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INFLUENCE OF ACTIVITY ON THE PASSIVE ELECTRICAL PROPERTIES OF DENERVATED SOLEUS MUSCLE FIBRES IN THE RAT

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SUMMARY

1. The technique of direct electrical stimulation of denervated muscle was used to study the role of muscle activity *per se* in controlling the passive electrical properties of muscle fibres.

2. Specific membrane resistance and capacitance of the denervated and the denervated-stimulated muscle fibres were measured by a sine-wave technique at frequencies between 5 and 240 Hz. The parameter values were constant at low frequencies up to a variable transition frequency and declined rapidly at higher frequencies.

3. Following denervation the low-frequency value of specific membrane resistance increased (2291 Ω cm^2 for 19-day denervated fibres *vs.* 766 Ω cm^2 for innervated fibres), the specific membrane capacitance declined (2·7 μF/cm^2 *vs.* 3·6 μF/cm^2) and the transition frequency shifted towards lower frequencies. The specific internal resistance was higher in denervated fibres (301 Ω cm for 19-day denervated fibres *vs.* 240 Ω cm in innervated fibres) apart from a transient decline after 5 days of denervation (164 Ω cm).

4. Direct electrical stimulation for 2 weeks beginning on the 5th day after denervation restored all parameters listed above to their original values before denervation.

5. Stimulation arrested in most cases further atrophy from the time of stimulation but did not restore normal fibre size.

INTRODUCTION

Loss of innervation leads to a number of changes in mammalian skeletal muscle fibres. After a few days of denervation the fibres are sensitive to acetylcholine (ACh) over their entire length (Axelsson &

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Thesleff, 1959), and may be innervated by a foreign nerve anywhere on their surface (Elsberg, 1917; Miledi, 1962; Guth & Zalewski, 1963). The resting membrane potential falls (Albuquerque, Schuh & Kauffman, 1971), the passive electrical parameters of the membrane, specific membrane resistance and capacitance, change (Nicholls, 1956; Albuquerque & McIsaac, 1970) and the fibres become atrophic (Tower, 1935).

The reason why denervation initiates these changes in the non-junctional part of the fibres is not well understood. They might be caused by the lack of some neurotrophic influence normally released from the nerve terminals (Miledi, 1960; Emmelin & Malm, 1965; Guth, 1968). Alternatively, the inactivity of the denervated muscle could be responsible. It has recently been shown that both extrajunctional ACh sensitivity (Lømo & Rosenthal, 1972) and innervation by a foreign nerve (Jansen, Lømo, Nicolaysen & Westgaard, 1973) may be largely prevented by prolonged direct stimulation of the denervated muscle. This indicates that activity plays an important role in the control of these properties of the membrane.

The main purpose of this work has been to study the effect of direct stimulation on resting membrane potential and passive electrical properties of the denervated membrane in order to determine whether these properties can become normal in the absence of neural influences.

METHODS

Soleus muscles of rats weighing 200–250 g were denervated under ether anaesthesia by resecting the sciatic nerve bilaterally. Five days later stimulating electrodes were implanted under barbiturate anaesthesia, leaving the opposite soleus as a denervated control. A few rats were denervated unilaterally to compare the membrane characteristics of denervated and normal muscles.

Chronic stimulation. The stimulating electrodes (*ca.* 2 × 10 mm) were cut from a platinum sheet, soldered to hearing-aid wires and covered with inert silicon rubber. A small opening (*ca.* 1 × 8 mm) was later cut in the insulating rubber. One electrode was implanted on each side of the soleus in adjacent leg muscles. The muscle was stimulated continuously with trains of stimuli at 10 Hz. Each train lasted 8 sec and was repeated every 12 sec (mean frequency 6·7 Hz). Stimulus polarity (square waves) was reversed between each train to reduce polarization of the electrodes. Stimulus duration was 2–2·5 msec and intensity 2–3 times threshold, judged by palpating the contracting muscles.

Acute experiments. After 1 or 2 weeks of stimulation the soleus muscles were removed from both legs and pinned out on a perforated wax plate which was kept in a Perspex bath perfused with oxygenated mammalian Ringer solution at room temperature (20–24° C). Mainly surface fibres were studied, but some experiments were also done on deeper lying fibres exposed by removing superficial tissue. Before any electrical measurements were attempted a 3% aqueous solution of Pontamine Sky Blue was injected by pressure into three to six fibres at random. The dye injection often caused the stained fibres to swell or shrink and experiments were therefore performed on neighbouring fibres. These could be readily identified

later in cross-sections of the muscle by their position relative to the stained fibres. Pl. 1 shows examples of such stained surface fibres with arrows indicating the fibres examined electrophysiologically.

Each fibre was penetrated by two micropipettes filled with potassium acetate (20–40 MΩ). One electrode recorded the membrane potential changes produced by sinusoidal current from the other electrode (Falk & Fatt, 1964). A grounded screen was positioned between the two electrodes to prevent capacitive coupling through the air. To avoid a non-linear voltage response peak-to-peak polarization at the site of current injection never exceeded 8 mV. An on-line computer (NORD-1) averaged consecutive (usually 100) current and voltage oscillations, determined the best fit of the averaged responses to a theoretical sine wave of the same frequency and calculated the amplitude and phase lag of the transfer impedance, i.e. the voltage-to-current ratio at a given electrode separation. In each fibre the transfer impedance was usually measured 4 times at different electrode separations.

Preliminary experiments showed that it was difficult to obtain stable conditions in standard mammalian Ringer solution (see *Sources of errors* in this section). All results reported here were therefore obtained in solutions containing a higher concentration of calcium (5 mM *vs.* 2 mM) and magnesium (5 mM *vs.* 1 mM) than normal which improved the stability of the electrical recordings (Eisenberg, Howell & Vaughan, 1971; DeMello, 1973). Control experiments on innervated muscles in normal Ringer solution showed that this modification had no significant effect on the electrical parameters of the muscle fibre.

The Ringer solution contained (mM): NaCl, 137; KCl, 4; $CaCl_2$, 5; $MgCl_2$, 5; KH_2PO_4, 1; $NaHCO_3$, 12; D-glucose, 10. The pH was kept at 7·0–7·2 by bubbling a gas mixture of 95 % O_2 and 5 % CO_2 through the solution.

Histology. At the end of each experiment the muscles were frozen in liquid N_2 whilst pinned out on the wax plate. In this way a minimum of dislocation in the shape and position of the individual fibres was ensured. A transverse segment containing the stained regions of the fibres was cut from the frozen muscle and mounted vertically on a cryostat block. Twenty μm sections were cut and mounted on standard slides with a water-soluble mounting medium (Aquamount). The unfixed and unstained sections were photographed through a microscope together with a micrometer scale. Mean values of fibre area and circumference in each fibre were obtained from measurements of five sections over a distance of 500 μm. Segments of representative cross-sections with one or two dye-injected surface fibres are shown in Pl. 1.

Calculation of electrical parameters. The input impedance ($R_{\text{in, a.c.}}$) was estimated by extrapolating in a semilogarithmic plot the transfer impedance to zero electrode separation (Text-fig. 3). The a.c. length constant ($\lambda_{\text{a.c.}}$) was then obtained from the slope of the straight line. By using the definition of input resistance ($R_{\text{in, d.c.}}$) and d.c. length constant ($\lambda_{\text{d.c.}}$) in terms of internal resistance (r_i) and membrane resistance (r_m) per unit length fibre (eqns. (1) and (2)), r_i (assumed frequency independent) may be determined if one knows $R_{\text{in, d.c.}}$ and $\lambda_{\text{d.c.}}$ (from eqn. (4) or Fatt & Katz, 1951):

$$R_{\text{in, d.c.}} = \tfrac{1}{2}\sqrt{(r_i\, r_m)}, \tag{1}$$

$$\lambda_{\text{d.c.}} = \sqrt{(r_m/r_i)}. \tag{2}$$

Hence,

$$r_i = \frac{2R_{\text{in, d.c.}}}{\lambda_{\text{d.c.}}}. \tag{3}$$

The input impedance and length constant were always measured at 12 Hz and as a first approximation, $R_{\text{in, a.c.}}$ and $\lambda_{\text{a.c.}}$ at this frequency were substituted for $R_{\text{in, d.c.}}$ and $\lambda_{\text{d.c.}}$ in eqn. (3) and the approximate value of r_i used in eqn. (4).

Membrane resistance (r_m) and capacitance (c_m) per unit fibre length at each frequency were calculated by an iterative fitting programme (Nelder & Mead, 1965), comparing the experimental input data with the theoretical values given by the formula (a modification of Falk & Fatt, 1964; eqn. (3)):

$$\hat{Z} = \frac{\sqrt{(r_m r_i)}}{2\sqrt{[(\omega r_m c_m)^2 + 1]}} (m - jn) \exp\{-jn\sqrt{(r_i/r_m)}x\} \exp\{-m\sqrt{(r_i/r_m)}x\}, \quad (4)$$

where $\hat{Z}$ = transfer impedance (a complex number), x = distance between current injecting and recording electrode:

$$m = \sqrt{\frac{\sqrt{[(\omega r_m c_m)^2 + 1]} + 1}{2}}, \quad (5)$$

$$n = \sqrt{\frac{\sqrt{[(\omega r_m c_m)^2 + 1]} - 1}{2}}. \quad (6)$$

Eqn. (4) applies to a simple core-conductor cable with one membrane time constant. By applying eqn. (4) to muscle fibres where more complex membrane models are required (Falk & Fatt, 1964; Adrian, Chandler & Hodgkin, 1969; Nicolaysen, 1975), r_m and c_m will by this procedure be frequency-dependent.

The validity of the substitution of $R_{in,a.c}$ and $\lambda_{a.c.}$ for $R_{in,d.c.}$ and $\lambda_{d.c.}$ in eqn. (3) may be examined through the relationship between $R_{in,a.c.}$ and $R_{in,d.c.}$ and between $\lambda_{a.c.}$ and $\lambda_{d.c.}$:

$$\lambda_{d.c.} = \lambda_{a.c.} m, \quad (7)$$

$$R_{in,d.c.} = [(\omega r_m c_m)^2 + 1]^{\frac{1}{2}} R_{in,a.c.} \quad (8)$$

These equations are easily derived from eqn. (4).

If the difference between $\lambda_{d.c.}$ and $\lambda_{a.c.}$ or between $R_{in,d.c.}$ and $R_{in,a.c.}$ exceeded 5%, a new estimate of r_i was made using the values of $\lambda_{d.c.}$ and $R_{in,d.c.}$ obtained from eqns. (7) and (8). The values of r_m and c_m were recalculated using the new estimate of r_i. $\lambda_{d.c.}$ and $R_{in,d.c.}$ were then calculated again, but the second estimates of these parameters were always close enough to the first values so a new estimate of r_i was not needed. After the final value of r_i was estimated, eqn. (4) could be used for calculating r_m and c_m from measurements at higher frequencies.

Finally specific internal resistance (R_i), specific membrane resistance (R_m) and specific membrane capacitance (C_m) were calculated by the equations

$$R_i = r_i A, \quad (9)$$

$$R_m = r_m S, \quad (10)$$

$$C_m = c_m / S, \quad (11)$$

where A is the area and S the circumference of the fibre.

Sources of errors. Leakage around the electrodes could potentially have been the largest source of error, as was suggested by an increase in transfer impedance of usually 10–50% during the first 5–30 min after each micro-electrode penetration. The largest increases were seen in muscle fibres denervated 12 and 19 days where the transfer impedance sometimes increased as much as 200%. The impedance was considered stable when three consecutive measurements, usually obtained within 5 min, showed less than 5% variation. In most fibres all the penetrations stabilized by this criteria. Moreover, the relationship between transfer impedance and electrode separation was linear when plotted using semilogarithmic co-ordinates. Leakage was therefore unlikely to have been a serious error in the measurements from these fibres. In normal and stimulated muscle fibres stable conditions usually occurred within a few minutes. In denervated fibres on the other hand, it was

sometimes necessary to postpone measurements until 2 hr after the penetration, especially when a full frequency analysis was attempted.

The calibration of current and voltage responses as well as the compensation for stray capacitance were checked against a calibrated RC-circuit several times during each experiment. Deviations of more than 2 % in amplitude and 0·5 degrees in phase lag (1 degree for frequencies over 100 Hz) were not accepted. After completion of a full frequency analysis the voltage recording electrode was withdrawn from the fibre and the residual voltage response was recorded. This response was usually less than 5 % of the voltage recorded in the fibre at the highest frequencies used (200–240 Hz) and less than 2 % at low frequencies, and no corrections were made. The linearity of the current and voltage responses were ensured by using the same procedure as used by Nicolaysen (1975).

Fibre area varied sometimes as much as 20 % over a distance of 500 μm in the same fibre. In contrast, isolated toad muscle fibres have nearly the same area along their entire length although their shape may change (Dulhunty & Gage, 1973). The close packing of the rat soleus muscle fibres may result in these variations which were especially pronounced near the local swellings and constrictions of neighbouring stained fibres. The histological procedures employed here may also contribute to this variability although the absence of gaps between individual fibres indicates that little shrinkage of the tissue occurred (Pl. 1). The irregular shape of the intact fibres makes it clear that estimates of area and circumference based on optical measurements of surface fibres seen through a dissection microscope would be subject to large errors.

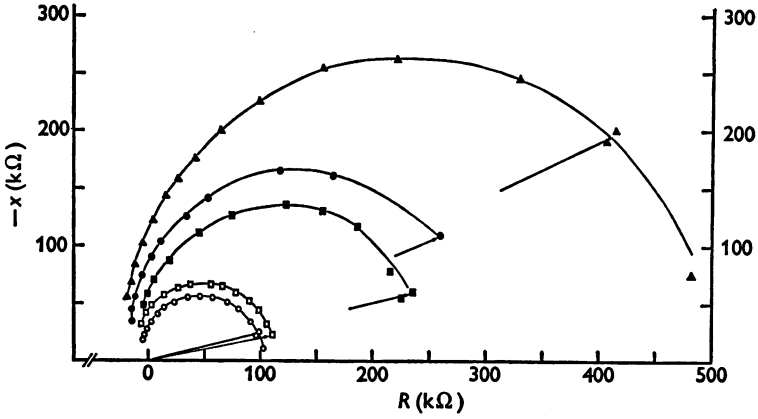
The histological sections were never cut at an angle of more than 15° from the transverse plane. At most this would increase fibre area and circumference about 4 and 2 % respectively, and does not introduce any significant error.

RESULTS

Frequency analysis of membrane parameters. Because of the tubular system, the muscle membrane has to be represented by a more complicated model than the standard core-conductor cable (Falk & Fatt, 1964; Adrian *et al.* 1969; Nicolaysen, 1975). The specific membrane resistance and capacitance will therefore vary with frequency and in initial experiments the frequency dependence of these parameters was studied. In Text-fig. 1 plots of impedance measurements with electrode separation 700–750 μm are given for frequencies between 5 and 240 Hz for representative fibres from muscles that were either normal, denervated (5, 12 and 19 days) or denervated 19 days and stimulated 14 days. The long distance between the electrodes was chosen to avoid non-linearities which may arise with small electrode separation (Falk & Fatt, 1964; appendix A). After 19 days denervation the transfer impedance was about five times larger than normal and the phase lag increased 100 %. In contrast, the values for the 19-day denervated, 14-day stimulated fibre were within the normal range.

In Text-fig. 2 the specific membrane resistance (A) and capacitance (B) are plotted as functions of frequency. Each value was calculated as

outlined in Methods from the transfer impedance data in Text-fig. 1. For any given fibre both specific membrane resistance and capacitance were frequency-independent at low frequencies. The low-frequency specific membrane resistance increased progressively with increasing time of denervation and after 19 days of denervation was more than double the value of the normal fibre. Stimulation reversed the effect of denervation on the specific membrane resistance and in the 19-day denervated,



Text-fig. 1. Reactance–resistance (X–R) plots of transfer impedances at different frequencies of soleus fibres which were either innervated (○), denervated 5 (■), 12 (●) and 19 days (▲) or denervated (19 days) and stimulated (14 days) (□). Frequency range 5–240 Hz. Electrode separation 700–750 μm. Arrows indicate the impedance vector at 12 Hz. Two data points are plotted at 12 Hz for the innervated and the 5- and 19-day denervated muscles. They indicate the rise in impedance at 12 Hz while the impedance at other frequencies were obtained.

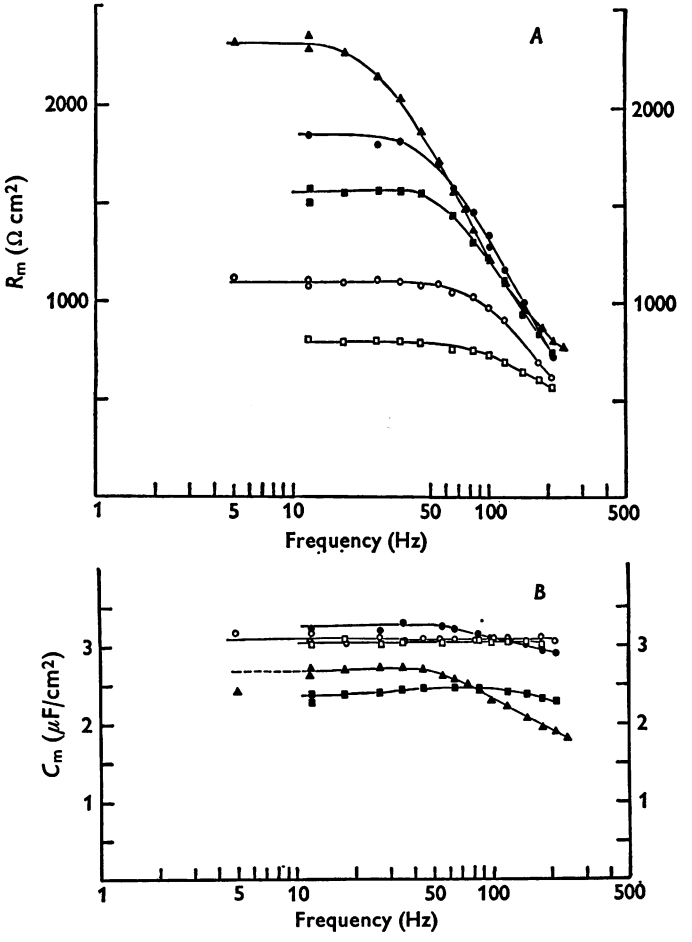
14-day stimulated fibre the specific membrane resistance was even lower than in the normal fibre (Text-fig. 2A). For the fibres illustrated in Text-figs. 1 and 2 there was no clear effect of either denervation or chronic stimulation on low-frequency specific membrane capacitance. However, it will be shown later that denervation led to a statistically significant fall in the specific membrane capacitance which was reversed by stimulation.

At higher frequencies both specific membrane resistance and capacitance declined. For the same fibre the decline in specific membrane resistance began at a lower frequency than the decline in specific membrane capacitance. Denervation led to a progressive fall in this transition frequency and chronic stimulation appeared to normalize the denervated membrane also in this respect.

It is evident from Text-fig. 2 that a frequency of 12 Hz, which was

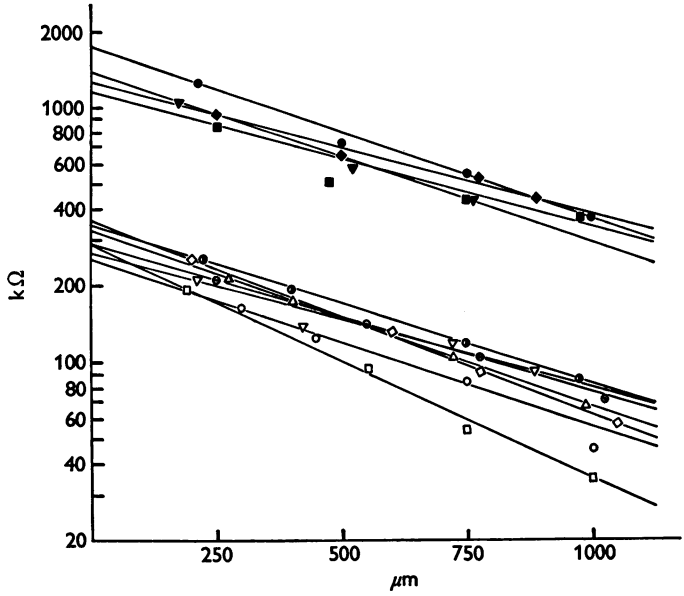
used exclusively in most fibres, measures the low-frequency value of specific membrane resistance and capacitance in all experimental groups. This frequency also gives a maximal capacitive component of the impedance vector and therefore permits the determination of specific membrane capacitance with least uncertainty.

Input impedance and length constant. Text-fig. 3 indicates the input impedance and length constant of individual fibres from the soleus muscles of a rat bilaterally denervated for 19 days and unilaterally



Text-fig. 2. Specific membrane resistance (*A*) and capacitance (*B*) calculated from the transfer impedance data in Text-fig. 1. Symbols are the same as in Text-fig. 1 and represent the same fibres. The decline in C_m (19 days denervated) at 5 Hz might be caused by an incorrect calibration of the circuit compensating for stray capacitances. The effect of such an error would be enhanced by the small phase lag at this frequency.

stimulated the last 14 days. The length of the transfer impedance vector at 12 Hz (Text-fig. 1) is plotted as a function of the distance between current and recording electrodes. Extrapolation of the straight line to zero electrode separation gives the input impedance at that frequency and the a.c. length constant is estimated from the slope of the line. From these a.c. values the corresponding d.c. values may be calculated as described under Methods.



Text-fig. 3. Semilogarithmic plots of transfer impedance *vs.* electrode separation for individual fibres from a 19-day denervated (filled symbols) and a 19-day denervated/14-day stimulated muscle (open and semifilled symbols). Both muscles are from the same rat. The straight lines were fitted by eye, ignoring in some fibres (●, ■, ○, ▽) an occasional low value presumably caused by the unstable penetrations at these electrode positions.

The stimulated fibres (open and semi-filled symbols) and the denervated controls (filled symbols) have separated into two groups with similar length constants but very different input impedance, whereas there is little variation between individual fibres in the same muscle. For these stimulated muscle fibres the input impedances and length constants were indistinguishable from those of normal fibres.

The complete data from this type of analysis are given in Table 1. There was a progressive increase in input resistance as the time of denervation was prolonged. After 19 days the mean input resistance was 5 times that of normal fibres. Stimulation had a pronounced effect

on this process. Some stimulated fibres had input resistance within normal range (Text-fig. 1). In other more atrophic fibres stimulation caused a less dramatic reduction in input resistance but the values were almost always less than in 5-day denervated fibres. After prolonged periods of denervation the variation in input resistance between individual fibres in the same experimental group was more pronounced. Some of this variability reflects the unstable penetrations that were more common in denervated fibres. However, most values were obtained after the transfer impedance had become stable, and there may well be real variations between individual fibres. Denervation alone or in combination with direct stimulation had little effect on the length constant (Table 1), apart from a small increase after 5 days of denervation.

Specific electrical parameters. Specific internal resistance, specific membrane resistance and specific membrane capacitance were calculated as outlined in Methods from the experimental data already presented. The results are presented in Table 1 as mean $\pm$ S.E. of mean with the number of muscles and muscle fibres in each experimental group indicated by the two numbers in parentheses in the first column. The mean specific internal resistance is $240 \pm 11\ \Omega$ cm (mean $\pm$ S.E. of mean, $n = 32$) in normal fibres. Small but statistically significant changes occurred after denervation. After 5 days the mean specific internal resistance was somewhat reduced ($164 \pm 8\ \Omega$ cm, $n = 13$). Thereafter it increased to $262 \pm 17\ \Omega$ cm ($n = 28$) and $301 \pm 25\ \Omega$ cm ($n = 18$) after 12 and 19 days of denervation. The specific internal resistance of the 14-day stimulated, 19-day denervated fibres ($237 \pm 19\ \Omega$ cm, $n = 22$) was very close to the normal value.

The changes in specific membrane resistance were very pronounced (Table 1), increasing from $766\ \Omega\ \text{cm}^2$ in normal fibres to 1409, 2369 and $2291\ \Omega\ \text{cm}^2$ for 5-, 12- and 19-day denervated fibres. Direct stimulation had a profound effect on these changes caused by denervation. After 7 days of stimulation (12 days of denervation) the specific membrane resistance was still higher than normal, but lower than in 5-day denervated muscles. Longer stimulation caused further reductions in specific membrane resistance. After 14 days of stimulation the fibres were indistinguishable from normal.

A small but statistically significant reduction in specific membrane capacitance occurred after denervation (Table 1), and stimulation normalized the membrane also in respect to this parameter.

Membrane potential. Denervation is known to lower resting membrane potential in rats (Albuquerque & McIsaac, 1970; Albuquerque *et al.* 1971). In the present experiments the membrane potential fell from $-72{\cdot}8 \pm 1{\cdot}4$ mV (mean $\pm$ S.E. of mean, $n = 32$) for normal fibres to

TABLE 1. Electrical membrane parameters of innervated, denervated and denervated-stimulated muscle fibres

Experimental group	RMP (mV)	R_{in} (kΩ)	$\lambda_{d.c.}$ (μm)	A (μm²)	R_i (Ω cm)	R_m (Ω cm²)	C_m (μF/cm²)
Innervated (7, 32)	$-72{\cdot}8 \pm 1{\cdot}4$	262 ± 10	670 ± 16	3078 ± 141	240 ± 11	766 ± 24	$3{\cdot}6 \pm 0{\cdot}07$
5-day denervated (2, 13)	$-67{\cdot}0 \pm 1{\cdot}1$ ($P < 0{\cdot}01$)	422 ± 17 ($P < 0{\cdot}001$)	897 ± 43 ($P < 0{\cdot}001$)	1752 ± 115 ($P < 0{\cdot}001$)	164 ± 8 ($P < 0{\cdot}001$)	1409 ± 79 ($P < 0{\cdot}001$)	$3{\cdot}3 \pm 0{\cdot}14$ ($P < 0{\cdot}1$)
12-day denervated (6, 28)	$-63{\cdot}8 \pm 1{\cdot}0$ ($P < 0{\cdot}001$)	866 ± 36 ($P < 0{\cdot}001$)	844 ± 37 ($P < 0{\cdot}001$)	1240 ± 48 ($P < 0{\cdot}001$)	262 ± 17 (n.s.)	2369 ± 156 ($P < 0{\cdot}001$)	$2{\cdot}8 \pm 0{\cdot}09$ ($P < 0{\cdot}001$)
19-day denervated (4, 18)	$-65{\cdot}7 \pm 1{\cdot}2$ ($P < 0{\cdot}001$)	1249 ± 48 ($P < 0{\cdot}001$)	717 ± 23 (n.s.)	833 ± 48 ($P < 0{\cdot}001$)	301 ± 25 ($P < 0{\cdot}05$)	2291 ± 109 ($P < 0{\cdot}001$)	$2{\cdot}7 \pm 0{\cdot}09$ ($P < 0{\cdot}001$)
12-day denervated, 7-day stimulated (3, 17)	$-71{\cdot}4 \pm 1{\cdot}3$ (n.s.)	428 ± 19 ($P < 0{\cdot}001$)	675 ± 19 (n.s.)	2118 ± 103 ($P < 0{\cdot}001$)	273 ± 17 (n.s.)	1126 ± 51 ($P < 0{\cdot}001$)	$3{\cdot}5 \pm 0{\cdot}19$ (n.s.)
19-day denervated, 14-day stimulated (3, 22)	$-78{\cdot}8 \pm 1{\cdot}2$ ($P < 0{\cdot}01$)	413 ± 25 ($P < 0{\cdot}001$)	556 ± 20 ($P < 0{\cdot}001$)	1646 ± 125 ($P < 0{\cdot}001$)	237 ± 19 (n.s.)	760 ± 38 (n.s.)	$3{\cdot}5 \pm 0{\cdot}09$ (n.s.)

Mean ± S.E. of mean in each experimental group for the following parameters: RMP, resting membrane potential; R_{in}, input resistance; $\lambda_{d.c.}$, d.c. length constant; A, cross-sectional fibre area; R_i, specific internal resistance; R_m, specific membrane resistance; C_m, specific membrane capacitance. The two numbers in parentheses in the first column indicate number of muscles and total number of muscle fibres examined in each experimental group. P indicates the probability that the mean of that experimental group is similar to the mean of innervated fibres for the same parameter. n.s., not significant, i.e. $P > 0{\cdot}1$. A modified t test is used where the data are assumed to have normal distribution but variable S.D. (Brownlee, 1960).

$-67{\cdot}0 \pm 1{\cdot}1$ mV ($n = 13$) and $-63{\cdot}8 \pm 1{\cdot}0$ mV ($n = 28$) for fibres denervated 5 and 12 days (Table 1). Direct stimulation not only arrested the fall in membrane potential after the 5th day when stimulation began, but eventually produced larger than normal membrane potentials ($-78{\cdot}8$ mV *vs.* $-72{\cdot}8$ mV for 14 days stimulated fibres). The mean membrane potential was still lower than normal after 7 days of stimulation ($-71{\cdot}4$ mV *vs.* $-72{\cdot}8$ mV). A similar effect of long term stimulation on the membrane potential of denervated soleus fibres has been reported by Lømo (1974).

Atrophy. One conspicuous result of denervation is the rapid atrophy of the muscle fibres. In Pl. 1B, the cross-sectional areas of 19-day denervated soleus fibres are much less than those of normal soleus fibres from a rat of similar weight (Pl. 1A). Table 1 shows that mean fibre areas decreases gradually during the first 19 days after denervation. This progressive atrophy is to a large extent arrested by direct stimulation. After 7 and 14 days of stimulation, i.e. 12 and 19 days after denervation, the mean fibre area is approximately the same as after 5 days of denervation when stimulation began (Table 1). This effect is illustrated by the denervated–stimulated fibres in Pl. 1C which are larger than the denervated fibres from the opposite soleus in the same rat (Pl. 1B), but not as large as the normal fibres (Pl. 1A). It appears that the direct stimulation in these experiments had a clear effect on denervation atrophy for most fibres, but it did not restore normal size. A few stimulated fibres had normal membrane characteristics even though the fibre area was similar to that of contralateral denervated fibres.

DISCUSSION

Passive electrical parameters of the denervated muscle

Specific membrane resistance. The specific membrane resistance is considerably increased after denervation in agreement with other reports (Nicholls, 1956; Albuquerque & Thesleff, 1968; Albuquerque & McIsaac, 1970). The absolute values, however, are difierent in different studies; Albuquerque & McIsaac (1970) found a mean specific membrane resistance of 515 $\Omega\,cm^2$ in normal rat soleus fibres and 1210 $\Omega\,cm^2$ in 19-day denervated fibres whereas in the present study the corresponding values were 766 and 2291 $\Omega\,cm^2$. The higher values seen in these experiments may be related to the use of increased concentrations of calcium and magnesium in the perfusion fluid and to the postponement of measurements until the input impedance became stable. These precautions probably caused the membrane to seal around the electrodes (Eisenberg *et al.* 1971; De Mello, 1973).

The specific membrane resistance (and capacitance) showed a clear-cut frequency dependence which differed in normal and denervated fibres. Although such changes can be predicted by changing the tubular properties in the models of the muscle membrane of Falk & Fatt (1964) or Nicolaysen (1975), in the absence of specific information on the tubular parameters in normal and denervated muscle fibres it is of little value to speculate on the mechanisms behind the change.

Specific membrane capacitance. In this study denervation also led to a gradual decrease in specific membrane capacitance. Specific membrane capacitance is proportional to fibre diameter, provided tubular membrane area per unit cross-sectional area is constant and current spreads to the middle of the fibre (Hodgkin & Nakajima, 1972). Denervated fibres become much smaller than normal and a reduction in specific membrane capacitance might therefore be expected, but denervation also leads to an overdevelopment of sarcotubular structures (Pellegrino & Franzini, 1963) which has been reported to increase the sarcotubular surface area relative to fibre volume by as much as 200–400 % (Engel & Stonnington, 1974). The actual change of the transverse tubular system and the spread of current along the tubuli is not known, however, and it is therefore difficult to predict how the overdeveloped sarcoplasmic reticulum would contribute to the specific membrane capacitance.

Previous studies have shown a small (Albuquerque & McIsaac, 1970) or a large (Albuquerque & Thesleff, 1968) increase in specific membrane capacitance. It is difficult to attribute this discrepancy to any particular cause as several factors affect the estimate of specific membrane capacitance, including (i) the method used for measuring the membrane time constant (Adrian & Almers, 1973), (ii) the value of the specific internal resistance and (iii) the estimate of fibre circumference.

Specific internal resistance. The present study presents clear evidence that denervation affects specific internal resistance. A transient increase in potassium concentration of the rat soleus following denervation has been observed (Drahota, 1960) and this might contribute to the reduction in specific internal resistance after 5 days of denervation. The mechanism behind the later increase in this parameter is unclear, but it might be related to the change in the relative proportions of myofibrils, sarcoplasm and sarcoplasmic reticulum (Pellegrino & Franzini, 1963; Engel & Stonnington, 1974). The mean value of specific internal resistance for innervated fibres, 240 Ω cm, is higher than for frog muscle fibres (170 Ω cm, Hodgkin & Nakajima, 1972).

Effects of electrical stimulation on denervated muscle

Atrophy. Stimulation arrested further atrophy in most cases, but did not restore normal fibre size in muscles denervated for 5 days before stimulation began. A few fibres with normal specific membrane parameters were nearly as atrophic as contralateral denervated muscle fibres. This indicates a differential effect of stimulation on fibre size and passive electrical membrane characteristics. Fibre size is influenced not only by activity (Tower, 1937) but also by the load on the muscle (Goldberg, 1967; Gutmann, Hájek & Horský, 1969). Tenotomized muscles are very atrophic despite intact innervation, near normal activity (Nelson, 1969) and normal sensitivity to acetylcholine (Lømo & Rosenthal, 1972).

A likely explanation for the persistent and variable atrophy is therefore the abnormal load on the soleus resulting both from denervation of antagonists and synergists and from a variable co-contraction of the faster gastrocnemius and the other flexors of the leg.

Electrical characteristics. In contrast to the incomplete effects of stimulation on fibre atrophy, stimulation for 2 weeks restored normal passive electrical characteristics in the denervated membrane and increased resting membrane potential above normal. These effects of stimulation developed slowly and were still incomplete after one week of stimulation. Thus, direct stimulation of the muscle appears to compensate fully for the absence of neural influences with respect to these membrane properties.

The amount and pattern of activity used in this study (10 Hz in 8 sec with 4 sec pause between the trains; mean frequency 6·7 Hz) is a reasonable approximation to that in a normally innervated rat soleus muscle where the soleus motoneurones appear to fire tonically at 5–20 Hz (Fischbach & Robbins, 1969).

As activity imposed by electrical stimulation appears to normalize the electrical membrane characteristics, it is interesting that the denervated membrane is abnormal in spite of fibrillatory activity. A possible explanation is that the fibrillatory activity, although comparable to the induced activity for short periods (Purves & Sakmann, 1974*b*), has a pattern which together with a low mean frequency is unable to maintain normal membrane properties. A similar explanation is suggested for extrajunctional ACh-sensitivity in the cultured diaphragm where fibrillation is self-regulating and shuts itself off before it has any significant effect on ACh-sensitivity (Purves & Sakmann, 1974*a*).

The results of the present study demonstrate that the effect of denervation on specific membrane resistance and capacitance is satisfactorily explained as a direct result of muscle inactivity. The same conclusion

has been reached with regard to extrajunctional ACh-sensitivity (Lømo & Rosenthal, 1972), and innervation of muscle by a foreign nerve (Jansen *et al.* 1973). However, none of these results excludes the possibility that neurotrophic factors may contribute to this control.

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REFERENCES

ADRIAN, R. H. & ALMERS, W. (1973). Measurement of membrane capacity in skeletal muscle. *Nature, New Biol.* **242**, 62–64.

ADRIAN, R. H., CHANDLER, W. K. & HODGKIN, A. L. (1969). The kinetics of mechanical activation in frog muscle. *J. Physiol.* **204**, 207–230.

ALBUQUERQUE, E. X. & MCISAAC, R. J. (1970). Fast and slow mammalian muscles after denervation. *Expl Neurol.* **26**, 183–202.

ALBUQUERQUE, E. X., SCHUH, F. T. & KAUFFMAN, F. C. (1971). Early membrane depolarization of the fast mammalian muscle after denervation. *Pflügers Arch. ges. Physiol.* **328**, 36–50.

ALBUQUERQUE, E. X. & THESLEFF, S. (1968). A comparative study of membrane properties of innervated and chronically denervated fast and slow skeletal muscles of the rat. *Acta physiol. scand.* **73**, 471–480.

AXELSSON, J. & THESLEFF, S. (1959). A study of supersensitivity in denervated mammalian skeletal muscle. *J. Physiol.* **147**, 178–193.

BROWNLEE, K. A. (1960). *Statistical Theory and Methodology in Science and Engineering*, 1st edn., pp. 235–239. New York, London: Wiley.

DE MELLO, W. C. (1973). Membrane sealing in frog skeletal-muscle fibers. *Proc. natn. Acad. Sci. U.S.A.* **70**, 982–984.

DRAHOTA, Z. (1960). Changes in the potassium content of various muscles immediately after denervation. *Physiologia bohemoslov.* **9**, 1–4.

DULHUNTY, A. F. & GAGE, P. W. (1973). Electrical properties of toad sartorius muscle fibres in summer and winter. *J. Physiol.* **230**, 619–641.

EISENBERG, R. S., HOWELL, J. N. & VAUGHAN, P. C. (1971). The maintenance of resting potentials in glycerol-treated muscle fibres. *J. Physiol.* **215**, 95–102.

ELSBERG, C. A. (1917). Experiments on motor nerve regeneration and the direct neurotization of paralyzed muscles by their own and by foreign nerves. *Science, N.Y.* **45**, 318–320.

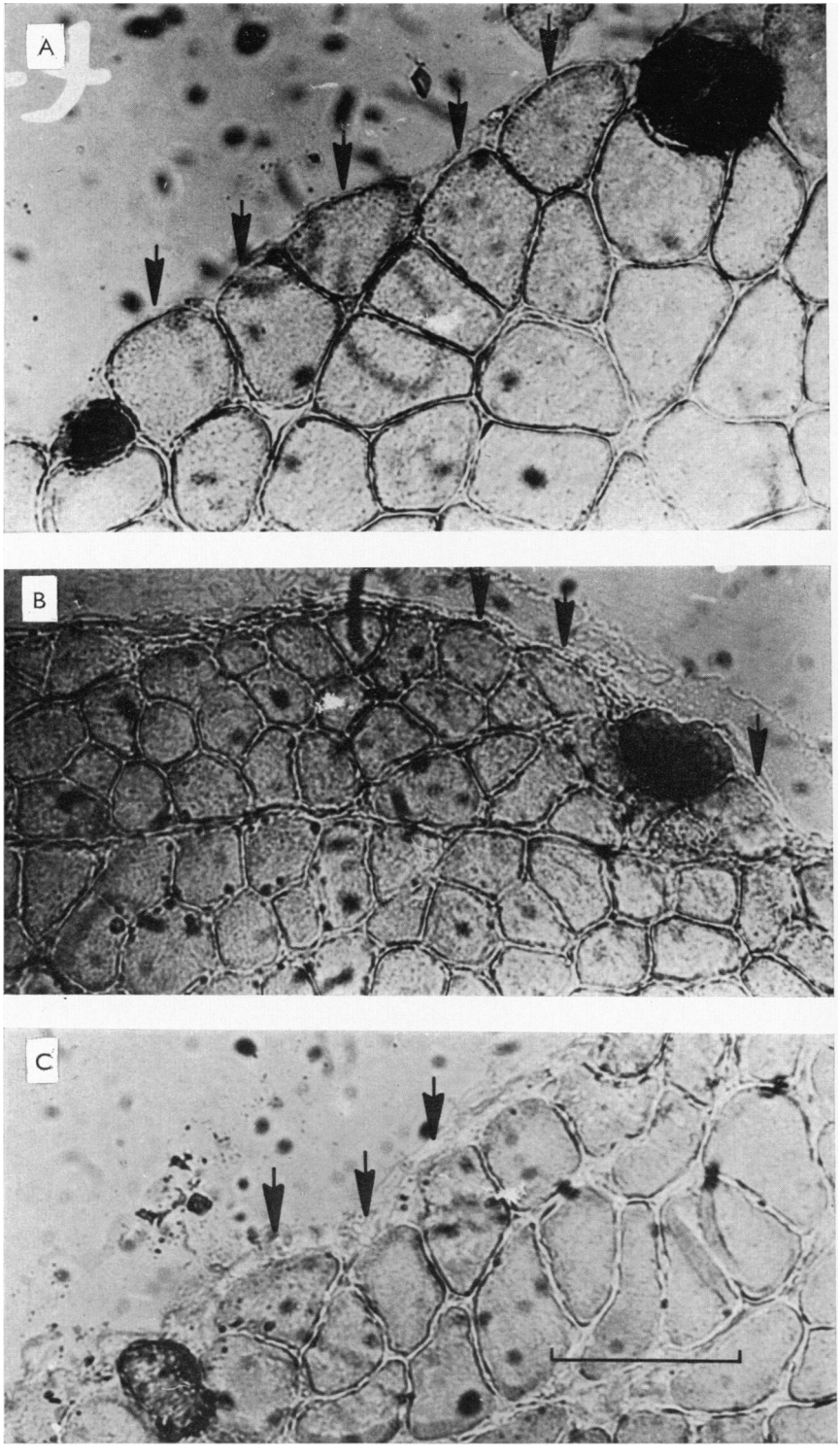
EMMELIN, N. & MALM, L. (1965). Development of supersensitivity as dependent on the length of degenerating nerve fibres. *Q. Jl exp. Physiol.* **50**, 142–145.

ENGEL, A. G. & STONNINGTON, H. H. (1974). Morphological effects of denervation of muscle. A quantitative ultrastructural study. *Ann. N.Y. Acad. Sci.* **228**, 68–88.

FALK, G. & FATT, P. (1964). Linear electrical properties of striated muscle fibres observed with intracellular electrodes. *Proc. R. Soc.* B. **160**, 69–123.

FATT, P. & KATZ, B. (1951). An analysis of the end-plate potential recorded with an intra-cellular electrode. *J. Physiol.* **115**, 320–370.

FISCHBACH, G. D. & ROBBINS, N. (1969). Changes in contractile properties of disused soleus muscles. *J. Physiol.* **201**, 305–320.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C

GOLDBERG, A. L. (1967). Work-induced growth of skeletal muscle in normal and hypophysectomized rats. *Am. J. Physiol.* **213**, 1193–1198.

GUTH, L. (1968). 'Trophic' influences of nerve on muscle. *Physiol. Rev.* **48**, 645–687.

GUTH, L. & ZALEWSKI, A. A. (1963). Disposition of cholinesterase following implantation of nerve into innervated and denervated muscle. *Expl Neurol.* **7**, 316–326.

GUTMANN, E., HÁJEK, I. & HORSKÝ, P. (1969). Effect of excessive use on contraction and metabolic properties of cross-striated muscle. *J. Physiol.* **203**, 46–47*P*.

HODGKIN, A. L. & NAKAJIMA, S. (1972). The effect of diameter on the electrical constants of frog skeletal muscle fibres. *J. Physiol.* **221**, 105–120.

JANSEN, J. K. S., LØMO, T., NICOLAYSEN, K. & WESTGAARD, R. H. (1973). Hyperinnervation of skeletal muscle fibres: Dependence on muscle activity. *Science, N.Y.* **181**, 559–561.

LØMO, T. (1974). Neurotrophic control of colchicine effects on muscle? *Nature, Lond.* **249**, 473–474.

LØMO, T. & ROSENTHAL, J. (1972). Control of ACh sensitivity by muscle activity in the rat. *J. Physiol.* **221**, 493–513.

MILEDI, R. (1960). The acetylcholine sensitivity of frog muscle fibres after complete or partial denervation. *J. Physiol.* **151**, 1–23.

MILEDI, R. (1962). Induced innervation of end plate free muscle segments. *Nature, Lond.* **193**, 281–282.

NELDER, J. A. & MEAD, R. (1965). A simplex method for function minimization. *Comput. J.* **7**, 308–313.

NELSON, P. G. (1969). Functional consequences of tenotomy in hind limb muscles of the cat. *J. Physiol.* **201**, 321–333.

NICHOLLS, J. G. (1956). The electrical properties of denervated skeletal muscle. *J. Physiol.* **131**, 1–12.

NICOLAYSEN, K. (1975). The spread of the action potential through the T-system in hagfish twitch muscle fibres. *Acta physiol. scand.* (In the press.)

PELLEGRINO, C. & FRANZINI, C. (1963). An electron microscope study of denervation atrophy in red and white skeletal muscle fibers. *J. Cell Biol.* **17**, 327–349.

PURVES, D. & SAKMANN, B. (1974*a*). The effect of contractile activity on fibrillation and extrajunctional acetylcholine-sensitivity in rat muscle maintained in organ culture. *J. Physiol.* **237**, 157–182.

PURVES, D. & SAKMANN, B. (1974*b*). Membrane properties underlying spontaneous activity of denervated muscle fibres. *J. Physiol.* **239**, 125–153.

TOWER, S. S. (1935). Atrophy and degeneration in skeletal muscle. *Am. J. Anat.* **56**, 1–43.

TOWER, S. S. (1937). Trophic control of non-nervous tissues by the nervous system: a study of muscle and bone innervated from an isolated and quiescent region of spinal cord. *J. comp. Neurol.* **67**, 241–268.

EXPLANATION OF PLATE

Segments of unstained, transverse sections of innervated (A) and 19-day-denervated (B, C) soleus muscles. B and C are from the same rat. In C the muscle was stimulated electrically from day 5 to day 19 after the denervation. Arrows indicate fibres examined electrophysiologically. Bar indicates 100 μm.