J. Physiol. (1971), **219**, *pp.* 331–343
With 4 text-figures
Printed in Great Britain

AGE CHANGES IN CROSS STRIATED MUSCLE OF THE RAT

BY E. GUTMANN, V. HANZLÍKOVÁ AND F. VYSKOČIL
From the Institute of Physiology, Czechoslovak Academy of Sciences, Prague, Czechoslovakia

(*Received 16 November 1970*)

SUMMARY

1. Senile muscle atrophy is characterized by a marked reduction in the frequency of spontaneous transmitter release with no electrophysiological evidence of denervation.

2. In spite of the reduced number of muscle fibres, there is no ultrastructural evidence for denervation at the end-plates. There is agglutination of synaptic vesicles, neurotubules and filaments, thickening of the basement membrane, widening of the primary synaptic cleft, and irregular branching of the junctional infoldings, but no axonal degeneration.

3. The contractile process in senile muscles is slowed down as is indicated by a prolongation of contraction time, latency period, maximum rate of twitch tension and relaxation time.

4. The muscle fibres show proliferation of the T system and increased SR but no fragmentation as is observed in denervation atrophy.

5. Senile muscle atrophy thus presents some specific features affecting both pre- and post-synaptic structures, related to a very slow process of deterioration of the neuromuscular contact.

INTRODUCTION

Senile muscle atrophy may represent a special type of muscle atrophy, as the total number of muscle fibres decreases in old age, without a corresponding reduction of spinal motor neurones (Gutmann & Hanzlíková, 1966). In an attempt to define the characteristics of senile muscle atrophy and to answer the question whether muscles undergoing this type of atrophy exhibit features of muscles after denervation, the following aspects were studied: frequency of spontaneous transmitter release, efficiency of neuromuscular transmission, basic characteristics of the post-synaptic membrane such as resting potential, action potential and chemosensitivity of the muscle membrane as well as the contractile properties and the ultrastructural picture of the muscle and the neuromuscular junction.

The aim of this paper was to correlate physiological and ultrastructural characteristics of muscles in young and old rats.

METHODS

Electrophysiological observations

The levator ani (LA) muscles of 2-month and 30-month-old male rats were dissected free and ligatures were applied to the bulbocavernosus ventralis muscle which overlaps the LA muscle. This thin muscle is very suitable for *in vitro* studies. The muscle was stretched to 130% of its resting length in a chamber containing an oxygenated perfusion solution (Liley, 1956) with the following composition (mM): Na^+ 149·8, K^+ 5·0, Ca^{2+} 2·0, Mg^{2+} 1·0, Cl^- 148·0, HCO_3^- 12·0, $H_2PO_4^-$ 1·0, glucose 11·0, pH 7·2, at 20° C. A sufficiently long stretch of the pudendal nerve was isolated and prepared for stimulation. Intracellular micro-electrodes filled with 3 M-KCl (resistance 10–20 MΩ) were used to examine the individual muscle fibres. The end-plates in this muscle are restricted to a narrow band in the middle of the lateral half of the muscle a few mm on either side of the nerve which can easily be detected using the stereomicroscope. In this region it is rather easy to find sites with small transient depolarizations (m.e.p.p.s.). The m.e.p.p.s were monitored on a Tektronix oscilloscope and their frequency per second was counted for 100–300 sec from each fibre by a Tesla decadic counter. To evoke action potentials the pudendal nerve was stimulated by pulses of supramaximal intensity (0·1–1·0 msec) at a frequency of 0·5/sec. The muscle fibres potentials were monitored on a Tektronix oscilloscope (Type 502). In this case 'floating' micro-electrodes were used (Vaughan Williams, 1959) enabling records from contracting fibres.

ACh sensitivity of the post-synaptic muscle membrane was tested by iontophoretic application from micropipettes filled with 4 M-ACh (resistance 10–15 MΩ), by a method similar to that described by Nastuk (1953) and del Castillo & Katz (1955). The ACh pipette was then moved in both directions from the end-plate region leaving the recording electrode in the focal end-plate region. ACh sensitivity was then at individual loci expressed as the amplitude of the transient membrane depolarization per unit charge passed through the ACh pipette, i.e. mV depolarization per nano-Coulomb (Miledi, 1960).

Contractility

The contractile properties of the LA and the extensor digitorum longus (EDL) muscles from young and old animals were studied *in vitro*. The muscles were first equilibrated for 10 min in oxygenated Liley solution containing 10^{-4} M tubocurarine chloride (Merck). The muscles were set up in a chamber with Pt electrodes for massive stimulation (Sandow & Brust, 1958) at a temperature of 37° C. Resting tension for isometric recording i.e. the initial tension, was preset so as to obtain maximal twitch tension to direct stimulation and varied from 0·5 to 2·0 g in individual cases. The twitch responses were monitored on an oscilloscope and recordings of full contraction time (time to peak), half contraction time, latency period (stimulus artefact to first mechanical response), half-relaxation time (from peak to half of amplitude of contraction) and of maximal rate of twitch tension development were made by an automatic analyser of muscle contraction properties (Rohlíček, 1968). The maximal rate of tension development is expressed as a time constant, describing the speed of tension development in msec in the middle part of the ascending contraction curve (25–75% of the ascending curve) where a linear slope can be assumed.

Ultrastructural observations

For electron microscopical observations the LA, EDL and soleus muscles of young and old rats were fixed for 2 hr in cold 3·5 % glutaraldehyde (0·1 M phosphate buffer, pH 7·2), or in 3·5 % glutaraldehyde ($CaCl_2$ 2mM and cacodylate buffer 0·1 M), and post-fixed (for 1 hr) in 1 % OsO_4 (0·1 M phosphate buffer at pH 7·2). After dehydration in alcohols the pieces were embedded in Epon 812. For better identification of motor end-plates the method for cholinesterase activity (Koelle & Friedenwald, 1949) was used. The pH of the incubation medium was 4·8 and the muscles were incubated for 15–30 min at room temperature. The parts of the muscles with clearly visible end-plates were cut using a stereomicroscope. Epon embedded pieces were cut for ultrathin sections on a Porter-Blum microtome, stained with uranyl-acetate and/or lead citrate and examined in a Tesla BS 813 microscope at 80 kV.

RESULTS

Resting potential and impulse transmission

Table 1 and Text-fig. 1 show that there is no significant difference in the resting membrane potential between LA muscle fibres of young and old animals. The muscle is a fast muscle (Čihák, Gutmann & Hanzlíková, 1970) and the values correspond to other fast muscles of the rat recorded by others (e.g. Albuquerque & McIsaac, 1970). All the muscle fibres of the old LA muscle tested responded with an action potential and contraction to stimulation of the pudendal nerve. Thus no denervated muscle fibres could be detected. There is also no difference in amplitude, rise time of the action potential (from 5 % of spike amplitude) and its duration at zero level.

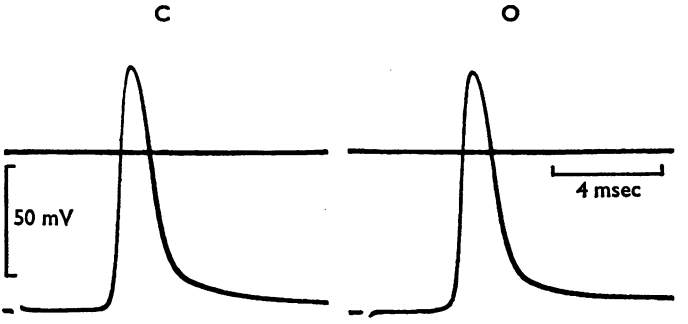
TABLE 1. Some electrophysiological characteristics of levator ani muscle fibres from normal (C) and old animals (O). Numbers in brackets indicate numbers of evaluated fibres or potentials

	C	O
Resting potential	78·7 ±0·59 (75)	77·3 ±0·5 (33)
Action potential		
a, amplitude (mV)	110·0 ±2·1 (22)	109·1 ±·8 (25)
b, rise time from 5 % of amplitude to pike (msec)	0·93±0·19 (22)	0·87±0·11 (25)
c, width at zero point (msec)	1·12±0·3 (22)	1·07±0·23 (25)
Min. e.p.p.s		
a, frequency (sec^{-1})	1·69±0·19 (5931)	0·30±0·05 (1888)
b, amplitude (mV)	0·78±0·20 (98)	1·69±0·23 (40)
c, rise time (msec)	0·90±0·17 (20)	0·92±0·28 (20)
d, half-decay time (msec)	1·70±0·25 (20)	1·52±0·29 (20)

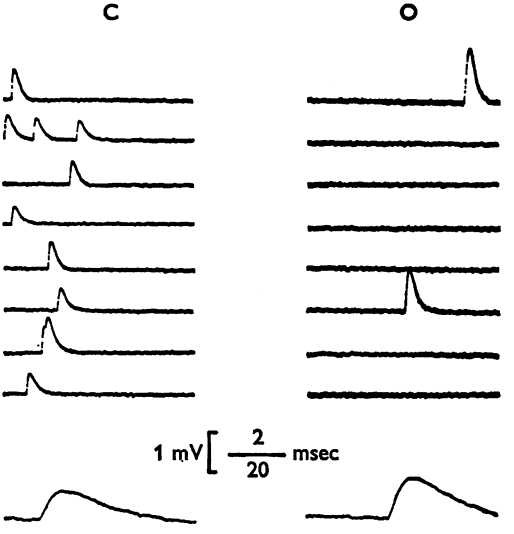
Spontaneous end-plate activity

The spontaneous activity of nerve terminals was studied on three LA preparations from 30-month-old rats. Miniature end-plate potential (m.e.p.p.s) characteristics of the neuromuscular junction were observed in

twenty out of twenty-two muscle fibres studied. However, as is shown in Table 1 and Text-fig. 2, there is a marked decrease in frequency of the m.e.p.p.s in the muscle fibres of 30-month-old animals in comparison with the young animals (fifteen fibres from three rats). This means that the

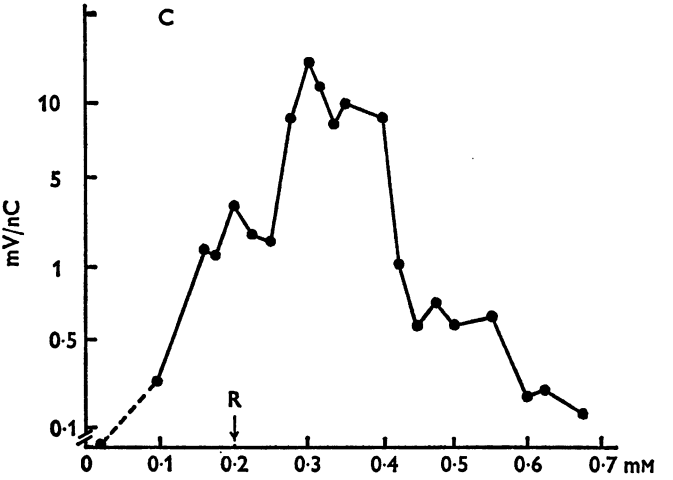


Text-fig. 1. Oscillographic recordings of the action potentials of muscle fibres of the levator ani muscle. C: control muscle (3 months). O: old animal (30 months). The line represents the zero potential difference between the inside and outside of the fibre. Recordings at room temperature (20° C).

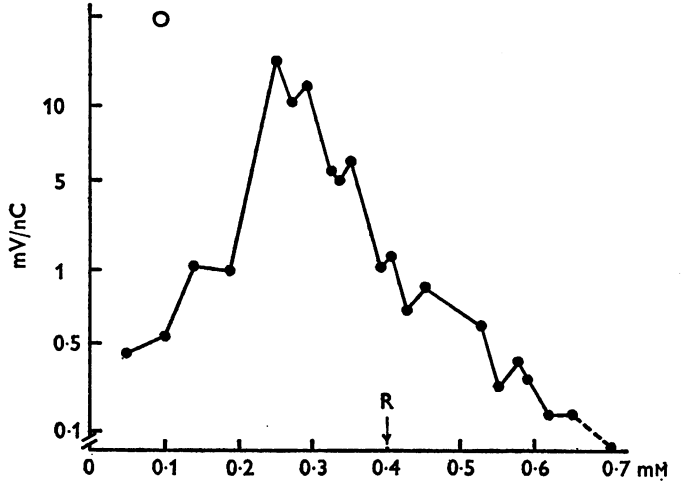


Text-fig. 2. Oscillographic recordings of m.e.p.p.s from a muscle fibre of a 3 month old (C) and 30 month old (O) animal. Recordings at room temperature (20° C).

release of quantal packets of ACh from nerve endings is markedly reduced but there is no complete arrest of spontaneous transmitter release, as observed in denervation (see Miledi & Slater, 1968; Albuquerque &



Text-fig. 3. Sensitivity of the surface of muscle fibres of the levator ani muscle to ACh. C: normal muscle fibre (3 month old animal). Abscissa: part of muscle fibre near the end-plate explored by the iontophoretic micropipette in mM. Ordinate: sensitivity expressed as ratio of amplitude of the ACh (responses to nC passing through the micropipette.) The same micropipette was used in both cases. R, relative position of the recording micro-electrode on the muscle fibre. Four fibres from the normal muscle and five fibres of the old animals were explored. The length of the sensitive zone does not exceed 1–1·2 mm in both cases.



Text-fig. 4. See legend to Text-fig. 3. O: muscle fibre of 30-months old animal.

McIsaac, 1970). The m.e.p.p.s have a tendency to appear in small bursts in muscle fibres of both young and old animals. Due to the decrease of muscle fibre diameter (see Rowe, 1969) the muscle fibres of old animals have a higher amplitude, but there are no differences between individual potentials as far as the rise or decay time is concerned in muscle fibres of young and old animals.

ACh sensitivity of the LA muscle fibres

The ACh sensitivity of the surface membrane was tested in four muscle fibres from two young animals and five fibres of three old animals. The area of maximal ACh sensitivity in the LA muscle was found at the point of branching of the pudendal nerve, i.e. at the end-plate region and was of the order of 10–12 mV/nC in both muscle fibres of old and young animals (see Text-figs. 3, 4). A similar loss of sensitivity was recorded in muscle fibres of young and old animals after moving away the ACh pipette to a distance of more than 1 mm from the recording electrode at the end-plate region. In muscle fibres of young animals, similarly as in those of old animals, the total ACh-sensitive zone did not exceed 1–2 mm. Thus it is evident that there was no change in sensitivity to ACh in the muscle fibres of old animals. In spite of the highly reduced frequency of transmitter release there is no spreading of the receptor area and no increase in ACh sensitivity as it is to be observed after denervation, i.e. after complete loss of transmitter release (Axelsson & Thesleff, 1959; Birks, Katz & Miledi 1960; Miledi, 1962).

TABLE 2. Twitch contraction properties of the levator ani (LA) and extensor digitorum longus (EDL) muscle of young (1 month) (C) and old (O) (30 months) animals. Full contraction time (FCT), half-contraction time (HCT), latency period (LP), maximal rate of twitch tension development (TCC) and half-relaxation time (HRT) in msec after direct stimulation *in vitro* at 37° C

		n	FCT	HCT	LP	TCC	HRT
LA	C	10	12·1 ± 0·1	6·7 ± 0·1	3·1 ± 0·1	3·5 ± 0·1	10·8 ± 0·3
	O	7	15·4 ± 1·0	8·0 ± 0·3	3·6 ± 0·1	4·3 ± 0·4	14·6 ± 1·3
EDL	C	12	11·6 ± 0·5	6·7 ± 0·2	2·9 ± 0·1	3·3 ± 0·2	11·8 ± 0·9
	O	8	17·6 ± 1·0	8·0 ± 0·4	3·1 ± 0·2	5·1 ± 0·4	19·2 ± 1·6

Contractile properties of muscle of old animals

The properties of a twitch contraction were determined in two muscles, the LA and the EDL in young (1 month) and old (30 months) animals. Table 2 shows, that there is a significant prolongation of full and half contraction time, latency period, maximal rate of tension development and half-relaxation time in old animals. The trend of the changes is very similar

in both muscles, though the latency period increases more in LA than in EDL muscle. The considerable prolongation of contraction time (see also Syrový & Gutmann, 1970), relaxation time and of maximal rate of twitch tension development appears to be a typical sign of senile muscle atrophy.

Electron microscopic structure of senile muscle

Characteristic feature of senile muscle atrophy is a marked reduction of muscle fibre and fibril size with occasional protrusion of sarcolemma into the extracellular space and proliferation of the sarcotubular system with the occurrence of pentads. The sarcolemma appears to be thickened and there is a considerable increase of collagenous fibrils and filaments of different width, lying in the extracellular space. The increased activity of the plasma membrane is evident, there is extensive proliferation of the tubular T-system beneath the sarcolemma (Pl. 1, fig. 1). Diadic couplings of SR elements with the sarcolemma, observed until now only in the developing stage (Schiaffino & Margreth, 1969) but never in mature rat skeletal muscles are also found in senile muscles (Pl. 1, fig. 2).

Transverse sections of the muscle fibre show the abnormal longitudinal distribution of flattened junctional cisternae, surrounding almost continuously whole myofibrillar units (Pl. 1, fig. 3). Parallel oriented multiple terminal cisternae of the SR form very close junctions (Pl. 2, fig. 4). Apparently at the periphery, rapid disorganization and later disintegration of myofilaments sets in coupled with marked proliferation of the SR and increased ribosomal activity (Pl. 2, fig. 5). In this chaotic picture conspicuous enlargement of SR cisternae is prominent, as is seen at later stages after denervation (Schiaffino & Settembrini, 1970), in periodic paralysis (Mussini, Di Mauro & Margreth, 1968) and also in other pathological conditions. High accumulation of ribosomes and polysomes is found in the subsarcolemmal zone. In some muscle fibres a small part of sarcoplasm beneath the sarcolemma contains free filaments, apparently newly formed myofilaments, triads, microtubules and developing Z lines resembling the embryonal type of muscle fibres (Pl. 2, fig. 6).

On the other hand there is considerable disintegration of cellular material at peripheral regions of the muscle fibre apparently related to a process of autophagy, associated with the appearance of autophagic vacuoles, multivesicular bodies, abundant vesicles and increased pinocytic activity (Pl. 2, fig. 7). In the centre of the fibres, however, the alignment and regularity of myofibrillar organization is remarkably well preserved. The Z line is regular with no disorder of adjacent filaments. In the large nuclei high condensation of chromatin is found at the periphery with frequent nucleoli and numerous pores in the nucleolemma. Satellite cells are seen fairly frequent.

End-plate changes in senile muscle fibres

Pl. 3, fig. 8 shows a normal LA end-plate in a young animal. No disintegration of the terminal axons could be found in the end-plates of senile muscle fibres. The number of synaptic vesicles appears to be increased in many terminals, the vesicles accumulate and a picture of 'agglutination' of vesicles is seen comparable to that observed in early stages of denervation before axonal disintegration sets in (Nickel & Waser, 1968). Circular myelin figures may be found in the terminal axons. Many Schwann cell processes surround the external axonal surface, but no interposition of Schwann cell between nerve and muscle, found in early stages of denervation (Miledi & Slater, 1970) is found. The junctional folds are more branched, the basement membrane in the enlarged synaptic clefts is thickened conspicuously in some places and filamentous structure can be recognized (Pl. 3, fig. 9). There are many neurotubules and some neurofilaments in the peripheral axons (Pl. 3, fig. 10). These are never found in normal adult end-plates. The primary synaptic clefts are considerably enlarged. Formation of collagen fibrils is increased at the end-plate region. The subplasmolemmal density is increased in all senile end-plates.

Thus we may conclude that no disintegration of axons can be found at the end-plate region; however, some synaptic disturbances are indicated by accumulation and 'agglutination' of synaptic vesicles, by the presence of neurotubules and neurofilaments in the terminal axons and by certain disturbances in the organization of synaptic infoldings. However, there is no indication of denervation as described after nerve section. The changes leading to loss of muscle fibres with the axons remaining intact for a long time, appear to be the result of a slow reduction of synaptic contact, a phenomenon possibly related to the changes in end-plates having fulfilled a limited life span (Barker, 1966).

DISCUSSION

Senile muscle atrophy presents apparently some specific features related to an extremely slow process of deterioration of neuromuscular contact. These features concern both pre- and post-synaptic structures. We did not find complete break-down of terminal axons and there is also no electrophysiological evidence of denervation, e.g. fibrillation potentials in muscles of old age (Carlson, Alston & Feldman, 1964) nor decrease of resting membrane potential or loss of neuromuscular transmission. The marked reduction of frequency of spontaneous transmitter discharge and some ultrastructural changes at the end-plates present disturbances, allowing senile muscle atrophy to be defined as a specific entity. There is no terminal axonal degeneration. However, the accumulation and 'agglutination' of

vesicles, the presence or neurotubules and filaments at the terminals, the enlargement of primary synaptic clefts, the thickening of the basement membrane and the irregular branching of the infoldings present characteristic features of the ultrastructure of the senile end-plates. There is also no extension of Schwann cells into the synaptic cleft as observed in early stages of denervation and no withdrawal of Schwann cells as observed in later stages of denervation (Miledi & Slater, 1968; Miledi & Slater, 1970). There is also no absolute loss of spontaneous transmitter release as in the denervated muscle (Miledi & Slater, 1963; Slater, 1966; Albuquerque & McIsaac 1970).

The m.e.p.p.s in the muscles of old animals have a larger amplitude and this is apparently due to an increase in the input impedance of the fibre owing to a reduction of its diameter. The quantal content of the end-plate potentials was not determined in this work. It is, however, unlikely that even a considerable reduction of quantal content could abolish the action potential in view of the decrease of muscle fibre diameter. These questions have still to be answered.

We have not been able to register the final event, i.e. the ultimate loss of neuromuscular contact, which might have been expected in the light of earlier findings showing some analogous changes in denervated and senile muscles (Gutmann, Hanzlíková & Jakoubek, 1968) and the decrease of number of muscle fibres in old age (Gutmann & Hanzlíková, 1966). However, the extreme slowness and the relatively small number of fibres involved at a given period may help to explain this discrepancy.

When discussing loss of neuromuscular contact it might be pointed out that transmitter release alone cannot be responsible for all 'trophic functions' of the nerve cell mediating maintenance of the innervated tissues (see Gutmann, 1962; Miledi 1963; Guth, 1968). Other so far not defined chemical agents synthesized in the nerve cell and transported to the muscle cell participate in these functions. This is also indicated, for instance, by the finding of normal spontaneous transmitter release in the highly atrophic LA muscle after castration (Vyskočil & Gutmann, 1969).

The process leading to reduction of number of muscle fibrils proceeds apparently very slowly until complete break-down of the contractile material in a single muscle fibre and applies especially to the peripheral myofibrils of the muscle fibre in which disorganization and loss can be detected.

Muscle changes in old age concern all structures, some of them being specific, some of them presenting general reactions of the muscle cell to neuromuscular disturbance. The proliferation of the T system tubules in senile muscle is morphologically similar to the overproduction of the T system tubules in denervated skeletal muscle (Pellegrino & Franzini, 1963; Muscatello, Margreth & Aloisi, 1965), but also to the development of

tubular units which are found in cultured skeletal muscle of the chick embryo (Ishikawa, 1968) and to a tubular system described during the early post-natal stages of development of the rat skeletal muscle (Schiaffino & Margreth, 1969). A peculiar overproduction of tubules of the T system, forming a labyrinthine structure was also observed in the LA muscle after castration (Gori, Pellegrino & Pollera, 1967).

We can thus see that the membrane reactivity is very high, not only during muscle differentiation but also after denervation, castration and in old age and is therefore a general reaction of the muscle cell. The system of sarcoplasmic reticulum in senile muscles appears to be more labile, as can be observed in the occurrence of parallel multiple terminal cisternae or flattened junctional cisternae. The formation of autophagic vacuoles and cytosegresomes (see Ericsson, 1969), however, does not represent a highly active mechanism in comparison with the lysosomal system found after perinatal denervation (Schiaffino & Hanzlíková, 1971).

Characteristic features resembling embryonal muscle are found in senile muscle. Newly formed free thin filaments, microtubules, many ribosomes, polysomes and satellite cells are found in the subsarcolemmal (so far not definitely differentiated) zone of the muscle fibre. They may present a reaction of compensatory 'abortive' regeneration, not seen in denervation atrophy. There was also no fragmentation of muscle fibres with subsequent degeneration of the fragments as observed in denervation atrophy (Bowden & Gutmann 1944; Miledi & Slater, 1969).

The increase in SR and the proliferation of the T system may lead to delayed Ca release and explain partly the increase of the latency period in senile muscle. Changes in contractile proteins found especially in the peripheral part of the muscle fibre apparently lead to a slowing of speed of contraction together with a decrease of ATPase activity in senile muscle (see Syrový & Gutmann, 1970).

Thus there are changes in pre- and post-synaptic structures resulting in senile muscle atrophy and explaining the typical disorders of the locomotor system in old age (see McKeown, 1965). This senile muscle atrophy presents, however, some specific features, characterized especially by a decrease of release of transmitter and of other neurotrophic agents.

The authors are grateful to Mrs L. Vedralová, Mr Z. Vít and Mrs A. Herbrychová for their technical assistance.

This work was aided by a grant from Muscular Dystrophy Association of America, Inc.

REFERENCES

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

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

BARKER, D. (1966). Sprouting and degeneration of mammalian motor axons in normal and de-afferentated skeletal muscle. *Proc. R. Soc.* B **163**, 538–554.

BIRKS, R., KATZ, B. & MILEDI, R. (1960). Physiological and structural changes at the amphibian myoneural junction, in the course of nerve degeneration. *J. Physiol.* **150**, 145–168.

BOWDEN, R. E. M. & GUTMANN, E. (1944). Denervation and reinnervation of human voluntary muscle. *Brain* **67**, 273–313.

CARLSON, K. E., ALSTON, W. & FELDMAN, D. J. (1964). Electromyographic study of aging in skeletal muscle. *Am. J. Phys. Med.* **43**, 141–145.

ČIHÁK, R., GUTMANN, E. & HANZLÍKOVÁ, V. (1970). Involution and hormone induced persistence of the M. sphincter (levator ani) in female rats. *J. Anat.* **106**, 93–110.

DEL CASTILLO, J. & KATZ, B. (1955). On the localization of acetylcholine receptors. *J. Physiol.* **128**, 157–181.

ERICSSON, J. L. E. (1969). Mechanism of cellular autophagy. In *Lysosomes in Biology and Pathology* (2), Frontiers of Biology: vol. 14B, ed. DINGLE, J. T. FELL, H. B. Amsterdam: North-Holland Research Monographs.

GORI, Z., PELLEGRINO, C. & POLLERA, M. (1967). The castration atrophy of the dorsal bulbocavernosus muscle of rat: an electron microscopic study. *Expl molec. Path.* **6**, 172–198.

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

GUTMANN, E. (1962). *The Denervated Muscle.* Prague: Czechoslovak Academy of Sciences.

GUTMANN, E. & HANZLÍKOVÁ, V. (1966). Motor unit in old age. *Nature, Lond.* **209**, 921–922.

GUTMANN, E., HANZLÍKOVÁ, V. & JAKOUBEK, B. (1968). Changes in the neuromuscular system during old age. *Expl Geront.* **3**, 141–146.

ISHIKAWA, H. (1968). Formation of elaborate networks of T-system tubules in cultured skeletal muscle with special reference to the T-system formation. *J. cell Biol.* **38**, 51–66.

KOELLE, G. B. & FRIEDENWALD, Y. (1949). A histochemical method for localizing cholinesterase activity. *Proc. Soc. exp. Biol. Med.* **70**, 617–622.

LILEY, A. W. (1956). An investigation of spontaneous activity at the neuromuscular junction of the rat. *J. Physiol.* **132**, 650–660.

MCKEOWN, F. (1965). *Pathology of the Aged.* London: Butterworths.

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

MILEDI, R. (1962). Induction of receptors. In *Ciba Foundation Symposium on Enzymes Drug Action*, pp. 220–235. : .

MILEDI, R. (1963). An influence of nerve not mediated by impulses. In *The Effect of Use and Disuse on Neuromuscular Functions*, ed. GUTMANN, E. & HNÍK, P. p. 41. Prague: Czechoslavak Academy of Sciences.

MILEDI, R. & SLATER, C. R. (1963). A study of rat nerve muscle junctions after degeneration of the nerve. *J. Physiol.* **169**, 23–24*P*.

MILEDI, R. & SLATER, C. R. (1968). Electrophysiology and electronmicroscopy of rat neuromuscular junctions after nerve degeneration. *Proc. R. Soc.* B **169**, 289–306.

MILEDI, R. & SLATER, C. R. (1969). Electron-microscopic structure of denervated skeletal muscle. *Proc. R. Soc.* B **174**, 253–269.

MILEDI, R. & SLATER, C. R. (1970). On the degeneration of rat neuromuscular junctions after nerve section. *J. Physiol.* **207**, 507–528.

MUSCATELLO, U., MARGRETH, A. & ALOISI, M. (1965). On the differential response of sarcoplasm and myoplasm to denervation in frog muscle. *J. cell Biol.* **27**, 1–24.

MUSSINI, I., DI MAURO, S. & MARGRETH, A. (1968). *Hypertrophy and Proliferative Changes of the SR in Periodic Paralysis.* Rome: IV. Europ. Reg. Conf. on Electron-microscopy.

NASTUK, W. L. (1953). Membrane potential changes at a single muscle end-plate produced by transitory application of acetylcholine with an electrically controlled microjet. *Fedn Proc.* **12**, 102.

NICKEL, E. & WASER, P. G. (1968). Electronmikroskopische Untersuchungen am Diaphragm der Maus nach einseitiger Phrenikotomie. *Z. Zellforsch. mikrosk. Anat.* **88**, 278–296.

PELLEGRINO, C. & FRANZINI, C. (1963). An electronmicroscope study of denervation atrophy in red and white skeletal muscle fibres. *J. cell Biol.* **17**, 327–349.

ROHLÍČEK, V. (1968). An automatic analyzer of muscle contraction. *SNTL tech. Dig.* **61**, 383–387.

ROWE, R. W. D. (1969). The effect of senility on skeletal muscles in the mouse. *Expl Geront.* **4**, 119.

SANDOW, A. & BRUST, M. (1958). Contractility of dystrophic mouse muscle. *Am. J. Physiol.* **194**, 557–561.

SCHIAFFINO, S. & HANZLÍKOVÁ, V. (1971). Studies on the effect of denervation in developing muscle. II. Lysosomal system (in the press).

SCHIAFFINO, S. & MARGRETH, A. (1969). Coordinated development of the sarcoplasmic and T system during postnatal differentiation of rat skeletal muscle. *J. cell Biol.* **41**, 855–875.

SCHIAFFINO, S. & SETTEMBRINI, P. (1971). Studies on the effect of denervation in developing muscle. I. Differentiation of the sarcotubular system. *Virchows Arch. path. Anat. Physiol.* **4**, 345–356.

SLATER, C. R. (1966). Time course of failure of neuromuscular transmission after motor nerve section. *Nature, Lond.* **209**, 305–306.

SYROVÝ, I. & GUTMANN, E. (1970). Changes in speed of contraction and ATPase activity in striated muscle during old age. *Expl Geront.* **5**, 31–35.

VAUGHAN-WILLIAMS, E. M. (1959). A method of mounting micro-electrodes for intracellular recording from contracting muscle. *J. Physiol.* **147**, 3*P*.

VYSKOČIL, F. & GUTMANN, E. (1969). Spontaneous transmitter release from motor nerve endings in muscle fibres of castrated and old animals. *Experientia* **25**, 945–946.

EXPLANATION OF PLATES

PLATE 1

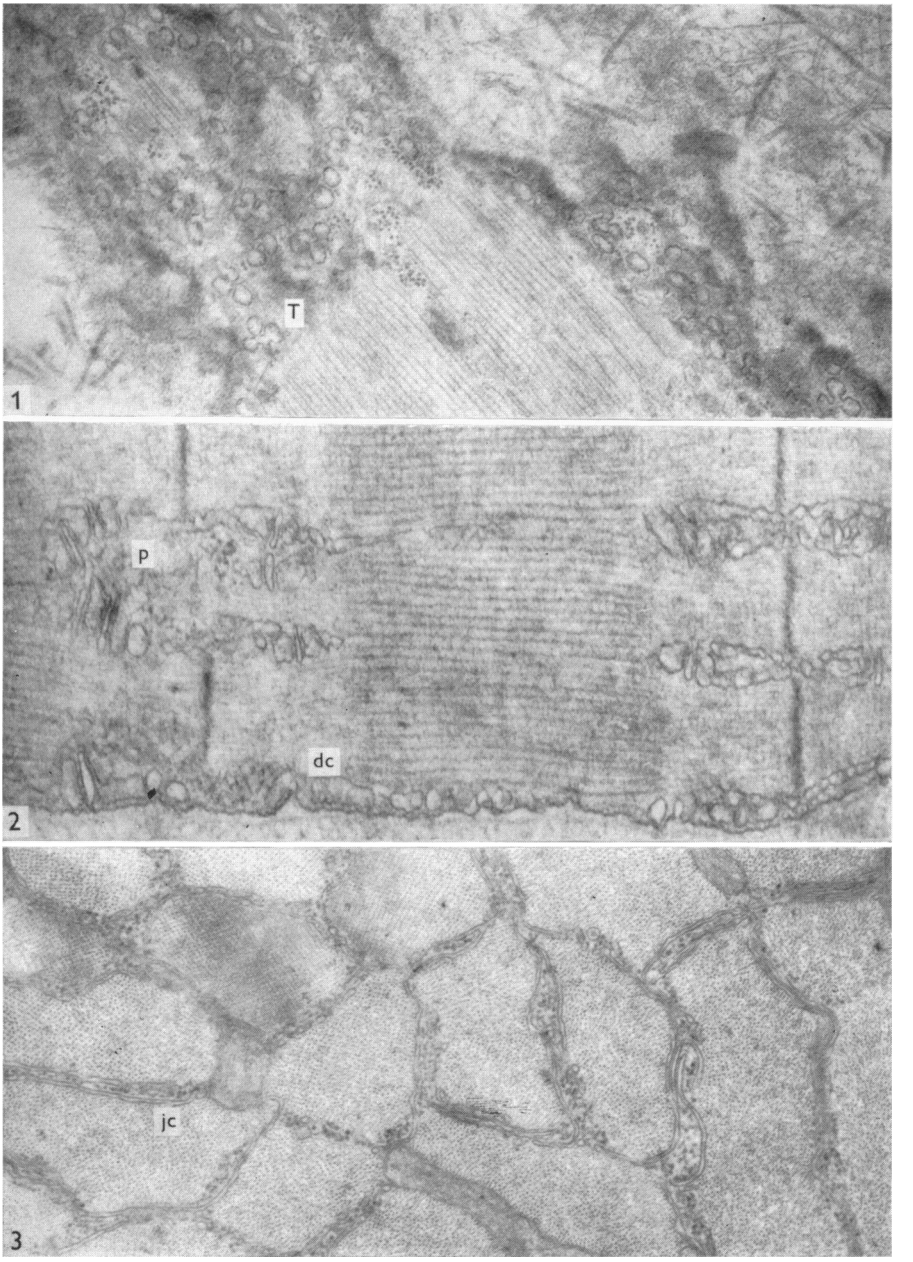
Fig. 1. Longitudinal section of senile LA muscle illustrating conspicuous activity of the tubular T system. Note trifoliate structures in the subsarcolemmal region. There are many disorganized collageneous fibres and various filaments near the muscle fibre; T, T system. Magnification, × 22,500.

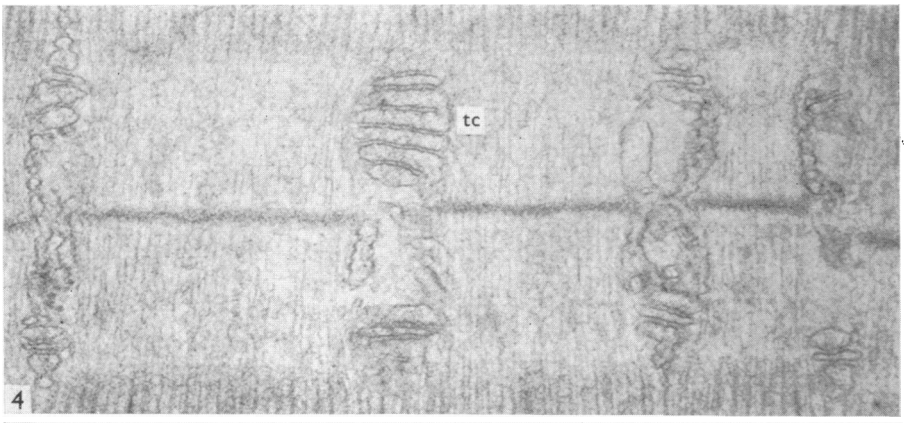
Fig. 2. Senile LA muscle. Diadic couplings of terminal cisternae of the sarcoplasmic reticulum with sarcolemma. Note pentad in neighbouring myofibril; dc, diadic coupling, p, pentad. Magnification, × 33,300.

Fig. 3. Transverse section of senile extensor digitorum longus muscle of 30-months old rat. Flattened junctional cisternae embracing myofibrils; jc, junctional cisterna. Magnification, × 22,500.

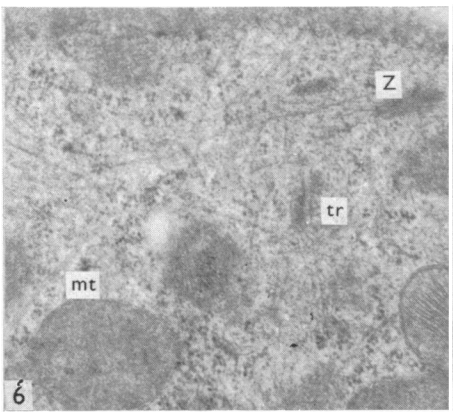
PLATE 2

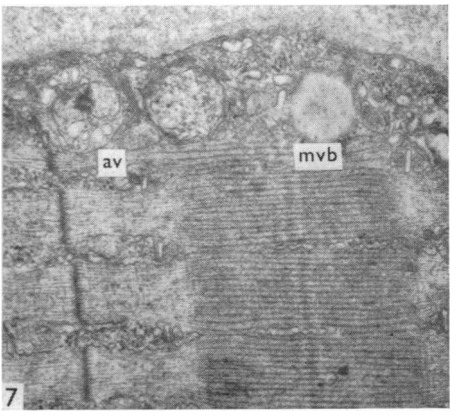
Fig. 4. Longitudinal section of senile LA muscle, showing parallel arrangement of the terminal cisternae; tc, terminal cisternae. Magnification, × 38,750.


T
1
P
dc
2
jc
3

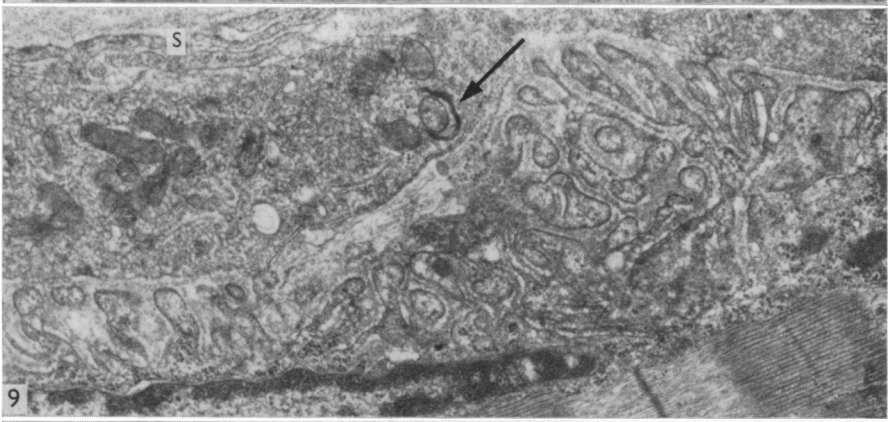

tc
4


tc
ps
sr
5


Z
tr
mt
6


av
mvb
7


ta
ji
8


S
9

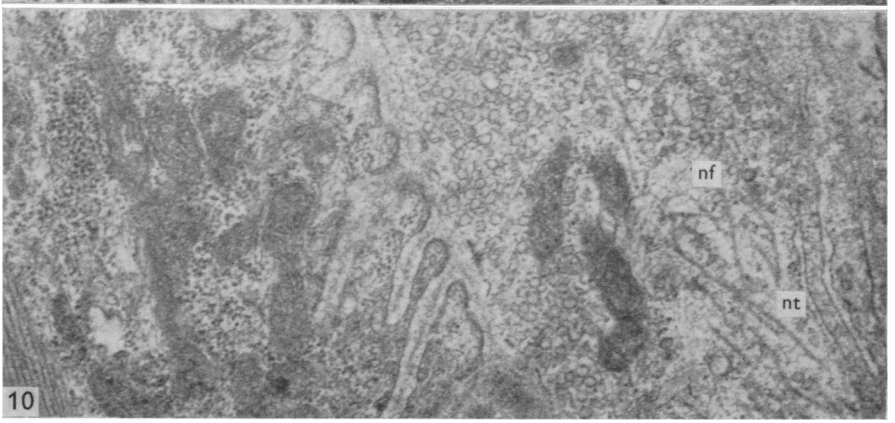

nf
nt
10

Fig. 5. Peripheral part of the senile LA muscle fibre with disorganized myofilaments in disrupted sarcomeres, abundant ribosomes and polysomes, enlarged terminal cisternae and network of tubules of the sarcoplasmic reticulum; ps, spiral configuration of polysomes; tc, terminal cisternae; sr, sarcoplasmic reticulum. Magnification, × 28,500.

Fig. 6. Subsarcolemmal part of senile soleus muscle resembling embryological growth. Sarcoplasm filled with many ribosomes, polysomes, thin free filaments, microtubules, triads, Z line and round mitochondria; mt, microtubule; tr, triad; Z, Z line. Magnification, × 22,500.

Fig. 7. Autophagic process in the peripheral part of the senile LA muscle fibre. Autophagic vacuole with membranous remnants, multivescular bodies, differing in size and number of vesicles; av, autophagic vacuole; mvb, multivesicular body. Magnification × 22,500.

PLATE 3

Fig. 8. Normal end-plate in LA muscle fibre of young animal (6 months) showing the characteristic components, Schwann cell processes enveloping terminal axons, basement membrane and regular infoldings into sarcoplasm of muscle fibre, Golgi apparatus and granular endoplasmic reticulum; ta, terminal axon: ji, junctional infoldings. Magnification, × 24,000.

Fig. 9. End-plate in senile LA muscle fibre. Axonal terminals, surrounded by Schwann cell processes show the synaptic vesicles, mitochondria and myelin figures (arrow). Junctional infoldings are more branched and the basement membrane is thickened; S, Schwann cell. Magnification, × 13,500.

Fig. 10. End-plate of senile LA muscle fibre. Terminal axons filled with neurotubules, some neurofilaments and vesicles. Enlargement of the primary synaptic cleft. Subsarcolemmal, many ribosomes and mitochondria; nt, neurotubules; nf, neurofilaments. Magnification, × 25,000.