonine and glycine. Thirty-two animals survived for experiment and in these there were fifteen cross-unions, five self-unions, six denervations, five sartorius transplantations and one PST transplantation. All except two of these animals remained in excellent condition and at the time of the experiment their mean body weight was 89 g following a post-operative weight loss of only about 4% over a period of about 4 months.

*Experimental arrangement.* Forty-four experiments were performed, including twelve on unoperated animals. Most of the experiments on the thirty-two operated animals were done about 4 months after operations but a few were performed 2 months and 6 months post-operatively.

The sciatic nerve and the sartorius and PST muscles were dissected from both the operated and unoperated limbs together with their bones of origin and all these were transferred to a bath containing Ringer solution (NaCl 90 mM, KCl 2 mM, CaCl<sub>2</sub> 2 mM, NaHCO<sub>3</sub> 2 g/l., glucose 2 g/l.) which was bubbled continuously with 95% O<sub>2</sub> and 5% CO<sub>2</sub> and maintained at room temperature (19.6–27.5°C). In setting up the preparation for stimulating and recording the bones of origin were clamped securely, the distal tendons of the sartorius and PST muscles of one limb were tied directly to strain gauges and the sciatic nerve was passed into a small bath of oxygenated paraffin oil and laid over platinum-wire electrodes set 10–15 mm apart (cathode proximal to muscle). This arrangement made it possible to stimulate the sciatic nerve and record simultaneously the responses of both the sartorius and PST muscles of one leg. All contractions were recorded with the muscles set at the *in situ* length (see below).

One of the strain gauges (Statham G1-4-250) was used as a DC bridge, the other (Statham G1-8-350) was used in conjunction with a carrier amplifier (Tektronix, Q). The outputs of the DC bridge and the Q unit were amplified and displayed on a dual-beam oscilloscope (Tektronix, 565) with plug-in amplifiers (Tektronix 3A3 and 72) operated in the chopped mode. The outputs of the amplifiers were also displayed on a slave oscilloscope and the traces were photographed with a Grass C4 camera. Time marks were triggered from a time-mark generator (Tektronix 180A).

A Grass S 4 stimulator with isolation unit was used for nerve stimulation; the stimulus was a square pulse of 20  $\mu$ sec duration and the amplitude was varied. For direct stimulation stimulation of curarized and denervated muscles the S 4 isolation unit was followed by a transistor current amplifier and the stimulus was a square pulse of 15 V and 0.3 msec duration. All tetanic contractions were elicited by stimulation at 80 c/s for 1 sec. In all the records shown below the first stimulus occurs at the start of the oscilloscope trace.

The responses of fast-twitch and slow-graded muscle fibres were distinguished on the basis of differences in rates of relaxation from isometric tetanic contractions. At the end of

a tetanus fast-twitch muscle fibres relax rapidly within about 200 msec after the last stimulus (Fig. 1*a, b*) whereas slow-graded fibres relax much more slowly over a period of about 30 sec (Fig. 1*f*). In heterogeneous muscles such as PST there is a well-defined inflexion in the tension-time curve for relaxation marking the end of relaxation in the fast-twitch fibres. Isometric tetanic contractions of sartorius and PST muscles from one leg were recorded simultaneously. The response of each muscle was displayed on both channels of one amplifier, with one channel at low gain for a record of the whole contraction and the other channel at 5 times greater amplification for a record of relaxation in slow-graded fibres. In this way four tension:time records were obtained simultaneously, similar to those shown in Fig. 1*e, f*. The tension developed by the slow-graded fibre component of PST at any time during contraction was estimated by subtracting the tension developed by the fast-twitch fibre component from that of the whole muscle. In some muscles, such as the cross-innervated PST, in which the response of the fast-twitch fibre component could not be measured separately, the maximum tension in the slow-graded component was measured at the point of inflexion in the tension-time curve which marks the end of relaxation in the fast-twitch fibres.

The nerve fibres which normally innervate fast-twitch muscle fibres and slow-graded muscle fibres were distinguished on the basis of differences in threshold for electrical stimulation. The relations between stimulus strength and both peak twitch tension and maximum isometric tetanic tensions were determined for each nerve-sartorius-PST muscle preparation using the method of simultaneous recording described above. In this way it was possible to identify the kind of nerve stimulated and the muscle fibres which they innervate both in normal and operated muscles.

Acetylcholine contractures of some muscles were recorded at the end of the experiment. The homonymous muscles were set at the *in situ* length and tied to strain gauges for simultaneous recording of tension. Acetylcholine chloride (B.D.H.) was added to the bath to a concentration of  $2 \times 10^{-5}$  g/ml. Ringer and the tension was recorded until the contracture disappeared or for a maximum period of 15 min.

*Definitions.* Maximum isometric twitch tension:  $P_t$ . The maximum tension in a twitch response to maximal stimulation of the sciatic nerve with the muscle at the *in situ* length.

Maximum isometric tetanic tension:  $P_0$ . The maximum tension in a tetanic response to maximum indirect stimulation at 80 c/s for 1 sec, with the muscle at the *in situ* length.

Isometric twitch contraction time:  $T_c$ . The time from the onset of contraction to the peak of the twitch.

Isometric twitch half-relaxation time  $T_{1/2}$ . The time for isometric twitch tension to decay from the peak tension to one half the peak tension.

Muscle length *in situ*. The length of the muscle measured during dissections with the thigh at right angles and the lower leg parallel to the mid line.

## RESULTS

*Properties of normal sartorius and PST muscles and their nerves.* Representative records of isometric twitch and tetanic contractions of normally innervated sartorius (N-SART) and posterior semitendinosus (N-PST) muscles are shown in Figs. 1 and 5, and the relations between stimulus strength and the maximum tension developed in twitch and tetanic responses of the same muscles are shown in Figs. 2 and 6. These diagrams show that N-SART contains only fast-twitch muscle fibres innervated by low-threshold nerve fibres (LTN) whereas N-PST contains both fast-twitch fibres and slow-graded muscle fibres innervated by LTN and high-threshold nerve fibres (HTN) respectively. As the results obtained from

all the normal muscles are essentially the same, only those shown in Fig. 1 *a-h* and Fig. 2*A, B* need be described.

The curves relating stimulus strength with maximum twitch and tetanic tensions for both N-SART and N-PST show well-defined thresholds for stimulation (Fig. 2*A, B*). In both muscles, no increase in twitch tension or alteration of twitch time course could be detected with further increase in stimulus strength up to 15 V (e.g. Fig. 1*c, d, g, h*). The tetanic response of N-SART is not altered by increase in stimulus strength between about 5 and 15 V, and in all eighteen N-SART muscles examined there was

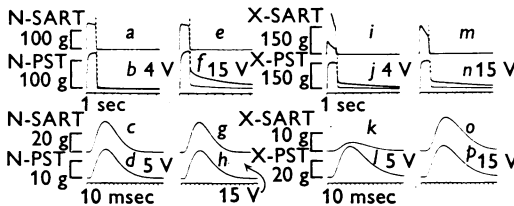


Fig. 1. Records of isometric twitch and tetanic contractions (lower and upper parts of the diagram respectively) of cross-innervated sartorius (X-SART) and posterior semitendinosus (X-PST) muscles (*i-p*) and the normally innervated contralateral control (N-SART, N-PST) muscles (*a-h*) from a toad 108 days after nerve cross-union operation. Each tetanus record was displayed on two beams of the oscilloscope on the same base line, one beam having a gain 5 times greater than the other to show the slow-graded component of tetanic tension in PST. The high-gain beams have been retouched in parts (interrupted lines). The tension calibration for tetanic contractions refer to the record of the whole response displayed on the low-gain beam. Records of contractions of the SART and PST muscles of one leg were recorded simultaneously, e.g. *a* and *b*, *c* and *d*, etc., and the strength of the stimulus applied to the nerve is shown in volts (V) alongside each set of records. Muscle weight: N-SART = 156 mg, N-PST = 117 mg, X-SART = 171 mg, X-PST = 122 mg; average muscle fibre length: N-SART = 39 mm, N-PST = 23 mm, X-SART = 38.5 mm, X-PST = 22.5 mm. Temperature = 23.5–24.5° C.

no evidence of slow-graded muscle fibre activity. The curve relating stimulus strength and maximum tetanic tension of N-PST (Fig. 2*A*) shows clear separation of the ranges of threshold for stimulation of LTN innervating fast-twitch fibres and the HTN innervating the slow-graded muscle fibres. This makes it possible to obtain a response of the whole fast-twitch fibre component of PST either alone (Fig. 1*b*) or in combination with the responses of the slow-graded muscle-fibre component (Fig. 1*f*). As the two components contract in parallel within the muscle, the tension developed by the slow fibres at any stage during contraction can be estimated by subtracting the response of the fast component from that of the whole muscle. In all preparations the slow-graded muscle fibre component of N-PST gave no detectable response to a single maximal stimulus (15 V, 20  $\mu$ sec) applied to the nerve.

Table 1 summarizes the properties of N-SART and N-PST muscles and the thresholds of their nerves. Overlap of the ranges of thresholds for repetitive stimulation of LTN and HTN in PST nerve probably results

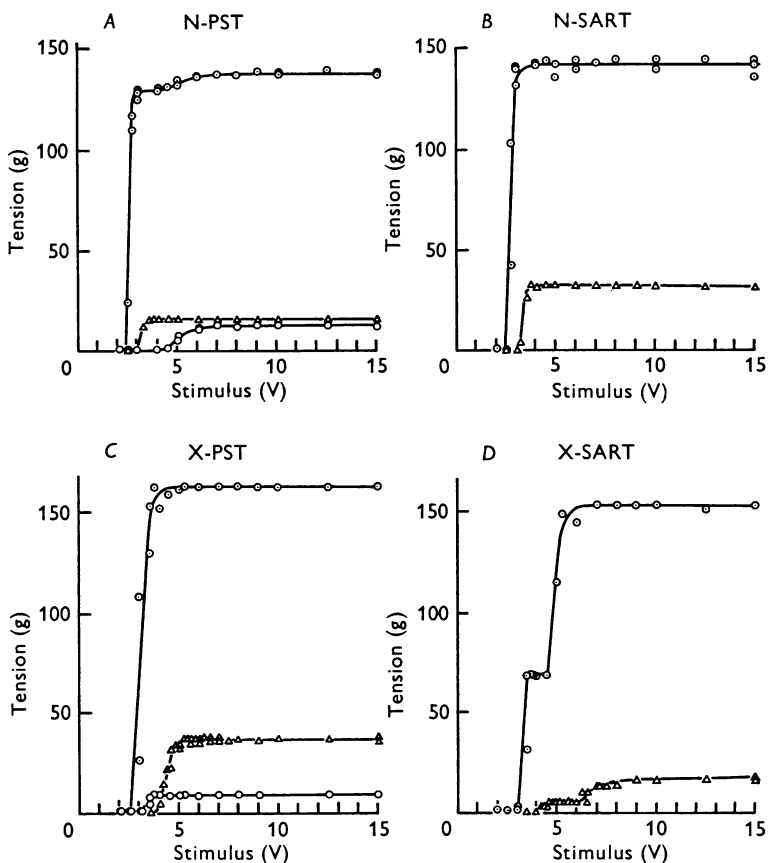


Fig. 2. Maximum tensions developed in isometric twitch ( $\Delta$ ) and tetanic (whole muscle =  $\odot$ , slow-graded component =  $\circ$ ) contractions, plotted against stimulus strength (V) applied to the sciatic nerve for the normally innervated PST muscle (A), the normally innervated sartorius muscle (B), the cross-innervated PST muscle (C) and the cross-innervated sartorius muscle (D) from a toad 108 days after the cross-union operation. Representative records of the series of contractions are shown in Fig. 1.

mainly from differences in amount of fluid between stimulating electrodes in different preparations, but with careful removal of excess fluid it was possible to separate the ranges of threshold for the two kinds of nerve fibre in every preparation. The mean difference between the maximum

threshold for LTN and the minimum threshold for HTN in PST nerve was 0.97 V (0.25–2.0 V,  $n = 11$ ). This difference in threshold results presumably from differences in diameter of the two kinds of nerve fibres (see Erlanger & Gasser, 1937; Kuffler & Vaughan Williams, 1953). The mean values for isometric twitch contraction time ( $T_c$ ), half-relaxation time ( $T_{\frac{1}{2}R}$ ) and the ratio of maximum twitch tension ( $P_t$ ) to maximum tetanic tension ( $P_0$ ) are greater for N-SART than for the fast-twitch fibre, component of N-PST. The results from eight pairs of muscles have been compared; records of simultaneous contractions were obtained for each pair of muscles, one N-SART and one N-PST in one leg, thereby eliminating differences due to temperature. Using the  $t$ -test it has been found that there are significant differences between N-SART and N-PST in the mean values for  $T_c$  (significance probability  $P < 0.025$ ) and  $P_t/P_0$  ( $P < 0.0005$ ) but not for  $T_{\frac{1}{2}R}$  ( $P < 0.1$ ) nor for the difference in maximum isometric tetanic tension developed per unit cross-sectional area of muscle ( $P < 0.4$ ).

The slow-graded component of PST does not develop its maximum tension within 1 sec in response to stimulation at 80 c/s. Higher frequencies of stimulation and longer durations of stimulation increase the response of the slow-graded component up to 10–15% of the maximum tension developed by the whole muscle.

*Properties of sartorius muscles and PST nerves following cross-union.* Twenty toads survived following operations in which the PST nerve was sutured to the distal stump of sartorius nerve. This group comprised fifteen animals with nerve cross-union and five with the sartorius muscle transposed. In twelve animals only the PST nerve appeared to have innervated the sartorius muscle functionally. The other eight sartorius muscle preparations were defective as a result of either denervation or reinnervation by collateral branches arising from the original sartorius nerve.

Figure 1 shows representative records of isometric twitch and tetanic contractions of a cross-innervated sartorius (X-SART) muscle and the contralateral control muscle from a toad examined 108 days after the operation. The curves relating stimulus strength and maximum tension in twitch and tetanic contractions of the same muscles are shown in Fig. 2. The twitch contractions of X-SART are similar in time course to those of the control muscle and records of tetanic contractions of both X-SART and N-SART show no phase of slow relaxation which could be attributed to activity of slow-graded muscle fibres. The curves in Fig. 2D show that sartorius muscle fibres were innervated by both LTN and HTN fibres of PST nerve following cross-union. The same result was obtained for seven X-SART muscles in which the contractile responses were studied in detail using graded nerve stimuli 55–134 days post-operatively. In the other three PST nerve–sartorius muscle preparations similarly studied the

TABLE 1. Summary of the properties of normal, self-innervated and cross-innervated sartorius (N-SART, S-SART and X-SART, respectively) and posterior semitendinosus (N-PST, S-PST and X-PST, respectively) muscles and the low threshold (LTN) and high threshold (HTN) nerves which innervate them. Mean values for the minimal (min.) and maximal (max.) thresholds are given in volts (V) with the range in parentheses. Mean values  $\pm$  s.e. are listed for the isometric twitch contraction time ( $T_c$ , msec), half-relaxation time ( $T_{1/2}$ , msec), maximum isometric tetanic tension of the whole muscle (Total  $P_0$ , in grams), tetanic tension of slow-graded fibre components (Slow  $P_0$ , % total  $P_0$ ), twitch:tetanus ratio of fast-fibre components ( $P_f/P_0$ ) and the maximum force developed by the whole muscle per unit cross-sectional area of muscle (Total  $P_0$  (kg/cm<sup>2</sup>)), of responses to nerve stimulation. The mean temperature and range in parentheses at which twitches were recorded (Twitch  $T^\circ$  C) are given and  $n$  is the number of observations on which each value is based

		Twitch responses					
		N-SART	S-SART	X-SART	N-PST	S-PST	X-PST
LTN							
Min.		2.80 ( $n = 9$ ) (2.25-4.25)	2.67 ( $n = 3$ ) (1.5-3.5)	3.08 ( $n = 8$ ) (2.0-4.8)	2.54 ( $n = 9$ ) (2.0-4.0)	2.75 ( $n = 3$ ) (1.75-3.5)	3.14 ( $n = 9$ ) (2.2-4.9)
Max.		3.91 ( $n = 9$ ) (2.75-6.5)	3.75 ( $n = 3$ ) (3.5-4.0)	3.95 ( $n = 8$ ) (2.6-5.5)	3.71 ( $n = 9$ ) (2.75-6.0)	3.67 ( $n = 3$ ) (3.5-4.0)	4.73 ( $n = 9$ ) (2.5-6.0)
HTN							
Min.		—	—	5.35 ( $n = 6$ ) (4.06-6.25)	—	—	—
Max.		—	—	9.00 ( $n = 6$ ) (6.0-15.0)	—	—	—
				Tetanic responses			
(i) Fast							
LTN							
Min.		2.44 ( $n = 9$ ) (1.5-3.5)	2.17 ( $n = 3$ ) (1.5-2.5)	2.50 ( $n = 8$ ) (1.5-4.0)	2.22 ( $n = 11$ ) (1.5-3.5)	2.25 ( $n = 3$ ) (2.0-2.5)	2.63 ( $n = 9$ ) (1.5-3.5)
Max.		3.56 ( $n = 9$ ) (2.5-4.5)	3.33 ( $n = 3$ ) (3.0-3.5)	3.79 ( $n = 8$ ) (2.5-5.5)	3.30 ( $n = 11$ ) (2.0-4.0)	3.17 ( $n = 3$ ) (3.0-3.5)	4.53 ( $n = 9$ ) (3.5-6.0)
HTN							
Min.		—	—	4.87 ( $n = 7$ ) (3.5-7.0)	—	—	—
Max.		—	—	9.86 ( $n = 7$ ) (4.0-15.0)	—	—	—



TABLE 1. (cont.)

	N-SART	S-SART	X-SART	N-PST	S-PST	X-PST
(ii) Slow-graded						
LTN						
Min.	—	—	—	—	2.5 (n = 1)	3.02 (n = 9) (2.0-4.5)
Max.	—	—	—	—	3.0 (n = 1)	4.53 (n = 9) (3.5-6.5)
HTN						
Min.	—	—	—	4.27 (n = 11) (2.5-7.5)	4.20 (n = 3) (3.0-5.5)	—
Max.	—	—	—	10.00 (n = 11) (7.0-15.0)	11.3 (n = 3) (7.0-12.0)	—
$T_e$ (msec)	46.9 ± 1.4 (n = 18)	47.0 ± 1.0 (n = 3)	54.7 ± 2.2 (n = 12)	41.6 ± 1.9 (n = 18)	44.3 ± 0.9 (n = 3)	47.1 ± 2.6 (n = 11)
$T_{1/2}$ (msec)	50.3 ± 2.7 (n = 18)	44.3 ± 1.20 (n = 3)	54.5 ± 3.2 (n = 12)	40.0 ± 2.5 (n = 18)	42.7 ± 0.7 (n = 3)	48.2 ± 3.8 (n = 11)
Total $P_0$ (g)	107.8 ± 6.7 (n = 18)	103.7 ± 24.4 (n = 3)	86.6 ± 14.0 (n = 12)	145.9 ± 10.2 (n = 19)	142.7 ± 36.2 (n = 3)	101.4 ± 12.9 (n = 11)
Slow $P_0$ (% total $P_0$ )	0	0	0	9.0 ± 0.7 (n = 13)	8.2 ± 0.9 (n = 3)	5.1 ± 0.6 (n = 11)
$P_t/P_0$ (fast)	0.45 ± 0.03 (n = 18)	0.46 ± 0.05 (n = 3)	0.36 ± 0.05 (n = 12)	0.28 ± 0.03 (n = 18)	0.24 ± 0.05 (n = 3)	0.32 ± 0.04 (n = 11)
Total $P_0$ (kg/cm <sup>2</sup> )	3.08 ± 0.12 (n = 16)	2.79 ± 0.24 (n = 3)	2.06 ± 0.30 (n = 10)	3.36 ± 0.12 (n = 14)	3.02 ± 0.14 (n = 3)	2.40 ± 0.19 (n = 8)
Twitch $T^{\circ}C$	22.8 (20.5-24.5)	22.7 (22.0-23.5)	21.7 (20.0-24.0)	24.1 (21.0-27.5)	22.7 (22.0-23.5)	22.4 (19.6-24.5)

muscle fibres were innervated by LTN fibres but the presence of HTN fibres could not be demonstrated.

Table 1 summarizes the properties of PST nerve-sartorius muscle preparations. There is separation of the thresholds of LTN and HTN and the mean of the difference between the threshold for single stimuli of the least excitable LTN and the threshold of the most excitable HTN was 1.32 V (range 0.75–1.75 V). The HTN of PST nerve innervated fast-twitch muscle fibres of sartorius muscle which contributed 37 % of the total twitch tension (range 9–73 %,  $n = 6$ ) and 32 % of the total tetanic tension (range 35–57 %,  $n = 7$ ). The extent of reinnervation of the whole muscle was determined for a number of X-SART muscles by comparing the maximum tension developed in tetanic contractions elicited by direct stimulation of the muscle and indirect stimulation through the PST nerve. Indirect stimulation produced on average only 59 % ( $\pm 14$  % s.e., range 13–96 %,  $n = 5$ ) of the tetanic tension which could be elicited by direct stimulation. Failure of the PST nerve fibres to innervate all the sartorius muscle fibres functionally probably accounts for the difference between X-SART and N-SART muscles in the maximum tetanic tension developed per unit cross-sectional area of the muscle (Table 1). The  $T_c$  and  $T_{\frac{1}{2}R}$  of X-SART exceed those of N-SART and both the  $P_i/P_0$  ratio and the maximum tetanic tension per unit cross-sectional area are less for X-SART than for N-SART. In order to test the statistical significance of these differences, the data from seven X-SART muscles and their contralateral controls were analysed. The means of the differences for  $T_c$ ,  $P_i/P_0$  and maximum force/unit area between these pairs of muscles are significant at the 1.25 % level of probability or less.

An interesting property of the PST nerve-sartorius muscle preparations is the fall in tetanic tension during indirect stimulation by way of the nerve. Direct stimulation of these muscles elicited the full tetanic contractions with the usual plateau of tension. The mean drop in tetanic tension was 39 % (range 11–97 %,  $n = 12$ ) when the nerve was stimulated maximally, and 42.5 % (range 22–72 %,  $n = 7$ ) when only the LTN were stimulated. In view of the wide range of values and the small difference between the means, it is likely that failure occurs to about the same extent during stimulation of both LTN and HTN.

The time course of the twitch contraction of X-SART is virtually the same for indirect stimulation of LTN alone or together with the HTN of PST nerve. In other words, the twitch time course is the same for muscle fibres innervated by LTN or HTN. This is shown more clearly in Fig. 3A for contractions of 2 X-SART muscles examined 55 days and 108 days (the latter described in Fig. 1) post-operatively. In this diagram the twitch responses of fibres innervated by HTN are shown as interrupted tension-

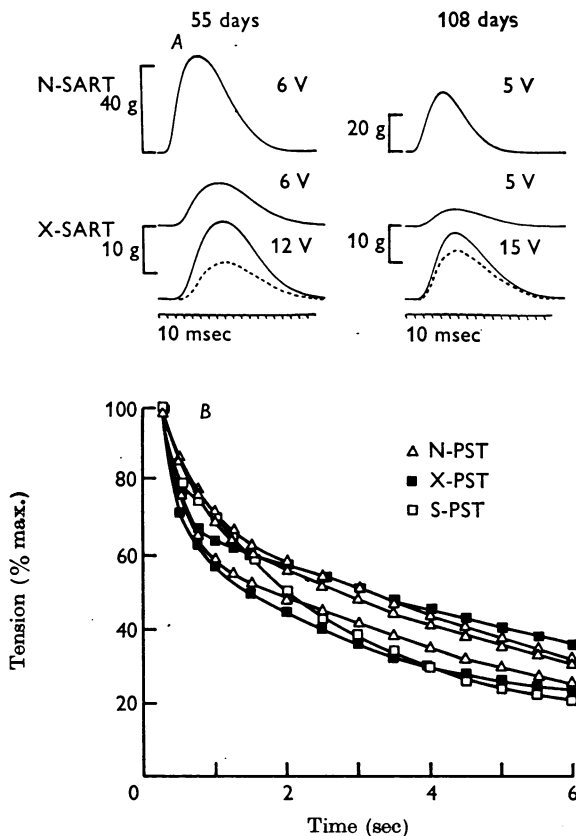


Fig. 3. (A) Isometric records of twitch contractions of normally innervated sartorius muscles (N-SART) and of cross-innervated sartorius muscles (X-SART) from two toads 55 days (left column) and 108 days (right column, from Fig. 1) after nerve cross-union, in response to the stimulating voltages indicated. Six volts excited all the low threshold nerve fibres to both the N-SART and X-SART muscles of the toad examined 55 days post-operatively while 12 V stimulated both the low threshold nerve fibres and the high threshold nerve fibres of the X-SART muscle of this animal; for the other animal the corresponding stimulus voltages are 5 V and 15 V. The time courses of the isometric twitch contractions of X-SART in response to stimulation of high threshold nerve fibres are shown as interrupted curves and were obtained by subtracting the responses to stimulation of low threshold nerves from the responses of the whole muscles. The tension calibrations of the two twitches of each X-SART are identical. (B) The time course of relaxation of the slow-graded component of isometric tetanic tension of three of the normal ( $\Delta$ ), two cross-innervated ( $\blacksquare$ ) and one self-innervated ( $\square$ ) PST muscles. These are from the two animals described in Figs. 1 and 5 and another with nerve cross-union examined 55 days post-operatively. Tension as percentage of the maximum tension developed by the slow-graded fibre component of each muscle is plotted against time in seconds. Temperature = 22.0–24.5° C.

time curves. These were obtained for each muscle by subtracting the twitch response of fibres innervated by LTN (6 V or 5 V) from the response of the muscle to maximal stimulation of the whole PST nerve (12 V or 15 V). The only apparent difference in the time courses of the responses of the two components of X-SART muscles innervated by LTN and HTN is the longer latency for muscle fibres innervated by HTN.

Table 1 shows that the means of the minimal and maximal thresholds of the LTN and HTN of the PST nerves innervating X-SART muscles are in nearly all cases higher than those for normal PST nerves. However, results of statistical analyses of the differences between the means of these thresholds for repetitive stimulation showed that these differences are not significant at the 5% level of probability.

*Properties of PST muscles and sartorius nerves following cross-union.* Sixteen toads survived for experiment with PST muscles which had been cross-innervated with the sartorius nerve. In one of these the PST muscle was transplanted from the contralateral side, and in the others the sartorius and PST nerves were cross-united. Nine PST muscles were successfully innervated by sartorius nerve. Four muscles failed to receive any nerve fibres, and in the remaining three preparations the PST muscle was wholly or partly reinnervated, by both LTN and HTN; the latter came presumably from the PST nerve.

Records of twitch and tetanic contractions of a cross-innervated PST (X-PST) muscle and those of contralateral control are shown below the corresponding records for the X-SART muscle in Fig. 1. The curves relating stimulus strength and isometric twitch and tetanic tensions for these muscles are shown in Fig. 2*A, C*. The diagrams show that slow-graded fibres persist in the PST muscle following innervation by the sartorius nerve and that the responses of the slow-graded muscle fibres are evoked by stimulation of LTN of sartorius nerve. This result has been confirmed in nine X-PST muscles examined 55–201 days post-operatively. Furthermore, the range of thresholds for stimulation of nerve fibres associated with slow-graded responses is almost identical with that for nerve fibres innervating the fast-twitch fibres of the same muscles (Table 1).

The mean values for the contractile properties of the X-PST muscles are not the same as those for N-PST muscles (Table 1) but comparison of results from five X-PST muscles and their contralateral controls shows that none of these differences is significant at a significance probability  $P \leq 0.05$ . X-PST muscles, in contrast with X-SART muscles, did not show a decline in tetanic tension during repetitive nerve stimulation. Furthermore, comparison of isometric tetanic tensions produced by direct stimulation and nerve stimulation showed that, in five out of six muscles examined, the sartorius nerve fully innervated the PST muscles (e.g. Figs. 1, 2). The

exception was the PST muscle examined 55 days after the operation and it is possible that in this instance insufficient time was allowed for the development of full, functional innervation. The insignificant difference between the maximum force developed per unit cross-sectional area of X-PST muscles and their contralateral controls when stimulated indirectly also points to complete innervation. Figure 3*B* shows that the time course of relaxation of the slow-graded muscle fibre component of two X-PST muscles examined 55 days and 108 days (the latter described in Fig. 1) post-operatively is unaltered following innervation by sartorius nerve. All other X-PST muscles show similar results.

The question arises whether slow-graded muscle fibres of X-PST are innervated by LTN of sartorius nerve and excited synaptically, or whether the slow fibres are denervated in these muscles and are merely excited ephaptically by the summed effect of action currents of neighbouring fast-twitch muscle fibres. An attempt has been made to answer this question by comparing the effects of direct stimulation of normal, cross-innervated and denervated muscles. In these experiments it has been found that N-PST and X-PST muscles show no slow-graded fibre response to direct stimulation in Ringer solution containing  $2 \times 10^{-5}$  g tubocurarine Cl/ml. with repetitive stimulation (80 c/s) of sufficient strength to excite all the fast-twitch muscle fibres. Five denervated PST (D-PST) muscles were also examined with direct stimulation. In two of these examined 62 and 138 days post-operatively, it was possible to obtain no slow-graded response with stimuli adequate to excite all the denervated fast-twitch muscle fibres. Four of these D-PST muscles denervated for periods ranging from 98 to 139 days developed a very prolonged contracture in response to direct stimulation with 15 V, 0.3 msec pulses. However, denervated sartorius (D-SART) muscles from the same animals did not show these prolonged contractures in response to direct stimulation and it is probable that the contracture of D-PST is the response of slow-graded fibres to direct stimulation after prolonged denervation. The time course of this contracture was not the same as that for innervated slow-graded muscle fibres; in some denervated muscles this contracture reached a peak about 1–3 sec after the end of the tetanus and the rate of subsequent relaxation was about 5 times slower than that for the slow-graded muscle fibres in N-PST or X-PST muscles stimulated via the nerve. The maximum contracture tension was about the same as the maximum tension developed by the slow-graded component of N-PST muscles, but varied according to the position of the stimulating electrodes, being maximal with the electrodes at the middle of the muscle and minimal at the ends. The strength of stimulus required to elicit the contracture response in D-PST muscles was higher than the threshold for stimulation of all or most of the fast-twitch fibres. In two cases 85–90% of the tension of the maximal fast tetanic tension could be elicited without any contracture. When the fast component of tetanic tension was fully elicited in these muscles, the accompanying contracture tension was 14–33% of the maximal contracture tension. These differences in threshold and the differences in the time course of tension changes of the slow-graded component of D-PST and X-PST argue against the possibility that slow-graded fibres of X-PST are denervated and excited ephaptically following nerve stimulation. The alternative view, that LTN of sartorius nerve actually forms functional synaptic connexions with the slow-graded fibres of PST, is supported by the results for the transplanted PST muscle reinnervated by sartorius nerve and of work on acetylcholine contractures described below.

The transplanted PST was very poorly reinnervated by the sartorius nerve. The total maximum isometric tetanic tension was only 15 g. Records of twitch and tetanic contractions of this muscle are shown in Fig. 4*A, B*. The curves relating stimulus strength and

sometric tetanic tensions for the fast-twitch and slow-graded components, shown in Fig. 4C, indicate two well-defined steps in both tension components and this is probably due to two motor axons innervating the muscle. These changes of tensions were always in step with each other despite attempts to separate them. However, towards the end of the experiment, a small pure slow-graded response was obtained at 2.1 V (Fig. 4C, inset).

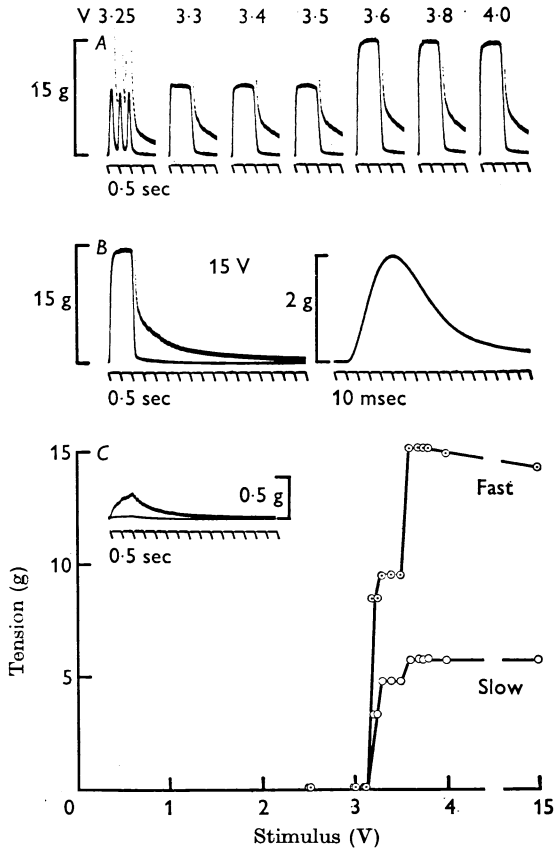


Fig. 4. (A) Records of isometric tetanic contraction of the PST muscle transplanted from the opposite side and cross-united with the sartorius nerve 119 days before the experiment. The records were displayed in the same way as described in Fig. 1, except that one beam of the oscilloscope has a gain 10 times the other. The tension calibration refers to the record of the whole response displayed on the low-gain beam. The strength of the stimulus applied to the sciatic nerve is shown in volts (V) above each record. (B) Records of isometric twitch (right) and tetanic (left) contractions of the same muscle in response to 15 V applied to the nerve. (C) Relation between strength of stimulus (V) applied to the sciatic nerve and the maximum isometric tetanic tension of the whole muscle ( $\odot$ ) and of the slow-graded component ( $\circ$ ). The tension scale on the Y-axis refers to the whole muscle response, while that for the slow-graded component is 1/10 of this scale. Inset, a pure slow-graded response obtained some time after the series of records were obtained. Muscle weight = 120 mg (muscle encapsulated in fibrous tissue); approximate average fibre length = 18 mm. Temperature = 23.5–24.5° C.

The simplest explanation for these observations is that this muscle was reinnervated by three low-threshold sartorius nerve fibres, one innervating only slow-graded fibres while the other two innervated both fast-twitch and slow-graded muscle fibres. However, further work is necessary to establish the point that both types of muscle fibre can be reinnervated by one foreign axon.

Table 1 shows that the means of the minimal and maximal thresholds of the sartorius nerves innervating X-PST are in all cases higher than those for normal sartorius nerves. Statistical analyses of the means of these thresholds for repetitive stimulation show that the maximal thresholds of sartorius nerve fibres innervating the fast-twitch ( $P < 0.025$ ) and the slow-graded ( $P < 0.05$ ) PST muscle fibres are significantly higher than corresponding maximal thresholds for normal sartorius nerves. Differences in the minimal thresholds of sartorius nerve fibres innervating the fast-twitch ( $P < 0.3$ ) and slow-graded ( $P < 0.1$ ) PST muscle fibres are not significantly higher than the corresponding minimal thresholds for normal sartorius nerves.

*Properties of self-innervated sartorius and PST muscles and their nerves.* Five animals survived for experiment following the self-union operation. Two of these were in very poor condition at the time of the experiment. The responses of the muscles of these two animals showed rapid failure of neuromuscular junctions even to low-frequency stimulation of normal muscles and were therefore discarded. Results were obtained for muscles of the other three animals 104–136 (mean 125) days after the operation and these are summarized in Table 1. Representative records of isometric twitch and tetanic contractions of self-innervated sartorius and PST (S-SART, S-PST) muscles and contralateral control muscles examined 104 days post-operatively are shown in Fig. 5 and the relations between stimulus strength and maximum tension for these muscles are present in Fig. 6.

The normal pattern of innervation, LTN innervating fast-twitch fibres and HTN innervating slow-graded fibres, redeveloped in all self-innervated muscles except one in which LTN of the PST nerve innervated some of the slow-graded muscle fibres (see Figs. 5, 6C).

The *t*-test was used to determine whether there were significant differences between the values for  $T_c$ ,  $P_i/P_0$  and the maximum force per unit cross-sectional area of muscle of self-innervated muscles and their contralateral controls. None of these differences is significant, the *P*-values being greater than 0.05. Unlike X-SART muscles (e.g. Fig. 1) the S-SART and S-PST muscles did not show a marked decline in tetanic tension during repetitive nerve stimulation. The self-innervated muscles were not tested systematically for completeness of reinnervation, but the fact that the maximum force developed per unit cross-sectional area is not significantly different

from that of the contralateral control muscles must mean that most, if not all, of the muscle fibres were re-innervated.

Table 1 shows that there are no consistent differences between the mean thresholds of self-united nerves and normal nerves. Statistical analyses of these thresholds for repetitive stimulation showed that none of these differences is significant ( $P > 0.2$ ).

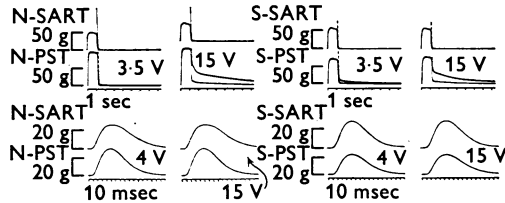


Fig. 5. Isometric records of tetanic and twitch contractions of the self-innervated sartorius (S-SART) and posterior semitendinosus (S-PST) muscles and their normally innervated contralateral controls (N-SART, N-PST) from a toad 104 days after the nerve self-union operation. The records were obtained and displayed in the same way as those described in Fig. 1. Muscle weight: N-SART = 89 mg, N-PST = 79 mg, S-SART = 100 mg, S-PST = 70 mg; average muscle fibre length: N-SART = 42 mm, N-PST = 25.5 mm, S-SART = 42 mm, S-PST = 25.5 mm. Temperature = 23.5–23.6° C.

*Acetylcholine contractures of normal, self-innervated, cross-innervated and denervated sartorius and PST muscles.* Contractures were recorded from six N-SART, one X-SART (described in Figs. 1, 2) one S-SART (described in Figs. 5, 6) and four denervated sartorius (D-SART) muscles in response to  $2 \times 10^{-5}$  g acetylcholine Cl/ml. Ringer. In all these muscles the response was transitory. The N-SART muscles developed a mean maximum contracture tension of 4.9 g (range 1–8.8 g) within a few seconds after adding the acetylcholine. The mean contracture half-relaxation time (i.e. time from peak contracture tension to the time when the tension falls to half this value) was about 20 sec and tension fell to zero between 20 and 120 sec after the onset of contracture. Similar responses were recorded from S-SART and X-SART muscles. D-SART muscles developed a higher mean contracture tension of 21.2 g (range 11.2–32.6 g) and the mean contracture half-relaxation time was 60 sec; zero tension was reached within 2–6 min after the onset of contracture, but in all cases the contracture tension was less than 5% of maximum within 3 min.

Contractures were recorded from six N-PST, one S-PST, one X-PST and four D-PST muscles in response to  $2 \times 10^{-5}$  g acetylcholine Cl/ml. Ringer. Contracture tension–time curves of some of these muscles are presented in Fig. 7. The N-PST muscle gave a sustained contracture with a mean maximum contracture tension of 7.9 g (range 7–12 g). The mean tetanic tension of the slow-graded fibre component of these muscles was 13.8 g



(range 11.5–16.7 g). In all but one muscle the maximum contracture tension was less than the tetanic tension of slow-graded component and had a mean value of 69% of the latter. The contracture tension fell gradually and at the end of 15 min after the onset of contracture the mean residual tension was 82% (range 76–88%) of the maximum contracture tension.

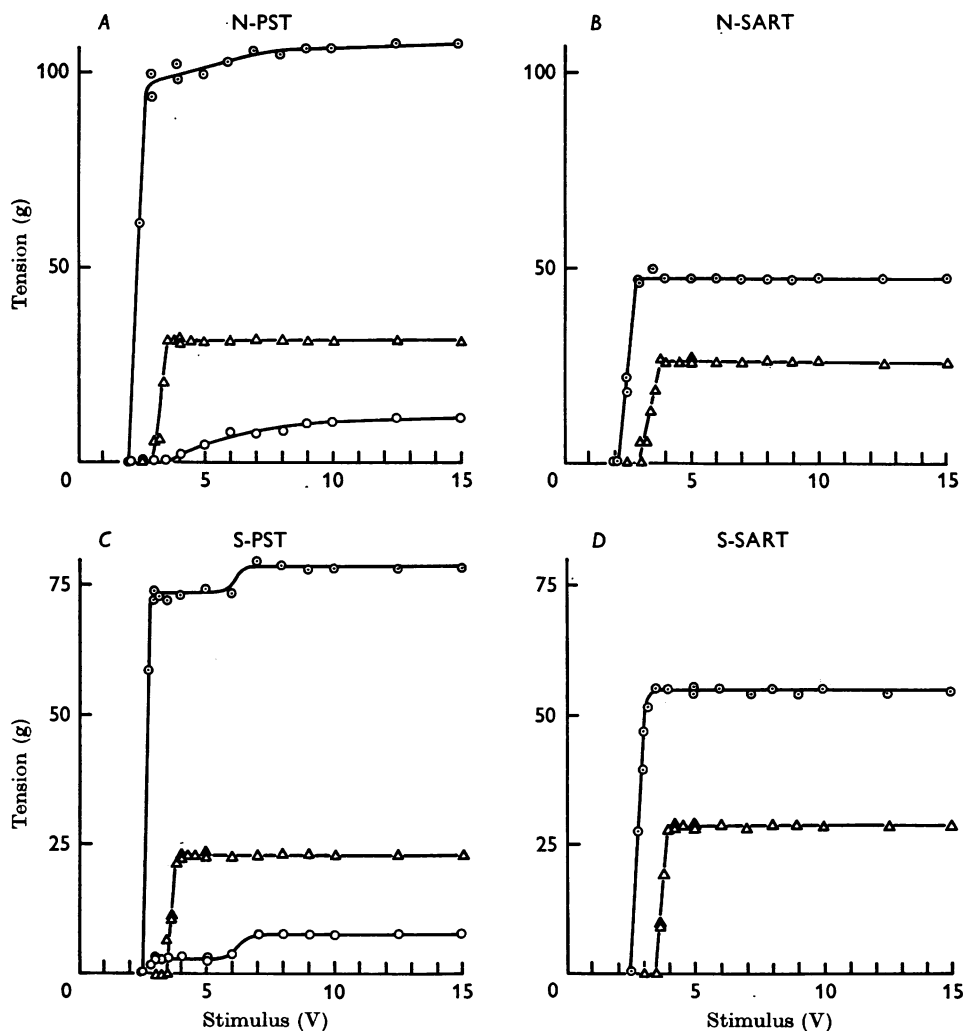


Fig. 6. Maximum tensions developed in isometric twitch ( $\Delta$ ) and tetanic (whole muscle =  $\odot$ , slow-graded component =  $\circ$ ) contractions, plotted against stimulus strength (V) applied to the sciatic nerve for the normally innervated PST muscle (A), the normally innervated sartorius muscle (B), the self-innervated PST muscle (C), and the self-innervated sartorius muscle (D) from a toad 104 days after the self-union operation. Representative records of the series of contractions are shown in Fig. 5.

The X-PST (described in Figs. 1, 2) and S-PST (described in Figs. 5, 6) muscles developed comparable maximum contracture tensions (6.7 g and 7.7 g respectively) but tension declined more rapidly than normal within the first 3 min and slowly thereafter to reach about 50–60% of the maximum contracture tension within 15 min (see Fig. 7).

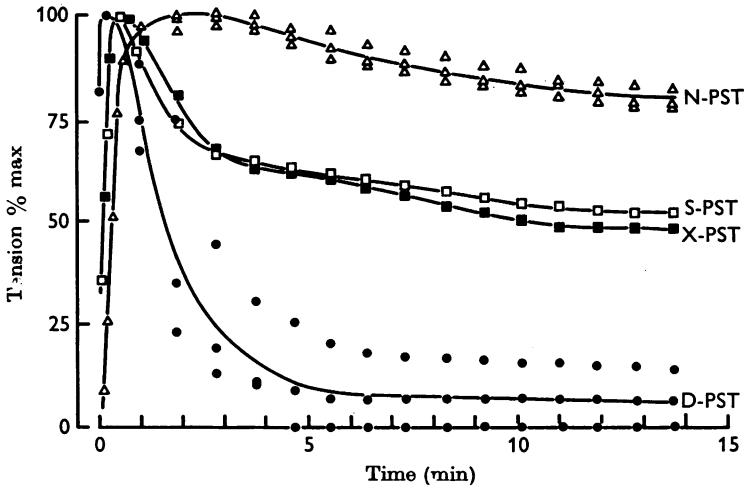


Fig. 7. Acetylcholine-contraction tension-time curves of PST muscles. Ordinate: contracture tension expressed as percentage of the maximum contracture tension of each muscle. Abscissa: time in minutes after addition of acetylcholine to the bath. Maximum contracture tensions for normal PST ( $\Delta$ ) = 8.1 g (mean; range 7.0–9.4 g); for self-innervated PST ( $\square$ ) = 6.7 g; for cross-innervated PST ( $\blacksquare$ ) = 7.7 g; for denervated PST ( $\bullet$ ) = 20.1 g (mean; range 17.5–23.4 g). Temperature = 20.2–24.5° C.

D-PST muscles differ markedly from N-PST, X-PST and S-PST muscles in having a high maximal contracture tension (mean 19.5 g, range 17.4–23.4 g) and a rapid drop of contracture tension. In one of these, studied only 62 days after denervation, the time course of the contracture was similar to those of the cross-innervated or self-innervated PST muscles and declined to 33% of the maximum contracture tension within 15 min after onset. The contracture curves of the other three D-PST muscles were obtained 98–138 days post-operatively (mean 120 days) and are shown in Fig. 7. There was a rapid fall of contracture tension in all these muscles. The contracture half-relaxation times ranged from 1.5 to 2.5 min and the tension at 15 min ranged from 0% to 13% of maximum contracture tension. In one of these muscles, examined 98 days post-operatively, the contracture disappeared within 5 min.

## DISCUSSION

*Innervation.* Two groups of nerve fibres with different thresholds form functional connexions with sartorius muscle fibres after reinnervation by PST nerve. There is no direct evidence which excludes the possibility that the HTN innervating X-SART were originally LTN innervating PST fast fibres and that these nerve fibres had undergone a marked increase in threshold as a result of innervating a foreign muscle. However, sartorius LTN do not become transformed into HTN after innervating the foreign PST muscle. Furthermore, the thresholds of HTN innervating X-SART are nearly the same as HTN in normal PST nerve and both are clearly separated from the thresholds of LTN. In view of these observations it is more likely that the HTN innervating X-SART were the small nerve fibres originally innervating the slow-graded muscle fibres of the PST.

The pure slow-graded contraction of transplanted X-PST in response to indirect stimulation shows that LTN of sartorius nerve are able to make functional connexions with slow-graded muscle fibres and indirect evidence indicates that this occurs in other X-PST. For example, it is possible to stimulate directly all, or nearly all, fast-twitch fibres of curarized X-PST or D-PST with little or no response of the slow-graded muscle fibres. Furthermore, the properties of slow-graded muscle fibres in X-PST and D-PST differ markedly in the rate of relaxation following a tetanus and in the time course of the contracture brought about by acetylcholine. Consequently some, if not all, the slow-graded muscle fibres of X-PST must have been reinnervated by LTN of sartorius nerve and were excited synaptically during indirect stimulation.

There are differences in degree and duration of functional reinnervation of cross-innervated muscles by alien nerves. The sartorius nerve innervates all, or nearly all, muscle fibres of the PST muscles, while the PST nerve functionally innervates an average of 60% of the total number of sartorius fibres as indicated by  $P_0$  of cross-innervated muscles. Some or all of the sartorius muscle fibres, which were not functionally reinnervated at the time of the experiments, may not have been anatomically denervated. During regeneration of a neuromuscular synapse there is a period of time between the arrival of an axon at the end-plate and the time when the regenerated junction can transmit an impulse (Miledi, 1960). It is possible that all X-SART muscle fibres were anatomically reinnervated but some regenerated junctions failed to develop the ability to transmit an impulse. The PST nerve maintains functional connexions with sartorius muscle up to 134 days after operations but the results obtained so far do not show clearly what happens to the new junctions after longer periods. Only two animals were examined about 200 days after the operation. In both of

these the PST nerve failed to innervate the sartorius muscles functionally whereas the sartorius nerves not only innervated the PST muscles, but in each case sent a collateral nerve to reinnervate the sartorius muscle functionally. These collateral nerves arose from the proximal stumps of the sartorius nerves near the site of cross-union and traversed in a direct path to reinnervate the sartorius muscle, the distal stumps of the sartorius nerves being atrophic in both cases. An axon reflex could be elicited in the X-PST muscle by stimulating the collateral nerve near the sartorius muscle. These observations indicate the existence of some kind of incompatibility between the PST nerve and the sartorius muscles, and also illustrate the remarkable preference of the sartorius muscle for its own nerve. The decline in tetanic tension during indirect stimulation of X-SART is similar to that of recently reinnervated rat muscles (Thomson, Morgan & Hines, 1950), and probably results from presynaptic failure such as that observed in the rat diaphragm (Krnjević & Miledi, 1958) and in the regenerating synapses of the frog sartorius muscle (Miledi, 1960), but it may also be due to progressive neuromuscular depression. This phenomenon has not been observed in X-PST or S-PST and may be another expression of the incompatibility between the PST nerve and the sartorius muscle fibres. These observations also point to the possibility that incompatibility and withdrawal of alien motor nerve fibres may lead to selective reinnervation such as that described for muscles in chick (Feng *et al.* 1965) and fish (Sperry & Arora, 1965; Mark, 1965). With regard to mammalian muscles, there is at present some doubt about whether there is selectivity in reinnervation (Elsberg, 1917; Weiss & Hoag, 1946; Bernstein & Guth, 1961).

Overlap in the normal innervation of the fast-twitch and slow-graded extrafusal muscle fibres has not been observed (Kuffler & Vaughan Williams, 1953; Gray, 1957; Hess, 1960). The results from the transplanted PST muscle suggest that an LTN fibre can innervate both fast-twitch and slow-graded muscle fibres at the same time, but further work is necessary to establish this point. No information is available regarding other possible patterns of abnormal innervation following cross-union.

Preliminary attempts to demonstrate end-plate morphology in cross-innervated muscles using the gold chloride method (Boyd, 1962) have been unsuccessful even though the same technique successfully stained *en plaque* and *en grappe* endings in normal fast-twitch and slow-graded muscle fibres respectively. Hník *et al.* (1967), using cholinesterase staining, have demonstrated in the chick that, following cross-union of nerves to fast and slow muscles, the end-plates of the foreign nerves were of the same morphological type as those in the original muscle.

*Interactions between nerve and muscle.* X-SART muscle fibres reinner-

vated by HTN of PST nerve gave only fast responses without any slow-graded component. The  $T_c$  of the twitch elicited by HTN was the same as the  $T_c$  of the twitch when only the LTN was stimulated. The small but statistically significant increase in  $T_c$  of X-SART may have resulted from the dispersal of the latency of muscle action potentials following the stimulus on the nerve because of a wider range of conduction velocities in the regenerated portion of the nerve. Asynchronous contraction of muscle fibres might also explain the observed decrease in the  $P_i/P_0$  of sartorius after nerve cross-union. All X-PST muscles successfully reinnervated by sartorius LTN showed persistence of slow-graded muscle fibres. The rate of relaxation of the slow-graded fibres following a tetanus was unaltered as a result of foreign innervation. It is not possible to determine directly from whole muscle and whole nerve preparations whether some X-PST slow-graded fibres had been transformed into fast-twitch fibres, but it is unlikely that this occurs to any appreciable extent because  $P_i/P_0$  of the X-PST are not significantly different from those of their contralateral controls. It may be concluded that cross-union of the nerves to PST and SART leads to reinnervation of the muscle fibres by alien nerve fibres but this does not cause any obvious change in the characteristic responses of fast-twitch and slow-graded components of these muscles which can be attributed to specific neural influences up to 134–200 days after operations. This does not mean that neural influences do not determine the characteristic fast-twitch and slow-graded properties at some stage in development. These results do not exclude the possibility that biochemical properties of these muscle fibre types (Lännergren, 1965; Lännergren & Smith, 1966) may change following nerve cross-union in the adult toad, as reversal of enzyme profiles of fast and slow mammalian muscles have been demonstrated following nerve cross-union (Romanul & Van der Meulen, 1967; Dubowitz, 1967).

Results from work on the contracture responses to acetylcholine support the conclusion that fast-twitch and slow-graded components of toad muscles are not transformed following nerve cross-union. The contractures of X-SART are larger and longer than normal but are still transitory, there being no sign of a prolonged contracture typical of slow-graded muscle fibres. Contractures of X-PST and S-PST are very similar and little different from the characteristic sustained contractures of N-PST. On the other hand, PST muscles denervated for 4 months show poorly sustained contractures which resemble those reported by Miledi & Orkand (1966) for frog iliofibularis muscles 6 months after cross-innervation by the sartorius nerve. They suggested that the sartorius nerve had transformed slow-graded muscle fibres into 'twitch-like' fibres but in the absence of information on mechanical responses to nerve stimulation the possibility

remains that the 'twitch-like' fibres were denervated slow-graded muscle fibres.

The importance of peripheral connexions in the maturation of regenerating nerve fibres in mammals has been shown by Weiss, Edds & Cavanaugh (1945), Sanders & Young (1946), Aitken, Sharman & Young (1947) and Aitken (1949). More recently Tomanek & Tipton (1967) have shown that there is a reduction in fibre diameter and number of myelinated nerve fibres in intact nerves to tenectomized rat muscles. It is not known whether there is a similar peripheral influence which affects the diameter of nerve fibres in the toad. However, the demonstration of a statistically significant increase in threshold of sartorius nerve fibres reinnervating PST muscles in contrast with the lack of significant differences in the thresholds of self-unioned and normal nerves may mean that nerve fibres central to the site of operation remain in their normal size after self-union, but become smaller after cross-union. This points to the possibility that toad muscles may exert an influence on nerve fibres, thereby affecting nerve fibre threshold and diameter, and, furthermore, that this influence from muscles may be specific for nerves normally innervating them.

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