Virchows Arch. A Path. Anat. and Histol. 386, 1–19 (1980)

Virchows Archiv A

Review

Pathology of Skeletal Muscle: Principles of Reaction Patterns and Histochemistry and Experience with 195 Biopsies*

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Summary. Technique and interpretation of muscle biopsies require the coordinated team work between referring clinician, surgeon, and pathologist so that optimal management of a patient with neuromuscular disease may be attained. Investigation of muscle diseases has advanced so much with an interdisciplinary approach that not only can accurate diagnosis be offered for the benefit of the patient in certain instances, but also genetic counselling provided and pre-natal diagnosis established. The principal reaction patterns and pathogenetic mechanisms of skeletal muscle as a contractile and metabolically active tissue are described; the diagnostic usefulness of enzyme histochemistry and the basic principles of the motor unit are discussed and illustrated. For the practicing pathologist, adequate tissue preparation of a muscle biopsy specimen requires interest and a willingness to dedicate time, effort and funds. While the paraffin-embedded section is still very valuable, enzyme histochemistry provides certain highly diagnostic information not otherwise obtainable. Likewise, there must be an interest in electron microscopy and appreciation for its value in depicting ultrastructural abnormalities when certain reaction patterns are apparent on light microscopic sections, enzyme histochemical stains, or Epon-embedded "thick" sections. Finally, concurrent sural nerve biopsy also requires optimal processing and interpretation.

Key words: Pathology of skeletal muscle – Muscle biopsy – Enzyme histochemistry – Diagnostic myopathology.

* Presented in part at the Canadian Association of Neuropathologists' annual meeting, September 29–October 1, 1977, Vancouver, Canada, and at the Virginia Society of Histology Technicians' Spring Workshop, March 29–31, 1979, Charlottesville, Virginia

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0340-1227/80/0386/0001/$03.80

Introduction

The skeletal musculature represents the bulk tissue (45% by weight) of the human body. Localized or generalized dysfunction – weakness, undue fatigue, pain, cramps, loss of bulk and abnormalities of tone – lead a patient to consult, or be referred to, a multitude of medical specialists: general practioners, pediatricians, neurologists, internists (including specialists in endocrinology, rheumatology, and infectious diseases, among others), orthopedic surgeons, and genetic counsellors; anesthesiologists, psychiatrists, cardiologists, and medical oncologists are acquainted with the side effects of anesthetic agents and medications on skeletal muscle (Lane and Mastaglia, 1978). Careful assessment of the personal and family history, thorough physical examination, appropriate laboratory data [ESR, serum levels of muscle enzymes and isoenzymes: aldolase, creatine phosphokinase (CPK), glutamic-oxalic transaminase (SGOT), and lactic dehydrogenase (LDH)], and electrophysiologic methods [electromyography (EMG) and nerve conduction studies] may provide clear evidence of a specific neuromuscular disorder. While muscle biopsy need not necessarily be performed under such circumstances, except as a confirmatory procedure, it may be essential in other cases where investigations have not provided an unequivocal answer. It is precisely in such cases that the pathologist or neuropathologist be apprised of all pertinent information. Coordinated team work between clinician, surgeon, and pathologist is one of the principles of sound medical management of a patient with neuromuscular disease. Certain diseases (polyarteritis, polymyositis, sarcoidosis, certain parasitic infections) may require histopathologic documentation before appropriate therapy can be instituted.

The primary physician through his evaluation should inform the surgeon that a certain group of muscles is likely to be involved by the disease process. The following guidelines should govern the choice of a muscle to be biopsied: it should not be 1. one totally unaffected but merely "convenient"; 2. an "end-stage" muscle, that is, one totally replaced by connective tissue; 3. the site of an intramuscular injection, EMG needling, or prior surgery. Good liaison between surgeon, operating room personnel, and pathologist further ensures that tissue is provided as fresh as possible for proper processing and optimal diagnostic usefulness. Recent advance in techniques and methodologies for studying diseases of the neuromuscular apparatus require of the pathologist expertise in enzyme histochemistry and electron microscopy for proper evaluation. This promotes accurate diagnosis which will best aid management, including genetic counselling, of the patient and his/her family.

Several textbooks (Adams, 1975; Dubowitz and Brooke, 1973; Hughes, 1974; Walton, 1974), review articles (Brooke and Kaiser, 1970; Close, 1972; Engel, 1962; Engel, 1970; Zacks, 1970), a published symposium (Pearson and Mostofi, 1973) and publications documenting the experience in other laboratories (Brooke and Engel, 1966; Brooke and Engel, 1969a and b; Brooke and Kaiser, 1974; Climie, 1973; Meijer et al., 1977) deal with the full range of muscular diseases and techniques in great detail. These references may be consulted for in-depth discussion of a multitude of topics only more superficially covered in this treatise.

In this communication I will briefly review the technical protocol for obtaining and processing a muscle biopsy; basic reaction pattern of the neuromuscular

apparatus; the background to the usefulness of enzyme histochemistry; and the experience with 195 muscle biopsies at Georgetown University Medical Center (GUMC) over the previous five years (October 1974 – September 1979).

Muscle Biopsy Technique and Processing

Due to its primary function as contractile tissue, skeletal muscle is highly irritable. In former times, pathologists often received a specimen of unnamed muscle in formalin for which orientation was not possible and from which little meaningful information could be gained. Several surgical colleagues are well acquainted with sound protocol, notifying the pathologist one day in advance of the biopsy and submitting specimens properly. As a surgical procedure, biopsy of a muscle requires sterile technique and attention to hemostasis and cosmetic appearance of the subsequent skin scar. At GUMC, the most commonly biopsied muscles are quadriceps femoris (36%), deltoid (30%), and gastrocnemius (17%). Optimally, two specimens of the same muscle (or occasionally of two different muscles), each approximately $1 \times 0.6 \times 0.5$ cm, should be obtained. One may be secured in an isometric clamp to prevent violent supercontraction, due to the agonal, writhing, brisk contraction when immersed in irritant formalin. The second specimen is best excised without a clamp. As a further precaution against artefact, both specimens are immediately wrapped in saline-moistened gauze to prevent drying. From the second specimen, a small piece is quickly procured for electron microscopy (diced into 1 mm cubes in 2.5% cacodylate-buffered glutaraldehyde); the remainder is snapfrozen at $-165°$ C in melting isopentane which had been brought to the freezing point in a container of liquid nitrogen. This virtually instantaneous freezing prevents formation of ice crystals and resultant holes in the tissue on subsequent thawing. This specimen is utilized for enzyme histochemistry after cutting cryostat sections. The specimen in the muscle clamp is placed into formalin after about 10–15 min; this deliberate delay permits slow dissipation of high energy compounds so that violent uncoordinated contraction is prevented when the muscle is exposed to the fixative. It is then processed in paraffin.

In approximately 10% of cases, a sural nerve biopsy is performed concurrently, particularly if clinical assessment and electrodiagnostic studies suggest neuropathy. Electron microscopy, osmic acid impregnation, and paraffin embedding with staining of sections with luxol-fast-blue hematoxylin-eosin (LFB-HE), Masson's trichrome, Bodian's protargol for axons, and Congo red for amyloid may be performed, depending on need.

Enzyme Histochemistry

Using appropriate substrates and methods of incubation, dozens of enzymes have been demonstrated in unfixed, cryostat sections of skeletal muscle. Dubowitz and Pearse (1960) demonstrated a reciprocal relationship between the staining intensity of oxidative enzymes in "red" fibers and a glycolytic enzyme, phosphorylase, in "white" fibers; these fibers were respectively labelled type 1 and type 2. Engel (1962) subsequently demonstrated a high concentration of myosin adenosine triphosphatase (ATP'ase) at an alkaline pH in type II fibers and a low concentration in type I fibers. This ATP'ase reaction is now the accepted standard for "typing" muscle fibers; but different classification schemes exist, utilizing other criteria. In experimental animals, the various proposed classification schemes do not entirely correspond to the classification of human skeletal muscle (Brooke and Kaiser, 1970; Close, 1972; Burke et al., 1973; Eisenberg, 1975).

In normal human muscles, there is a mosaic or roughly checkerboard pattern of distribution of type I and type II fibers; these demonstrate a reciprocal

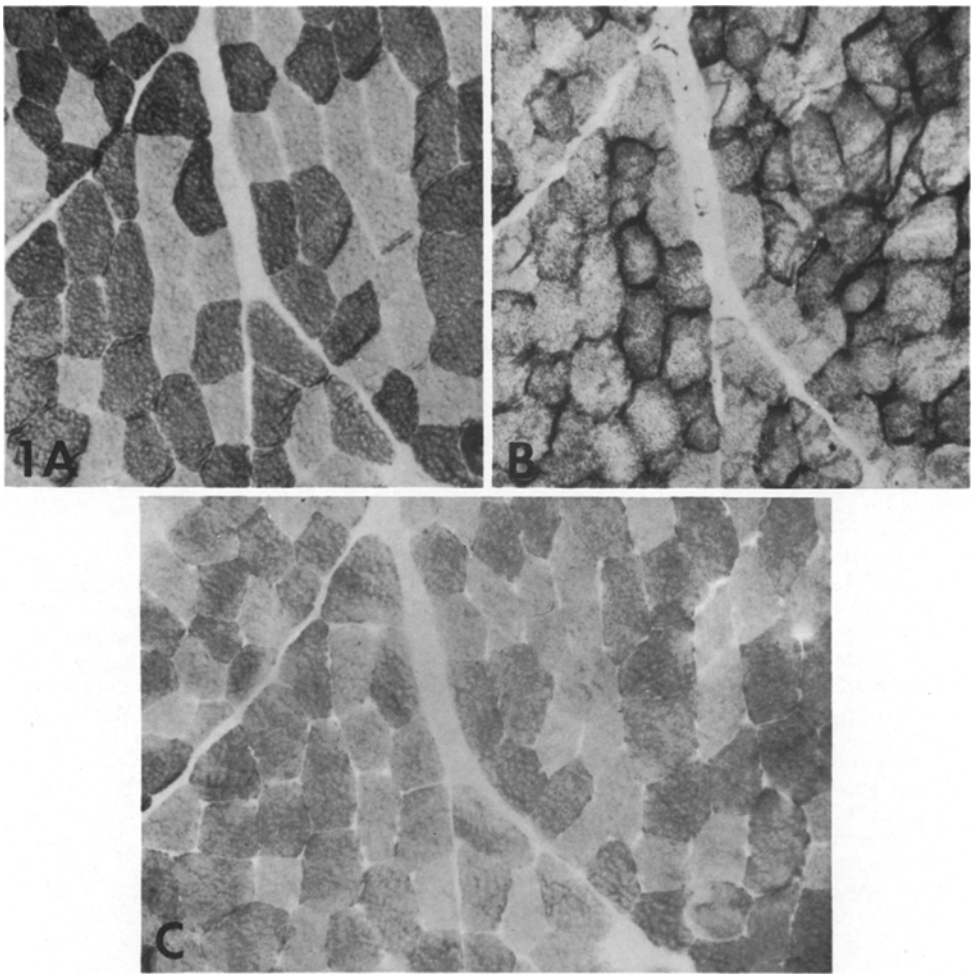

Fig. 1A–C. Serial sections, stained for ATP'ase (dark fibers type II) (**A**), DPNH (**B**) (darker fibers type I, especially subsarcolemmal deposits) and phosphorylase (**C**) from patient without neuromuscular disease. A reciprocal pattern of staining intensity for ATP'ase and DPNH can be appreciated, while the phosphorylase activity tends to resemble the ATP'ase reaction (× 165)

staining pattern with ATP'ase and an oxidative enzyme, reduced diphosphopyridine nucleotide (DPNH), (Fig. 1). This distribution pattern is determined by a presumed type I and type II anterior horn cell, which imprints the unique enzyme histochemical, electrophysiologic and functional characteristics on the respectively innervated muscle fibers (Table 1). A motor unit comprises an anterior horn cell, its axon, the final intramuscular arborizations of the axon, and the specifically innervated or synaptically-linked muscle fibers. Depending on the functional significance of the particular muscle, a motor unit may vary in size from 15 muscle fibers in the delicate and intricate extraocular muscles

Table 1. Morphologic, histoenzymatic/histochemical, metabolic, and electrophysiological/functional characteristics of human skeletal muscle.

	Skeletal muscle fibers		
	Type I	Type IIA	Type IIB
1. Morphologic			
mitochondrial number and size	large	intermediate	small
Z-line width	broad	intermediate	narrow
neuromuscular junction	small, simple	intermediate	large, complex
sarcoplasmic reticulum	simple, scant	intermediate	complex, abundant
2. Histoenzymatic/chemical			
ATP'ase (pH 9.4) activity	low	high	high
ATP'ase (pH 4.6 pre-incubation)	high	low	high
phosphorylase activity	low	high	intermediate
DPNH-TR activity	high	intermediate/ high	low
SDH-TR activity	high	intermediate/ high	low
glycogen (PAS stain)	low	high	intermediate
lipid (oil-red-O)	high	low	low
3. Metabolic			
oxidative	high	intermediate	low
glycolytic	low	high	intermediate
4. Electrophysiologic/functional			
contraction time	slow twitch/ fatigue resistant	fast twitch/ fatigue resistant	fast twitch/ fatigue sensitive
dynamic use	postural activity	phasic, volitional contraction	fast, phasic, volitional contraction

to almost 2000 for the massive gluteus maximus. Muscle fibers of different motor units interdigitate to give the mosaic pattern. In muscles commonly biopsied in humans, the ratio between type I and type II fibers happens to be, fortuitously, 1 to 2. Utilizing a technique of pre-incubating the sections for ATP'ase at an acid pH and then incubating them at the standard (alkaline) pH, the type II fibers may be further divided into types IIA and IIB; again, fortuitously, type IIA and IIB fibers are present in approximately equal numbers, so that type I, type IIA, and type IIB fibers each represent about one-third of the total (Dubowitz and Brooke, 1973). This fiber subtyping is becoming general practice in all diagnostic laboratories. Through a precedural modification, the three fiber types can be identified simultaneously on a single cryostat section (Tunell and Hart, 1977). Further, recently human type I fibers have been subdivided on the basis of various oxidative enzyme stains (Askanas and Engel, 1975), a classification scheme long in vogue among experimentalists.

Alterations in the ratio of type I and type II fibers, i.e. predominance of one or other fiber type, may occur in congenital fiber type disproportion

but type I fiber predominance tends to be associated with myopathies while type II fiber predominance tends to occur with motor neuron diseases (Brooke and Kaiser, 1970). If more than 55% of all fibers are type I or more than 80% are type II, the respective fiber type is said to dominate (Dubowitz and Brooke, 1973). Selective involvement of one fiber type in atrophy, hypertrophy, or intracellular staining intensity or specific patterns of distribution of fiber types may occur in specific processes. At times, only the histochemical pattern is abnormal, indicating the presence of a myopathy or prior denervation with successful reinnervation. Specific enzyme deficiencies may be detected, such as myophosphorylase deficiency in McArdle's glycogenosis or myoadenylate deaminase deficiency associated particularly with muscle cramps (Fishbein et al., 1978). On the other hand, not every muscle biopsy requires the performance of enzyme histochemistry, since certain disease processes (arteritis, sarcoidosis, amyloidosis, etc.) are not really intrinsic diseases of muscle (Brooke and Kaiser, 1974).

At GUMC in about 90% of cases we perform the following enzyme histochemical reactions and histochemical and histologic stains on cryostat sections:

A. Enzymes Involved in the Contractile Process. Alkaline (pH 9.4) myofibrillar ATP'ase without and with acid preincubation (pH 4.6), the latter also referred to as "reverse ATP'ase".

B. Oxidative Enzymes. 1. reduced diphosphopyridine nucleotide tetrazolium reductase (DPNH-TR) or also called reduced nicotinamide adenine dinucleotide tetrazolium reductase (NADH-TR), 2. occasionally succinic dehydrogenase (SDH).

C. Glycolytic Enzymes. Myophosphorylase, in selected cases in which McArdle's disease is clinically suspected.

D. Deaminating Enzymes. Myoadenylate deaminase.

E. Non-Specific Esterase

F. Histochemical Stain for Glycogen. Periodic acid Schiff (PAS) with and without diastase.

G. Histochemical Stain for Lipid: oil-red-O.

H. Histologic Stains. 1. Hematoxylin-eosin (HE), 2. modified Gomori trichrome.

I. Occasionally, immunofluorescent studies in suspect cases of polymyositis or other collagen-vascular diseases.

J. In certain cases, we collaborate with other laboratories by providing them with fresh frozen tissue for specific biochemical analysis or special enzyme histochemical reactions.

While enzyme histochemistry plays a prominent role in the evaluation and interpretation of a muscle biopsy and necessitates careful assessment (Brooke and Kaiser, 1974; Engel, 1970; Meijer et al., 1977), a systematic examination of all constituent tissues must be made also on paraffin-embedded longitudinal and cross sections of the muscle. The muscle fibers are carefully studied for uniformity or variation in size; alterations in the cross striations, location and arrangement of nuclei (i.e., displacement from their normal subsarcolemmal position; or clusters or chains); vacuolation or storage deposits in the sacroplasm; necrosis; and degeneration and regeneration of fibers. The relative size of muscle fibers is assessed or even accurately quantitated by the use of an ocular micrometer (and a histogram established for the different fiber types on the enzyme histochemical preparations) (Brooke and Engel, 1969a and b; Dubowitz and Brooke, 1973). With biopsies from children one must keep in mind that the cross-sectional diameter of fibers varies with age. The average diameter of fibers at one year of age is 15 μm, with a regular, yearly increment of 2 to 4 μm until adult size is attained at about puberty (Brooke and Engel, 1969b). In adults, size variations occur between the two sexes; while the average diameter for type I fibers is approximately equal for males and females, there is an appreciable difference for the type II fibers. In males, the cross sectional diameters of types I, IIA, and IIB fibers on cryostat sections are 62, 71 and 66 μm; in females they are 58, 57, and 47 μm (Dubowitz and Brooke, 1973).

The endomysium or ordinarily delicate connective tissue between muscle fibers, the blood vessels, the intramuscular nerves and their terminal ramifications, the neuromuscular junctions (when present), and the muscle spindles must each in turn be conscientiously evaluated. Each component may demonstrate pathologic changes which are specific, e.g., vasculitis, or deposits of amyloid or immunoglobulins. Most often, however, proper evaluation of a muscle biopsy requires a synthesis of all changes, including the distribution pattern of the morphologic alteration, and utilizing sound judgment based on knowledge of the literature and past experience. This also obviously implies that on review of biopsies posing a diagnostic dilemma, or with a subsequent biopsy, the pathologic diagnosis may have to be altered.

Depending on the interest and anticipated yield in a particular case, electron microscopic study is undertaken (Mair and Tomé, 1972; Neville, 1973). This aspect of diagnostic muscle biopsy has provided at times exciting and even unexpected information. The diagnostic yield of a sural nerve biopsy is also augmented by electron microscopic evaluation.

Discussion of Biopsy Material

Three major diagnostic categories of muscle biopsies were determined, each representing one-third of the total (Table 2); morphologically normal; specific or diagnostic abnormality; and non-specific myopathy.

Several explanations can be offered for the inclusion of a case in the normal category. Some biopsies were performed incidentally during an unrelated surgical procedure; the specimen might be a non-representative sample, e.g., for

Table 2. Diagnostic categories of muscle biopsies of 195 patients, October 1974 – September 1979

1.	Morphologically normal		68
2.	Specific ("diagnostic") abnormality		65[a]
	denervation	31	
	myositis	18	
	dystrophy	12	
	vasculitis	3	
	hemangioma	1	
3.	Non-specific "myopathy"		66[a]

[a] Note: Four patients had two distinct processes: one had a vasculitis and features of denervation; two had myositis and denervation, the latter corroborated by concurrent sural nerve biopsy in each patient; and one had denervation atrophy and hemosiderosis

suspected vasculitis; some biopsies were submitted only in formalin; some patients might be psychoneurotic, but the clinican was willing to give the patient the benefit of another diagnostic procedure; others might have a musculoskeletal, rheumatic, or central nervous system disorder which may not have a concomitant morphologic abnormality of skeletal musculature; finally, the pathologist may have misinterpreted the biopsy.

The largest proportion of cases with specific abnormalities represents changes due to denervation (Table 2). As a contractile tissue, skeletal muscle is responsible for movement, but under the influence of the nervous system. Loss of innervation, whether due to loss of anterior horn cells (infantile spinal muscular atrophy, poliomyelitis, or amyotrophic lateral sclerosis) or due to one of the peripheral neuropathies, results in trans-synaptic degeneration or denervation atrophy of the muscle fibers of that motor unit. There is no inflammatory or necrotizing response; rather, the sarcoplasmic contents diminish in size, causing the configuration of the fibers to become more angular; the nuclei cluster more closely as clumps or subsarcolemmal chains. Characteristically, both fiber types are involved. The denervated or orphaned fibers are able to resume synthesis of extrajunctional acetylcholine receptor (EJR) diffusely along the sarcolemma (Ringel et al., 1976) and this signal is recognized by normal intramuscular nerve fibers. These latter may heed this call for help and "adopt" the orphans by collateral sprouts, but imprint their own characteristic "name" on these re-innervated muscle fibers. This results in the association of small or large fiber groups of the same enzyme histochemical type, or "fiber type grouping", a characteristic feature of denervation with successful re-innervation (Fig. 2). When reinnervation occurs, receptor protein becomes restricted to the motor endplate and the diffuse EJR is eliminated (Ringel et al., 1976). The fibers are of normal size and by routine H & E the muscle would appear entirely normal. In a peripheral neuropathy the process may be progressive, so that successive axons degenerate with resultant atrophy of the previously enlarged motor units. Thus, by enzyme histochemistry, large foci of "group atrophy"

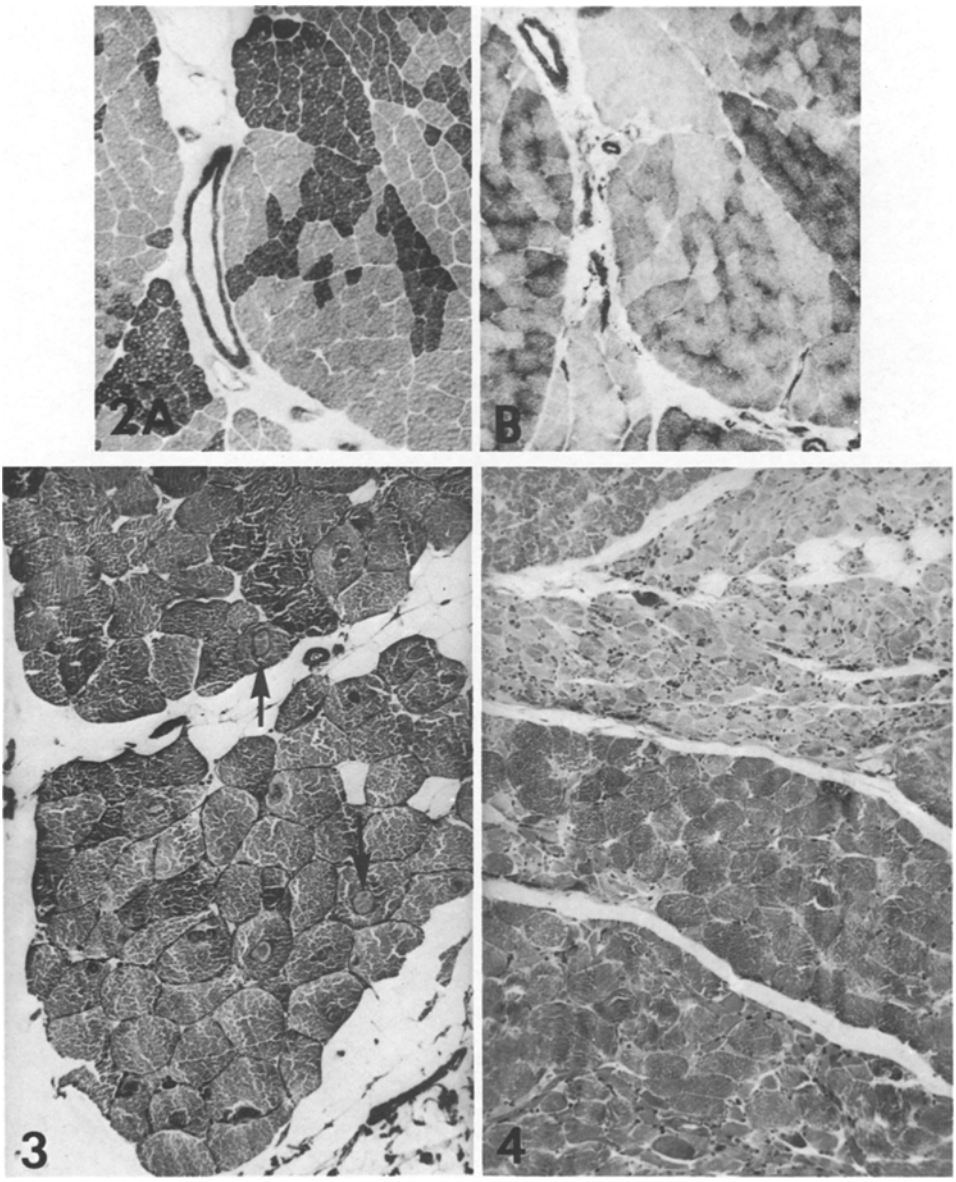

Fig. 2. Serial sections from a patient with peripheral neuropathy. Successful collateral reinnervation resulted in fiber type grouping, obliterating the normal "checkerboard" pattern of staining with the reciprocal enzymes, ATP'ase and DPNH. (×130)

Fig. 3. Several target fibers (arrows) are present in this cross section of muscle. (PTAH; ×165)

Fig. 4. Fascicular atrophy is seen at the top and grouped, angular fibers in a motor unit pattern of distribution are scattered among normal-sized fibers at the bottom. (Trichrome; ×130)


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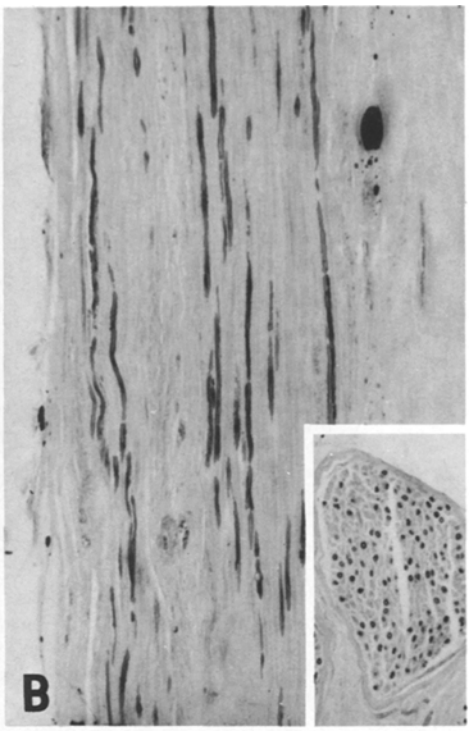
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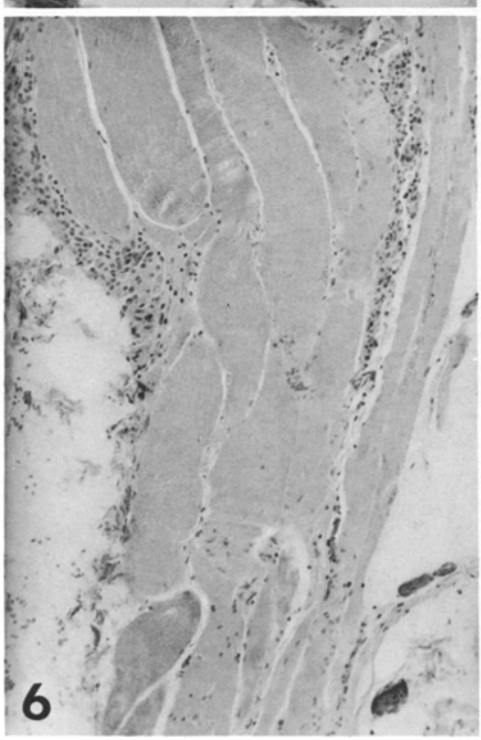
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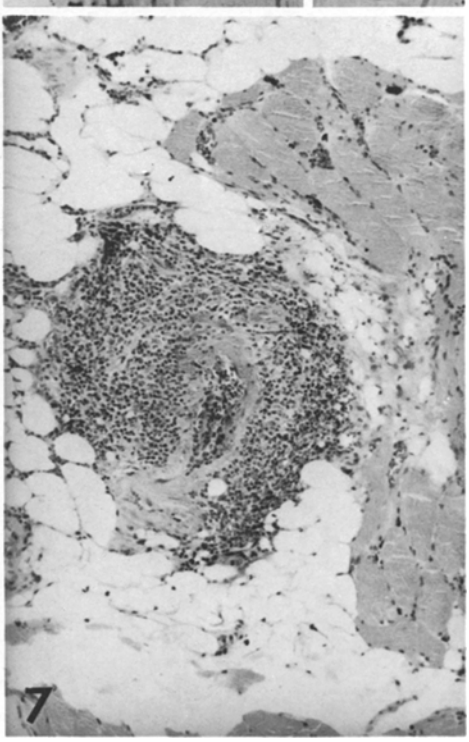
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appear, again diagnostic of denervation. In approximately 50% of cases with a peripheral neuropathy, target fibers are seen in the muscle biopsy; these occur virtually exclusively in type I fibers, and can be recognized on H & E stained sections (Kovarsky et al., 1973) (Fig. 3). If anterior horn cell disease or radiculopathy are present, major portions of, or entire, fascicles of muscle may be atrophic, while adjacent fascicles have fibers of normal size with or without small numbers of angular atrophic fibers in a motor unit distribution pattern (Fig. 4). Biopsy of sural nerve will commonly demonstrate loss of myelinated fibers in a peripheral neuropathy (Fig. 5A and B).

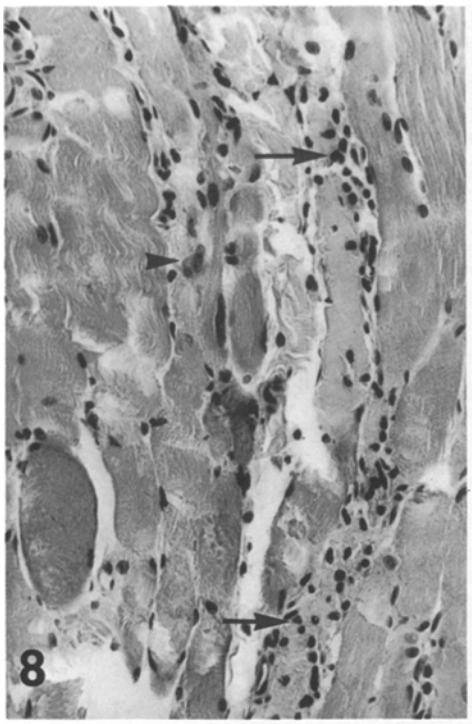
The inflammatory myopathies or myositis include viral infections, poly/dermatomyositis (Bohan and Peter, 1975), sarcoidosis, and bacterial infection and parasitic infestations. The specific infectious myopathies will not be further discussed. Since only one muscle is generally biopsied, a histopathologic diagnosis of inflammatory myopathy or "myositis" may be interpreted as "polymyositis" by the clinician who has all other pertinent information available. Classically, there will be muscle fiber degeneration and necrosis with macrophage activation (myophagocytosis), an infiltrate of inflammatory cells between muscle fibers and about vessels (Fig. 6), and often an atrophy of muscle fibers at the periphery of fascicles (Banker and Victor, 1966). In some cases, immunoglobulins can be demonstrated (Banker, 1975; Carpenter et al., 1976). A vasculitis is more commonly an arteritis (Fig. 7) or arteriolitis, as in polyarteritis nodosa, but may also be encountered in rheumatoid arthritis and childhood dermatomyositis (Banker, 1975). The accompanying fibrinoid necrosis of vessel walls may permit demonstration of fibrin and immunoglobulins by the immunofluorescent technique (Whitaker and Engel, 1972).

The muscular dystrophies represent a group of progressive, genetically determined, primary, degenerative myopathies in which the disease process is essentially one of the muscle itself (Appenzeller and Ogin, 1975; Rowland, 1976; Walton, 1977; Furukawa and Peter, 1978), although a minority of workers subscribes to a neural hypothesis, with progressive loss of functioning motor units (Sica and McComas, 1978). Various types are recognized on the basis of age at onset, distribution and severity of muscle weakness, and the pattern of inheritance. The pathogenesis of at least some of the dystrophies involves the cell surface membrane (Schotland et al., 1977; Bonilla et al., 1978; Carpenter and Karpati, 1979) as indicated biochemically by loss of muscle enzymes into

Fig. 5.A Sural nerve from patient with peripheral neuropathy demonstrates markedly reduced numbers of myelinated axons and corresponding increase in endoneural connective tissue and Schwann cells. (LFB-HE; ×165). **B** Sural nerve from another patient with peripheral neuropathy was suspended over osmic acid in a sealed Petri dish. Osmium tetroxide fumes fix, impregnate, and oxidize lipids. Scant numbers of myelin sheaths are seen in longitudinal and cross (insert) sections. Intervening unstained tissue represents endoneural fibrosis (osmic acid impregnation; ×225)

Fig. 6. Longitudinal section illustrates intense inflammatory cell infiltrate and phagocytosis of necrotic fibers in a patient with myositis (HE; ×165)

Fig. 7. Small intramuscular artery demonstrating acute necrosis with extensive inflammatory cell infiltrate in a patient with panarteritis nodosa (HE; ×130)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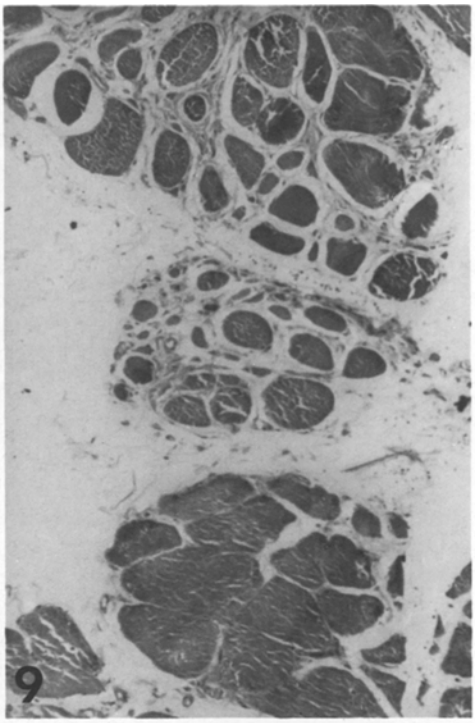
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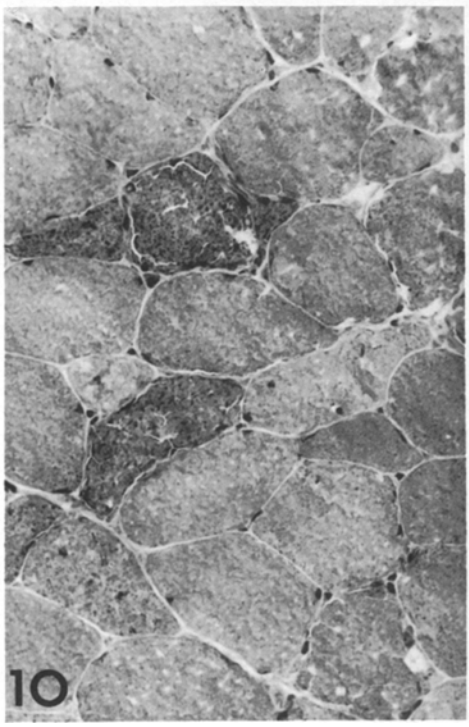
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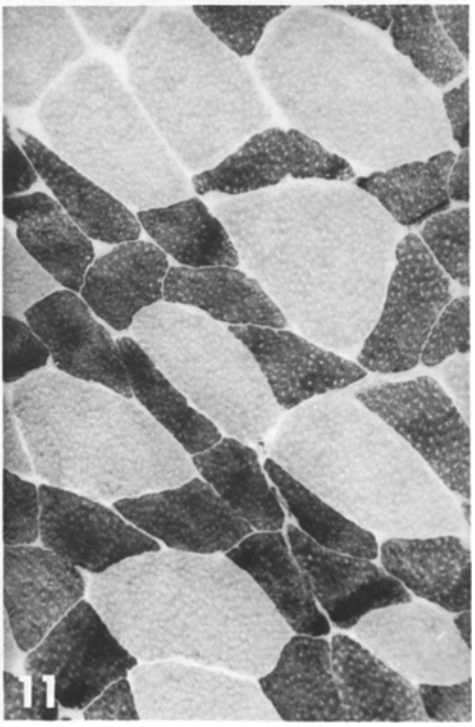
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the blood (Luif et al., 1971), metabolically by abnormalities of the adenyl cyclase system (Mawatari et al., 1974), morphologically by rounding of muscle fibers from their generally polygonal outline[1] and ultrastructurally by breaks in the plasma membrane (Mokri and Engel, 1975) and accompanying intracellular calcium accumulation, at least in Duchenne muscular dystrophy (Bodensteiner and Engel, 1978).

Characteristic morphologic changes in the muscular dystrophies depend on the specific subtype of dystrophy, the individual muscle, the tempo of disease progression, and the stage at which the biopsy is obtained. Thus, there are various degenerative and necrotizing changes with myophagocytosis; early on a significant regenerative response (Fig. 8); slight to marked variation in muscle fiber sizes; fiber splitting and internal location of nuclei; proliferation of endomysial and perimysial connective tissue; and in the end stage, a virtually complete replacement of the muscle, except for the muscle spindles, by dense fibrous tissue and adult adipose tissue (Fig. 9). In the facioscapulohumeral dystrophy, a marked inflammatory reaction may be present (Munsat et al., 1972), while in myotonic dystrophy aberrant myofibrils are aligned circumferentially rather than longitudinally (Schroeder and Adams, 1968) and the number of intrafusal fibers in muscle spindles is markedly increased (Swash and Fox, 1975). With enzyme histochemical reactions there is frequently a poor differentiation of fiber types (especially Duchenne dystrophy) or else a predominance of type I fibers and relative deficiency of type IIB fibers. In myotonia congenita, the specific abnormality is an absence of type IIB fibers (Crews et al., 1976). In the oculo-cranio-somatic syndrome ("ophthalmoplegia plus"), the Gomori trichrome stain reveals "ragged-red fibers" (Olson et al., 1972) (Fig. 10); these represent accumulations of mitochondria, which, at the ultrastructural level, contain paracrystalline inclusions (Olson et al., 1972; Kamieniecka, 1976) (as in Fig. 16).

The last category, non-specific myopathies, includes a variety of subtle or striking morphologic changes in standard sections, in enzyme histochemical reaction patterns, or at the ultrastructural level. Thus, there may be isolated myofiber necrosis, a focal perivascular cuffing by chronic inflammatory cells,

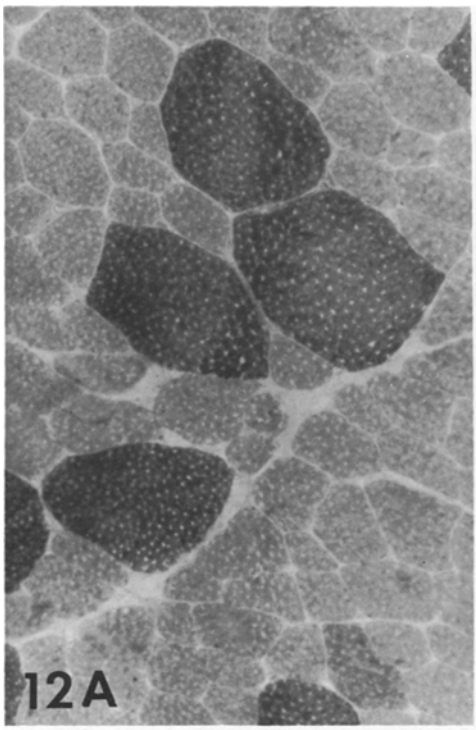
[1] Conceptually, a circular outline exposes the smallest surface for the largest volume, or analogously, it requires more energy to maintain a polygonal configuration

Fig. 8. Biopsy from young boy with Duchenne muscular dystrophy. In the early phases, individual necrotic muscle fibers are phagocytosed by macrophages (*arrows*); concurrently, there is significant regenerative activity as demonstrated by large, vesicular nuclei containing prominent nucleoli (*arrow head*). (HE; ×390)

Fig. 9. In late stages of muscular dystrophy, there is great variation in muscle fiber size and a considerable degree of endomysial fibrosis. (Trichrome; ×130)

Fig. 10. Several dark-staining "ragged red" fibers are scattered among light-staining fibers. The "red raggedness" is the result of the accumulation of abnormally large and inclusion-bearing mitochondria throughout the myofiber. (Modified Gomori trichrome; ×390)

Fig. 11. The darkly-stained type II fibers are uniformly smaller than the lightly-stained (and normal-sized) type I fibers, indicative of disuse atrophy. (ATP'ase; ×390)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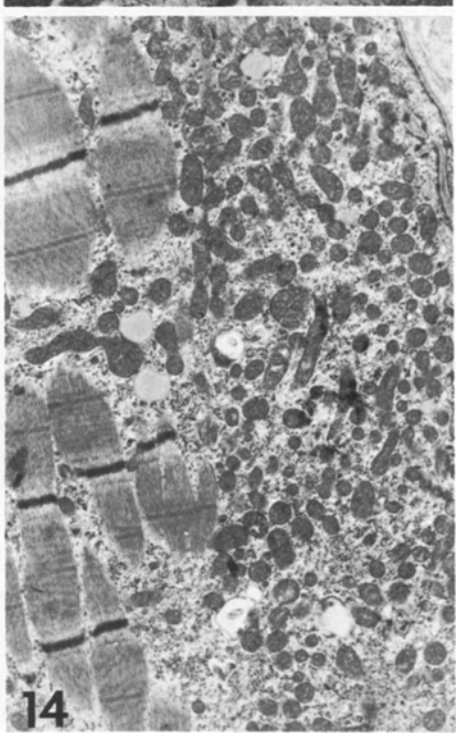
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or a variation from the accepted norm in the ratio of enzyme histochemically defined fiber types. These changes may not be quite within the range for one of the subcategories in the "diagnostic" group, and a comment to that effect is made on the pathology report. The lymphorrhages and dysplastic neuromuscular junctions (Drachman, 1978; Engel and Santa, 1971) of myasthenia gravis or the vacuolar degeneration of periodic paralyses (Engel, 1966) would be included in this category. Somewhat more specific is the selective atrophy of type II (specifically IIB) fibers referred to as disuse atrophy (Fig. 11), and seen with cancer cachexia (Engel and Askanas, 1976), myasthenia gravis, the collagen-vascular disease, upper motor neuron lesions and chronic steroid intoxication (Engel, 1970), including Cushing's syndrome (Pleasure et al., 1970). The basis for this phenomenon is considered to be related to the fact that type II fibers subserve fast, phasic, and more highly dextrous muscular activity (Close, 1972) (Table 1). Local or systemic diseases limiting volitional motor activity would thereby reduce trophic and phasic synaptic input by anterior horn cells on the type II fibers, resulting in atrophy. The converse also applies, namely hypertrophy of type II fibers with excessive use (Bernat and Ochoa, 1978). This factor of exercise is also invoked as the explanation for the observed appreciably larger size of type II fibers than type I fibers in males, whereas in the female the type II fibers tend to be smaller (Brooke and Engel, 1969a; Dubowitz and Brooke, 1973). For this reason, diagnosis of disuse atrophy is more difficult to make in females. A toxohormone has been isolated from malignant neoplasms of animals and found to cause skeletal muscle atrophy, particularly depletion of contractile proteins by inhibiting protein synthesis (Goodland and Raymond, 1973). On the other hand, in human patients with cancer, intramuscular distal axons show morphologic abnormalities (Barron and Heffner, 1978). Recently, we reported the myopathic changes of chronic organophosphate insecticide toxicity (Ahlgren et al., 1979) and of iron overload in patients on chronic hemodialysis (Bregman et al.).

Also included in the category of non-specific myopathy are the congenital (generally non-progressive) myopathies – nemaline, central core, and myotubular myopathy, congenital fiber type disproportion (Fig. 12), and the mitochondrial myopathies (Kamieniecka, 1976; Shapira and Harel, 1975) and lipid-storage myopathies (Editorial, 1973). A number of cases has been encountered in which the oxidative enzyme activity is focally excessive (Fig. 13). At the ultrastructural level, essentially normal-appearing mitochondria are clustered in large numbers either beneath the sarcolemma and adjacent to nuclei or between myofibrils

Fig. 12A and B. Myofibrillar ATP'ase (**A**) and DPNH (**B**) reactions illustrate the dramatic hypertrophy and small number of the type II fibers and the hypotrophy and large number of type I fibers, from a patient with congenital fiber type disproportion. (×390)

Fig. 13. Large accumulations of mitochondria in a subsarcolemmal position are suggested by the dense accentuation of reaction product. (DPNH-TR; ×390)

Fig. 14. Unduly large clusters of morphologically unremarkable mitochondria are present beneath the sarcolemma in this patient with mitochondrial myopathy (uranylacetate and lead citrate; ×10,400)

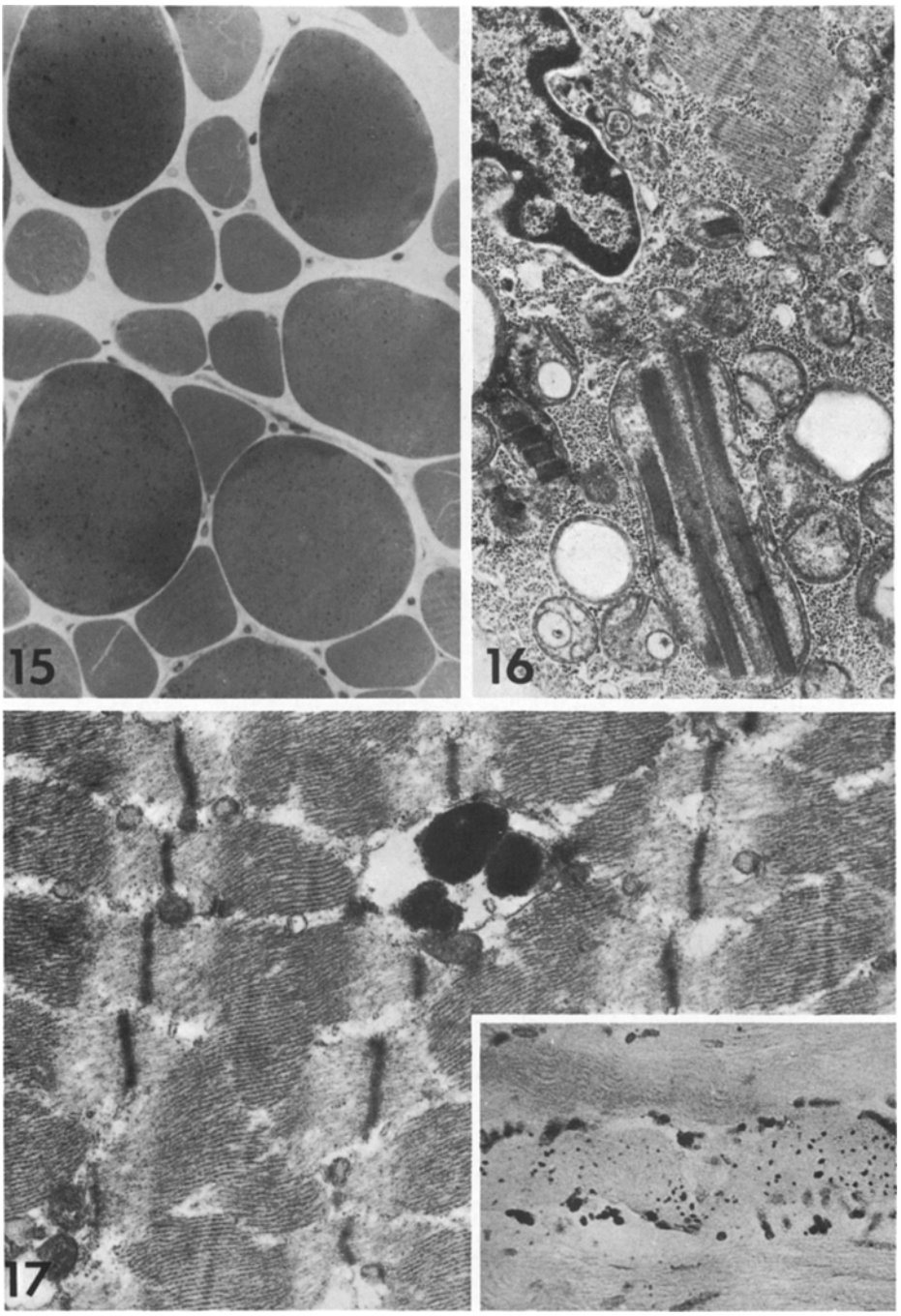

Fig. 15. Epon-embedded, toluidine-blue stained section of muscle from a patient with pleoconial (mitochondrial) myopathy and disuse atrophy demonstrates the accumulation of large numbers of mitochondria scattered haphazardly throughout the larger type I myofibers. (× 390)

Fig. 16. In another patient, with a pleoclonial myopathy, crystalline inclusions, sectioned both longitudinally and transversely, are present in some of the mitochondria (uranyl acetate and lead citrate; ×16,500)

Fig. 17. Electron-dense deposits in mitochondria at the actin/myosin junction represent hemosiderin in a chronic hemodialysis patient on oral iron supplementation and proximal myopathy. (Uranyl acetate and lead citrate; ×24,000). Inset depicts hemosiderin in degenerate myofiber (Perl's iron; ×165)

(Fig. 14); other mitochondria may be unduly large and bizarre (Fig. 15); still others contain paracrystalline arrays (Fig. 16) or electron-lense deposits representing hemosiderin (Fig. 17). These morphologic changes represent either the substrate for, or are an accompaniment of, abnormal metabolic function of these generators of intracellular high-energy compounds. One should also keep in mind the experimental studies that atypical mitochondria may represent a compensatory and reactive process, not a primary abnormality (Reznik and Hansen, 1969). In other instances, peculiar inclusions or alterations in structure of sarcoplasmic constituents or the subcellular contractile elements appear (Mair and Tomé, 1972; Neville, 1973).

In this highly condensed review I have attempted to familiarize physicians with some of the exciting aspects of muscle pathology in the hope that a better understanding of the team approach and of the principles of histopathologic and enzyme histochemical reaction will aid interpretation and diagnosis and thus patient management.

Acknowledgments. I express sincere gratitude for competent assistance in various phases of this study to: Mrs. Shirley Pulley, HT (ASCP), Mr. Dale Gibson, HT (ASCP) and Mrs. Rhea Bick with enzyme histochemistry; Ms. Eileen Rusnock and Mr. Newton More with electron microscopy; and Mrs. Marilyn Davis and Mrs. Teresa Compton for typing the manuscript.

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Received October 6, 1979