INTERACTIONS BETWEEN NERVE AND MUSCLE

by

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Some of the investigations described in this thesis have been done in collaboration with Dr. R. Close, and these have all been published or accepted for publication and are listed in the following page. All other investigations described in this thesis are my own original work.

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As a result of investigations in which I have collaborated during the tenure of my Scholarship at the Australian National University, the following papers have appeared or have been accepted for publication:-

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SECTION I

GENERAL INTRODUCTION : INTERACTIONS BETWEEN NERVE AND MUSCLE

The functional relationships between the skeletal muscle and its nerve supply has been extensively studied with electrophysiological techniques so that there exists now a fairly clear understanding of the phenomena associated with the transmission of electrical impulses to and from skeletal muscle. There are other relationships between muscle and nerve which are as yet poorly understood, such as their mutual interaction during morphogenesis, the long-term "trophic" relations and the specificity of their connexions. These phenomena have been studied in a wide range of vertebrates, mainly during development, following denervation and during nerve regeneration. A brief survey of this field is presented here from the following points of view:-

1. Effects of the nervous system on muscle.
2. Effects of muscle on the nervous system.
3. Specificity of nerve-muscle connexions.

1. Effects of the nervous system on muscle.

(a) Muscle differentiation.

In the last century, it was thought that the nervous system played a decisive role in the initial differentiation of muscle from undifferentiated blastema (Zelená, 1962). This view was disproved by experimental studies on embryos of amphibians (Harrison, 1907; Hooker, 1911; Hamburger, 1939a; Piatt, 1942, 1952) and birds (Hoadly, 1925a, 1925b; Hunt, 1932; Hamburger, 1939a; Eastlick, 1943; Eastlick and Wortham, 1947) in which innervation of premuscle tissue was avoided by operative procedures. Muscle fibres with myofibrils developed in the absence of innervation even though uninnervated muscle tissue was atrophic and might finally degenerate. No comparable experiments have been done on mammals. However, Zelená (1962) has been able to denervate developing muscles at the myotube stage and has shown that mature extrafusal muscle fibres can develop in the absence of reinnervation. Tissue culture experiments (Shimada, Fischman & Moscona, 1967) have also demonstrated that muscle fibres can develop from myoblasts in the absence of innervation.

The differentiation of the subneural apparatus of

the extrafusal muscle fibres is directly dependent on the influence of the nerve (Zelená & Szentágothai, 1957). Denervation of developing rat muscles at the myotube stage when neuromuscular junctions are just being formed lead to rapid disappearance of cholinesterase activity which characterizes the subneural apparatus.

Extrafusal muscle fibres of a wide range of adult vertebrates can be differentiated into fast and slow types having distinct contractile properties with or without distinct morphological features and patterns of innervation (for literature on fish, see Barets, 1961; anurans: Peachey, 1961; birds: Ginsborg, 1960b; mammals: Close & Hoh, 1967). At the present time, it is not known whether these muscle fibre types differentiated spontaneously or as a result of some influence originating from the nerve, though it has been suggested that both mechanisms play a part in the differentiation of cat fast and slow muscles (Buller, Eccles & Eccles, 1960a).

The role of the nervous system in the development of muscle receptors has been studied in the rat (Zelená, 1957). Muscle spindles differentiate from myotubes in the vicinity of sensory nerve fibres 3 days before birth.

If muscles are denervated at this stage, no muscle spindles are formed. If denervation of muscles is carried out at birth, when the nuclear bag of muscle spindles has just been formed, muscle spindles do not differentiate further and disintegrate within a few days (Zelená, 1957). Similarly, the differentiation of tendon organs is completely arrested after this post-natal denervation (Zelená and Hník, 1963).

(b) Trophic influences of the motoneurone.

It has long been known from clinical observations that the nervous system is necessary for the maintenance of normal structure and function of muscles. For example, if the nerve supply to a muscle is cut, it gradually undergoes atrophic changes which are reversed following reinnervation. This long-term influence of the nervous system on muscle is referred to as "trophic influence", and it is mediated by motor nerve fibres (Tower, 1935) while sensory and sympathetic nerve fibres play no significant role (Tower, 1931a, 1931b).

Histological, biochemical and physiological changes in muscle following denervation and their reversal during regeneration have been studied extensively, and there

exists now an extensive literature on this subject (for reviews, see Tower, 1939; Gutmann & Hník, 1962; Gutmann, 1964). Most of the histological and biochemical changes in denervated muscle possibly do not differ significantly from those occurring in muscle atrophy induced by disuse or tenotomy (Tower, 1937; Žák, 1962), but a few changes in membrane properties, such as fibrillation (Hník & Škorpil, 1962), lengthening of electrical time constants (Desmedt, 1950a, 1950b) and increase and spreading of acetylcholine sensitivity (Kuffler, 1943; Miledi, 1960; Thesleff, 1960, 1961) in some mammalian and amphibian muscles, are specific changes of muscle fibres to denervation.

The weight of present evidence suggests that the trophic influence of the motoneurone which prevents changes that occur following denervation does not depend on impulse transmission. The onset of post-denervation changes in muscle depend on the length of the distal stump of the severed nerve; these changes commence earlier in muscles with shorter distal stumps (Luco & Eyzaguirre, 1955; Emmelin & Malm, 1965). When motoneurones are silenced by surgically isolating a segment

of the spinal cord, there is atrophy from disuse, but fibrillation and spreading of acetylcholine sensitivity do not occur (Tower, 1937; Johns & Thesleff, 1961). Interruption of nerve conduction by a local anaesthetic does not lead to the rapid biochemical changes in muscle which follows denervation (Gutmann & Žák, 1961). Prolonged curarization does not produce changes in contractile properties which occur following denervation of the same duration (Lanari & Lopez Amalfará, 1966). On the other hand, neuromuscular block produced by botulinum toxin lead to changes which are indistinguishable from denervation (Thesleff, 1960, 1961; Josefsson & Thesleff, 1961; Jirmanová, Sobotková, Thesleff & Zelená, 1964). Since botulinum toxin blocks even the spontaneous quantal release of acetylcholine which is not blocked by other measures of preventing neuromuscular transmission (Thesleff, 1960, 1961; Josefsson & Thesleff, 1961), it has been suggested that acetylcholine released spontaneously at the neuromuscular junction may normally prevent fibrillation and the increase and spreading of acetylcholine sensitivity. Recent experiments on developing chicks in which marked atrophy of skeletal muscles

observed when botulinum toxin, curare and hemicholinium were used to block the neuromuscular junction have been interpreted as supporting the view that acetylcholine released spontaneously at the neuromuscular junction may function as the transmitter of the motor nerves' trophic influence (Drachman, 1964, 1967, 1968). These results did not rule out the possibility that disuse of muscles during development without neuromuscular block could lead to the observed degree of atrophy even though it was shown that tenotomy resulted in only a mild degree of atrophy.

A new approach to the study of trophic influences of the motoneurone on skeletal muscle was introduced by Buller, Eccles and Eccles (1960b) who cross-united nerves to fast and slow muscles in cats and found that the contraction times of cross-innervated fast muscles was increased and that of cross-innervated slow muscles was reduced. As one of the principal differences in the dynamic properties between fast and slow mammalian muscles is in the force:velocity properties (Close, 1964, 1965a; Close & Hoh, 1967) it would be of interest to know if these properties are altered following nerve cross-union.

Further work on cross-innervated cat muscles in which force:velocity properties were not directly measured suggested that these properties were changed only in the originally fast muscles (Buller & Lewis, 1965b). In contrast, cross-innervation of fast and slow muscles in the rat leads to the reversal of force:velocity properties in both fast and slow muscles (Close, 1965b).

Two possible mechanisms whereby motoneurones mediate the influence on the speed of contraction of the muscle fibres they innervate have been suggested. These are the pattern of nerve impulses passing down the motor nerve and a hypothetical trophic substance secreted by the motoneurone and passing to the muscle fibres (Buller, Eccles & Eccles, 1960b). Several attempts have been made to elucidate the role of the pattern of nerve impulses. These experiments have either produced small changes in contraction times (Eccles, Eccles & Kozak, 1962; Vrbová, 1966) or large changes in contraction time accompanied by proportional changes in the twitch:tetanus ratio (Salmons & Vrbová, 1967). However, there is as yet no evidence that these changes are the same as those which follow nerve cross-union as they

could occur with an increase in the duration of the active state of the twitch with no change in the force:velocity properties (Hill & Macpherson, 1954).

Nerve cross-union has been used increasingly to investigate other possible neural influences on various properties of fast and slow mammalian muscles which may account for the differences between them. It has been shown that some biochemical differences between these muscles, such as intracellular glycogen and potassium levels (Drahota & Gutmann, 1963), electrophoretic pattern of soluble proteins (Guth & Watson, 1967), soluble enzymes (Prewitt & Salafsky, 1967; Guth, Watson & Brown, 1968), the principal pathway of energy metabolism (Romanul & Meulen, 1966, 1967; Dubowitz & Newman, 1967; Dubowitz, 1967) and the ratio of the concentration of myoglobin in fast and slow muscles (McPherson & Tokunaga, 1967) are changed or reversed following nerve cross-union. The manner in which motoneurones exert these influences is not known, but the possibilities are presumably the same as those described above for the neural influence on the speed of muscle contraction.

2. Effect of muscle on the nervous system.

(a) During development.

It has been recognized for some time that the non-nervous periphery exerts a profound influence on the development of nervous elements innervating it. Thus, when the peripheral field of innervation is reduced, there is a reduction in the number of motoneurones innervating that field. This has been shown in urodeles (Stulz, 1942), anurans (May, 1930; Beaudoin, 1955; Flanigan, 1960; Hughes, 1962; Prestige, 1967b), birds (Hamburger, 1934; Bueker, 1943; Barron, 1948; Mottet, 1952; Dunnebacke, 1953) and mammals (Curtis & Helmholz, 1911; Barron and Barcroft, 1938). Conversely, the number of motoneurones are increased in animals with supernumerary limbs or digits (Hamburger, 1939b; Tsang, 1939; Baumann & Landauer, 1943; Bueker, 1945). In many different vertebrates spinal ganglion cells have been found to be similarly affected by a decrease or increase in the peripheral field of innervation (Detwiler, 1920, 1924; May, 1930; Hamburger, 1934, 1939b; Barron, 1945; Hall & Schneiderhan, 1945; Prestige, 1967a). Both muscle and skin exert this influence on ganglion cells (Detwiler, 1926, 1927).

The nature of this peripheral influence on the differentiation of nerve cells is not known. Since the number of cells in a given region of the nervous system at any stage of development is the result of cellular proliferation, migration, maintenance and degeneration (Hamburger & Levi-Montalcini, 1950), an adequate analysis of this mechanism must take these factors into account. Recent attempts in this direction (Hughes & Tschumi, 1958; Hughes, 1962, 1964; Prestige, 1967a, 1967b) suggest that ganglion cells and motoneurones undergo a labile stage in their development during which they depend for their further differentiation and maintenance on essential factors carried centripetally in their axons. Failure to make adequate contact with the periphery at this stage leads to degeneration of the neuron. This concept provides a plausible explanation of cell deaths previously observed during normal neurogensis (Romanes, 1946; Hamburger & Levi-Montalcini, 1949; Glücksman, 1951; Hughes, 1961, 1962; Prestige, 1965).

(b) Nerve regeneration.

Studies on the regeneration of peripheral nerves have revealed the influence of peripheral connexions on

the diameter of nerve fibres. When peripheral nerve fibres are crushed and permitted to reinnervate the original end-organs, a normal fibre size spectrum is regained (Gutmann & Sanders, 1943). If, however, regenerating nerve fibres are prevented from entering an end-organ (Weiss, Edds & Cavanaugh, 1945; Cavanaugh, 1951) or are directed into end-organs with which they do not make functional connexions (Simpson & Young, 1945; Sanders & Young, 1946; Aitken Sharman & Young, 1947) both the regenerated and the proximal segments of sensory and motor nerve fibres suffer marked reduction in size. Muscle nerve fibres regenerating into denervated muscles mature more rapidly than those regenerating into normally innervated muscles (Aitken, 1949). Separation of the nerve from its muscle periphery at an early age results in complete cessation of nerve growth (Evans & Vizoso, 1951). Chronic disconnexion of an axon from its periphery leads to a permanent reduction in the size of its cell body, nucleus and nucleolus (Cavanaugh, 1951).

3. Specificity of nerve-muscle connexions.

(a) Specificity of synaptic connexions.

Highly specific connexions between muscle and nerve are established during development. Not only are different components in a muscle nerve connected to their appropriate end-organs, but also individual muscles are connected to appropriate centres in the nervous system to enable innervated organs to function in a coordinated manner. The current concepts underlying the establishment of these specific connexions between nerves and appropriate end-organs are matters of long-standing controversy.

Ramón y Cajal (1928, 1960) introduced the concept of "neurotropism" to account for highly specific contacts between axons and end-organs during development and nerve regeneration. He postulated that an end-organ could specifically attract, or at least selectively contact its appropriate nerve fibre, and speculated that the forces responsible were probably physico-chemical in nature.

Attempts to demonstrate neurotropism in tissue culture experiments have not been successful (Weiss, 1934,

1941). These experiments pointed to the importance of the orientation of the structural matrix of the growth medium in determining the orientation of growing nerve fibres. Weiss postulated that peripheral tissues have no influence on the direction of nerve growth, but that mechanical factors in the path of growing axons are decisive in this respect. Nerve regeneration experiments in mammals (Weiss & Taylor, 1944; Weiss & Hoag, 1946) show that peripheral tissues do not influence the direction of growth of regenerating nerve fibres.

On the other hand, there is now overwhelming evidence in favour of selective forces operating during the re-establishment of synaptic contacts between nerves and end-organs. The development of the current concepts of selective synaptic contacts was closely intertwined with the related physiological controversy centred around the phenomenon of "homologous response".

When a supernumerary limb is grafted in a larval amphibian and is innervated by nerves derived from the nearby normal limb, individual muscles in the transplanted limb move with the same timing as muscles of the same name in the normal limb. This phenomenon has been referred

to as "homologous response" (Weiss, 1936). To explain it, Weiss proposed the theory of "myotypic specification" or "nerve modulation" which may be summarized as follows: (i) each individual muscle has some constitutive specificity by which it is distinguished from all other muscles (except homologous ones), (ii) each muscle is reinnervated in a random, non-specific way, following which it imparts its specificity to the motoneurones and thereby determines the properties of the motoneurones and in some way enables them to become selectively sensitive to impulse patterns intended for that muscle. According to this view, the pattern of central and peripheral synaptic connexions has no relevance to function; nevertheless, motoneurones connected to a given muscle are able to respond selectively to appropriate impulses by a process analogous to "resonance" which cannot be accounted for in terms of current neurophysiological concepts (Weiss, 1952).

A more plausible alternative explanation for homologous response is that the pattern of central and peripheral synaptic connexions are decisive in bringing about coordinated function. During the innervation of the transplant, the appropriate central-peripheral relations

are re-established either by specific changes in the pattern of synapses on the motoneurone induced by changes in peripheral connexions (Sperry, 1941, 1951a, 1951b, 1955, 1958, 1965), or by selective reinnervation of muscles (Sperry & Arora, 1965; Mark, 1965; Mark, Campenhausen & Lischinsky, 1966). It is assumed that lasting functional synaptic connexions are established only between cells with matching chemical affinities. The significance of these concepts is that they have general application not only in connexion with homologous response, but also in the formation of specific synaptic contacts during neurogenesis and regeneration of the nervous system.

The early experimental evidence for selectivity in the establishment of synaptic contacts was derived chiefly from behavioural studies in lower vertebrates which showed that many fibre systems in the central nervous system were capable of regeneration with recovery of function (for review, see Sperry, 1950a). More recently, demonstrations of selective synaptic contacts during regeneration of the nervous system using electrophysiological (Gaze, 1959, 1967; Gaze & Jacobson, 1963; Gaze,

Jacobson & Szekely, 1963; Guth & Bernstein, 1961; Westerman, 1965) and anatomical (Attardi & Sperry, 1960, 1963; Arora & Sperry, 1962; Arora, 1963) methods have become available. Muscle reinnervation experiments have also provided evidence for selective synaptic contacts (see below).

The results of these studies provide strong evidence that there are selective forces in the formation of synaptic contacts and it would seem impossible to interpret selective phenomena purely in terms of mechanical factors as proposed by Weiss (1955). The nature of these selective forces is an extremely important problem in neurobiology, but presumably owing to the complexity of interconnexions in the nervous system, these forces are at present very poorly understood. The neuromuscular junction offers a simple and accessible system for the analysis of these selective forces.

(b) Affinities between nerves and muscles.

Early interest in the problem of affinities between nerves and muscles stem from studies in the comparative anatomy of muscles and nerves of tetrapods. The pervading pattern of homologous muscles and nerves led Fürbringer

(1888) and Cunningham (1882, 1890) to postulate their theories of nerve-muscle specificity, according to which there exists an inherent, specific ontogenetic and phylogenetic relationship between individual striated muscles and nerves. Results of studies in experimental embryology in which attempts were made to alter the normal relationship between muscles and nerves (reviewed by Straus, 1946) are against the existence of a rigid specificity between individual muscles and nerves.

Recent studies on the transplantation of the spinal cord in the chick embryo (Székely & Szentágothai, 1962; Straznicky, 1963, 1967) reveal complex patterns of nerve-muscle affinity. These experiments show that while the brachial and the lumbar segments of the spinal cord are interchangeable as far as the establishment of neuromuscular end-plates and maintenance of muscles are concerned, the thoracic cord segment cannot innervate wing muscles, and brachial and lumbar cord segments cannot innervate thoracic musculature.

Muscle reinnervation experiments in general show that nerve fibres can reinnervate foreign muscles in the same or in another limb. Functional neuromuscular

junctions are re-established following nerve cross-union (for review of early literature see Sperry, 1945; Buller, Eccles & Eccles, 1960b; Close, 1965b; Buller & Lewis, 1965b). Forelimb nerves can reinnervate hindlimb muscles and hindlimb nerves can reinnervate forelimb muscles in the rat (Barron, 1934). In fish, pelvic nerves can innervate pectoral muscles (Sperry & Deupree, 1956), but pectoral muscles receiving the foreign nerve showed atrophy while those reinnervated by pectoral nerves are normal.

Attempts to demonstrate stronger affinities between muscles and their original nerves have produced conflicting results in mammals. Elsberg (1917) reported that when a rabbit muscle was given its own and a foreign nerve, it was selectively reinnervated by its original nerve. No selective reinnervation was observed in the rat by Weiss and Hoag (1946) and Bernstein and Guth (1961). However, selective reinnervation was reported in fish extraocular (Sperry & Arora, 1965) and pectoral fin (Mark, 1965) muscles. In the chick, Feng, Wu and Yang (1965) showed that nerve fibres which normally innervated fast and slow muscles selectively reinnervate the original muscles.

However, both fish and chick muscles, which show selective reinnervation, could also be reinnervated by foreign nerves (Sperry & Deupreé, 1956; Mark, 1965; Feng, Wu & Yang, 1965; Hník, Jirmanová, Vyklický & Zelená, 1967) when innervation by their original nerves is prevented.

In summary, it may be said that a rigid nerve-muscle specificity as originally conceived by Fürbringer and Cunningham is untenable since nerve fibres generally show the ability of innervating muscles other than those they normally innervate. Nevertheless, limb muscles show greater affinity for limb nerves than for nerves to the trunk, and some nerve fibres show greater affinity for its original muscles than for another. The pattern of affinities between nerves and muscles appear to be rather complex and require further elucidation.

Scope of investigations reported in this thesis

This thesis examines some aspects of the interactions between nerves and muscles during nerve regeneration in toads and rats. Section II deals principally with the question whether nerve cross-union in a lower vertebrate would reveal neural influences on the speed

of muscle contractions as described previously for mammals. Section III describes the differences in the responses of fast and slow rat muscles to repetitive stimulation and to changes in temperature, and examines the question whether these differences are under neural control by studying the effects of nerve cross-union on these properties. Sections IV and V deal with the question of selective reinnervation on the fast-twitch and slow-graded muscle fibres of the toad (Section IV) and of the fast and slow muscle fibres of the rat (Section V) by nerve fibres which normally innervate these muscle fibres. Section VI contains a general discussion.

SECTION II

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

The demonstration of neural control of the speed of contraction of mammalian skeletal muscles (Buller, Eccles & Eccles, 1960b; Buller & Lewis, 1965b; Close, 1965b) has raised the question whether similar influences are exerted through motoneurones innervating muscle fibres with different speeds of contraction in other vertebrates.

In anurans there are fast-twitch and slow-graded muscle fibres which differ in structure, innervation and function. Fast-twitch muscle fibres show Fibrillen-struktur in cross-sectional appearance (Krüger, 1952; Gray, 1958) and are focally innervated by low threshold nerve fibres of large diameter (Tasaki & Mizutani, 1944; Kuffler & Vaughan Williams, 1953a; Gray, 1957) with nerve endings of the en plaque type (Gray, 1957). These muscle fibres respond to direct or indirect stimulation with a propagated action potential followed by an all-or-nothing twitch contraction, but when the muscle fibre

membrane is subjected to persistent depolarization, there is a transitory contracture response (Kuffler & Vaughan Williams, 1953a; Hodgkin & Horowicz, 1960; Miledi & Orkand, 1966).

Anuran slow-graded muscle fibres show Felderstruktur in cross-sectional appearance (Krüger, 1952; Gray, 1958) and show many ultrastructural differences from fast-twitch muscle fibres (Peachy & Huxley, 1962; Page, 1965). These muscle fibres are innervated by high threshold nerve fibres of small diameter (Tasaki & Mizutani, 1944; Kuffler & Vaughan Williams, 1953a; Gray, 1957) with nerve endings of the en grappe type which occur diffusely along the muscle fibres so that each muscle fibre is innervated by several nerve fibres (Gray, 1957; Hess, 1960). Slow-graded muscle fibres respond to stimulation of their nerves with a locally spreading small-nerve junctional potential, but are unable to respond with a propagated action potential to either indirect or direct stimulation (Kuffler & Vaughan Williams, 1953a; Burke & Ginsborg, 1956). These fibres give an almost imperceptible mechanical response to a single stimulus, but produce a slow, graded contraction in response to repetitive stimuli (Tasaki &

Mizutani, 1944; Kuffler & Vaughan Williams, 1953b). Depolarizing agents acting on these fibres cause a persistent contracture tension (Kuffler & Vaughan Williams, 1953b; Nasledov Zachar & Zacharová, 1966; Miledi & Orkand, 1966).

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. The results of these investigations have been published and are presented as a paper (Close & Hoh, 1968c).

SECTION III

THE EFFECTS OF REPETITIVE STIMULATION AND TEMPERATURE ON ISOMETRIC CONTRACTIONS OF NORMAL AND CROSS-INNERVATED RAT FAST AND SLOW MUSCLES

INTRODUCTION

Recent experiments have shown that some physiological and biochemical properties of mammalian muscles are under neural control. Cross-union of nerves to fast and slow muscles have been shown to result in a reversal of a number of properties characteristic of mammalian fast and slow muscles, such as the speed of contraction (Buller, Eccles & Eccles, 1960b; Close, 1965b; Buller & Lewis, 1965b), the electrophoretic pattern of soluble proteins (Guth & Watson, 1967), and enzyme profiles (Romanul & Meulen, 1966, 1967; Dubowitz, 1967).

A brief period of repetitive stimulation causes transitory post-tetanic potentiation (PTP) of the isometric twitch contractions of fast skeletal muscles in several mammals, whereas under similar conditions

there is usually a post-tetanic depression (PTD) of slow muscles (Brown & Euler, 1938; Euler & Swank, 1940; Bernhard, Euler & Skoglund, 1941; Bowman, Goldberg & Raper, 1962; Standaert, 1964; Buller & Lewis, 1965a; Desmedt & Hainnaut, 1968; Close & Hoh, 1968b). The possibility arises that this difference between fast and slow muscles is also under neural control. This possibility was explored by examining the effects of repetitive stimulation on cross-innervated fast and slow muscles in the rat and preliminary results have been reported elsewhere (Close & Hoh, 1968a).

Before the experiments on cross-innervated muscles were performed, it was deemed desirable to investigate in detail the effects of repetitive stimulation on isometric contractions of normal rat muscles in order to provide some information which may be useful in planning the experiments and interpreting the findings on cross-innervated muscles. For this purpose, the fast extensor digitorum longus (EDL) muscle of juvenile rats was studied in vitro. The results of these investigations are submitted in the form of a publication (Close & Hoh, 1968b). These results indicated that there were two distinct

aspects of potentiation of the isometric twitch of rat EDL following repetitive stimulation at 35°C. With repetitive trains of up to about 200 stimuli, there was an increase in the post-train peak tension with little or no change in time course of the twitch. When the number of stimuli exceeded that required to give maximum PTP, there occurred a prolongation in both the contraction and relaxation phases of the post-train twitch. It was suggested that PTP with little or no change in the post-train twitch time course resulted from an increase in the degree of activation of muscle fibres, and that changes following prolonged stimulation resulted from an increase in the duration of the active state.

During the course of this work it was found that the peak twitch tension of EDL increased approximately two-fold with a fall in temperature from 35 to 20°C, whereas the peak twitch tension of the slow soleus (SOL) muscle fell slightly over the same change in temperature. A similar difference between the temperature dependence of isometric twitch tensions of cat fast and slow muscles has recently been reported by Buller, Ranatunga and Smith (1968a). The possible relationship between the

differences in the temperature dependence of twitch contractions of rat EDL and SOL muscles, and the differences in the effects of repetitive stimulation on these muscles were examined using in vitro preparations. The results of these investigations are also submitted in the form of a publication (Close & Hoh, 1968a). It was shown that, with a fall in temperature, PTP in EDL decreased as the peak twitch tension increased until there was little or no PTP at 20°C, at which temperature the peak twitch tension was about the same as the peak tension of the maximally potentiated twitch at other temperatures between 20 and 35°C. It was suggested that a fall in temperature increased the degree of activation of muscle fibres in EDL in a manner similar to that postulated to follow repetitive stimulation (Close & Hoh, 1968b), whereas neither a fall in temperature nor repetitive stimulation seems to alter the degree of activation of SOL muscle fibres.

As a result of the investigations outlined above, observations on the effects of repetitive stimulation on isometric twitch contractions of cross-innervated EDL and SOL muscles were made at various temperatures. Similar

observations were also made on normal and self-innervated EDL and SOL muscles.

METHODS

Operations. Twelve female rats of the Wistar strain were used. In 10 of these animals, the EDL and SOL muscles in one hindlimb were cross-innervated when the animals were 3 weeks old. The contralateral limbs of these animals were either left intact (4 animals) or the SOL and EDL nerves were sectioned and resutured in turn (self-union: 6 animals). The operations were performed under aseptic conditions and the anaesthetic was 40-50 mg sodium pentobarbital/kg body weight injected intraperitoneally). No operation was performed on the other two animals which were 18-19 weeks old at the time of the experiment.

Dissections. The experiments were carried out 140 to 346 days after operations. The average body weight of the animals at the time of the experiment was 229 g (range 180 g - 266 g). Sodium pentobarbital was used as anaesthetic; initially 50 mg sodium pentobarbital/kg body weight was injected intraperitoneally,

followed by 20-30% of the initial dose every 1-1½ hr. The trachea was cannulated in all animals and atropine sulphate 0.1 mg/kg was given intraperitoneally to suppress excessive secretions in the respiratory tract. The EDL and SOL muscles in both legs were dissected free except for their attachments through the proximal tendons. The main blood supplies to these muscles were kept intact. The tibial and peroneal branches of the sciatic nerve were separated and transected near the sciatic notch. All branches of these nerves were cut except those to EDL and SOL muscles.

Equipment. The nerves and muscles in situ were placed in a Perspex bath in the way described previously (Close, 1967a). The muscles were bathed in about 120 ml of Ringer's solution (NaCl 137 mM, KCl 5 mM, $CaCl_2$ 2 mM, $MgCl_2$ 1 mM, NaH_2PO_4 1mM, $NaHCO_3$ 2 g/l., glucose 2 g/l.). This solution was aerated with a mixture of 95% O_2 and 5% CO_2. The solution in the bath was replaced at the rate of about 2-3 ml/min.

Isometric contractions of the muscles were recorded with the proximal tendon clamped securely to a rigid frame and the distal tendon attached directly to a strain gauge

(Statham G1-8-350 or G1-80-350), the total compliance of the recording system was no more than 3.3×10^{-5} cm/g. The strain gauge was used in conjunction with a carrier amplifier (Tektronics, Q), the output of which was amplified and displayed on a dual beam oscilloscope (Tektronics, 565) with plug-in amplifiers (Tektronics 3A3 and 72). One beam of the oscilloscope was used for displaying responses to single stimuli while the other beam, on a different time base and with a different gain, was used for displaying responses to repetitive stimulation. 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 (Tektronics 180A) and were displayed simultaneously with the tension records on either beam with the plug-in amplifiers in the chopped mode.

A Grass S4 stimulator with isolation unit was used for indirect stimulation of the muscles via the nerves in the Ringer's solution. The stimulus was a square pulse of 20 μsec duration and supramaximal in intensity, usually 15 V, through platinum wire electrodes. In all the records shown below, the 1st stimulus occurred at the

beginning of each sweep.

Procedure for determining PTP or PTD. The optimal length for twitch contractions was determined for every muscle at 35°C and all subsequent measurements were made at this length. In a few preparations the optimal length at 20°C was also determined and in every case the value was identical to that determined at 35°C. For each muscle a series of measurements of the effect of repetitive stimulation were made at 35°C. In some muscles, measurements were made also at 30, 25 and 20°C in a random sequence and finally again at 35°C. Changes in temperature were brought about by replacing the muscle bath with Ringer's solution at the desired temperature which was then maintained by a temperature regulating circuit. Following each change of temperature a period of equilibration lasting at least 10 minutes was allowed before measurements at that temperature began. The muscles remained in good condition at the end of the experiment as indicated by the ratio of the final peak twitch tension at 35°C to the initial peak twitch tension at 35°C, the mean for all the muscles studied was 1.015. At each temperature the standard procedure used to study the

after-effects of repetitive indirect stimulation was to record a series of 2 or 3 pre-train responses to single stimuli at intervals of 20 sec followed by responses to a train of repetitive stimuli which were timed to end 20 sec after the last pre-train stimulus, and the responses to single post-train stimuli beginning 10 seconds after the end of the train and thereafter every 20 seconds for 5 minutes. The number of stimuli in the train was always 200 and the frequency of stimulation was 200 c/s at 35°C, 154 c/s at 30°C, 100 c/s at 25°C and 80 c/s at 20°C. This number of stimuli was chosen because it produced nearly maximal PTP in normal EDL muscles *in vitro* with little or no change in the time course of the post-train twitch (Close & Hoh, 1968b). The frequency used for stimulation at 35°C is close to the optimal frequency for isometric contractions of both normal EDL and SOL muscles (Close, 1964). The lower frequencies used at lower temperatures were based on the assumption that optimal frequency for isometric contractions of these muscles is halved for a 10°C drop in temperature.

Definitions. Optimal length (L_o) is the length

of the muscle at which the peak twitch tension, in excess of initial tension, is maximal at 35°C.

Maximum isometric twitch tension (P_t) at any temperature is the peak twitch tension in excess of initial tension at L_o.

Maximum isometric tetanic tension (P_o) is the maximum tension in excess of initial tension at L_o during repetitive stimulation using 200 c/s at 35°C, 154 c/s at 30°C, 100 c/s at 25°C and 80 c/s at 20°C.

Contraction time (T_c) at any temperature is the time from onset of contraction to the peak of the isometric twitch at L_o.

Half-relaxation time ($T_{\frac{1}{2}R}$) at any temperature is the time for decay of tension from the peak of the isometric twitch to one half of the peak tension at L_o.

Cross-sectional area of muscle in square centimetres was estimated by dividing the weight of the muscle (M) in grams by the average fibre length (L) at L_o in centimetres.

The degree of potentiation or depression at any temperature is the ratio of the peak tension of a post-train twitch (P_t*) to the peak tension of the pre-train

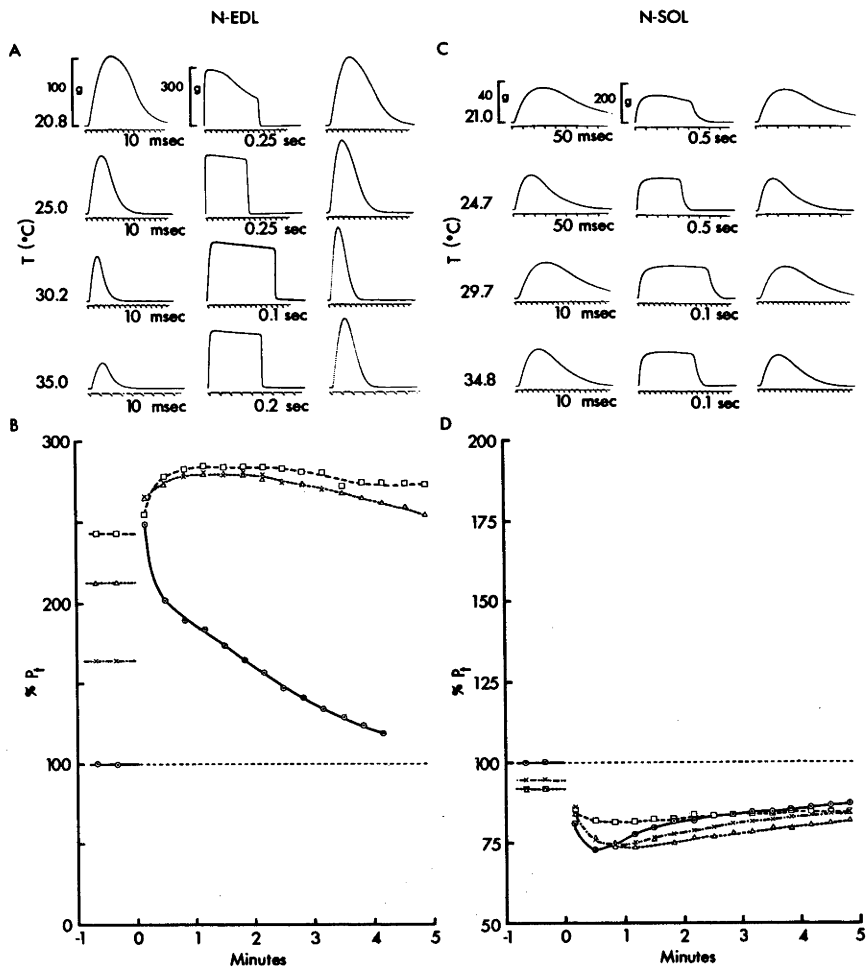

Fig. 1. A, records of contractions of normal extensor digitorum longus (N-EDL) muscle at various temperatures (°C) indicated to the left of each row of records. The column of records from left to right are pre-train twitch contractions, tetanic contractions and post-train twitch contractions at 10 sec after the end of the tetanus. The tension calibration for all records in each column is the same. Tension and time calibrations for each post-train twitch contraction are the same as for the corresponding pre-train twitch. B, time course of changes in peak tension of post-train twitches of the N-EDL described in A. The peak tensions of pre-train and post-train twitches at 20°C (□), 25°C (△), 30°C (×) and 35°C (⊙) expressed as percentages of the peak tension of the pre-train twitch at 35°C (% P_t), are plotted against time after the end of the tetanus in minutes. C, D, results from normal soleus (N-SOL) muscle of the same animal displayed as in A and B. Muscle weights: N-EDL = 111 mg, N-SOL = 97 mg; mean fibre lengths: N-EDL = 10.5 mm, N-SOL = 16 mm.

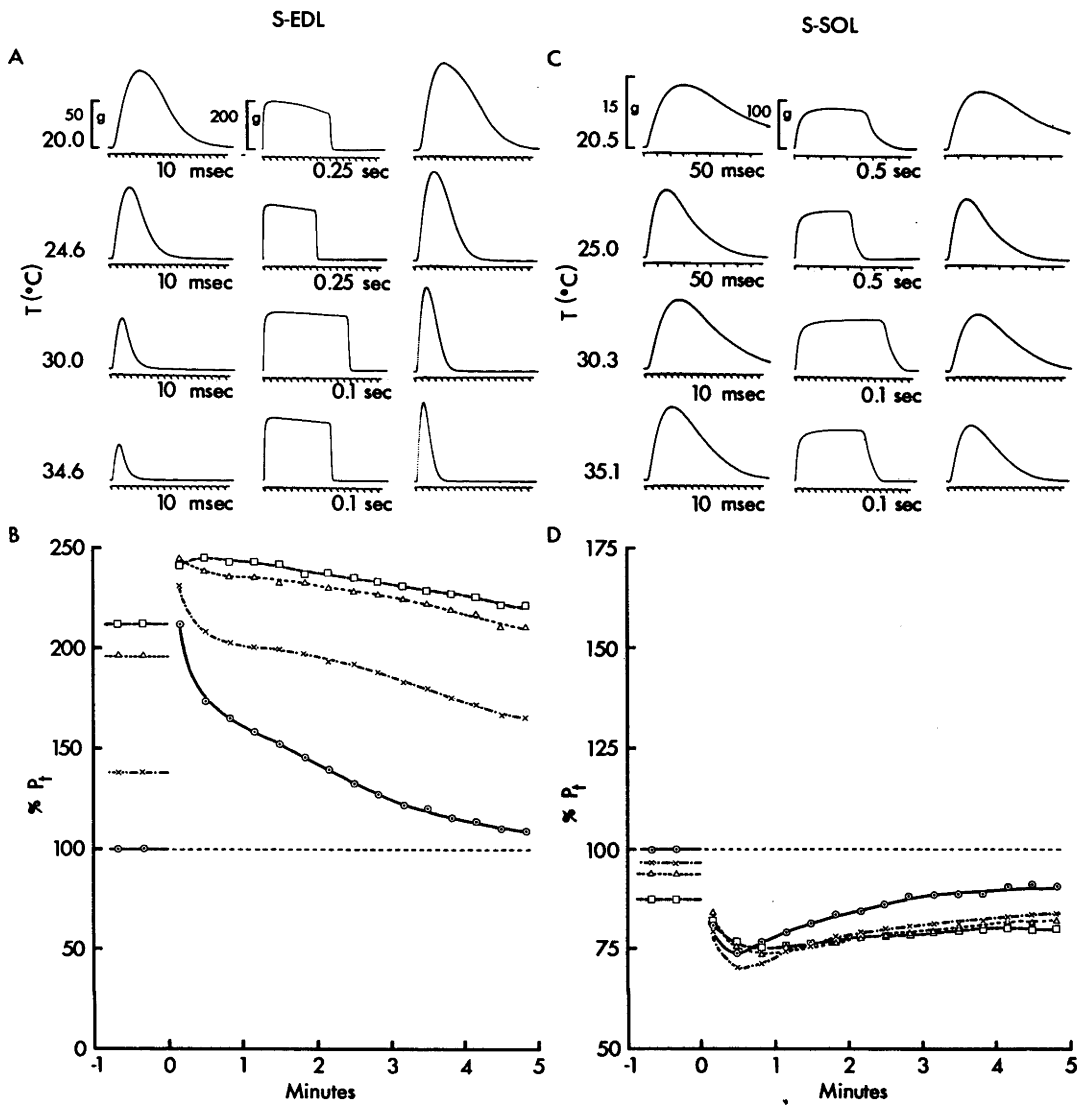

Fig. 2. Records of contractions and time course of changes in peak tension of post-train twitches at various temperatures for self-innervated extensor digitorum longus (S-EDL) muscle (A, B) and self-innervated soleus (S-SOL) muscle (C, D) displayed as in Fig. 1. The experiment was done 346 days after operation. Muscle weight: S-EDL = 95 mg, S-SOL = 63 mg; mean fibre lengths: S-EDL = 12 mm, S-SOL = 16 mm.

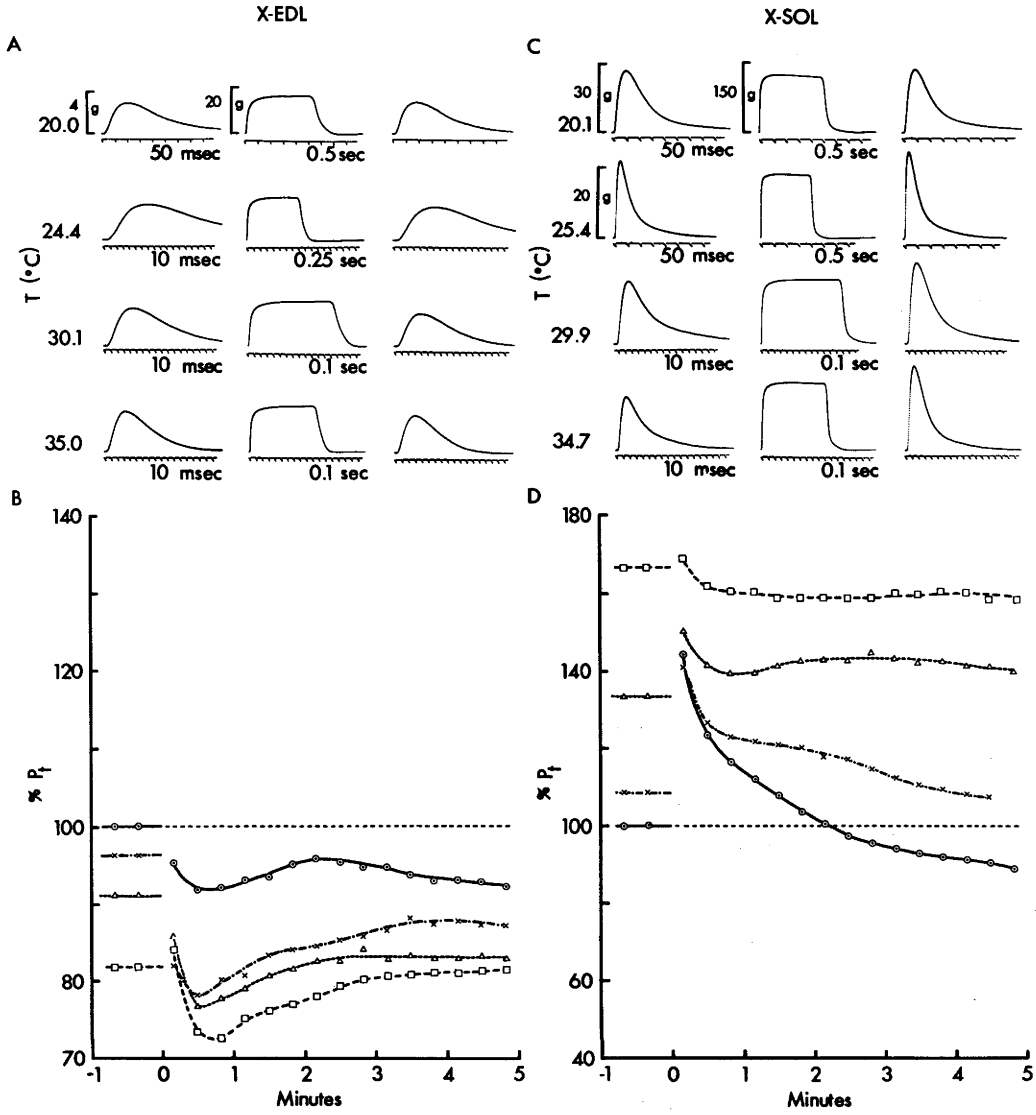

Fig. 3. Records of contractions and time course of changes in peak tension of post-train twitches at various temperatures for cross-innervated extensor digitorum longus (X-EDL) muscle (A, B) and cross-innervated soleus (X-SOL) muscle (C, D), displayed as in Fig. 1 except that the tension calibration for X-SOL twitch records at 20.1°C differ from that at other temperatures. These muscles and the normally innervated muscles described in Fig. 1 are from the same animal examined 338 days after the operation. Muscle weights: X-EDL = 69 mg (this muscle was reinnervated by nerve fibres from soleus and peroneal nerves) X-SOL = 87 mg; mean fibre lengths: X-EDL = 12.5 mm, X-SOL = 17 mm.

twitch (P_t), i.e. P_t^*/P_t.

RESULTS

Representative records of isometric responses of normal (N-EDL, N-SOL), self-innervated (S-EDL, S-SOL) and cross-innervated (X-EDL, X-SOL) EDL and SOL muscles at various temperatures are shown in A and C of Figs. 1-3. For each muscle, each row of records are, from left to right, the control twitch recorded about 10 sec before the tetanus, the tetanic contraction in response to 200 stimuli, and the post-tetanic twitch recorded 10 sec after the end of the tetanic train. The records shown for each muscle are part of a series described in the graphs below them (i.e. in B and D of Figs. 1-3) in which the peak twitch tensions before and after repetitive stimulation at 20°C (□), 25°C (△), 30°C (×) and 35°C (⊙), expressed as percentages of the pre-train P_t at 35°C, are plotted against time after the end of repetitive stimulation.

A comparison of the pre-train twitch and tetanic records at 35°C of these muscles show that while the speeds of contraction of self-innervated muscles (Fig. 2) are

the same as those of corresponding normal muscles (Fig. 1), the speed of contraction of X-EDL (Fig. 3A) is decreased and that of X-SOL (Fig. 3C) is increased. These effects of nerve cross-union on the speed of muscle contraction in mammals have already been described in detail (Buller, Eccles & Eccles, 1960b; Close, 1965b; Buller & Lewis, 1965b).

At 35°C, the post-train records of muscles innervated by the EDL nerve (i.e. N-EDL, S-EDL and X-SOL shown in Figs. 1A, 2A & 3C, respectively) show PTP with little or no change in the post-train contraction time. The degree of potentiation for N-EDL and S-EDL is between 2.1-2.5, and this is comparable to the maximal value of 1.9 reported for EDL muscles from juvenile rats stimulated <u>in vitro</u> (Close & Hoh, 1968b), but the value for X-SOL is much less, being only 1.45. At lower temperatures, the pre-train P_t of these muscles rise, and at 20°C the pre-train P_t is approximately the same as the peak tension for the post-train twitch at 10 sec after the end of the tetanus ($P_t*x=10$) of the same muscle at 35°C. In contrast, the $P_t*x=10$ of these muscles are not temperature dependent between 20 and 35°C. In other words, the peak twitch

tensions of these muscles are raised to about the same level by repetitive stimulation using 200 stimuli at temperatures between 20 and 35°C or by lowering the temperature from 35 to 20°C, and that PTP is maximal at 35°C and disappears at 20°C. These features, which are essentially the same as those in EDL muscles *in vitro* (Close & Hoh, 1968a), are clearly shown in the graphs below the records for each muscle (i.e. Figs. 1B, 2B & 3D for N-EDL, S-EDL & X-SOL, respectively). These graphs also show that the time course of decay of PTP at 35°C for N-EDL and S-EDL is about the same while that for X-SOL is more rapid and is followed by a transient phase of depression which probably results from superimposed PTD. At lower temperatures, PTP decays more slowly in all muscles.

The peak twitch tensions of muscles innervated by SOL nerve (i.e. N-SOL, S-SOL and X-EDL, shown in C & D of Figs. 1 & 2 and A & B of Fig. 3) are depressed by a fall in temperature as well as by repetitive stimulation. In N-SOL and S-SOL, PTD is more pronounced at 35°C and the P_t*x=10 is relatively temperature independent. At 35°C, the peak tensions of post-train twitches in all these

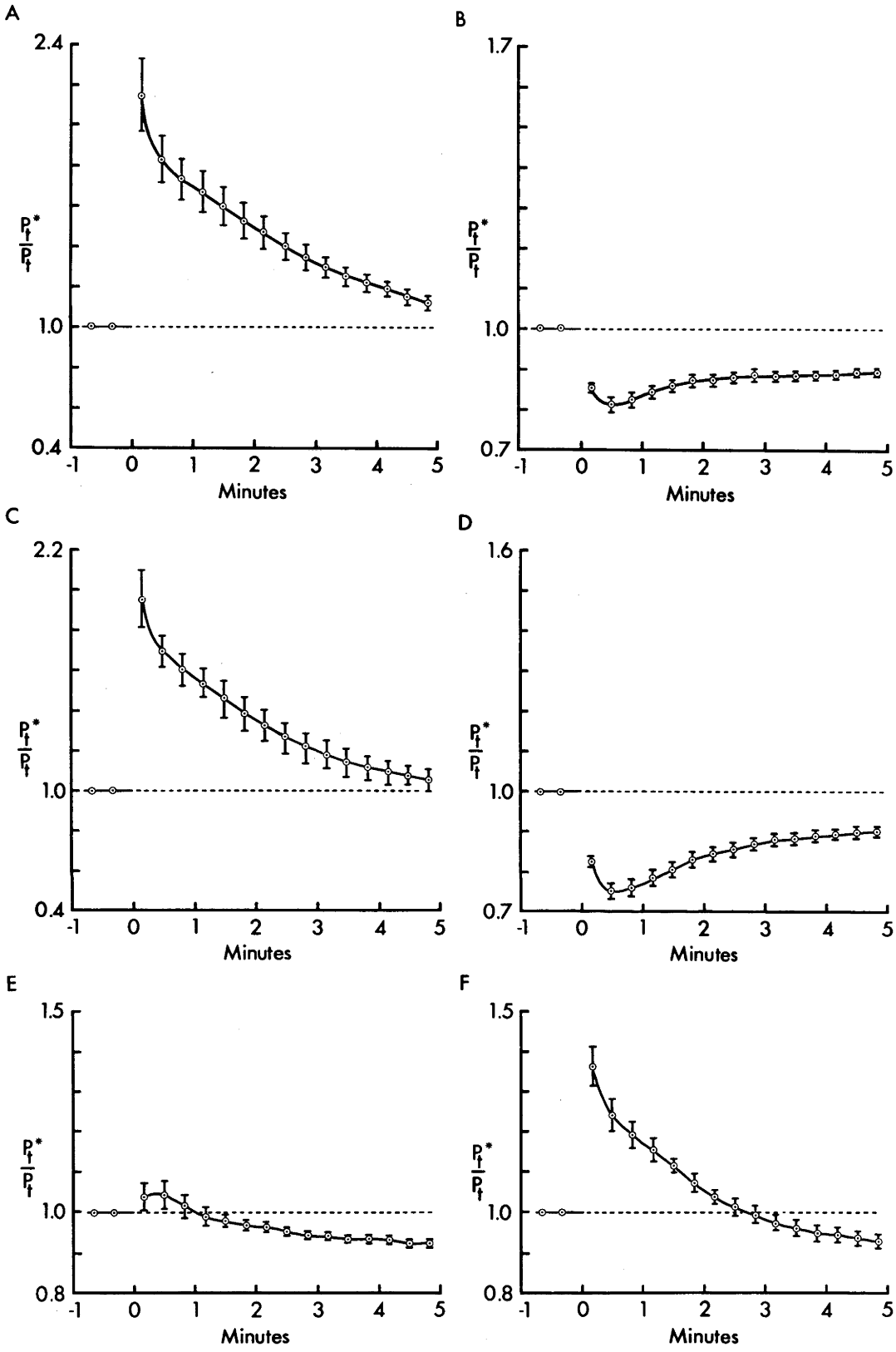
A
2.4
1.0
0.4
$\frac{P_f^*}{P_f}$
-1 0 1 2 3 4 5
Minutes
B
1.7
1.0
0.7
$\frac{P_f^*}{P_f}$
-1 0 1 2 3 4 5
Minutes
C
2.2
1.0
0.4
$\frac{P_f^*}{P_f}$
-1 0 1 2 3 4 5
Minutes
D
1.6
1.0
0.7
$\frac{P_f^*}{P_f}$
-1 0 1 2 3 4 5
Minutes
E
1.5
1.0
0.8
$\frac{P_f^*}{P_f}$
-1 0 1 2 3 4 5
Minutes
F
1.5
1.0
0.8
$\frac{P_f^*}{P_f}$
-1 0 1 2 3 4 5
Minutes

Fig. 4. Averaged time course of PTP or PTD at 35°C following 200 stimuli at 200c/s in 4 N-EDL (A), 5 N-SOL (B), 3 S-EDL (C), 5 S-SOL (D), 7 X-EDL (E) and 9 X-SOL (F) muscles. Ordinates: ratio of peak tension of post-train twitch (P_t*) to peak tension of pre-train twitch (P_t), i.e. P_t*/P_t; abscissae, time after the end of repetitive stimulation in minutes. Each point and vertical bar give the mean ± S.E.

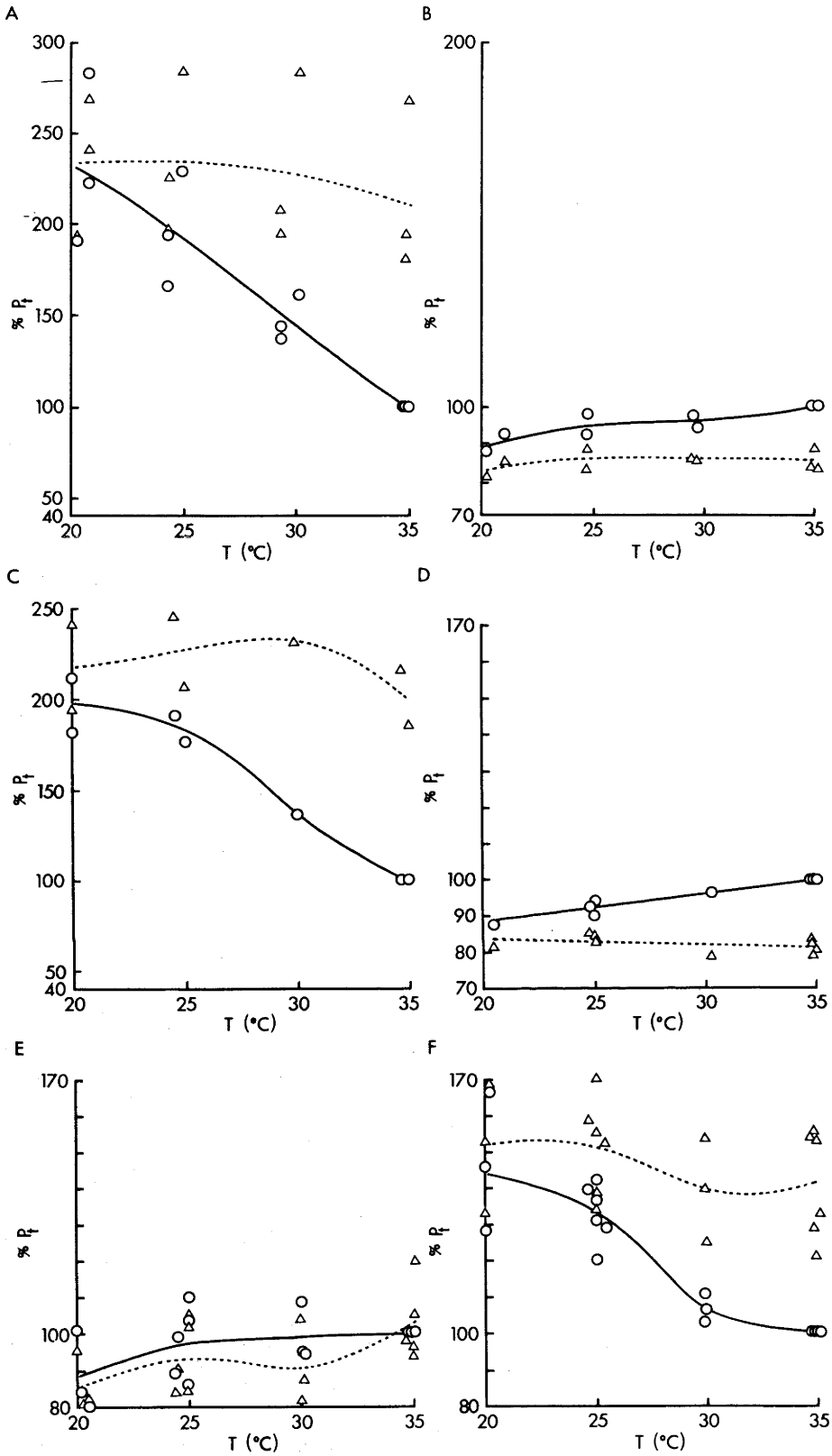
A
B
C
D
E
F
% P_f
T (°C)
20
25
30
35
40
50
70
80
90
100
150
170
200
250
300

Fig. 5. Relation between peak tension of pre-train and post-train twitches and temperature for N-EDL (A), N-SOL (B), S-EDL (C), S-SOL (D), X-EDL (E) and X-SOL (F). The peak tension of pre-train (○) and the first post-train (△) twitches for each muscle at different temperatures are expressed as percentages of the peak tension of the pre-train twitch at 35°C (% P_t) and plotted against temperature in degrees Centigrade (°C).

TABLE I

	N-EDL	S-EDL	X-EDL
No. of muscles	4	3	8
Pre-train T_c (msec)	11.3 ± 0.5	11.2 ± 0.3	27.9 ± 1.2
$T_{½R}$ (msec)	8.8 ± 0.3	9.0 ± 0.0	40.9 ± 2.7
Post-train T_c (msec)	12.1 ± 1.0	11.5 ± 0.8	26.3 ± 0.9
$T_{½R}$ (msec)	9.3 ± 0.3	9.3 ± 0.3	35.1 ± 2.2
$(P_t{}^*x=10)/P_t$	2.143 ± 0.187	1.958 ± 0.146	1.021 ± 0.034
P_t/P_o	0.155 ± 0.010	0.147 ± 0.002	0.309 ± 0.028
P_o (g)	283.8 ± 9.3	237.5 ± 5.4	51.6 ± 6.8
L (mm)	11.0 ± 0.2	12.8 ± 0.6	12.2 ± 0.6 (n = 6)
M (mg)	109.3 ± 9.3	104.0 ± 6.9	54.0 ± 4.5 (n = 7)
P_oL/M (kg/cm^2)	2.90 ± 0.17	2.91 ± 0.10	1.35 ± 0.16 (n = 6)

Table I. Summary of properties for normal, self-innervated and cross-innervated extensor digitorum longus (N-EDL, S-EDL & X-EDL, respectively) and soleus (N-SOL, S-SOL & X-SOL, respectively) muscles at 35°C. Each value gives the mean ± S.E., the number of observations (n) upon which each value is based is the same as the number of muscles studied except where n is given in parentheses below the mean. The post-train T_c and $T_{½R}$ are from twitch responses obtained

TABLE I (continued)

N-SOL	S-SOL	X-SOL
5	5	10
37.8 ± 2.4	41.2 ± 1.0	14.2 ± 0.4
51.8 ± 1.6	52.8 ± 1.4	20.1 ± 1.3
33.6 ± 2.0	36.6 ± 1.0	13.3 ± 0.4
43.4 ± 2.3	45.5 ± 1.1	15.7 ± 0.7
0.854 ± 0.011	0.825 ± 0.013	1.348 ± 0.05
0.213 ± 0.021	0.208 ± 0.018	0.117 ± 0.006
172.4 ± 6.8	153.9 ± 22.8	160.2 ± 8.0
14.6 ± 0.6	15.2 ± 0.3	17.3 ± 0.5 (n = 9)
96.4 ± 8.6	76.6 ± 6.3	88.9 ± 3.9
2.65 ± 0.13	2.58 ± 0.10	3.18 ± 0.12 (n = 9)

10 sec after the end of repetition stimulation at 200c/s for 1 sec. The mean values for post-train T_C and $T_{\frac{1}{2}R}$ which are significantly different from corresponding pre-train values, and those for all other properties of self-innervated and cross-innervated muscles which are significantly different from corresponding mean values for normal muscles, as indicated by the t-test ($P < 0.05$), are underlined.

muscles are minimal at about 30 sec after the end of repetitive stimulation and return to pre-train values over a period of about 10 minutes. The time course of recovery from PTD at lower temperatures is very similar to that at 35°C.

The results obtained from other normal, self-innervated and cross-innervated EDL and SOL muscles are very similar to those described above. A summary of the contractile properties of these groups of muscles is shown in Table I. Fig. 4 shows the averaged time courses of PTP or PTD of these groups of muscles at 35°C and Fig. 5 shows the relations between temperature and pre-train and post-train peak twitch tensions of these groups of muscles.

The t-test shows that self-innervation of EDL and SOL muscles produced no significant changes in all the properties listed in Table I except that the mean P_o of S-EDL is less ($P < 0.0125$), and the mean fibre length of S-EDL is longer ($P < 0.05$), than that for N-EDL. Fig. 4 shows the similarity between the averaged time course of PTP for N-EDL (A) and S-EDL (C), and of PTD for N-SOL (B) and S-SOL (D). The curves for the decay of PTP in N-EDL and S-EDL deviate from exponential decay observed in

massively stimulated juvenile EDL muscles *in vitro* (Close & Hoh, 1968b), and this may be due to PTD or neuromuscular depression superimposed on PTP in these muscles. Fig. 5 shows that the relations between temperature and the pre-train and post-train peak twitch tensions for N-EDL (A) and S-EDL (C), and for N-SOL (B) and S-SOL (D), are the same.

PTP in N-EDL and S-EDL is accompanied by small increases in T_c and $T_{\frac{1}{2}R}$, but t-tests show that these are not significant ($P > 0.20$). PTD in all N-SOL muscles is accompanied by a decrease in T_c and $T_{\frac{1}{2}R}$, and t-tests indicate that the post-train T_c of S-SOL ($P < 0.01$) and post-train $T_{\frac{1}{2}R}$ of N-SOL ($P < 0.025$) and S-SOL ($P < 0.05$) are significantly reduced. These changes are probably due to a decrease in the duration of the active state as suggested by Bowman, Goldberg and Raper (1962) who reported a decrease in T_c associated with PTD in cat SOL muscle.

All the 8 X-EDL muscles studied were innervated accidentally by nerve fibres from the peroneal nerve in addition to the intended innervation by SOL nerve fibres. However, only those muscle fibres innervated by the SOL

nerve were excited during these experiments, and this accounts for the low mean values for $\frac{P_oL}{M}$ and P_o. The P_o is low also because the diameter of X-EDL muscle fibres innervated by SOL nerve fibres is about half of that for muscle fibres innervated by peroneal nerve fibres (Close, personal communication). The smaller size of X-EDL muscle fibres innervated by the SOL nerve would partly account for significant differences in the mean muscle weight between X-EDL and N-EDL ($P < 0.0005$) and between X-EDL and S-EDL ($P < 0.0005$).

The mean pre-train T_c and $T_{\frac{1}{2}R}$ of X-EDL are significantly greater than those for N-EDL (T_c, $P < 0.0005$; $T_{\frac{1}{2}R}$, $P < 0.0005$) and S-EDL (T_c, $P < 0.0005$; $T_{\frac{1}{2}R}$, $P < 0.0005$), but these values are also significantly lower than those for N-SOL (T_c, $P < 0.0025$; $T_{\frac{1}{2}R}$, $P < 0.01$) and S-SOL (T_c, $P < 0.0005$; $T_{\frac{1}{2}R}$, $P < 0.01$). Four X-EDL muscles whose pre-train T_c range from 29 to 31 msec show PTD at 35°C, while 4 others with pre-train T_c ranging from 23.5 to 26 msec show a small degree of PTP at 35°C. The $(P_t * x=10)/P_t$ for all these muscles range from 0.91 to 1.21. The P_t of those X-EDL muscles which show PTD are depressed by a fall in temperature while those of X-EDL muscles which

show PTP are raised by a fall in temperature from 35 to 30°C or 25°C, but further cooling lowers the P_t (see Fig. 5E). The mean P_t/P_o of X-EDL is significantly higher than that for N-EDL ($P < 0.01$) and S-EDL ($P < 0.01$). P_t/P_o of X-EDL show no correlation with T_c. The mean fibre length of X-EDL is not significantly different from that of N-EDL ($P > 0.1$) and of S-EDL ($P > 0.3$).

All the 10 X-SOL muscles studied were innervated only by fibres from the EDL nerve, there being no accidental innervation from the SOL nerve. The mean pre-train T_c and $T_{\frac{1}{2}R}$ of S-SOL are significantly less than those for N-SOL (T_c, $P < 0.0005$; $T_{\frac{1}{2}R}$, $P < 0.0005$) and S-SOL (T_c, $P < 0.0005$; $T_{\frac{1}{2}R}$, $P < 0.0005$), but these values are also significantly higher than those for N-EDL (T_c, $P < 0.0025$; $T_{\frac{1}{2}R}$, $P < 0.0005$) and S-EDL (T_c, $P < 0.0025$; $T_{\frac{1}{2}R}$, $P < 0.0025$). While the mean post-train T_c is not significantly different from the pre-train value ($P > 0.05$), mean post-train $T_{\frac{1}{2}R}$ is significantly lower than the pre-train value ($P < 0.005$), and in these respects X-SOL muscles behave in the same way as EDL muscles <u>in vitro</u> (Close & Hoh, 1968b).

$(P_t{}^*x=10)/P_t$ of X-SOL muscles at 35°C range from

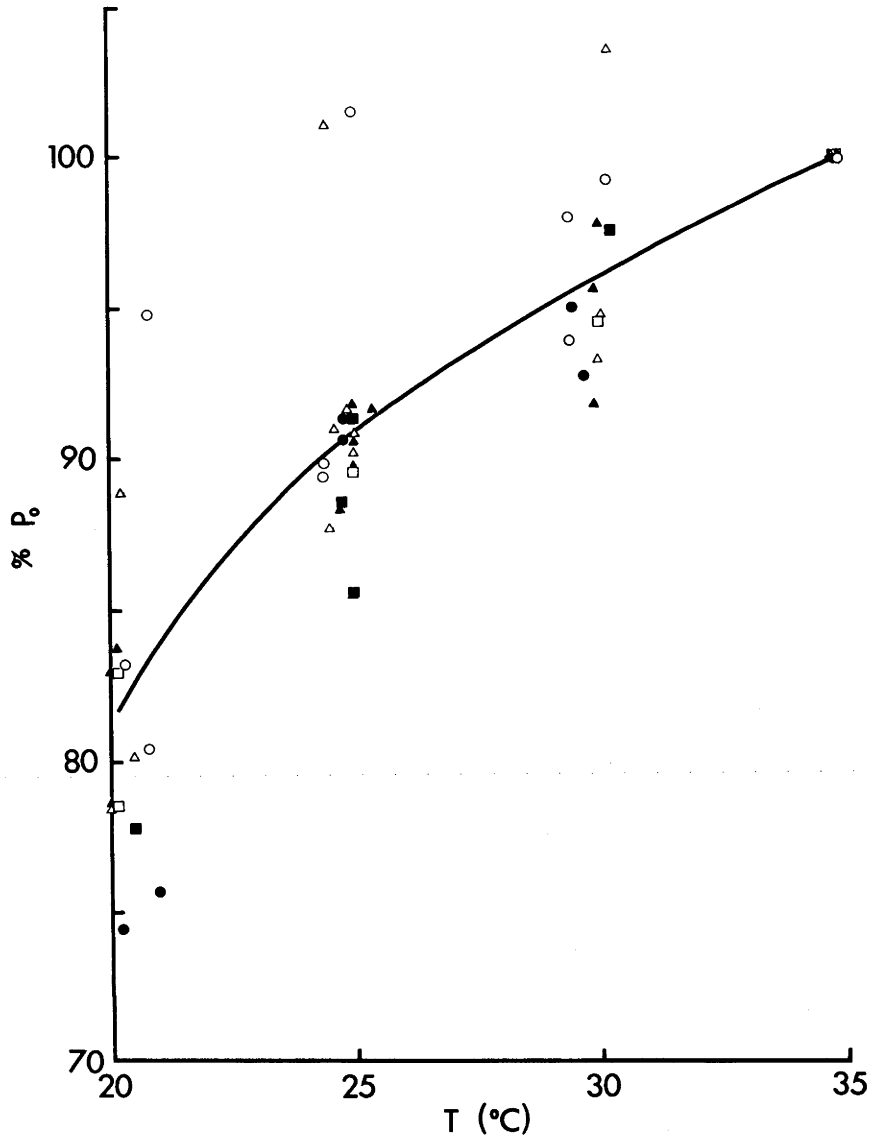

Fig. 6. Relation between maximum isometric tetanic tension (P_o) and temperature for N-EDL (○), N-SOL (●), S-EDL (□), S-SOL (■), X-EDL (△) and X-SOL (▲). For each muscle, the P_o at various temperatures is expressed as percentages of the mean of the initial and final P_o at 35°C (% P_o) and plotted against temperature (°C).

1.18 to 1.59 and tends to show an inverse relationship to the pre-train T_c and P_t/P_o. The mean $(P_t{*}x{=}10)/P_t$ for X-SOL is significantly different from that of N-EDL ($P < 0.0005$) and S-EDL ($P < 0.0005$). Fig. 4 shows that the rate of decay of PTP in X-SOL (F) is more rapid than that in N-EDL (A) or S-EDL (C), and that PTP in X-SOL is followed by a phase of depression. The mean P_t/P_o of X-SOL is about half of that for N-SOL or S-SOL, and in each case this difference is statistically significant ($P < 0.005$). The mean fibre length of X-SOL is significantly longer than that for N-SOL ($P < 0.005$) and for S-SOL ($P < 0.01$). The mean $\frac{P_oL}{M}$ of X-SOL is significantly higher than that of N-SOL ($P < 0.0125$) and S-SOL ($P < 0.005$).

The effect of temperature on the maximum isometric tetanic tensions (P_o) of all the muscles studied is shown in Fig. 6. The P_o for each muscle at various temperatures are plotted as percentages of the mean of the initial and final P_o at 35°C ($\%P_o$). As the initial and final P_o at 35°C of each normal, self-innervated, cross-innervated EDL or SOL muscle is about the same, this diagram shows that the P_o for all these muscles fall to about an equal extent with an equal drop in temperature. At 25°C, the

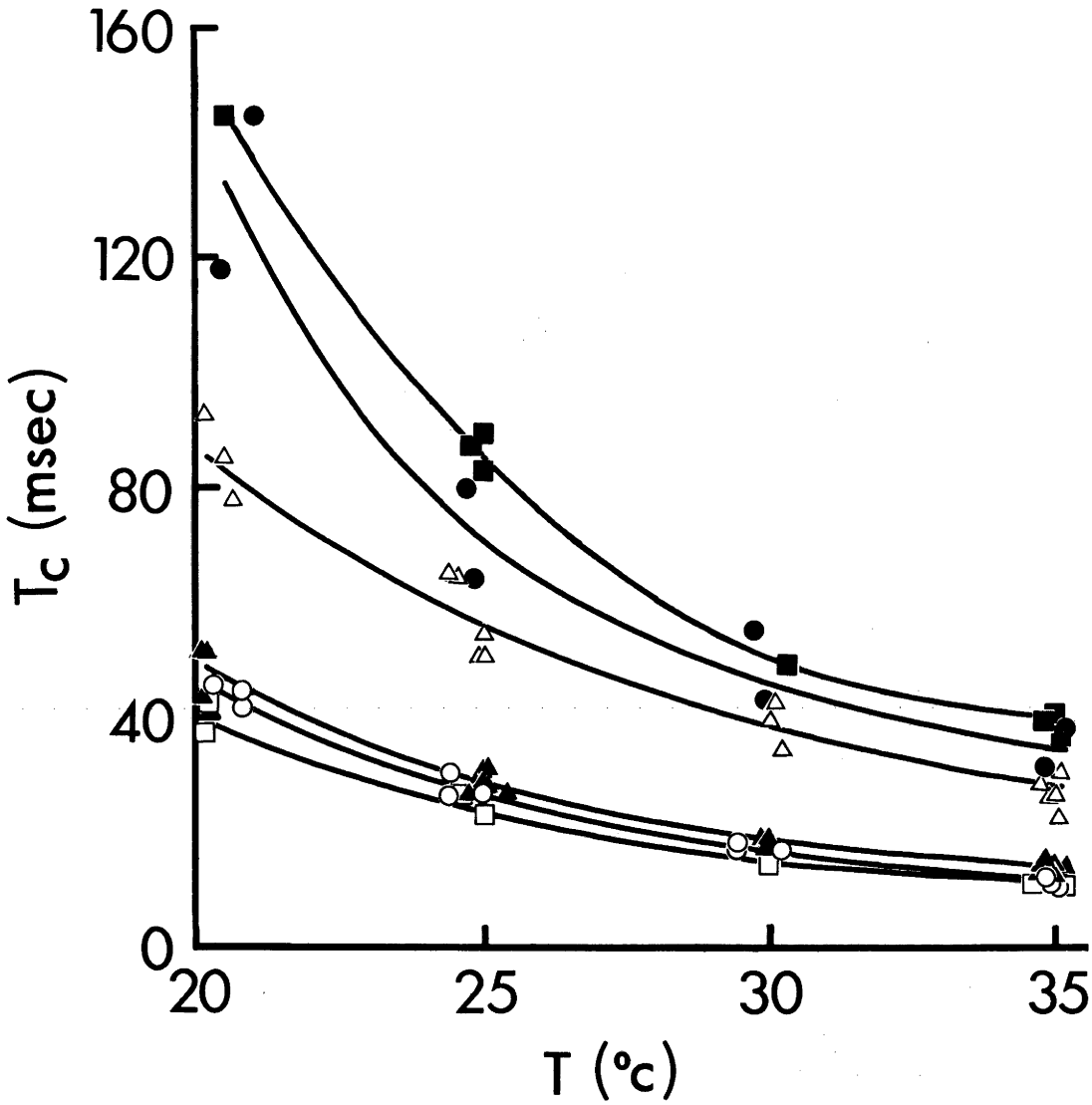

Fig. 7. Relation between contraction time (T_c) and temperature. T_c in msec for N-EDL (○) N-SOL (●), S-EDL (□), S-SOL (■), X-EDL (△) and X-SOL (▲) are plotted against temperature (°C).

TABLE II

		Pre-train		Post-train	
	n	T_c	$T_{½R}$	T_c	$T_{½R}$
N-EDL	3	28.2 ± 0.9	22.8 ± 1.1	26.3 ± 0.6	<u>29.0 ± 0.3</u>
S-EDL	2	25.3 ± 1.8	21.0 ± 2.0	23.7 ± 2.3	25.5 ± 4.0
X-EDL	5	57.4 ± 3.2	88.8 ± 8.3	51.6 ± 2.7	76.9 ± 7.2
N-SOL	2	77.5 ± 2.5	99.0 ± 4.0	<u>64.0 ± 1.0</u>	90.0 ± 5.0
S-SOL	3	86.8 ± 2.0	118.0 ± 8.7	80.2 ± 3.7	108.0 ± 8.5
X-SOL	6	29.1 ± 0.9	38.5 ± 1.3	<u>26.2 ± 0.9</u>	<u>33.5 ± 1.7</u>

<u>Table II.</u> T_c and $T_{½R}$ (msec) of pre-train and post-train twitch contractions at 25°C of normal, self-innervated and cross-innervated extensor digitorum longus (N-EDL, S-EDL & X-EDL, respectively) and soleus (N-SOL, S-SOL & X-SOL, respectively) muscles. Each value gives mean ± S.E., based on the number of observations (n) as indicated. The post-train values are from twitch responses obtained 10 sec after the end of repetitive stimulation at 100c/s for 2 sec, and those values which are significantly different ($P < 0.05$) from corresponding pre-train values are underlined.

mean $\%P_o$ of all the muscles studied is 90.8.

Fig. 7 shows the relation between pre-train T_c and temperature for N-EDL (○), S-EDL (□), X-EDL (△), N-SOL (●), S-SOL (■) and X-SOL (▲). Table II gives the mean T_c and $T_{½R}$ at 25°C of these groups of muscles before, and 10 sec after, stimulation at 100 c/s for 2 sec. The mean post-train T_c for all these groups of muscles are lower than the corresponding pre-train values; these differences are statistically significant for N-SOL ($P < 0.05$) and X-SOL ($P < 0.05$). The mean post-train $T_{½R}$ of N-EDL ($P < 0.01$) is significantly higher, and that of X-SOL is significantly lower, than corresponding pre-train values.

There appears to be an inverse relationship between T_c and $(P_t*x=10)/P_t$ at 35°C for all the muscles studied. As changes in temperature also lead to inverse changes in T_c and $(P_t*x=10)/P_t$, the possibility exists that there is a common inverse relationship between T_c and $(P_t*x=10)/P_t$ for rat muscles irrespective of the way in which changes in these properties are brought about. This possibility is examined for all the muscles which give PTP in Fig. 8, in which the ratio $P_t/(P_t*x=10)$ at 35°C for X-EDL (△),

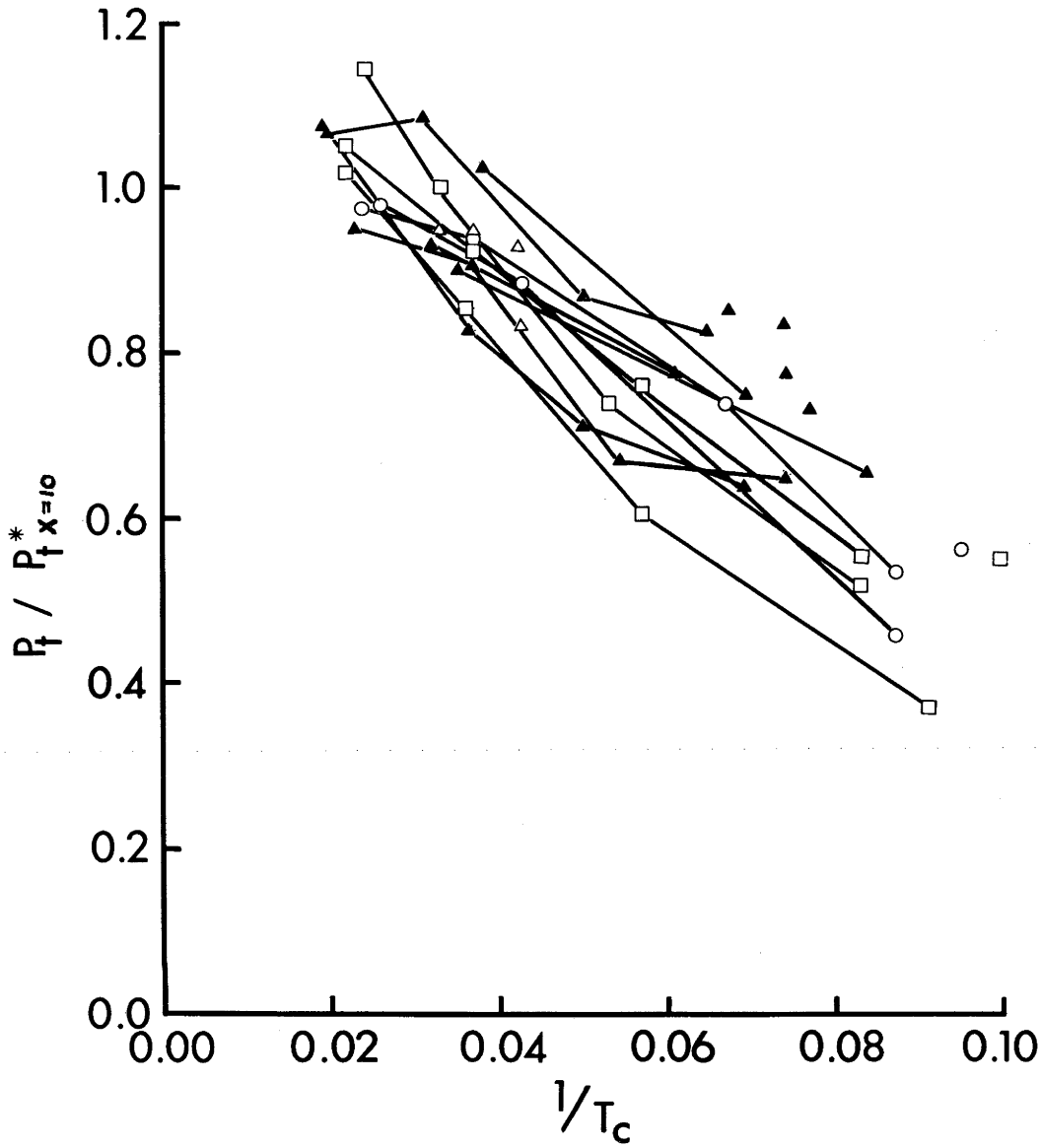

Fig. 8. Relation between the ratio of the peak tension of the pre-train twitch at various temperatures (P_t) and the peak tension of the post-train twitch at 35°C ($P^*_{tX=10}$), i.e. $P_t/P^*_{tX=10}$, and the reciprocal of the contraction time in msec ($1/T_c$). N-EDL = ○ , S-EDL = □ , X-SOL = ▲ and X-EDL = △ . Points joined by lines are for the same muscle.

X-SOL (▲ with $1/T_c > 0.06$), S-EDL (□ with $1/T_c > 0.08$) and N-EDL (○ with $1/T_c > 0.08$), and the ratio of P_t at other temperatures to $P_t{}^*x{=}10$ (which is virtually temperature independent) at 35°C for X-SOL (▲ with $1/T_c < 0.06$), S-EDL (□ with $1/T_c < 0.08$) and N-EDL (○ with $1/T_c < 0.08$) are plotted against $1/T_c$. All the points in Fig. 8 are described approximately by

$$\frac{P_t}{(P_t{}^*x{=}10)} = 1.29 - \frac{9.09}{T_c}$$

suggesting that T_c and $(P_t{}^*x{=}10)/P_t$ are related in the same way whether these changes are brought about by nerve cross-union or by changes in temperature.

DISCUSSION

The results show that cross-union of nerves to EDL and SOL muscles in the rat leads to a reversal of the effects of repetitive stimulation on the isometric twitch contractions of these muscles. Soleus muscles, which normally show PTD, exhibit PTP after reinnervation by EDL nerves. PTP in EDL muscles is drastically reduced or replaced by PTD after reinnervation by SOL nerves. These observations indicate that the property of rat

muscles which allows them to undergo PTP is under neural control.

The results further show that all muscles which show PTP (all N-EDL, S-EDL and X-SOL muscles and X-EDL muscles with $T_c \leqslant 26$ msec) also show twitch enhancement with a fall in temperature, and that all muscles which show PTD (all N-SOL and S-SOL muscles, and X-EDL muscles with $T_c \geqslant 29$ msec) also show a fall in peak twitch tensions with decrease in temperature. In this respect, temperature dependence of isometric twitch tensions of fast and slow rat muscles are also controlled by a neural influence.

It has been suggested that muscle fibres which give PTP are incompletely activated during the isometric twitch, and that both PTP and decrease in temperature increase the degree of activation of these muscle fibres (Close & Hoh, 1968a, 1968b). It follows from this hypothesis that if the degree of PTP of a muscle is changed, the degree of twitch enhancement with a decrease in temperature should be changed to an equal extent. The muscles described above show a wide range of values for the degree of PTP and in every case the degree of PTP

matches the degree of twitch enhancement with a decrease in temperature, and in this respect, these results support the hypothesis. According to this hypothesis, neural control of PTP and of temperature dependence of the isometric twitch tension of skeletal muscles would entail essentially the control of the degree of activation of muscle fibres.

Different results have been obtained from similar studies on cat muscles. Buller, Ranatunga and Smith (1968b) showed that peak twitch tensions of cross-innervated cat fast muscles fell with decrease in temperature. This agrees with the results described above for X-EDL muscles which show PTD. However, cross-innervated cat SOL muscles behaved very differently from rat X-SOL muscles with respect to the effects of repetitive stimulation and decrease in temperature on peak twitch tensions. Buller (1963) reported that cross-innervated cat SOL muscles did not show PTP. Buller, Ranatunga and Smith (1968b) showed that peak twitch tensions of cross-innervated cat SOL muscles at 28^{o}C were lower than those at 38^{o}C, though some of these muscles showed a transient increase in peak twitch tension with

gradual cooling from 38 to 35°C. A possible explanation for this difference between the behaviour of cross-innervated SOL muscles in cat and rat will be discussed in Section V in connexion with the question of selective reinnervation of mammalian muscles.

PTP and twitch enhancement with decrease in temperature appear to be inversely related to the speed of contraction of muscle fibres. Neonatal rat EDL muscle, whose intrinsic speed of shortening is virtually identical to that of neonatal or adult SOL muscle (Close, 1964), shows no twitch enhancement with decrease in temperature (Close, 1965c) and no PTP of the kind described above, though it shows an increase in the isometric twitch tension with an increase in the duration of the active state after repetitive stimulation (Close, personal communication). PTP in cat fast muscles appears during development along a time course similar to that for the increase in their intrinsic speed of shortening (Buller & Lewis, 1965a). The loss of twitch enhancement with decrease in temperature in cross-innervated cat fast muscles (Buller, Ranatunga & Smith, 1968b) is associated with a decrease in the intrinsic speed of shortening (Buller & Lewis,

1965b). Cross-innervated cat SOL muscles, which appear to undergo little change in the intrinsic speed of shortening (Buller & Lewis, 1965b) show no PTP (Buller, 1963) together with little or no change in temperature dependence of the isometric twitch tensions (Buller, Ranatunga & Smith, 1968b). On the other hand, SOL muscles of newborn kittens, which have speeds of contraction similar to SOL muscles in adult animals, show potentiation of twitch contractions following indirect stimulation (Buller & Lewis, 1965a), but it is not clear whether this was the result of recruitment of muscle fibres, repetitive firing or potentiation of the response of individual muscle fibres.

The results presented above suggest that when the speeds of contraction of rat muscle fibres are altered by nerve cross-union or by changes in temperature, the degrees of PTP and twitch enhancement with decrease in temperature are changed in such a way as to have the same inverse relationship to the speeds of contraction. Further work is necessary to investigate the possibility that the speed of muscle contraction and the degree of activation of muscle fibres are causally related, in

other words, the possibility that the same neural influence on speed of contractions of mammalian muscles regulates PTP and temperature dependence of the isometric twitch tension of these muscles.

SECTION IV

SELECTIVE REINNERVATION OF FAST-TWITCH AND SLOW-GRADED MUSCLE FIBRES IN THE TOAD

INTRODUCTION

The question whether nerve fibres show a stronger affinity for skeletal muscle fibres they normally innervate than for other skeletal muscle fibres during the process of reinnervation has been investigated in a number of vertebrates. It is currently believed that reinnervation of mammalian skeletal muscles is non-selective (Weiss & Hoag, 1946; Bernstein & Guth, 1961), even though Elsberg (1917) had much earlier reported selective reinnervation of rabbit skeletal muscle. In the fish, selective reinnervation has recently been demonstrated for extraocular (Sperry & Arora, 1965) and pectoral fin muscles (Mark, 1965). In the chick, Feng, Wu and Yang (1965) demonstrated selective reinnervation of the fast, focally innervated posterior latissimus dorsi muscle and the slow, diffusely innervated anterior latissimus dorsi muscle. This demonstration of selective

reinnervation of fast and slow chick muscles raises the possibility that fast and slow types of skeletal muscle fibres of other vertebrates may also show selective reinnervation by their original nerve fibres.

In the toad, skeletal muscle fibres are of two types (see Section II), the fast-twitch type which is focally innervated by low-threshold nerve fibres (LTN) and the slow-graded type which is diffusely innervated by high threshold nerve fibres (HTN). It has been shown above (Section II) that following cross-union of nerves to a homogeneous fast-twitch muscle and a heterogeneous muscle, nerve fibres which normally innervate muscle fibres of one type could functionally reinnervate those of the other type. These observations were made under restricted conditions imposed by nerve cross-union whereby two types of nerve fibres were allowed to reinnervate only one type of muscle fibre, or only one type of nerve fibre was allowed to reinnervate both types of muscle fibres. In the experiments described below, nerves to the toad sartorius muscle (homogeneous fast-twitch) and the anterior semitendinosus muscle (heterogeneous) were allowed to reinnervate both muscles in order to determine

whether LTN and HTN would selectively reinnervate fast-twitch and slow-graded muscle fibres respectively, and whether LTN from the two different muscles would selectively reinnervate fast-twitch muscle fibres in the original muscle.

METHODS

The experiments were carried out on adult toads (Bufo marinus) of body weight ranging from 75 g to 95 g obtained from Queensland, kept alive in the laboratory at room temperature and force-fed with minced liver every 1-2 weeks.

The nerves supplying the sartorius and anterior semitendinosus (AST) muscles occur as a common trunk deep in the thigh. Near the AST muscle, this common trunk gives rise to 1-4 nervules at various points along it to supply the AST muscle before continuing distally as the sartorius nerve in a single bundle.

Operations were performed in 18 toads on the nerves to the sartorius and AST muscles in one leg, the contralateral nerves and muscles were kept intact as controls. Ether was used as anaesthetic and all operations were

performed in aseptic conditions. All the nerve fibres to sartorius and AST muscles were transected and the proximal and distal stumps of the nerve fibres were reunited with fine silk. In most of these animals it was possible to tie together the nervules which make up the AST nerve. In these animals the AST and sartorius nerves were cut and the proximal stumps of these nerves were tied together, following which the distal stumps were united, and finally the proximal and distal stumps were tied together. Extreme care was taken to ensure that all AST nervules were transected in order to avoid collateral nerve regeneration from intact axons (Edds, 1953). For the subsequent identification of the proximal stumps of AST and sartorius nerves, a loose loop of silk was tied around one of these nerves, or the proximal stump of the sartorius nerve was looped around a neighbouring blood vessel before uniting with the proximal stump of the AST nerve. In a few of these animals, it was not possible to tie the AST nervules together because they were short and far apart. In these animals the AST and sartorius nerve fibres were cut and retied at the level of the common trunk.

Because of high mortality rate, only 5 animals survived for experiment 5-7 months after the operation. These include 2 animals in which the AST and sartorius nerves were transected at the common trunk. All 5 animals were in excellent condition at the time of experiment and had a mean body weight gain of 22% during the post-operative period.

For each experiment, the sartorius and AST muscles, sciatic nerve and ventral roots on both sides were carefully dissected and transferred to a bath containing Ringer's solution (NaCl 90 mM, KCl 2 mM, $CaCl_2$ 2 mM, $NaHCO_3$ 2 g/l., glucose 2 g/l.) which was bubbled continuously with 95% O_2 and 5% CO_2 at room temperature (19.5 - 22.7^{o}C).

The equipment and the methods for setting up the preparation for simultaneous recording of isometric tensions of the two muscles, for stimulating the LTN and HTN, for distinguishing responses of fast-twitch and slow-graded muscle fibres and for direct stimulation of (uncurarized) muscles were the same as described above (Section II) for sartorius and posterior semitendinosus muscles. Care was taken to tie muscles securely to the transducers to avoid accidental free shortening during

tetanic contractions which could lead to the appearance of a slow-relaxing component of isometric tetanic tension indistinguishable from the slow-graded response.

Motor units were isolated in the operated muscles by splitting the ventral roots into small filaments which contained only one axon innervating the AST or sartorius muscle. Simultaneous records were obtained from both muscles. The motor axons stimulated were identified as AST or sartorius axons by cutting the proximal stump of one of the two nerves before recording. This was, however, not possible in the two animals in which AST and sartorius nerve fibres were transected at the level of the common trunk.

Definitions. Maximum isometric twitch tension (P_t), maximum isometric tetanic tension (P_o), isometric twitch contraction time (T_c) and isometric twitch half-relaxation time ($T_{\frac{1}{2}R}$) are defined as in Section II above.

Muscle length in situ is defined as the length of the muscle measured during dissection with the thigh at right angles to the midline and the leg at 45° to the thigh.

Total cross-sectional area of muscle in square

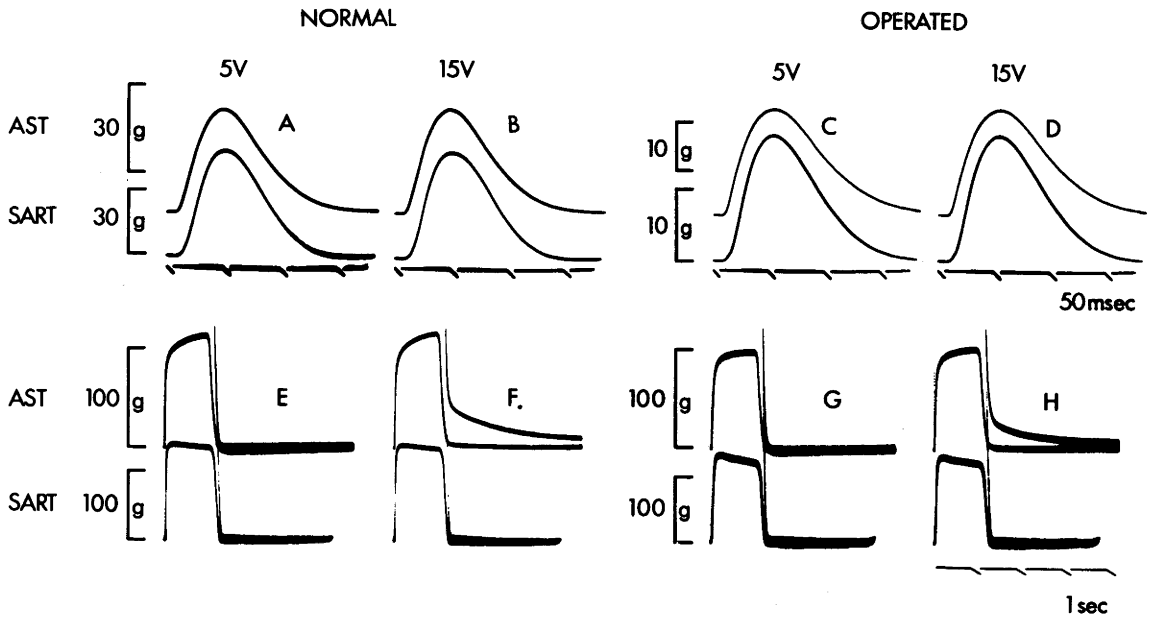

Fig. 9. Records of isometric twitch (A-D) and tetanic (E-H) contractions of normal (A, B, E, F) and operated (C, D, G, H) anterior semitendinosus (AST) and sartorius (SART) muscles from a toad 217 days after operation. The pairs of records of contractions of AST and SART in A-H were recorded stimultaneously. Each tetanic record was displayed on two beams of the oscilloscope on the same base line, one beam having a gain 10 times greater than that of the other to show the slow-graded component of tetanic tension. The tension calibrations for tetanic contractions refer to records of whole muscle responses displayed on the low-gain beams. Time calibrations for records shown in E-H are the same. The strength of the stimulus applied to the sciatic nerve is 5 volts for A, C, E & G and 15 volts for B, D, F & H. Repetitive stimulation was 80c/s for 1 sec in all cases. Muscle weights: normal AST = 97 mg, normal SART = 157 mg, operated AST = 76 mg, operated SART = 150 mg; mean muscle fibre lengths: normal AST = 19 mm, normal SART = 35 mm, operated AST = 18.5 mm, operated SART = 35 mm. Temperature: 21.0°C - 22.0°C.

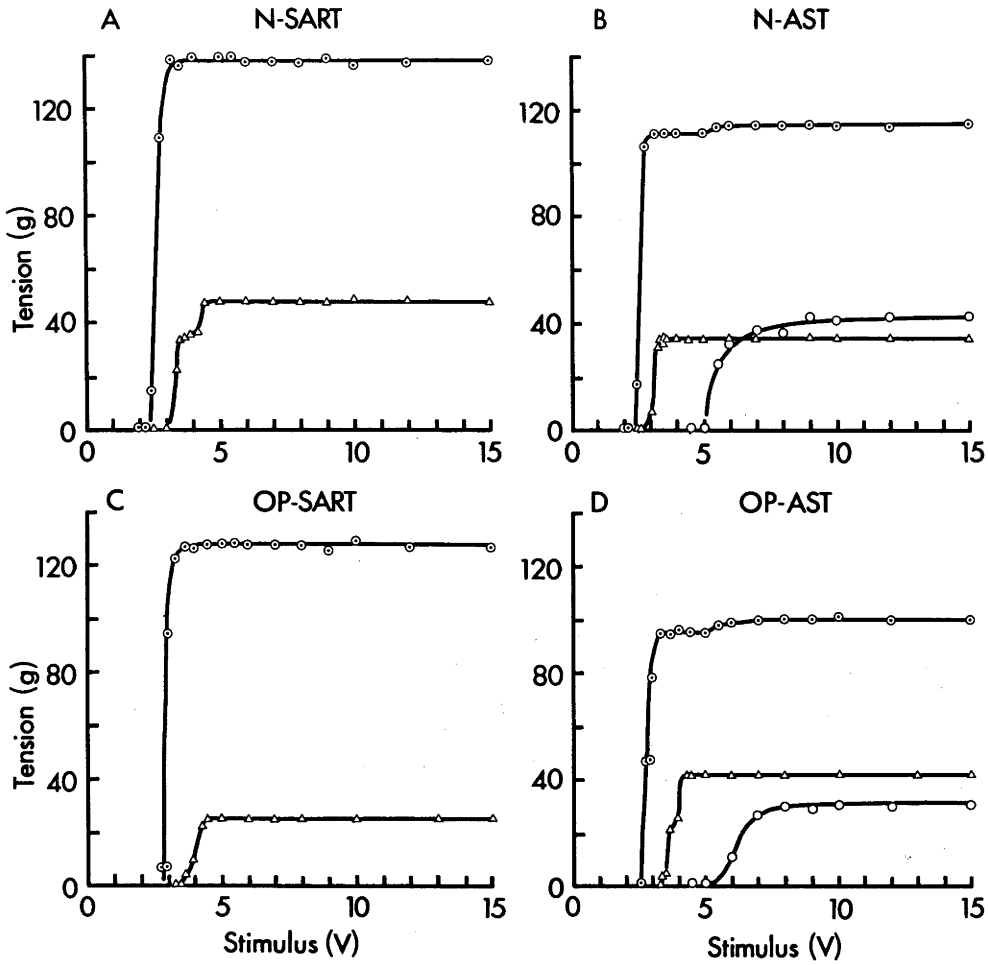

Fig. 10. Maximum tensions developed in isometric twitch (△) and tetanic (whole muscle = ⊙ , slow-graded component = ○) contractions, plotted against stimulus strength in volts (V) applied to the sciatic nerve for normal sartorius muscle (A), normal AST muscle (B), operated sartorius muscle (C) and operated AST muscle (D) from a toad 217 days after operation. The tension scale for slow-graded component of isometric tetanic tension is one tenth of that shown on the ordinate. Representative records of the series of contractions are shown in Fig. 9.

centimetres was estimated by dividing the weight of the muscle in grams (M) by the average fibre length *in situ* in centimetres (L).

RESULTS

Representative records of isometric twitch and tetanic contractions of normal and operated sartorius and AST muscles from a toad 217 days after operation in response to sciatic nerve stimulation are shown in Fig. 9. The curves relating stimulus strength and maximum tensions in twitch and tetanic contractions of the same muscles are shown in Fig. 10. These diagrams show that whereas normal sartorius muscle is a homogeneous fast-twitch muscle innervated only by LTN, normal AST is very similar to normal posterior semitendinosus muscle (Section II), being a heterogeneous muscle with a large fast-twitch component innervated by LTN and a small slow-graded component innervated by HTN. Results from normal muscles from all 5 preparations show the same pattern of innervation of fast-twitch and slow-graded muscle fibres. Twitch threshold curves for normal muscles occasionally show a hump as in Fig. 10A, but there is no corresponding hump in the

TABLE III

	N-SART	N-AST
Thresholds (V)		
LTN: twitch		
min	3.00 ± 0.26	2.90 ± 0.20
max	4.12 ± 0.39	3.66 ± 0.26
LTN: tetanus		
min	2.44 ± 0.24	2.50 ± 0.15
max	3.32 ± 0.35	3.20 ± 0.27
HTN: tetanus		
min	-	5.00 ± 0.42
max	-	9.50 ± 1.20
T_C (msec)	39.8 ± 0.5	41.0 ± 2.2
$T_{½R}$ (msec)	35.8 ± 1.8	41.6 ± 2.7
Total P_O (g)	86.2 ± 18.9	104.4 ± 12.1
Slow P_O (% total P_O)	-	5.3 ± 0.8
P_t/P_O (fast)	0.37 ± 0.06	0.28 ± 0.04
P_OL/M (kg/cm^2)	2.62 ± 0.28	2.68 ± 0.30

Table III. Summary of properties of normal and operated sartorius (N-SART, OP-SART) and anterior semitendinosus (N-AST, OP-AST) muscles and the low threshold (LTN) and high threshold (HTN) nerves which innervate them, obtained from the stimulation of the sciatic nerve. Mean values ± S.E. are listed for the minimal (min) and maximal (max) thresholds for twitch (for LTN) and tetanic (for LTN and HTN) responses in volts (V), isometric twitch contraction time (T_C, msec), half-relaxation time ($T_{½R}$, msec), maximum

TABLE III (continued)

OP-SART	OP-AST
3.54 ± 0.07	3.50 ± 0.05
4.20 ± 0.12	4.20 ± 0.09
2.96 ± 0.13	2.86 ± 0.07
3.84 ± 0.10	3.90 ± 0.14
-	6.10 ± 0.25
-	11.20 ± 0.49
49.0 ± 1.6	45.6 ± 1.2
48.6 ± 2.5	47.2 ± 2.4
93.7 ± 15.8	93.4 ± 10.9
-	3.7 ± 0.6
0.29 ± 0.07	0.29 ± 0.03
3.17 ± 0.20	2.57 ± 0.20

isometric tetanic tension of the whole muscle (Total P_o in grams), tetanic tension of slow-graded fibre component (Slow P_o, % total P_o), twitch:tetanus ratio of the fast component (P_t/P_o), and the maximum force developed by the whole muscle per unit cross-sectional area of muscle (P_oL/M, kg/cm^2); n = 5 for all values. Values for operated muscles which are underlined are significantly different from those for corresponding normal muscles ($P < 0.05$). Temperature: 19.5 - 22.7°C.

tetanus threshold curves for fast-twitch responses in the same preparation to suggest that this may be due to HTN. Furthermore, there is a clear separation in every preparation between the maximum threshold for LTN and the minimum threshold for HTN, the mean of the difference between these thresholds being 1.6 V (range: 0.4 - 2.7 V).

Figs. 9 & 10 also show that fast-twitch responses in both operated muscles are associated exclusively with LTN, and slow-graded responses are found only in the operated AST and are associated exclusively with HTN. These results are confirmed in 5 preparations. The mean of the difference between the maximum threshold for LTN and the minimum threshold for HTN innervating these muscles is 2.2 V (range: 1.2 - 3.0 V).

Table III summarizes the properties of the normal and operated sartorius and AST muscles and their nerves. The thresholds of nerve fibres innervating operated muscles are higher than those innervating the corresponding normal muscles, and the t-test indicates that some of these differences for operated AST muscles are statistically significant ($P < 0.05$), as shown in Table III. The T_C ($P < 0.0025$) and $T_{\frac{1}{2}R}$ ($P < 0.005$) of operated sartorius

muscles are significantly higher than those for normal sartorius muscles. The mean of the ratios of P_o from nerve stimulation to P_o from direct stimulation for all operated muscles is 0.96 and this indicates that nearly all the fibres in these muscles were reinnervated.

The question whether fast-twitch muscle fibres in the operated AST and sartorius muscles were selectively reinnervated by LTN from their original nerves was examined in three preparations in which it was possible to cut one of the proximal stumps. In one preparation, the ratio of the P_o obtained from the stimulation of the sciatic nerve after cutting the AST nerve to the P_o before the procedure was 1.00 for sartorius muscle and 0.78 for AST muscle. No slow-graded response was observed in the AST muscle from the stimulation of the sartorius nerve. This indicates that the sartorius nerve fibres in this preparation innervated all the sartorius muscle fibres and a large proportion of the fast-twitch but none of the slow-graded muscle fibres of the AST. However, it does not necessarily mean that the sartorius muscle was exclusively reinnervated by sartorius nerve fibres, since double innervation of some

sartorius muscle fibres by fibres from sartorius and AST nerves was not excluded. The proximal stump of the sartorius nerve was cut in two other preparations, and the average of the ratios of P_o for fast-twitch responses after cutting the nerve to the P_o before the procedure was 0.58 (0.41 - 0.74) for sartorius and 0.75 (0.65 - 0.85) for AST muscles. This indicates that the AST nerve fibres innervated a larger proportion of the AST muscle than of the sartorius muscle. For these three preparations the mean of these ratios for muscles stimulated through their own nerve is 0.83 ± 0.11 (S.E.), compared with 0.64 ± 0.12 (S.E.) for muscles stimulated through the foreign nerve, but the t-test indicates that the differences between the means are not statistically significant ($P > 0.1$).

Since the operated sartorius and AST nerves innervated both muscles, the question arises as to whether nerve fibres branched at the site of union and regenerated down the distal stumps of both nerves. To test this possibility, attempts were made in three preparations to elicit axon reflexes in the AST muscle by stimulating the distal stump of the sartorius nerve in paraffin oil

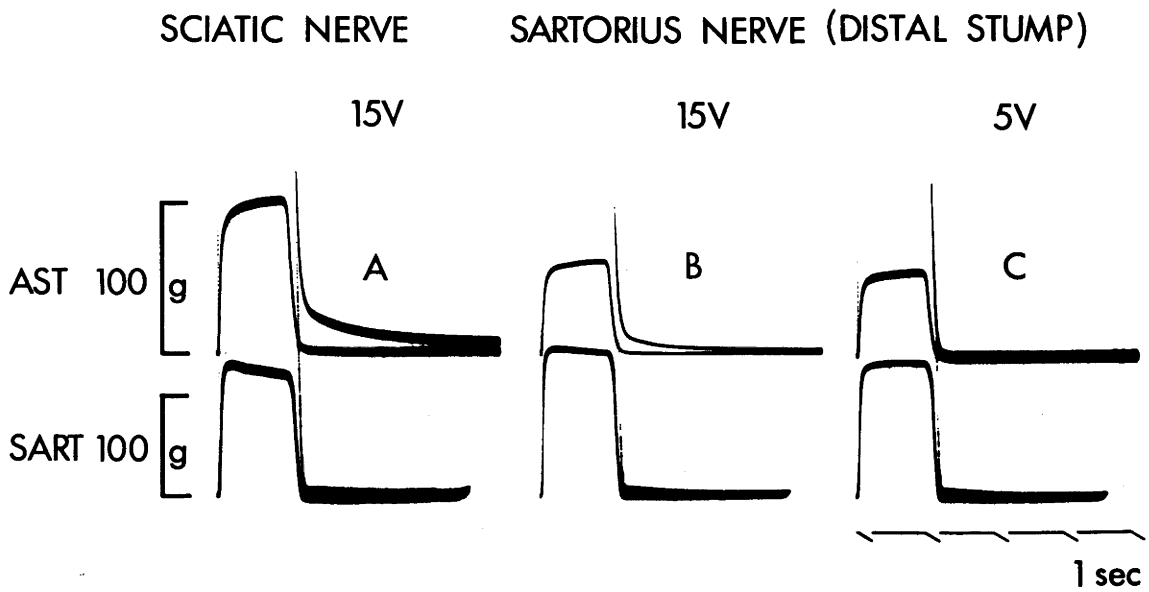

Fig. 11. Records of isometric tetanic contractions of operated AST (upper records) and sartorius (lower records) muscles of the same toad as that described in Figs. 9 & 10 from the stimulation of the sciatic nerve (A) and the distal stump of the sartorius nerve (B, C). The stimulus strength used is shown in volts (V) above each set of records, which are displayed in the same way as tetanic records in Fig. 9.

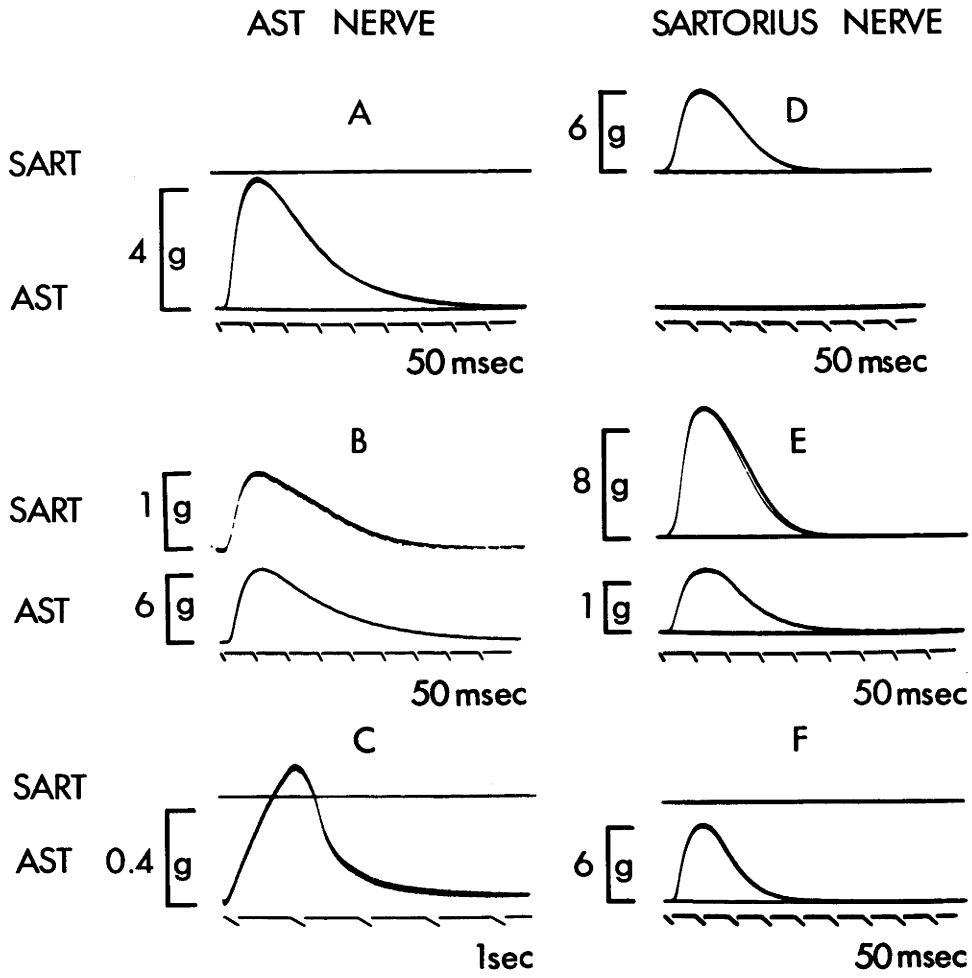

Fig. 12. Records of isometric twitch (A, B, D, E & F) and slow-graded tetanic (C) contractions, in the operated sartorius (SART) and AST muscles, of motor units isolated from the proximal stump of the AST nerve (A-C) of a toad 151 days after the operation and from the sartorius nerve (D-F) of another toad 142 days after operation. For each motor unit, responses in both muscles were recorded simultaneously. A, D, E & F show 10 superimposed traces of twitch responses at the threshold for stimulation of these units. The P_t of whole SART and AST muscles from which A-C were recorded were 25.2 g and 30.3 g, respectively, and the P_t of those from which D-F were recorded were 30.0 g and 18.2 g, respectively. Temperature: 19.5°C - 21.0°C.

near its point of entry into the sartorius muscle, which was about 8-10 mm from the site of operation. Fig. 11 shows records of isometric tetanic contractions of the operated sartorius and AST muscles described in Figs. 9 & 10 in response to repetitive stimulation of the sciatic nerve (A) and the distal stump of the sartorius nerve using 15 V (B) and 5 V (C). These records show large axon reflexes in the AST muscle mediated by both LTN and HTN. Similar results were obtained in all the three preparations tested, and the mean of the ratios of the reflex P_o to the P_o from sciatic nerve stimulation was 0.79 (range: 0.63 - 1.00) for fast- twitch responses and 0.54 (range: 0.44 - 0.72) for slow-graded responses.

The patterns of innervation of the operated muscles by axons from their own or foreign nerves were studied by recording motor unit responses in both muscles simultaneously. Twenty motor units were obtained from four preparations, and Fig. 12 illustrates the types of motor unit responses obtained from two preparations in which the axons stimulated were from the proximal stump of the AST nerve (A-C) or the sartorius nerve (D-F). The twenty motor units consisted of 7 with fast-twitch responses in

TABLE IV

	AST RESPONSES		SARTORIUS RESPONSES	
T_c (msec)	49.8 ± 1.8	(n=13)	52.9 ± 1.7	(n=9)
$T_{½R}$ (msec)	72.8 ± 5.9	(n=13)	70.9 ± 5.5	(n=9)
P_tu/P_ou	0.299 ± 0.049	(n=7)	0.219 ± 0.070	(n=4)
P_tu/P_tm	0.114 ± 0.034	(n=14)	0.132 ± 0.034	(n=9)
P_ou/P_om	0.123 ± 0.039	(n=7)	0.242 ± 0.105	(n=4)

Table IV. Summary of properties of 16 motor units with fast-twitch responses in sartorius and/or anterior semitendinosus (AST) muscles. Mean values ± S.E. are listed for isometric twitch contraction time (T_c, msec), half-relaxation time ($T_{½R}$, msec), ratio of peak twitch of unit to maximum tetanic tension of unit (P_tu/P_ou), ratio of peak twitch tension of unit to peak twitch tension of whole muscle (P_tu/P_tm), ratio of maximum tetanic tension of unit to maximum tetanic tension of fast-twitch component of whole muscle (P_ou/P_om); n is the number of observations upon which each value is based.

both muscles, 2 with fast-twitch responses in the sartorius muscle, 7 with fast-twitch responses in the AST muscle and 4 with slow-graded responses in the AST muscle. The characteristics of the responses in the sartorius and AST muscles of the 16 units with fast-twitch responses are given in Table IV. The average ratio of the P_o of the 4 slow-graded units to the P_o of the slow-graded responses of whole muscles is 0.216 (range: 0.05 - 0.34). None of the slow-graded units has any fast-twitch component, and none of the 7 units with fast-twitch responses tested with repetitive stimulation showed any slow-graded component.

Motor units with responses in both muscles showed a marked asymmetry in the amount of tension elicited in each muscle. In one preparation, 3 such sartorius units (i.e. those with sartorius axons) were isolated, the twitch records for one of these being shown in Fig. 12E. For these units, the mean of the ratios of the P_t of the unit ($P_t u$) to the P_t of the whole muscle ($P_t m$), i.e. $P_t u/P_t m$, was 0.237 ± 0.038 (S.E.) for sartorius muscles and 0.035 ± 0.016 (S.E.) for AST muscles. The t-test shows that the differences between these ratios are

statistically significant ($P < 0.01$). In another preparation 2 AST units (i.e. those with AST axons) with responses in both muscles were isolated. In one of these units which is shown in Fig. 12B, the P_tu/P_tm ratio was 0.200 for AST and 0.047 for sartorius, and in the other the corresponding ratios were 0.004 and 0.025 respectively. In these AST units the mean P_tu/P_tm for responses in the sartorius muscle was 0.036 ± 0.011 (S.E.) and this is significantly lower than the mean P_tu/P_tm for responses in the sartorius muscle found for sartorius units ($P < 0.025$).

DISCUSSION

The results described for whole preparations and motor units show that the exclusive association of LTN with fast-twitch responses and the HTN with slow-graded responses are re-established within 5-7 months after an operation which permitted both types of nerve fibres to reinnervate either type of muscle fibre. This could have arisen either from random reinnervation followed by appropriate changes in muscle or nerve fibres, or from selective reinnervation of each type of muscle

fibres by the appropriate type of nerve fibres. It has been shown in nerve cross-union experiments (Section II) that LTN can innervate slow-graded muscle fibres and HTN can innervate fast-twitch muscle fibres, there being no transformation of mechanical properties of the muscle fibres and no transformation of one type of nerve fibre into the other type. It may, therefore, be concluded that LTN selectively reinnervated fast-twitch muscle fibres and HTN selectively reinnervated slow-graded muscle fibres.

Although available data from the stimulation of the proximal stump of the sartorius or AST nerves indicate that LTN do not show a clear-cut selective reinnervation of their original muscle fibres, there is a trend for whole nerves to reinnervate their original muscles more extensively. Motor unit studies show that sartorius axons which branched and reinnervated both muscles show significantly more extensive reinnervation of the sartorius muscle. Furthermore, AST axons which reinnervated both muscles, reinnervated the sartorius muscle less extensively than branched sartorius axons reinnervated it. These observations suggest that LTN show a stronger affinity

for fast-twitch muscle fibres of the original muscle than for those of a foreign muscle. Further work is necessary to establish this point and to investigate further the possibility that the original central-peripheral relations of LTN and fast-twitch muscle fibres in both AST and sartorius muscles may be re-established eventually.

It is well recognized that branching of regenerating axons occur in experimental animals (Ramón y Cajal, 1928; Feiss, 1912; Greenman, 1913; Weiss & Campbell, 1944; Weiss, Edds & Cavanaugh, 1945; Hughes, 1964) and in man (Esslen, 1960; Fullerton & Gilliatt, 1965; Gilliatt, 1966). The experiments described above show that branching occurs in both regenerating LTN and HTN, since axon reflexes in the AST muscle mediated by LTN and HTN could be elicited by stimulating the distal stump of the sartorius nerve. LTN, therefore, regenerated down the distal stump of the sartorius nerve, but failed to make functional synaptic contacts with the fast-twitch muscle fibres. A similar situation was observed in the chick by Feng, Wu & Yang (1965), who found that nerve fibres normally supplying the slow

anterior latissimus dorsi muscle regenerated down the distal stump of the nerve to the fast posterior latissimus dorsi muscle, but failed to innervate this muscle.

Muscle fibres in fish (Takeuchi, 1959) and salamanders (Mark, Campenhausen & Lischinsky, 1966) are multiply innervated. It has been suggested that this type of innervation, in which one muscle fibre will accept several nerve endings, favour selective reinnervation of muscles by their original nerves by allowing prolonged competition between nerve fibres of different central origins for control of the muscle (Mark, 1965; Mark, Campenhausen & Lischinsky, 1966). On the basis of this hypothesis, it would be difficult to explain the observed selective reinnervation of fast-twitch muscle fibres in the toad by LTN, and of fast muscle fibres in birds by their original nerves (Feng, Wu & Yang, 1965), since these muscle fibres are usually focally innervated (Section II; Ginsborg, 1960a). Furthermore, this hypothesis would exclude the possibility of re-establishing the original central-peripheral relations of focally innervated anuran, avian and mammalian muscle fibres and the type of nerve fibres which normally innervate them

during reinnervation.

An alternative hypothesis which would not exclude selective reinnervation of focally innervated muscle fibres is that branching of the axon of a regenerating motoneurone enables it to reinnervate, at first indiscriminantly, fibres of different types or from different muscles. Whether muscle fibres are multiply innervated or not, this would allow for prolonged interaction between the motoneurone and muscle fibres, whereby nerve sprouts connected to the appropriate muscle fibres mature and are retained, while those connected to inappropriate muscle fibres atrophy and thus allow these muscle fibres to be reinnervated by other regenerating nerve fibres. A similar mechanism has been suggested for the development of specific nerve-muscle relations during development in an anuran (Hughes, 1965). Appropriate peripheral connexions are necessary for the maturation of regenerating axons (see Section I, p 11). If this peripheral influence from a given muscle is specific for nerve fibres normally innervating this muscle, as suggested in Section II, it may play a very significant role in selective reinnervation.

SECTION V

THE PROBLEM OF SELECTIVITY IN THE REINNERVATION OF FAST AND SLOW RAT MUSCLES

INTRODUCTION

There is disagreement in the literature on the question whether reinnervation of mammalian skeletal muscle is selective or random. The current view, based primarily on the experiments of Weiss and Hoag (1945) and Bernstein and Guth (1961), is that it is non-selective. Weiss and Hoag (1945) allowed tibial and peroneal nerves in the rat to regenerate into the distal stump of the tibial nerve, and found that the plantar extensors were reinnervated with equal probability by either tibial or peroneal nerves. Bernstein and Guth (1961) found that, in normal rats in which both soleus and plantaris muscles received innervation from L4 and L5 ventral roots, the ratio of the tension produced by the stimulation of L4 to that of L5 is higher for plantaris than for soleus, but following transection and regeneration of the sciatic nerve, this difference is lost. None of these experiments

in which nerve trunks were used refutes earlier observations carried out on nerves to individual muscles which show selective reinnervation in rabbits (Elsberg, 1917). In the present work, the question whether there is selective reinnervation of skeletal muscles in the rat is re-investigated using nerves to extensor digitorum longus (EDL) and soleus (SOL) muscles.

METHODS

Twenty-one three-week-old female rats (Wistar) were operated on in one or both lower limbs under aseptic conditions using pentobarbital sodium (40-50 mg/kg body weight injected intraperitoneally) as anaesthetic. Nerves to EDL and SOL muscles were transected and one of these muscles was completely excised. The ends of the proximal stumps of the transected nerves were tied together with fine silk and the distal stump of the nerve to the remain- muscle was united with the two proximal stumps. Extreme care was taken during the operation to avoid injury to the nerves proximal to the site of union. In order to avoid collateral nerve regeneration from the terminals of intact motor axons (Edds, 1953), care was taken to ensure that

all nervules entering each muscle were transected. As a further precaution, muscles were carefully dissected at the end of the experiments to look for accessory nerves which might not have been transected at the operation. One SOL muscle was found to have such an accessory nerve from the nerve to the plantaris muscle, and all data from this muscle were rejected.

Experiments were performed 22-162 days after operation. The methods and equipment used for setting up muscles for in situ isometric tension recording in response to indirect stimulation were the same as those described in Section III. For each muscle, the optimal length (L_o) for isometric twitch contractions in response to stimulation of its original nerve was found and all subsequent measurements on this muscle were made at this length. A series of twitches and responses to repetitive stimulation at various frequencies were recorded for each muscle from the stimulation of each of the two nerves.

Direct stimulation was also carried out on uncurarized muscles examined in the early post-operative period. The S4 stimulator was followed by a transistor current amplifier, and stimulus pulses used were of

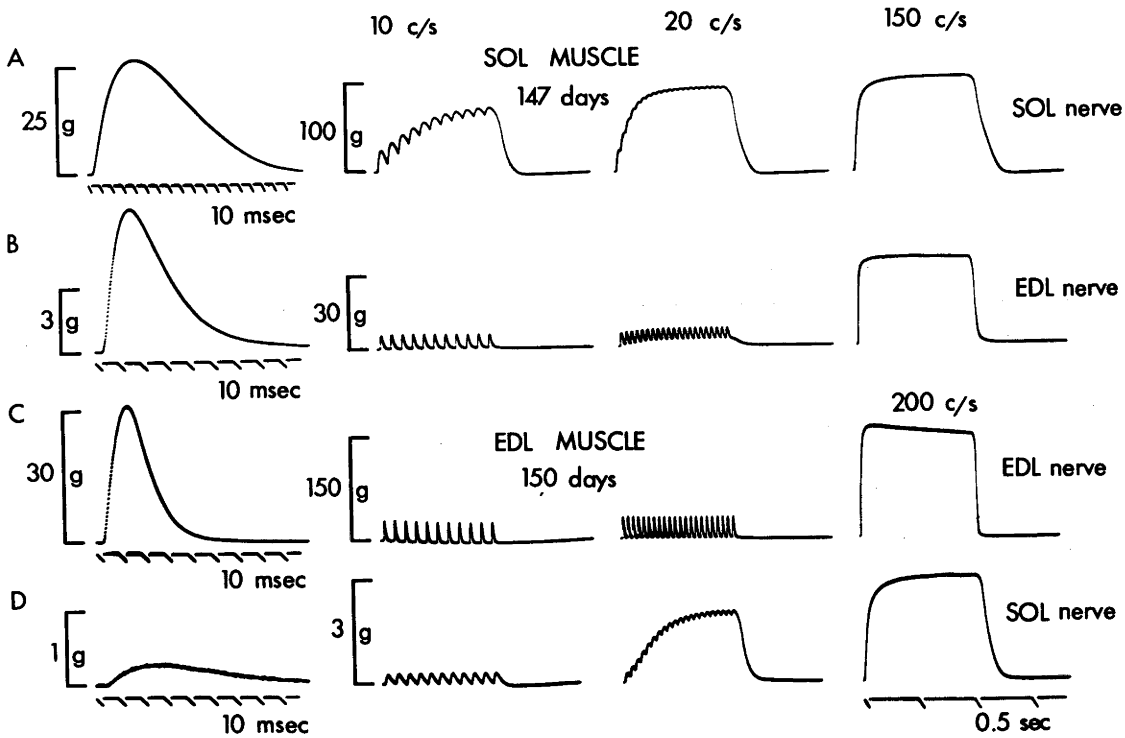

Fig. 13. Records of contractions of a SOL muscle examined 147 days (A, B) and of an EDL muscle 150 days (C, D) after operation, in response to stimulation of SOL nerve (A, D) and EDL nerve (B, C). These records are from left to right, twitch contraction, responses to repetitive stimulation at 10c/s, 20c/s and 150c/s (A, B) or 200c/s (C, D). Muscle weights: SOL = 83.5 mg, EDL = 105 mg; mean fibre lengths: SOL = 15.5 mm, EDL = 12 mm. Temperature: 34.8°C - 35.4°C.

0.2-0.3 msec duration and of supramaximal strength (about 24 V). Platimum wire electrodes were used on both sides of the muscle in a paraffin bath. In order to avoid mechanical interference from neighbouring muscles, as much of these muscles as possible was excised during the initial dissection for the experiment.

All measurements were made at 35°C.

The definitions for T_c, $T_{\frac{1}{2}R}$, P_t and P_o are the same as in Section III except that all measurements are based on L_o as defined above.

RESULTS

Fig. 13 shows records of contractions elicited from SOL and EDL nerves of a SOL muscle (A, B) and an EDL muscle (C, D) examined 147 and 150 days respectively, after operation. These records show that for each muscle, fibres reinnervated by the foreign nerve differ in the speed of their contractions from that of fibres reinnervated by the original nerve, as would be expected from the neural influence on the speed of muscle contraction (Buller, Eccles & Eccles, 1960b; Close, 1965b, Buller & Lewis, 1965b; Section III). These records also show

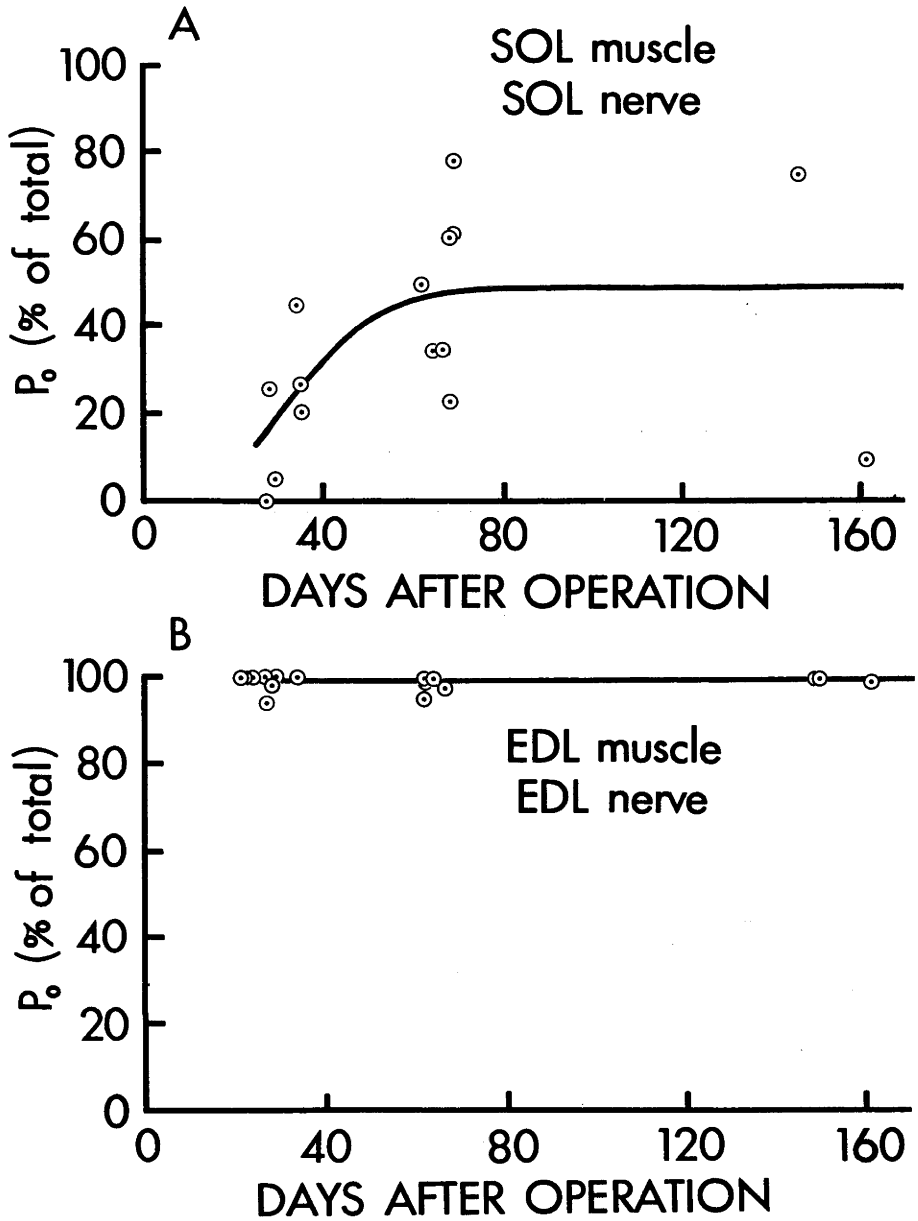

Fig. 14. Reinnervation of SOL (A) and EDL (B) muscles by their own nerves. Ordinates: maximum isometric tetanic tension (P_o) from the stimulation of SOL nerve (A) or EDL nerve (B) as percentage of the total P_o from the stimulation of EDL and SOL nerves separately. Abscissae: days after operation.

that both muscles are reinnervated to a greater extent by their original nerves and that this is more marked in the case of the EDL muscle.

Fig. 14 shows the extent of reinnervation of 15 SOL muscles (A) and 16 EDL muscles (B) by their original nerves at various times after operation. For each muscle, the P_o obtained from the stimulation of the original nerve, expressed as a percentage of the total P_o (sum of the P_o from the stimulation of each of the two nerves), i.e. P_o(% of total), is plotted against days after operation. Fig. 14A shows that for SOL muscles the values for P_o(% of total) are low during the early post-operative period, but show a subsequent rise to an average of about 50% within 60-70 days. The degree of reinnervation, by both nerves, of the 6 SOL muscles examined 27-35 days after operation, was tested by comparing the P_o from the simultaneous stimulation of both nerves with the P_o obtained from direct stimulation. With the exception of the two muscles examined 27 and 28 days after operation, which were 38% and 62% functionally reinnervated, all the muscles in this group were fully reinnervated. The mean P_o(% of total) for the stimulation of the SOL nerve

for these fully reinnervated muscles examined in the early post-operative period is 24.0 ± 8.3 (S.E.), and the t-test shows that this is significantly different from the mean of 48.8 ± 7.2 (S.E.) for the 7 muscles examined 62-69 days after operation ($P < 0.05$).

Fig. 14B shows that EDL muscles are reinnervated almost exclusively by their original nerves over the whole of the post-operative period examined. The mean value of P_o(% of total) from the stimulation of EDL nerve is 98.9% (n = 16). In 5 preparations, stimulation of SOL nerve produced no detectable tension. The degree of functional reinnervation was determined for the group of 8 EDL muscles examined 22-34 days after operation in the same way as described for SOL muscles by comparing P_o obtained from direct stimulation of each muscle and from indirect stimulation of both nerves. One preparation showed 45% reinnervation 27 days after operation while all the others showed degrees of reinnervation ranging from 86% - 100% (mean: 93.8%).

In 6 other EDL preparations not represented in Fig. 14B attempts were made to increase the degree of reinnervation of EDL muscles by SOL nerves by crushing

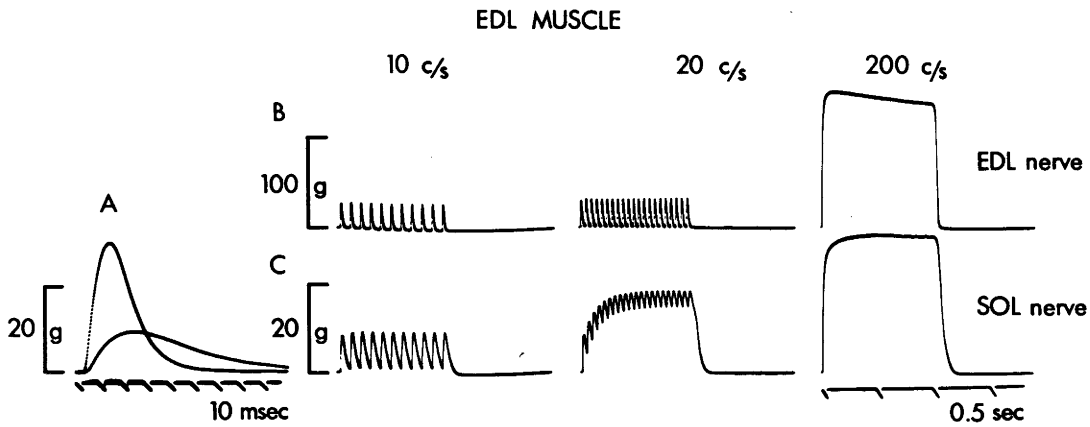

Fig. 15. Records of contractions of an EDL muscle from a preparation in which the peroneal nerve was crushed 32 days after the original operation and the experiment performed 36 days after the nerve crush. A, superimposed records of twitch contractions with identical amplifier gains in response to stimulation of EDL nerve (the faster response) and SOL nerve. B and C show responses to repetitive stimulation at 10c/s, 20c/s and 200c/s of EDL nerve (B) and SOL nerve (C). Muscle weight = 80 mg. Mean fibre length = 12 mm. Temperature: 34.9°C.

the peroneal nerves about 3 mm proximal to the level of transection of EDL nerves at various times after the original operation. In 4 of these preparations the peroneal nerves were crushed 12 days after the original operation and experiments performed 37-39 days later. All these EDL muscles were fully reinnervated and stimulation of SOL nerves in these preparations gave no more than 0.25% (mean; range: 0.0% - 1.0%) of the total P_o. In the other 2 preparations the peroneal nerves were crushed at 30-32 days after the original operation and experiments performed 36-42 days after. These EDL muscles also were fully reinnervated, and stimulation of SOL nerves gave 8.4% - 17.3% of the total P_o. Representative records of contractions of one of these muscles from the stimulation of EDL and SOL nerves are shown in Fig. 15.

The question whether some muscle fibres were reinnervated by fibres from both nerves was investigated in 7 SOL muscles. The ratio of the total P_o from the stimulation of the two nerves separately to the P_o obtained from the simultaneous stimulation of both nerves, at the same frequency, was calculated for these muscles.

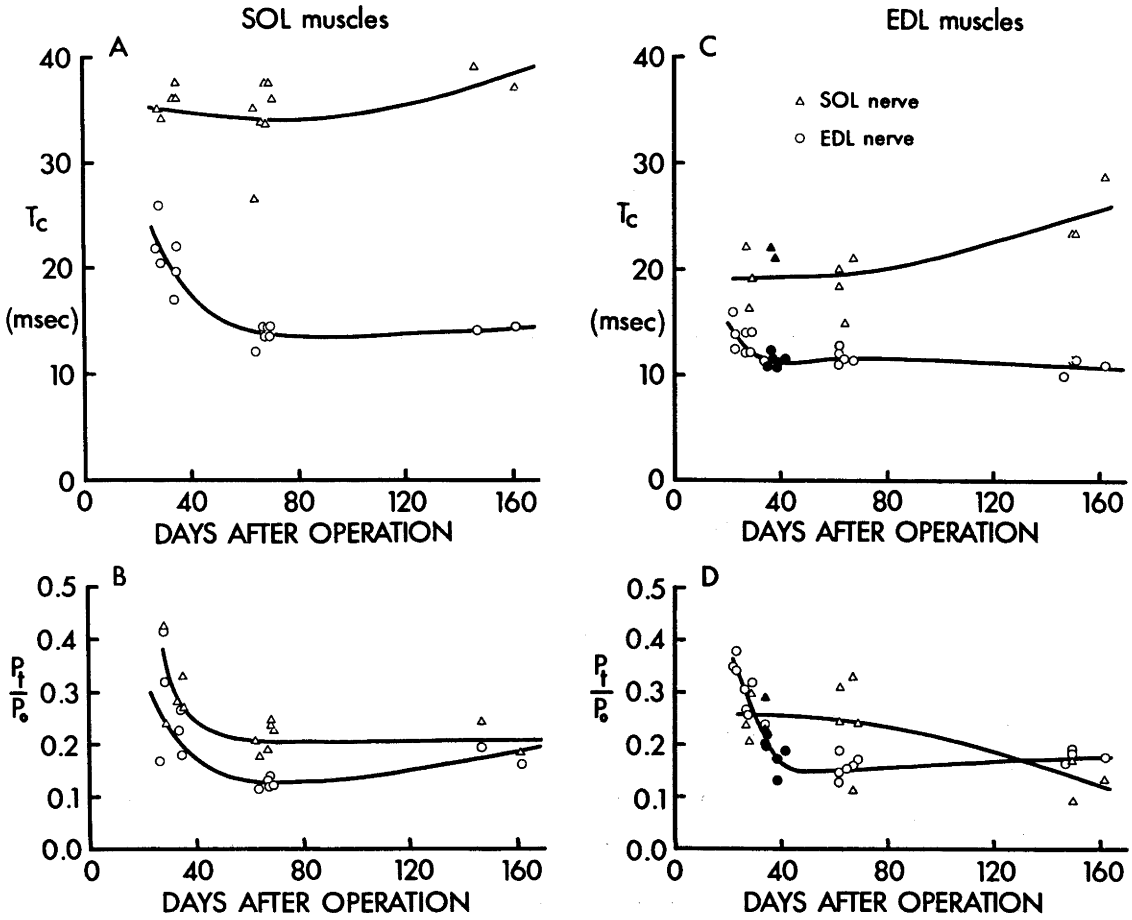

Fig. 16. Contraction times (T_c, msec) and twitch:tetanus ratios (P_t/P_o) of SOL (A, B) and EDL (C, D) muscles reinnervated by SOL (△) and EDL (○) nerves plotted against days after operation. Results from EDL preparations in which the peroneal nerves had been crushed are plotted (filled symbols) against days after the nerve crush.

Overlap of innervation would lead to a ratio in excess of unity. The ratios for these muscles range from 0.95 to 1.05, the average being 1.003. These results do not indicate a significant degree of overlap in the innervation of SOL muscles by SOL and EDL nerves, but do not exclude hyperneurotization of muscle fibres by nerve fibres of the same type. This test is not sufficiently sensitive for detecting overlap of innervation in EDL muscles because of the large disparity in the values for P_o from SOL and EDL nerves.

Fig. 16 shows T_c and P_t/P_o of SOL (A & B) and EDL (C & D) muscles obtained from the stimulation of SOL (△) and EDL (○) nerves plotted against days after operation. These diagrams show the time courses for the reversal of the effects of denervation on these properties by their original nerves and the time courses for the transformation of these properties by foreign nerves. The time courses of changes in these properties are similar to those in self-innervated and cross-innervated rat SOL and EDL muscles (Close, personal communication). At more than 140 days after operation, the values for these properties are comparable to those of self-innervated

and cross-innervated SOL and EDL muscles described in Section III, except that the P_t/P_o of EDL muscles innervated by SOL nerves shown in Fig. 16D are lower than those of cross-innervated EDL muscles described in Section III.

DISCUSSION

The experiments described above show that when SOL and EDL nerves are given equal chances to reinnervate the SOL muscle, stimulation of the EDL nerve during the early post-operative period produces a larger tetanic tension than stimulation of the SOL nerve, but that subsequently the tetanic tensions produced by the stimulation of these nerves tend to be the same. A possible explanation for these observations is that SOL muscle fibres are reinnervated with equal probability by fibres from either nerve, but those muscle fibres reinnervated by SOL nerve fibres recover from denervation atrophy more slowly than those reinnervated by EDL nerve fibres. An alternative explanation is that EDL nerve fibres regenerate to the end-plate region more rapidly and consequently reinnervate a larger proportion of SOL muscle fibres

initially, but following this there is a period of time during which some of the functional foreign nerve endings are withdrawn and replaced by SOL nerve endings. In this connexion, it should be mentioned that Guth (1962) observed persistence of collateral sprouts formed as a result of collateral nerve regeneration in partially denervated rat SOL muscle even after regeneration of nerve fibres into collaterally reinnervated muscle fibres. However, these collaterals were from fibres in the SOL nerve, and therefore were not foreign to SOL muscle fibres. Gutmann and Hanzlíková (1967) reported persistence of foreign neuromuscular junctions in hyperneurotized rat SOL muscle fibres formed by implanting the peroneal nerve into SOL muscle denervated by crushing the tibial nerve, but it was not clear whether the tibial nerve fibres which hyperneurotized the SOL muscle fibres were the original SOL nerve fibres. However, electrophysiological evidence for the withdrawal of inappropriate preganglionic nerve endings on mammalian sympathetic ganglion cells and their replacement by appropriate ones has been presented by Guth and Bernstein (1961).

When SOL and EDL nerves are given equal chances to reinnervate the EDL muscle, it becomes reinnervated almost exclusively by EDL nerve fibres. A possible explanation for this observation is that rate of regeneration of the EDL nerve fibres to the end-plate region is faster than that of the SOL nerve fibres, as suggested above in connexion with the reinnervation of the SOL muscle, so that EDL nerve fibres reinnervated nearly all the muscle fibres before the arrival of SOL nerve fibres at the end-plate region. Since SOL nerve fibres reinnervated EDL muscle fibres very poorly even in preparations in which peroneal nerves were crushed at various times after the initial operation, this difference in the rate of regeneration would need to be considerable. An alternative explanation is that there are selective forces operating which favour the formation of junctions between EDL nerve fibres and denervated EDL muscle fibres or cause newly formed foreign junctions to be withdrawn and replaced by those from EDL nerve fibres.

The foregoing discussion indicates that available data are consistent with the hypothesis that fast and

slow mammalian muscle fibres have a stronger affinity for their original nerve fibres than for foreign nerve fibres, though further work is necessary to investigate other possible explanations and the possibility that the different rates of regeneration of nerve fibres innervating fast and slow muscle fibres may, in conjunction with this hypothesis, account for the apparent selective reinnervation of the fast muscle and the apparent non-selective reinnervation of the slow muscle.

Cross-innervated EDL muscles described in Section III were reinnervated by nerve fibres from the peroneal nerve in addition to those from the SOL nerve, even though during the cross-union operation only the SOL nerve was tied to the distal stump of the EDL nerve. This observation probably reflects the greater affinity between fast muscle fibres and fast nerve fibres, and emphasizes the need to take special precautions to prevent selective reinnervation in studies on the effects of nerve cross-union. In their studies on the neural regulation of intracellular potassium and glycogen levels in rat EDL and SOL muscles, Drahota and Gutmann (1963) joined the proximal stump of the tibial nerve to the

distal stump of the peroneal nerve, and found no change in the potassium level in EDL muscle after reinnervation. However, SOL muscles after reinnervation by peroneal nerve fibres showed increases in potassium and glycogen levels. It is probable that in these experiments EDL muscle fibres were selectively reinnervated by fast fibres in the tibial nerve.

Motor unit studies (Close, 1967a) reveal that rat EDL and SOL muscles are virtually homogeneous fast and slow muscles, respectively. Similar studies in the cat show that while SOL is also a homogeneous slow muscle (McPhedran, Wuerker & Henneman, 1965), flexor digitorum longus (FDL) muscle is heterogeneous (Olson & Swett, 1966), and about 20% of the motor units of this muscle have contraction times which are within the range of values for the contraction times of motor units from cat SOL muscles (McPhedran, Wuerker & Henneman, 1965). Histochemical studies on cat FDL and flexor hallucis longus (FHL) muscles (Olson & Swett, 1966; Romanul & Meulen, 1966, 1967; Dubowitz & Newman, 1967; Dubowitz, 1967) show that both these muscles have a large proportion (20% - 55%) of muscle fibres with high activity of enzym

of lipid and oxidative metabolism which characterize slow muscle fibres.

In view of possible selective reinnervation of mammalian fast and slow muscle fibres by the type of nerve fibres which normally innervate them, the question arises whether nerve cross-union experiments involving nerves to cat FDL or FHL muscles have been complicated by selective reinnervation. In this connexion, it is of interest that several contractile properties (Buller, 1963; Buller & Lewis, 1965b; Buller, Ranatunga & Smith, 1968b; see Discussion in Section III) and histochemical properties (Dubowitz & Newman, 1967; Dubowitz, 1967) of cat SOL muscles reinnervated by FDL or FHL nerve are not altered, or less completely altered than FDL or FHL muscles reinnervated by SOL nerves. This situation would be expected from the cross-union of a homogeneous SOL nerve with a heterogeneous FDL or FHL nerve followed by selective reinnervation, since fast muscle fibres in the heterogeneous muscle would be reinnervated only by slow SOL nerve fibres and be transformed into muscle fibres resembling normal slow muscle fibres, whereas the

homogeneous SOL muscle could selectively favour re-innervation by slow nerve fibres from the heterogeneous nerve, and fibres so reinnervated would not be transformed. Whether this is the explanation for the different degrees of transformation in cross-innervated cat fast and slow muscles on the one hand, and for the differences between cross-innervated cat SOL and rat SOL muscles (Section III) on the other, remains to be clarified by future work.

SECTION VI

GENERAL DISCUSSION

Neural regulation of the speed of muscle contraction.

In many mammals, all limb muscles at an early stage of development contract at about the speed of contraction of slow muscles in the adult, but changes which occur within the first few weeks of life lead to a 2-3 fold increase in the speed of contraction of muscle fibres destined to be fast (see Close, 1967b; Close & Hoh, 1967). The myosin adenosinetriphosphatase (ATPase) activity of developing (Villafranca, 1954; Trayer & Perry, 1966) and adult (Bárány, Bárány, Reckard & Volpe, 1965; Sreter, Seidel & Gergely, 1966; Bárány, 1967) mammalian muscles are correlated with their speeds of shortening. As this correlation is also found for muscles from a wide range of vertebrates and invertebrates (Bárány, 1967; Bárány, Conover, Schliselfeld, Gaetjens & Goffart, 1967), it appears likely that myosin ATPase activities for fast and slow mammalian muscles after nerve cross-union would be changed in proportion to changes in the speed of their

contractions.

There are differences in biochemical properties other than the ATPase activity between foetal and adult myosins and between myosins of fast and slow muscles (Trayer & Perry, 1966; Sreter, Seidel & Gergely, 1966) which suggest structural differences between them. There are also antigenic differences between foetal and adult myosins (Varga, Kövér, Kovács, Jókay & Szilágyi, 1962). These observations suggest that differentiation of the speed of contraction of fast and slow muscles and the neural control of the speed of contraction of these muscles following nerve cross-union involve the syntheses of new species of myosins. This would mean that mammalian motor nerves have the remarkable ability of controlling the expression of specific genetic information in muscle cells. Future investigations into the detail mechanisms of this control would be very important to molecular biology.

If mammalian motoneurones can indeed control the expression of specific genetic information in muscle cells, the transfer of a highly specific message from motoneurone to muscle would be necessary. Chemical

substances such as ribonucleic acids, "repressors" or "inducers" (Jacob & Monod, 1961) would have the required specificity. These substances may represent part of the flow of neuroplasmic material from the motoneurone down the axon (for review see Weiss, 1961; Waelsch & Lajtha, 1961; Lubińska, 1964) and across the neuromuscular junction into the muscle cell (Korr, Wilkinson & Chornock, 1967).

Specificity of synaptic connexions.

The molecular basis for the specificity of synaptic connexions has been the subject of much speculation. Sperry (1965) suggested that specific molecular interaction between biochemically matching cell surfaces may be the basis for their specificity. Ungar (1968) suggested that at the synapse, neuronal surfaces are held together by a specific connective material in the synaptic cleft synthesized by the presynaptic or postsynaptic cell. A protein, known as the nerve growth factor, which has the remarkable property of stimulating specifically the growth of sympathetic nerve cells and some embryonic sensory nerve cells at minute concentrations, has been isolated and purified from a variety of biological sources

(for review, see Levi-Montalcini, 1966). It has been suggested that there may exist other nerve growth factors which act specifically on other types of nerve cells (Levi-Montalcini, 1964), and that these factors may play a very important part in selective synaptic contacts (Strumwasser, 1965). A nerve growth factor hypothesis for the specificity of neuromuscular connexions is outlined below.

Motoneurones are maintained in the normal trophic state by specific nerve growth factors produced by muscle fibres they innervate and transported centripetally along the axons. During reinnervation, each motoneurone by branching of its regenerating axon may come into contact with fibres in many muscles. Those axon branches innervating appropriate muscle fibres mature under the influence of specific nerve growth factors, while those with inappropriate connexions eventually atrophy, thereby permitting muscle fibres initially reinnervated by them to be reinnervated by appropriate nerve fibres. Motoneurones which have no access to their original muscle fibres may form persistent functional connexions with other muscle fibres but in the absence of specific nerve

growth factors, these motoneurones may not regain their normal trophic state. Mammalian motoneurones may control the type of nerve growth factor produced by muscle fibres they innervate in much the same way as they control other biochemical properties of muscle fibres.

The hypothesis outlined above may account for the influence of muscle fibres on growth of normal nerve fibres and maturation of regenerating nerve fibres (Section I, p.11). Nerve growth factors may be released by denervated muscle fibres and this may account for sprouting of nerve terminals from neighbouring intact nerve fibres in partially denervated muscles (Harreveld, 1947). Hypertrophy of nerve fibres which collaterally reinnervate denervated fibres in partially denervated muscles (Edds, 1949) may result from an increase in the supply of nerve growth factors to these motoneurones.

There is evidence for the postulated existence of some mechanism for the transfer of substances from muscle fibres to motoneurones. Axoplasmic streaming in regenerating and in normal nerve fibres is bidirectional (for review see Lubińska, 1964; Miani, 1964; Burdwood, 1965; Dahlström & Haggendal, 1966; Lasek, 1967). Kerkut,

Shapiro & Walker (1967) have shown migration of labelled substances from muscle to nerve cells along axons. Future investigations into the nature of substances which migrate from muscle to nerve and into the nature and specificity of chemical factors which have growth promoting effects on motoneurones may contribute significantly to the molecular basis for the specificity of synaptic connexions.

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Influence of Temperature on Isometric Contractions of Rat Skeletal Muscles

THE influence of temperature on isometric contractions of rat skeletal muscles *in vitro* has been determined for fast extensor digitorum longus muscles (EDL) and slow soleus muscles (SOL) from 4 week old female Wistar rats. Isometric contractions were recorded with the long axis of the muscle vertical, with one tendon tied to the frame below and the other tendon tied to a short steel wire connexion which linked it with the tension transducer above. The compliance of the transducer (Statham, *Gl*-80-350) and the steel wire connexion to the muscle was $4{\cdot}5 \times 10^{-5}$ cm/g and the natural frequency of vibration was 2 kHz. The muscle was immersed in about 100 ml. of fluid (137 mM NaCl; 5 mM KCl; 2 mM $CaCl_2$; 1 mM $MgCl_2$; 1 mM NaH_2PO_4; 2 g/l. of $NaHCO_3$ and 2 g/l. of glucose) which was bubbled continuously with 95 per cent O_2 and 5 per cent CO_2. Neuromuscular transmission was blocked by adding $2{\cdot}0 \times 10^{-5}$ g of tubocurarine chloride/ml. of bath fluid. The muscles were stimulated directly by a transverse electrical field of 15 V/cm for 0·2 or 0·3 ms applied to the bath fluid by "massive" bright platinum electrodes set about 1 cm apart[1]. All recordings were made with the muscle set at the optimal length determined for twitch contractions at 20° C.

Fig. 1 shows representative records of isometric twitch and tetanic contractions of an EDL muscle and a SOL muscle from one animal. Three twitches were recorded (1/10 s) about 10 min after each change in temperature for a series in which the temperature was altered in 5° C steps from 35° C to 20° C and from 20° C to 35° C. For both muscles the maximum twitch tension (P_t) at a given temperature did not differ by more than 2 per cent for the ascending and descending series of measurements. The isometric tetanic contractions were recorded 10 min after each change in temperature in a separate series of measurements at 35° C, 20° C, 25° C, 30° C and 35° C in that order. The muscles remained in good condition throughout the experiment and the ratio of the maximum isometric tetanic tension (P_o) for the final contraction at 35° C to P_o for the initial contraction at 35° C was 1·05 for SOL and 0·985 for EDL. The results show that decrease in temperature from 35° C to 20° C decreased P_t and P_o of SOL and P_o of EDL, whereas P_t of EDL was increased 1·7 times. The decrease in temperature from 35° C to 20° C increased the isometric twitch contraction time (T_c) in both muscles from 10·75 ms to 37·5 ms for EDL and from 28 ms to 122 ms for SOL and also in-

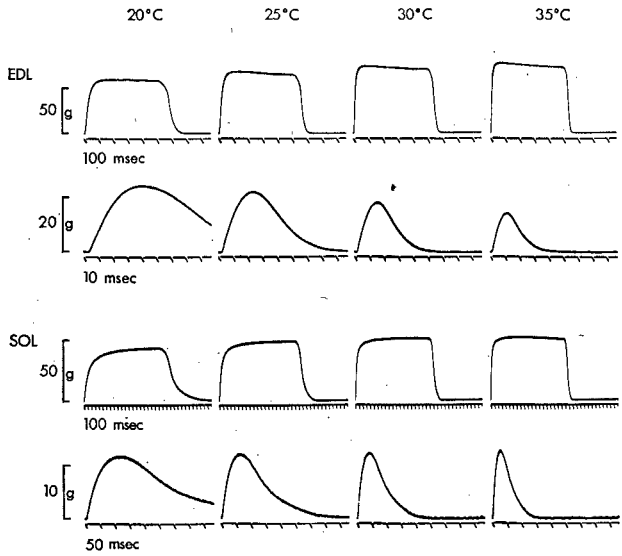

Fig. 1. Records of isometric twitch (10 ms and 50 ms time marks) and tetanic (100 ms time marks) contractions of extensor digitorum longus (EDL) and soleus (SOL) muscles at different temperatures. The frequencies of repetitive stimulation for tetanic contractions of EDL were 300 Hz at 35° C, 210 Hz at 30° C, 150 Hz at 25° C and 105 Hz at 20° C and the frequencies of stimulation of SOL were 250 Hz at 35° C, 180 Hz at 30° C, 125 Hz at 25° C and 90 Hz at 20° C. The rat was 4 weeks old and weighed 70 g. Muscle weights: EDL = 32 mg, SOL = 27·5 mg. Average muscle fibre length: EDL = 9·5 mm, SOL = 10·5 mm.

creased the time for half relaxation from 9·25 ms to 42 ms for EDL and from 36 ms to 170 ms for SOL.

The effects of temperature on P_t and P_o of fourteen EDL muscles and three SOL muscles are shown in Fig. 2. The linear changes in P_t and P_o of EDL are almost the same as those described before for rat triceps surae[2]. A similar influence of temperature has been described for amphibian muscle[3]. Recent measurements on the sartorius muscle of *Rana temporaria* which were stored at 3° C in October, using "massive" direct stimulation *in vitro*, show that P_t increases from 0·7 kg/cm² (0·5–0·96, $n = 5$) at 20°–22° C to 1·42 kg/cm² (1·21, 1·74, $n = 2$) at 2° C and that P_o decreases from 3·5 kg/cm² (3·37–3·63, $n = 5$) to 2·38 kg/cm² (2·06, 2·69, $n = 2$) for the same range of temperature (unpublished results of Close). The effect of temperature on contractions of SOL is similar to that described for neonatal rat EDL[4].

The differences in temperature dependence of contractions of EDL and SOL muscles may be related to differences in the effects of repetitive stimulation on these muscles. Post-tetanic potentiation (PTP) is usually absent in neonatal EDL and SOL and adult SOL, but appears in EDL during the first few weeks after birth. The peak

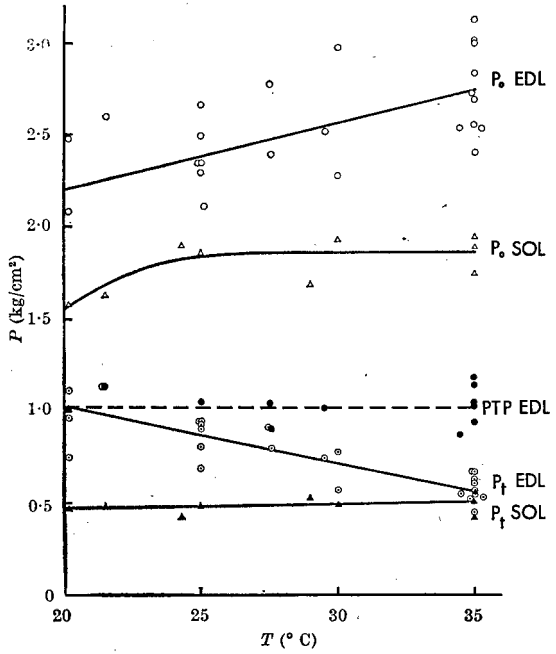

Fig. 2. The relation between maximum tension, P (ordinate in kg/cm² cross-sectional area of muscle) and temperature (abscissa in ° C) for isometric twitch (P_t) and tetanic (P_o) contractions of fourteen extensor digitorum longus (EDL) and three soleus (SOL) muscles from four-week old rats (P_t of EDL = ⊙, P_t of SOL = ▲, P_o of EDL = ○, P_o of SOL = △). Responses of individual muscles at different temperatures were recorded for three SOL and five EDL muscles. Of the other nine EDL muscles, two were examined at 25° C only, two at 27·5° C only and 5 at 35° C only. Post-tetanic potentiation (PTP) was recorded at various temperatures in ten of the fourteen EDL muscles. The peak tensions of potentiated twitches recorded 10 s after the end of repetitive stimulation which produced maximum PTP have been plotted as filled circles (●) for comparison with the control pretetanic P_t. The average value for peak twitch tension in the potentiated twitches was 1·05 kg/cm² and is indicated in the diagram by the interrupted line.

twitch tension of EDL during maximum PTP at different temperatures has been plotted in Fig. 2 for comparison with the pretetanic P_t. The results show that there is little or no post-tetanic potentiation in EDL at 20° C and P_t at that temperature is about the same as the peak tension of the maximally potentiated twitch at other temperatures between 20° C and 35° C. In other words, the influence of temperature on contractions of EDL in the fully potentiated state is similar to the effect of temperature on contractions of SOL and neonatal EDL and involves a change in time course of the twitch response with little or no change in P_t. At 35° C maximum post-tetanic potentiation leads to a two-fold increase in the peak twitch tension with little or no change in the time

course of the response. A similar degree of maximum post-tetanic potentiation has been observed in cat fast muscles at 37° C and frog twitch muscle at about 20° C and has been shown to occur in single muscle fibres[5-7]. A possible explanation for the differences in temperature dependence of frog twitch muscle and rat EDL muscle on one hand and rat SOL on the other is that a decrease in temperature decreases the intrinsic speed of shortening and the rate of removal of activator in all these muscles, thereby causing an inversely proportional increase in T_c with little or no change in P_t (ref. 4), but also increases P_t of frog twitch muscle and adult rat EDL, in much the same way that repetitive stimulation increases it, by increasing the degree of activation of individual muscle fibres with little or no change in the time course of the response. This interpretation of the results follows an earlier suggestion[4] that differences in isometric twitch contraction times of different muscles may be due largely to differences in intrinsic speed of shortening and that differences in P_t/P_o, or P_t as kg/cm^2, may result mainly from differences in the degree of activation of single muscle fibres.

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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 motoneurones 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 pyriformis 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, $CaCl_2$ 2 mM, $NAHCO_3$ 2 g/l., glucose 2 g/l.) which was bubbled continuously with 95 % O_2 and 5 % CO_2 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 Gl-4-250) was used as a DC bridge, the other (Statham Gl-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 μsec 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 2×10^{-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 maximal 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 $T_{\frac{1}{2}R}$. 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

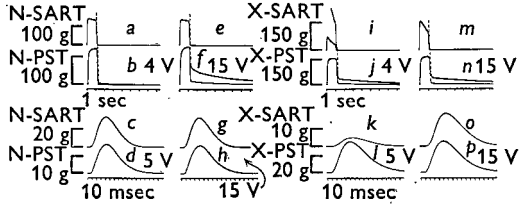

Fig. 1. Records of isometric twitch and tetanic contractions (lower and upper parts of the diagram respectively) of cross-innervated sartorius (X-SART) and posterior semitendinosus (X-PST) muscles (*i–p*) and the normally innervated contralateral control (N-SART, N-PST) muscles (*a–h*) from a toad 108 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 μsec) 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

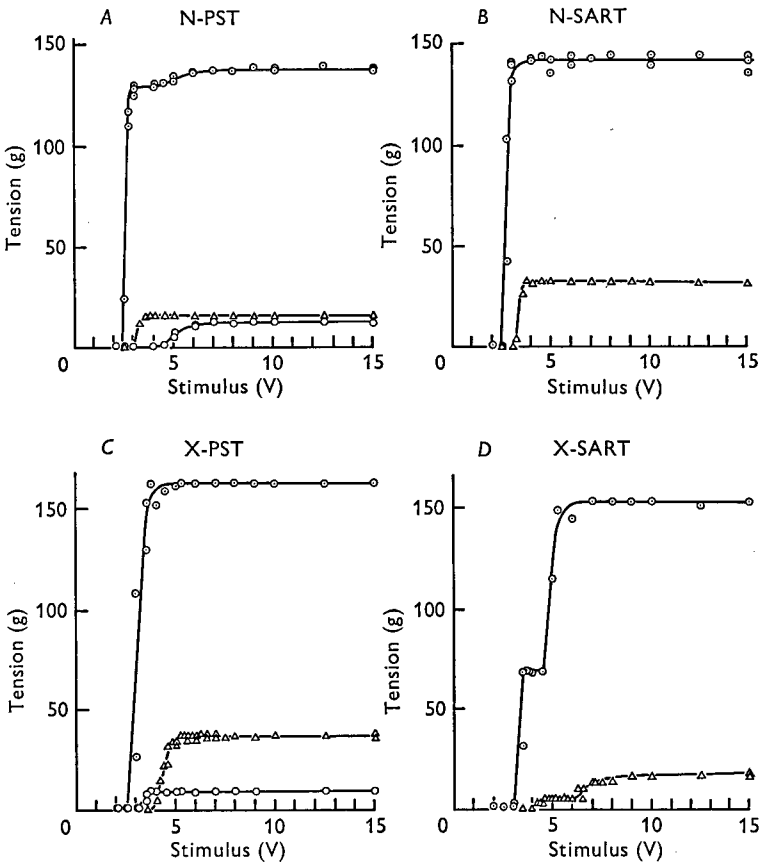

Fig. 2. Maximum tensions developed in isometric twitch (△) and tetanic (whole muscle = ⊙, slow-graded component = ○) contractions, plotted against stimulus strength (V) applied to the sciatic nerve for the normally innervated PST muscle (*A*), the normally innervated sartorius muscle (*B*), the cross-innervated 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 $P < 0{\cdot}025$) and P_t/P_0 ($P < 0{\cdot}0005$) but not for $T_{\frac{1}{2}R}$ ($P < 0{\cdot}1$) nor for the difference in maximum isometric tetanic tension developed per unit cross-sectional area of muscle ($P < 0{\cdot}4$).

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. 2*D* 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 ± S.E. are listed for the isometric twitch contraction time (T_c, msec), half-relaxation time ($T_{\frac{1}{2}R}$, 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_t/P_0) and the maximum force developed by the whole muscle per unit cross-sectional area of muscle (Total P_0 (kg/cm²)), of responses to nerve stimulation. The mean temperature and range in parentheses at which twitches were recorded (Twitch T° C) are given and n is the number of observations on which each value is based

	N-SART	S-SART	X-SART	N-PST	S-PST	X-PST
Twitch responses						
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_c (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)
$T_{\frac{1}{2}R}$ (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 (n = 18)	0 (n = 3)	0 (n = 12)	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/cm²)	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° 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 % (± 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_l/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_l/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. 3*A* 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–

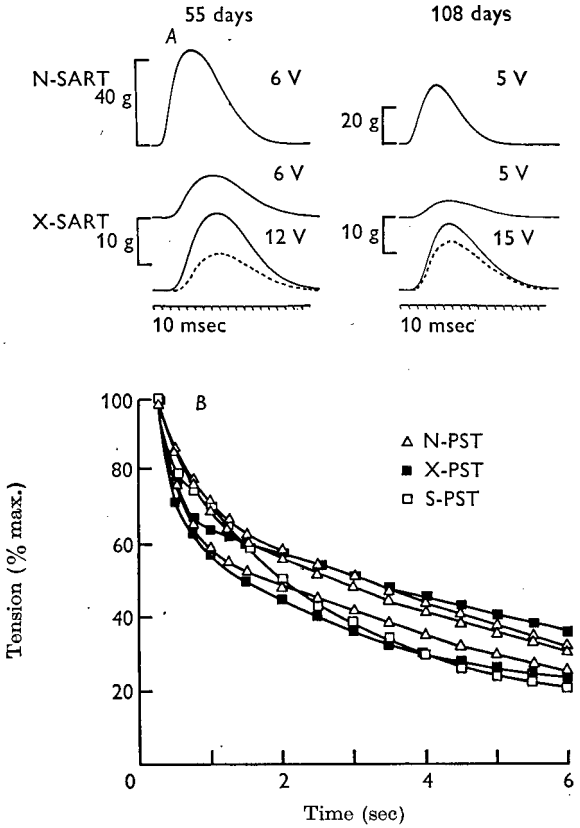

Fig. 3. (*A*) Isometric records of twitch contractions of normally innervated sartorius muscles (N-SART) and of cross-innervated sartorius muscles (X-SART) from two toads 55 days (left column) and 108 days (right column, from Fig. 1) after nerve cross-union, in response to the stimulating voltages indicated. 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 (△), two cross-innervated (■) and one self-innervated (□) 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 $P \leqslant 0{\cdot}05$. 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 2×10^{-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. 4*C*, 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. 4*C*, inset).

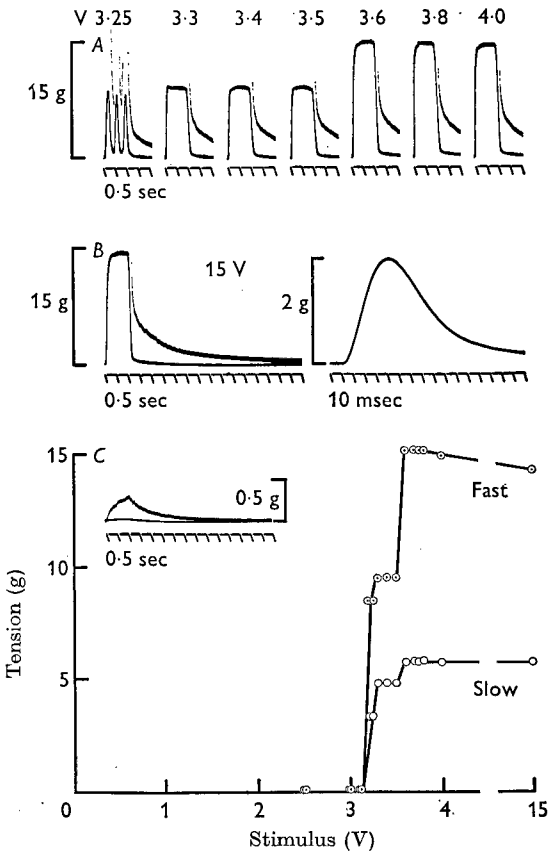

Fig. 4. (*A*) Records of isometric tetanic contraction of the PST muscle transplanted from the opposite side and cross-united with the sartorius nerve 119 days before the experiment. The records were displayed in the same way as described in Fig. 1, except that one beam of the oscilloscope has a gain 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 (⊙) and of the slow-graded component (○). 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 ($P < 0{\cdot}025$) and the slow-graded ($P < 0{\cdot}05$) 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 ($P < 0{\cdot}3$) and slow-graded ($P < 0{\cdot}1$) 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, 6*C*).

The *t*-test was used to determine whether there were significant differences between the values for T_c, P_t/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 ($P > 0{\cdot}2$).

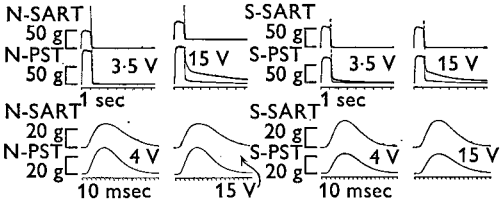

Fig. 5. Isometric records of tetanic and twitch contractions of the self-innervated sartorius (S-SART) and posterior semitendinosus (S-PST) muscles and their normally innervated contralateral controls (N-SART, N-PST) from a toad 104 days after the nerve self-union operation. The records were obtained 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 2×10^{-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 2×10^{-5} g acetylcholine Cl/ml. Ringer. Contracture tension–time curves of some of these muscles are presented in Fig. 7. The N-PST muscle gaves 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.

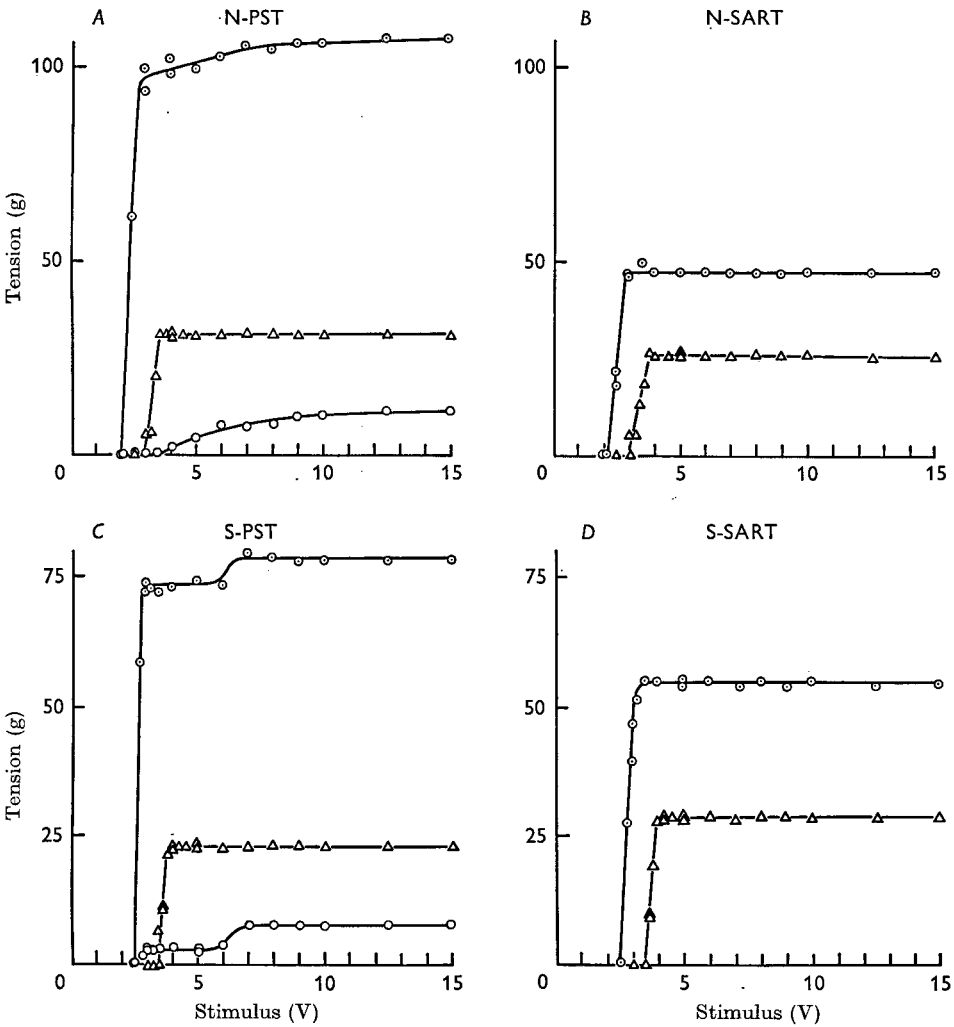

Fig. 6. Maximum tensions developed in isometric twitch (△) and tetanic (whole muscle = ⊙, slow-graded component = ○) contractions, plotted against stimulus strength (V) applied to the sciatic nerve for the normally innervated PST muscle (*A*), the normally innervated sartorius muscle (*B*), the self-innervated 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).

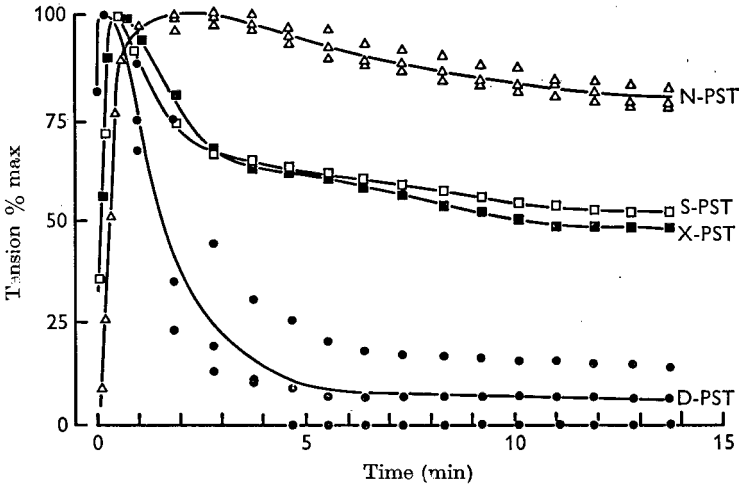

Fig. 7. Acetylcholine-contracture tension–time curves of PST muscles. Ordinate: contracture tension expressed as percentage of the maximum contracture tension of each muscle. Abscissa: time in minutes after addition of acetylcholine to the bath. Maximum contracture tensions for normal PST muscles (△) = 8·1 g (mean; range 7·0–9·4 g); for self-innervated PST (□) = 6·7 g; for cross-innervated PST (■) = 7·7 g; for denervated PST (●) = 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 phemomenon 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_t/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_t/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-united 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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THE AFTER-EFFECTS OF REPETITIVE STIMULATION ON THE ISOMETRIC TWITCH CONTRACTION OF RAT FAST SKELETAL MUSCLE

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SUMMARY

1. The peak tension and time course of isometric twitch contractions of rat extensor digitorum longus muscle *in vitro* (35° C) have been measured at various stages of potentiation following repetitive stimulation at 20 c/s and 300 c/s.

2. Potentiation of the peak twitch tension increases with an increase in the number of repetitive stimuli up to a maximal level of about 1·9 times the control value. The relation between potentiation and numbers of stimuli is dependent on the frequency of stimulation.

3. Potentiation of peak twitch tension is maximal shortly after the end of repetitive stimulation and subsequently decays exponentially at a rate which is dependent on the number of stimuli in the train and the frequency of stimulation.

4. Short trains of stimuli bring about nearly maximal potentiation with little or no change in contraction time and a small decrease in half-relaxation time.

5. Long trains of stimuli increase the contraction time, the half-relaxation time and the twitch duration in addition to potentiating the peak tension. The changes in twitch time course are dependent on the number of repetitive stimuli and the frequency of stimulation.

6. The results are discussed in relation to possible mechanisms of post-tetanic potentiation and the degree of activation of mammalian and amphibian muscle fibres.

INTRODUCTION

Repetitive stimulation of mammalian fast skeletal muscle causes a transitory increase in the peak tension of the isometric twitch response (Lee, 1907; Brown & Euler, 1938; Euler & Swank, 1940; Bernhard, Euler & Skoglund, 1941; Standaert, 1964; Buller & Lewis, 1965). In the present work the influence of repetitive stimulation on peak tension and time

course of the isometric twitch of rat extensor digitorum longus muscles has been determined for various stages of potentiation. The abbreviation PTP is used throughout to refer to post-tetanic potentiation of peak twitch tension following a train of stimuli at 300 c/s and to post-train potentiation after stimulation at 20 c/s.

METHODS

The muscles used were extensor digitorum longus (EDL) muscles from hind limbs of 4-week old female rats (Wistar) which had an average body weight of 67·5 g ($\pm$2·95 g S.D.).

Dissection. EDL muscles were obtained from rats anaesthetized with pentabarbitone sodium (50 mg/kg body wt.). After cutting the blood vessels to EDL the muscle was transferred immediately to a bath of oxygenated Ringer solution at room temperature.

Tension recording. The muscle was held with its long axis vertical, with the proximal tendon tied directly to the frame below and the distal tendon tied directly to a short steel wire connexion which linked it with the tension transducer above the bath. The muscle was immersed in about 100 ml. of fluid (NaCl, 137 mM; KCl, 5 mM; $CaCl_2$, 2 mM; $MgCl_2$, 1 mM; NaH_2PO_4, 1 mM; $NaHCO_3$, 2 g/l.; glucose, 2 g/l.), which was bubbled continuously with a mixture of 95 % O_2 and 5 % CO_2, and replaced at the rate of a few millilitres per minute. The compliance of the transducer (Statham G1-80-350) plus the steel wire connexion was $4{\cdot}5 \times 10^{-5}$ cm/g and the natural frequency of vibration was 2 kc/s. The transducer was used in conjunction with a carrier amplifier (Tektronix Q) the output of which was displayed on an oscilloscope (Tektronix 565) and recorded photographically (Grass C4 camera). The oscilloscope amplifier (Tektronix 3A3 or 72) was used in the chopped mode; one of the channels carried the tension/time record and the other carried time marks from a pulse generator triggered by a time-mark generator (Tektronix 180A). All recordings were made with the muscle at the optimal length for twitch contractions at 20° C. The temperature of the fluid surrounding the muscle was maintained between 34·6 and 35·5° C in all experiments on PTP. The average temperature at the beginning of each series of measurements on PTP was 35·02° C ($\pm$0·2° C S.D., n = 35).

Stimulation. Neuromuscular transmission was blocked by adding tubocurarine chloride (2×10^{-5} g/ml.) to the Ringer fluid. The muscles were stimulated directly in a transverse electrical field applied to the bath fluid through 'massive' platinum electrodes (Gutmann & Sandow, 1965) set about 1 cm apart on either side of the muscle and extending beyond its ends. The stimulus amplitudes given below are the voltages applied to the electrodes.

Figure 1 shows records and graphs which describe the relation between mechanical response and stimulus strength for single stimuli applied to a curarized EDL muscle at 25° C. The curves relating stimulus duration and peak tension of the mechanical response are similar for all voltage gradients between 5 and 25 V but the threshold duration for the response is higher for low voltages. The curves show that increase in stimulus duration increases the peak tension up to a plateau followed by a decrease and then by a further increase in the maximum response. The plateau tension represents the maximum twitch response of the muscle to a single stimulus and this is only slightly greater for massive stimulation than for either indirect stimulation via the nerve *in situ* or direct stimulation at a point midway along the muscle fibres *in vitro*. The depression of the twitch response, shown for most voltage gradients with stimuli of several milliseconds duration, is not associated with a change in the time course of the twitch. This depression has been observed not only with massive stimulation but also with direct stimulation at a point and probably results from stimulus currents preventing the generation or conduction of the action potential in some muscle fibres. The large twitch-like responses to stimuli of long duration (10–20 msec) show an inflexion in the tension/time record shortly after the end of the stimulus (Fig. 1A, arrow) and there is a marked increase in both the contraction time and the half-relaxation time. It

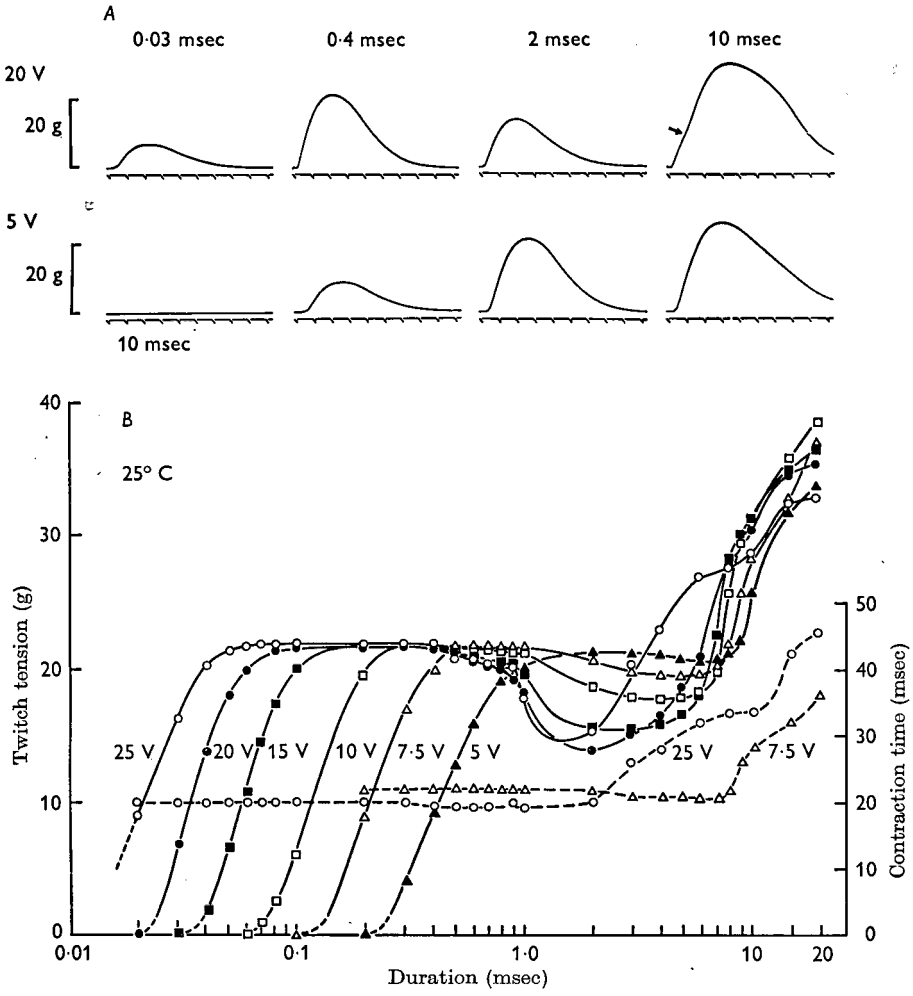

Fig. 1. The records in *A* are for isometric contractions of an EDL muscle in response to 'massive' direct stimulation *in vitro*. The amplitude of the stimulus was 20 V for the upper series and 5 V for the lower series. The stimulus durations shown above the records are for both series. The arrow over the large twitch-like response indicates an inflexion which occurs shortly after the end of a 10 msec stimulus. The records in *A* form part of the series in *B* which show the relations between peak tension (continuous curves) and stimulus duration (abscissa, log scale) for stimulus amplitudes of 25 V ○, 20 V ●, 15 V ■, 10 V □, 7·5 V △, and 5 V ▲. The contraction times for twitch responses to 25 V and 7·5 V are shown by the interrupted curves. The amplitude of the stimulus is given as the voltage applied to platinum electrodes placed 1 cm apart. Muscle weight = 22 mg. Average fibre length = 9·5 mm. Temperature: 24·6° C to 25·3° C.

seems likely that in these large responses some of the muscle fibres are excited twice, once during the stimulus and again at the end of the stimulus.

Figure 2 shows that both the peak twitch tension, indicated by the plateau values, and the threshold duration for excitation are increased by a decrease in temperature in the range 20–35° C.

The stimulus used throughout this work was 15 V for 0·2 msec or 0·3 msec. The heating effect of the current during one stimulus has been estimated to raise the temperature of the fluid around the muscle by less than 4×10^{-4}° C.

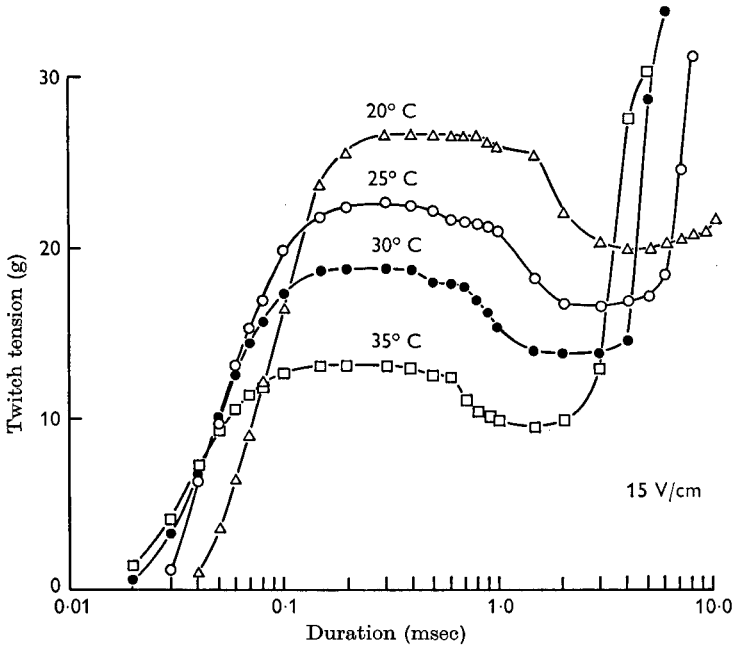

Fig. 2. The relations between peak tension (ordinate) of isometric contractions and duration (abscissa, log scale) of direct 'massive' stimuli (15 V) at different temperatures. Muscle weight = 25 mg. Average muscle fibre length = 10·5 mm.

Procedure for determining PTP. The optimal length for twitch contractions was determined for each muscle at room temperature. The temperature of the bath fluid was raised subsequently and maintained at 35 °C during measurements on PTP. In every series of measurements control twitches were elicited at 0·05 c/s followed by the response to a train of stimuli at either 20 c/s or 300 c/s timed to end 30 sec after the last control twitch, followed by the post-train potentiated responses to single stimuli at 0·05 c/s beginning 10 sec after the end of repetitive stimulation. The influence of frequency of testing on the disappearance of PTP was not studied extensively but there seemed to be no difference between results obtained for testing at 0·1 c/s and 0·05 c/s. Usually only one or two series were obtained for each muscle before the peak tension of the control, pre-train, twitch had decreased to below 90 % of the original value (see below), but up to four series were obtained for small numbers of repetitive stimuli.

Condition of muscles in vitro. Isometric contractions have been recorded for several preparations in which each muscle was stimulated maximally first by indirect stimulation of the nerve *in situ*, then by direct stimulation with platinum wire electrodes placed midway along

the muscle fibres *in situ* and finally by direct stimulation through massive platinum electrodes *in vitro*; for each muscle the maximum isometric twitch tension was almost exactly the same for all methods of stimulation. Consequently all, or nearly all the muscle fibres of EDL remained excitable when the muscle was excised. The length of time these muscles remained fully excitable at 35 °C *in vitro* depended on the rate of stimulation and the activity of the muscle. For example, the twitch response of one muscle did not change during 6 hr of continuous stimulation once every 10 min and the maximum isometric tetanic tension of another muscle showed no decline over a period of 1 hr in response to stimulation at 300 c/s for 1 sec every 10 min. On the other hand there is usually a small reduction (*ca.* 5 %) in the peak twitch tension after the disappearance of PTP following a maximum tetanic contraction to stimulation at 300 c/s for 1 sec. All the results on PTP described below were obtained from those series of measurements in which the peak tension of the control, pre-train, twitch exceeded 90 % of the original peak tension of the initial control twitch.

Definitions. Optimal length (L_0) is the length of the muscle at which the peak twitch tension, in excess of initial tension, is maximal at 20 °C. The optimal length is the same for twitch and tetanic contractions at 20 °C.

Maximum isometric twitch tension (P_t) at 35 °C is the peak twitch tension in excess of initial tension at L_0.

Maximum isometric tetanic tension (P_0) at 35 °C is the maximum tension in excess of initial tension at L_0 during stimulation at 300 c/s.

Contraction time (T_c) at 35 °C is the time from onset of contraction to the peak of the isometric twitch at L_0.

Half-relaxation time ($T_{\frac{1}{2}R}$) at 35 °C is the time for decay of tension from the peak of the isometric twitch to one half of the peak tension at L_0.

Cross-sectional area of muscle in square centimetres was estimated by dividing the weight of the muscle in grams (M) by the average fibre length at L_0 in centimetres (L).

The degree of potentiation is the ratio of the peak tension of a post-train potentiated twitch (P_t*) to the peak tension of the pre-train twitch (P_t), i.e. P_t^*/P_t.

RESULTS

Some of the properties of EDL muscles *in vitro* are listed in Table 1. The results do not differ greatly from those described previously for juvenile rat EDL muscles *in situ* (Close, 1964). The values listed for the twitch are for responses before repetitive stimulation in the first series of measurements on PTP in each muscle. The ratios of values for pre-train twitches in second and subsequent series to the values obtained for the initial series were 0·95 ($\pm$ 0·03 S.D., n = 18) for maximum twitch tension, 0·986 ($\pm$ 0·034 S.D., n = 18) for contraction times and 0·96 ($\pm$ 0·06 S.D., n = 18) for half-relaxation times. These small changes in the twitch were not due to differences in temperature; the average temperature was 35·07° C ($\pm$ 0·2° C, S.D., n = 17) for the initial series and 34·97° C ($\pm$ 0·2° C, n = 18) for subsequent series.

An example of PTP of the twitch contraction of EDL is shown in Fig. 3; the records are for the control pre-train twitch contraction (A) and the potentiated response (B) recorded 10 sec after the end of a train of 300 stimuli at 300 c/s. The time course of the two contractions is almost

the same except that relaxation is a little more rapid in the potentiated twitch.

Figure 4 *A* and *B* show a few examples of the time course of decline of potentiation following repetitive stimulation at 20 c/s and 300 c/s and

TABLE 1. Properties of EDL muscles from 4-week old rats. The values are the mean values ± the standard deviation for a number (n) of muscles. All measurements were made at 35° C.

Muscle weight (mg)	$26{\cdot}2 \pm 2{\cdot}4$, $n = 15$
Muscle fibre length (mm)	$9{\cdot}5 \pm 0{\cdot}4$, $n = 16$
P_t(g)	$15{\cdot}25 \pm 2{\cdot}45$, $n = 17$
P_t/P_0	$0{\cdot}21 \pm 0{\cdot}03$, $n = 17$
P_0(g)	$73 \pm 8{\cdot}2$, $n = 17$
$P_0.L/M$ (kg/cm²)	$2{\cdot}57 \pm 0{\cdot}286$, $n = 15$
T_c (msec)	$9{\cdot}8 \pm 0{\cdot}7$, $n = 17$
$T_{\frac{1}{2}R}$ (msec)	$9{\cdot}4 \pm 0{\cdot}6$, $n = 17$

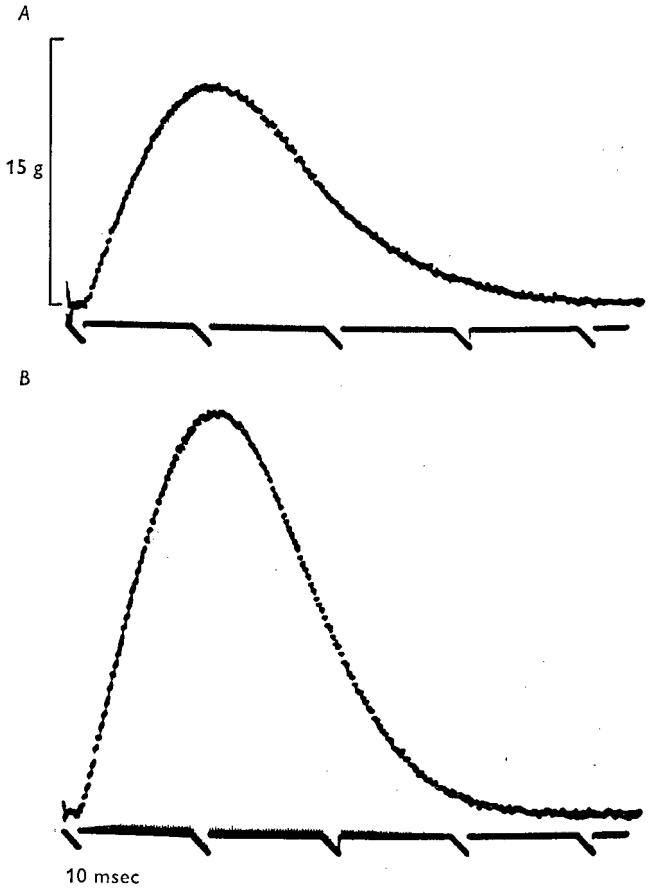

Fig. 3. Isometric responses of an EDL muscle to a single stimulus, *A* before and *B* 10 sec after a train of 300 stimuli at 300 c/s. Temperature: 35° C.

different numbers of stimuli; the degree of potentiation is shown as the ratio of the peak tension of the post-train twitch to the peak tension of the pre-train twitch. The decay of PTP is approximately exponential though in some instances (e.g. Fig. 4 *B*), particularly following several hundred stimuli at 300 c/s, the experimentally determined values for potentiation

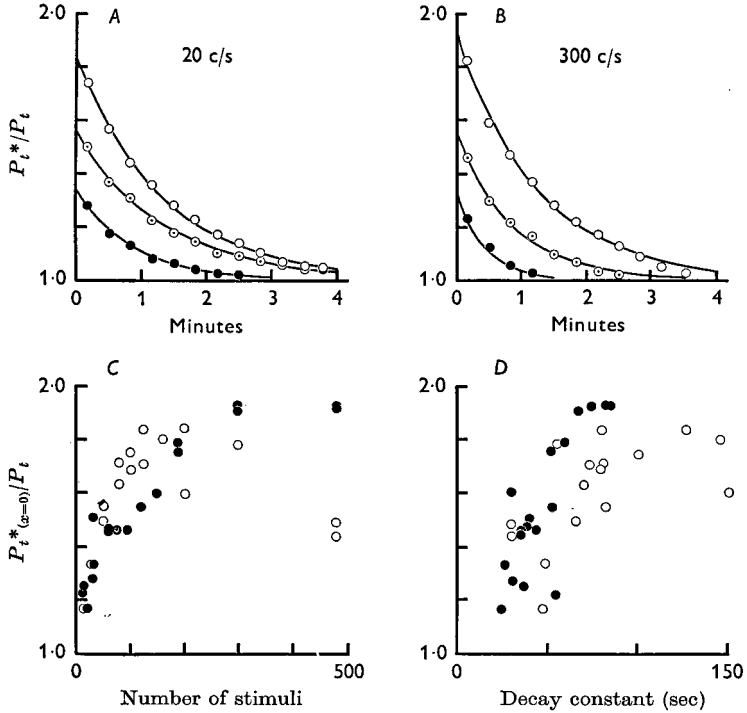

Fig. 4. The time course of decay of PTP after 126 (○), 50 (⊙) and 26 (●) stimuli at 20 c/s (*A*) and 300 (○), 120 (⊙) and 30 (●) stimuli at 300 c/s (*B*). In *A* and *B* the degree of potentiation P_t^*/P_t of post-train twitches (ordinates) is plotted against the time after the end of repetitive stimulation (abscissae). *C* shows the relation between maximum degree of potentiation at the end of repetitive stimulation $P_t^*{}_{(x=0)}/P_t$ estimated as described in the text, and the number of repetitive stimuli at 20 c/s (○) and 300 c/s (●). *D* shows the relation between the estimated maximum potentiation at the end of repetitive stimulation and the time constant (τ in seconds) for decline of PTP after stimulation at 20 c/s (○) and 300 c/s (●). The results shown in *C* and *D* are for seventeen EDL muscles the properties of which are summarized in Table 1. Temperature: 35 °C.

fall below the fitted curves in the region where potentiation is less than 1·1 P_t due to decline in P_t after the disappearance of PTP (see above). It has been assumed that potentiation is maximal soon after the end of repetitive stimulation at 35° C and a value for maximum potentiation has been estimated for each series of measurements by extrapolating the

exponential decay curve to zero time. The degree of potentiation of the twitch at any time later than 10 sec after the end of repetitive stimulation is given by

$$\frac{P_t^{*}{}_{(x)}}{P_t} = \left(\frac{P_t^{*}{}_{(x=0)}}{P_t} - 1\right) e^{-x/\tau} + 1,$$

where P_t is the peak tension of the control twitch, $P_t^{*}{}_{(x=0)}$ is the estimated peak tension of the potentiated twitch at zero time, $P_t^{*}{}_{(x)}$ is the peak tension in the potentiated twitch at any time x sec after the end of repetitive stimulation and τ is the time constant in seconds for the decline of PTP.

Figure 4 *C* shows the relation between the estimated maximum degree of potentiation ($P_t^{*}{}_{(x=0)}/P_t$) and the number of stimuli (S) for thirty-five series of measurements following repetitive stimulation at either 20 c/s (○) or 300 c/s (●). The general form of the relation is described approximately by

$$\frac{P_t^{*}{}_{(x=0)}}{P_t} = 0{\cdot}93(1 - e^{-0.012S}) + 1$$

for all points except those for 480 stimuli at 20 c/s. Repetitive stimulation at 20 c/s is a little more effective in potentiating the twitch than stimulation at 300 c/s for trains of less than 150 to 200 stimuli and more accurate descriptions of the relation between maximum potentiation and numbers of stimuli are given by

$$\frac{P_t^{*}{}_{(x=0)}}{P_t} = 0{\cdot}069\sqrt{S} + 1$$

for 20 c/s and 10 to 150 stimuli and

$$\frac{P_t^{*}{}_{(x=0)}}{P_t} = 0{\cdot}055\sqrt{S} + 1$$

for 300 c/s and 10 to 300 stimuli. For larger numbers of stimuli the maximum degree of potentiation decreased following stimulation at 20 c/s whereas it continued to increase for stimulation at 300 c/s up to a maximum of about 1·93 for trains of 300–500 stimuli.

Figure 4 *D* shows that the time constant (τ) for decay of PTP increased with increase in the degree of maximum potentiation $P_t^{*}{}_{(x=0)}$. Furthermore for a given maximum degree of potentiation the rate of decline of PTP was usually less following stimulation at 20 c/s than following stimulation at 300 c/s. The relation between τ in seconds and the number of stimuli (S) is approximately linear and is given by

$$\tau = 0{\cdot}55\,S + 35$$

for 10–200 stimuli at 20 c/s and

$$\tau = 0{\cdot}12\,S + 32$$

for 10–500 stimuli at 300 c/s. The decreased degree of maximum potentiation following 480 stimuli at 20 c/s (Fig. 4 *C*) was accompanied by a decrease in τ to about 30 sec (Fig. 4 *D*).

Figure 5 shows the relations between the number of repetitive stimuli and both the degree of potentiation and the time course of isometric

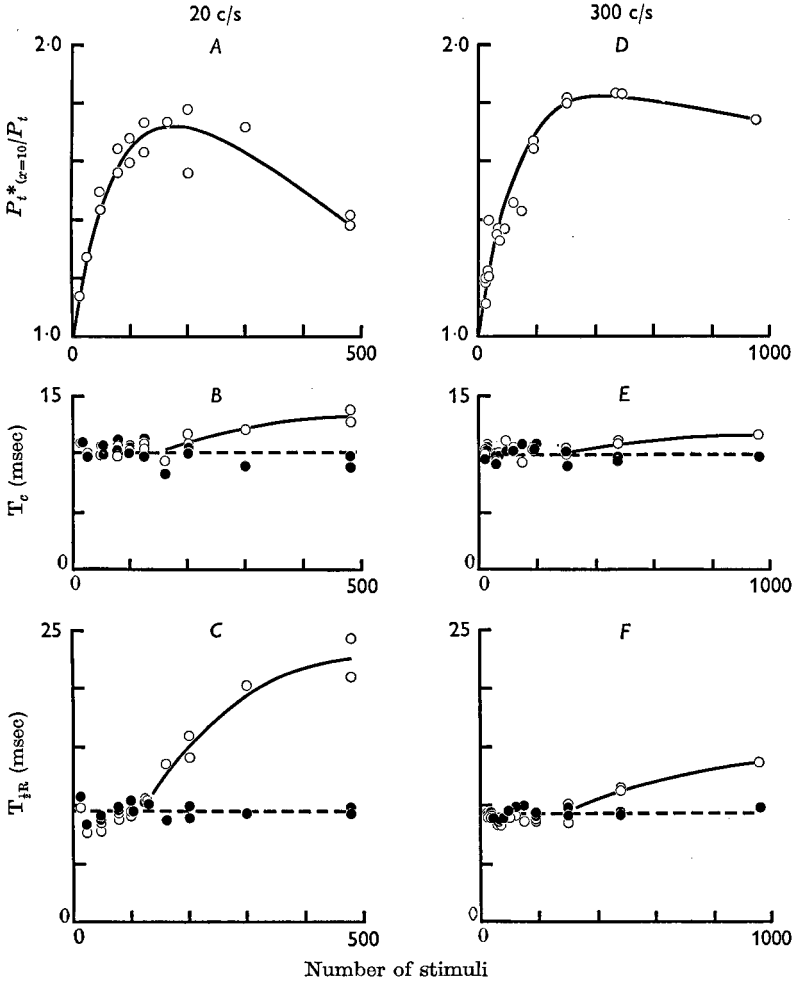

Fig. 5. The relations between number of repetitive stimuli (abscissae) and the degree of potentiation $P_t^*{}_{(x=10)}/P_t$ (*A*, *D*), contraction time (*B*, *E*), and half-relaxation time (*C*, *F*) on ordinates for twitches recorded 10 sec after the end of repetitive stimulation at 20 c/s (*A*, *B*, *C*) and 300 c/s (*D*, *E*, *F*). The contraction times and half-relaxation times of both the pre-train (●) and post-train (○) twitches have been plotted at the same position along the abscissa in *B*, *C*, *E* and *F* and the mean pre-train value is shown by the interrupted line. The results were obtained from seventeen EDL muscles described in Table 1.

twitches recorded 10 sec after the end of repetitive stimulation at 20 c/s and 300 c/s. The peak tension of the first post-train twitch in each series has been plotted as a multiple of the pre-train value (i.e. $P_t^*{}_{(x=10)}/P_t$); mean $P_t.L/M$ was 0·55 kg/cm^2 for muscles stimulated at 20 c/s (Fig. 5 *A*) and 0·58 kg/cm^2 for muscles stimulated at 300 c/s. T_c and $T_{\frac{1}{2}R}$ for both the pre-train twitch (filled circles) and the post-train twitch (unfilled circles) are plotted for comparison in Fig. 5 *B*, *C*, *E*, *F*, and in each of these graphs the average value for the pre-train twitch is indicated by the interrupted

TABLE 2. Contraction times and half-relaxation times before, and 10 sec after, the end of short trains of stimuli which caused PTP with little or no change in time course of post-train twitches. The values given are mean values ± the standard deviation for a number (n) of series. The probability values (P) were obtained from t-values calculated from the ratio of the mean difference between pre-train and post-train values of T_c and $T_{\frac{1}{2}R}$ and the standard error of this mean difference. The mean values have been plotted in Fig. 5

Frequency of stimulation (c/s)	Number of stimuli	T_c (msec)		$T_{\frac{1}{2}R}$ (msec)	
		Pre-train twitch	Post-train twitch	Pre-train twitch	Post-train twitch
20	12 to 126	10·3 ± 0·664	10·24 ± 0·476	9·68 ± 0·731	9·15 ± 0·89
		$n = 10$, $P > 0·5$		$n = 10$, $P < 0·02$	
300	10 to 300	9·8 ± 0·441	10·06 ± 0·489	9·25 ± 0·45	8·85 ± 0·53
		$n = 14$, $P < 0·05$		$n = 14$, $P < 0·01$	

line. The graphs show that there is little or no change in the time course of the isometric twitch with increase in PTP up to nearly maximum levels of potentiation brought about by 126 stimuli at 20 c/s and 300 stimuli at 300 c/s. The mean values for T_c and $T_{\frac{1}{2}R}$ for pre-train twitches and post-train twitches after 12–126 stimuli at 20 c/s and 10–300 stimuli at 300 c/s are listed in Table 2. The probability values (P) given in Table 2 were obtained from t-values calculated from the ratio of the mean difference between pre-train and post-train values of T_c and $T_{\frac{1}{2}R}$ and the standard error of this mean difference. T_c is only slightly altered with increase in PTP up to the maximum level in both series. The small change in T_c following stimulation at 300 c/s is significant at the 5% level but the values show no obvious trend with increase in the number of stimuli up to about 300 stimuli. In contrast, $T_{\frac{1}{2}R}$ of the post-train twitch decreased progressively with increase in the number of stimuli in the train to a minimum of about 0·9 times the control value of $T_{\frac{1}{2}R}$ following 50–70 stimuli at 20 c/s and 150 stimuli at 300 c/s but increased gradually with further increase in the number of repetitive stimuli and was approximately the same as $T_{\frac{1}{2}R}$ of the pre-train twitch after 100–126 stimuli at 20 c/s and

300 stimuli at 300 c/s. Both T_c and $T_{\frac{1}{2}R}$ of the post-train twitch are increased markedly following trains in which the number of stimuli exceeds about 126 stimuli at 20 c/s and 300 stimuli at 300 c/s and these changes are much more pronounced following stimulation at the lower frequency.

Figures 6 and 7 show examples of the time course of change of degree of potentiation and of T_c and $T_{\frac{1}{2}R}$ during the decline of PTP. Figure 6 shows representative records of twitch contractions and graphs illustrating the effects of trains of 100 and 300 stimuli at 20 c/s on contractions of two muscles and Fig. 7 shows similar results obtained from two other muscles after trains of 300 and 960 stimuli at 300 c/s. The degree of potentiation and the time course of decay of PTP are similar in the four series (Fig. 6 *B*, 7 *B*). The short trains of stimuli, 100 at 20 c/s and 300 at 300 c/s, caused potentiation of twitch tension with little or no alteration of the time course of the twitch. Prolonged stimulation by 300 stimuli at 20 c/s and 960 stimuli at 300 c/s markedly increased both T_c and $T_{\frac{1}{2}R}$ in addition to potentiating the peak twitch tension (Figs. 6 *B*, 7 *B*). The increase in T_c and $T_{\frac{1}{2}R}$ after prolonged stimulation declines along a time course which depends on the number of stimuli in the train and the frequency of repetitive stimulation. For example, after 160 or 200 stimuli at 20 c/s T_c and $T_{\frac{1}{2}R}$ decreased to pre-train values at a time when the peak tension of the twitch was still 20–40 % potentiated and the twitch time course remained virtually unaltered thereafter (not shown in Fig. 6). The disappearance of the increase in T_c and $T_{\frac{1}{2}R}$ after 300 stimuli at 20 c/s followed a time course which was almost the same as that for the decline of PTP (Fig. 6). The effects of prolonged stimulation at 300 c/s were less marked and even after trains of 480 or 960 stimuli the increase in T_c and $T_{\frac{1}{2}R}$ disappeared before the end of PTP. More observations are required to define precisely the time course of disappearance of changes in twitch duration which occur following prolonged repetitive stimulation.

DISCUSSION

The results described above have revealed two distinct aspects of potentiation of the twitch of rat EDL following repetitive stimulation at 35° C. These are the increase in peak tension with little or no change in time course of the twitch and prolongation of both the contraction and relaxation phases. These effects have not been examined in detail for other muscles but the results available show that PTP of the twitch of mammalian fast skeletal muscle at 35° C is similar to that observed in frog twitch muscle at 20 °C not only in the degree of maximum potentiation but also in changes in time course of twitches during potentiation. For example, short trains of stimuli increase peak tension with little or no change in

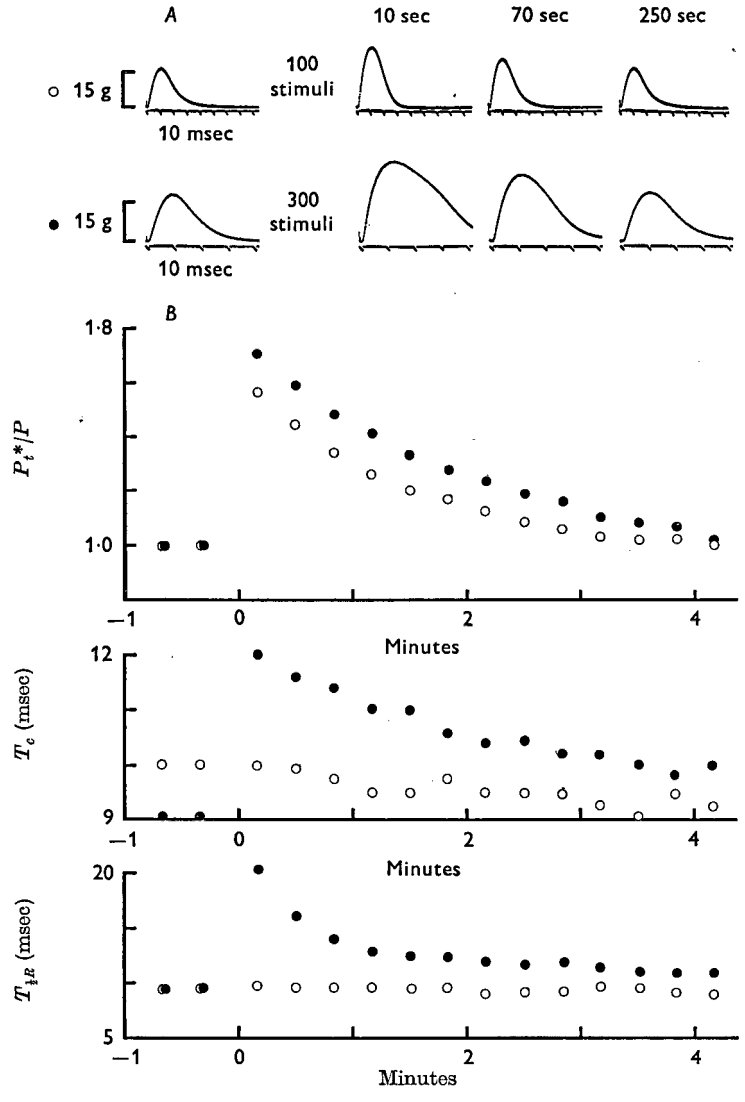

Fig. 6. The two series of records in *A* are, from left to right, a pre-train twitch and post-train twitches recorded 10, 70 and 250 sec after 100 stimuli at 20 c/s in one muscle (○) and 300 stimuli at 20 c/s in the other muscle (●). The records in *A* form part of the series in *B* which show the degree of potentiation P_t^*/P_t, the contraction time and half-relaxation time of post-train twitches at different times after 100 stimuli at 20 c/s (○) and 300 stimuli at 20 c/s (●). Values for pre-train twitches are shown between −1 and 0 on abscissae and P_t is expressed as 1·0. The time scale on the abscissa refers to time before and after the end of repetitive stimulation at 20 c/s. Muscle weights ○ = 30 mg. ● = 28 mg. Average muscle fibre lengths ○ = 9·5 mm, ● = 9·0 mm. Temperature: 35 °C.

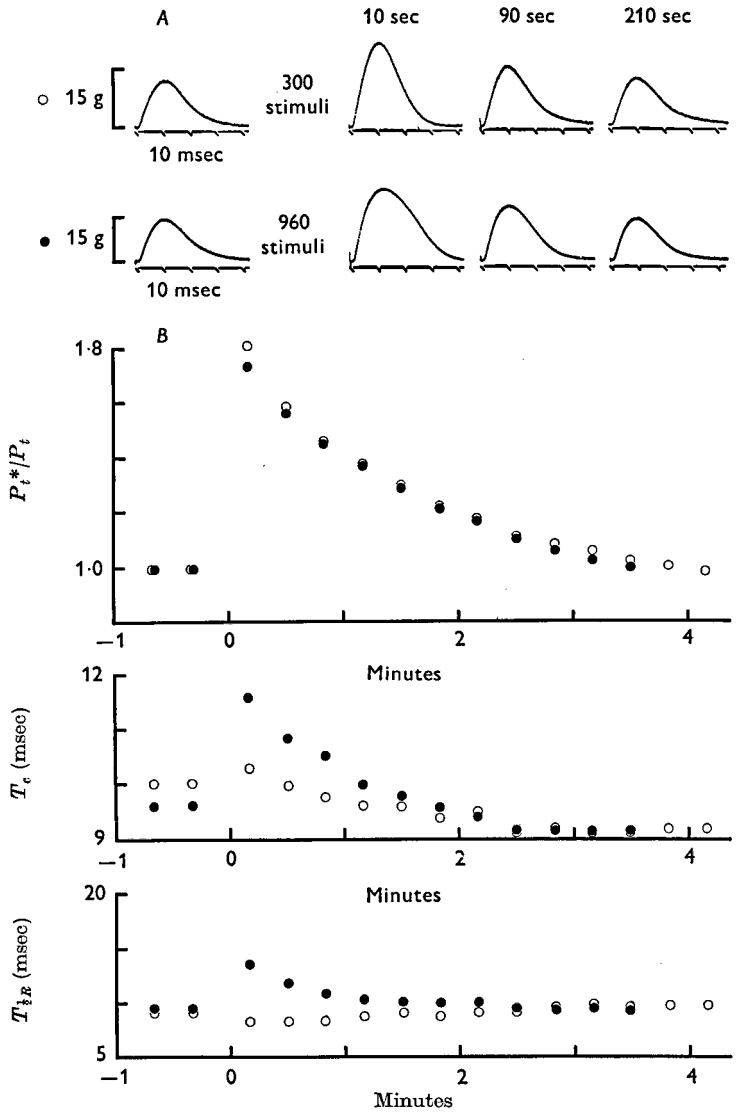

Fig. 7. Representative records (*A*) and graphs (*B*), similar to those in Fig. 6, showing the degree of potentiation, contraction time and half-relaxation time of post-train twitches after 300 stimuli at 300 c/s in one muscle (○) and 960 stimuli at 300 c/s in another muscle (●). Muscle weight ● = 27 mg. Average muscle fibre length ● = 10 mm. The weight and average fibre length of the other muscle (○) are not available. Temperature: 35 °C.

contraction time, depending on the number of stimuli in the train, the frequency and method of stimulation and the time after the end of the train of stimuli, in cat (Brown & Euler, 1938; Standaert, 1964), rat (above), human (Desmedt & Hainnaut, 1968) and frog muscles (Colomo & Rocchi, 1965) and this is usually accompanied by either no change or a small decrease in the half-relaxation time. Following prolonged stimulation there are marked increases in contraction time, half-relaxation time and total duration of the twitch in both rat (above) and frog (Ramsey & Street, fig. 7, 1941) muscles.

PTP has been observed in single fibres of frog muscles (Ramsey & Street, 1941; Colomo & Rocchi, 1965) and indirect evidence indicates the same for mammalian muscles. It has been shown above that all, or nearly all, muscle fibres must have been excited in the isolated EDL muscles. Consequently the increase in peak tension during maximum PTP of whole EDL muscle could not have resulted merely from an increase in the number of muscle fibres excited, with every muscle fibre responding with an all-or-nothing twitch. This is consistent with the observation of Brown & Euler (1938) and Bernhard *et al.* (1941) that the compound action potential of whole cat muscle is not increased during PTP. On the other hand the results obtained for whole rat muscle must be regarded as providing only average values for the whole population of muscle fibres with no indication of the range of P_t/P_0 and the degree of PTP of individual fibres. Ramsey & Street (1941) observed a considerable range of P_t/P_0 for frog single muscle fibres and found that the extent to which the twitch was potentiated after repetitive stimulation was inversely related to P_t/P_0 of the pre-train twitch (fibres for which P_t was about 0·1 P_0 showed a fivefold increase in peak twitch tension during PTP whereas those with P_t about 0·64 P_0 showed little or no increase in P_t) and that the twitch to tetanus ratio of maximally potentiated twitches never exceeded the maximum P_t/P_0 of 0·64 recorded for control twitches.

The question arises whether PTP results from a decrease in the compliance of series elastic elements within muscle fibres, an increase in the response of the contractile material itself, or both. The properties of the series elastic elements have not been determined during PTP but it is important to note that a decrease in series compliance would probably lead to either no change in T_c or a decrease in T_c, depending on the time course of decay of the active state, whereas in the results shown above T_c was usually increased in twitches recorded 10 sec after a tetanus. Furthermore, a decrease in compliance of the elastic elements would not cause prolongation of the twitch duration which occurs after prolonged stimulation. The effects of temperature on PTP are also interesting in this connexion. The peak tension of the maximally potentiated twitch of rat EDL

is about twice that of the normal twitch at 35° C and is independent of temperature from 35 to 20° C despite a twofold increase in the peak tension of the normal twitch as temperature is decreased over that range (Close & Hoh, 1968). The same appears to hold for cold-adapted frog sartorius muscle in the range 20–0° C (Hill, 1951; also unpublished observations) even though the properties of the series elastic elements in that muscle are only slightly altered by change in temperature (Jewell & Wilkie, 1958). So far as can be judged from these observations PTP does not result merely from changes in passive series elastic elements but it remains for measurements to be made to determine whether the properties of the elements are altered in any way by repetitive activity. On the other hand, all the observations listed above, as well as that of Ramsey & Street (1941) on the relation between PTP and P_t/P_0 of frog muscle fibres at 20 °C are entirely consistent with the view that many muscle fibres in mammalian fast muscles near body temperature and some frog twitch muscles at 20 °C are only partially activated, and that PTP at these temperatures leads to an increase in the response of the contractile material itself (Close, 1965; Colomo & Rocchi, 1965; Close & Hoh, 1968; Desmedt & Hainnaut, 1968). On this basis PTP with little or no change in twitch time course may be regarded as being due largely to an increase in the degree of activation of individual muscle fibres whereas prolonged stimulation, in which the number of stimuli exceeds that required for full potentiation, leads to an increase in the duration of the twitch presumably as a result of prolongation of the active state as described by Ritchie & Wilkie (1955) for frog muscle at 0°C.

As regards a change in the response of the contractile material it is possible that PTP results from an increase in the number of fully activated myofibrils contracting in individual muscle fibres. This hypothesis has the advantage that potentiation would result simply from addition of the same kind of response of elements contracting in parallel. It has been reported that even the central myofibrils of frog fibres are activated following a single stimulus at 20° C and undergo shortening (Gonzalez-Serratos, 1966) but in the absence of a mechanical record of the contraction which was photographed there remains the possibility that the fibre was one of the few which appear to be fully activated and show little or no PTP (Ramsey & Street, 1941). In any event the shortening of fibrils observed by Gonzalez-Serratos was free shortening with no external load and those results do not preclude the possibility that the response of individual fibrils may be increased during PTP, either by an increase in the number of active filaments or cross-bridges or by some other means.

Alternatively, all the cross-bridges of a fibre may contribute to the normal twitch contraction of rat EDL at 35° C and frog muscle at 20° C, but the amount of activator liberated following the action potential may

be submaximal and limit the average rate of cycling of each cross-bridge at any particular load. PTP may then result simply from an increase in the amount of activator liberated in all regions of the fibre following a single action potential, thus raising the activator concentration throughout the fibre, with a corresponding increase in the average rate of cycling of individual cross-bridges at a particular load and an increase in the number of bridges formed and tension developed at any given time in the twitch. According to this hypothesis the degree of activation or potentiation would increase with increased liberation of activator up to a maximum corresponding to the maximum rate of cycling of cross-bridges for each load and this would not necessarily alter either the intrinsic speed of shortening or the isometric twitch contraction time.

A possible explanation for the effects of prolonged stimulation increasing the twitch duration is that there is an increase in the amount of activator liberated, thereby exceeding the amount which saturates and fully activates the contractile material. As a consequence it would take some time for the concentration of activator to fall below the saturation level, as suggested by Hodgkin & Horowicz (1960*b*) for potassium contractures, and this may result in delayed decline of the active state, thereby increasing T_c, $T_{\frac{1}{2}R}$ and twitch duration, and the appearance of a hump on the record of relaxation (Fig. 6) normally seen in rat and frog muscles only at low temperature.

It has been suggested that PTP results from accumulation of a substance in some part of the muscle fibre (Lee, 1907; Brown & Euler, 1938; Martini, 1939; Giachetti, 1950; Zingoni, 1954). Brown & Euler (1938) pointed out the similarity in potentiation of the twitch of mammalian muscle by potassium ions and PTP but there is as yet no direct evidence that accumulation of this ion in, or about, the muscle fibre actually causes PTP. Diffusion of excess external potassium from frog single muscle fibres occurs within about 10 sec after a sudden decrease in external potassium ion concentration (Hodgkin & Horowicz, 1960*a*) whereas PTP in frog single muscle fibres persists much longer (Ramsey & Street, 1941; Colomo & Rocchi, 1965). Nevertheless, it is possible that potassium ions accumulate in a space such as the transverse tubules during repetitive excitation, thereby affecting excitation–contraction coupling and enhancing activation, and that diffusion of these ions out of the tubules is retarded following repetitive stimulation. There is also the possibility that the mechanical threshold (Hodgkin & Horowicz, 1960*b*) is lowered during PTP, as suggested by Desmedt & Hainnaut (1968), or that the muscle membrane is altered in some other way. Some changes in the action potential after repetitive stimulation disappear with a time course similar to that for PTP (Persson, 1963), but it is not known whether these electrical changes are causally

related to the potentiation of twitch tension or whether they are independent effects of repetitive stimulation.

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FORCE:VELOCITY PROPERTIES OF KITTEN MUSCLES

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SUMMARY

1. The characteristics of isometric contractions and force:velocity properties of the extensor digitorum longus (EDL) and soleus (SOL) muscles of neonatal kittens were determined *in situ*.

2. The mean contraction time is 51 msec for EDL and 70 msec for SOL and the half-relaxation time is 51 msec for EDL and 109 msec for SOL.

3. The average maximum isometric tetanic tension per unit cross-sectional area of muscle is 1·27 kg/cm^2 for EDL and 1·17 kg/cm^2 for SOL.

4. The average twitch:tetanus ratio is 0·28 for EDL and 0·119 for SOL; the low value for SOL was found for both indirect and direct stimulation.

5. The average maximum speed of shortening of a sarcomere is 22·8 μ/sec for EDL and 12·7 μ/sec for SOL.

6. These properties of neonatal muscles are compared with those of adult cat muscles and discussed in connexion with differentiation of mammalian muscles into fast and slow types.

INTRODUCTION

In some mammals all the limb muscles are slow at birth. Subsequent changes, which occur during the first few weeks of life, lead to differentiation of fast muscles and slow muscles. The pattern of differentiation, as indicated by changes in the time course of isometric contractions, is similar in cat, rabbit, rat and mouse (Banu, 1922; Denny-Brown, 1929; Koschtojanz & Rjabinowskaja, 1935; Buller, Eccles & Eccles, 1960; Close, 1964, 1965*b*; Buller & Lewis, 1965). A more detailed description has been obtained for rat and mouse muscles by determining the relation between speed of shortening and load in isotonic contractions for a fast muscle, the extensor digitorum longus (EDL), and a slow muscle, the soleus (SOL), at different stages of development (Close, 1964, 1965*a*). In these rodents the force:velocity properties of EDL and SOL are virtually identical at birth, thereafter the speed of shortening of sarcomeres of EDL increases two to threefold within 3–4 weeks, whereas SOL undergoes

little or no change in this respect. It has been suggested that this increase in intrinsic speed of shortening is the principal change leading to differentiation of the two kinds of muscles in rat and mouse (Close, 1965*b*). In view of the similarity between rodents and some other mammals in the pattern of changes in the isometric twitch contraction time during development, the possibility arose that differentiation of fast and slow muscles is brought about in the same way in different animals through changes in the speed of shortening of sarcomeres of the fast muscles. This possibility has been investigated for the cat in the present work. The force:velocity properties of a fast muscle and a slow muscle have been determined for new-born kittens and compared with those reported previously for adult cats.

METHODS

The dynamic properties of the EDL and SOL muscles of one limb were examined in three 1- to 2-day-old kittens. The animals were anaesthetized with 20 mg pentobarbital sodium/kg body wt. injected intraperitoneally, followed by one quarter of the initial dose every 1–2 hr. The preparations were set up with the muscles *in situ* and with the major blood vessels supplying the muscles intact. The lower limb was immersed in about 150 ml. of Ringer fluid (NaCl 137 mM; KCl 5 mM; $CaCl_2$ 2 mM; $MgCl_2$ 1 mM; NaH_2PO_4 1 mM; $NaHCO_3$ 2 g/l.; glucose 2 g/l.) which was bubbled continuously with 95 % O_2 and 5 % CO_2 and maintained at temperatures between 34·5° and 36·0° C. The muscles were stimulated indirectly with 20 μsec pulses through platinum wire electrodes on the tibial nerve for SOL and the common peroneal nerve for EDL. Reflex activation of the muscles was prevented by cutting the sciatic nerve central to the point of stimulation, and mechanical interference through contraction of neighbouring muscles was avoided by transecting the nerves to all muscles of the lower limb except SOL and EDL. The dynamic properties of the muscles were determined using the methods and equipment employed previously for work on adult rat muscles (Close, 1964). At the end of each experiment the muscles were fixed at optimal length, macerated in acid and stored in 50 % glycerol (Close, 1964). Fibres were dissected from the muscles and examined under the light microscope at a magnification of × 450. The average sarcomere length was determined for 5–7 fibres from each muscle by counting the number of sarcomeres in a 145 μ length of fibre in regions every 1·25 mm along the fibre. In two other 1-day-old kittens the twitch:tetanus ratio of SOL was determined for contractions elicited by both indirect and direct stimulation.

RESULTS

Representative records of isometric contractions of EDL and SOL of a new-born kitten are shown in Fig. 1 together with the force:velocity curves determined from isotonic contractions of the same muscles. Table 1 summarizes the results obtained for three EDL and three SOL muscles from 1- to 2-day-old kittens.

The average isometric twitch contraction time for the EDL muscles was 51 msec recorded at a mean temperature of 34·7° C (34·5–34·8° C). This value for the contraction time (T_c) of EDL is shorter than those reported previously for neonatal flexor hallucis longus (FHL) muscle (Buller & Lewis, 1965), but 2–3 times longer than the values obtained for adult

fast muscles (Buller *et al.* 1960; Buller & Lewis, 1965). The mean contraction time for the SOL muscles was 70 msec at 35·5° C. (35·0–36·0° C), which is nearly the same as the times previously reported for both new-born and adult cat soleus muscles (Buller *et al.* 1960). The ratio of half-relaxation time to contraction time ($T_{\frac{1}{2}R}/T_c$) is 1·0 for EDL and about 1·5 for SOL (Table 1), and in this respect these muscles show little or no

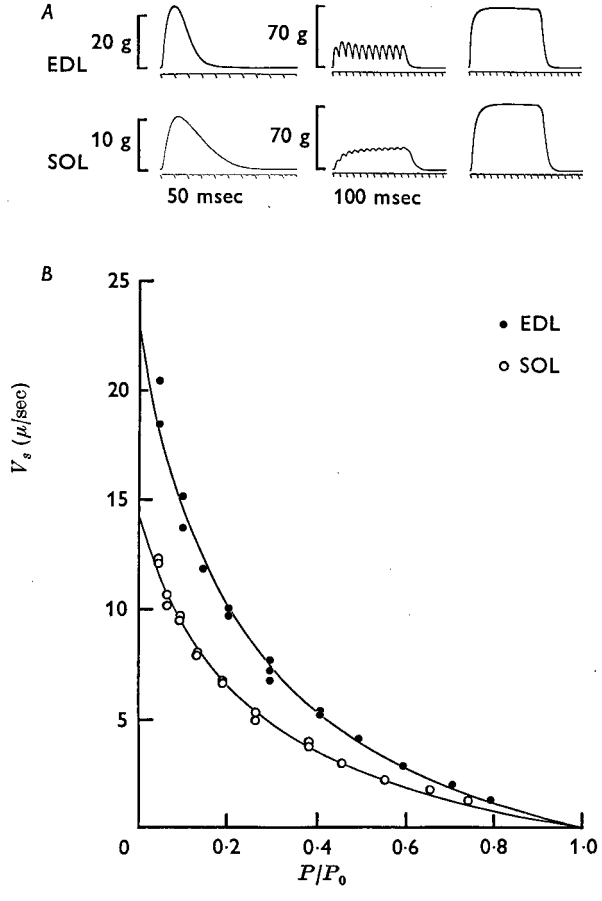

Fig. 1. *A*, Representative records of isometric contractions of extensor digitorum longus (EDL) and soleus (SOL) muscles of a new-born kitten. The records are, from left to right, the isometric twitch and responses to repetitive stimulation at 10 c/s and 100 c/s. *B*, Force–velocity curves for the same extensor digitorum longus (●) and soleus (○) muscles. The speed of shortening of a sarcomere (V_s in μ/sec) is plotted against the isotonic load expressed as a fraction of the maximum isometric tetanic tension; the curves were fitted using Hill's (1938) equation. Muscle weights: EDL = 54 mg, SOL = 56 mg; average muscle fibre length: EDL = 10·1 mm, SOL = 9·5 mm; average sarcomere length: EDL = 3·32 μ, SOL = 3·27 μ.

change during development from birth to maturity. These results show that the isometric twitch of EDL is considerably faster than the twitch of SOL at birth.

The maximum isometric tetanic tension (P_0) was determined at the optimal frequency (Close, 1964), which is about 100 c/s for both EDL and

TABLE 1. Average values for properties of three EDL muscles and three SOL muscles from three new-born kittens. The ranges of values are indicated in parentheses

	EDL	SOL
Muscle wt. (mg)	53·7 (49–58)	57 (50–65)
Fibre length (mm)	10·6 (9·75–12·0)	9·5 (9·27–9·75)
Sarcomere length (μ)	3·25 (3·15–3·32)	3·24 (3·16–3·28)
Maximum isometric tetanic tension (g)	64 (47–76)	69·8 (61·0–74·5)
Twitch:tetanus ratio	0·28 (0·19–0·333)	0·119 (0·103–0·14)
Maximum force/area (kg/cm^2)	1·27 (0·94–1·57)	1·17 (1·12–1·25)
Contraction time (msec)	51 (50–53)	70 (67–74)
Half-relaxation time (msec)	51 (46–56)	109 (101–126)
Maximum speed of shortening of a sarcomere (μ/sec)	22·8 (20·0–25·6)	12·7 (10·0–14·15)
a/P_0	0·265 (0·23–0·295)	0·315 (0·285–0·375)

SOL at birth for a tetanus of 1 sec duration. The arrangement of muscle fibres in kitten EDL and SOL is very similar to that described for the corresponding muscles of the rat (Close, 1964) and all the muscle fibres have about the same length. The average cross-sectional area of the muscles calculated from the fibre length and the muscle weight, assuming the density of the muscle to be unity, was $5{\cdot}3 \times 10^{-2}$ cm^2 for EDL muscles and $6{\cdot}0 \times 10^{-2}$ cm^2 for SOL muscles. The maximum tension developed for unit cross-sectional area of muscle was calculated for each muscle and the average values were 1·27 kg/cm^2 for EDL and 1·17 kg/cm^2 for SOL. Thus the intrinsic strength is probably about the same for the two kinds of muscles and is within the usual range (i.e. 1–2 kg/cm^2) found for most adult skeletal muscles. The tension developed per gram of muscle is about the same for EDL and SOL at birth and in this respect the results obtained in the present work differ from those reported by Buller & Lewis (1965) for new-born FHL and SOL muscles. The average of the ratios of the isometric twitch tension to the maximum isometric tetanic tension, P_t/P_0, was 0·28 for EDL, whereas the average ratio was only 0·119 for the SOL muscles. As the maximum isometric tetanic tension is about the same for EDL and SOL the difference in P_t/P_0 is attributable to the difference in peak tension developed in twitches of the two muscles. In two other experiments the P_t/P_0 was determined for two neonatal SOL muscles for contractions elicited first by indirect stimulation, *in situ* in one preparation and *in vitro* in the other. The muscles were subsequently curarized *in vitro* ($2{\cdot}0 \times 10^{-5}$ g of (+)-tubocurarine chloride/ml.) and stimulated

directly with massive platinum electrodes (Mostofsky & Sandow, 1951) using pulses of about 20 V/cm and 0·5 msec duration. P_0 and P_t/P_0 were nearly the same for the two kinds of stimulation at 35° C and in both preparations P_t/P_0 did not exceed 0·1. Consequently the low P_t/P_0 for SOL is a property of the muscle itself and is not attributable to failure of some of the muscle fibres to be excited when the muscle is stimulated indirectly by way of the nerve.

The sarcomere length is about the same in neonatal EDL and SOL and the average values obtained were 3·25 μ for EDL and 3·24 μ for SOL. The average number of sarcomeres/fibre was estimated for each muscle from the sarcomere length and the fibre length and ranged from 2975 to 3040 sarcomeres/fibre for EDL muscles and from 2830 to 3085 sarcomeres/fibre for the SOL muscles.

The speed of shortening of the musles was determined for different loads and distance–time curves of after-loaded isotonic contractions to repetitive stimulation at the optimal frequency (100 c/s). In order to compare the force:velocity properties of small neonatal muscles and large muscles from older animals it is necessary to express the speed of shortening and the load in terms of unit amounts of contractile material in series and parallel, respectively. For this purpose the speed of shortening of a sarcomere was estimated by dividing the speed of the whole muscle by the average number of sarcomeres per fibre, and the load has been expressed as a fraction of the maximum isometric tetanic tension. Hill's equation (Hill, 1938), $(P+a)V = b(P_0-P)$, in which P = load, V = speed of shortening, P_0 = maximum isometric tetanic tension and a and b are constants, was used to fit the force:velocity data obtained for each muscle. The constants describing the force:velocity curves for new-born kitten EDL and SOL muscles are listed in Table 1. In every preparation the EDL was between 1·6 and 2 times faster than SOL with respect to the speed of shortening in isotonic contractions for any given load. The average value for the intrinsic speed of shortening (V_s^{max}), i.e. the maximum speed of shortening of a sarcomere when the load is zero, was 22·8 μ/sec for EDL muscles and 12·7 μ/sec for SOL muscles. In the kitten therefore EDL is faster than SOL at birth whether the speed of isotonic shortening or the isometric twitch contraction time is taken as the criterion of speed of contraction.

DISCUSSION

In order to compare the properties of fast and slow muscles of new-born kittens and adult cats the speed of shortening of sarcomeres of adult cat muscles has been estimated as described previously (Close, 1965*b*) from the results of work by Fenn & Marsh (1935) and Rosenblueth & Rubio

(1959), except that the sarcomere length was taken to be 2·7 μ for fast muscle and 3·1 μ for SOL (Buller & Lewis, 1965). This gives a maximum speed of shortening of sarcomeres of about 31 μ/sec for cat fast muscle and 13 μ/sec for cat SOL. This estimate for adult SOL is about the same as the value obtained for new-born kitten SOL (Table 1). The results show that the intrinsic speed of shortening of SOL does not change during development whereas the intrinsic speed of EDL is increased. In this respect the pattern of change in the speed of cat muscles during differentiation into fast muscles and slow muscles is the same as that for rat and mouse muscles. However, unlike rat and mouse muscles, the speed of shortening of kitten EDL is higher than that of SOL at birth. Nevertheless it is possible that the force:velocity properties of cat EDL and SOL are identical at some earlier stage during foetal development and that the difference between cat and rodent muscles arises because in the kitten the onset of muscle speed differentiation occurs before birth.

The time course of developmental changes in force:velocity properties of cat muscles has not been determined directly in the present work. It is therefore of interest to examine the time course of other changes which may be brought about by changes in the intrinsic speed of shortening. It is known, for example, from the work of Hill (1949) and Jewell & Wilkie (1958) that the time course of tension increase in an isometric tetanus is partly determined by the force:velocity properties of the muscle. The relation between rate of rise of isometric tetanic tension and speed of shortening is not fully understood but the two appear to be approximately proportional in rat muscles (Close, 1964). In this connexion the results of Buller & Lewis (1965) are interesting because they show that the maximum rate of rise of isometric tetanic tension ($\%P_0$/msec) for cat EDL at birth is almost twice as great as the rate for SOL, and that during postnatal development this rate increases in EDL but is not changed in SOL. This pattern of change is similar to that for changes in the maximum speed of shortening of sarcomeres (V_s^{max}). A further observation indicating a correspondence between $\%P_0$/msec and V_s^{max} is seen in the relation between each one of these properties and the isometric twitch contraction time. It has been shown for a number of muscles that there is a hyperbolic relation between V_s^{max} and T_c (Close, 1964, 1965*b*) and a similar inverse relation between $\%P_0$/msec and T_c is evident in Fig. 7 of the paper by Buller & Lewis (1965). It seems likely therefore that in cat muscles $\%P_0$/msec and V_s^{max} are approximately proportional and that developmental changes in V_s^{max} largely determine the changes in the time course of tension development in isometric tetanic contractions. The data presented by Buller & Lewis (1965) on the time course of changes in $\%P_0$/msec (Fig. 4*B*, 1965) show that fast muscles attain adult values for $\%P_0$/msec within

5–6 weeks after birth and it is likely that the changes in force:velocity properties of EDL follow a similar time course.

The developmental changes in isometric twitch contraction time of rat muscles have been accounted for in terms of changes in both V_s^{max} and P_t/P_0 (Close, 1964) and it is of interest to ascertain whether the changes in the cat muscles can be explained similarly. In rat muscles a change in P_t/P_0 leads to an approximately proportional change in T_c and a change in V_s^{max} brings about an inversely proportional change in T_c. For newborn cat fast muscle P_t/P_0 is approximately 0·3 (Table 1 above; Buller & Lewis, 1965) and V_s^{max} is 22·8 μ/sec, whereas for adult fast muscle P_t/P_0 is about 0·24 (Buller & Lewis, 1965) and V_s^{max} is about 31 μ/sec (above). These changes in P_t/P_0 and V_s^{max} may be expected to alter the contraction time from about 51 msec at birth to about 30 msec for adult muscles. This estimated time for contraction of adult fast muscle is larger than the values reported by Buller & Lewis (1965) but corresponds fairly well with the contraction times of about 27 msec recorded by Buller *et al.* (1960) for several fast muscles. The pattern of change in various properties of cat fast muscles therefore resembles closely the patterns described previously for rat and mouse muscles.

The development of cat SOL is similar to that of rat and mouse SOL muscles in that there are no changes in V_s^{max}, but it differs from the others with respect to changes in P_t/P_0 and T_c. In rodent SOL muscles there are proportional decreases in P_t/P_0 and T_c during the first few weeks after birth, whereas in cat SOL there is little or no change in T_c but there is a twofold increase in P_t/P_0 from about 0·119 (Table 1) at birth to about 0·26 within 2 weeks. It is unlikely that this change is due to a decrease in the relative compliance of the passive series elastic elements because both % P_0/msec and V_s^{max} remain unchanged throughout development. The possibility that the low P_t/P_0 of SOL at birth results from failure of some muscle fibres to be excited when the muscle is stimulated indirectly has been excluded because the responses of the muscle to direct and indirect stimulation are the same. An alternative explanation is that at birth the individual muscle fibres of cat SOL are incompletely activated either because the muscle fibre action potential fails to activate all the myofibrils and cross bridges or because the tension-generating sites fail to develop maximum tension in the twitch.

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