Muscle Enzymes & Serum Markers

Last updated: December 22, 2020

MUSCLE BIOPSY

If patient is free of muscle weakness, muscle biopsy is unlikely to show any significant changes!

MUSCLE SELECTION

Myopathic processes do not affect all skeletal muscles equally! → risk of sampling error

1) **degree** of muscle involvement
   - avoid clinically ***unaffected*** muscle (may not be involved pathologically).
   - avoid ***severely affected*** muscle (may only show *endstage* features - atrophy, fat, fibrosis).

   Muscles that are ***moderately weak*** should undergo biopsy

   - use Medical Research Council (MRC) strength grading and/or electrodiagnostic testing.
   - best is muscle with MRC grade 4/5 strength vs. muscle with MRC grade 3/5 strength is often too severely affected, with extensive non-specific end-stage changes (up to lack of muscle fibers).

2) **rapidity** of onset of disease process
   - slowly progressive disorder - use moderately affected muscle.
   - acute disorder – use more severely affected muscle.

3) **muscle's history** - chosen muscle should neither be involved with *another disease process* (e.g. neuropathy) nor have suffered recent (i.e. within 1 month) *injection* or *needle EMG*.
   needle electrode examination should be performed on only one side of body (and this should be clearly labeled in chart!) - homologous muscle in contralateral extremity is sampled.

4) **pathological familiarity** of muscle

5) **accessibility** of muscle

6) **EMG** findings.

7) **MRI** findings.

MRI of the lower limbs in the case of a toxic myopathy. T2 images show asymmetric involvement affecting only the left lower limb. Had a muscle biopsy been taken from the right gastrocnemius, the pathologic tissue would have been missed.

A. B.

Most frequently biopsied muscles:

lower extremity - **quadriceps** (e.g. vastus lateralis), **tibialis anterior**; avoid gastrocnemius (type 1 muscle fiber predominance, greater susceptibility to random pathological changes, pennate nature*); **peroneus brevis**, located in close proximity to the superficial peroneal nerve, is a favored biopsy site when a nerve biopsy is also indicated.

*inadvertent sampling near myotendinous junction can occur (tends to have more central nucleation, muscle fiber size variability, and split muscle fibers); however, **gastrocnemius** and **tibialis anterior** muscles are appropriate choices in diseases with distal limb signs and symptoms

upper extremity - **deltoid** and **biceps brachii**; deltoid muscle normally has 60-80% predominance of type 1 fibers.

N.B. pathologist will need to be informed about biopsy site - muscles vary in their normal ratio of type I to type II fibers making this information necessary

TECHNIQUE

OPEN BIOPSY

- carefully *avoid muscle infiltration* during local anesthesia.
- small incision in belly region (i.e. away from myotendinous junction) along long axis of muscle; incision is extended only to fascia.
- **quadriceps** has a thick fascia.
- sectioned fascia should be sutured to prevent muscle herniation - chronic nuisance for the patient.
- site is wrapped with an elastic wrap, a light pressure for a few hours.
- no follow-up visits after the muscle biopsy are normally necessary.

SPECIMENS

Fixed specimens should be shipped separately from **frozen** specimens!

include: patient's name, sampled muscle, procurement time, brief note (detailing clinical presentation and workup findings to date + list of pending studies).

1. Unclamped specimen for **histochemistry** (most important piece)
   - 2-3 cm in length; about as round as pencil.
   - handle gently by its ends using tweezers.

- place in cool, normal saline-moistened piece of gauze to prevent drying out (soaked gauze may interfere with freezing and produce artifacts).
- gauze-wrapped specimen is placed in screw-cap vial.
- transported rapidly to PATHOLOGY LABORATORY (otherwise it may lose enzymatic activity) → ***freezing*** by immersion in liquid nitrogen-cooled isopentane → immediately placed in previously cooled specimen container → sent to OUTSIDE REFERENCE LABORATORY.

2. Clamped specimen for **electron microscopy**
- can be slightly smaller.
- gently raised (e.g. with Metzenbaum scissors) just high enough to permit placement of muscle clamp.
- clamp is locked → muscle specimen cut just *outside* clamped sites.
  Clamping helps avoid CONTRACTION ARTIFACT.
- alternative - suturing muscle tissue specimen (e.g. with 3-0 silk) to piece of tongue depressor before excising it (i.e. it is sutured in situ).
- once removed, specimen is placed in 4% ***buffered glutaraldehyde*** (or ***Karnovsky fixative***) → embedded in plastic for electron microscopy.

N.B. EM is important in only certain diseases - congenital myopathies, mitochondrial disorders.

3. Clamped specimen for **histopathology** - similar to specimen for electron microscopy, with exception that it is fixed in ***formalin*** → embedded in ***paraffin*** for light microscopy.

4. Fourth specimen is ***frozen*** in event further studies are deemed necessary.

When **specialized studies** are planned (e.g. mitochondrial DNA studies), larger tissue specimens may be necessary!

NEEDLE BIOPSY

Advantages of open biopsies - larger specimen can be obtained, specimen can be fixed at its in situ length (preventing contraction artifact).
Advantages of needle biopsy - limited scarring, ability to sample multiple sites (in either same or different muscles) in single session.
Disadvantages of needle biopsy - smaller specimen size, greater orientation difficulty.

Diagnostic Staining Methods

STAINS	
Hematoxylin & eosin (H & E)	Hematoxylin: nuclei, cross-striations (purple) Eosin: cytoplasm (red), connective tissue (darker red)
	general morphology
Modified Gomori trichrome	Nuclei, mitochondria, T-tubules, sarcoplasmic reticulum (red); myocytes (blue-green)
	identifying ***ragged-red fibers***.
Periodic acid-Schiff (PAS)	**Glycogen** (purple; type 1 > 2; ***glycogen storage disorders***)
Oil red O	**Lipid** (orange; type 1 > 2; ***lipid storage disorders***)
Sulfonated Alcian blue	stains ***amyloid*** ("sea foam" green), **mast cells** (red).
Alkaline Congo red	stains ***amyloid*** (red; apple green birefringence under polarized light).
REACTIONS	
NADH-TR (NAD dehydrogenase-tetrazolium reductase)	oxidative enzyme - reflects concentration of **mitochondria**; also **T-tubules**, **sarcoplasmic reticulum** - sarcoplasm appears granular.
	Type 1 (dark); type 2A (intermediate); type 2B (light) N.B. atrophied type 2 fibers appear darker!
Succinate dehydrogenase	Krebs' cycle enzyme - selective stain for **mitochondria**; tubular elements are not highlighted.
Cytochrome-c oxidase	respiratory chain enzyme (orange-brown; type 1 > 2) - selective stain for **mitochondria**; tubular elements are not highlighted.
Myofibrillar ATPase	- most accurate method of muscle fiber typing:
ATPase (at pH 4.3) ATPase (at pH 4.6) ATPase (at pH 9.4)	type 1 (dark); type 2A, B (light); type 2C (intermediate) type 1 (dark); type 2A (light); type 2B, C (intermediate) type 1 (light); type 2 (dark)
Acid phosphatase	lysosomal enzyme - ***degeneration*** (stains red; background fir green), inflammatory cells, ***lysosomal storage disorders***
Alkaline phosphatase	***regeneration*** (stains black; background yellow)
Nonspecific esterase	acetylcholinesterase (yellow-red; type 1 >2) - endplates, lysosomes, macrophages, recently (i.e. within 6 months) denervated muscle fibers (appear smaller and darker).
Immunologic techniques	stain proteins that are deficient in some ***muscular dystrophies***.

NORMAL FINDINGS

- cross section - muscle fibers appear polygonal and their diameters vary (within given section, they are somewhat uniform).
- ***intermyofibrillar pattern*** (best demonstrated with reactions for oxidative enzymes) should appear uniform.
- muscle fibers of different motor units are interspersed - normal muscle shows *checkerboard pattern* of light and dark fibers.

ATROPHY

A. **Denervation atrophy**:
- ***decrease in cell size*** (down-regulation of myosin and actin synthesis, resorption of myofibrils), but cells remain viable.
- atrophic fibers in cross-section have roughly triangular shape ("***angulated***").
- some fibers develop cytoskeletal reorganization - rounded zone of disorganized filaments ("***target fiber***").
- while Acch receptors are normally located in center of length of muscle fibers, after denervation, fibers develop supersensitivity throughout their course.
- during reinnervation, *checkerboard pattern* of type 1 and type 2 fibers is altered - fibers of same staining type are grouped (due to collateral sprout-related reinnervation); adjacent atrophied myocytes are of same fiber type ("**fiber type grouping**").
- with reinnervation, motor fibers reform neuromuscular junctions at original end plates.
- if fibers are not reinnervated within ≈ 20 months, they will be replaced by connective tissue.

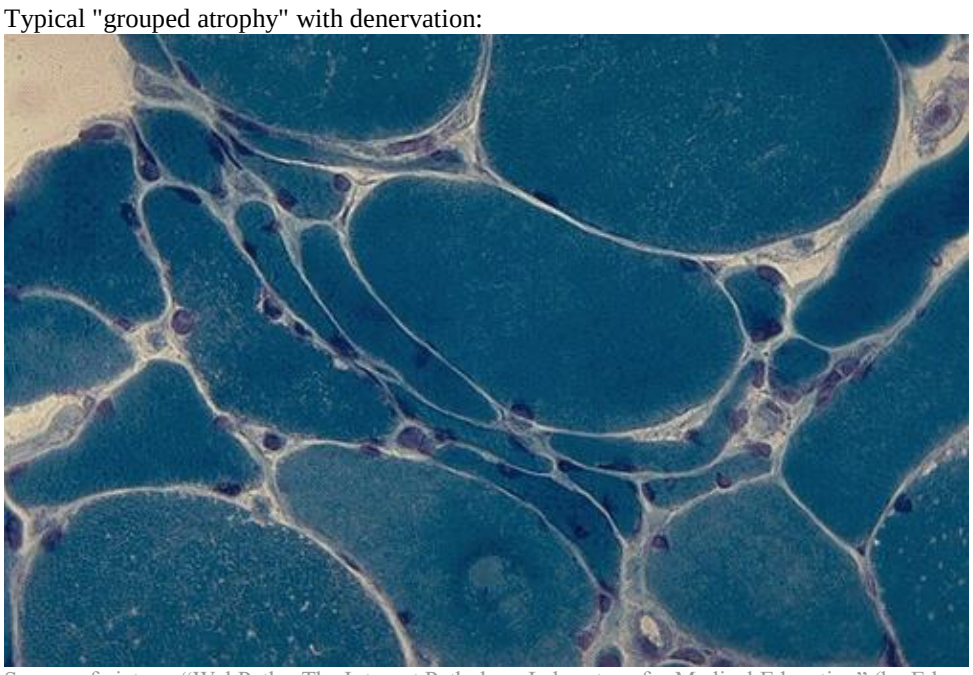
B. Disuse atrophy - *checkerboard arrangement* is maintained; mostly affected are type 2 fibers.

Preferential atrophy:

Type 1 fibers: myotonic dystrophy (prominent), nemaline myopathy, centronuclear myopathy, congenital fiber type disproportion.
Type 2 (especially 2B) fibers: disuse, corticosteroid excess (exogenous, endogenous).

Perifascicular atrophy (fibers near edges of fascicle are atrophied) - hallmark of ***dermatomyositis***.
Panfascicular atrophy - indicative of ***Werdnig-Hoffmann disease*** (spinal muscular atrophy type I).

Typical "grouped atrophy" with denervation:



Source of picture: "WebPath - The Internet Pathology Laboratory for Medical Education" (by Edward C. Klatt, MD) >>

CYTOARCHITECTURAL ABNORMALITIES

Preferential involvement:

Type 1 fibers: target fibers, central cores (central core disease), rod bodies (nemaline myopathy), mitochondrial abnormalities.
Type 2 fibers: tubular aggregates.

Target fibers (cardinal feature of ***neurogenic disorders***)

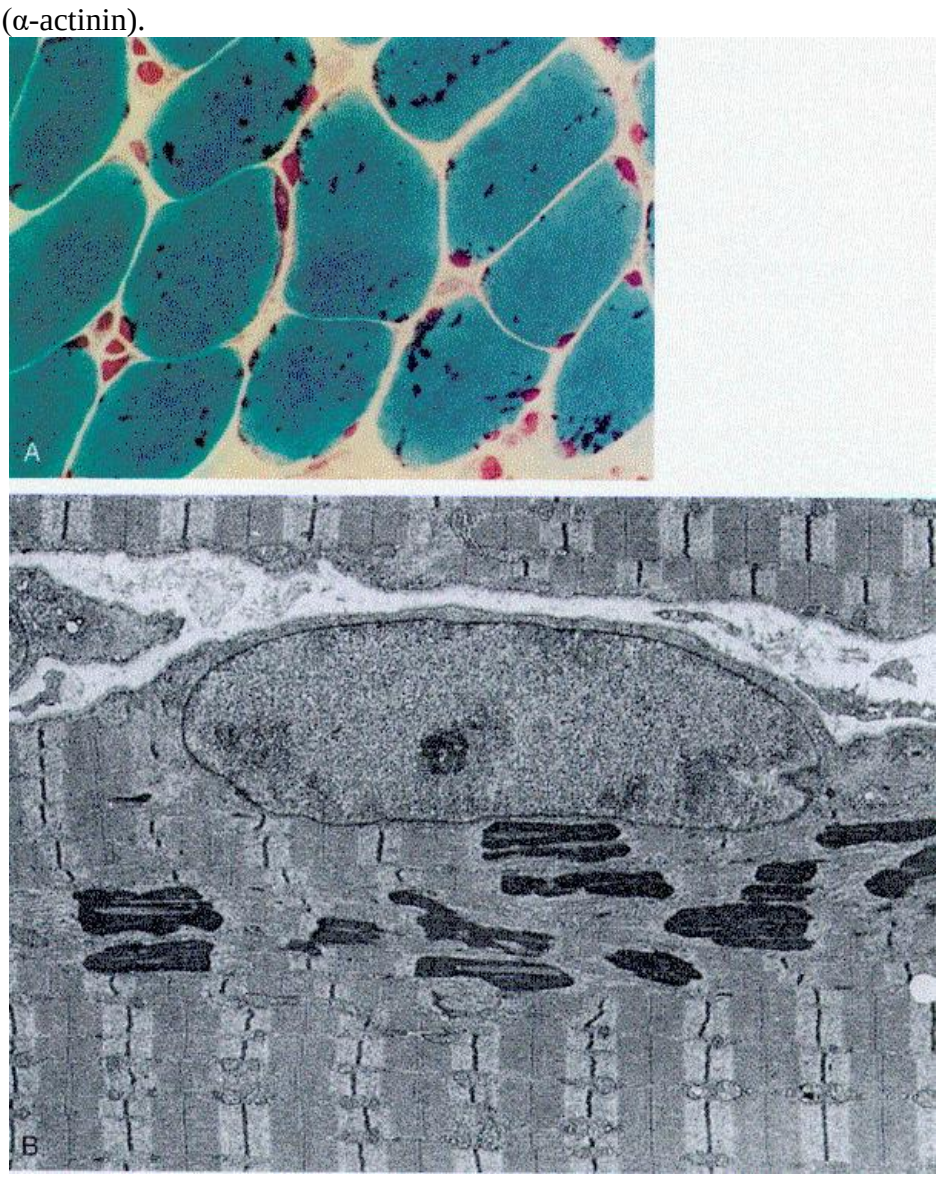
- predominant among type 1 fibers.
- composed of three "rings":
  1) central light-staining ring
  2) intermediate dark-staining ring
  3) peripheral normal-staining ring.

Central cores (***central core disease***)

- only in type 1 fibers, which usually predominate.
- CENTRAL CORE - amorphous area in center* of fiber – ***devoid of enzymatic activity***, ***lacks myofibrils and mitochondria*** - does not stain for NADH-TR, glycogen, but sometimes stains with ATPase; stains blue with Gomori trichrome stain.
  *surrounded by normally staining periphery.
- central cores resemble target fibers, but cores run whole length of fiber.

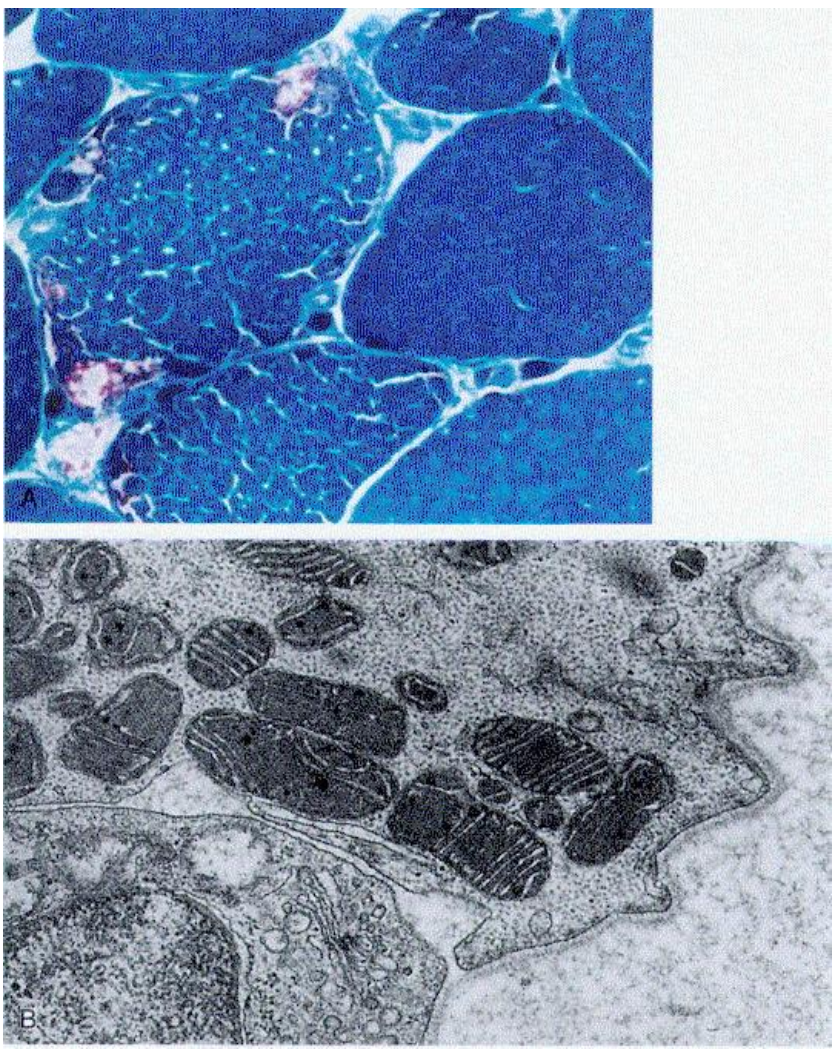
Rod bodies (***nemaline myopathy*** - numerous subsarcolemmal rod bodies in many muscle fibers; small numbers of rod bodies may be found in muscular dystrophy, polymyositis, HIV-related myopathy, muscle injured by tenotomy).

- spindle-shaped threadlike appearance (Gr. *nema* – thread).
- predominantly, but not exclusively, in type 1 fibers.
- reddish purple in modified Gomori trichrome stain; difficult to demonstrate with conventional H&E stain.
- electron microscopy shows that rods represent abnormal deposition of ***Z-band material*** (α-actinin).



Ragged-red fibers (***mitochondrial myopathies***)

- subsarcolemmal* collections of ***mitochondria*** (enlarged, bizarrely shaped, with paracrystalline "parking-lot" inclusions).
  *with severe involvement, may extend throughout fiber.
- mitochondria distort muscle fiber contour (irregular on cross-section – "ragged").
- stain red with modified Gomori trichrome stain.



Tubular aggregates (frequently seen with ***hyperkalemic periodic paralysis***) - faintly basophilic deposits in both interior and periphery of muscle fibers.

- ***sarcoplasmic reticulum***-derived collections.
- ultrastructure - fascicular arrays of parallel double-walled 60-90 nm tubules with hexagonal array in transverse section.
- stain red with modified Gomori trichrome stain.
- demonstrated with NADH-TR but not highlighted by succinate dehydrogenase (vs. mitochondrial aggregates)!

Rimmed vacuoles (inclusion body myopathy, oculopharyngeal muscular dystrophy, distal myopathy, denervation)

- blue margins with H&E; red margins with modified Gomori trichrome stain.

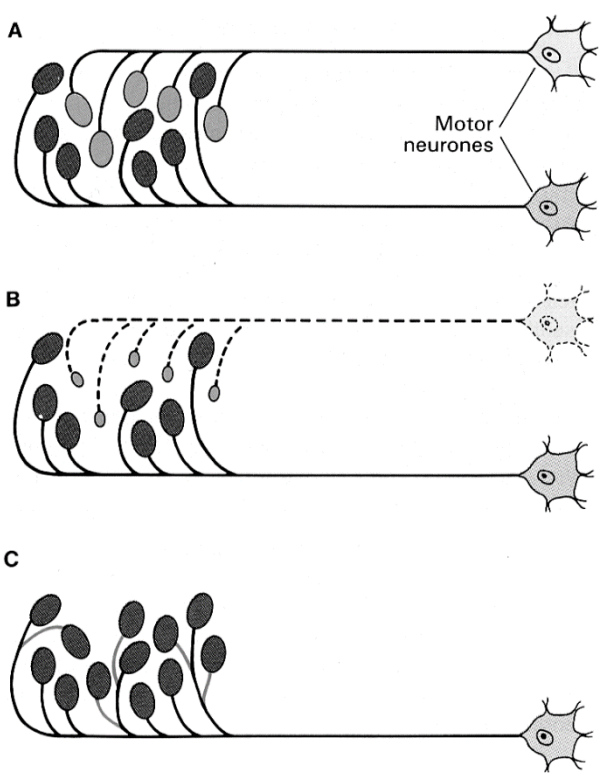
Differentiating MYOPATHIC and NEUROPATHIC changes

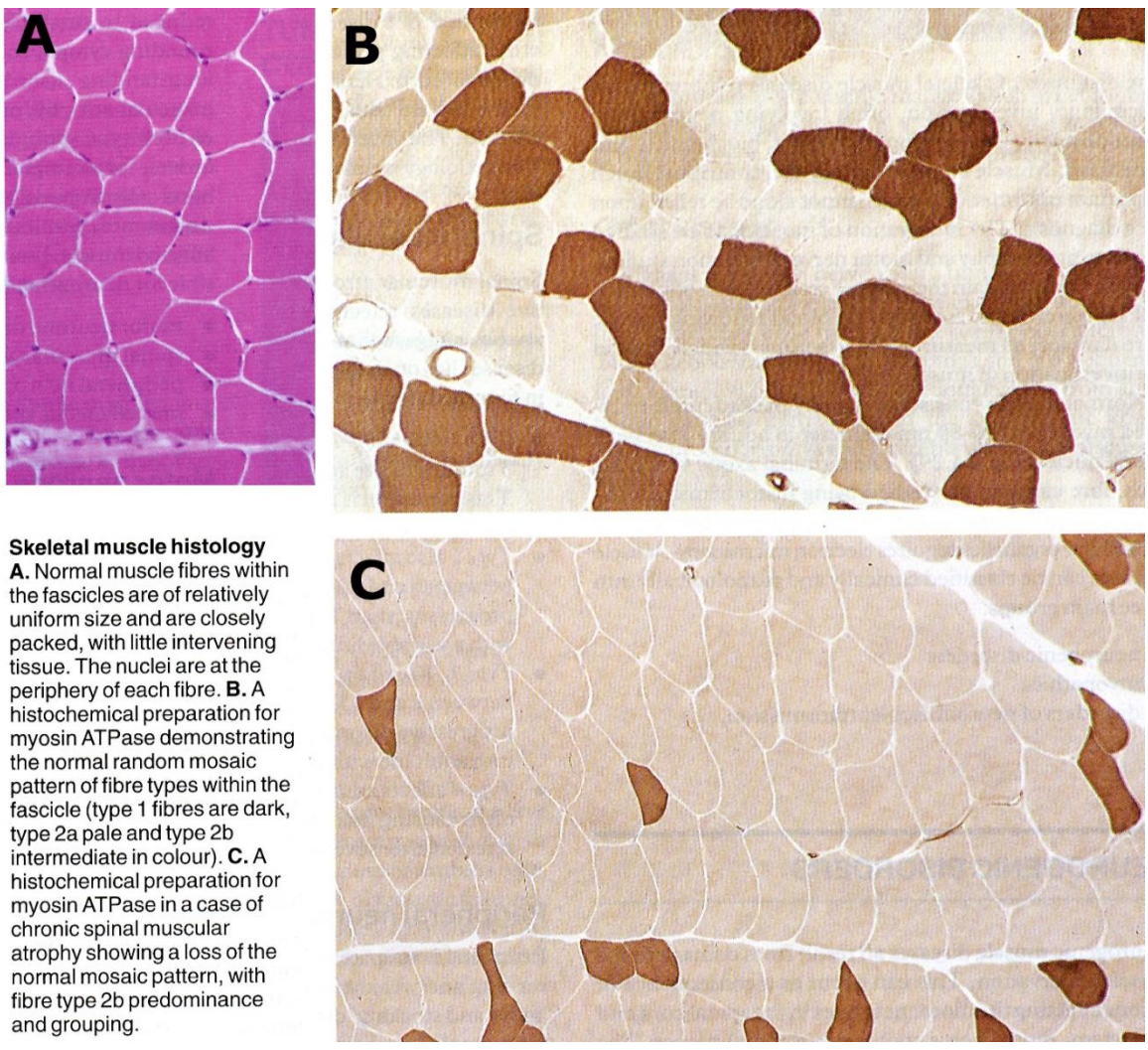
NEUROPATHIC PROCESSES

Most typical attribute is atrophy!

1) small **angulated** (in cross section) **fibers** - may be earliest sign!
   - not selective for fiber types, scattered throughout specimen.
   - denervated fibers appear darker (e.g. nonspecific esterase).
2) **fiber type grouping** (sine qua non of reinnervation) - enlarging groups of contiguous fibers of same type due to collateral sprouting reinnervation → diminished normal checkerboard staining pattern.
   - must be distinguished from fiber type predominance.
3) **grouped atrophy** (hallmark of ***chronic denervation***) - atrophy of these reinnervation groups.
   - extreme version of grouped atrophy is panfascicular atrophy (in ***Werdnig-Hoffmann disease***).
4) target fibers
5) nuclear bags
6) minimal interstitial fibrosis.

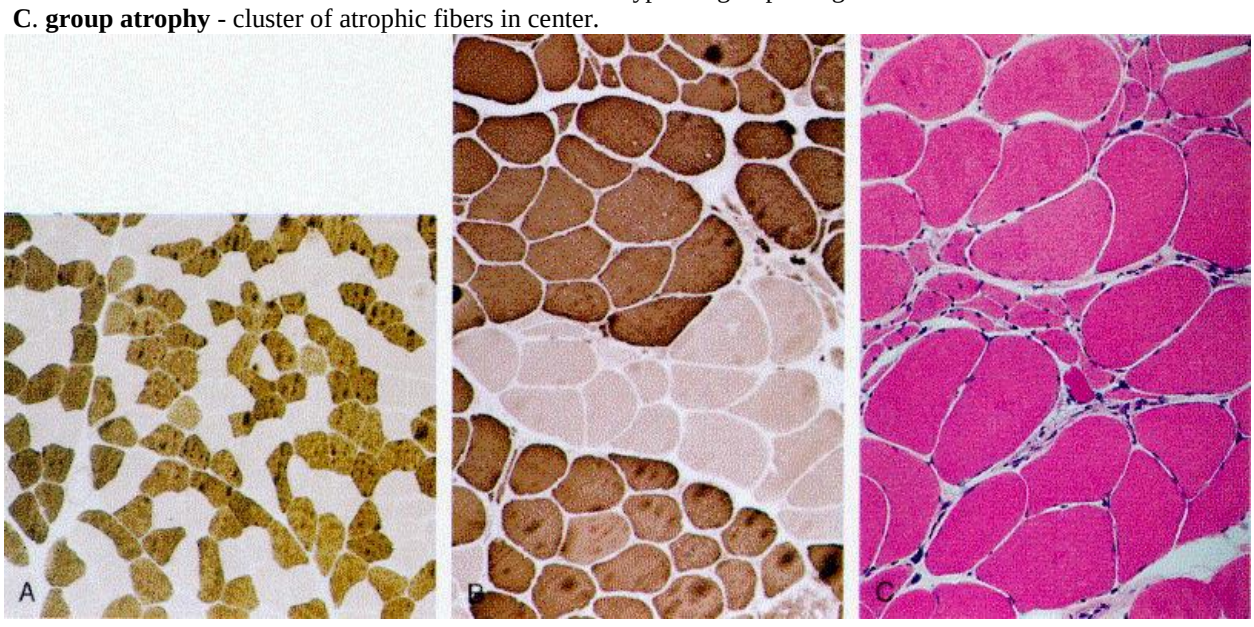
Skeletal muscle: effects of denervation
A. In normal muscle, the two main fibre types are distributed in a mosaic pattern. The muscle fibre type is determined by its innervation from a motor neurone. A single motor neurone can supply many muscle fibres. **B.** Damage to a single motor neurone or its axon results in neurogenic atrophy of muscle fibres; each affected fibre is of the same fibre type. **C.** The atrophied denervated fibres can be re-innervated by axons from other motor neurones supplying adjacent fibres. This process can change the fibre type of the re-innervated muscle fibres, resulting in fibre type grouping with loss of the normal mosaic arrangement





Skeletal muscle histology
A. Normal muscle fibres within the fascicles are of relatively uniform size and are closely packed, with little intervening tissue. The nuclei are at the periphery of each fibre. **B.** A histochemical preparation for myosin ATPase demonstrating the normal random mosaic pattern of fibre types within the fascicle (type 1 fibres are dark, type 2a pale and type 2b intermediate in colour). **C.** A histochemical preparation for myosin ATPase in a case of chronic spinal muscular atrophy showing a loss of the normal mosaic pattern, with fibre type 2b predominance and grouping.

ATPase histochemical staining, at pH 9.4:
A. **normal muscle** showing checkerboard distribution of intermingled type 1 (light) and type 2 (dark) fibers.
B. **muscle reinnervation** - fibers of either histochemical type are grouped together.
C. **group atrophy** - cluster of atrophic fibers in center.



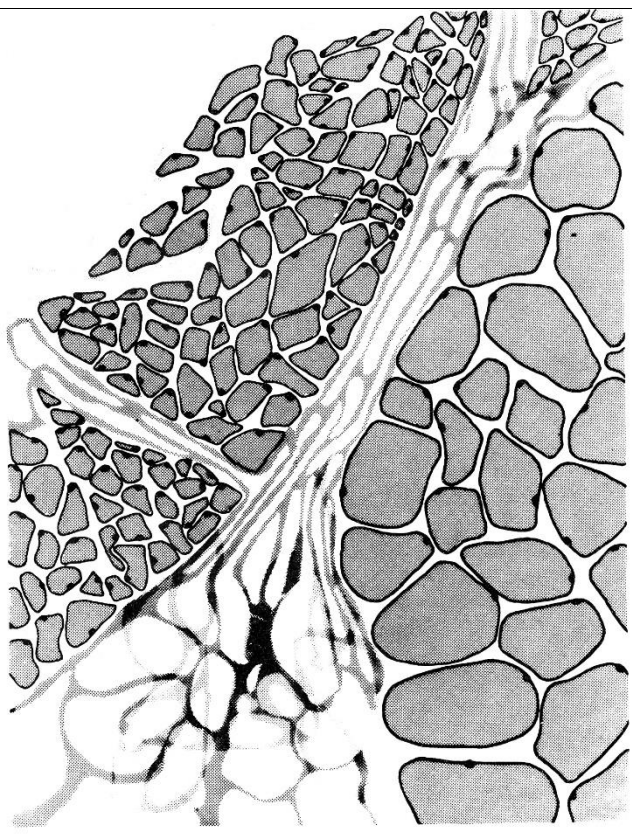


Figure 15.14–2. Neurogenic atrophy in muscle biopsy (transverse section). Groups of atrophic (small) muscle fibers are shown above to left. Group of muscle fibers to right is or normal size. Muscle nuclei remain peripheral in position. Degenerative changes are absent.

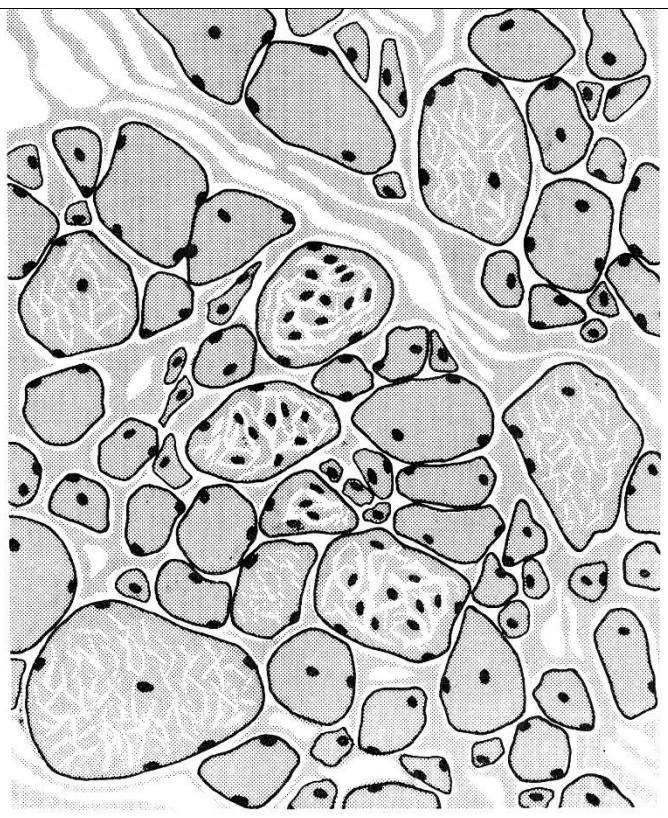


Figure 15.14–1. Drawing of myopathic changes in muscle biopsy (transverse section). There is random variation in muscle fiber size. Sarcolemmal nuclei are placed centrally rather than in their normal position immediately under sarcolemmal membrane. Several fibers are undergoing floccular degenerative changes. There is increase in endomysial connective tissue.

see p. A46 (5a) >>

MYOPATHIC PROCESSES

1) **random fiber loss** (vs. loss of whole motor unit territories).
   - if portion of muscle fiber is degenerated (SEGMENTAL NECROSIS), muscle fiber functions as two separate fibers - portion with motor endplate (i.e. innervated fiber) and portion without it (i.e. denervated fiber).
     – precursor (satellite) cells can regenerate destroyed portion.
     – denervated portion can be adopted by collateral sprouting → small foci of fiber type grouping

     N.B. small patches of fiber type grouping should not be considered synonymous with neuropathic process!
   - not reinnervated fibers undergo degeneration → extensive collagen deposition and fatty infiltration.
2) **central nucleation** - centrally located nuclei (normally observed in < 3% normal muscle fibers).
   - especially prominent in ***myotonic dystrophy***.
   - *single* (para)central nucleus in every myocyte - ***centronuclear myopathy***.
3) **rounded fibers**
4) **fiber size variability** - combination of atrophy and hypertrophy.
5) **fiber necrosis** (degeneration)
6) cellular **infiltration with myophagocytosis**
   a) ***perivascular*** collections - collagen vascular disorder, dermatomyositis
   b) most pronounced ***intracellularly*** - facioscapulohumeral dystrophy.
7) **fiber regeneration** - basophilic sarcoplasm (rich RNA content), large internalized nuclei with prominent nucleoli.
8) **fiber splitting** (normally occurs near myotendinous junctions - muscle biopsies from this region may appear myopathic!) - large fibers divide along segment so that, in cross-section, single large fiber contains cell membrane traversing its diameter, often with adjacent nuclei.
9) various **structural changes** (e.g. rod bodies, central cores, ragged-red fibers, vacuoles).
10) **microorganisms** (e.g. toxoplasmosis, trichinosis).

Active myopathic process - muscle fiber necrosis, basophilia, myophagocytosis.

Chronic myopathy - muscle fiber splitting and fibrosis.

SERUM MARKERS

Many diseases of motor unit may not cause elevated enzymes!

Creatine phosphokinase (CPK or CK)
- lysosomal enzyme released by damaged / degenerating muscle fibers.

- found in only three organs – different isozymes:
  MM for skeletal muscle
  MB for cardiac muscle
  BB for brain.
  N.B. in differential diagnosis, *isoenzyme study is not helpful* - appearance of "cardiac isoenzyme" MB does not necessarily implicate heart when there is limb weakness!
- normal maximum is 50 units.
- characteristically elevated in certain diseases and magnitude of CK increase is characteristic for particular diseases:
  1) very high levels (at least 20 times normal) – ***dystrophinopathies***; attacks of ***myoglobinuria***.
  2) high levels - interictal ***phosphorylase deficiency*** or ***acid maltase deficiency***; men with ***nonvacuolar form distal myopathy***, dermatomyositis, polymyositis,
  3) some ***spinal muscular atrophies*** (esp. Werdnig-Hoffmann disease, Kugelberg-Welander syndrome, ALS) – usually < 500 U.
  4) **NORMAL PEOPLE**:
     a) *idiopathic hyperCKemia*, some African individuals
     b) for days *after strenuous voluntary exercise*!
     c) generalized motor *seizure or tetany*
     d) minor muscle *trauma* (e.g. EMG).

Other sarcoplasmic enzymes (AST or SGOT, ALT or SGPT, LDH) – increased in myogenic disorders together with CK, but less sensitive than CK.

Elevated AST and ALT → differentiate between: **hepatic disease** → liver-specific enzyme GGT **muscle disease** → muscle-specific enzyme CK

Creatinine↓ - useful indicator of diseased muscle mass.

Serum **myoglobin** has same diagnostic significance as serum **CK**.

URINARY MARKERS

3-methyl His - quantitative measurement of ***muscle breakdown***.
- some of His residues of actomyosin complex are methylated after their incorporation.

Quantitative **creatinine** excretion – index of ***muscle mass***.
- requires meat-free diet.
- must be done over period of ≥ 72 hours.

BIBLIOGRAPHY for ch. "Diagnostics" → follow this LINK >>