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THE EFFECTS OF DENERVATION ON CONTRACTILE PROPERTIES OF RAT SKELETAL MUSCLE

BY H. J. FINOL*, D. M. LEWIS AND ROSEMARY OWENS†
From the Department of Physiology, University of Bristol, Bristol BS8 1TD

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SUMMARY

1. Isometric contractions of fast and slow twitch muscles of rats were recorded 1–42 days after denervation.

2. The major changes occurred over the period from 2 to 6 days after denervation. These changes were qualitatively similar in the two types of muscle. The most important effects were on the twitch: times to peak and half relaxation were prolonged, active tension and peak rate of rise of tension were increased. Tetanic tension per unit cross-sectional area and the maximum rate of rise of tension decreased during this period but, in the second week, the tension recovered substantially and the maximum rate recovered completely.

3. Very small differences were seen between muscles denervated with short and long nerve stumps at day 4.

4. In the late stage of denervation (7–42 days) twitch and tetanic tension fell more than cross-sectional area, but this may have been due to greater atrophy of fibres compared with other muscle tissue.

5. Apart from this tension fall, there were only small changes in the fast muscle in the late stage of denervation. These were a fall in twitch-tetanus ratio and a prolongation of relaxation.

6. In more than half of the soleus muscles there was a late reversal of some of the denervation changes, and these muscles showed a greater degree of atrophy. The less atrophied soleus muscles maintained a prolonged twitch and a low rate of development of tension.

7. It is concluded that denervation affects the contractile properties of muscle as early and as abruptly as it does the membrane properties, and that most of the contractile changes are a direct consequence of changes in excitation-contraction coupling alone.

INTRODUCTION

The isometric twitch contraction of skeletal muscle becomes prolonged following denervation. One hypothesis (Lewis, 1972) to explain the contractile changes is that prolongation of the action potential is the primary event, bringing about the release

* On leave of absence from Universidad Central, Caracas, Venezuela.
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of more intrafibrillar calcium and, therefore, the more complete and prolonged activation of the contractile proteins. This hypothesis predicts that the changes in the action potential and the twitch will occur at the same time following nerve section. The time course of membrane changes has been defined in detail, with studies of the effects of denervation on the resting potential (Albuquerque, Schuh & Kaufman, 1971), the action potential (Redfern & Thesleff, 1971*a*, *b*) and the development of tetrodotoxin resistant action potentials (Harris & Thesleff, 1972).

Although several workers have shown that the change in the mechanical response occurs within the first week after nerve section in the rat (e.g. Harris & Miledi, 1972; Gutmann, Melicha & Sorovy, 1976) there have been no full, systematic observations. The most detailed work (Gutmann *et al.* 1976) studied the onset but not the full time course of denervation, and so does not allow a complete comparison of mechanical and membrane properties for an adequate test of the hypothesis outlined above. We have followed, therefore, the mechanical changes in sufficient detail for comparison to be made with reports on changes in surface membrane properties. We examined a fast twitch muscle (extensor digitorum longus) and a slow twitch muscle (soleus). A preliminary report of these results has been presented (Finol & Lewis, 1975).

A further prediction of the hypothesis is that the length of nerve stump connected to the muscle following neurotomy should influence the onset of mechanical changes as it does on the electrical changes (Albuquerque *et al.* 1971; Harris & Thesleff, 1972). We have tested this, supplementing the observations of Gutmann *et al.* (1976).

METHODS

Adult male Wistar rats of mean body weight 268 g (S.D. = 33 g) were anaesthetized with I.P. sodium pentobarbitone (40 mg/kg body wt.). Operations were performed under aseptic conditions. In experiments to test the effects of nerve stump length both hind limbs were dissected; in one the denervation was remote from the muscle and in the other it was close. Neurotomy remote from the muscle was performed by section of the sciatic nerve as it emerged from the sciatic notch. Close section was made of the nerve to extensor digitorum longus by transection of the lateral popliteal nerve immediately above the point at which it runs deep to peroneus longus muscle (about 12 mm from the muscle). A dummy operation exposing extensor digitorum longus nerve proximally was made on the side of the remote denervation. On the side of the proximal denervation, the sciatic nerve was sectioned just below the sciatic notch, in order to ensure that the limb muscle paralysis was symmetrical. This symmetry was necessary because Gutmann, Schiaffino & Hanzlikova (1971) have suggested that the extent of the response to denervation depends on whether antagonists or synergists are denervated. The proximal and distal sections were randomly assigned to left and right limbs. In other animals only one limb was denervated in mid-thigh. For long periods of denervation the sciatic nerve above the section was tied and drawn through the hamstring muscles to delay regeneration.

Control measurements were made on ten or twelve indirectly stimulated muscles of normal animals with body weights similar to those chronically denervated. At the end of this recording, three animals were curarized and soleus stimulated directly as described below. No significant differences were found between the mechanical responses to indirect and direct stimulation.

From 1 to 64 days after the initial operation the animals were anaesthetized with sodium pentobarbitone (50 mg/kg I.P.) supplemented as necessary with additional doses of 5 mg/kg. One or both limbs were prepared for tension recording. The skin of the lower leg was incised along a posterolateral line. Soleus and/or extensor digitorum longus muscles were exposed and freed from adjacent muscles until only connected to the body by their blood vessels and perivascular connective tissue. The upper tendons were cut for later fixation but were held in place temporarily by sutures so as to reduce the possibility of trauma to the blood vessels. The distal tendons were

bound to metal rods for later attachment to the tension transducers via insulating connectors. The tibia and fibula were held by clamps at the ankle and knee, and the skin flaps were pulled apart by cotton thread. The resulting pools were built up around their edges by laying cotton wool soaked in 5% agar-saline on the skin flaps and threads. The pools were filled with light mineral oil which was maintained at 37·5 ± 0·2 °C by radiant heat. The proximal tendons were fixed by clamping in box-jointed artery forceps supported on an insulating rod. Electrical stimulation pulses were applied between the two tendons via their metallic attachments. Methods of stimulation were similar to those described by Kean, Lewis & McGarrick (1974). Body temperature was maintained at about 37·5 °C by an electric blanket.

Tension was recorded by unbonded wire strain gauge dynamometers (Ether UF2-16 or 4) which had resonant frequencies of 780 and 380 Hz respectively. The amplified outputs were analysed on line by a digital computer (Ranatunga, 1972). In proximal-distal denervation comparisons, the order in which the muscles were tested was varied at random.

Total muscle length (between tendons) was measured at optimum length in all experiments. About two thirds of the muscles were removed at the end of the experiment, blotted dry with filter paper and weighed after cutting off both tendons. The other muscles were held at optimum length at the end of the experiment. Connexions with the animal were severed and a dish of 10% formal-saline was brought up to immerse the muscle. After fixation for one day the muscles were blotted dry and weighed. Fixation continued for 2 days; length changed less than 5% during fixation. The muscles were prepared for dissection by treatment with 20% nitric acid for 3–4 days and were kept in 50% glycerol. Each muscle was teased longitudinally into eight approximately equal parts, from each of which a bundle of one to five fibres was dissected with blunt needles. The bundle was laid straight on a microscope stage, the vernier movement of which was used to measure fibre length. In the bundles the lines of origin and insertion were not perpendicular to the longitudinal axis, but the tapered ends of the fibres were visible under the microscope so that no systematic errors were introduced. If some of the fibre lengths within a muscle were very different from the average for that muscle, an additional bundle was dissected from the appropriate part. Muscle cross-sectional area was calculated as muscle weight divided by mean fibre length. It was found that fibre length was a consistent fraction of total muscle length throughout the period of denervation (0·709, S.D. 0·035 in soleus and 0·417, S.D. 0·017 in extensor digitorum longus). In those muscles in which fibre length was not measured, over-all muscle length was multiplied by one of the factors above to estimate fibre length.

RESULTS

The effects of denervation were followed daily over 5 days (averaging five animals for each period of denervation) and then at 1, 2 and 6 weeks. Some intermediate and later periods were used; the results are not reported in detail here, but they fit in with the trends described. The fundamental changes in the isometric myograms are illustrated in Fig. 3 and the time course of the changes in Figs. 1 and 2. Total muscle fibre cross-sectional area (calculated as wet weight/fibre length) was also followed, and is illustrated in Fig. 4*A*. Both muscles atrophied progressively although there was a difference between the two muscles, soleus showing rapid atrophy in the first week during which period extensor digitorum longus changed little. It is convenient to describe the effects of denervation on muscle contractile properties as occurring in two phases, the early phase occupying the first week after nerve section.

The early phase began with a fall in tetanic tension per unit muscle fibre area (specific tension), which was almost fully developed on day 2 (the difference seen in Fig. 1*D* between values at days 2 and 3 in extensor digitorum longus was not significant). Towards the end of the early phase there was a recovery of specific tetanic tension, complete in soleus and to more than 80% of normal in extensor digitorum longus by day 7.

In contrast, specific twitch tension increased in the early phase (Fig. 1*C*). The

increase was so great in extensor digitorum longus that there was also a 20 % increase of absolute tension (Fig. 3, myograms). The discrepancy between changes in twitch and tetanic tensions is most clearly seen in the graphs of the ratio of twitch tension to tetanic tension (Fig. 1*A*); the increase in the twitch–tetanus ratio was greater in extensor digitorum longus than soleus. The increase in twitch tension was accomp-

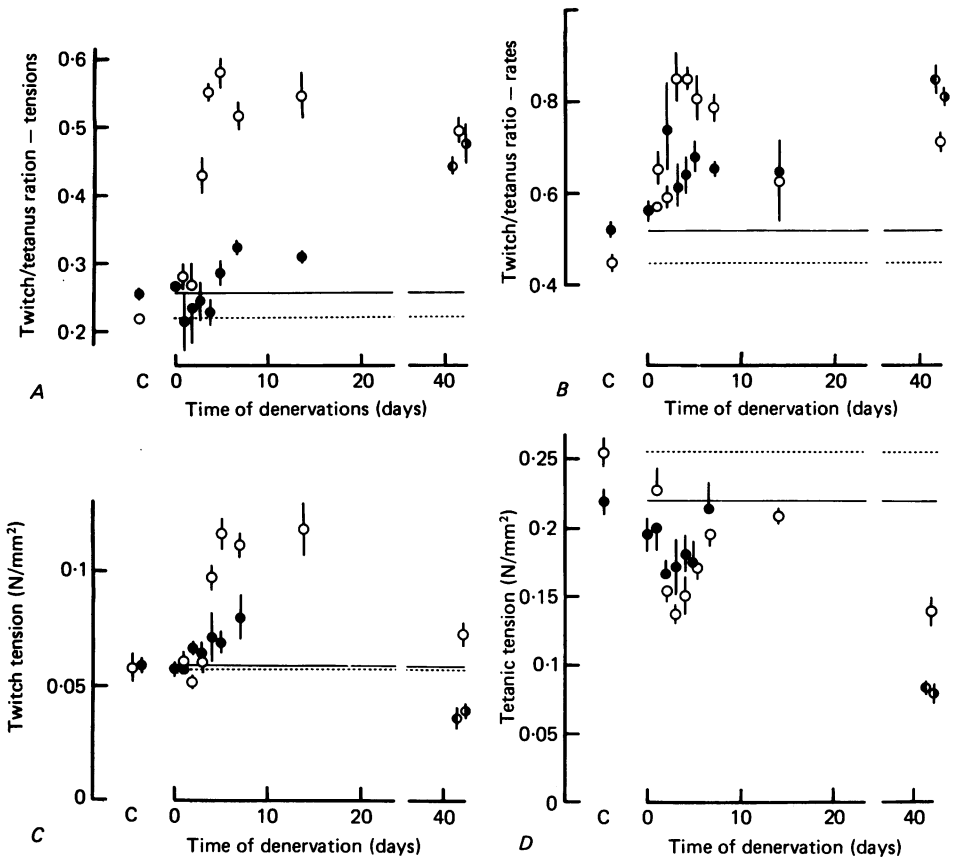

Fig. 1. Changes in contractile properties of denervated rat muscles; ○ extensor digitorum longus; ● soleus (at 42 days, ◐ indicates muscles with a long twitch time-to-peak and ◑ those with a short time-to-peak: see Fig. 4*B*). S.E. of mean is indicated by vertical bars, unless smaller than the symbol (n = 3–14). Indirectly stimulated control values (n = 10–12) shown by *C* on the abscissa and the horizontal lines. Directly stimulated, curarized controls (n = 3) are shown at day 0. *A* is the ratio of peak active twitch tension to maximum tetanic tension. *B* is the ratio of the maximum rate of rise in a twitch to the maximal rate of rise in a fully activated tetanus. In *C*, *D* tensions are normalized by estimated muscle cross-sectional area (see Fig. 4*A*).

anied by a prolongation of the time to peak (Fig. 2*A*) and the time to half relaxation. The lengthening of the twitch was approximately the same in both muscles, although this represented a larger proportional increase in extensor digitorum longus. Generally, changes in this group of contractile characteristics were relatively larger in the fast twitch muscle and were seen earlier than in the slow muscle. The first major change was in the twitch time to peak of extensor digitorum longus which was

significantly different from that of the controls at 2 days ($t = 4{\cdot}06$, degrees of freedom = 12, $P < 0{\cdot}005$) and appeared to reach a maximum at 4 days (no significant differences from value at 7 days; $t = 1{\cdot}2$, d.f. = 12). Changes in half relaxation time were very similar, but the specific twitch tension did not increase significantly until day 4 ($t = 4{\cdot}87$, d.f. = 15, $P < 0{\cdot}001$) which may also represent the maximal effect

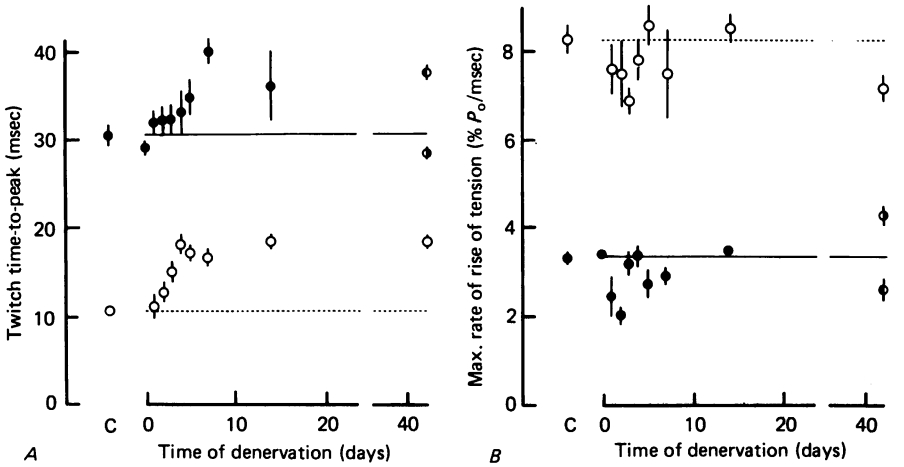

Fig. 2. Changes in the time-to-peak of the twitch (*A*) and in the maximum rate of development of tension in a fully activated tetanus expressed as a percentage of the maximum tetanic tension (*B*). Symbols as in Fig. 1.

since the difference between days 4 and 5 was not significant ($t = 1{\cdot}96$, d.f. = 14, $P < 0{\cdot}1$). None of these variables was significantly different from the control values in denervated soleus until day 4 when twitch tension and times were altered. Another difference was that the changes in soleus progressed between days 4 and 7.

The maximum rates of development of tension in twitches and tetani were also measured. The maximum rate in the tetani, expressed as a percentage of maximum tetanic tension (Buller & Lewis, 1965), showed changes (Fig. 2*B*) which had a time course similar to those of specific tetanic tension. There was a transient fall around days 2 and 3 which recovered almost to normal by day 7. These changes in the rate of tension development were small and only significant at day 2 in soleus and day 3 in extensor digitorum longus. Although the maximum rates of tension development were not greatly changed they were achieved at lower rates of stimulation in the denervated muscles. Normal extensor digitorum lobgus requires a stimulation rate of 600–800/sec to reach its maximum rate. After denervation stimulation at 400–500/sec was sufficient. The corresponding figures for soleus were 400/sec before and 300/sec after denervation.

The rate of tension development in the twitch increased after denervation. In order to compare muscles with differing tensions, the peak rate in the twitch was expressed as a fraction of the maximum rate of tension development in the tetanus (Fig. 1*B*), this ratio increased very early (on day 2 in both muscles). In one muscle the twitch rate was as much as 0·93 of the maximum tetanic rate.

There were some difficulties in measurements of twitch characteristics on day 2, in that the duration of the twitch was dependent on the duration of the stimulus pulse. Fig. 3 illustrates the problem. On the left the amplitude and time to peak of a directly stimulated, curarized control muscle are plotted against stimulus voltage. Different symbols indicate stimulus duration (from 0·1 to 1 msec). For durations of 0·1–0·5 msec the twitch tension increased with voltage up to a maximum which was close to the tension of the indirectly elicited twitch. Over most of this range

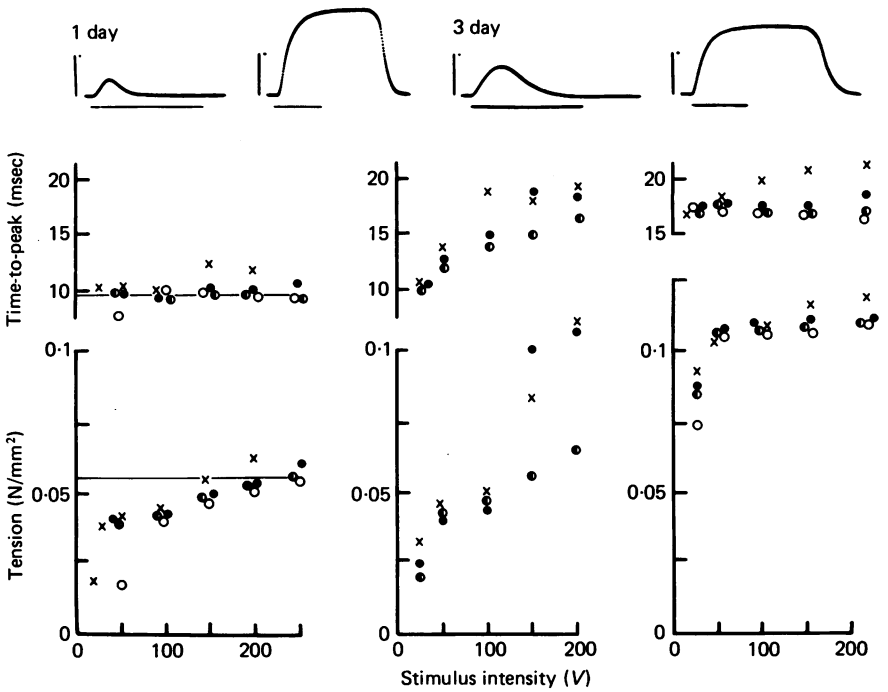

Fig. 3. *Above*: isometric twitch and tetanus myograms from extensor digitorum longus muscles denervated 1 and 3 days. Calibration bars indicate 1 N and 100 msec. *Below*: effect of stimulus voltage on twitch tension and time to peak of three extensor digitorum longus muscles: left, curarized control; centre, denervated 2 days; right, denervated 7 days. In each muscle stimulus durations were ○ 0·1 msec, ◐ 0·2 msec. ● 0·5 msec, × 1·0 msec. The continuous lines (left) show results obtained by indirect stimulation before curarization.

twitch time to peak did not vary systematically. Higher voltage stimuli of 1 msec duration gave a secondary increase in tension accompanied by an increase in twitch time-to-peak, which was probably due to repetitive excitation (muscle refractory period is less than 1 msec). The results at 7 days denervation (Fig. 3 right) were very similar, although twitch tension and time to peak were both increased. At 2 days (Fig. 3 centre), however, there was no plateau of tension at any stimulus duration, and twitch time to peak depended on stimulus intensity and duration. We assume that this behaviour results from asynchrony of the denervation changes between fibres of a muscle. It is know that denervation increases the chronaxie of muscle (Adrian, 1916), so in a partially converted muscle the fibres with the greatest increase in time-to-peak would tend to be recruited by longer duration pulses. This phenomenon was seen in three out of five extensor digitorum longus and two of four soleus muscles at day 2. Similar but much smaller effects (less than 5% differences) were seen in a minority of muscles recorded at days 3 and 4. The variability with stimulus duration and voltage was not a consequence of stimulation of residual motor nerve terminals since the phenomenon was as extensive after curarization of one 2 day denervated muscle. In calculations for Figs. 1 and 2, each muscle value was estimated, when necessary, as the mean of the maximal (or 200 V, if there was no maximum) responses to stimuli of 0·1, 0·2 and 0·5 msec duration. Such variability made the statistical calculations less certain but, if the explanation suggested above is accepted, reinforce the idea that the onset of denervation effects was early.

In nine animals extensor digitorum longus was denervated close to the muscle in one limb and with a maximal length of nerve stump in the other limb (remote denervation). The muscle twitches were examined 3–5 days later, using maximal stimuli of 0·1, 0·2 and 0·5 msec duration. No significant differences were found between the effects of close or remote denervation for any of the twitch characteristics when comparing mean values for each day of denervation. (The two sets are therefore combined in Figs. 1 and 2.) It was clear, however, that in addition to differences between animals there was variation due to small effects of stimulus duration on twitch response (see above).Taking the mean of the responses to three stimulus durations, while adequate for representing the overall time course of changes, would take no account of the extent of the denervation change in individual muscles. A more complicated analysis was therefore undertaken to look for effects of nerve stump length. For each animal, a ratio was calculated (close denervation muscle response divided by remote denervation muscle response) for the maximal responses to a 0·1, 0·2 and 0·5 msec stimulus. The twitch responses tested were tension, time to peak and maximum rate of tension development. At each day of denervation this ratio was compared with unity, which value would have been found if there were no differences between close and remote denervations. Significant differences were only found if every ratio calculated for each animal was used as if it were an independent measurement: a total of 15 at day 4, 3 at day 3 and 9 at day 5. At day 4 the ratio of twitch tensions (1·093, S.E. of mean 0·0208, $t = 4·46$, $P < 0·001$) and the ratio of maximum rates of development of twitch tension (1·183, S.E. of mean 0·0483, $t = 3·78$, $P < 0·005$) were significantly different from unity. In both cases the close denervation (short nerve stump) muscle had progressed further in the denervation process, and had a larger twitch developing tension at a higher rate. The twitch time to peak did not show a significant difference between close and remote denervations (mean = 0·97, S.E. of mean = 0·068, $t = 0·42$). No ratios were significantly different from unity on either day 5 or day 3.

In the late phase of denervation (between 7 and 42 days) tetanic tension fell in both muscles to 22 % of control values in extensor digitorum longus and 10 % in soleus. Even allowing for the atrophy (Fig. 4*A*) specific tension appeared to fall in both muscles (Fig. 1*D*). In most other contractile properties there were qualitative as well as quantitative differences between the two muscles. The changes in extensor digitorum longus were simpler and will be outlined first. The specific twitch tensions declined from the potentiation of the early phase of denervation to a value little greater than that of the controls (Fig. 1*C*). Twitch tension fell slightly more than tetanic so that the twitch-tetanus ratio was less than that at days 4 and 5 ($t = 2·66$, $n = 23$, $P < 0·02$). The rate of rise of tension in the tetanus fell slightly ($t = 2·31$, $n = 11$, $P = 0·05$). Although the twitch time to peak did not change, the twitch time to half relaxation, which had increased from 9·4 (S.E. of mean 0·60) msec in the controls to 19·2 (0·61) msec at days 4 and 5, was prolonged further to 26·3 (1·17) msec at day 42 ($t = 5·9$, $n = 23$, $P < 0·001$).

In soleus the fall in twitch tension was smaller than in extensor digitorum longus, so that twitch-tetanus ratio rose markedly (Fig. 1*A*) and by day 42 was not different from the value for extensor digitorum longus. The rise in the twitch-tetanus ratio was accompanied by an increase in the ratio of the rates of rise of twitch and tetanic tension (Fig. 1*B*).

The other changes in soleus are more difficult to describe because the scatter of values at day 42 was very great: the twitch times-to-peak, for example, ranged from 26 to 41 msec. The distribution of twitch time to peak appeared to be discontinuous with no values being measured between 30 and 36 msec in present series of twelve solei (Fig. 4*B*), and if these muscles are considered to be divided into two groups then

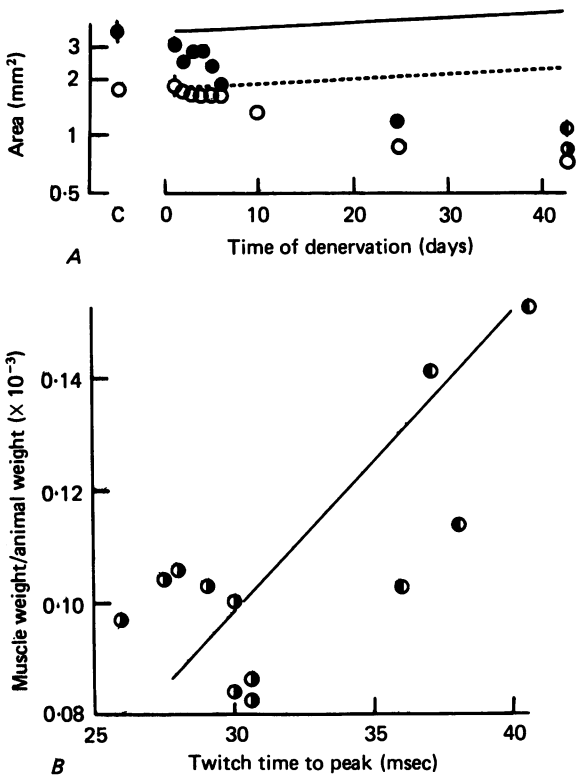

Fig. 4. *A*, change in muscle cross-sectional area (estimated as wet muscle weight divided by fibre length). Symbols as Fig. 1, except the lines are estimates of changes in normal muscle area made from the regressions of muscle area on body weight in the control animals. *B*, relation between twitch time to peak and degree of atrophy in soleus muscles denervated for 42 days. Symbols indicate the division into the two groups used in Figs. 1 and 2. Muscle weight has been expressed as a percentage of animal weight to allow for different sizes of animals. $r = 0{\cdot}71$, $P = 0{\cdot}01$ (graphs using wet weight directly are similar but show slightly more scatter).

the variances within the groups were no greater than those at earlier periods of denervation, or than those of extensor digitorum longus. Moreover this grouping was valid for those other characteristics of soleus which had a wide range of values at day 42 of denervation (twitch time to half relaxation and maximum rate of rise of tetanic tension). The two groups are shown by different symbols in Figs 1, 2 and 4. It can be seen that the twitch times of one of the day 42 groups were indistinguishable from those of soleus at the end of the first phase; for the other group there had been a shortening of the time course of the twitch. Even in this second group of soleus muscles the twitch was significantly longer than that of extensor digitorum longus.

The two groups of solei at day 42 did not differ in specfic tension or twitch-tetanus ratio. There was, however, a relationship with the degree of atrophy of the muscles: the more atrophied muscles had briefer twitches. The relationship was most clearly seen if muscle weight was normalized to allow for different sizes of animals, and is shown in Fig. 4*B*.

DISCUSSION

The results clearly indicate that the changes which denervation brings about in many of the contractile properties of skeletal muscle are early in onset and rapid in transition. Further, in extensor digitorum longus, the changes occurring within the first week comprise the major component of the response to denervation. The modification of the twitch seemed to involve no substantial contribution from changes in myofibrillar protein isoenzyme type because there was no consistent change in the rate of development of tetanic tension after 14 days (the early, transient decrease in rate of tension of development is ignored in this argument and discussed below in more detail). This is consistent with the very small fall in myosin ATPase activity at day 7 of denervation suggested by Gutmann, Melichna & Syrovy (1972: a decrease of 12% was quoted without any indication of variability).

Such observations are compatible with the hypothesis presented in the Introduction, that the changes of the isometric twitch in denervation are a consequence of the prolongation of the surface action potential of muscle fibres. The observations, of course, are equally compatible with alternative hypotheses suggesting early changes in one or more elements involved in excitation-contraction coupling, bringing about higher levels of intrafibrillar calcium ions following a single stimulus. The published data are not uniformly precise in describing the time course of changes in the electrical properties. The rate of rise of voltage in the action potential is reduced 36 hr after denervation, and this change is complete in 48 hr (Redfern & Thesleff, 1971*a*). Tetrodotoxin resistant action potentials also appear in muscle at 30–36 hr near the end-plate (Thesleff, 1974), although it is a day or more before the whole length of the fibre is involved (Redfern & Thesleff, 1971*b*). The duration of the action potential increases over the same period. Details of the last process are not published, but inspection of the figures in Redfern & Thesleff (1971*a*, *b*) indicate that prolongation may lag behind the fall in the rate of change of voltage in the action potential and would then match the change in the twitch more accurately.

There is evidence of changes in the sarcoplasmic reticulum over the same period. Caffeine contractures are thought to be due to direct release of calcium from the sarcoplasmic reticulum, and are an index of the state of the sarcoplasmic reticulum. Gutmann & Sandow (1965) found that rat exensor digitorum longus at room temperature became more sensitive to caffeine and developed contractures more rapidly within 24 hr of denervation, and the effect were greater at days 3 and 6. Increased sensitivity to caffeine has also been seen at 37 °C one day after denervation and possibly was maximal at day 4 (H. Browne & S. Pritchard, unpublished observations). Such changes indicate an increased ability of the sarcoplasmic reticulum in denervation to release calcium, which could be a factor in the potentiation of the twitch response. Other elements involved in excitation-contraction coupling such as T-tubule conduction, have not been investigated in denervation.

It is concluded that the changes in the twitch of fast muscle after denervation occur with a time course similar to that of the changes in the duration of the surface action potential and in the sarcoplasmic reticulum, but that it is impossible to distinguish between possible factors, several of which may be involved.

The twitch changes in soleus were slower to develop, the early phase of denervation was probably not complete until day 7. It is impossible to comment further since the only detailed observations on the time course of membrane changes in soleus are for membrane potential and the spread of acetylcholine receptors, although these do not indicate any great difference between muscle types.

There were transient falls in tetanic tension and in the rate of development of tension which were seen at days 1–3 and had recovered by day 7. We would suggest that these falls are due to failure of propagation along muscle fibres. D. M. Lewis, M. J. Pardoe & S. N. Webb (unpublished observations) have observed in cat muscle that, in the early phase of denervation, there may be failure to propagate along fibres with decreasing resting potentials. We suggest that there may be a transient phase when the muscle membrane depolarization impairs conduction and that propagation returns to a safe level again once the action potential is prolonged. It is known that repetitive activation decreases the excitability of cells, so it is reasonable to suppose that during this transient state the denervated muscle responds maximally to a single stimulus but fails in a proportion of fibres during a tetanus. This would cause transient falls in the specific tetanic tension and in the maximum rate of rise of tension. This view is reinforced by the finding that caffeine contracture tension may be greater than tetanic tension in early denervation (unpublished observations with H. Browne & S. Pritchard).

If failure of propagation in the tetanus occurs in the early phase of denervation it must affect the twitch-tetanus ratio. Acceptance of this hypothesis removes some of the differences between the two muscles which complicate the present findings. The transient fall of tetanic tension was almost twice as large in extensor digitorum longus as in soleus. At day 6 the twitch-tetanus ratio would have been 0·46 in extensor digitorum longus if there had been no transient failure of the tetanus, and both muscles would have shown an increase in twitch-tetanus ratio in the late phase of denervation (still larger in soleus).

The late phase of denervation involved a fall in specific tension in both types of muscle which was approximally linear from the first day of denervation if the transient changes in the first week are ignored. Muscle area was calculated conventionally as mass over fibre length and would include the area of non-muscular elements (such as connective tissue and blood vessels) which appear in histological preparations to occupy a greater proportion of the muscle after long periods of denervation. Indeed the estimated change of areas (to 42% in extensor digitorum longus and 27% in soleus at day 42) are considerably less than directly measured changes of fibre area (to about 15%: Engel & Stonnington, 1974). If the values of fibre area of Engel & Stonnington (1974) are used to estimate specific tensions from the changes in absolute tension reported here, then there was a rise (to 147%) in extensor digitorum longus and a fall (to 76%) in soleus. Clearly these values are very imprecise, being taken from different series of experiments, but they do suggest that there may be little change in specific tetanic tension in denervation.

The late changes in soleus were complicated by the apparent separation into two groups of muscles. Those muscles which had shorter twitch contractions and a higher rate of rise of tetanic tension had atrophied more than the other group of soleus muscles. One possible explanation is that the atrophy is differential. Rat soleus contains a mixture of motor units with slow and intermediate twitch contractions (Close, 1967). If the slow ones atrophy more rapidly, those muscles which atrophy least would have the slower contractions. In relation to this possibility there is conflicting evidence from histochemistry. Most authors suggest that fibres staining intensely for myosin ATPase (Type II, presumed fast) atrophy more rapidly than Type I (slow) fibres. In contrast Jaweed, Herbison & Ditunno (1975) found 2 weeks after crush injury to the sciatic nerve that, although Type II fibres atrophied more in a fast muscle, Type I fibres showed the greatest atrophy in soleus. If this last result were confirmed for full denervation, it would support the idea that the group of soleus muscles which became faster in the late stage of denervation did so because of differential atrophy. It may be noted that cat soleus, which contains only Type I fibres remains slow contracting after very long periods of denervation (Lewis, 1972).

There was some indication that the changes in the contractile response developed earlier when the muscle was denervated with a short nerve stump which is in conflict with an earlier report by Gutmann *et al.* (1976) who found no effect of nerve stump length on contractile properties. The positive result, although small, would be important for acceptance of the idea that the mechanical changes following denervation are a consequence of primary changes in the excitable membranes, many of which are dependent on stump length. The effect cannot be regarded as proved by the present experiments, only reopened. In retrospect the largest difference would be expected on an earlier day of denervation. At that stage, however, the problems of deciding on an appropriate stimulus would have added to the variability. If fibres do transform asynchronously any method which can record individual fibre responses will allow a much better estimate of a mean value to be made than a method which records the mass response of a fraction of fibres selected in a non-random manner: estimates of electrical changes will be less affected by variability and more likely to detect small differences than mechanical responses.

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