Muscle weakness with ageing is almost inevitable, generally beginning to manifest beyond the age of 40, and is usually unstoppable. It can lead to reduced mobility, increased risk of falling, injury, and even death. But “you’re just getting old” is not a sufficient diagnosis. Specific causes of neuromuscular symptoms may explain progressive muscle weakness, and should be investigated for potential treatment.

Muscle Ageing, Inclusion-Body Myositis and Myopathies explores the clinical and pathological expression of muscle weakness in aging persons. Case studies demonstrate how physicians can more accurately diagnose weakening elderly patients and make better management decisions.

It also explores sporadic inclusion-body myositis and hereditary inclusion-body myopathies. The former, the most common progressive muscle disease in the over 50s, is frequently under-diagnosed and, with the increasing population of aged individuals, is presenting a greater challenge. This disease of muscle has pathological similarities with the well-known Alzheimer and Parkinson brain diseases.

Edited and written by a leading international cast of authors, Muscle Ageing, Inclusion-Body Myositis and Myopathies provides a state-of-the-art guide to ageing-associated neuromuscular disorders. It should be in the hands of all those involved in the care of aging and muscle-weakened patients.

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Preface

This book contains chapters by internationally recognized experts and covers two currently very important topics: muscle aging, and sporadic inclusion-body myositis, the most common aging-associated muscle disease. Also described are hereditary inclusion-body myopathies, which are genetically determined disorders pathologically rather similar to sporadic inclusion-body myositis but which become clinically manifest in early, or sometimes later, adulthood.

Human muscle aging causes a gradual enfeeblement of older persons, and is progressively evident from about age 40 onwards. It causes muscle weakness and frailty of the elderly, resulting in falling and ensuing complications. The cost of caring for weakening older persons in the USA and around the world is escalating. There is a striking paucity of information related to human muscle aging.

This book is focused on various aging-associated neuromuscular disorders at the cellular and clinical levels. These are exemplified by the characteristic histochemical abnormalities seen in older patients’ muscle biopsies, and in a number of clinical vignettes of representative aging patients.

Emphasis is given to various treatable, and not-yet-treatable, human conditions associated with aging. We stress that the phrase “you are just getting old” is not an acceptable medical diagnosis, and that aging is not a satisfactory explanation for the cause of neuromuscular symptoms. Nevertheless, aging certainly is a risk factor for a number of disorders. A full neuromuscular evaluation is needed to properly analyze and diagnose a neuromuscular problem in an aging patient, as the basis for providing the best possible treatment. Herein we describe some examples of successful treatments, but much more remains to be done to help aging neuromuscular patients. Presented are the current knowledge of these aspects and new concepts intended to help development of innovative treatments. For the not-yet-treatable neuromuscular disorders associated with aging, the goal should be to not only stop their relentless progressive weakness, but to provide enduring improvement.

Sporadic inclusion-body myositis is the most common progressive muscle disease of older persons, age 50 and above. As the world population ages, sporadic inclusion-body myositis is becoming more prevalent and a significant health hazard. It causes increasingly severe muscle weakness, leading relentlessly to pronounced disability, including frequent falls and resultant injuries, inability to arise from a chair or toilet, or to grip a fork, spoon, or drinking glass. Swallowing difficulties and choking can occur. Sporadic inclusion-body myositis is generally underdiagnosed, and it is often misdiagnosed.

In this book are presented numerous details of the newest molecular mechanisms involved in the pathogenesis of sporadic inclusion-body myositis. These remarkable discoveries have not yet led to enduring treatment, but they are providing important leads toward that goal. Of urgent importance, therefore, is further clarification of the molecular pathogenesis of this disease, including learning the ultimate upstream cause, as well as details of the downstream muscle-destroying cellular molecular mechanisms.

Also of special interest – to general neurologists, neuroscientists, and gerontologists, as well as to internists and general physicians, nurses, and physical therapists, and to especially-curious patients, caregivers, and members of the general public – are the very intriguing, remarkable similarities between the special pathologic features of muscle fibers in sporadic inclusion-body myositis and pathologic...
features of brains of patients with Alzheimer disease and Parkinson disease. The similarities between sporadic inclusion-body myositis and those two most common neurodegenerative diseases of older persons suggest that aspects of the molecular pathogenic mechanisms may be extremely similar, or even in some aspects the same.

Specifically, the similarities of sporadic inclusion-body myositis with the Alzheimer brain include accumulations of amyloid-β, phosphorylated tau and numerous other “Alzheimer disease-characteristic” proteins. The similarities of inclusion-body myositis with Parkinson disease include accumulations of α-synuclein, parkin, DJ-1, and other abnormalities. These similarities suggest that (a) the aging-associated degenerative-muscle and degenerative-brain diseases may share certain pathogenic steps and (b) knowledge of one disease might help elucidate the cause and treatment of the others. And, despite the remarkable molecular similarities in those very different tissues, muscles and brain (the movers and the thinker), the separate muscle and brain diseases appear to never cross into the territory of the other. What protects the sporadic inclusion-body myositis patient’s brain from succumbing to the same degeneration as in his muscle fibers, and what protects Alzheimer and Parkinson patients from having the same abnormalities in their muscles as in their brain? These are dramatic, very intriguing phenomena, understanding of which might very well contribute to finding cures.

The Editors are pleased to acknowledge their gratitude to various collaborators, including many clinical and research fellows, whose dedication and hard work greatly contributed to the results described in this book.

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PART 1

Muscle Aging
CHAPTER 1
Aging of the human neuromuscular system: pathological aspects

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Introduction

This chapter discusses both our original findings and concepts, as well as some data of others from the literature. It is not able to cover all aspects of this broad topic. Selected references are presented to stimulate further exploration of the various points discussed.

Succinct introduction to the biology of the neuromuscular system, for clinicians

Aging persons often have progressive fatigability, weakness, slowness, and general frailty, accompanied by visible atrophy of limb muscles. The weakness frequently is a cause of falling, which can result in serious injury, and sometimes death. Healthy muscle is maintained by: (a) its own salutary trophic metabolic processes; (b) multifactorial trophic influences dispensed from its innervating lower motor neuron (LMN) that are received at each muscle fiber’s single neuromuscular junction; and (c) circulating trophic influences. The LMN itself is interdependent both (a) on normal trophic factors from the numerous myelin-containing Schwann cells surrounding its long axonal process like oblong beads on a string, and (b) on retrograde trophic influences acquired from its numerous muscle fibers at the neuromuscular junctions. Each LMN in the human biceps is responsible for activating about 200 muscle fibers and for the continuing trophic nurturing of good health of those muscle fibers. A motor unit refers to one LMN, its Schwann cells, and the muscle fibers it innervates. A neuromuscular disorder, or disease, is one arising from abnormality of any part of the motor unit.

The LMNs and lower sensory neurons have a vital interdependence with the Schwann cells that coat and nurture their axonal extensions: the neurons cannot survive without the Schwann cells, and vice versa. Just as trophic factors “emitting” from LMNs induce and control the special type-1 versus type-2 characteristics of the muscle fibers they innervate, the LMNs probably also induce and maintain hypothetically different sets of “type-1” and “type-2” Schwann cells, respectively, on themselves. And, probably the Schwann cells on sensory neurons are different from ones on motor neurons, because clinically there can be anti-Schwann-cell dysimmune diseases that rather preferentially affect either motor or sensory neurons, and even preferentially involve selectively large-diameter sensory nerve fibers (faster-conducting, conveying position, vibration, and touch sensations) or small-diameter sensory nerve fibers (slower-conducting, conveying pain signals).

A motor unit, with its arborizations, has been likened to a tree, the leaves being compared to the muscle fibers (I think that I shall always see, a motor unit as a tree; with apologies to Joyce Kilmer).
In regard to its loss of “leaves,” a tree in autumn, or a waning motor unit, can be affected in toto or in portio. In toto reflects all of the leaves becoming “malnurtured” at about the same time, and in portio is manifested as leaves on the more distal twigs being affected first, showing the first autumnal color changes (as is characteristic of maple trees).

The clinically evident muscle atrophy of elderly persons, which we call atrophy of aging muscle (AAM) (an intentionally general descriptive term), is often assumed to be strictly myogenous (defined as meaning a process involving only muscle, but not LMNs or their peripherally extending axons). However, based on our evidence, it is likely that in a number of circumstances AAM is ultimately neurogenic, i.e. caused by malfunction of the LMNs, or antecedently by impaired trophic influence of the Schwann cells on their LMNs. Because of its clinical, social, and economic importance, AAM will be discussed in regard to some facets of the known and putative malfunctions of the motor unit components, their causes, and their possible treatments.

Note that we use AAM instead of the term sarcopenia. “Sarcopenia” sounds like a definitive diagnosis but it is not. It is often erroneously interpreted as designating a singular pathogenesis. Sarcopenia simply refers, imprecisely, to muscle atrophy in aged animals; it does not indicate or imply any pathogenic mechanism, of which there are a number of possibilities. AAM is usually manifest as type-2 fiber atrophy. A further critique of “sarcopenia” is presented below.

AAM is not a definitive clinical diagnosis, no more than is anemia, or jaundice, or stroke; it is a reason to look carefully, in each individual patient, for a cause, and especially for a treatable cause. Several known causes are described below and in Chapters 2 and 3 in this volume. Whether there is also an as-yet-unidentified general pervasive cause (or causes) that eventually harms the muscles of every aging person is not known. Biochemical studies seeking a general, nearly universal cause typically do not intensively seek, in individual patients and in experimental animals, the possible presence of an identifiable and potentially treatable primary cause (such as peripheral neuropathy, nerve-root radicopathy, malnutrition, hyperparathyroidism, or a myovascular component).

Aging is a risk factor for AAM, but it is not an ultimate cause. “You’re just getting old” is not a cause of AAM, and clinically it certainly should not be used as a dismissive diagnosis of an older patient.

**Cellular aging, in general**

Despite a vast literature on cellular aging, the causes and mechanisms are still poorly understood, and treatment non-existent. Mature, post-mitotic muscle fibers, similarly to post-mitotic neurons, seem to be more susceptible to a chronic cellular aging than are dividing cells. Cellular aging involves abnormalities of various subcellular aspects, such as nuclei, mitochondria, endoplasmic reticulum, Golgi, and structural and aqueous components. Proteasome and lysosome degradations are especially important. Oxidative stress and endoplasmic reticulum stress are also proposed to play important roles. The “proteome” designates the large and varied family of proteins of a cell, the profile of which is cell-type-specific.

One can wonder whether the general aging changes of cells are due to effects of a still-obscure omnipotent “master vitalostat,” such as a “master gene” acting like a rheostat that gradually turns down the vitality of the cell. If there is a master vitalostat. What initiates the turning-down, what are the key steps by which it executes that turn-down, and how can it be controlled? What are the underlying genetic factors, and/or important epigenetic mechanisms? (Philosophically, why are all living creatures programmed from “conception” to die?) In the atrophy process, there might be multiple stages and pathways participating, some of which, if identifiable, could become amenable to not-yet-developed treatments. Hypothetically, for skeletal muscle there might be at least two so-called master genes, Fiber2atrophin and Fiber1atrophin, that are normally inhibited, but when activated by an atrophy-promoting factor they instigate cascades of other genetic activations and inhibitions, resulting in preferential atrophy of type-2 or type-1 muscle fibers respectively. Preferential type-2 fiber atrophies are discussed below. (Preferential atrophy of type-1 fibers is seen in myotonic dystrophy type-1, a disease caused by...
expansion of CTG trinucleotide repeats of the gene DMPK; and in preferential “congenital type-1 fiber hypotrophy with central nuclei” [3], which in some patients is attributable to a genetic mutation of myotubularin, myogenic-factor-6, or dynamin-1.)

**Some unanswered questions**

Is the muscle frailty associated with AAM universally inevitable, like the aging-related, more-visible frailty and atrophy of skin, like the failure of estrogen in menopausal women and the gradual petering-out of testosterone in aging men, like scalp follicles disappearing or producing only non-pigmented hairs, like vascular sclerosis, like accumulation of “wear-and-tear” lipofuscin pigment within lower-motor neurons, other neurons, and muscle fibers? What is the most essential mechanism that starts and perpetuates AAM? Is it something we all ingest, or do not ingest; is it the cumulative solar or cosmic irradiation, or Mother Earth’s constant radon emission; or perhaps there is something else to which we all are exposed? Is there a gradual cellular accumulation of something cumulatively more toxic than the accumulating lipofuscin – such as oxidatively damaged or otherwise-toxified misfolded proteins – that gradually “rusts” beneficent cellular functions and activates “atrophy processes”? Why can’t any of the pathogenic mechanisms putatively contributing to AAM be prevented or treated now? Much work needs to be done before we can prescribe an elixir to make the elderly intellectually brilliant and vigorous.

Indefinable are the terms “normal aged person” or “normal-control aged person.” In muscle biopsies of aging persons we nearly always have observed different combinations and various degrees of denervation atrophy and/or type-2 fiber atrophy (see below).

**Neuromuscular histology**

Normal skeletal muscle is the most abundant of human tissues. It is composed of muscle fibers that are very long cylinders. Their length is about 1000 times their typical diameter of about 45–65 μm (in the biceps). Transverse histochemical sections of muscle biopsies are diagnostically more informative than longitudinal ones. The universally used stain for general evaluation of muscle-biopsy histochemistry is the Engel trichrome [4, 5]. (It stains myofibrils green and their Z-disks red; mitochondria, t-tubules, longitudinal endoplasmic reticulum, and plasmalemma red; and DNA and RNA dark blue. It also stains the protein component of Schwann cell myelin red and neuronal axons green.) The histochemical types of human muscle fibers are most distinctively delineated by two myofibrillar ATPase reactions: (a) the regular ATPase (reg-ATPase) incubated at pH 9.4 [6], and (b) the acid-preincubated reverse-ATPase (rev-ATPase) [7]. (Some myopathologists also use antibodies against different types of myosin for fiber-type definition.) In normal adult human and mammalian animal muscle, fibers lightly stained with reg-ATPase and reciprocally dark with rev-ATPase are arbitrarily designated type-1 fibers [4, 7–10], while the fibers oppositely stained are type-2 fibers. The type-1 fibers are high in most of the mitochondrial oxidative enzyme activities (e.g. cytochrome oxidase (COX), succinate dehydrogenase (SDH), and hydroxybutyrate dehydrogenase), as well as myoglobin and triglyceride droplets; and they are low in the anaerobic glycolysis enzymes myophosphorylase and UDPG-glycogen transferase, in glycogen, and in the aqueous sarcoplasmic enzyme lactate dehydrogenase. The type-2 fibers are oppositely stained with those reactions. (Interestingly, the very useful mitochondrial oxidative enzyme menadione-mediated α-glycerophosphate dehydrogenase (men-αGPDH) is stronger in type-2 fibers.) Type-1 fibers have more capillaries adjacent to them and are better equipped for oxidative metabolism, and clinically are utilized for prolonged muscle activity. The type-2 fibers are better equipped for anaerobic glycolysis, and clinically are utilized for short bursts of more intense activity. In some neuromuscular disorders there is a rather selective involvement of one fiber type, and in other disorders the involvement is nonselective [11]. (A muscle biopsy, done as an outpatient procedure with local, not general anesthesia, must be from a muscle not recently needled...
for electromyography, therapeutic injection, or “acupuncture therapy”: these can produce confounding focal myopathy [12].

During normal development, each LMN induces and trophically maintains the distinctiveness and uniformity of histochemical and functional fiber type and subtype of its approximately 200 muscle fibers controlled by it as members of its motor unit. We have therefore hypothesized that there are, respectively, type-1 and type-2 LMNs, and A and B subtypes of each. (In the cat anterior horns, we were not able to histochemically distinguish the different types of LMNs from each other [13–15] but we could demonstrate that the large α-motor neurons are rich in phosphorylase and glycogen and poor in mitochondrial SDH, while the small neurons, namely the gamma efferents, renshaw neurons and interneurons have the opposite histochemical profile.) Muscle fibers denervated from any cause gradually atrophy. If only some fibers in a muscle are denervated, they become small angular fibers when viewed in transverse sections (Figure 1.1a–e), progressing to become pyknotic nuclear clumps (Figure 1.2a). At some indefinable point, the atrophying fibers become incapable of attracting and/or accepting reinnervating nerve sprouts, but before that point of no return, they can be rescued by reinnervation. Muscle fibers can “switch” their histochemical type when denervated and then reinnervated by the type of LMN opposite to their original type of innervating LMN (i.e., foreign reinnervation) [16]. Denervated muscle fibers apparently can promiscuously accept comforting reinnervation from any type or subtype of LMN, a phenomenon commonly occurring in chronic neurogenic diseases that we have demonstrated experimentally in nerve-crush + reinnervation experiments [16–20]. In human muscle, This seemingly random foreign reinnervation results in type-grouping (Figure 1.3), which is evident as smaller or larger groups of the same histochemical fiber type replacing the normal, rather even inter-mixture of type-2 and type-1 fibers. When seen in a patient’s diagnostic muscle biopsy, type-grouping is considered a manifestation of “established reinnervation” (Figure 1.3), namely previously denervated orphaned muscle fibers having been successfully reinnervated by neurite sprouts from nearby relatively healthy LMN axons of the opposite (foreign) type.

In abnormal human muscle, two situations produce muscle fibers of intermediate degree of staining with both ATPases: (a) partially converted fibers that are in the process of being “switched” due to foreign reinnervation, which is typically a neuropathic phenomenon (although in a myopathy there can sometimes be “myogenous de-innervation” due to muscle-fiber abnormality at or near the neuromuscular junction with survival of the more distal portion of the fiber thereby cut off from the innervation influence and thus able to accept foreign reinnervation); and (b) regenerating/degenerating (“regen-degen”) muscle fibers (RNA-positive, often alkaline-phosphatase-positive, and sometimes slightly acid-phosphatase-positive), which are usually evident in the setting of a myopathy, although a few of them can occur in the setting of prominent denervation (Engel, unpublished results) and in infantile spinal muscular atrophy [21].

Physiologically, type-1 fibers are considered to be slow-twitch and rather fatigue-resistant, while the type-2 fibers are fast-twitch and fast-fatiguing, as found in normal mammalian muscle [22, 23] and corroborated in human muscle [24, 25]. The designations slow-twitch and fast-twitch were introduced [4, 8, 9, 26] to distinguish the twitch properties of mammalian twitch-muscle fibers from amphibian non-twitch, extremely slow tonic fibers of the thigh adductor clasp muscles.

Relative paucity of one type of muscle fiber in a patient’s biopsy can be caused, hypothetically, by (a) preferential impairment of the corresponding type of LMNs or Schwann cells; (b) if both LMN types are equally abnormal, preferentially more successful sprouting and reinnervation ability of the opposite type of LMNs; (c) if there are large groups of both types of muscle fibers in a chronic reinnervation situation, biopsy sampling could produce a non-representative impression of paucity; or (d) preferential myopathic loss of that type of muscle fiber. (We use the term fiber-type paucity and not fiber-type predominance because it is more likely that the muscle fibers that are too few reflect the abnormal status.)

In our muscle biopsies of elderly patients, we have observed that type-1 fiber paucity (Figure 1.3d) is
Figure 1.1 (a–e) Recent denervation without innervation, evidenced by small dark angular muscle fibers; (a,b) amyotrophic lateral sclerosis; (c–e) dysimmune peripheral neuropathy. Also, moderately atrophic muscle target fibers each with a pale small central regions and often having three concentric zones of staining; (d,e) one large two-zoned muscle targetoid-core fiber (possibly a pre-target fiber);

Dark dots within some normal fibers in (a) indicate esterase-positive lipofuscin collections. (a–c) Pan-esterase staining; (d,e) NADH-tetrazolium reductase staining. Magnification: (a) ×2000; (b) ×1330; (c) ×1830; (d) ×4330; (e) ×4170. Note that Figures 1.1–1.4, except Figure 1.2b, are transverse sections of fresh-frozen human biopsies. Muscle fibers are stained with various histochemical reactions.
evident more often than type-2 fiber paucity. In aging humans it is uncertain whether there is a gradual loss of spinal cord LMNs. If there is, possibly the type-1 fiber paucity could be due to an aging-associated gradual loss preferentially of type-1 LMNs.

More structurally labile are the type-2 muscle fibers, and especially the type-2B fibers. In human skeletal muscle: (a) they are more prone to selectively atrophy, which occurs in various conditions such as experimental pan-denervation [19, 20] (Figure 1.2b), glucocorticoid toxicity, disuse, cachexia, remote-effect of a neoplasm, and male castration; and (b) they are more prone to hypertrophy with work, especially in men. In normal young adult men the diameter of type-2 fibers is larger than of type-1 fibers, but in normal young adult women type-2 fibers are smaller (the gender difference has been attributed to more testosterone in men). There are two subtypes of normal type-2 fibers, 2A and 2B [27]. The 2B fibers are the more labile regarding atrophy and hypertrophy. At a less acidic acid-pre-incubation for rev-ATPase staining, the 2B fibers have properties intermediate between the 2A fibers and the type-1 fibers. In some type-2-fiber atrophies the subtype-2B fibers are the more prone to atrophy. There are also two subtypes of type-1 fibers, 1A and 1B [28].

**Atrophy in aging human muscle: description and new concepts**

The topic of type-2 fiber atrophy is large, multifaceted, and complex, and the subject of numerous experimental animal studies (selected references are given). Some of our personal concepts and general principles will be discussed here, but this is not a complete review of all possibly pertinent studies. The causes of type-2 atrophy are multiple, and even in an individual patient the cause can be multifactorial.

In contrast to a large body of literature regarding muscle aging in animals, there is a paucity of information regarding human muscle aging. The clinical and experimental muscle atrophies associated with cachexia, hyponutrition (starvation), remote (paraneoplastic) neoplasm, experimental (and probably human) total denervation without successful reinnervation (Figure 1.2b), glucocorticoid “myotoxicity”, and disuse all involve preferential type-2 fiber atrophy, and they have a number of their molecular degradative steps in common [29–37].

General questions include: what makes the type-2 fibers relatively susceptible to these atrophogenic processes? And what relatively protects type-1 fibers from them? Another question is why do muscle fibers (cells) have such an elaborate protein-catabolizing
Figure 1.3  (a–c) Established reinnervation, indicated by muscle fiber type-grouping, in three adult males with chronic dysimmune peripheral neuropathy. Darkly-stained type-2 fibers and lightly-stained type-1 fibers are type-grouped, in contrast to what normally would be a rather even intermixture of fiber types (not illustrated). The successfully “foreign reinnervated” fibers among the type-grouped type-1 fibers have retained, or re-achieved, their normal diameter. In (a,b) there is also rather diffuse type-2 fiber atrophy of moderate degree, because in these adult males the dark type-2 fibers normally would have been of somewhat larger diameter than the light type-1 fibers. (c) Very large type-groupings, and also a very few tiny atrophic muscle fibers. (d) Prominent paucity of type-1 fibers (dark), possibly caused by a sampling phenomenon of a very large type-grouping (or, hypothetically, by a selective loss of type-1 lower motor neurons or type-1 Schwann cells). (a–c) Regular ATPase, incubated at pH 9.4 [4–6]; (d) acid-preincubated at pH 4.35 and then the ATPase staining at pH 9.4 [7]. Magnification: (a,b) ×830; (c) ×1500; (d) ×1330.
complex, namely to unleash “atrogens” controlling self-destructing molecular systems? Is it to provide rapid-response hypertrophy, or atrophy martyring? Most important medically, can we prevent or treat the crippling atrophy in aging persons?

There are two major categories of muscle-fiber atrophy: (a) ordinary denervation atrophy (Figures 1.1–1.3) and (b) type-2 muscle fiber atrophy (Figure 1.4), and other less frequently seen atrophies, including vacuolar atrophies. Conceptually, type-2 fiber atrophy can be either neurogenous or myogenous, or both. Determining which applies to each specific patient is essential to establishing patient-specific treatments. We propose that the putatively neurogenous type is much more common (see below).

Mechanisms of muscle-fiber atrophy involve:

- Greater catabolism than synthesis of muscle-fiber proteins, especially catabolism of myofibrillar proteins, which occurs mainly through two subsystems:

**Figure 1.4** Type-2 fiber atrophy in four males. Many of the darker-stained type-2 fibers are of smaller diameter than the lighter-stained type-1 fibers, whereas in normal men the type-2 fibers should have a larger diameter than the type-1 fibers. There is also a concurrent slight type-grouping in (a) and slight paucity of type-1 fibers in (d). The patients in (a–c) have a chronic dysimmune peripheral neuropathy, and in (d) a chronic glucocorticoid toxicity. Regular myofibrillar ATPase, pH 9.4. Magnification: (a) ×830; (b) ×2000; (c) ×830; (d) ×2500.
proteasomes involving ubiquitinated proteins and autophagy/lysosomes (see Chapter 7 for details).

- Less certain is the possibility that there might also be decreased synthesis of muscle protein in the atrophy of aging, as occurs in some forms of “cellular senescence.”

**Neurogenic atrophy**

Neurogenic changes include denervation, “dysinnervation,” and reinnervation. While these are not restricted to older persons, they are the most common pathologic changes found in the atrophying muscle of aging persons.

**Denervation**

This is a complete loss of LMN influences on its muscle fibers, and that produces weakness.

**Dysinnervation**

This is our hypothetical concept of only partially impaired, incomplete loss of neural influence, especially of molecular neurotrophic factors, some of which are still able to be produced from crippled but alive motor neurons. Our putative “dysinnervation” phenomenon can be conceptualized as having some aspects similar to a persistence of the early stages of ordinary “recent denervation,” to which it appears histochemically similar.

**Pan-denervations and pan-dysinnervations in regard to type-2 fiber atrophy**

These are postulated as adversely influencing mainly the type-2 fibers (or sub-preferentially the type-2B fibers). Hypothetically, “pan-denervations” are due to (a) abnormality of both type-2 and type-1 LMNs or of their intimately related, respectively type-2 and type-1 Schwann cells (which are nurturing the LMNs and being nurtured by them). This results in lack of trophic influence on “all” the muscle fibers, either: (i) fully (in pan-denervations) or (ii) partially deficient – quantitatively or qualitatively – (in pan-dysinnervations), to which the type-2 muscle fibers (or sub-preferentially type-2B fibers) are more susceptible; or (b) hypothetically, relatively selective abnormality at the level of the presumed type-2 LMNs, or of their closely associated Schwann cells that we designate as “type-2 (or type-2B) Schwann cells.” Whereas in denervation diseases the loss of each individual LMN’s trophic influence on its muscle fibers is, by this definition, total. In dysinnervations there can be a hypothetical quantitative partial loss impairing all of the LMN’s trophic influences to some degree or qualitative loss affecting only a fraction of the presumably several “trophic factors” originating from the affected LMNs. Denervation always produces weakness, the degree being related to the number of muscle fibers denervated.

The dysinnervations can occur in disorders of the following.

- LMNs, at the level of the soma, axon, root, proximal axon or distal axonal twigs. One putative example is “axonal hyperactivity, which produces fasciculations, macrocramps, and multi-microcramps [38]. The discomforting and disabling multi-microcramps are presumably due to lability and persistent aberrant firings of distal axonal twigs – each twig innervating a few abnormally contracting/microcramping muscle fibers – caused by molecular abnormality essentially at (a) the axons themselves, or (b) at their enveloping Schwann cells. Dysinnervations can produce fatigue and weakness in relation to the quantity and quality of the neural impairment.

- Schwann cells: Schwann cell trophism to LMNs is vital for the normal function and survival of those LMNs. Dysschwannian peripheral neuropathies are the result of abnormal Schwann cells causing a secondary involvement of the proximal or distal portions of their encompassed axons, retrograde of the neuronal somas. Examples of dysschwannian neuropathies include: (a) diabetes-2 (type-2 diabetes) dysimmune neuropathies, (2) genetico-diabetoid-2 dysimmune neuropathies, (3) other dysimmune dysschwannian neuropathies, and (4) various non-dysimmune neuropathies (such as genetic and toxic ones). The first three are types of chronic immune dysschwannian polineuropathy (CIDP).

**Recent denervation without reinnervation compared to type-2 fiber atrophy**

When slightly to moderately evolved, recent denervation without reinnervation (Figure 1.1a–e) is manifest (in transversely cut muscle fibers) as small
angular-contoured (“angular”) fibers, which are often, but not always, excessively dark with the NADH-TR and/or pan-esterase and/or the men-
\( \alpha \)GPDH reaction; sometimes those fibers are low in myophosphorylase and/or COX reactivity (Askanas and Engel, unpublished results) [4]. (The denervated type-1 fibers are more likely to be excessively dark with the NADH-TR, SDH, and pan-esterase reactions and the denervated type-2 fibers more likely excessively dark with the men-
\( \alpha \)GPDH reaction.) Slightly or moderately small “roungulated” (shape between rounded and angulated) (Figure 1.4a–c), often “pre-
angular,” muscle fibers can indicate either early recent denervation or type-2 fiber atrophy, the latter evidenced in its early and mid stages as more roun-
gulated than angular atrophy. Three-zone “target fibers” (Figure 1.1d,e) in muscle are another sign of impaired innervation [4, 39], and they are often associated with an improvable dyschwannian neu-
ropathy (Engel, unpublished results). Two-zone “targetoid fibers” (Figure 1.1e) are probably of the same neurogenic pathogenesis as target fibers but, because they are, individually, often histochemical-
lly indistinguishable from central-core disease fibers, they are called “targetoid-core fibers.”

In early and mid stages of recent denervation, e.g. from amyotrophic lateral sclerosis (ALS) or periph-
eral neuropathy, on transverse sections typically there are scattered (not grouped) small, angular-
contoured fibers, whose angularity seems to be due to their being slightly indented by the adjacent normal fibers, which apparently have greater inter-
nal hydrostatic turgidity pressure than the denerv-
ated fibers. By contrast, in early and mid stages of type-2 fiber atrophy, many of the type-2 fibers (or the 2B subset of fibers) are in about the same stage atrophy, and they are more likely to be roun-gulated. In nearly total recent denervation (pan-denerva-
tion), e.g. in the acute neuropathy of Guillain–Barré disease, the denervated fibers are roungulated, probably because there are no normally turgid mus-
cle fibers to compress them. The ultrastructure of type-2 fiber atrophy resembles that of denervation atrophy [40].

(Regarding type-2 fiber atrophy, in what seem to be an advanced stage of atrophy, some fibers have become very small, dark and angular. Arbitrarily, in a setting of type-2 fiber atrophy we consider those small angular fibers and pyknotic nuclear clumps as evidence of a denervation aspect. In the advanced stage of type-2 fiber atrophy associated with small dark angular muscle fibers, the situation in that biopsy sample can be proposed to reflect (a) that all those atrophic type-2 fibers are the result of a dys-
nervation process, which we favour or (b) it is “strictly a myogenous” process (if such actually ex-
ists) eventuating into atrophic fibers with denerva-
tion-like properties.) Seemingly relevant is that Goldberg et al. has reported that in rodents biochem-
ic changes are similar between denervation atro-
phy and atrophy caused by cancer cachexia, starva-
tion, disuse, and corticosteroid atrophy [29–37]. It
should also be emphasized that experimental surgi-
cal pan-denervation of a muscle, plus preventing reinervation, produces type-2 fiber atrophy, as we have shown [19, 20] (see below).

Accordingly, the neurogenic kind of type-2 fiber atrophy is proposed to be a dysinnervation evolving into denervation.

**Pan-denervation or pan-dysinnervation hypothetically can be manifest as type-2 fiber atrophy**

This can be without or with manifestation of asso-
ciated ordinary recent denervation and/or estab-
lished reinervation. When there is coexisting type-2 fiber atrophy and atrophic small dark angular fibers like those of recent denervation, it can be difficult to decide whether the interpretation is: (a) two separate processes consisting of type-2 fiber atrophy plus recent denervation, or (b) the small angular fibers represent the advanced state of the type-2 fiber atrophy. The latter interpretation would be especially likely if that patient’s type-2 fiber atrophy is considered to be the result of a neurogenic pan-denervation or pan-dysinnervation process. In the early and mid stages of type-2 fiber atrophy the atrophying type-2 fibers retain their normal, relative lighter-staining with NADH-TR vis-à-vis the darker type-1 fibers, for example in glucocorticoid-induced atrophy of humans, and they retain their distinctive reg-ATPase and rev-ATPase appearances throughout the type-2 atrophy [4, 9, 41–43].
Both type-2 fiber atrophy and recent denervation

Both type-2 fiber atrophy (which often seems to be due to dysinnervation) and recent denervation (with or without established reinnervation) exist concurrently in muscle biopsies of many aging patients (see details above and below).

Established reinnervation following previous denervation

This is manifest by muscle fiber type-grouping (detailed above) [4, 9, 16–18, 42, 43].

End-stage non-reinnervation following previous denervation

This is evident as “pyknotic nuclear clumps” (Figure 1.2a) of extremely atrophic muscle fibers. With the NADH-TR stain such “end-stage” atrophic fibers typically show high activity, indicating that they are still alive. These end-stage, apparently alive atrophic fibers can have long-persisting pyknotic nuclei, some of which can show certain features of apoptosis, such as DNA fragmentation by Tunel staining [44]. Because this atrophying process is extremely slow compared to the rapid cellular deterioration of ordinary apoptosis, we have called it “apoptosis lente.”

Hypoactivity (“disuse atrophy”) is manifest as type-2 fiber atrophy

This atrophy can be attributed to net reduction of overall neural activation, which triggers catabolic processes within the muscle fibers. Causes include: supra-segmental central nervous system disorders, experimental de-afferentation of LMNs, general illnesses, cast on a limb, arthritic joint pains, and psychosocial factors, such as depression or interminable television.

General neuropathic mechanisms that could cause type-2 fiber atrophy

Because the neuromuscular system is complex, there are several hypothetical neuropathic mechanisms:

1 unlimited pan-neuropathic: disorders (including supra-segmental disorders) affecting all type-2 LMNs plus all type-1 LMNs, but a disorder to which the type-2 fibers are more susceptible;

2 unlimited dysschwannian pan-neurogenic: LMN malfunction secondary to disorders affecting all type-2 plus type-1 Schwann-cells, but disorders to which the type-2 muscle fibers are more susceptible (Figure 1.2b) [19, 20];

3 limited type-2 neurogenic: disorders affecting only type-2 LMNs;

4 limited dysschwannian neurogenic: secondary to disorders affecting only type-2 Schwann-cells.

In each of these four situations, the disorder of each individual cell involved (LMN or Schwann cell) can be complete (resulting in denervation) or partial (resulting in dysinnervation).

Type-2 fiber atrophy is, after ordinary denervation and reinnervation, the second most common pathology we find in muscle atrophy of the aging. Different human conditions are associated with type-2 fiber atrophy, implying various possible pathogenic mechanisms. In the individual patient, determining which cause of the type-2 atrophy is most influential might lead to an appropriate treatment.

In the various conditions, is there a “final common path” to the type-2 fiber atrophy? This is not certain, but several of the conditions associated with type-2 fiber atrophy have the same major players, such as: ubiquitin ligases and the ubiquitin-proteasome proteolytic system; the autophagy proteolytic system; the FoxO3 system that coordinates the two proteolytic systems [32, 34, 35, 45]; JunB [29]; and myostatin (see below). Even if there is a final common pathogenic path, finding a final common elixir must be a long and winding road.

Experimentally, certain maneuvers have been reported to allegedly prevent or retard, or even reverse, type-2 fiber-associated atrophy, such as: peroxisome proliferator-activated receptor (PPAR) co-activator 1α or 1β overexpression [32]; probably increasing puromycin-sensitive aminopeptidase [30]; decreasing insulin-like growth factor 1 (IGF-1)-phosphinositide 3-kinase (PI3K)-Akt signaling and its activation of mammalian target of rapamycin (mTOR) and FoxO3 pathways [33]; increasing peroxisome proliferator-activated receptor γ coactivator 1α (PGC-1α); by suppressing FoxO3 action and atrophy-specific
gene transcriptions [34]); enhancing JunB transcription factor [29]; and antagonizing ActRIIB [31]. If confirmed, these might provide clinical therapeutic leads.

**Type-2 fiber atrophy: further comments**

In an individual patient the cause of type-2 fiber atrophy can be multifactorial. For example, possibilities are: (a) in arthritic muscle atrophy, in which the commonly associated muscle-fiber atrophy is typically type-2 fiber atrophy [46], but whether the mechanism(s) is, speculatively, related to hypofunction/disuse, or a putative pain reflex decreasing LMN function, and/or, in rheumatoid arthritis, a concomitant dysimmune mechanism; (b) in HIV the atrophy could be dyschwannian dysimmune neuropathic, dysneuronal neuropathotoxic, cachectic, or possibly viral-myotoxic, or a combination of these.

Neuropathic mechanisms causing type-2 fiber atrophy are discussed above.

**“Myopathic” mechanisms causing type-2 fiber atrophy**

**General comments**

“Atrogenes” are a common set of genes whose expression is coordinately induced or suppressed in muscle during generalized wasting states (such as fasting, cancer cachexia, renal failure, and diabetes) [36, 45]. These can be activated by intrinsic or extrinsic muscle-fiber abnormalities.

**Atrophy: “Protectosome” Versus Atrogenes**

Hypothetically, aging changes might be considered a “wearing out” or a “weariness” of the cellular protective mechanisms. The normal muscle fiber, like all cells, has what we are calling a protectosome, i.e. a group of factors that normally inhibit expression of atrophy-producing “atrogenes;” thereby the protectosome is holding in abeyance the atrophogenic mechanisms with which the muscle fibers of all of us are normally equipped. However, those atrophogenic factors are constantly ready to be unleashed to produce self-erosion, self-catabolization, a sort of self-cannibalization; this putatively occurs when a beneficent protective system falters in an aging cellular environment, or other circumstances that cause type-2 fiber atrophy. The myofiber’s internal protective systems include control of endogenous free radicals and of misfolded proteins.

**Myostatin**

Myostatin is a negative regulator of muscle mass in normal development, and it is an important factor limiting the size of mature muscle fibers [47–53]. A normal level of myostatin is sufficient to inhibit myofibrillar synthesis rate and phosphorylation of S6K and rpS6 [54]. In normal human muscle, it is not known which fiber type expresses more myostatin, because with the antibodies used no immunoreactive myostatin was detectable in normal fibers of either type. In human type-2 muscle fiber atrophy associated with aging, myostatin protein/precursor–protein (Mstn/MstnPP), but not the mRNA, was quantitatively increased, and it was immunohistochemically increased preferentially in the atrophying type-2 muscle fibers [55]. It was also increased quantitatively in the weakening muscle of sporadic inclusion-body myositis (s-IBM), which is an aging-associated myopathy (see [55]). We propose that increased myostatin is an important pathogenic component of the type-2 fiber atrophy associated with “aging,” but of yet-undetermined mechanism. Mstn/MstnPP might play an adverse role in the pathogenic cascade of type-2 muscle fiber atrophy in various situations. Quantitative increase of myostatin and its mRNA has been reported by others in human atrophic muscle associated with arthritis, with HIV, and with glucocorticoid myotoxic type-2 fiber atrophy (see [55]). Elevated serum myostatin levels occur in end-stage liver diseases, in which patients have profound muscle wasting [56]. In normal animal muscle there seems to be a pool of extracellular pro-myostatin [57]. In animal models of chronic heart failure, skeletal muscle myostatin is increased, and treadmill exercise can mitigate that myosin protein expression [58]. The actual mechanisms by which myostatin protein is pathologically increased could be a therapeutic target. IGF-1 inhibits the effects of myostatin and tends to preserve skeletal muscle in mouse models of cachexia. Administration of