A Study of the Reinnervation of Fast and Slow Mammalian Muscles

JOSEPH J. McARDLE and EDSON X. ALBUQUERQUE

From the Department of Pharmacology, Schools of Medicine and Dentistry, State University of New York at Buffalo, Buffalo, New York 14214

ABSTRACT Miniature end plate potential (mepp) frequency in innervated extensor muscle is significantly higher than in soleus muscle. 9 days after nerve crush mepps of low amplitude and prolonged duration reappeared at a frequency of 2% of control and were similar to normal muscles after 35 days. Membrane potential began to increase 9–10 days after nerve crush and at 30 days was similar to controls. The region most sensitive to ACh in denervated and reinnervated muscles was the end plate. Caffeine (20 mM, 23°C) induced contracture in innervated soleus but not in extensor muscles. After denervation the extensor became sensitive to caffeine while the soleus muscles decreased in sensitivity to the drug; 4–5 days after reinnervation the effect of caffeine on these muscles was similar to control. The events during reinnervation are: (a) reappearance of mepps at the same time as end plate potential and muscle twitch; (b) partial restoration of the membrane potential; (c) return of caffeine-induced contracture to normal levels in the soleus and its absence in the extensor muscles; (d) return of membrane resistance to normal values in both muscles at about 25 days; and (e) return of ACh-sensitivity to control levels at about 30 days in both muscles. Although these results suggest that the membrane potential and sarcoplasmic reticulum are under neural influence, it remains to be established whether or not separate neurotrophic factors are involved.

INTRODUCTION

Denervation of the “fast” extensor digitorum longus (extensor) and the “slow” soleus muscles of the rat produces marked alterations in the electrical and chemosensitive properties of the membranes of individual muscle fibers (Albuquerque and Thesleff, 1968; Lømo and Rosenthal, 1972). Within 24 h after section of the nerves at about 1.2 cm from their entrances into the extensor and soleus muscles, membrane depolarization and cessation of spontaneous transmitter release are observed (Albuquerque and McIsaac, 1970). The values of the passive electrical properties of the membranes of these muscle fibers are increased 3–4 days after denervation (Albuquerque et al., 1971; Albuquerque and McIsaac, 1970). 2–3 days after denervation, hypersensitivity to microiontophoretically applied acetylcholine (ACh) appears.
scattered along the entire muscle fiber with regions of highest receptor density at end plate and myotendinous regions (Albuquerque and McIsaac, 1969). Denervation also induces vesiculation of the terminal cisternae and a relative overdevelopment of the sarcoplasmic reticulum in fast and slow muscles (Pellegrino and Franzini, 1963). Simultaneously with these alterations of the sarcoplasmic reticulum, the fast muscles become sensitive to caffeine while in the slow muscles a decrease in caffeine-induced contracture is observed (Gutmann and Sandow, 1965; Miledi and Stefani, 1969; McArdle and Albuquerque, 1970). These contractures induced by caffeine may result from a direct action of the drug on the sarcoplasmic reticulum (Hasselbach, 1964; Weber and Herz, 1968; Warnick et al., 1971).

The first observable effect of muscle denervation produced by either nerve section or application of botulinum toxin was the disappearance of spontaneous miniature end plate potentials (mepps) (Axelsson and Thesleff, 1959; Birks et al., 1960; Stater, 1966; Thesleff, 1960 a) and simultaneous alterations of the postsynaptic membrane, specifically, the appearance of extra-junctional sensitivity to ACh. These observations led to the suggestion that the transmitter might also be the neurotrophic factor (Axelsson and Thesleff, 1959; Thesleff, 1960 b). However, recent studies using silastic cuffs embedded with local anesthetic around the nerves, showed that the frequency of the spontaneous transmitter release remained practically unaltered at a time when extra-junctional ACh-sensitivity in both extensor and soleus muscles reached values similar to those observed under denervation condition (Lømo and Rosenthal, 1972). Furthermore, evidence has been obtained to support the view that substances in addition to the cholinergic transmitter exert a neurotrophic influence upon the muscles (Gutmann and Hník, 1962; Miledi, 1962; Gutmann, 1969; Albuquerque and McIsaac, 1970; Albuquerque et al., 1971; Harris and Miledi, 1971).

The experiments reported in the present study were undertaken to analyze quantitatively the time-course of muscle reinnervation in the extensor and soleus muscles of the rat and to correlate further electrophysiological and pharmacological characteristics of these muscles with the influence of some putative neurotrophic factors. Short reports of these results have been presented in abstract form (McArdle and Albuquerque, 1970, 1971).

M E T H O D S

Preparation

All experiments were performed in vitro on the extensor and soleus muscles of male Wistar rats weighing 300–400 g. The rats were anesthetized with ether, and the denervations were performed by either of two methods. In one series of experiments, a segment of nerve approximately 1.5 cm long was removed from the left deep peroneal and soleus nerves approximately 1.2 cm from the nerve's entrance into the
muscle. This surgical procedure precludes reinnervation within the time-course of this study (Albuquerque and McIsaac, 1970). In the experiments involving denervated muscles, electrical stimulation was routinely applied to the distal stump of the nerves and on no occasion up to 60 days after nerve section were muscle twitches or end plate potentials (epps) observed. For the reinnervation studies, a 1.0 mm segment of the peroneal or soleus nerves was crushed 1.0 to 1.2 cm from the nerve's entrance into the muscle. Special care was taken not to damage the circulation to the muscles during denervation procedures. The wound was closed and the animals maintained for varying intervals on a normal laboratory diet with water ad lib. On the selected day after denervation the extensor and soleus muscles, and when necessary a 1.0-2.0 cm length of the attached nerve, were removed under continuous flow of Krebs-Ringer solution.

Electrophysiological Measurements

The isolated extensor and soleus muscles were stretched slightly beyond their resting length and secured by means of stainless steel pins inserted through the tendons, to a paraffin-lined plexiglass plate. The electrophysiological measurements were made in fibers of the dorsal surface of both muscles by means of glass microelectrodes filled with 3 M KCl, having resistances of 5–15 MΩ. The recording circuit had a time constant of about 40 μs when a 15 MΩ recording electrode was used. Intracellular recording of resting membrane potential (RMP) and the determination of electrical constants of the membrane and sensitivity to microiontophoretically applied ACh were made as previously described (Albuquerque and Thesleff, 1968; Albuquerque and McIsaac, 1970; Albuquerque and Warnick, 1972). The lower limit of our technique for detection of ACh-sensitivity was approximately 1 × 10⁻⁴ mV/nC. End plate regions were located by inserting the recording microelectrode at the apparent terminal portion of each axon arborization visible under the microscope; the spontaneous mepps thus detected were displayed on an oscilloscope and regarded as focal when their rise time, or the time required for the potential to reach its summit, was less than 1.0 ms. After locating the end plate region, the recording microelectrode was left in position for at least 5 min so that spontaneous transmitter release at very low frequency could be detected. Since the mepp frequency observed in both muscles had a similar frequency distribution to that of the diaphragm muscle (Gage and Quastel, 1966), i.e. the frequency distribution of the mepps was wide and skewed (see Fig. 1), the treatment of the data was approached in a manner similar to that described by Gage and Quastel (1966). In order to evaluate the mepp frequency in the control innervated muscles these potentials were recorded from 255 extensor fibers (28,000 mepps) and 227 soleus fibers (25,000 mepps) in 30 pairs of muscles. At the intervals after nerve crush shown in Table I, mepp frequency was evaluated in at least 35 fibers from six pairs of extensor and soleus muscles. However, fewer fibers had mepps at the early times after nerve crush and on the first few days of reinnervation. Therefore, about 200 mepps were recorded at 12 and 9–11 days after crushing the extensor and soleus muscles. At times later than 25 days a minimum of 2000 mepps from the extensor and soleus muscles were counted in determining mepp frequency. For the recording of epps and muscle action potentials (MAPs), a micro-
### Table I

FREQUENCY OF MINIATURE END PLATE POTENTIALS (mepps) RECORDED IN CONTROL DENERVATED, AND REINNERVATED EXTENSOR AND SOLEUS MUSCLES*

| Time after nerve crush | Extensor | | | Soleus | | | |
|------------------------|----------|----------|----------|----------|----------|----------|----------|
|                        | % of fibers with mepps | Geometric mean | Arithmetic mean | % of fibers with mepps | Geometric mean | Arithmetic mean | P (%) |
| 0                      | 98       | 0.48±0.01 | 3.02      | 3.42      | 97       | 0.07±0.01 | 1.18 | 1.53 <0.00 |
| 6 h                    | 80       | 0.57±0.03 | 3.72      | 4.68      | 67       | 0.25±0.04 | 1.79 | 2.14 <0.00 |
| 12 h                   | 12       | -0.16±0.26 | 0.68      | 1.37      | 21       | -0.12±0.10 | 0.76 | 0.95 >0.05 |
| 21 h                   | 0        |           |           |           | 0        |           |     |         |
| 72 h                   | 0        |           |           |           | 0        |           |     |         |
| 5 days                 | 0        |           |           |           | 0        |           |     |         |
| 7 days                 | 0        |           |           |           | 0        |           |     |         |
| 9 days                 | 23       | -1.03±0.35 | 0.08      | 0.04      | 23       | -1.52±0.11 | 0.03 | 0.02 >0.05 |
| 11 days                | 31       | -1.52±0.05 | 0.03      | 0.05      | 44       | -1.38±0.05 | 0.04 | 0.06 >0.05 |
| 13 days                | 59       | -0.77±0.05 | 0.17      | 0.22      | 69       | -0.92±0.08 | 0.12 | 0.10 >0.05 |
| 15 days                | 72       | -0.48±0.06 | 0.33      | 0.51      | 70       | -0.75±0.06 | 0.18 | 0.21 <0.01 |
| 21 days                | 74       | -0.15±0.04 | 0.71      | 0.83      | 74       | -0.73±0.10 | 0.19 | 0.40 <0.00 |
| 25 days                | 84       | 0.09±0.05  | 1.24      | 1.47      | 86       | -0.49±0.07 | 0.33 | 0.42 <0.00 |
| 30 days                | 88       | 0.06±0.04  | 1.14      | 1.48      | 93       | -0.42±0.07 | 0.38 | 0.51 <0.00 |
| 50 days                | 98       | 0.46±0.04  | 2.88      | 2.16      | 99       | -0.22±0.08 | 0.60 | 1.38 <0.00 |

* The total number of mepps, muscle fibers and muscles are described in detail in the Methods section.
† (log f) indicates the mean of the logarithms of mepp frequencies and its standard error (SE).
§ The geometric mean is the antilog of (log f) of the mepp frequencies.
|| The value of P for the difference between the geometric means of the mepp frequencies of the extensor and soleus muscles.

electrode was inserted at the end plate region and a conditioning pulse was applied to the nerves supplying the extensor and soleus muscles by means of extracellular bipolar platinum electrodes. On some occasions during study of the epps and MAPs, a second microelectrode was introduced into the same fiber as near as possible to the recording microelectrode and a constant anodal current was passed across the membrane to maintain a membrane potential of -90 mV (Narahashi, 1964; Albuquerque and Warnick, 1972). All potentials were displayed on the beams of 502 and 565 Tektronix (Tektronix, Inc., Beaverton, Oreg.) oscilloscopes, photographed with a Grass (Grass Instrument Co., Quincy, Mass.) C4 camera and/or recorded on paper with the use of a Minograf (Elema-Schönander, Sweden) 800 ink-jet writing polygraph having a flat frequency response from DC up to 500 cycle/s. All values are expressed as the mean ± standard deviation with the exception of the data on mepp frequency in Table I which is expressed as the mean ± SEM.

For the determination of ACh-sensitivity, electrical constants of the membrane and RMP of reinnervating surface fibers of the extensor and soleus muscles the following criteria were applied: (a) only fibers located within about 2.0 mm proximal to the nerve’s entrance into the muscle were used; and (b) the frequency and time-course of the spontaneous mepps of the fibers had to be similar to that previously determined and shown in Tables I and II. The data shown in these tables represent pooled data from randomly sampled surface fibers. In arriving at the mean mepp frequency one
Reinnervation of Mammalian Muscles

### Table II

| Time after nerve crush | Time-course | Duration | Time-course | Duration |
|------------------------|-------------|----------|-------------|----------|
| **Extensor**           |             |          |             |          |
| 0                      | 0.58 ± 0.12 | 3.50 ± 0.45 | 0.64 ± 0.11 | 3.39 ± 0.64 |
|                        | (125)       | (975)    | (613)       | (449)    |
| 11                     | 1.08 ± 0.43 | 8.58 ± 2.41 | -           | -        |
|                        | (14)        | (13)     |             |          |
| 13                     | 1.00 ± 0.43 | 5.53 ± 1.64 | 1.20 ± 0.41 | 6.04 ± 1.13 |
|                        | (10)        | (10)     | (10)        | (6)      |
| 17                     | 1.14 ± 0.38 | 5.14 ± 1.72 | 1.27 ± 0.67 | 4.82 ± 0.67 |
|                        | (32)        | (32)     | (32)        | (30)     |
| 21                     | 0.87 ± 0.22 | 4.75 ± 0.95 | 0.93 ± 0.14 | 4.83 ± 0.71 |
|                        | (102)       | (102)    | (92)        | (50)     |
| 30                     | 0.72 ± 0.10 | 4.46 ± 0.46 | 0.82 ± 0.11 | 4.69 ± 0.55 |
|                        | (839)       | (597)    | (61)        | (65)     |
| 40                     | 0.64 ± 0.12 | 3.63 ± 0.27 | 0.60 ± 0.04 | 4.12 ± 0.57 |
|                        | (31)        | (51)     | (120)       | (88)     |
| 45                     | 0.61 ± 0.03 | 3.54 ± 0.62 | 0.72 ± 0.16 | 4.09 ± 0.81 |
|                        | (154)       | (112)    | (127)       | (93)     |

* Values presented are the mean ± standard deviation; numbers in parentheses are the total number of individual potentials studied.

should realize that this represents fibers already reinnervated for a few days as well as those just reinnervated. These frequencies are therefore understandably different, as illustrated by the large standard deviation. Under these conditions only fibers which had a mepp frequency at or near the upper limit of the mean ± standard error for each respective day of reinnervation were used for the determination of ACh-sensitivity, electrical constants of the membrane, and RMP. Statistical analysis of the data was performed by using the Student's *t* test. A *P* value of < 0.01 was considered statistically significant.

**Histological Procedures**

The transverse diameter of individual fibers was measured in all muscles which were used for the calculation of the passive electrical properties. The muscles were fixed in Bouin's solution and then dehydrated in ethanolyxene before embedding in paraffin. Transverse sections 10 μm in thickness were made from surface fibers located at the center of the muscle. By means of a micrometer coupled to a camera lucida, about 30 surface muscle fibers were measured per muscle.

**Recording of Muscle Tension**

The isometric contracture and twitch tension developed by control and reinnervated extensor and soleus muscles when exposed to 5–20 mM caffeine were recorded using two methods. In the first, tension was recorded by means of an RCA (RCA Electronic
Components, Harrison, N. J.) 5734 transducer whose output was displayed on an oscilloscope. The second method employed a Grass (Grass Instrument Co.) FT.03 force displacement transducer connected to a Grass model 5 polygraph. The distal tendon of the excised muscle was tied to a glass rod and the muscle was then inserted into a chamber containing Krebs-Ringer solution which was continuously equilibrated with a mixture of 95% O\textsubscript{2}-5% CO\textsubscript{2}. The initial tension of 2 g was subtracted from all values of tension reported. The muscles were allowed to equilibrate for 30 min before exposure to caffeine. All experiments were performed at 23°C.

**Solutions and Drugs**

The Krebs-Ringer solution had the following composition in millimoles/liter: NaCl, 135; KCl, 5.0; MgCl\textsubscript{2}, 1.0; CaCl\textsubscript{2}, 2.0; NaHCO\textsubscript{3}, 15.0; Na\textsubscript{2}HPO\textsubscript{4}, 1.0; glucose, 11.0. The osmolarity was kept constant in the presence of increased KCl concentration by reducing the concentration of NaCl. Solutions were continuously bubbled with a mixture of 95% O\textsubscript{2}-5% CO\textsubscript{2} and had a pH of 7.1–7.2. Caffeine (mol wt 194.19) and tubocurarine chloride (mol wt 695.67) were purchased from K & K Laboratories, Inc. (Plainview, N. Y.). Anhydrous acetylcholine chloride (mol wt 181.68) was obtained from Sigma Chemical Company (St. Louis, Mo.).

**RESULTS**

*Frequency, Amplitude, and Duration of the Spontaneous Miniature End Plate Potentials Recorded from Surface Fibers of Innervated, Denervated, and Reinnervated Extensor and Soleus Muscles*

In the innervated extensor and soleus muscles the geometric mean (antilog of (log f)) frequency of the mepps was 3.02 and 1.18, respectively, and this difference was found to be significant (P < 0.001) (Table I). 55% of the extensor fibers had mepp frequencies greater than the modal value of 3.0 s\textsuperscript{-1}, whereas in the soleus muscles 99% of the fibers had mepp frequencies below this value (Fig. 1). The mean amplitude of the mepps was 0.50 mV for the extensor and 0.46 mV for the soleus muscle; this difference was not

![Figure 1. Frequency distribution of the miniature end plate potentials (mepps) recorded from the end plates of the innervated extensor (solid line) and soleus muscles (broken line). Each histogram represents at least 180 fibers from more than 23 different muscles.](image-url)
statistically significant ($P > 0.05$). There was also no significant difference between the mean duration of the mepps of the extensor and soleus muscles (Table II) and between the mean time-course of the mepps of the two muscles (Table II).

6 h after crushing the nerves supplying the extensor and soleus muscles, the mean frequencies of the mepps in most of the surface fibers were significantly increased ($P < 0.001$) (Table I). However, within 5 min after raising the concentration of KCl in the bathing medium ($[K^+]_o$) from 5 to 30 mM, an increase in the frequency of mepps to only $16.5 \text{ s}^{-1}$ occurred in the extensor and soleus muscles, while in the control muscles such treatment caused an increase of spontaneous mepp frequency to about $80 \text{ s}^{-1}$. The number of fibers in which mepps could be detected was reduced 12 h postoperatively and these potentials could no longer be observed at 18 h. At 12–15 h after nerve crush, high $[K^+]_o$ had little or no effect on the frequency of mepps recorded from either the extensor or soleus muscles. A similar result with high $[K^+]_o$ was obtained by Miledi and Slater (1968). Once spontaneous transmitter release had ceased, neither 30 mM $[K^+]_o$ nor hypertonic sucrose solution was able to reestablish the spontaneous transmitter release.

When the nerves were crushed at 1.0–1.2 cm from their entrances into the muscles, reinnervation began 8–9 days postoperatively. This was indicated by a gain of a few millivolts in RMP and the appearance of spontaneous mepps of a very low frequency (Table I). At this time the spontaneous mepps in the extensor muscle were not significantly different ($P > 0.05$) from soleus muscle and the potentials were prolonged in duration and time-course (Table II). The frequency of these potentials was increased by elevating $[K^+]_o$ to 20 mM. Spontaneous mepps were first observed in those fibers of the extensor and soleus muscles which were located near the nerve's entrance into the muscle. Reinnervation of the surface fibers started at the nerve's entrance into the muscle and progressed in a proximo-distal direction across the muscle. 11 days after nerve crush 87% of the surface fibers located within 2.0 mm of the nerve's entrance into the muscle were reinnervated, as signaled by the appearance of spontaneous mepps. About 4 days later, i.e. 15 days after nerve crush, these potentials were found with an equal probability in cells which were more distal to the entrance of the nerve into the muscle (see Methods and Table I). At this period of reinnervation the mepp frequency of the extensor muscle was significantly higher ($P < 0.01$) than that of the soleus (Table I).

The mepp amplitudes recorded 13–15 days after crushing the nerves to the extensor and soleus muscles showed a shift to the left, indicating that there was a greater number of very low amplitude mepps in these muscles. Occasionally, however, spontaneous mepps which had an amplitude about 6 times greater than the mean amplitude of the normal mepps were also recorded.
There was a gradual increase in mepp frequency in all surface fibers in both muscles so that 30–50 days after nerve crush, mepp frequencies were similar to the control extensor and soleus muscles. The alterations in fiber diameter may in part explain the presence of low amplitude mepps (Katz and Thesleff, 1957). It is conceivable that a slight increase in the spacing between pre- and postsynaptic membranes might exist in the reinnervated muscles similar to that seen in developing muscles (Teräväinen, 1968). While at 50 days after nerve crush one could still detect fibers with low amplitude mepps, the histograms of mepp amplitudes showed a pattern similar to control for both muscles. At this time the values of mepp duration and time-course were similar to those of the control innervated muscles (Table II).

Response of the Reinnervated Extensor and Soleus Muscles to Nerve Stimulation

9 days after nerve crush, a time when only 22% of the surface fibers showed spontaneous mepps (see Table I), stimulation of the nerves supplying the extensor and soleus muscles resulted in the simultaneous appearance of epps and MAPs in the muscles. In about 25% of the surface fibers the epps failed to initiate a MAP (Fig. 2 A); this may not reflect an alteration of the

![Figure 2](image-url)

**Figure 2.** Response of the postjunctional membrane of single fibers in the extensor and soleus muscles to indirect stimulation at 9 days after nerve crush. In (A) a single microelectrode for recording was inserted into a focal end plate region. In (B) a second microelectrode was inserted into the same fiber at an interelectrode distance of 100 μm and an anodal current, sufficient to hyperpolarize the membrane to −90 mV, was passed through this electrode. The top line in each trace indicates zero potential. The voltage and time calibrations are 50 mV and 10 ms, respectively.
total quanta of the epps but rather a partial inactivation of the sodium-ac-
tivation mechanism resulting from the large membrane depolarization present
at this stage of muscle reinnervation (Hodgkin and Huxley, 1952; Julian et al.,
1962; Albuquerque and Warnick, 1972). In fact, indirect stimulation con-
sistently produced a MAP when the postjunctional membrane at the end
plate region was hyperpolarized to −90 mV (Fig. 2 B). The alteration of the
rate of rise of these action potentials was similar to that of the intracellularly
recorded spike of the 5–7 day chronically denervated muscles, i.e., the rate of
rise of action potentials in the reinnervated muscles at 9 days after nerve
crush was reduced to 50–60% of control values in both muscles (Albuquerque
and Thesleff, 1968; Albuquerque and Warnick, 1972).

It is not within the scope of this study to investigate the mechanical prop-
erties of the innervated and reinnervated muscles, but to verify whether or
not at early times after nerve crush, indirect stimulation could elicit muscle
twitch and sustained tension. Although in most cases indirect stimulation
produced a muscle twitch at 9 days after nerve crush, the twitch amplitude
of the extensor and soleus muscles was reduced to 8 and 9%, respectively, of
control values. This is somewhat similar to the reduction in twitch amplitude
observed when direct stimulation was applied to denervated muscles. The
time-course of the twitch was prolonged to 250% of control values for the
extensor and 178% of control for the soleus (Fig. 3). In many preparations,
the reinnervated extensor muscles were not capable of maintaining as pro-
longed a tetanus when indirectly stimulated as were the control muscles.
In the 1 day reinnervated muscles, i.e. 9 days after nerve crush, there was a
more rapid development of a fused tetanic tension upon indirect stimulation
than in the control muscles, and the contractile properties of these muscles
are interestingly similar to those observed by Close (1964) in newborn rats.

The possibility that direct stimulation of the reinnervated muscles could
occur by means of current leakage from the stimulating microelectrode was
excluded since the responses of the muscles to indirect stimulation were
abolished by section of the nerves supplying these muscles or the addition of
tubocurarine (1.0 × 10⁻⁵ M) to the bathing medium.

Resting Membrane Potential of the Innervated, Denervated, and Reinnervated
Extensor and Soleus Muscles

Although the mean RMP in the control innervated extensor muscles was
−79.6 ± 2.9 mV and −77.0 ± 2.7 mV for the innervated soleus muscles,
this difference was not statistically significant (P < 0.05).

During the period of denervation, i.e. up to 8 days after nerve crush, the
RMP was reduced in both muscles to about 73% of control (Albuquerque et
al., 1971).

11–13 days after nerve crush, the RMP of the muscle fibers that showed
spontaneous mepps and produced an action potential in response to indirect stimulation was 7% greater than that recorded in fibers where neuromuscular transmission was not yet reestablished. On the following days there was a continuous increase in RMP until values equivalent to control were recorded from both muscles 30–35 days postoperatively (Fig. 4).

**Electrical Constants of the Membrane of the Innervated, Denervated, and Reinnervated Extensor and Soleus Muscles**

No significant differences were found between control fibers of the extensor and soleus muscles in specific resistance of a unit area ($R_\text{s}$), capacitance ($C_\text{m}$), input resistance ($R_\text{in}$), space constant ($\lambda$), or time constant ($T_\text{m}$) of the membrane. Although in previous reports (Albuquerque and Thesleff, 1968; Albuquerque and McIsaac, 1970; Albuquerque et al., 1971) the values obtained for control $R_\text{in}$ were greater than those shown in Tables III and IV, this difference can be accounted for by the larger size of the animals used in the present study and consequently larger muscle fibers. Thus, the values
shown in Tables III and IV are understandably lower than the values for $R_{in}$ previously reported. The $R_m$ of fibers in the extensor muscle (Table III) and in the soleus muscle (Table IV) 5 days after crushing the nerves was significantly increased ($P < 0.01$). The $R_m$ of the membrane continued to increase until peak values were attained for both muscles 7–15 days postoperatively. Although the first sign of membrane reinnervation was evident by 9 days after nerve crush, only after 17 days did the $R_m$ in both muscles begin to decrease towards control values (see Methods). In fact, there was no significant difference ($P < 0.5$) between the values for $R_m$ obtained at the beginning of reinnervation, i.e. 9 days after nerve crush, and those recorded through 15 days after nerve crush. Similarly, there was no significant difference between the mean values of $R_{in}$ for the same period of time after nerve crush in 250 surface fibers of 37 pairs of extensor and soleus muscles. At 25–30 days after nerve crush, values equivalent to those of normal innervated muscles were obtained (see Tables III and IV).

Acetylcholine-Sensitivity of the Innervated, Denervated, and Reinnervated Extensor and Soleus Muscles

Values similar to those reported in an earlier study (Albuquerque and McIsaac, 1970) were obtained for the sensitivity of the end plate regions of the extensor and soleus muscle fibers to microiontophoretically applied ACh. Similarly to that found by Lømo and Rosenthal (1972), the ACh-sensitivity
| Days after nerve crush | Days after reinnervation | $R_{in}$ (MΩ) | $\lambda$ (mm) | $T_m$ (ms) | $\rho_{real}$ (µm) | $\rho_{exp}$ (µm) | $C_m$ (Ω cm²) | $\mu F/cm^{2}$ |
|------------------------|-------------------------|----------------|-------------|-----------|-----------------|----------------|----------------|-----------------|
| 0                      | --                      | 0.21±0.05      | 0.55±0.11  | 1.3±0.3   | 27.9±4.6        | 21.7±4.6       | 385±100        | 3.6±0.8         |
| 1.5                    | --                      | 0.25±0.02      | 0.57±0.10  | --        | 25.6±2.8        | --             | 461±82         | --              |
| 3                      | --                      | 0.34±0.03      | 0.47±0.03  | 1.7±0.3   | 20.0±1.3        | --             | 423±47         | 3.9±0.7         |
| 5                      | --                      | 0.38±0.12      | 0.59±0.14  | 2.3±0.5   | 22.7±4.3        | 20.5±3.9       | 392±147        | 3.9±0.8         |
| 7                      | --                      | 0.47±0.12      | 0.82±0.21  | 3.7±0.6   | 22.7±4.8        | 15.6±1.5       | 1024±281       | 3.6±0.6         |
| 9                      | 2                       | 0.66±0.19      | 0.67±0.17  | 3.8±0.5   | 17.1±2.7        | --             | 923±207        | 4.1±0.5         |
| 11                     | 4                       | 0.53±0.13      | 0.89±0.14  | 4.6±0.7   | 22.0±2.8        | --             | 1239±420       | 3.7±0.6         |
| 13                     | 6                       | 0.52±0.21      | 0.70±0.11  | 3.2±0.8   | 20.6±4.5        | 16.7±3.9       | 888±277        | 3.7±0.9         |
| 15                     | 8                       | 0.40±0.09      | 0.61±0.11  | 2.3±0.6   | 21.2±3.3        | --             | 657±197        | 3.5±0.9         |
| 21                     | 12                      | 0.33±0.09      | 0.58±0.10  | 2.1±0.3   | 22.8±3.8        | 18.0±2.4       | 516±139        | 4.2±0.6         |
| 25                     | 16                      | 0.32±0.08      | 0.51±0.08  | 1.9±0.5   | 21.7±3.8        | 17.4±2.1       | 428±102        | 4.6±1.1         |
| 30                     | 21                      | 0.25±0.04      | 0.55±0.10  | 1.5±0.4   | 26.5±4.8        | 21.1±4.1       | 388±62         | 3.8±0.9         |
| 35                     | 26                      | 0.23±0.08      | 0.55±0.11  | 1.8±0.4   | 27.0±6.2        | --             | 423±193        | 4.2±0.9         |
| 40                     | 31                      | 0.30±0.11      | 0.50±0.09  | 1.8±0.4   | 22.9±4.1        | 20.1±4.1       | 430±155        | 4.2±1.0         |
| 45                     | 36                      | 0.22±0.05      | 0.54±0.05  | 1.6±0.1   | 26.6±3.1        | --             | 402±90         | 4.0±0.2         |
| 50                     | 41                      | 0.24±0.07      | 0.51±0.10  | 1.5±0.3   | 24.9±4.9        | --             | 377±87         | 3.9±0.8         |

*The figures in parentheses are the number of fibers studied in at least five muscles. $R_{in}$, input resistance; $\lambda$, space constant; $T_m$, time constant; $p$, fiber radius; $R_m$, transverse resistance of a unit area; $C_m$, membrane capacitance per unit area. The myoplasm resistance ($R_p$) was assumed to be 180 Ω cm.

† Reinnervation begins on this day.

recorded at the end plate region of the muscles varied from 25 to 470 mV/nC. However, we have eliminated all fibers having values below 100 mV/nC because most certainly they did not reflect the real ACh-sensitivity of the end plate region, but rather, they indicated either that the ACh-pipette was not
# Table IV

**EFFECT OF DENERVATION AND REINNERVATION UPON THE ELECTRICAL PROPERTIES OF THE MEMBRANE OF SURFACE FIBERS OF THE SOLEUS MUSCLE**

| Days after | Days after | $R_{in}$ | $\lambda$ | $T_m$ | $\rho_{scal}$ | $\rho_{esp}$ | $R_m$ | $C_m$ |
| nerve crush | reinnervation | MΩ | mm | mm | μm | μm | Ω cm$^{-1}$ | μF/cm$^2$ |
|---|---|---|---|---|---|---|---|---|
| 0 | — | 0.28±0.06 (59) | 0.52±0.09 | 1.5±0.3 | 24.4±4.8 | 22.7±3.4 | 421±101 | 3.6±0.7 |
| 1.5 | — | 0.26±0.08 (6) | 0.50±0.05 | — | 24.3±3.6 | — | 366±89 | — |
| 3 | — | 0.42±0.07 (12) | 0.58±0.10 | 2.7±0.3 | 20.0±2.5 | 17.2±1.6 | 607±157 | 4.5±0.5 |
| 5 | — | 0.51±0.04 (17) | 0.71±0.15 | 2.7±0.3 | 19.7±2.6 | 15.3±3.3 | 861±168 | 3.1±0.4 |
| 7 | — | 0.59±0.11 (10) | 0.86±0.20 | 4.4±1.0 | 20.4±2.9 | 14.1±2.0 | 1072±370 | 4.1±1.0 |
| 9 | ‡ | 0.72±0.12 (15) | 0.65±0.07 | 4.7±1.0 | 16.3±2.3 | — | 880±100 | 5.3±1.1 |
| 11 | 2 | 0.64±0.12 (15) | 0.68±0.11 | 4.5±1.0 | 17.3±2.3 | — | 945±208 | 4.8±1.0 |
| 13 | 4 | 0.53±0.10 (16) | 0.74±0.16 | 3.8±0.9 | 20.2±3.4 | — | 937±185 | 4.1±1.0 |
| 15 | 6 | 0.49±0.10 (18) | 0.70±0.10 | 3.4±1.2 | 23.1±3.5 | 13.8±3.2 | 788±250 | 4.3±1.5 |
| 17 | 8 | 0.38±0.08 (18) | 0.68±0.14 | 2.7±0.4 | 22.9±3.7 | 15.0±2.5 | 727±217 | 3.8±0.6 |
| 21 | 12 | 0.27±0.05 (18) | 0.59±0.07 | 2.4±0.4 | 25.0±2.1 | 17.7±3.6 | 516±110 | 4.6±0.8 |
| 25 | 16 | 0.37±0.08 (7) | 0.59±0.08 | 1.9±0.5 | 21.8±3.4 | 20.2±1.9 | 564±86 | 3.4±1.0 |
| 30 | 21 | 0.27±0.04 (13) | 0.59±0.10 | 1.6±0.3 | 25.0±3.3 | — | 494±117 | 3.2±0.6 |
| 35 | 26 | 0.32±0.07 (11) | 0.54±0.09 | 2.1±0.2 | 22.5±2.7 | — | 536±124 | 3.9±0.5 |
| 40 | 31 | 0.27±0.05 (9) | 0.64±0.15 | 1.6±0.3 | 25.3±5.6 | 17.8±1.8 | 524±90 | 3.0±0.6 |
| 45 | 36 | 0.29±0.02 (6) | 0.64±0.07 | 1.9±0.2 | 25.0±2.4 | 20.1±3.7 | 596±53 | 3.2±0.2 |
| 50 | 41 | 0.24±0.04 (9) | 0.54±0.13 | 1.4±0.3 | 25.9±4.8 | 20.2±3.8 | 406±97 | 3.4±0.8 |

*The figures in parentheses are the number of fibers studied in at least five muscles. $R_{in}$, input resistance; $\lambda$, space constant; $T_m$, time constant; $\rho$, fiber radius; $R_a$, transverse resistance of a unit area; $C_m$, membrane capacitance per unit area. The myoplasm resistance ($R_i$) was assumed to be 180 Ω cm.

† Reinnervation begins on this day.

---

inserted properly in the end plate region or that the end plate region was not located on the upper surface of the fiber. Furthermore, even using values from 25 to 470 mV/nC for the ACh-sensitivity of the end plate regions of the muscles, the mean value was significantly higher ($P < 0.001$) than the values obtained at the extra-junctional regions of the denervated muscles. The rise
time of the ACh potentials recorded at the end plate regions of the innervated extensor and soleus muscles was 3.1 and 3.5 ms, respectively. A second site of high ACh-sensitivity was found at the myotendinous region of the soleus muscle, with “spots” of low ACh-sensitivity distributed randomly over the entire muscle fiber between this region and the end plate area. In contrast, the extensor muscle was completely unresponsive to ACh when it was applied 500–750 μm from the end plate or at the myotendinous region (Fig. 5, 7 A). The profile of the ACh-receptive areas in the control at 8, 15, and 35 days after crushing the nerves to the extensor and soleus muscles is shown in Figs. 6, 7, and 8.

**Figure 5.** Typical response of the extensor muscle to microiontophoretically applied acetylcholine at 0 days (control) (A), 8 (B), 15 (C), and 35 days (D) after nerve crush. In each row the upper trace is the muscle membrane response to applied acetylcholine, the bottom gives the current applied to the acetylcholine pipette, and the line below the current trace indicates the time calibration. The numbers at the bottom of the figure are the distances in millimeters from the end plate region toward the tendon (T). Time calibration for the end plate region (0 mm) applies to all of the potentials recorded from this region.
5 and 6, respectively. (For details of the technique used in the study of ACh-sensitivity of the reinnervated muscles see Methods.)

8 days after crushing the nerves to the extensor and soleus muscles, sensitivity to ACh was observed over the entire surface with the highest sensitivity usually located at the end plate and myotendinous regions (Figs. 7 A and 8 A). This distribution of ACh-sensitivity was similar to that previously observed in denervated muscles (Albuquerque and McIsaac, 1970). A decline in ACh-sensitivity was observed for both muscles about 15 days after nerve crush which corresponds to 6 days after the beginning of reinnervation (see

![Figure 6](image-url)

**Figure 6.** Typical response of the soleus muscle to microiontophoretically applied acetylcholine at 0 days (control) (A), 8 (B), 15 (C), and 35 days (D) after nerve crush. In each row the upper trace is the muscle membrane response to applied acetylcholine, the bottom trace gives the current applied to the acetylcholine pipette, and the line below the current trace indicates the time calibration. The numbers at the bottom of the figure are the distances in millimeters from the end plate region toward the tendon (T). The time calibration for the end plate region (0 mm) applies to all of the potentials recorded from this region.
Figs. 7 and 8). The ACh-sensitivity at myotendinous regions of both muscles continued to decrease, while no alteration at the end plate region was observed. 21 days after crushing the nerves, the myotendinous regions of the extensor muscle were still sensitive to ACh and "spots" of low ACh-sensitivity were scattered along the muscle fibers (see Fig. 7 B). The myotendinous region of the extensor muscle remained sensitive to ACh up to 35 days postopera-

**Figure 7.** ACh-sensitivity at the end plate region and postsynaptic membrane of surface fibers of the extensor muscles of the rat. In (A), innervated muscles (●—●), 8 days (▲—▲), and 11 days (■—■); (B), 13 days (●—●), 15 days (▲—▲), and 21 days (■—■); and (C), 30 days (●—●), 35 days (▲—▲), and 40 days (■—■) after nerve crush. Abscissa denotes the distance in millimeters from the end plate along the fiber to the myotendinous region of the muscles; ordinate, the acetylcholine-sensitivity in millivolts/nanocoulombs plotted logarithmically. Each curve represents the mean of at least five individual fibers ± standard deviation from three different muscles.

**Figure 8.** ACh-sensitivity at the end plate region and postsynaptic membrane of surface fibers of the soleus muscles of the rat in (A), innervated muscles (●—●); (B), 8 days (●—●), 11 days (▲—▲), and 15 days (■—■); and (C), 21 days (●—●) and 25 days (▲—▲) after nerve crush.
Reinnervation of Mammalian Muscles

J. J. McArdle and E. X. Albuquerque

At this time a few "spots" of low ACh-sensitivity were found along the muscle fiber between the end plate and myotendinous regions. Only at 40 days after nerve crush did the ACh-sensitivity of the myotendinous region of the extensor muscles decrease to a level too low to be measured by our technique (Fig. 7C). It is noteworthy that 35 days after nerve crush most of the electrogenic properties of both muscles were similar to those for control innervated muscles.

The entire membrane of the surface fibers of the soleus muscle between the end plate and myotendinous regions was still more sensitive to ACh up to 21 days after nerve crush (Fig. 8B). Although 25-40 days after nerve crush the soleus muscle exhibited a pattern of ACh-sensitivity similar to that observed in control muscles (see Fig. 8C), some fibers, even at 40 days after nerve crush, showed higher sensitivity to ACh, applied extra-junctionally.

Response of the Innervated, Denervated, and Reinnervated Extensor and Soleus Muscles to Caffeine

In the control unstimulated soleus muscle kept in a muscle bath at 23°C, caffeine (20 mM) produced an increase in muscle tension which reached a maximum of 25 g 2 hr after the drug had been admitted to the experimental chamber. Under identical experimental conditions, the extensor muscle never developed contracture in the presence of 20 mM caffeine. Muscle fasciculations were observed in the extensor and soleus muscles while exposed to 5-20 mM caffeine. These fasciculations were blocked by previous treatment of the muscles with 1.0 × 10^-5 M tubocurarine. 18 h after crushing the nerves supplying the extensor and soleus muscles, caffeine failed to induce fasciculations in these muscles. 11 days after nerve crush, caffeine induced fasciculations in both muscles. The return of muscle fasciculations induced by caffeine was preceded by the reappearance of spontaneous transmitter release and epps in these muscles.

1 1/2 days after nerve crush the extensor muscle developed a contracture of 3 g in the presence of 20 mM caffeine. The peak tension developed by the extensor muscle in response to caffeine was increased at later times, while the response of the soleus muscle to caffeine progressively decreased after nerve crush (Fig. 9). At 9 days the maximal increase in tension of the soleus muscles produced by caffeine was significantly less (P < 0.01) than that observed in the extensor muscles.

During the first few days of reinnervation there was a progressive increase in the response of the soleus muscles to caffeine (20 mM), and 30 days after nerve crush the contracture of these muscles was equivalent to that of the control muscles. Between 11 and 13 days postoperatively, there was a sudden reduction in the amplitude of the contracture developed by the extensor muscle in the presence of caffeine (Fig. 9). At 13 days after nerve crush, expo-
Figure 9. Maximal tension developed by the extensor and soleus muscles after exposure to 20 mM caffeine (23°C) in controls (0 days) and at various intervals after denervation (open bars), i.e. without reinnervation, and after crushing the nerves supplying the muscles (solid bars) which allows reinnervation. Each bar is the mean of five muscles.

sure to 20 mM caffeine caused practically no contracture of the extensor muscle.

If muscle reinnervation was not allowed to occur by excising a 1.0-2.0 cm section of the nerves, the response of the extensor muscle to caffeine reached a maximum value 7-10 days after denervation while the soleus progressively decreased in sensitivity to the drug. 25-30 days after denervation the contractures produced by the extensor and soleus muscles in the presence of 20 mM caffeine decreased to a level of 5 g (Fig. 9).

DISCUSSION

The present study revealed that the frequency of the spontaneous mepps is significantly greater in the “fast” extensor muscle than in the “slow” soleus muscle of the rat. Our mean value for mepp frequency of the soleus muscle is similar to that reported by others (Miledi and Zelená, 1966; Robbins and Fischbach, 1971) for the same muscle. The lower frequency of the spontaneous transmitter release observed in the soleus vs. extensor muscles may be due to the possible differences which exist in connection with the types of motor units supplying the “fast” and “slow” muscles (Eccles et al., 1958; Buller et al., 1960 a, b; Close, 1964, 1972; Padykula and Gauthier, 1970). That the frequency of spontaneous transmitter release appears to be correlated with the size of the end plate region has been discussed by other investigators (Duchen, 1970; Kuno et al., 1971) who have found that fibers with small nerve terminals do in fact have lower mepp frequency than the fibers with
larger nerve terminals. Kuno et al. (1971) showed a significant correlation between the mean mepp frequency and the end plate area. The findings of these investigators on frog muscle and our results in the rat suggest that the differences between extensor and soleus muscles, with respect to the frequency of transmitter release, may result from differences in the size of end plate regions and the density of synaptic vesicles in the nerve terminals (Padykula and Gauthier, 1970).

At the beginning of reinnervation, spontaneous mepps in the extensor and soleus muscles had a low amplitude, a prolonged time-course and duration, and a low frequency of occurrence. Although no large alteration of the subneural apparatus occurs during the short period of denervation (Csillik, 1967) some shrinkage of the end plate region and increase in the space between pre- and postsynaptic membrane may have developed in association with the overall atrophy of the muscle fiber (Gutmann and Young, 1944; Teräväinen, 1968). If one assumes that no alteration occurs in the ACh storage in the presynaptic terminal, the postsynaptic changes mentioned above may be responsible for the low amplitude of the mepps observed at the beginning of the reinnervation period. In fact, electrophysiological investigations of intercostal muscles taken from myasthenic patients (Elmqvist et al., 1964) have shown a decreased mepp amplitude and morphological studies of such tissues have revealed alterations in the synaptic clefts (Zacks et al., 1966; Santa et al., 1972). The prolonged time-course and duration of the mepps recorded on the early days of muscle reinnervation is expected in view of the reduced activity of cholinesterase (Csillik, 1967) and the increase in the electrical constants of the denervated muscle (Albuquerque and McIsaac, 1970; Albuquerque et al., 1971; Fischbach and Robbins, 1971). In spite of the low mepp frequency recorded on the early days of muscle reinnervation, transmitter release could be increased by elevating the [K+]o indicating that the nerve terminal was capable of releasing numerous quanta of transmitter when depolarized by potassium. This response to [K+]o excludes the possibility of release of ACh from the Schwann cell at the end plate of denervated muscles, since under this condition potassium would not increase the frequency of transmitter release (Miledi and Slater, 1968). Furthermore, 9 days after nerve crush, the muscles also exhibited epps when their nerves were stimulated (see Fig. 2).

As reinnervation of the muscles progressed, there was a gradual increase in mepp frequency and an increase in the membrane potential of the muscle fibers. A partial repolarization of the muscle fibers occurred within 3 days after reinnervation, but the membrane resistance began to decrease towards control values only at 17 days after nerve crush. It seems that the nerve may normally release a regulatory factor that directly influences the membrane potential of the muscle fibers (Albuquerque et al., 1971).
The ACh-sensitivity of the denervated and reinnervated extensor and soleus muscles was higher at the end plate than at extra-junctional regions (see Figs. 7 and 8), and this confirms the previous findings of Albuquerque and McIsaac (1969, 1970). This suggests that if reinnervation is to occur during a period of 9–10 days after nerve crush, the motor nerve would reinnervate the muscle by establishing contact with the old end plate region (Gutmann and Young, 1944; Csillik, 1967; Lüllmann-Rauch, 1971). Although the increase or appearance of extra-junctional ACh-sensitivity occurs 24–48 h after denervation (Albuquerque and McIsaac, 1970), the disappearance and decrease of ACh-sensitivity along the muscle fiber occurs very slowly. The extra-junctional sensitivity to ACh remained very high for over 26 days after the return of the spontaneous and evoked transmitter release. However, one can still find sensitivity to ACh at a few myotendinous regions of the 35 day reinnervated extensor muscles (Fig. 5). Thus, it seems that the presence of extra-junctional ACh-sensitivity may be controlled by various neurotrophic factors which will bring about normal muscle activity, rather than a single specific factor (Miledi, 1960; Diamond and Miledi, 1962; Duchen and Stefani, 1971; Gutmann, 1969; Gutmann and Hnik, 1962).

Another important difference between the extensor and soleus muscles was the alteration in their response to caffeine at the beginning of reinnervation. Once the muscles were reinnervated, the caffeine-induced contracture of the reinnervated extensor muscle remained unaltered until a drastic decrease in the contracture tension was observed between 12 and 13 days after nerve crush. At the time when the extensor muscle becomes insensitive to caffeine and the soleus muscle returns to control levels, the electrical constants of the postsynaptic membrane were almost twice that of control, the spontaneous transmitter release was still significantly altered, and the extra-junctional membrane was still highly sensitive to ACh.

Since most of these effects of caffeine may result from an action of the drug on calcium stores of the sarcoplasmic reticulum (Thorpe and Seeman, 1971; Weber and Herz, 1968; Warnick et al., 1971) it may be inferred that the nerve might exert a regulatory effect upon the sarcoplasmic reticulum (Isaacs and Sandow, 1967).

Note Added in Proof While this manuscript was in press, E. X. Albuquerque, J. E. Warnick, J. R. Tasse, and F. M. Sansone (1972. Effects of vinblastine and colchicine on neural regulation of the fast and slow skeletal muscles of the rat. Exp. Neurol. 37. In press) observed that chronic application of vinblastine or colchicine to the nerves supplying the extensor and soleus muscles of the rat caused no apparent alterations in muscle activity per se or in evoked and spontaneous transmitter release. Despite the apparent retention of normal activity in both muscles, denervation-type alterations which included a membrane depolarization of 15 mV, appearance of extrajunctional ACh-sensitivity, and tetrodotoxin-resistant action potentials resulted...
from chronic application of these drugs for 7–10 days. Values for extrajunctional ACh-sensitivity as high as 310 mV/nC were observed. The mean twitch and tetanus tensions of the extensor muscles were about 35 g and 160 g, respectively, both before and during chronic exposure to either drug for 7 days. Thus, neuromuscular transmission in general appeared to be unaltered when changes in the RMP and extrajunctional ACh-sensitivity occurred. Light and electron microscope analyses of the drug-treated portion of the nerve revealed axoplasmic degeneration with little effect on the axolemma. These results are in agreement with the suggestions made above that some trophic substance other than ACh is released from the nerve and controls muscle membrane properties.

The authors wish to thank Miss Mabel A. Zelle for expert technical and secretarial assistance during the course of this study. We are also indebted to Dr. Jordan E. Warnick and to Dr. René Tassé for critical and technical help, to Mr. E. Novak for photographic assistance, and to Dr. Norman Robbins for critical evaluation of the manuscript and helpful comments. This study was supported by Grants NS-08233 and GM-00107 from the National Institutes of Health.

Received for publication 30 May 1972.

REFERENCES

ALBUQUERQUE, E. X., and R. J. MCISAAC. 1969. Early development of acetylcholine receptors in fast and slow mammalian skeletal muscle. Life Sci. 8:409.

ALBUQUERQUE, E. X., and R. J. MCISAAC. 1970. Fast and slow mammalian muscles after denervation. Exp. Neurol. 26:183.

ALBUQUERQUE, E. X., F. T. SCHUH, and F. C. KAUFFMAN. 1971. Early membrane depolarization of the fast mammalian muscle after denervation. Pfluegers Arch. Eur. J. Physiol. 320:36.

ALBUQUERQUE, E. X., and S. THESELEFF. 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.

ALBUQUERQUE, E. X., and J. E. WARNICK. 1972. The pharmacology of batrachotoxin. IV. Interaction with tetrodotoxin on innervated and chronically denervated rat skeletal muscle. J. Pharmacol. Exp. Thr. 180:683.

AXELSON, J., and S. THESELEFF. 1959. A study of supersensitivity in denervated mammalian skeletal muscle. J. Physiol. (Lond.) 147:178.

BIRKS, R., B. KATZ, and R. MILEDI. 1960. Physiological and structural changes at the amphibian myoneural junction, in the course of nerve degeneration. J. Physiol. (Lond.) 150:145.

BULLER, A. J., J. C. ECCLES, and R. M. ECCLES. 1960 a. Differentiation of fast and slow muscles in the cat hind limb. J. Physiol. (Lond.) 150:399.

BULLER, A. J., J. C. ECCLES, and R. M. ECCLES. 1960 b. Interactions between motoneurones and muscles in respect of the characteristic speeds of their responses. J. Physiol. (Lond.) 150:417.

CLOSE, R. 1964. Dynamic properties of fast and slow skeletal muscles of the rat during development. J. Physiol. (Lond.) 173:74.

CLOSE, R. I. 1972. Dynamic properties of mammalian skeletal muscles. Physiol. Rev. 52:129.

CSILLIK, B. 1967. Functional Structure of the Postsynaptic Membrane in the Myoneural Junction. Akademiai Kiado, Publishing House of the Hungarian Academy of Sciences, Budapest.

DIAMOND, J., and R. MILEDI. 1962. A study of foetal and new-born rat muscle fibres. J. Physiol. (Lond.) 162:393.

DUCHEN, L. W. 1970. Changes in motor innervation and cholinesterase localization induced by botulinum toxin in skeletal muscle of the mouse: differences between fast and slow muscles. J. Neurol. Neurosurg. Psychiat. 33:40.
DUCHEK, L. W., and E. STEFANI. 1971. Electrophysiological studies of neuromuscular transmission in hereditary “motor end-plate disease.” J. Physiol. (Lond.) 212:535.

ECCLES, J. C., R. M. ECCLES, and A. LUNDBERG. 1958. The action potentials of the alpha motoneurones supplying fast and slow muscles. J. Physiol. (Lond.) 142:275.

ELMOVIST, D., W. W. HOPMANN, J. KUGELBERG, and D. M. QUASTEL. 1964. An electrophysiological investigation of neuromuscular transmission in myasthenia gravis. J. Physiol. (Lond.) 174:417.

FISCHBACH, G. D., and N. ROBBINS. 1971. Effect of chronic disuse of rat soleus neuromuscular junctions on postsynaptic membrane. J. Neurophysiol. 24:562.

GAGE, P. W., and D. M. J. QUASTEL. 1966. Competition between sodium and calcium ions in transmitter release at mammalian neuromuscular junctions. J. Physiol. (Lond.) 142:275.

GUTMANN, E. 1969. The trophic function of the nerve cell. Scientia (Milano). 54:1.

GUTMANN, E., and P. HNIK. 1962. Denervation studies in research of neurotrophic relationships. In The Denervated Muscle. E. Gutmann, editor. Academia, Publishing House of the Czechoslovak Academy of Sciences, Prague. 13.

GUTMANN, E., and A. SANDOW. 1965. Caffeine induced contracture and potentiation of contraction in normal and denervated muscle. Life Sci. 4:1149.

GUTMANN, E., and J. Z. YOUNG. 1944. The re-innervation of muscle after various periods of atrophy. J. Anat. 78:15.

HARRIS, A. J., and R. MILLED. 1971. The effect of type D botulinum toxin on frog neuromuscular junctions. J. Physiol. (Lond.) 217:497.

HASSLEBACH, W. 1964. Relaxation and the sarcotubular calcium pump. Fed. Proc. 23:909.

HODGKIN, A. L., and A. F. HUXLEY. 1952. Currents carried by sodium and potassium ions through the membrane of the giant axon of Loligo. J. Physiol. (Lond.) 116:449.

ISAACSON, A., and A. SANDOW. 1967. Caffeine effects on radiocalcium movements in normal and denervated rat skeletal muscle. J. Pharmacol. Exp. Ther. 155:376.

JULIAN, F. J., J. W. MOORE, and D. E. GOLDMAN. 1962. Current-voltage relations in the lobster giant axon membrane under voltage clamp conditions. J. Gen. Physiol. 45:1217.

KATZ, B., and S. TESLEFF. 1957. A study of the “desensitization” produced by acetylcholine at the motor end-plate. J. Physiol. (Lond.) 138:63.

KUNO, M., S. A. TURKANIS, and J. N. WEAKLY. 1971. Correlation between nerve terminal size and transmitter release at the neuromuscular junction of the frog. J. Physiol. (Lond.) 213:545.

Löffło, T., and J. ROSENDAL. 1972. Control of ACh sensitivity by muscle activity in the rat. J. Physiol. (Lond.) 221:493.

LÜLLMANN-RAUCH, R. 1971. The regeneration of neuromuscular junctions during spontaneous re-innervation of the rat diaphragm. Z. Zellforsch. Mikrosk. Anat. 121:593.

McARDLE, J., and E. X. ALBUQUERQUE. 1970. Regeneration of fast and slow mammalian muscle. Pharmacologist. 12:224.

McARDLE, J., and E. X. ALBUQUERQUE. 1971. Regeneration of fast and slow mammalian muscle. Pharmacologist. 13:217.

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

MILEDI, R. 1962. Induction of receptors. Ciba Found. Symp. Enzymes Drug Action. 220.

MILEDI, R., and C. R. SLATER. 1968. Electrophysiology and electron-microscopy of rat neuromuscular junctions after nerve degeneration. Proc. R. Soc. Lond. B Biol. Sci. 169:289.

MILEDI, R., and L. STEFANI. 1969. Non selective reinnervation of slow and fast fibers in the rat. Nature (Lond.) 222:569.

MILEDI, R., and J. ZELENÁ. 1966. Sensitivity to acetylcholine in rat slow muscle. Nature (Lond.) 210:855.

NARAHASHI, T. 1964. Restoration of action potential by anodal polarization in lobster giant axons. J. Cell. Comp. Physiol. 64:73.

PADIJKULA, H., and G. GAUTHIER. 1970. The ultrastructure of the neuromuscular junctions of mammalian red, white, and intermediate skeletal muscle fibers. J. Cell. Biol. 46:27.
PELLEGRINO, C., and C. FRANZINI. 1963. An electron microscope study of denervation atrophy in red and white skeletal muscle fibers. J. Cell. Biol. 17:327.

ROBBINS, N., and G. D. FISCHBACH. 1971. Effect of chronic disuse of rat soleus neuromuscular junctions on presynaptic function. J. Neurophysiol. 34:570.

SANTA, T., A. G. ENGEL, and E. H. LAMBERT. 1972. Histochemical study of neuromuscular junction ultrastructure I. Myasthenia gravis. Neurology. 22:71.

SLATER, C. R. 1966. Time course of failure of neuromuscular transmission after motor nerve section. Nature (Lond.). 209:305.

TERÄVÄINEN, H. 1968. Development of the myoneural junction in the rat. Z. Zellforsch. Mikrosk. Anat. 87:249.

THORPE, W. R., and P. SEEMAN. 1971. The site of action of caffeine and procaine in skeletal muscle. J. Pharmacol. Exp. Ther. 179:324.

THESLEFF, S. 1960a. Supersensitivity of skeletal muscle produced by botulinum toxin. J. Physiol (Lond.) 154:598.

THESLEFF, S. 1960b. Effects of motor innervation on the chemical sensitivity of skeletal muscle. Physiol. Rev. 40:734.

WARNICK, J. E., E. X. ALBUQUERQUE, and F. M. SANSONE. 1971. The pharmacology of batrachotoxin I. Effects on the contractile mechanism and on neuromuscular transmission of mammalian skeletal muscle. J. Pharmacol. Exp. Ther. 176:497.

WEBER, A., and R. HERZ. 1968. The relationship between caffeine contracture of intact muscle and the effect of caffeine on reticulum. J. Gen. Physiol. 52:750.

ZACKS, S. I., D. R. SHIELDS, and S. A. STEINBERG. 1966. A myasthenic syndrome in the dog: a case report with electron microscopic observations on motor end-plates and comparisons with the fine structure of end-plate in myasthenia gravis. Ann. N. Y. Acad. Sci. 135:79.