Electron Microscopy and Myopathology

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Disclosures

- I have the following relevant financial relationships to disclose:
  - Consultant for Astellas Gene Therapies

Learning objectives

- Summarize normal skeletal muscle structures and organelles that can be assessed by electron microscopy.
- Identify common and diagnostic abnormalities in skeletal muscle ultrastructure.
- Compare ultrastructural findings with light microscopic features in skeletal muscle biopsies.

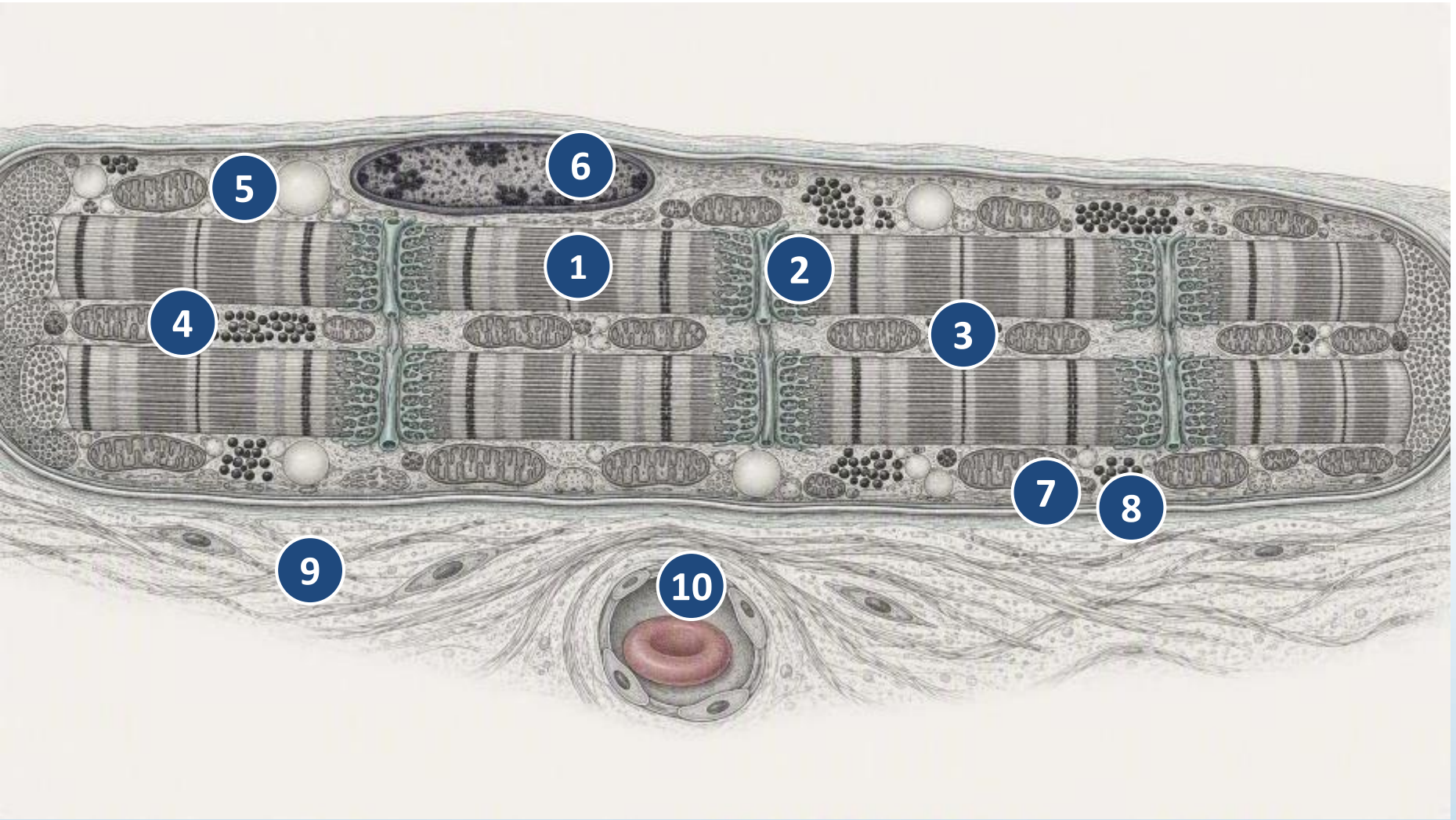
Why Learn Muscle EM?

- Muscle biopsy is a limited, precious specimen — EM shows detail no other modality can
- Resolves structures below the light microscope's limit: sarcomeres, mitochondria, membranes, inclusions
- Can be diagnostic on its own: nemaline rods, tubular aggregates, tubuloreticular inclusions, etc
- Can assist with resolution of variants are of uncertain significance

Myopathology requires integration of multiple complementary modalities

Light microscopy H&E	Histochemistry/IHC/IF	Electron microscopy
Fiber shape/size	Hints regarding myofibrillar architecture	Definitive sarcomeric and myofibrillar structure
Placement of nuclei	Abnormal aggregation of proteins/material or inclusions	Nuclear abnormalities
Hints about mitochondria, glycogen, and lysosomes	Distribution of mitochondria, lipid, glycogen	Organelle structure and location
Vacuoles	Enzymatic activity	Vacuole contents/associated material
Necrosis/regeneration	Protein expression	Membrane systems
Inflammation	Subtyping of inflammatory cells	Interstitial and blood vessel changes

Electron Microscopy approach in myopathology: think in compartments/structures, not diseases



1. Sarcomeres / myofibrils
2. Triads / SR
3. Mitochondria
4. Glycogen
5. Lipid
6. Nucleus
7. Sarcolemma
8. Basal lamina
9. Interstitium
10. Capillaries

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The challenge in EM is not recognizing abnormalities, it is knowing which abnormalities matter

1. Diagnostic findings:
   - Nemaline rods
   - Tubuloreticular or tubulofilamentous inclusions
   - Curvilinear bodies
   - Structured coress
2. Supportive findings:
   - Mitochondrial inclusions
   - Autophagic vacuoles
   - Abnormal accumulation of glycogen or lipid
   - Tubular aggregates
3. Nonspecific findings:
   - Z-line streaming
   - Myofibrillar disorganization
   - Mitochondrial aggregation

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Electron Microscopy approach in myopathology: think in compartments/structures, not diseases

- Start with normal ultrastructure — you cannot call an abnormality without a baseline
- Work through the fiber compartment by compartment, from sarcomere to interstitium
- For each: normal appearance, common artifacts, then diagnostic abnormalities
- **Correlate EM findings back to the frozen section histopathologic findings**

A note about review of semithin (thick) sections

1. First ask yourself what abnormalities are you expecting based on the frozen section review
2. If looking for alterations in sarcomeric structure, myofibrils, glycogen, or specific inclusions it's best to use a longitudinal section for EM ultrathin sections
3. If looking for changes in mitochondria, lysosomes, or vacuoles, you can consider transverse sections (look for the vacuoles!)
4. If it's an inflammatory myopathy and you are looking for inclusions, pick a section with an inflammatory infiltrate
5. If there are preservation issues, pick the best preserved tissue sections

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Practical EM checklist for muscle biopsies:

1. Myofibrils and the contractile apparatus
Are the myofibrils organized? Sarcomeric structure intact? Z-lines normal?

2. Membrane systems
Any obvious abnormalities in the T-tubules or SR?

3. Organelles
Are mitochondria, lysosomes, glycogen and lipid normal?

4. Nucleus
Is the nucleus appropriately positioned and structurally intact? Any inclusions?

5. Subsarcolemmal space and basal lamina

Any abnormal material accumulating or inclusions? Is the basal lamina abnormal?

6. Extracellular compartment
Is the interstitum and vasculature normal? Any abnormal material, inclusions, or cellular infiltrates?

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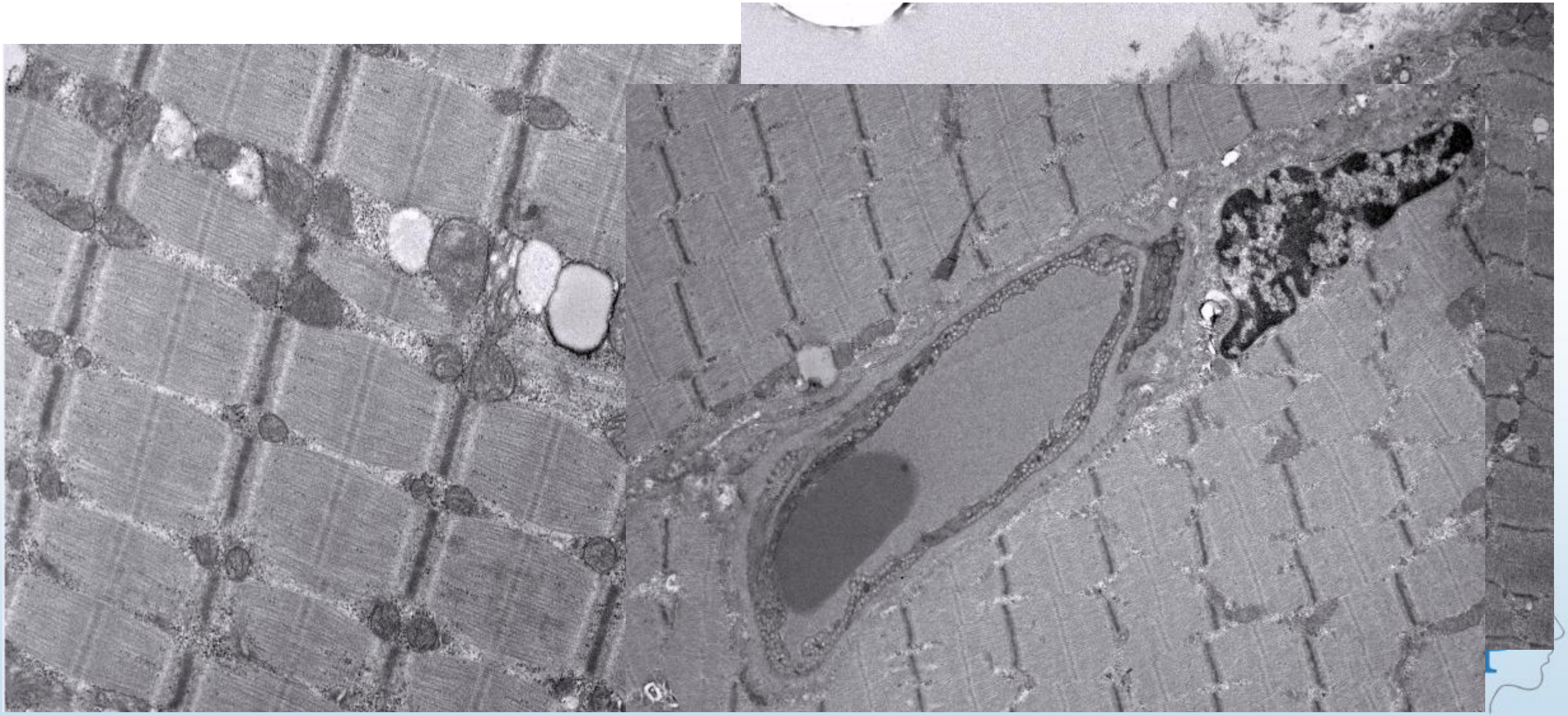

MYOFIBRILS, THE CONTRACTILE APPARATUS, AND MEMBRANE SYSTEMS

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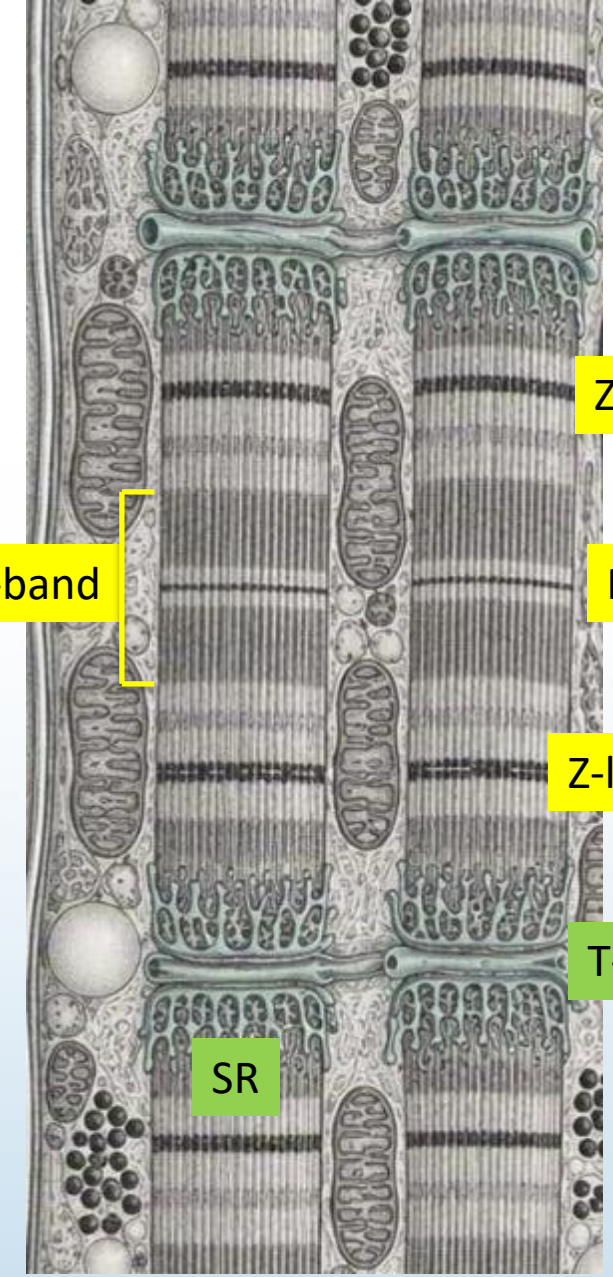

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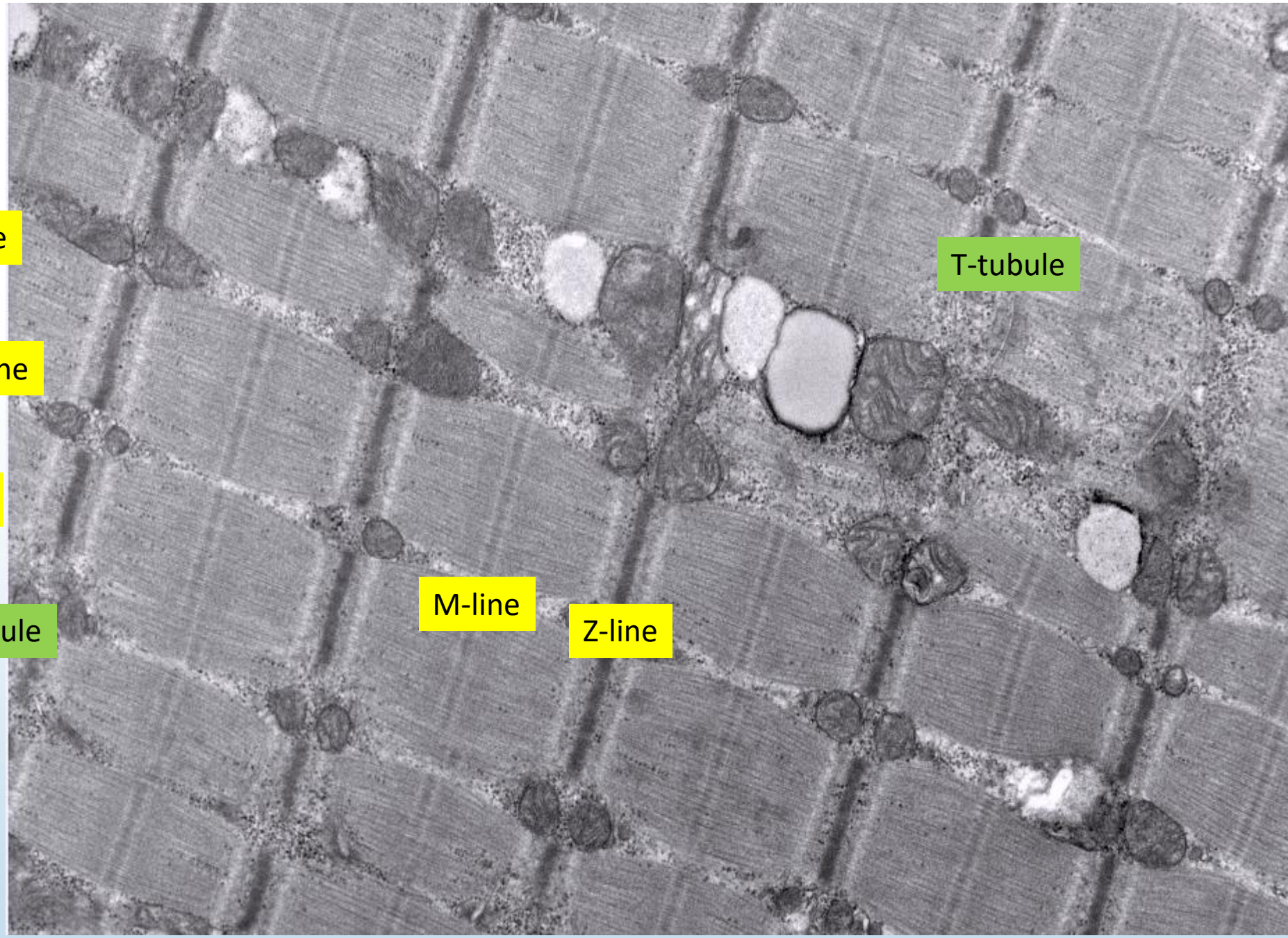
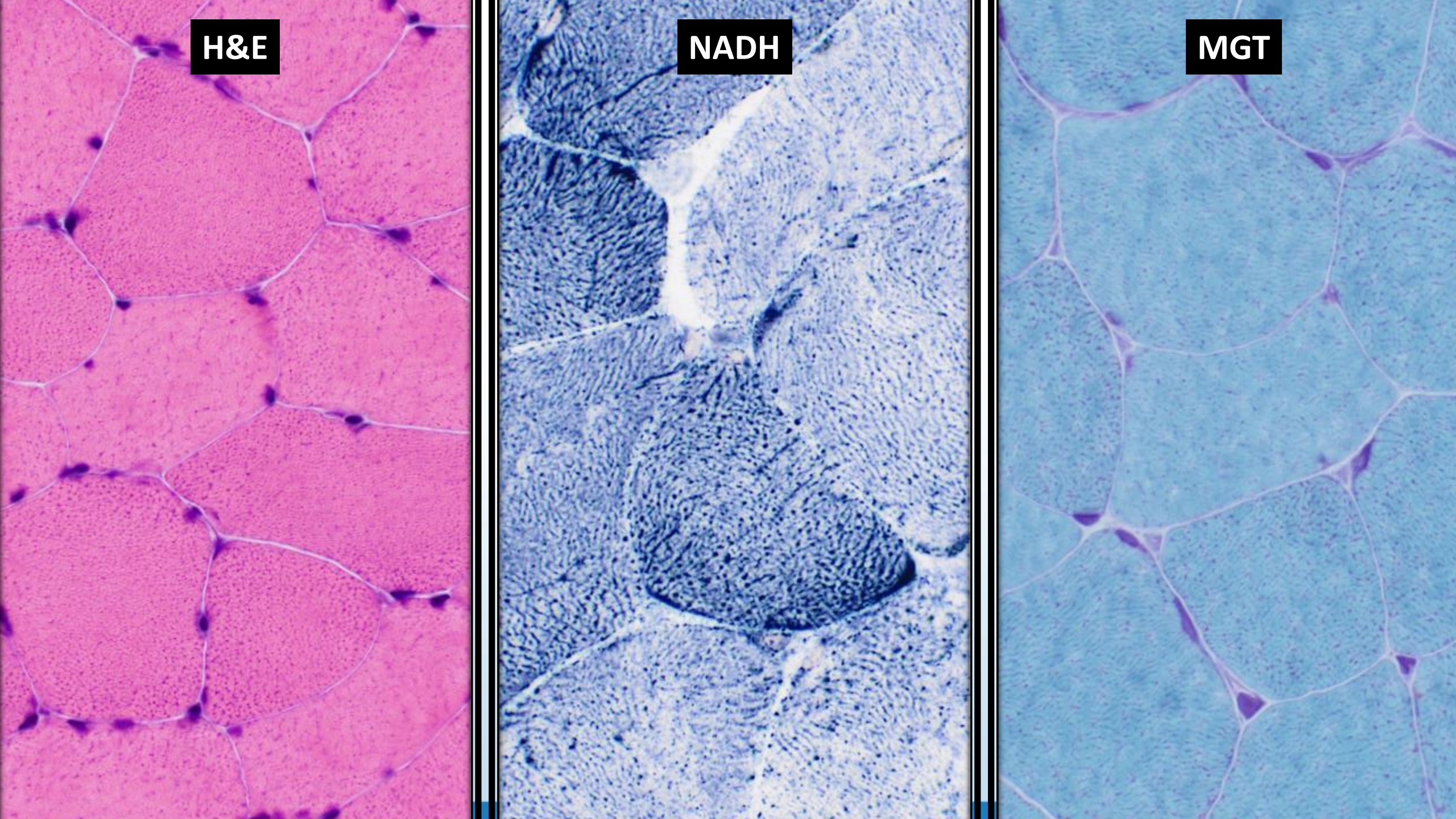
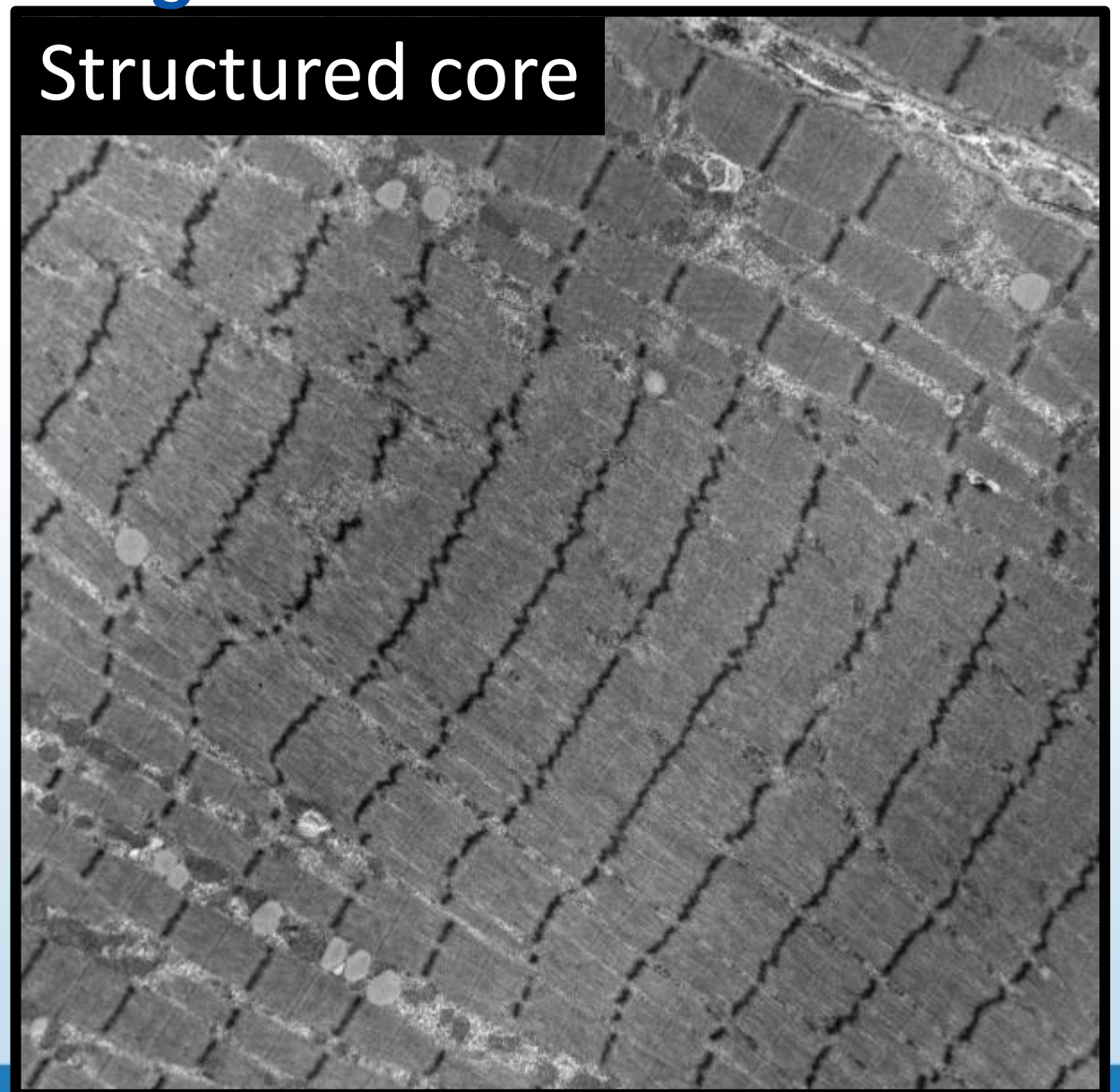
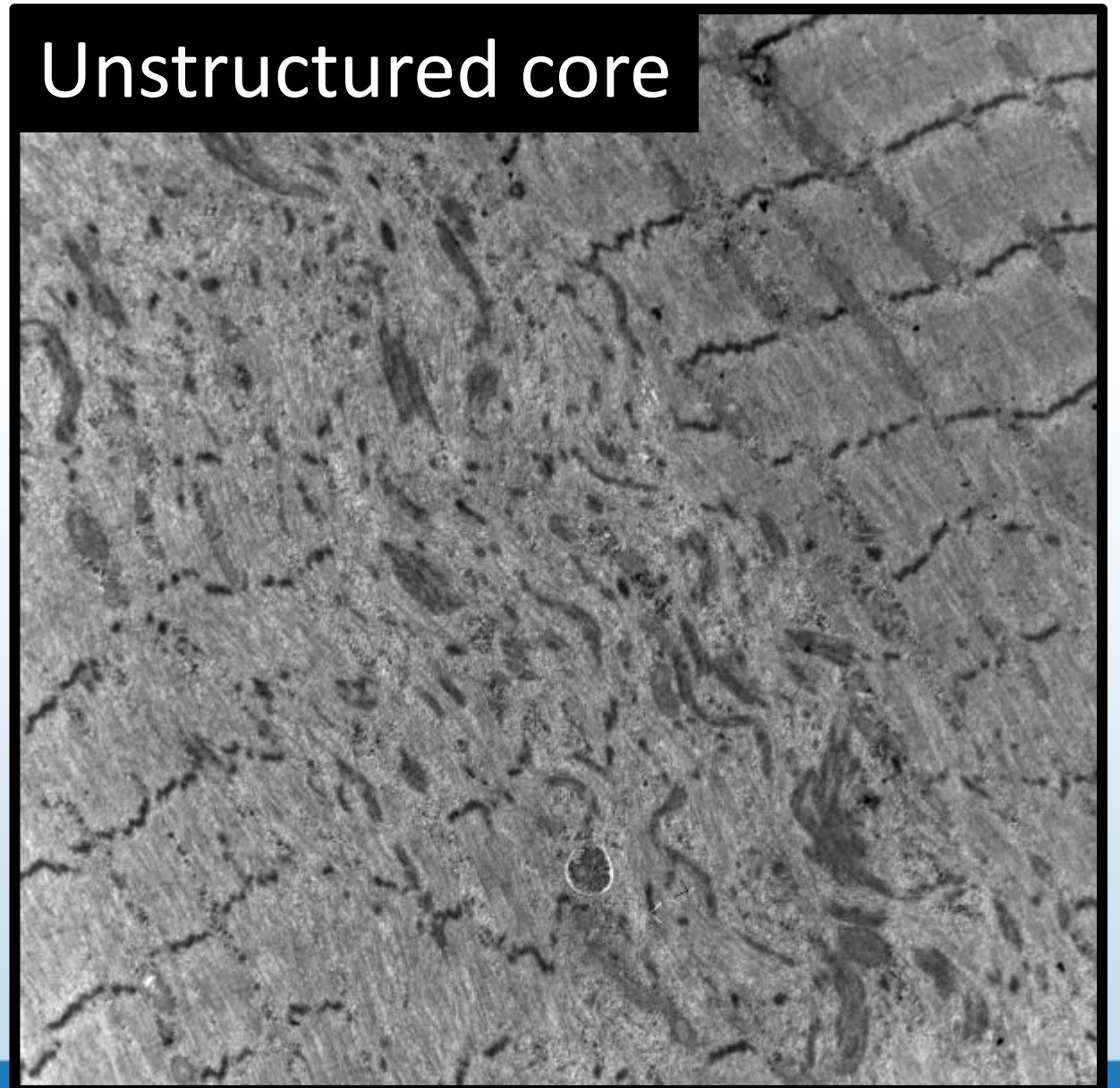


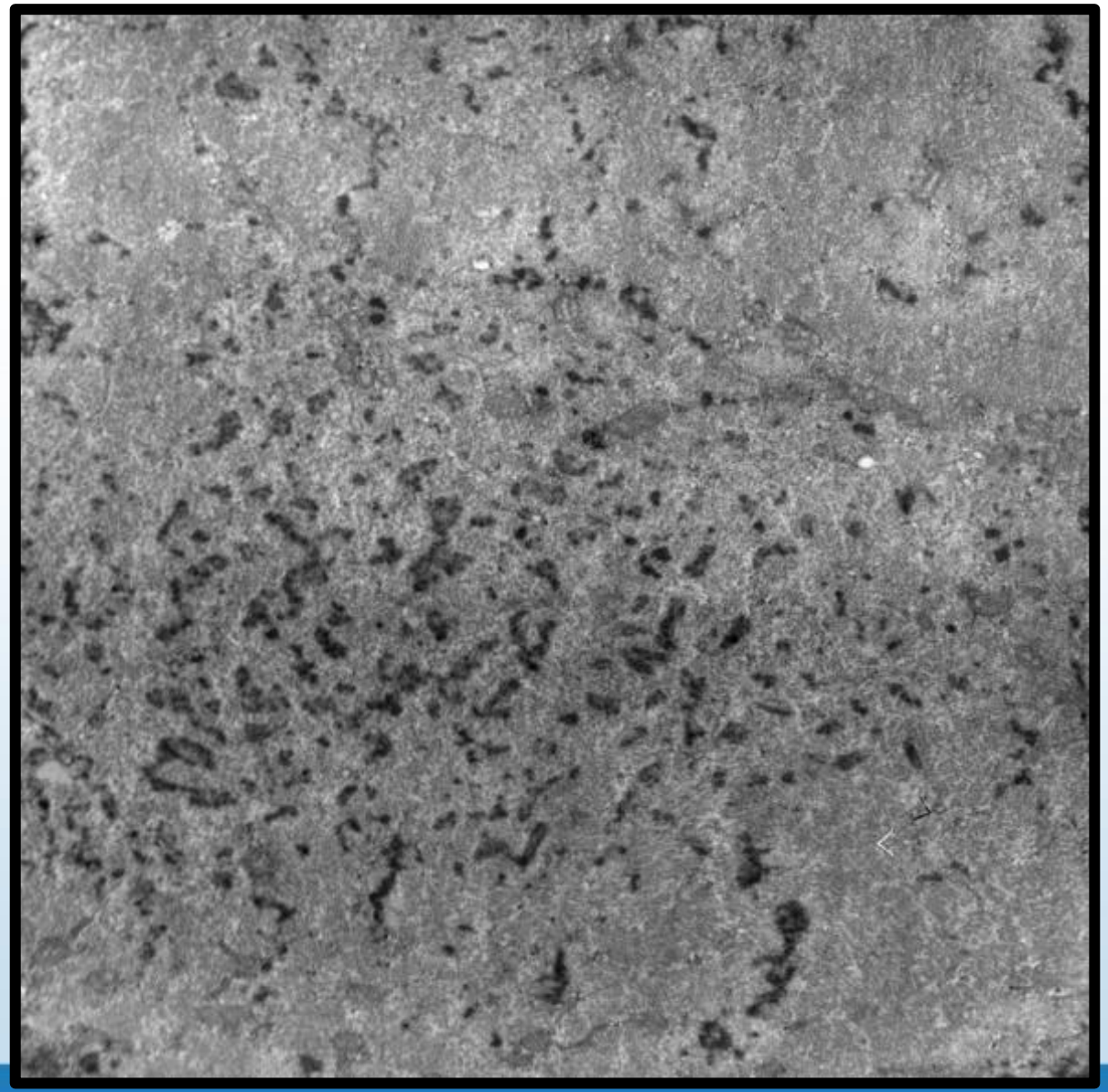

H&E
NADH
MGT

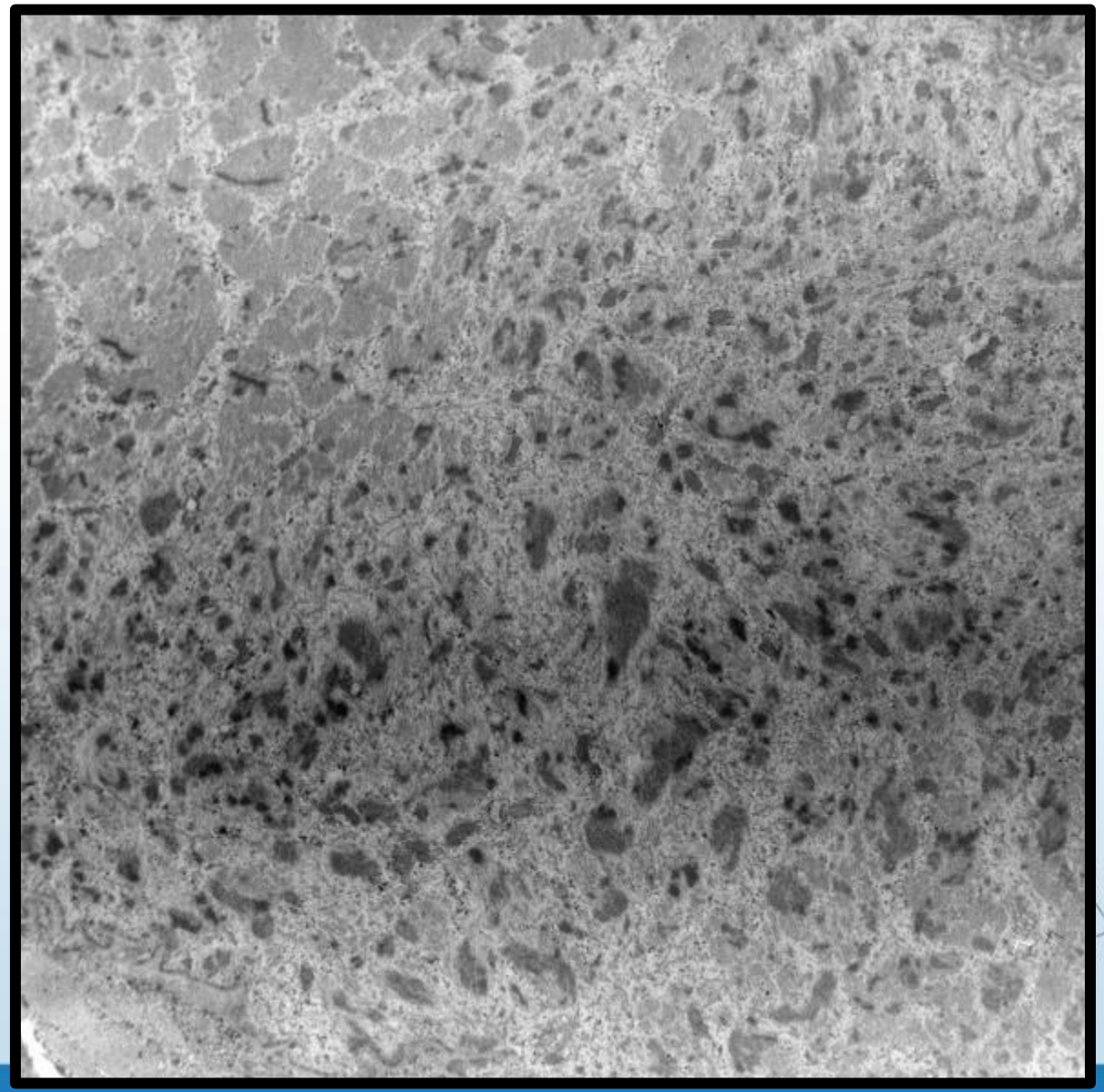
Classic sarcomeric abnormality: Cores longitudinal section



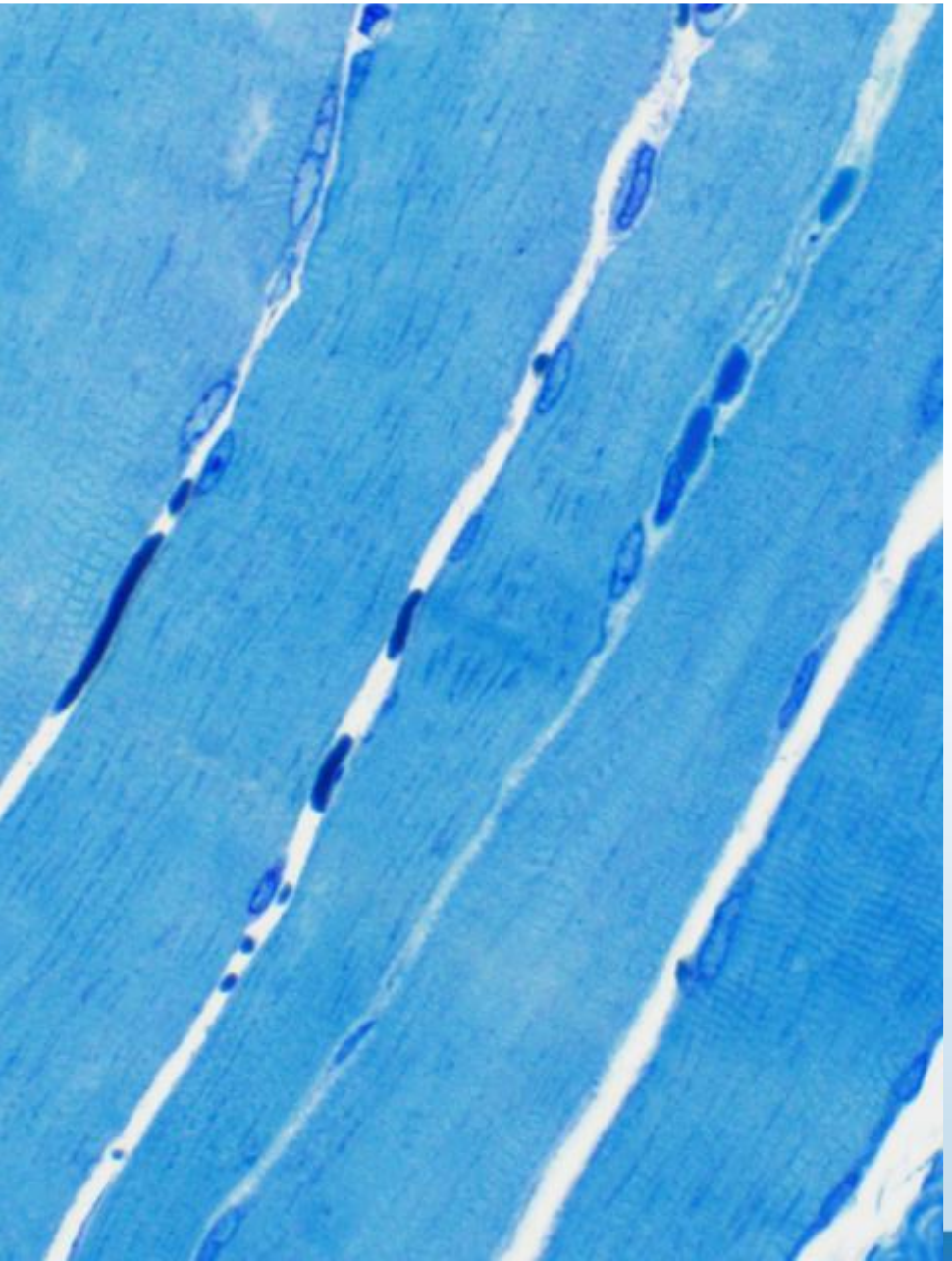


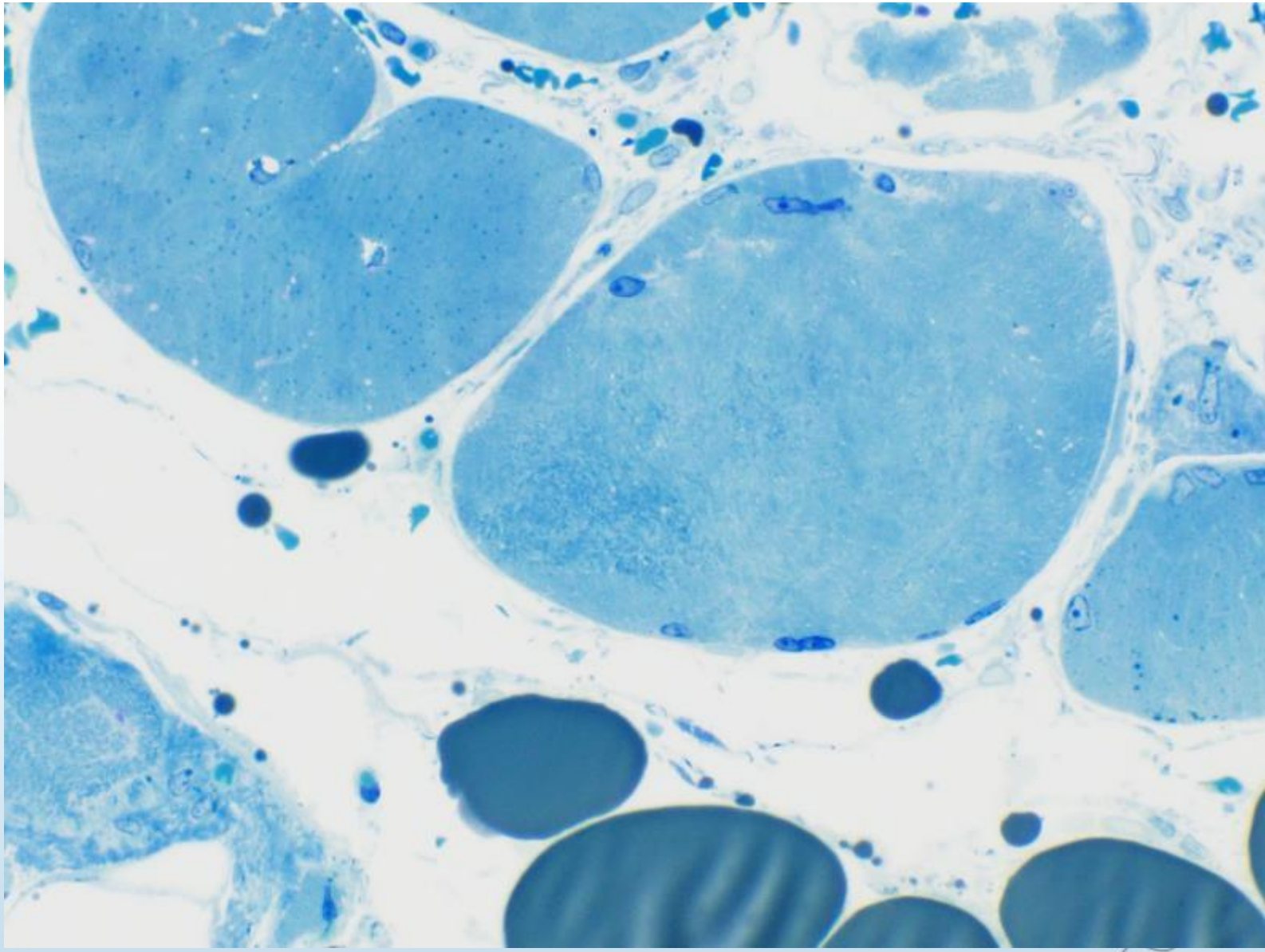
Classic sarcomeric abnormality: Cores transverse section



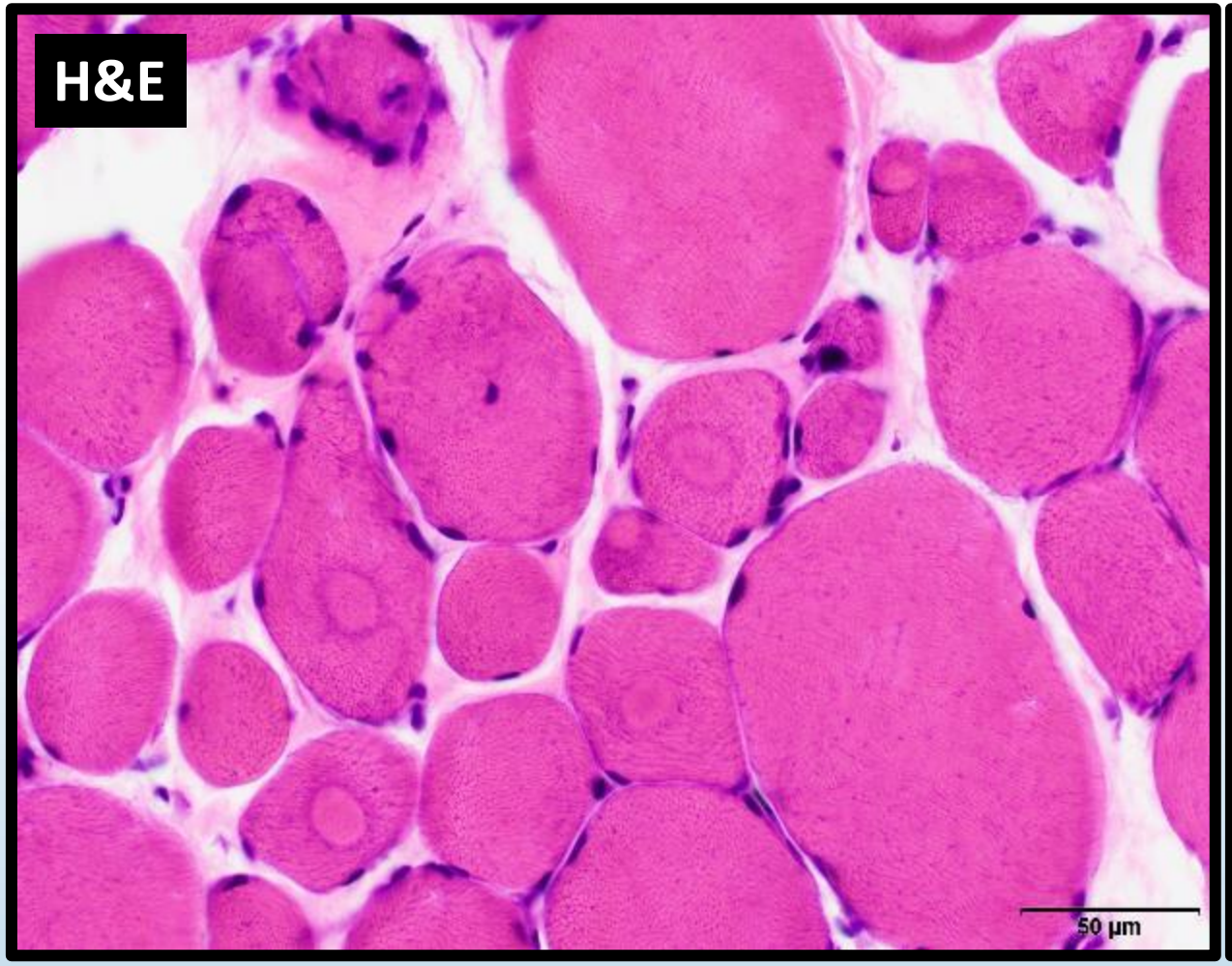


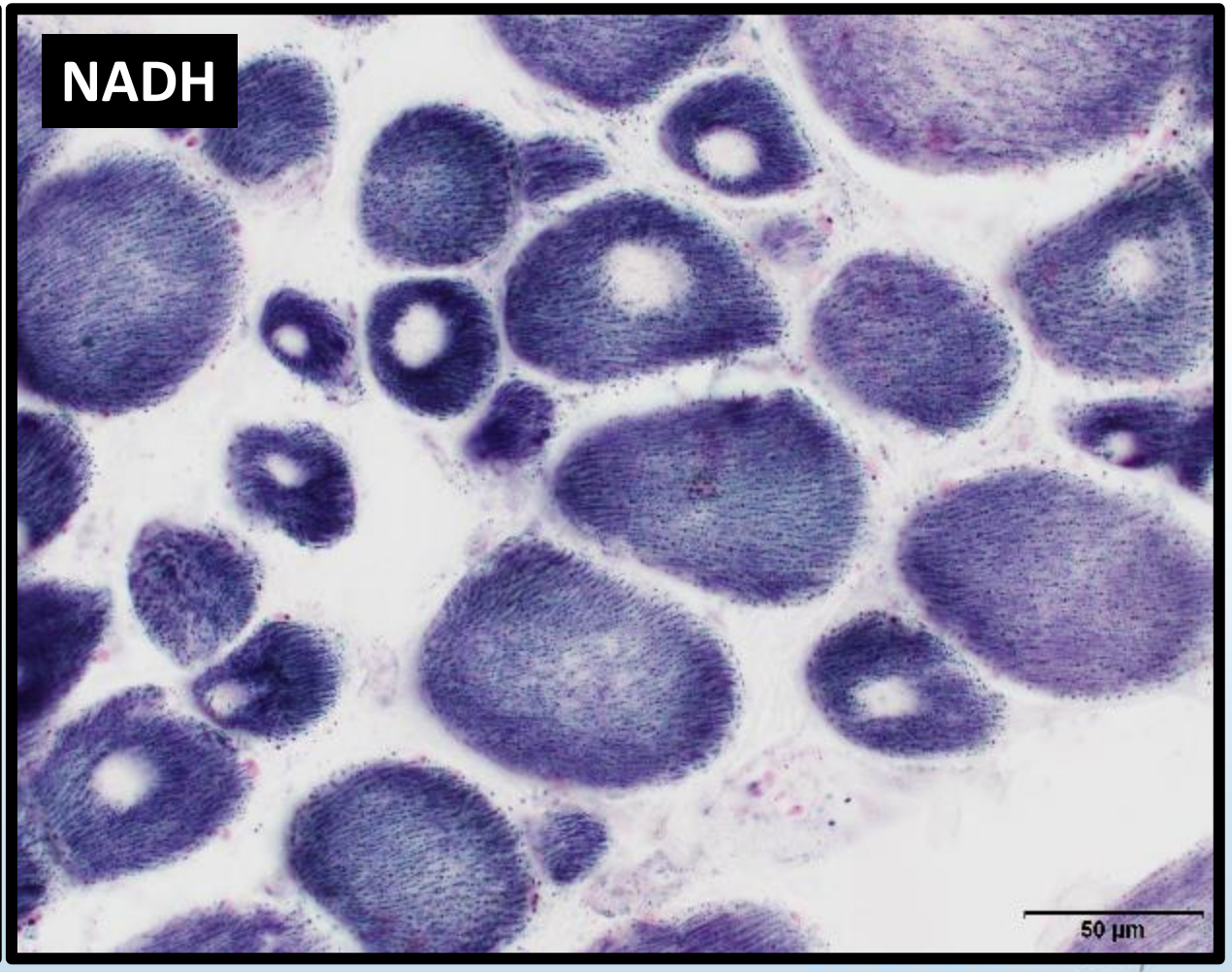
Cores on toluidine blue stained epon sections



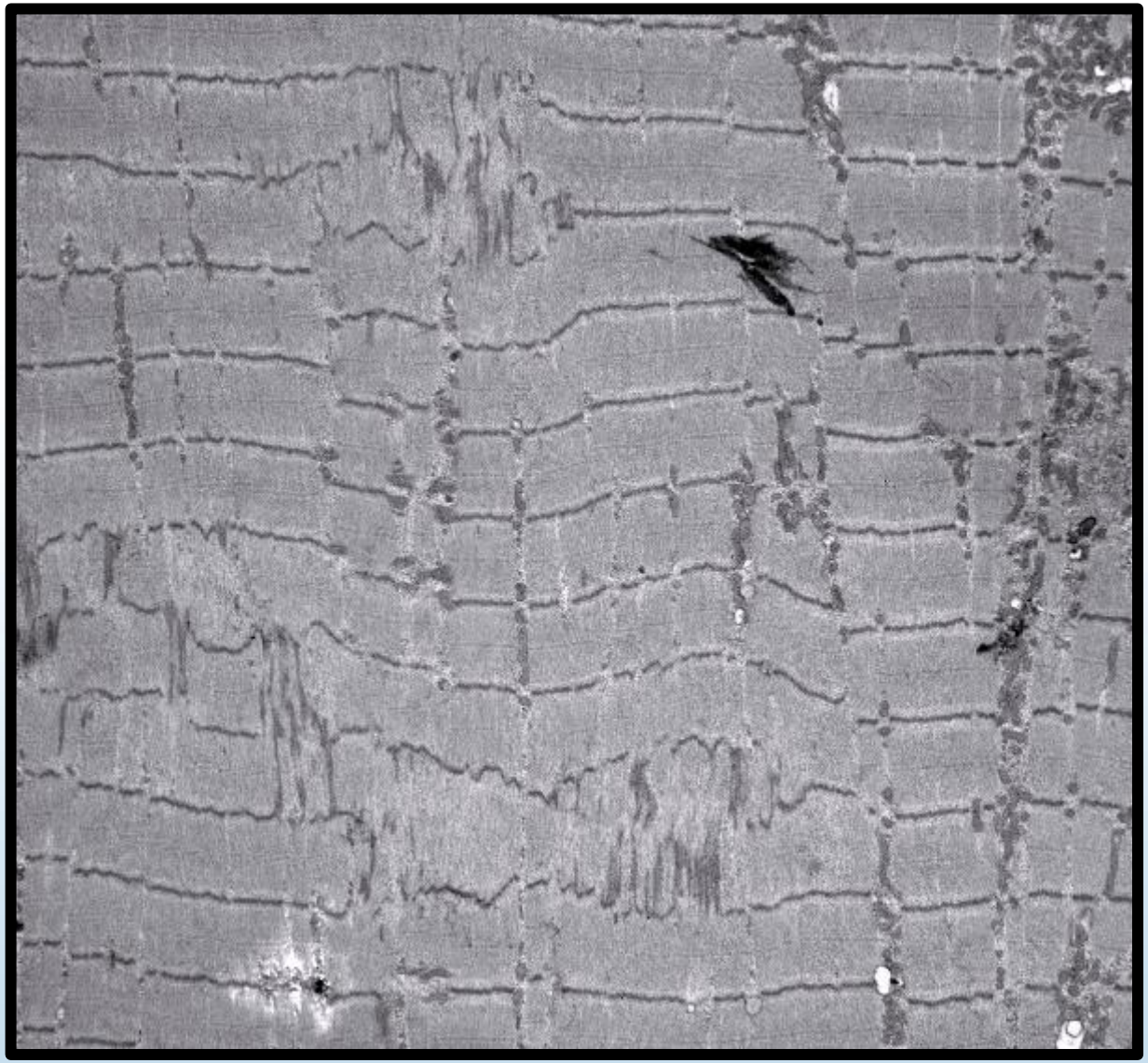


Central/structured cores on frozen sections: *RYR1*-related central core myopathy

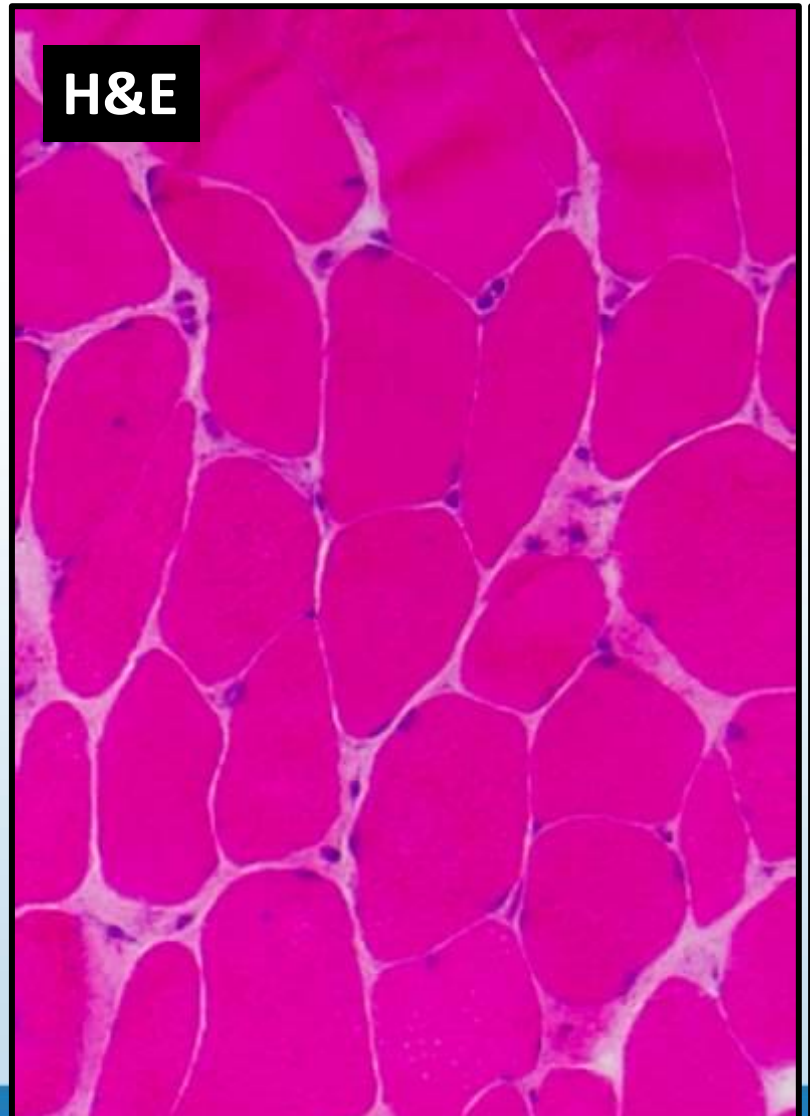


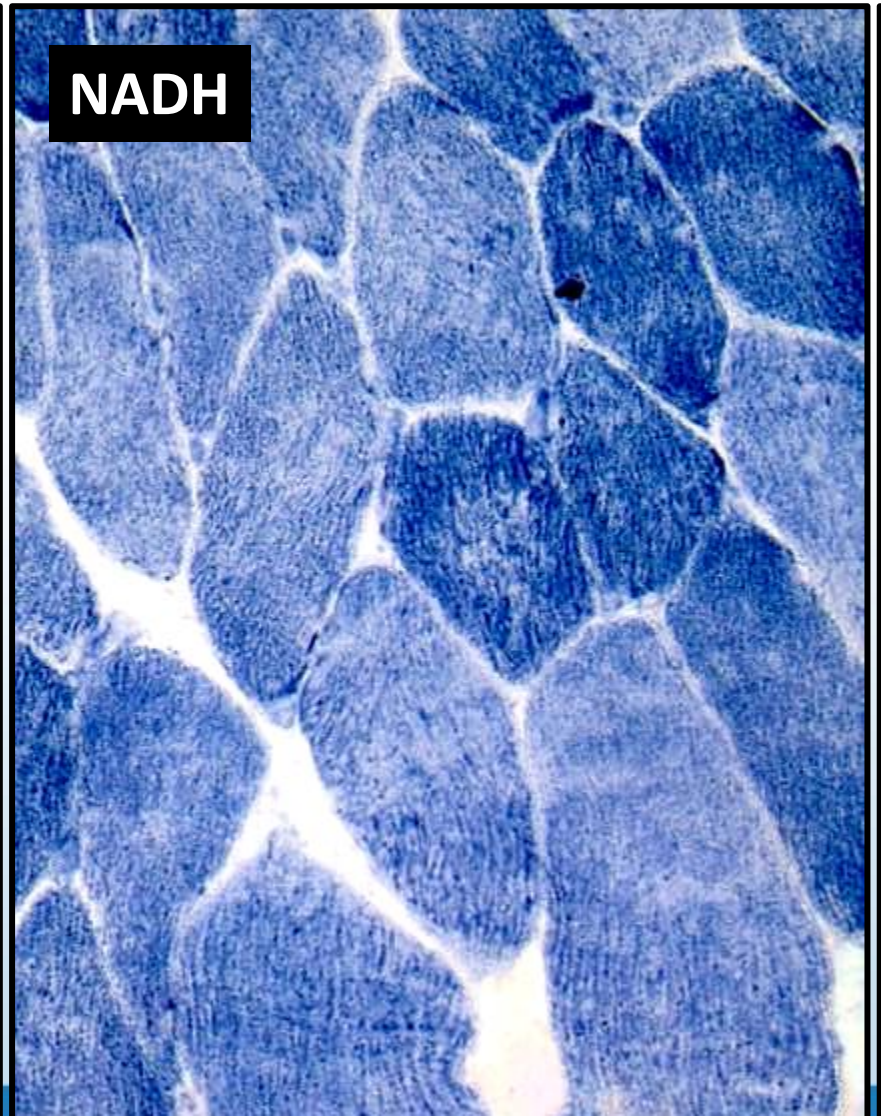


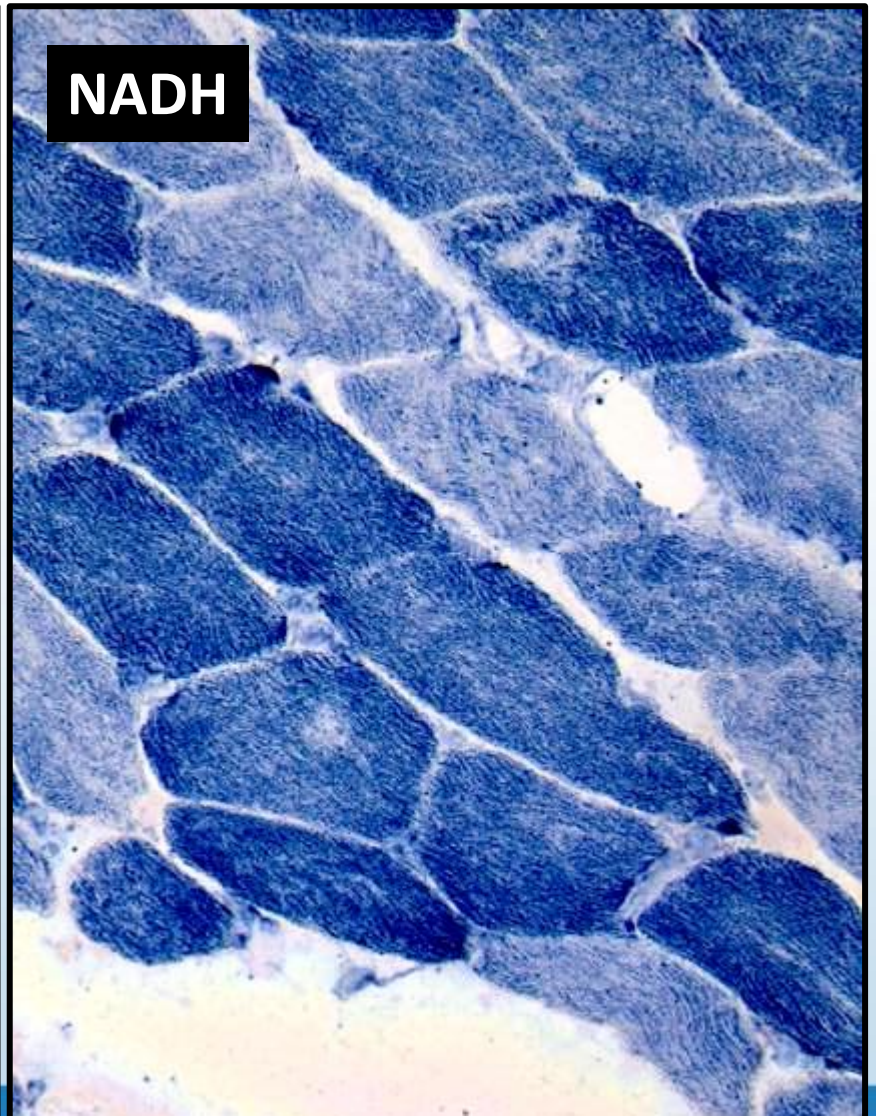
Classic sarcomeric abnormality: Minicores longitudinal section



Minicores on frozen sections:
SEPN1-related multi minicore myopathy

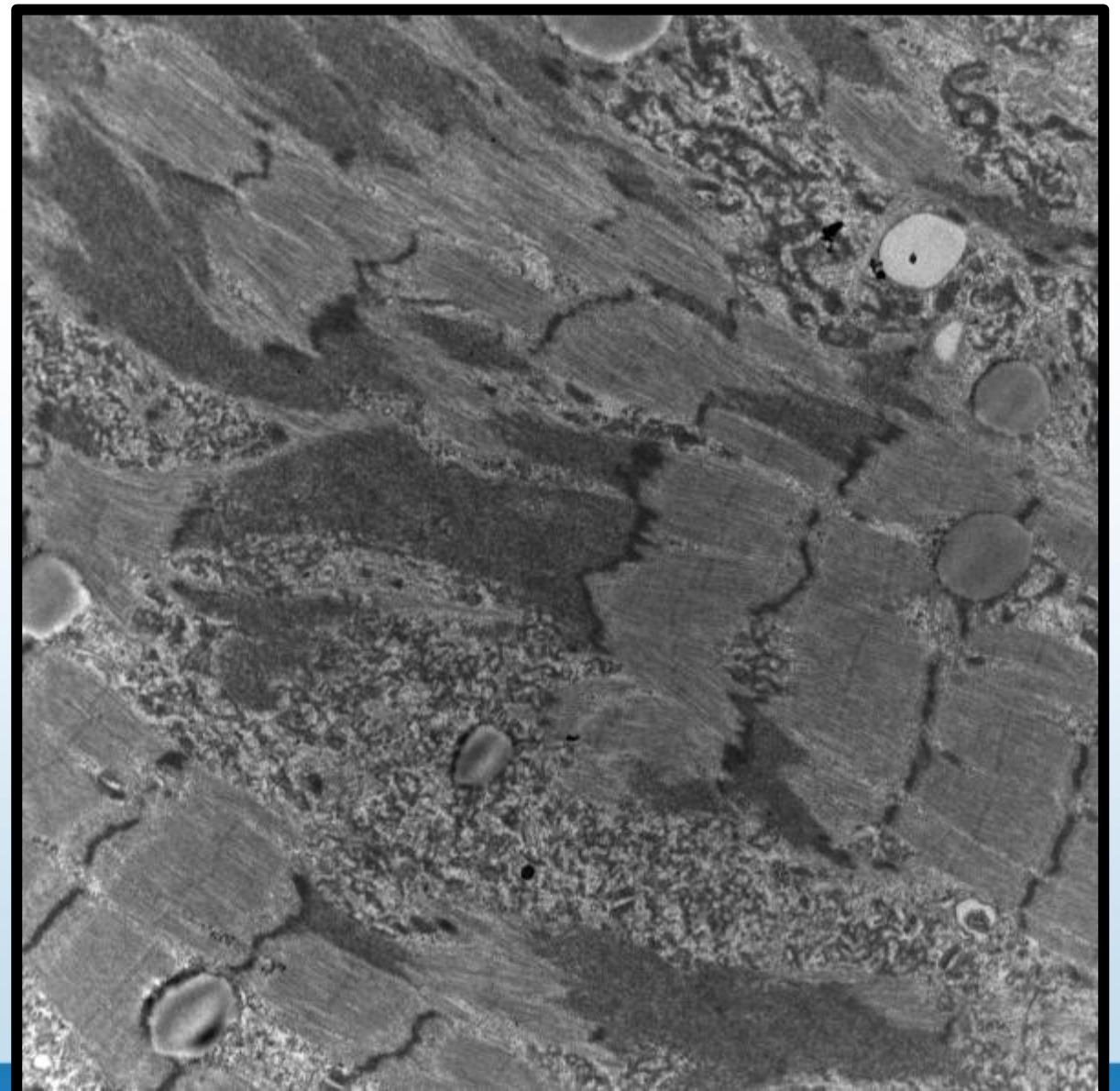


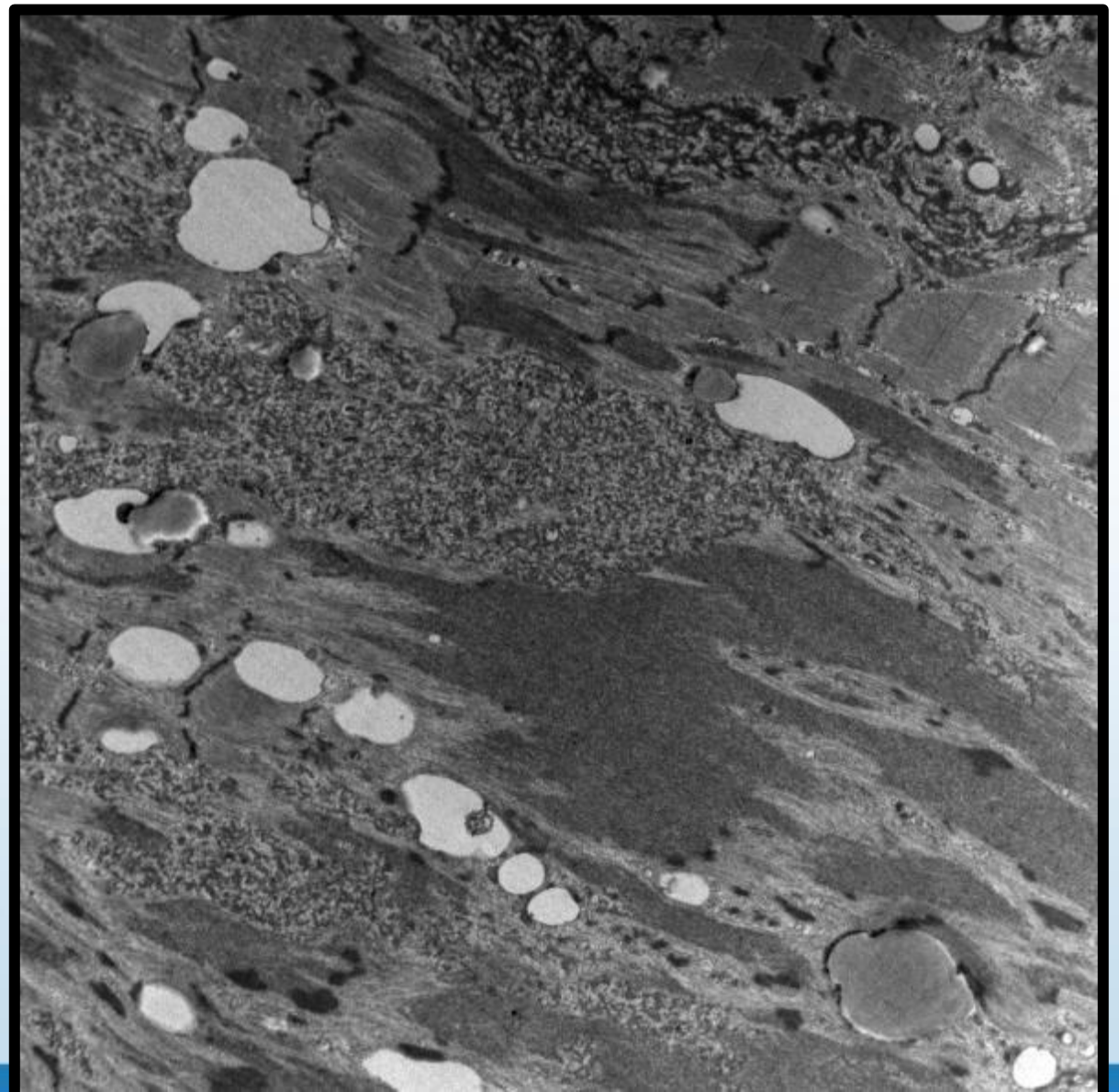




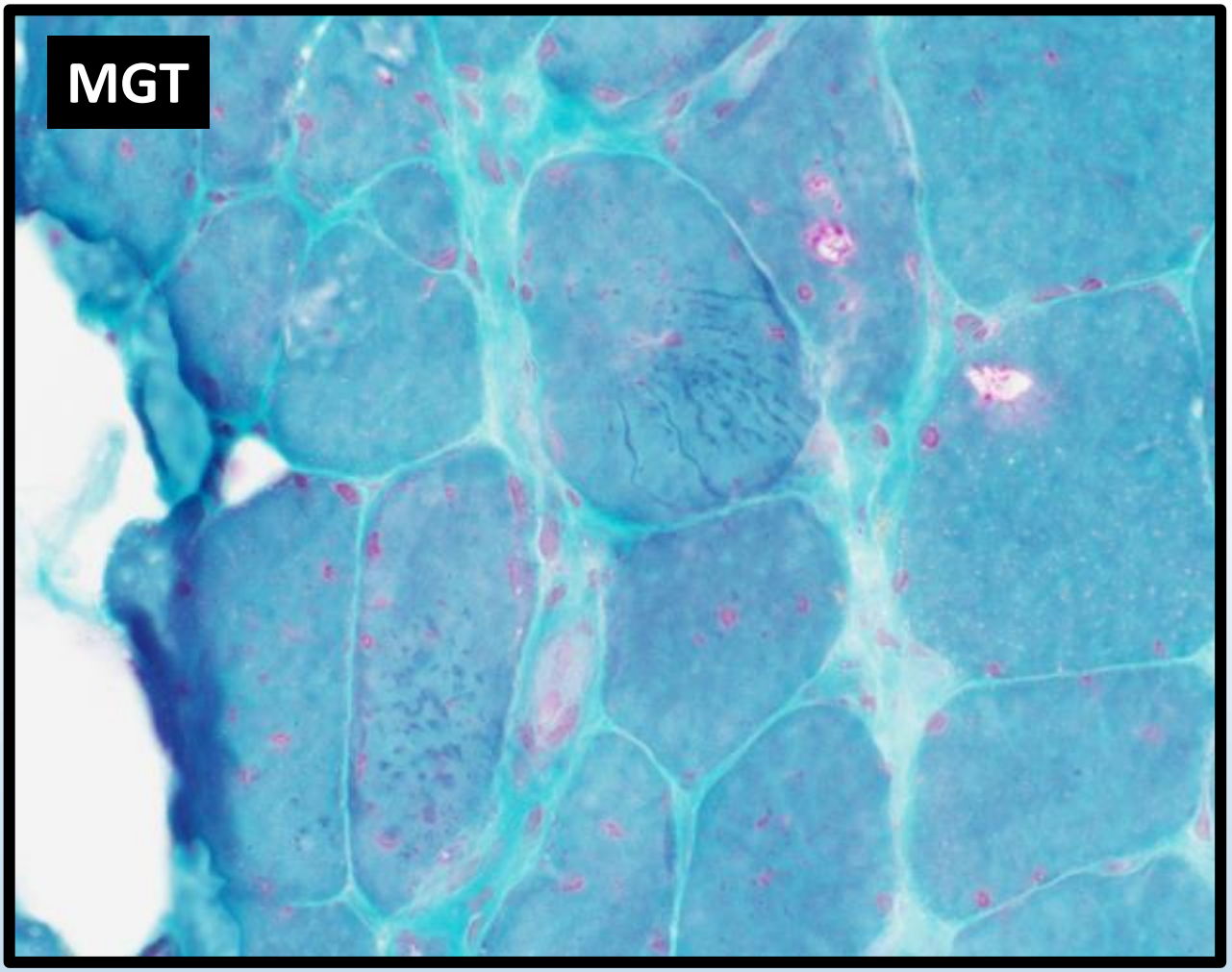
Target fibers in neurogenic disease – identical to cores by EM

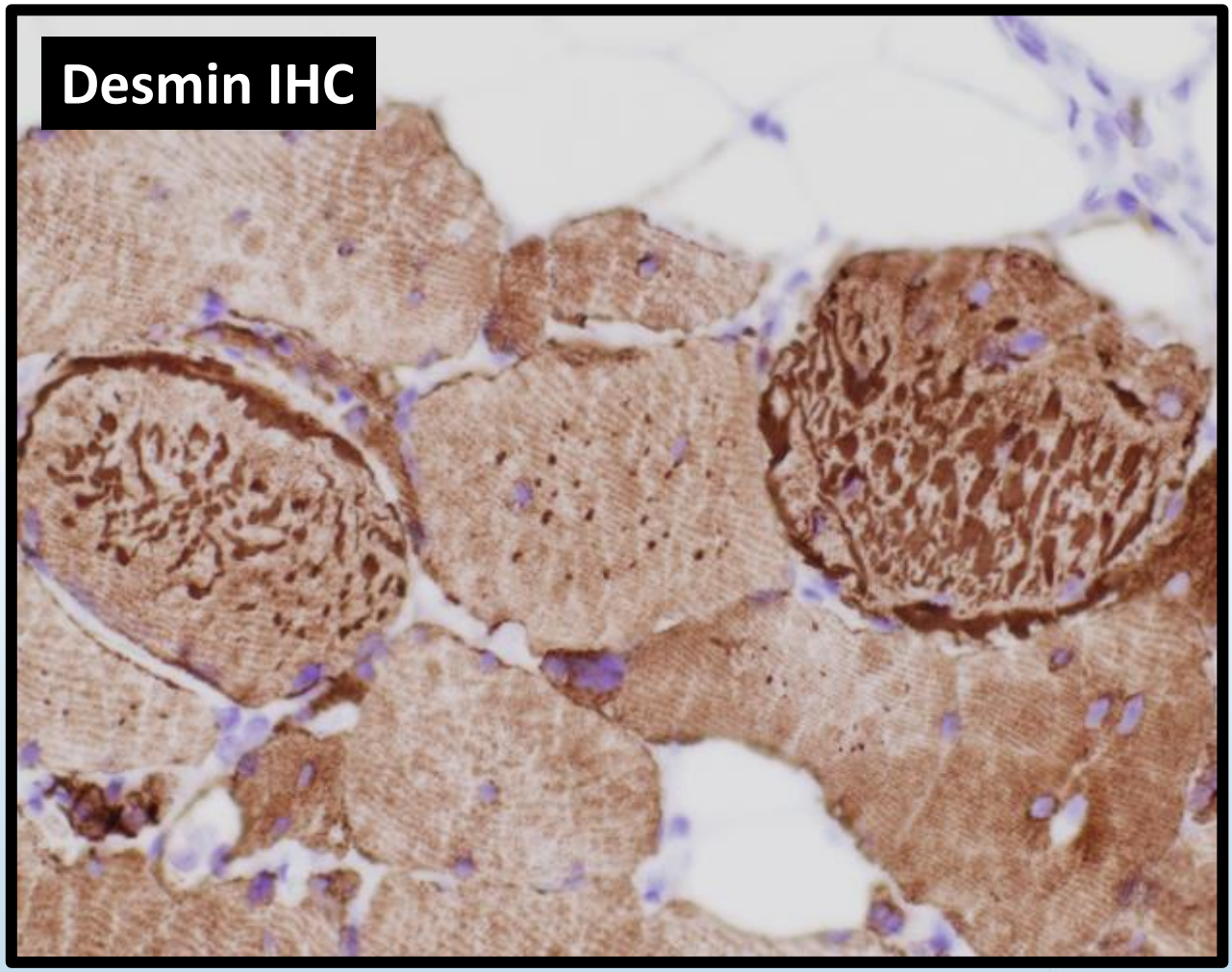
Z-line streaming in myofibrillar myopathies - associated with granulofilamentous inclusion material



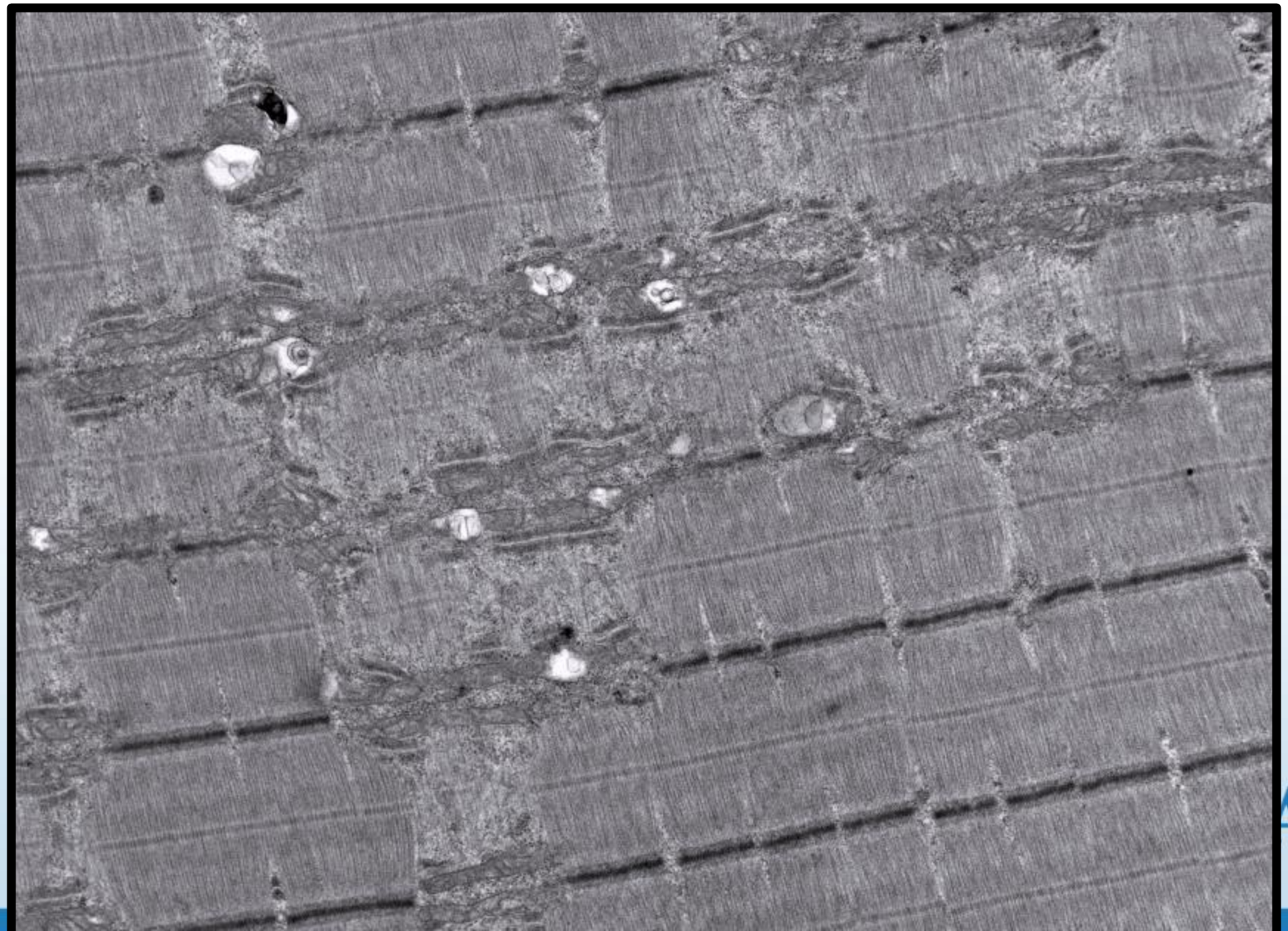


Myofibrillar inclusions on frozen sections: *DES*-related myofibrillar myopathy

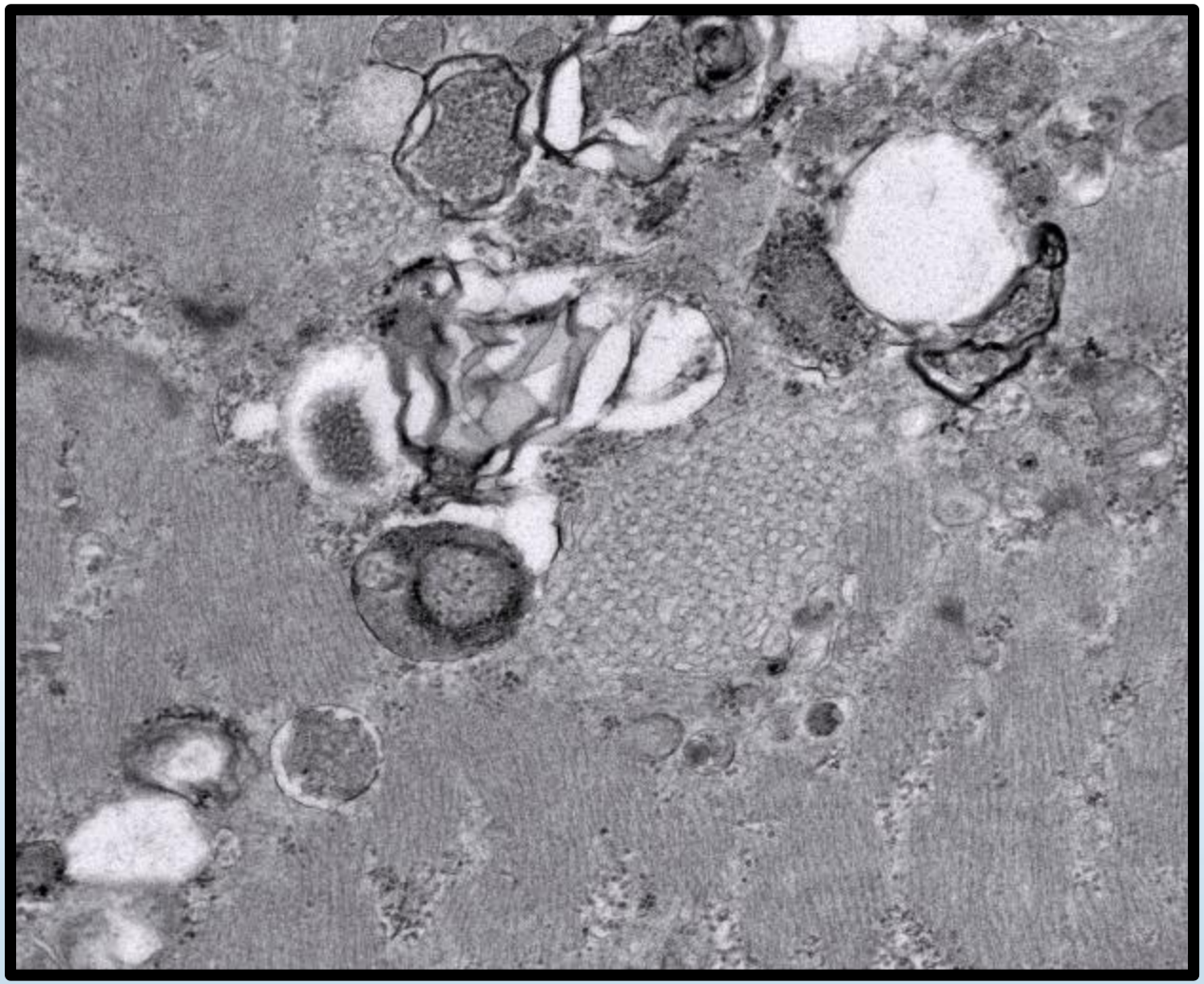


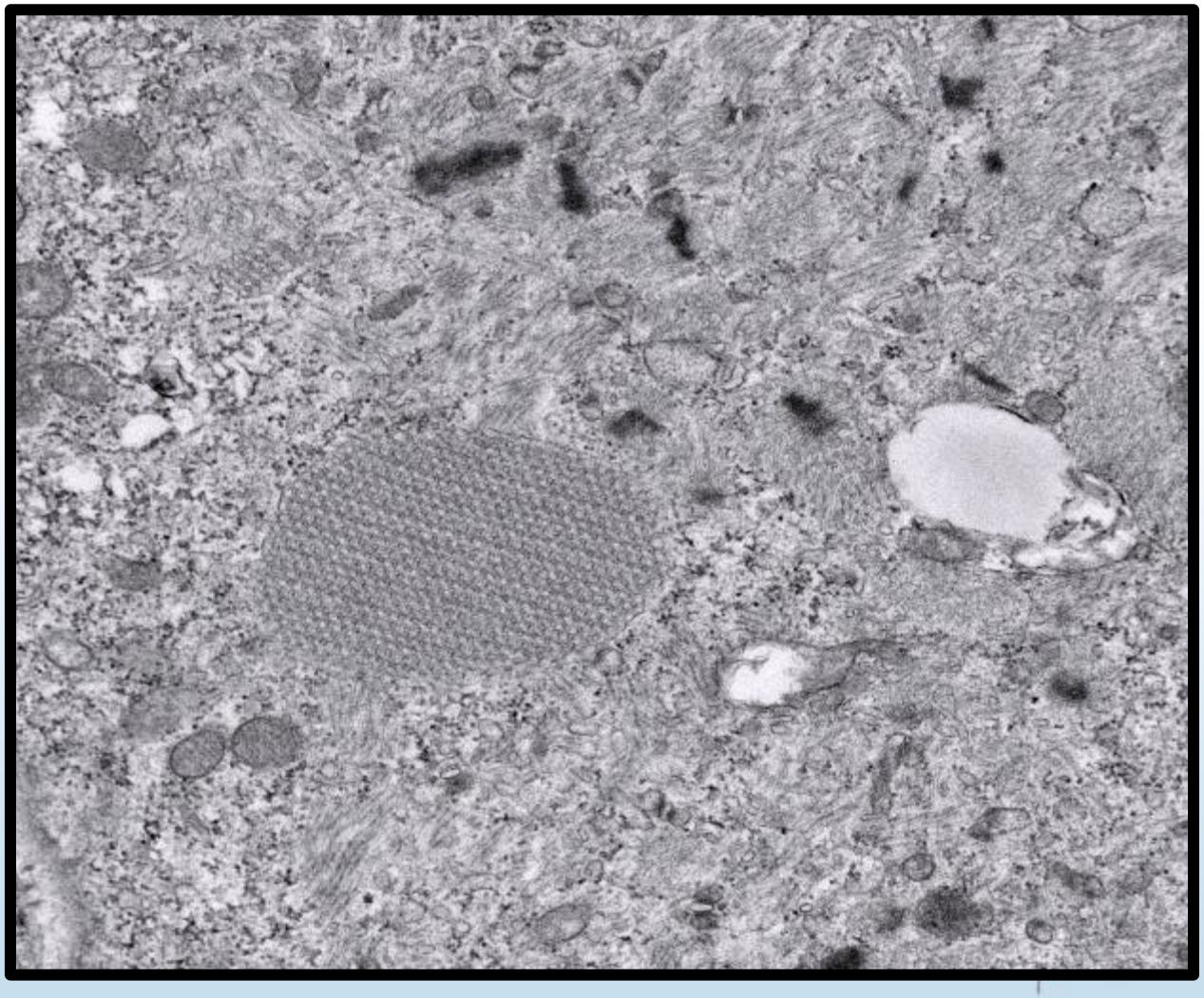


Prominent T-tubules - nonspecific



Honeycomb structures: evidence of regeneration





MITOCHONDRIA AND LIPID

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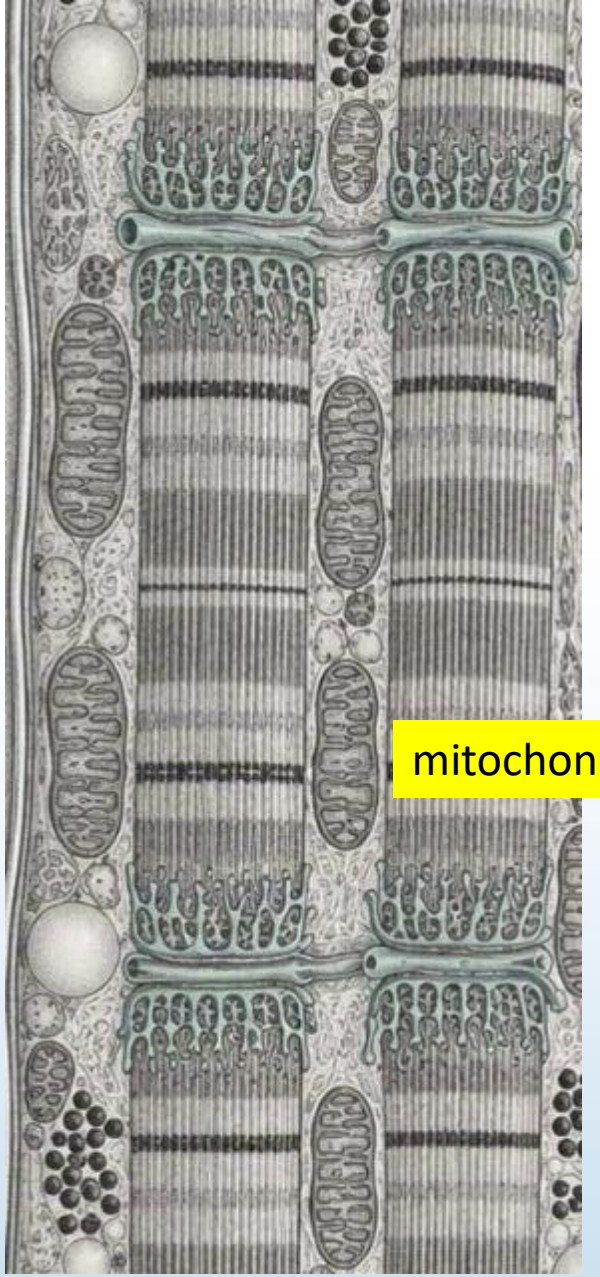

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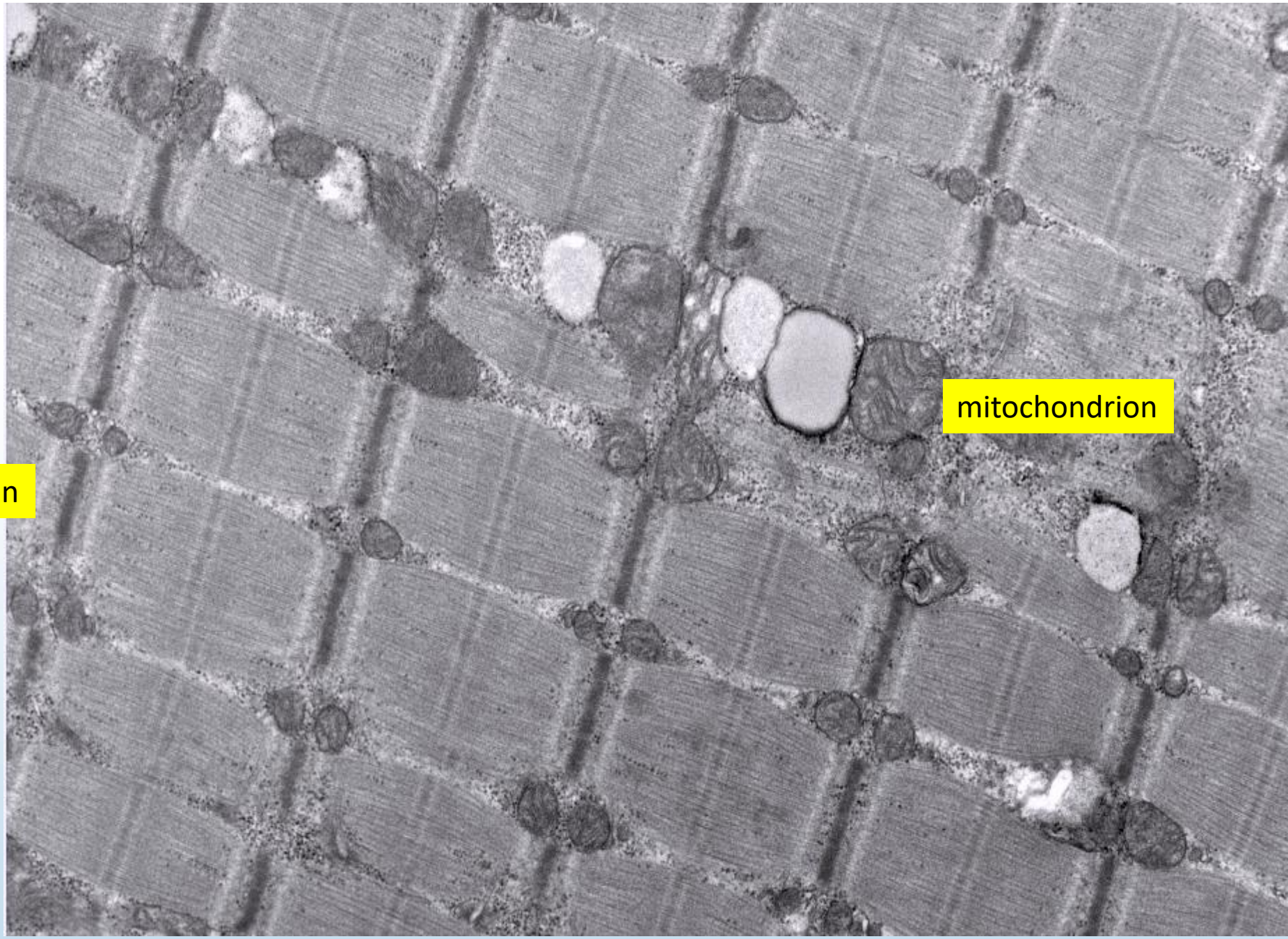
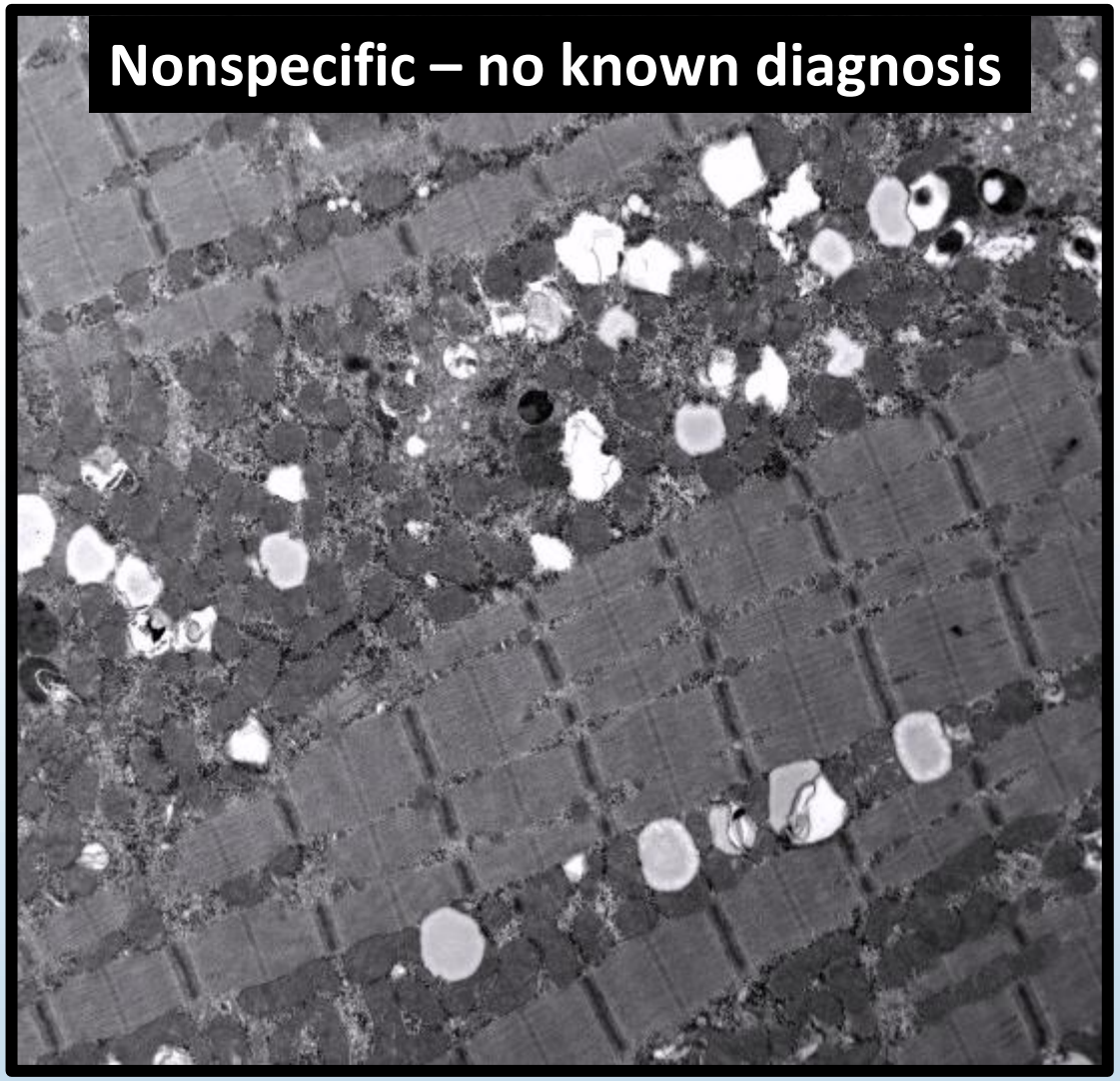
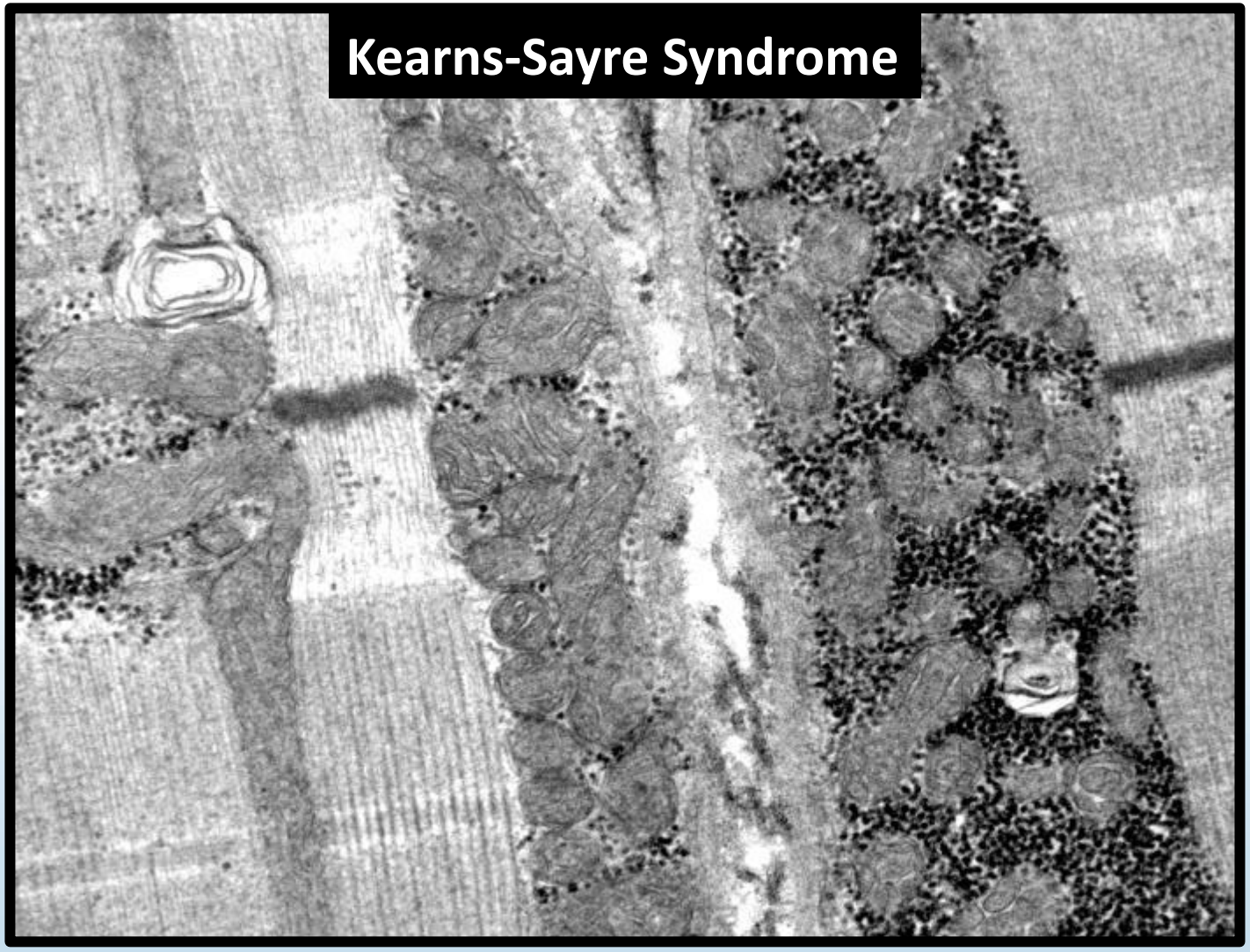
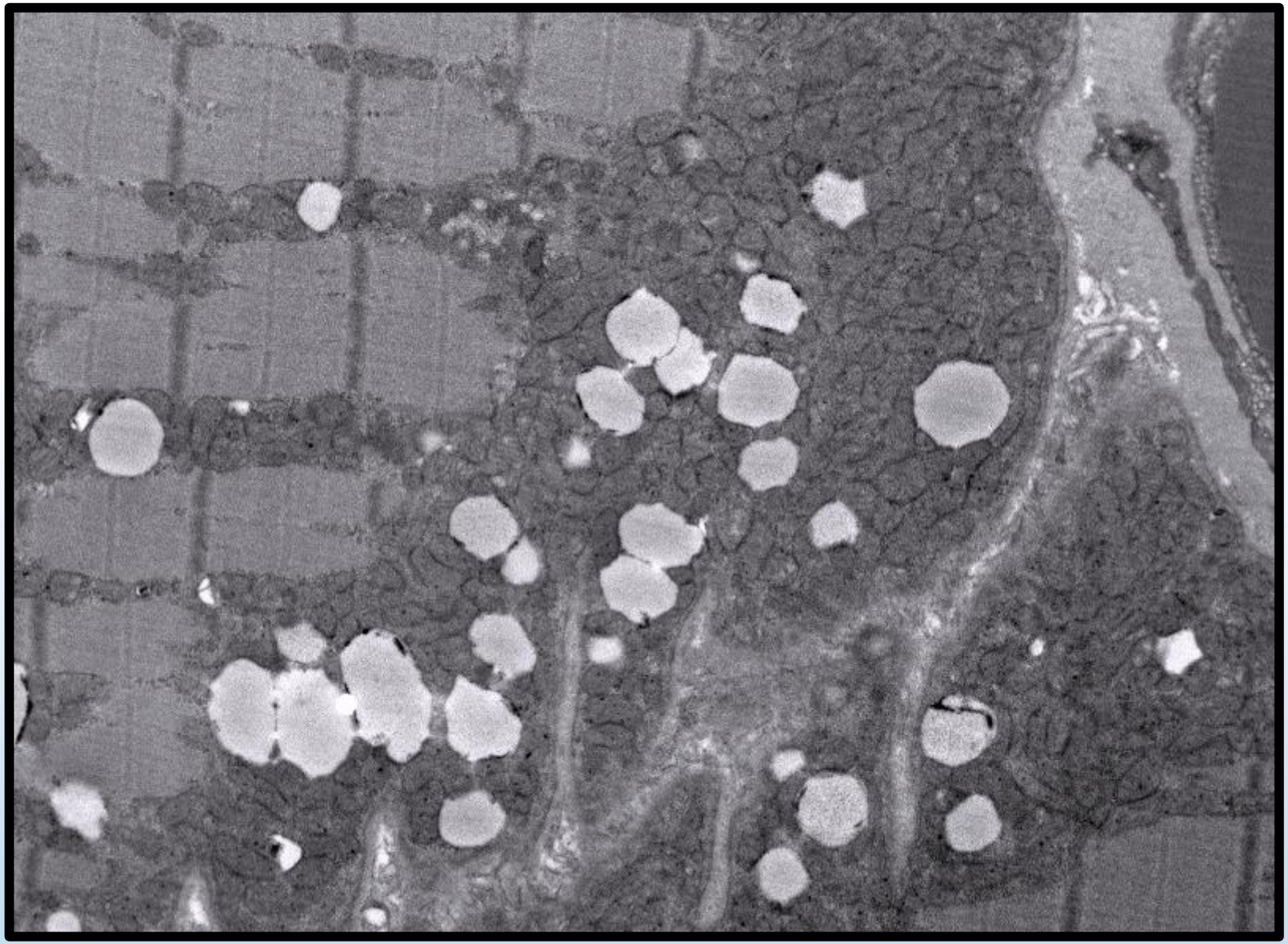


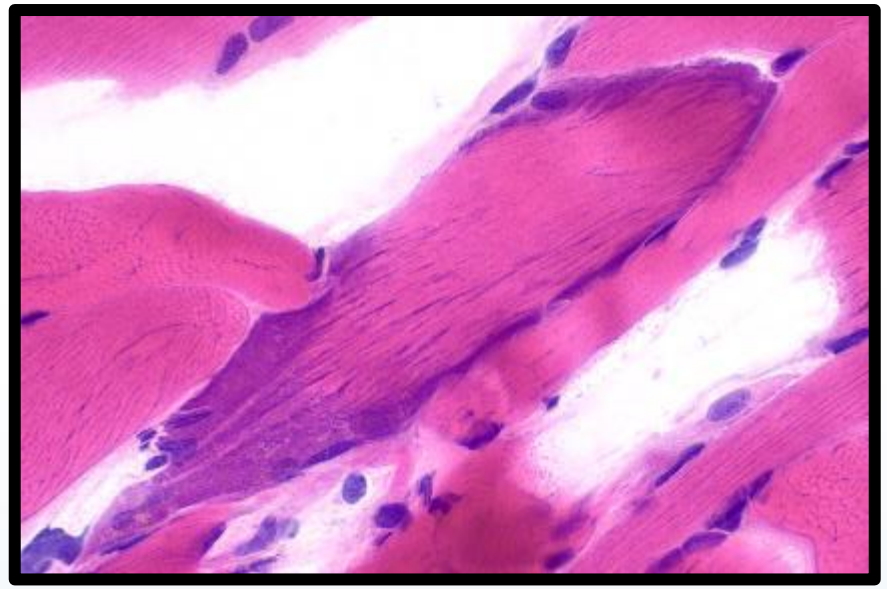
Mitochondria: increased numbers, pooling, aggregation, branching

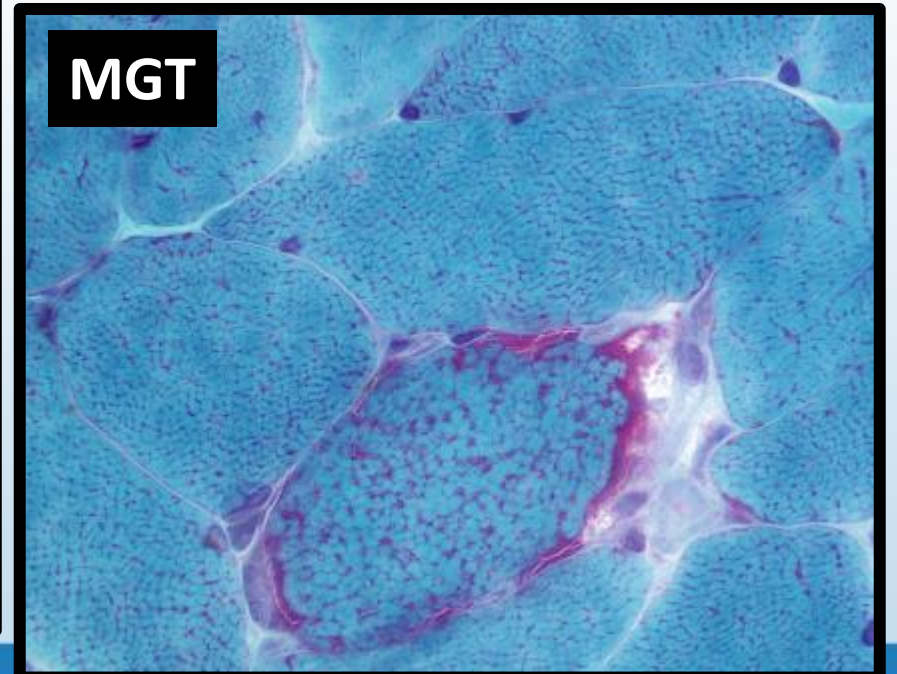




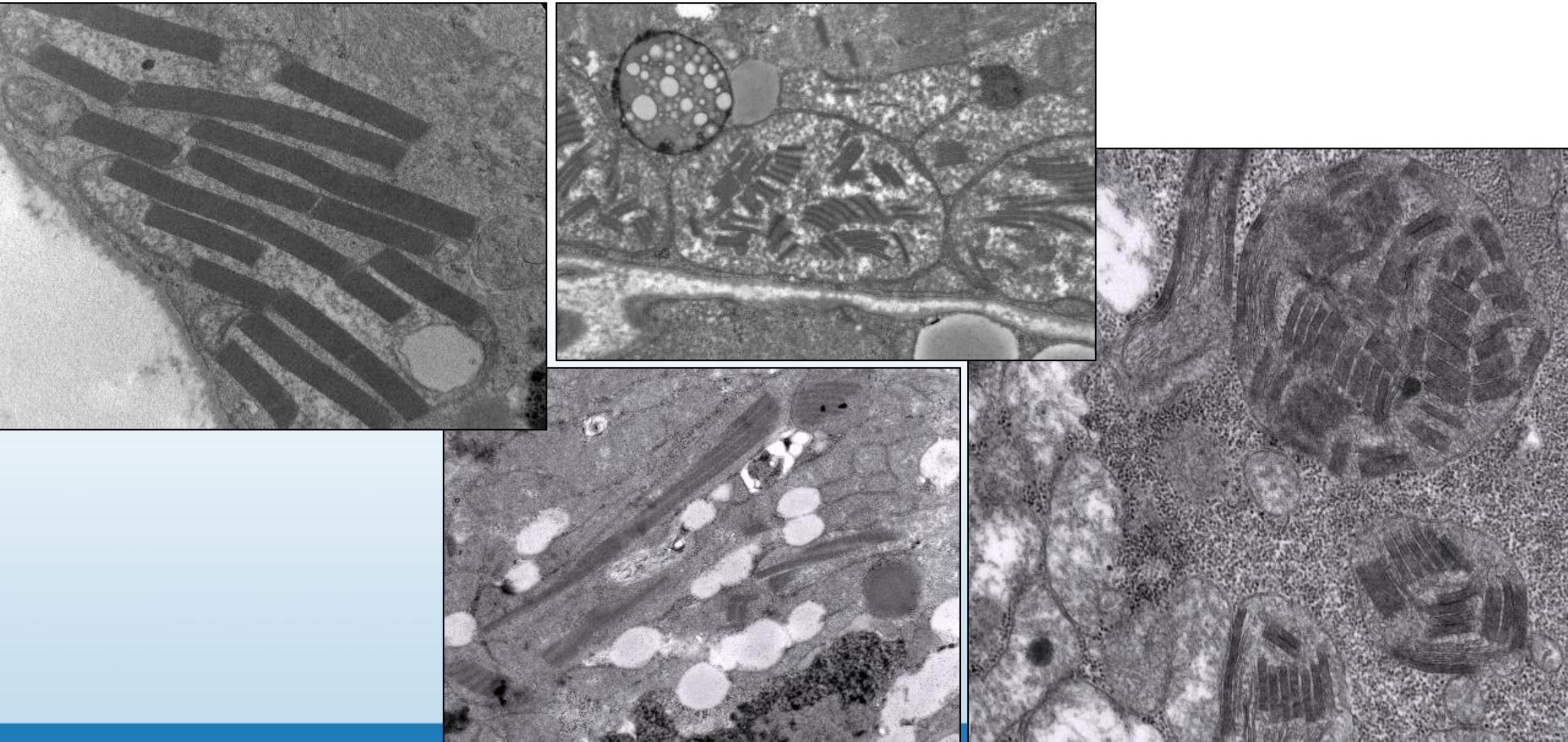
Mitochondria: ragged red fibers



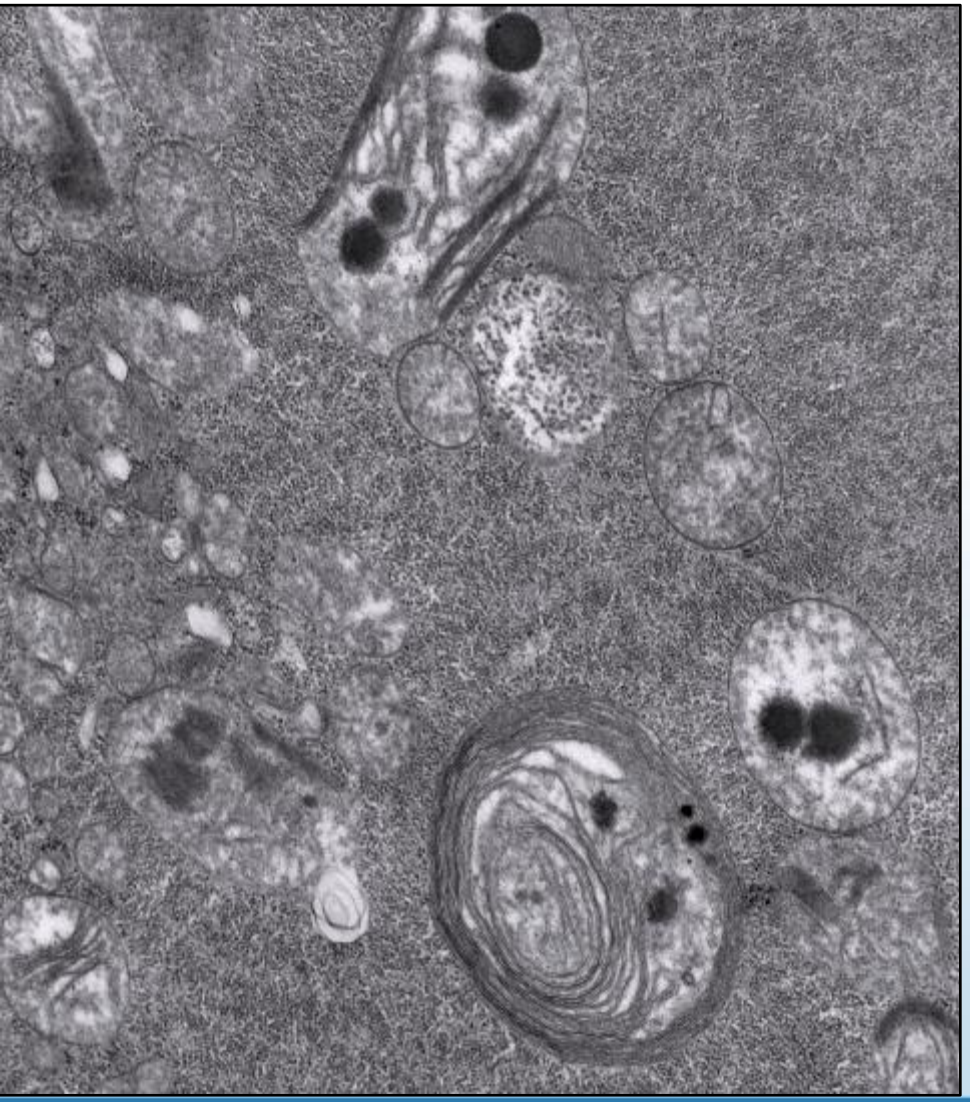


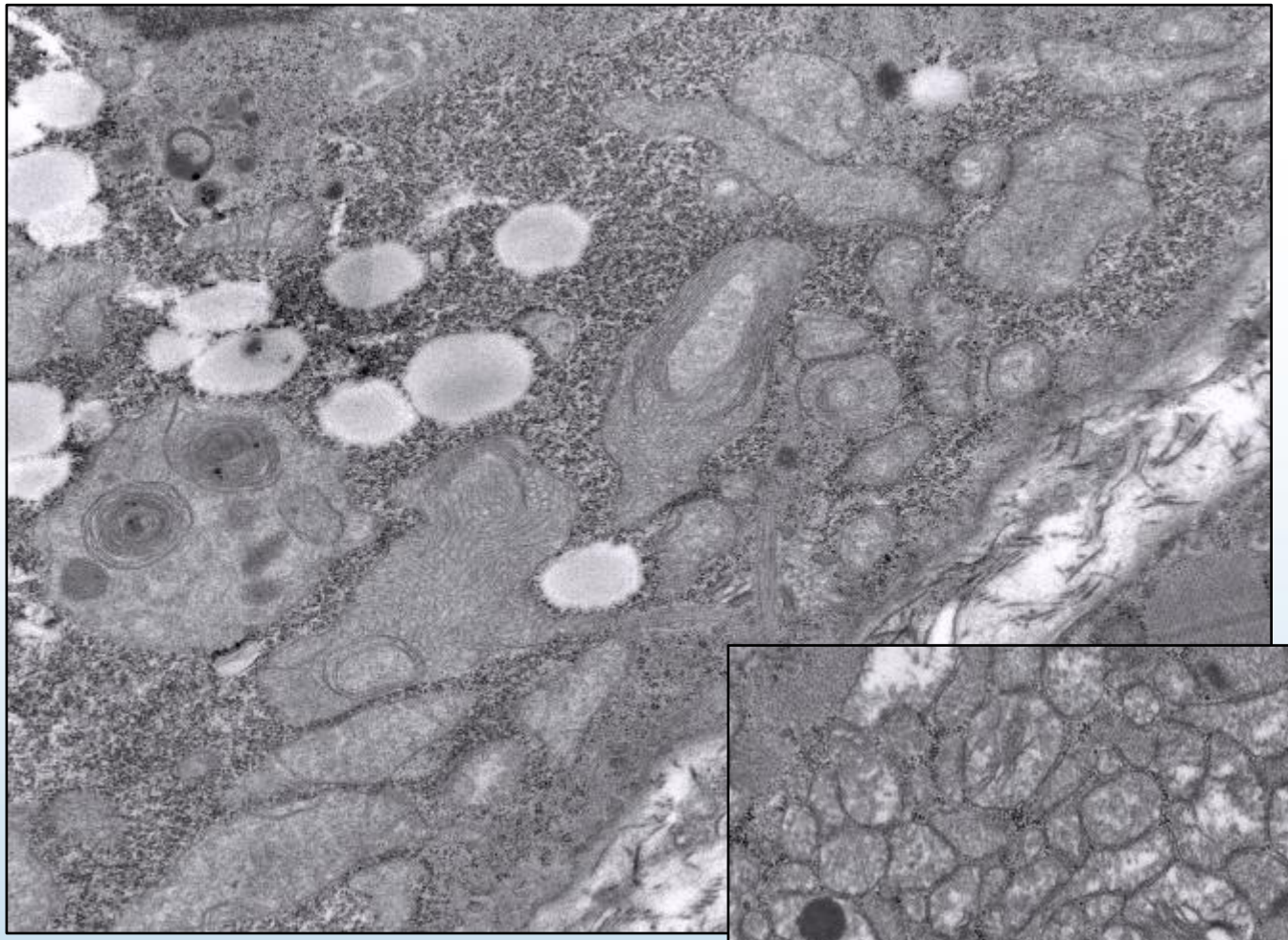


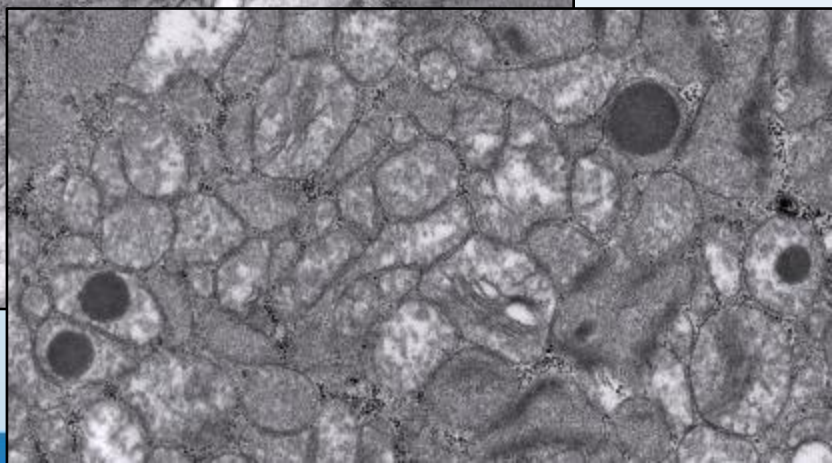
Mitochondria: paracrystalline, rectilinear, or parking lot inclusions



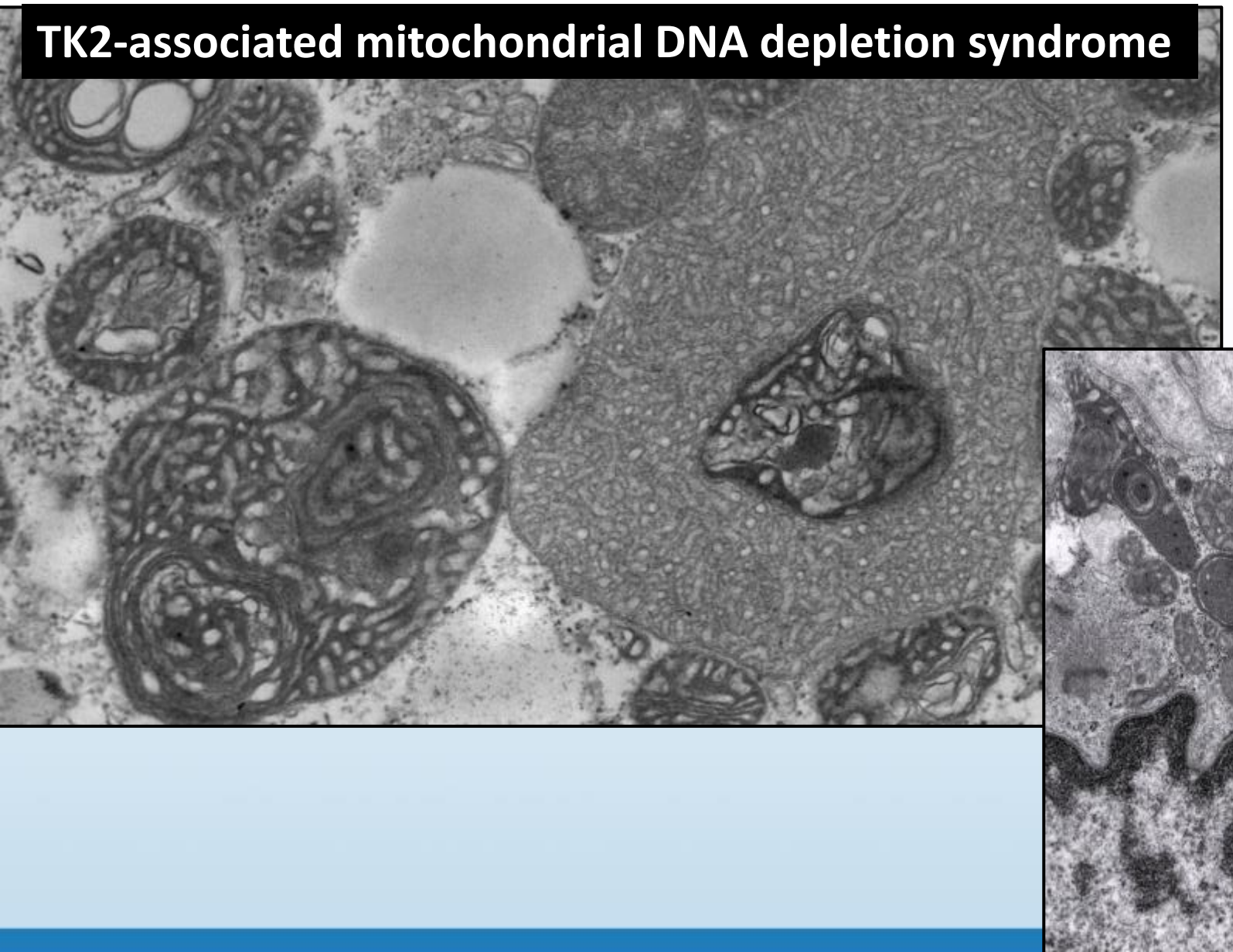
Mitochondria: concentric cristae and rounded electron dense inclusions

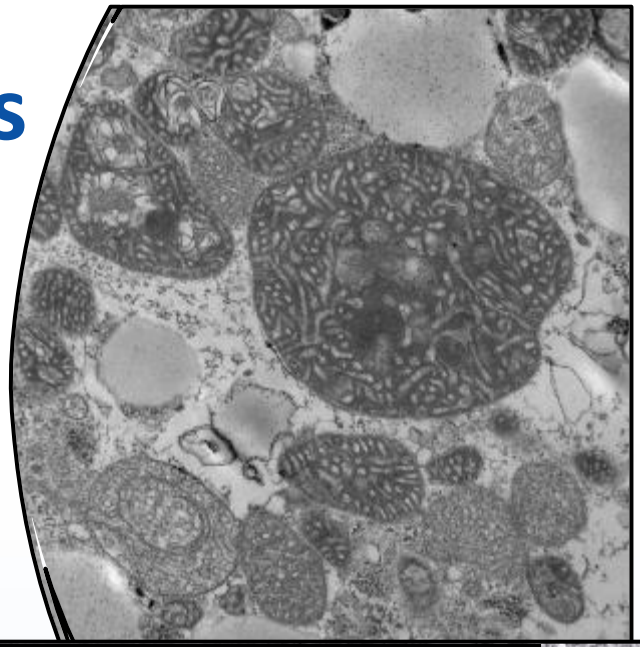


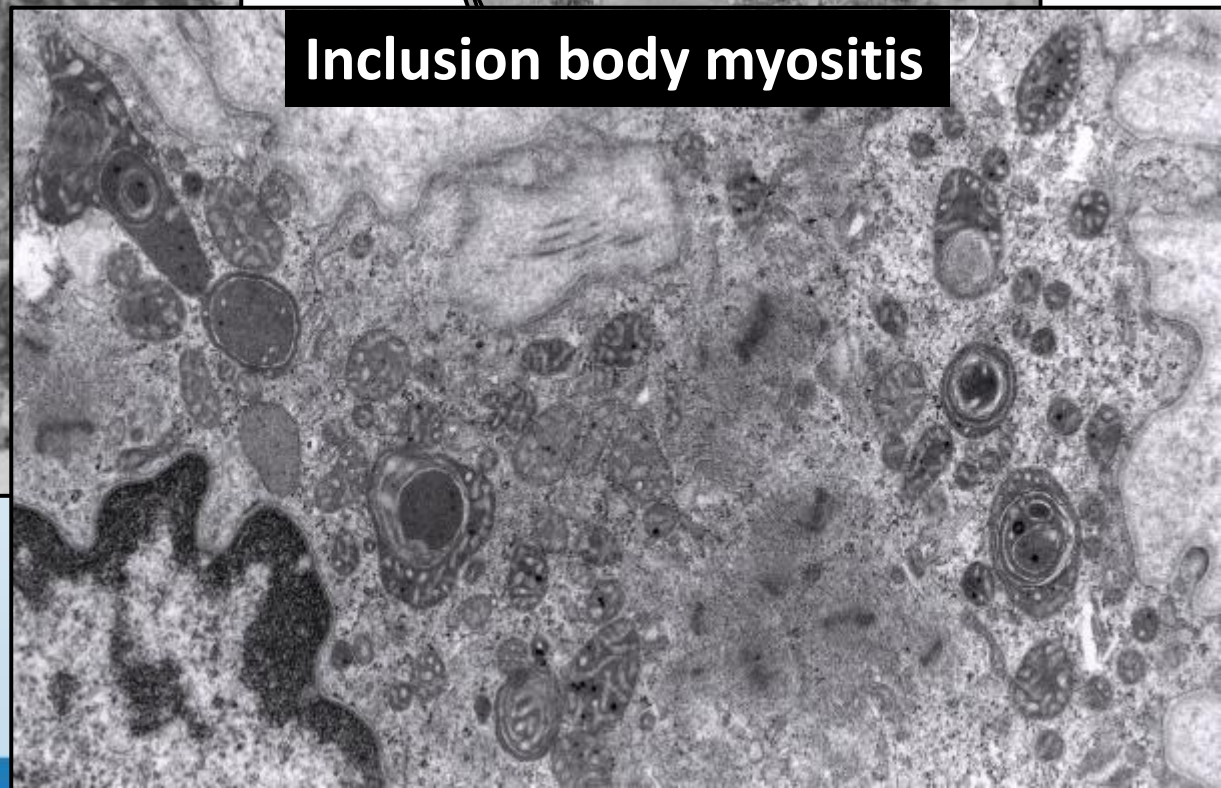




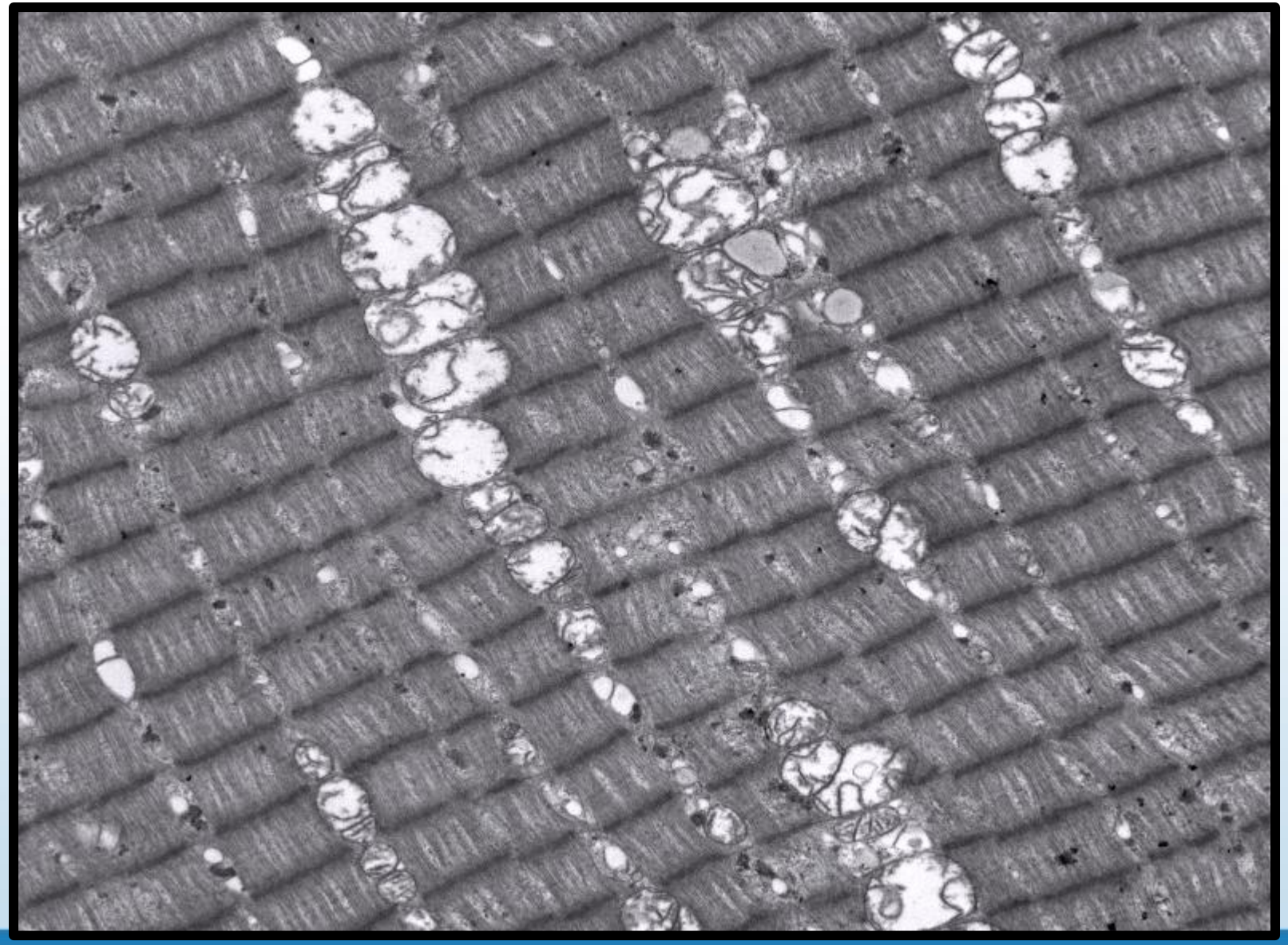
Mitochondria: unusual cristae/inclusions



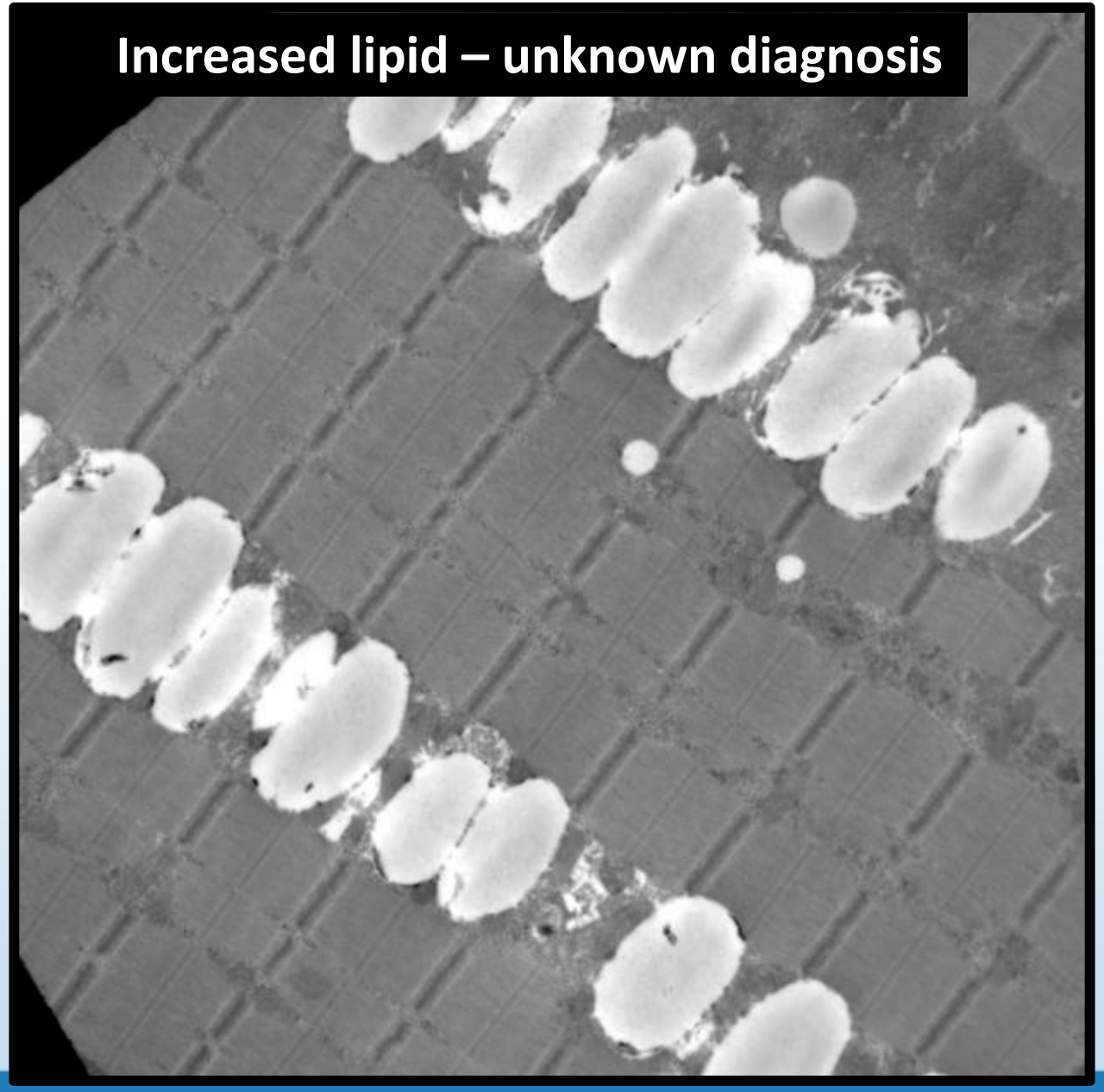




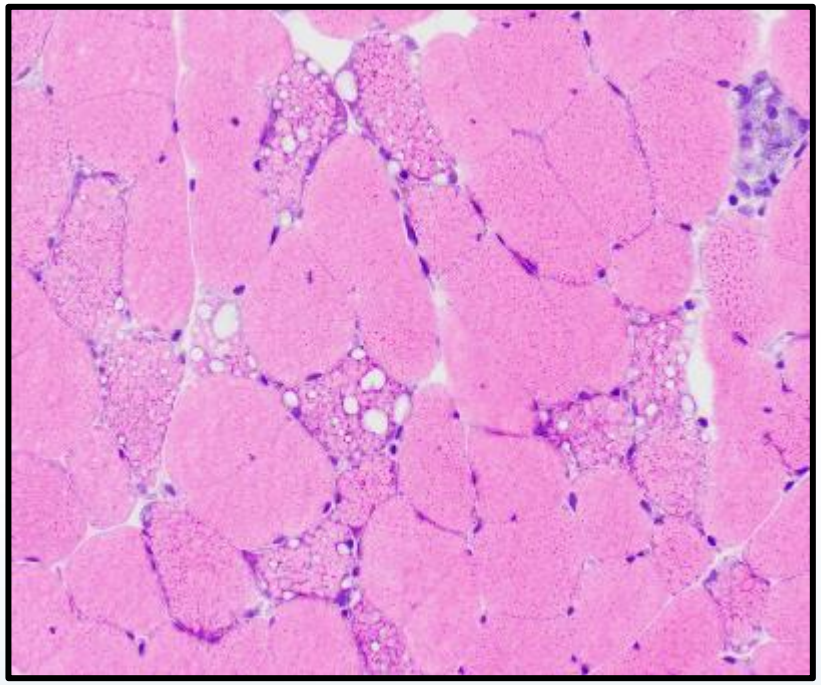
Mitochondria: poor preservation (“blown out”)

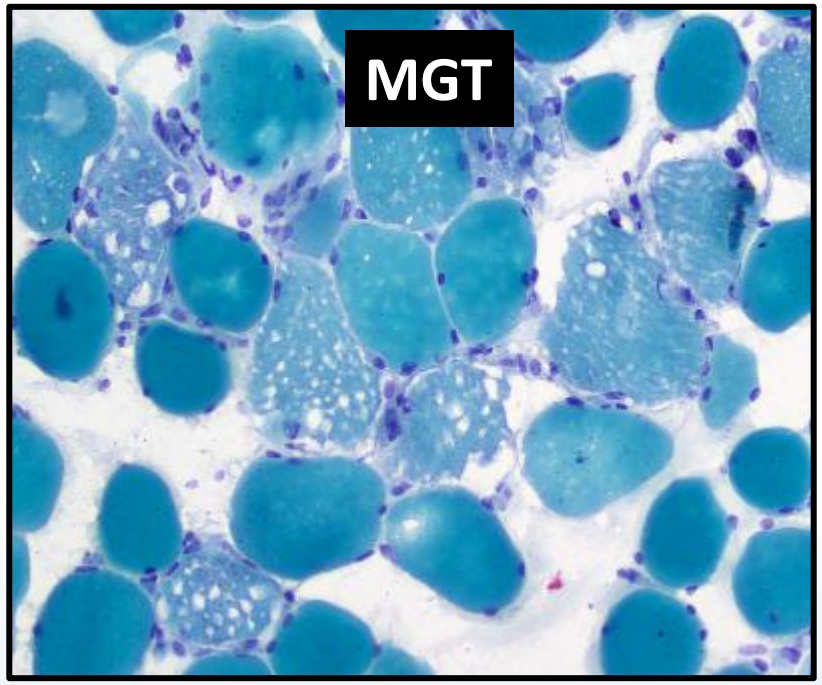


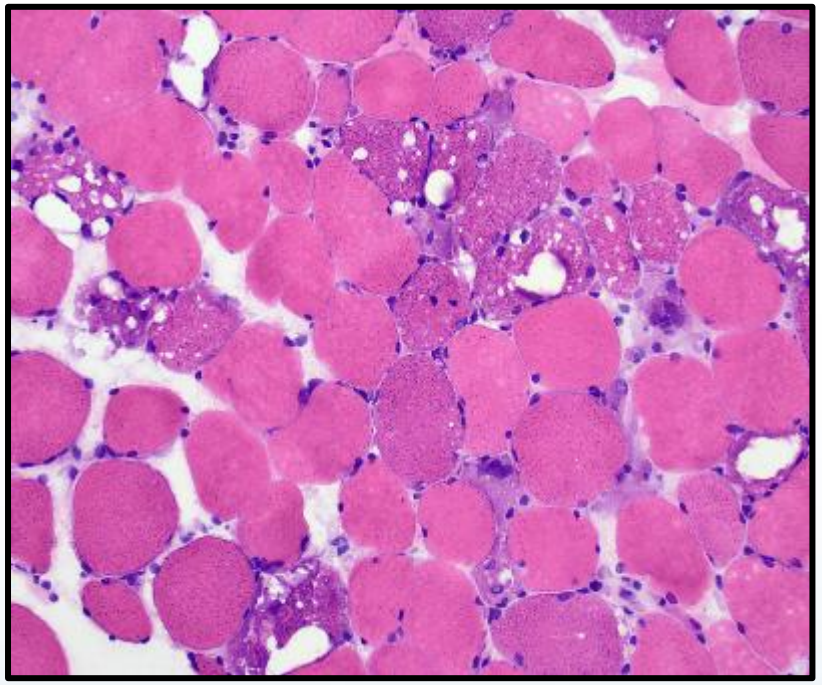
Lipid: increased numbers, enlargement, conglomeration

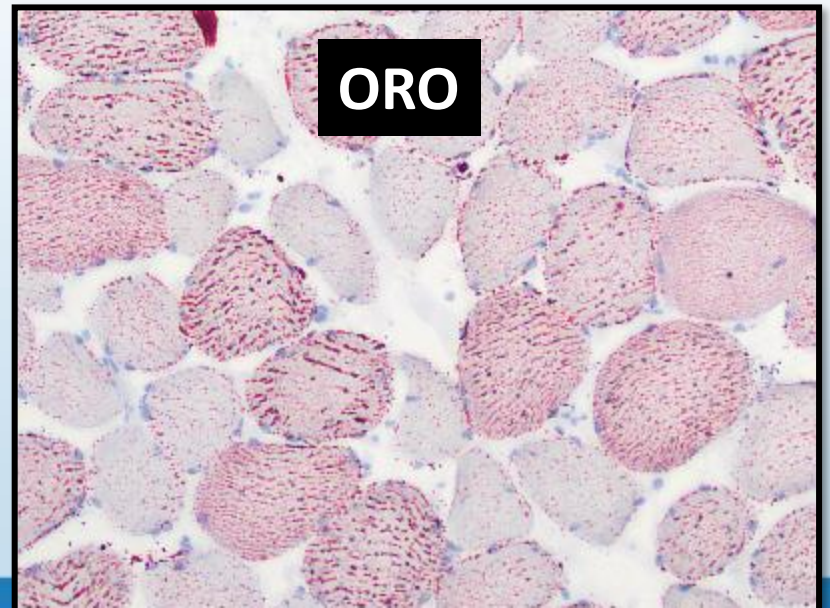


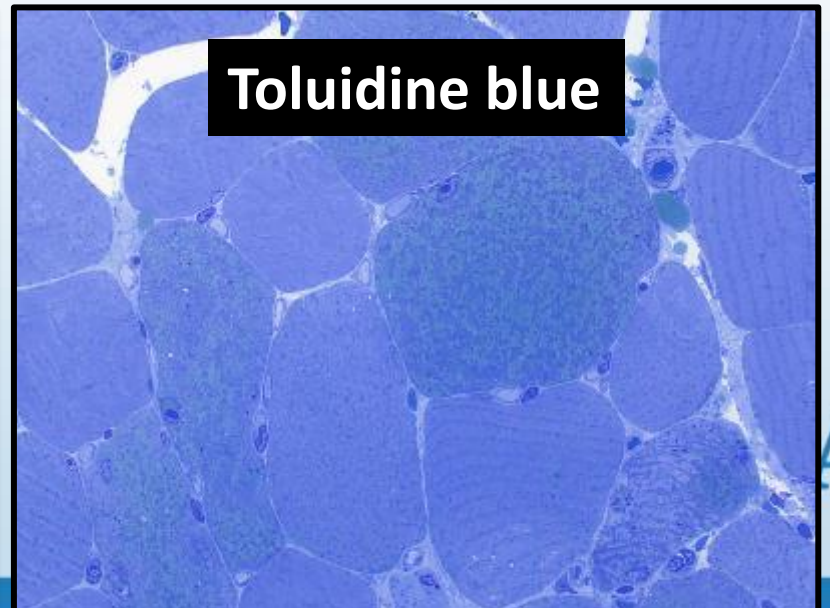
Lipid on frozen sections and semithin sections











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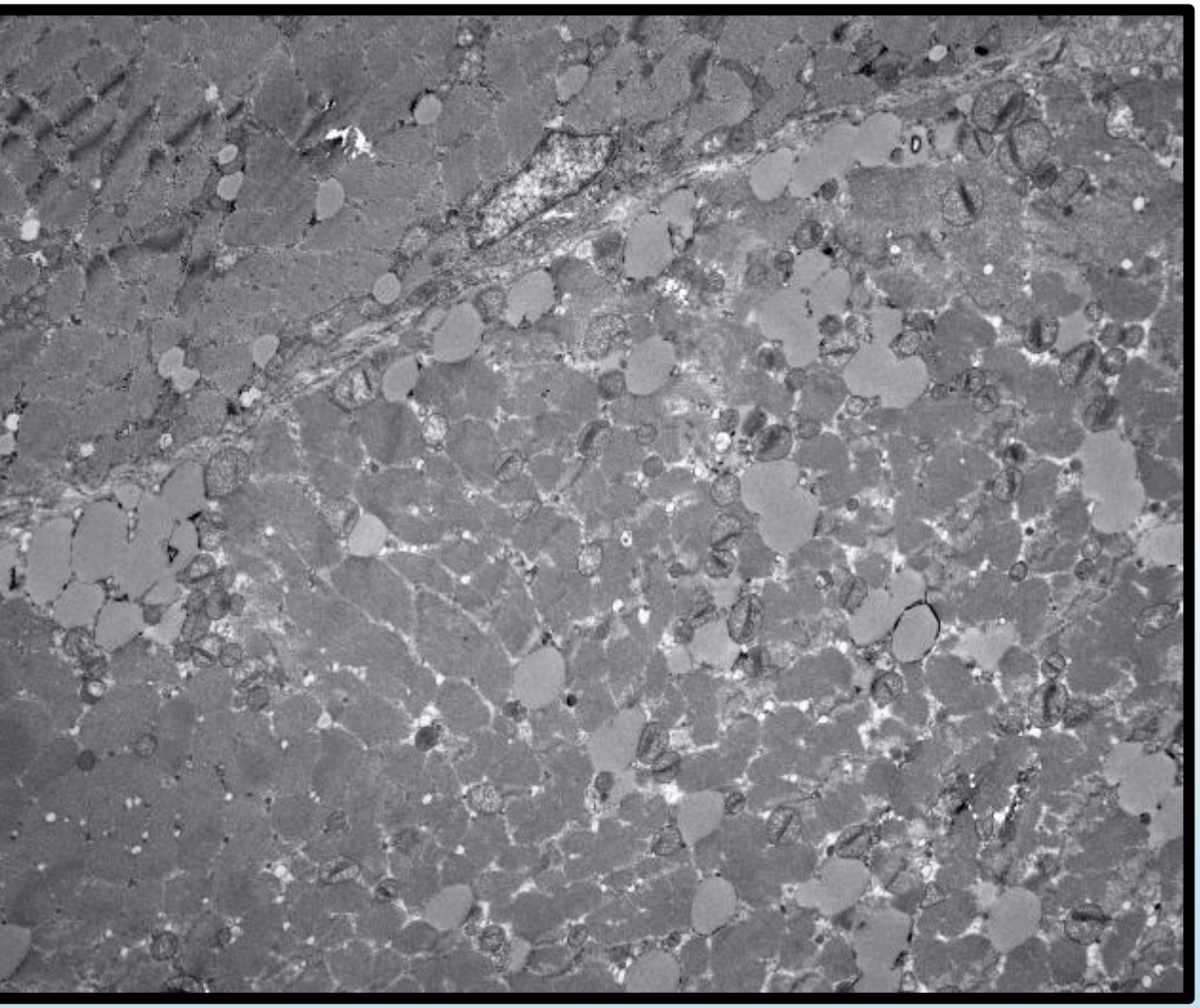
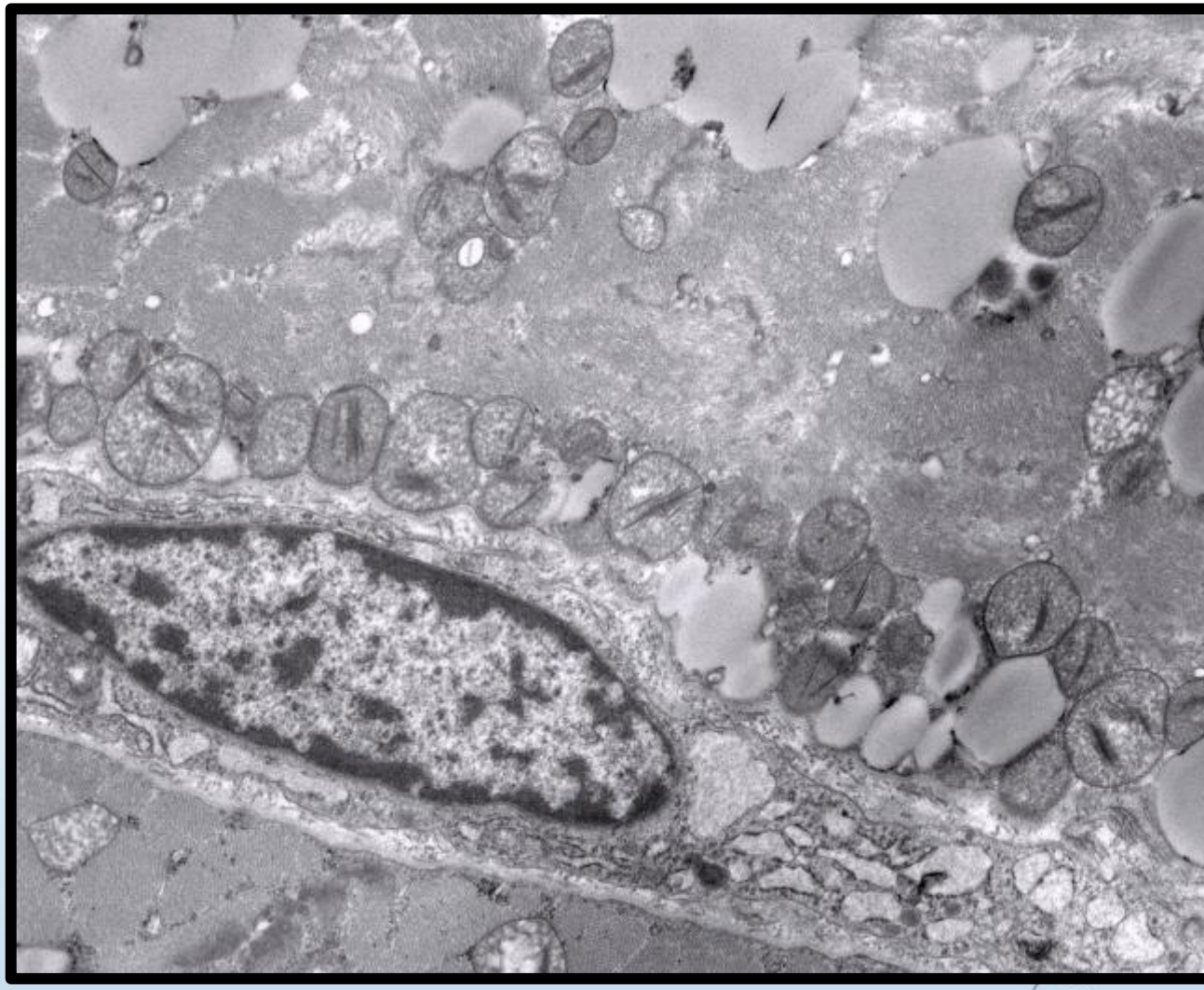
Lipid: increased in primary mitochondrial myopathies





GLYCOGEN

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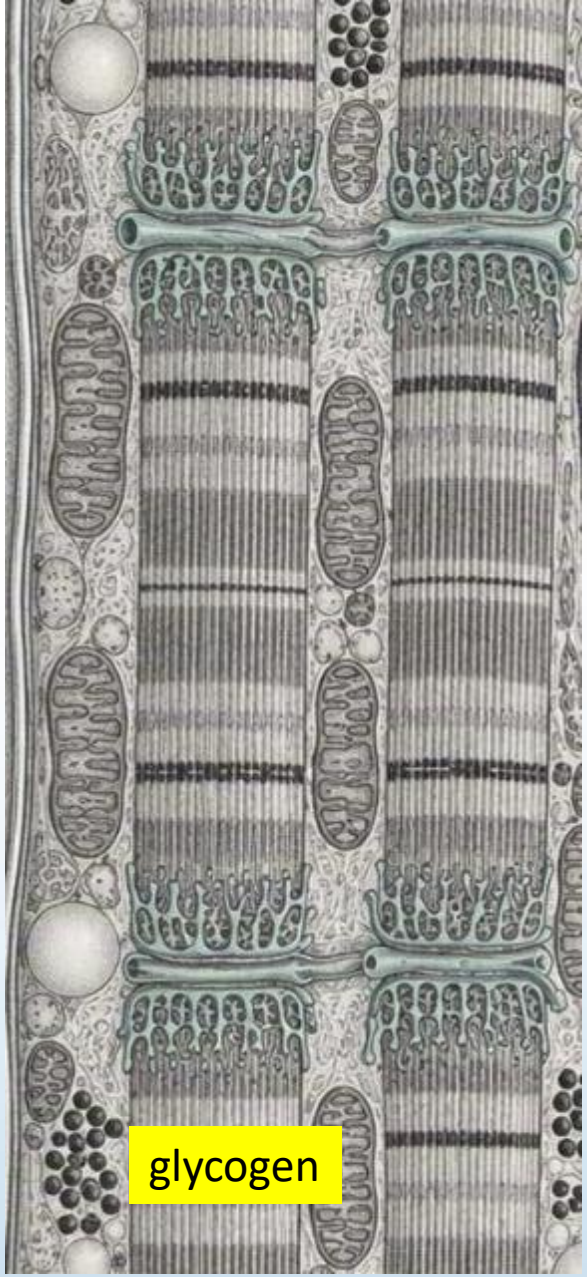
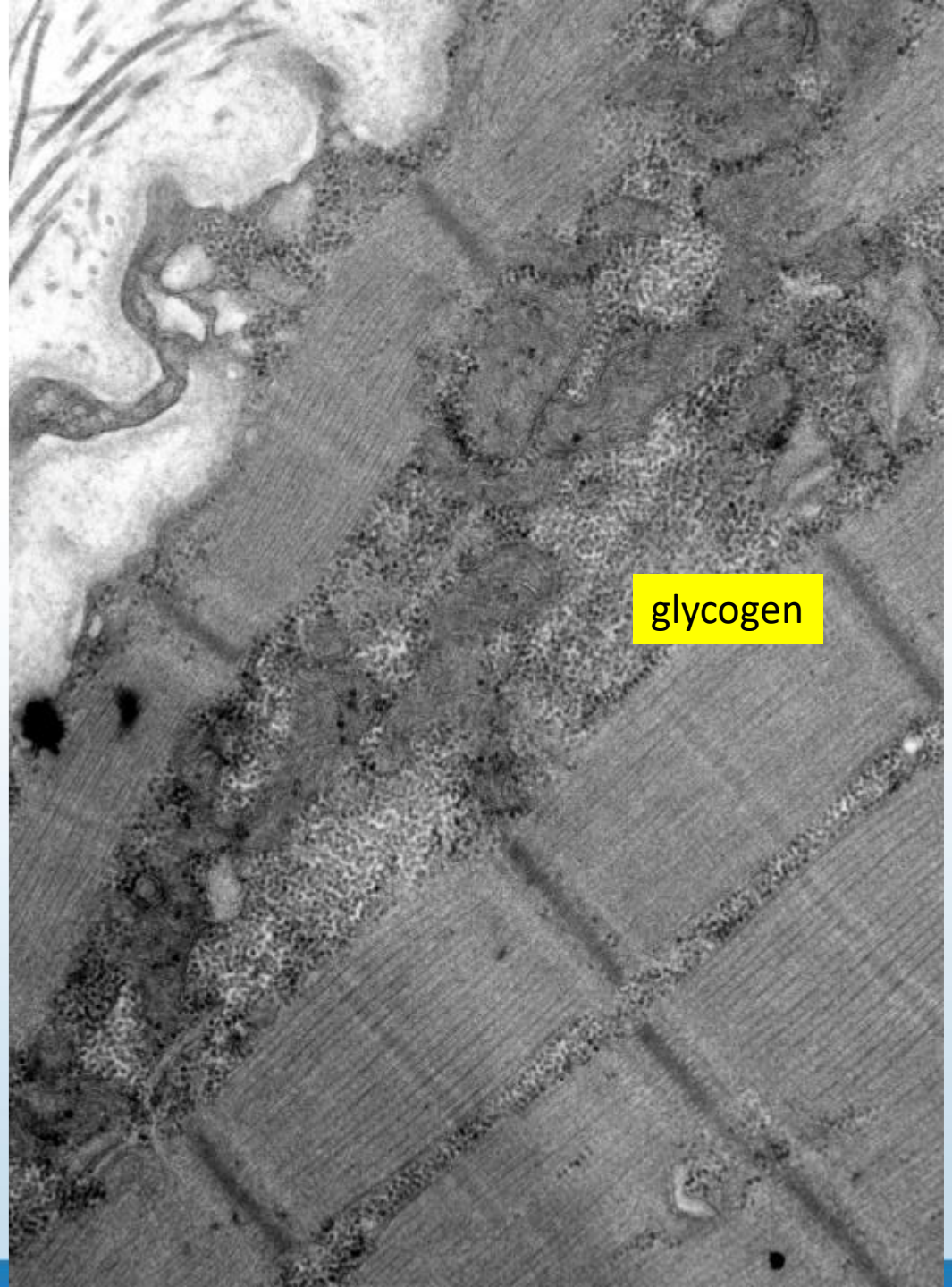
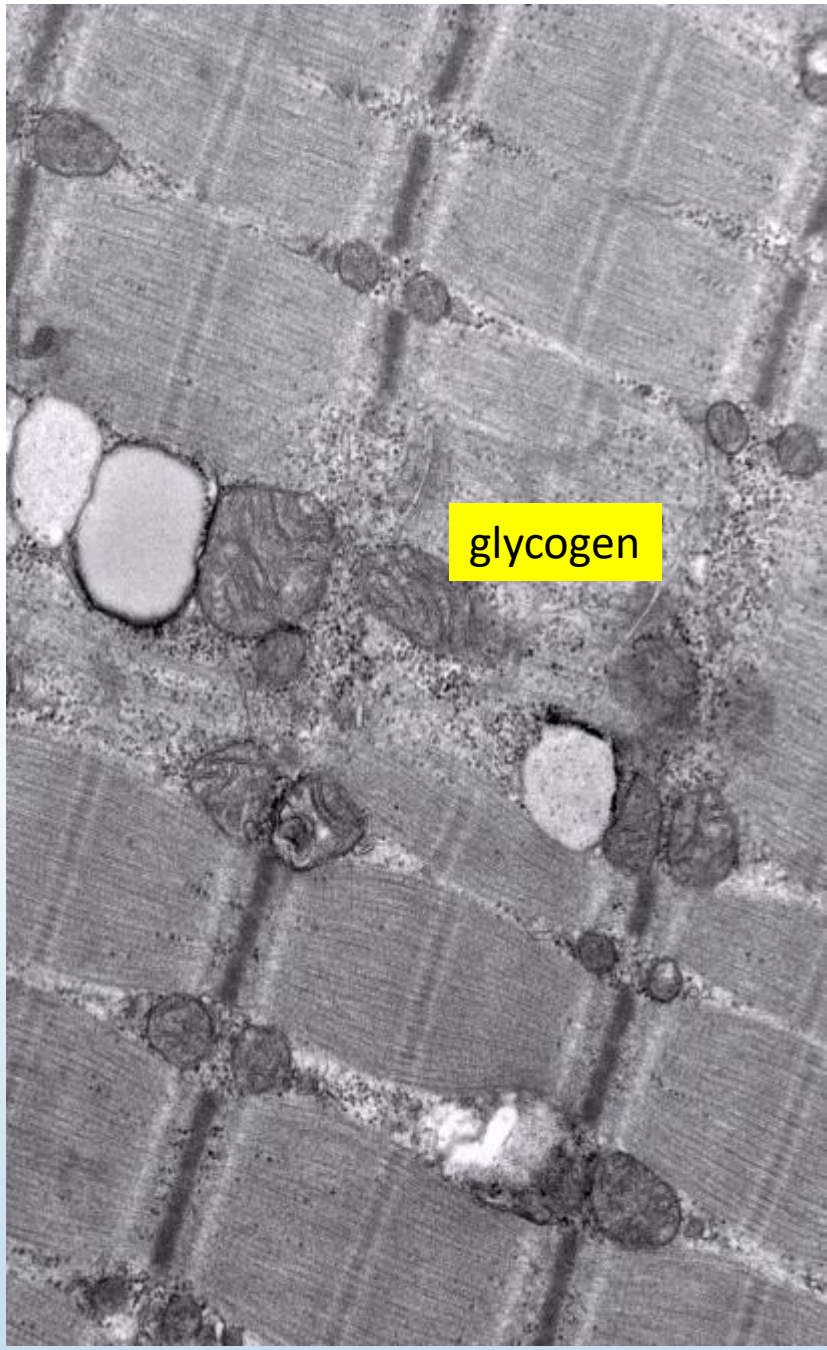
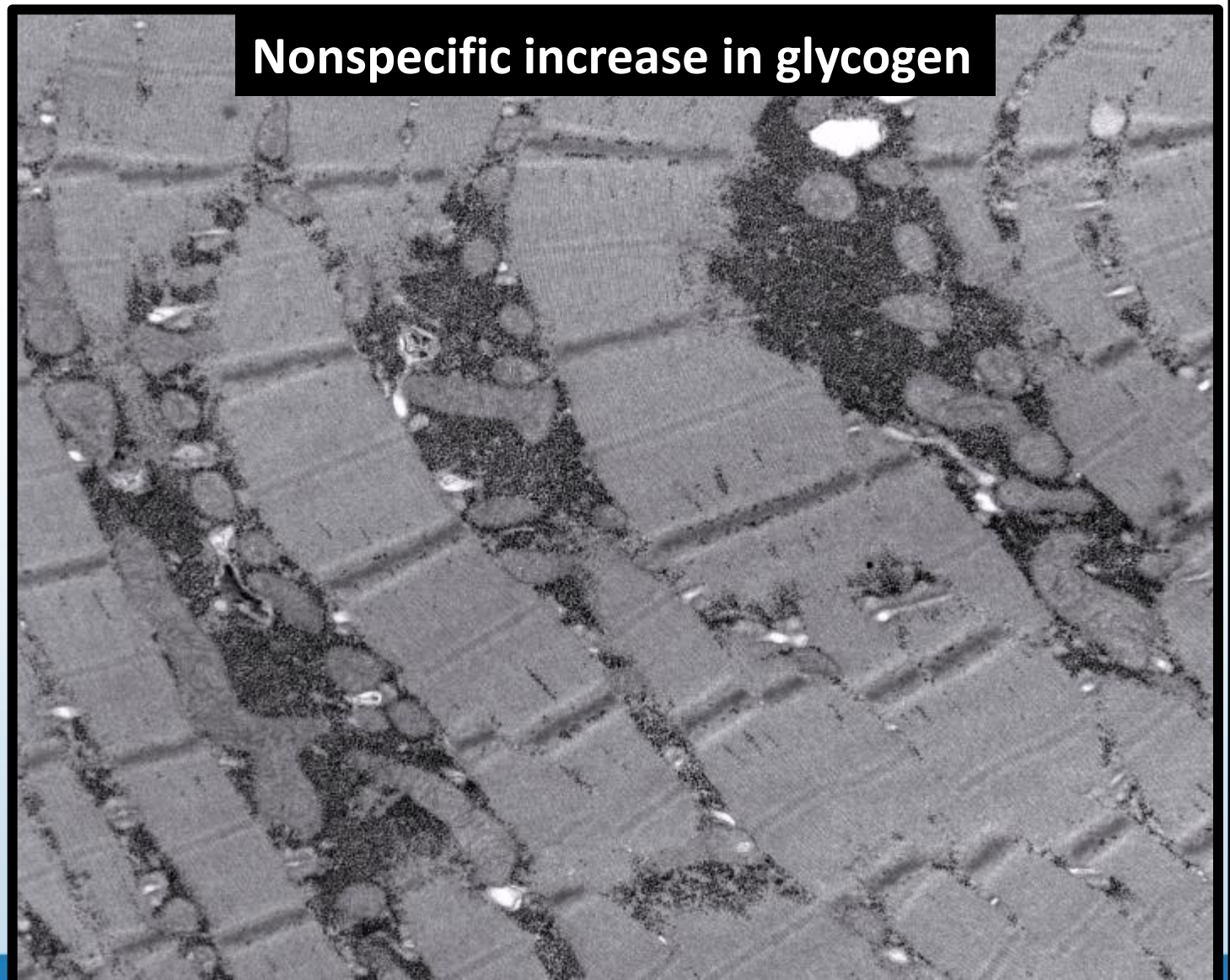


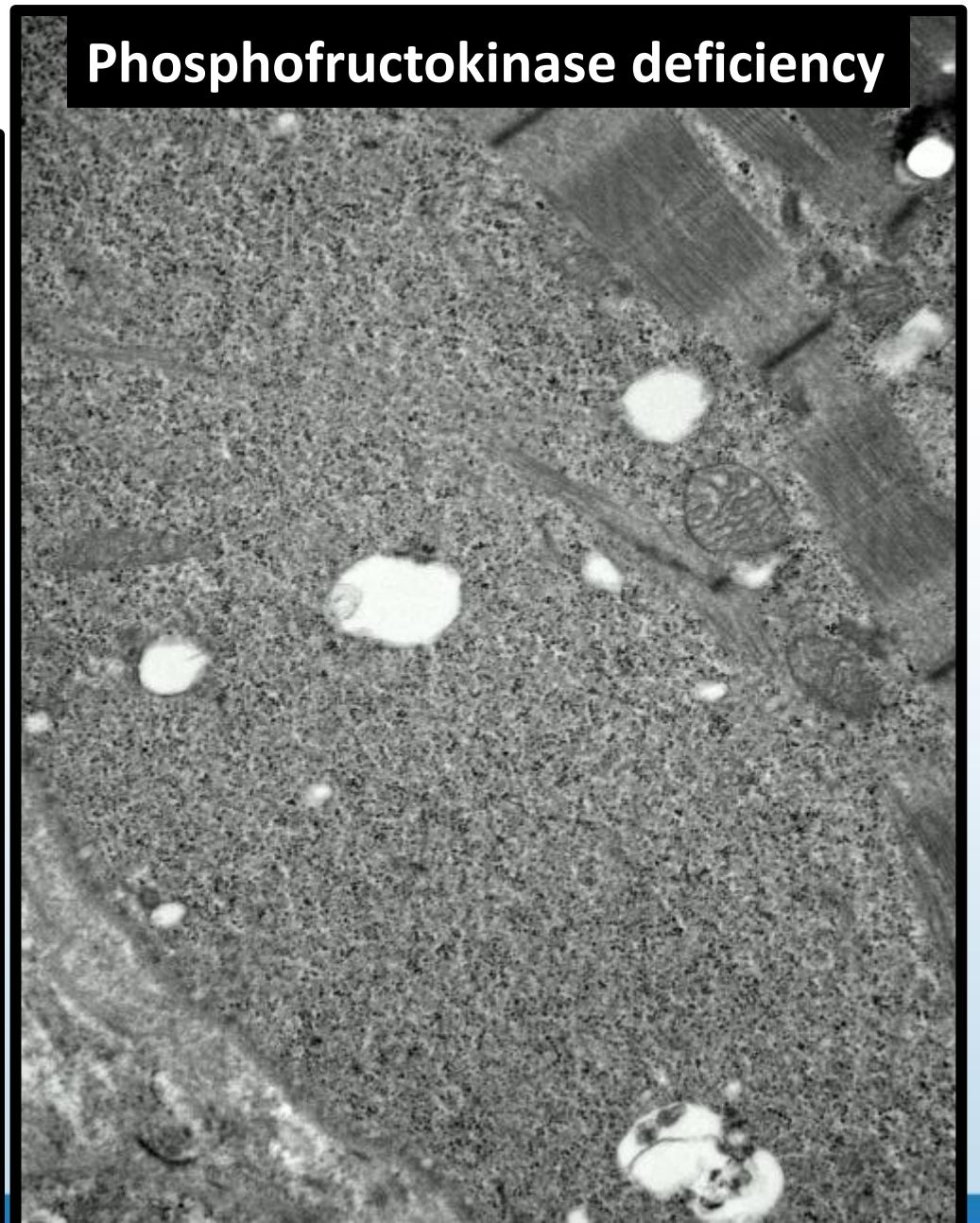
Stylized illustration, Microsoft Copilot-generated and edited by K Jones, August 2026.



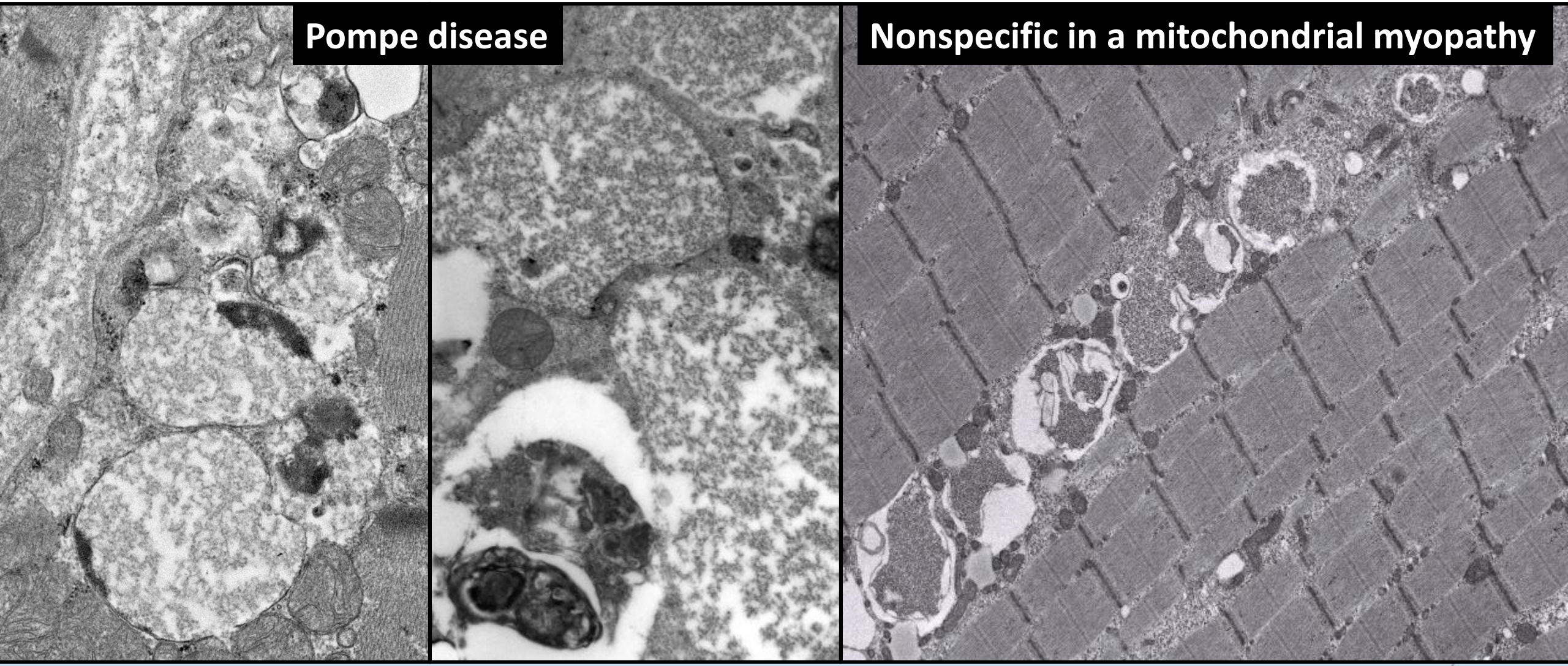


Glycogen: excess free glycogen

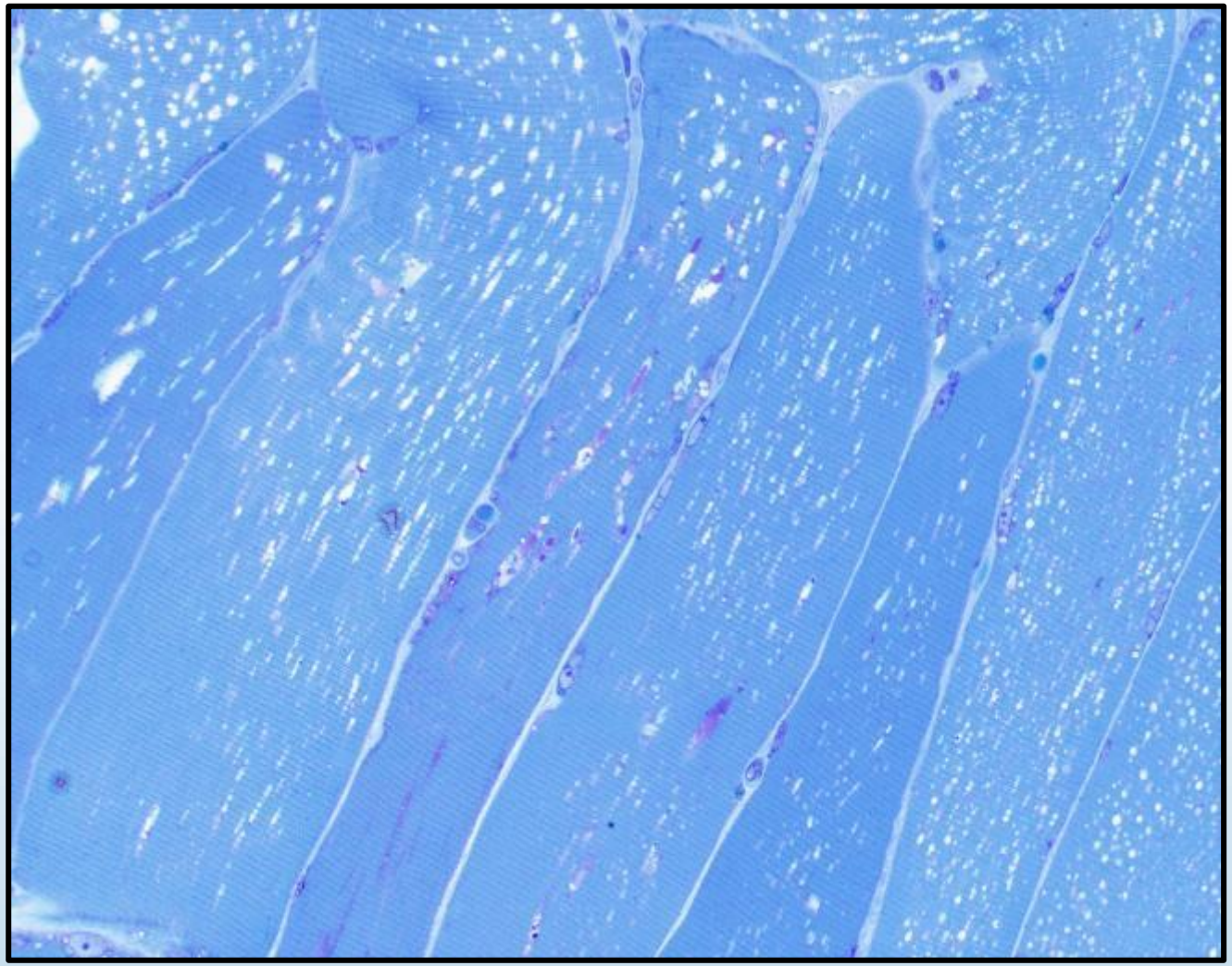


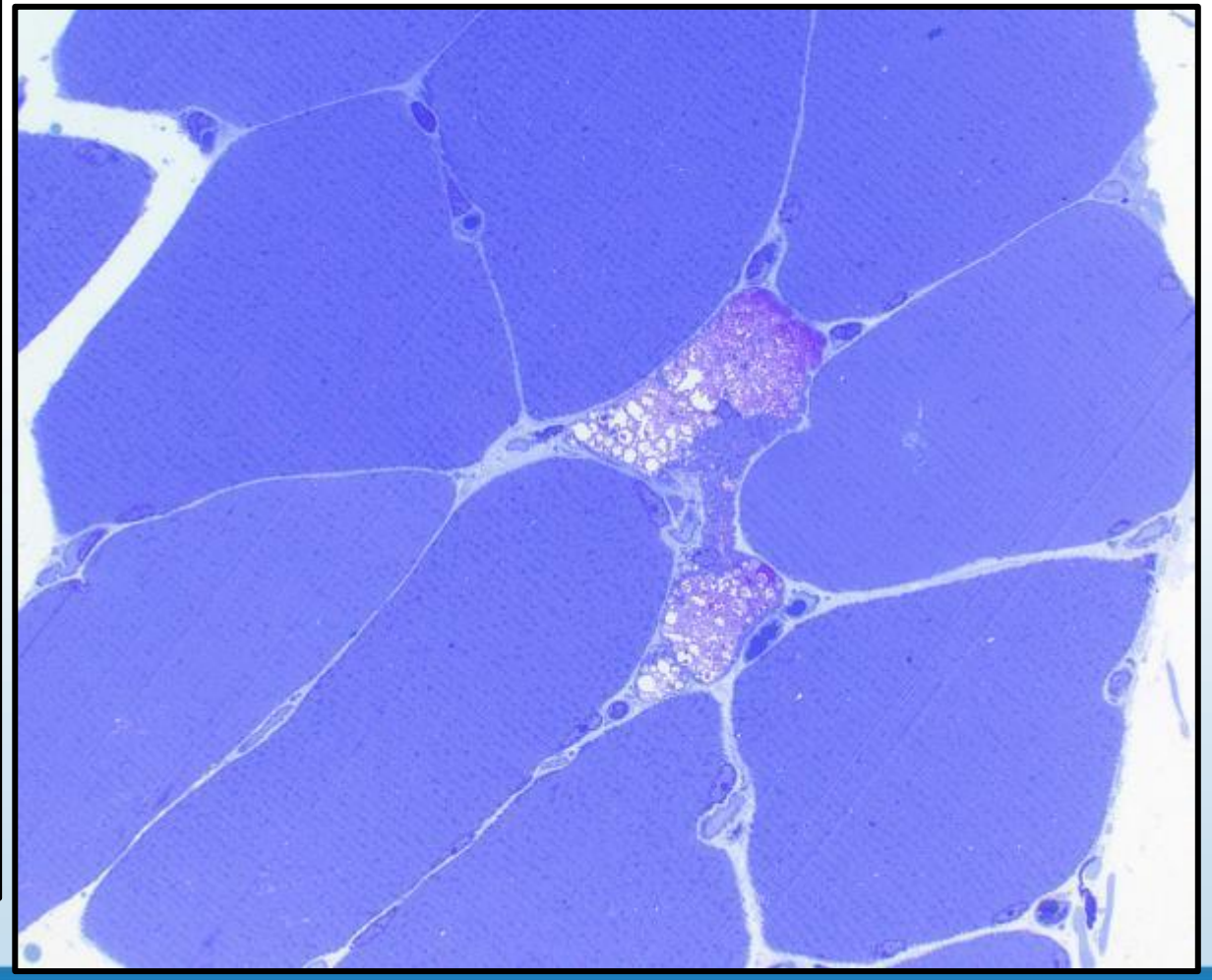


Glycogen: membrane-bound glycogen

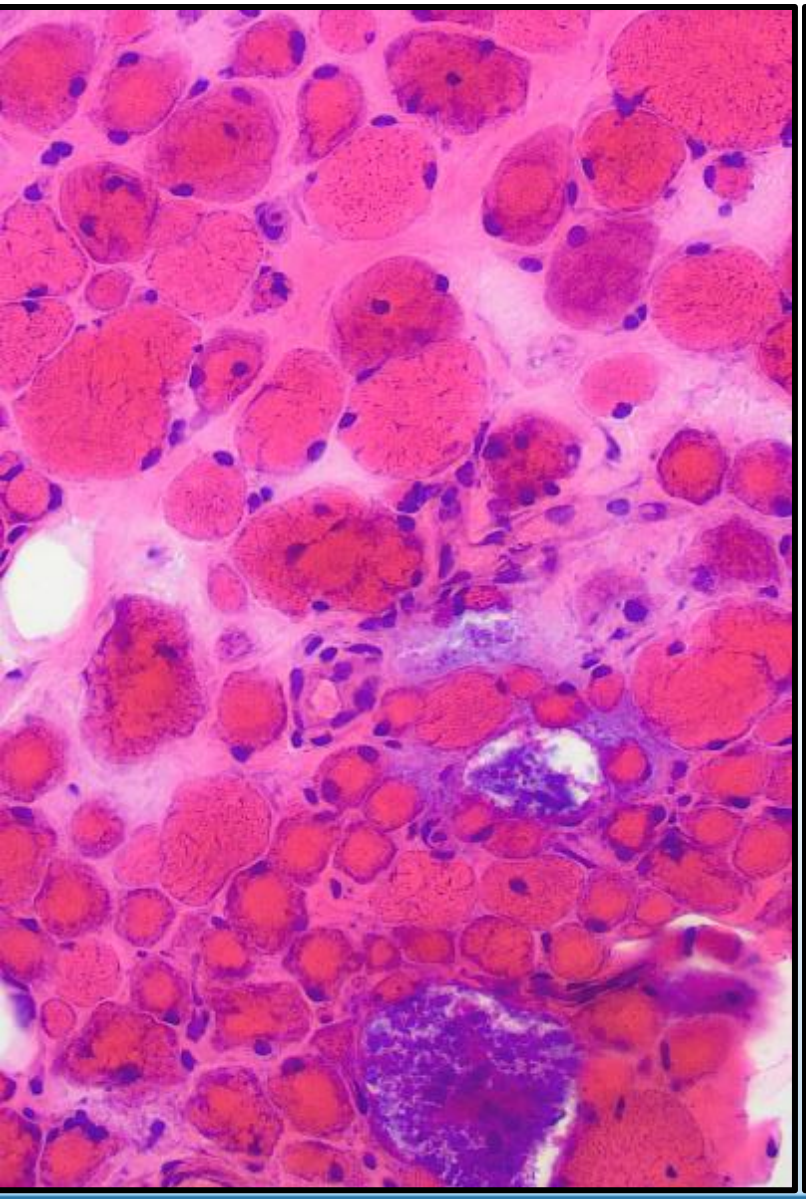


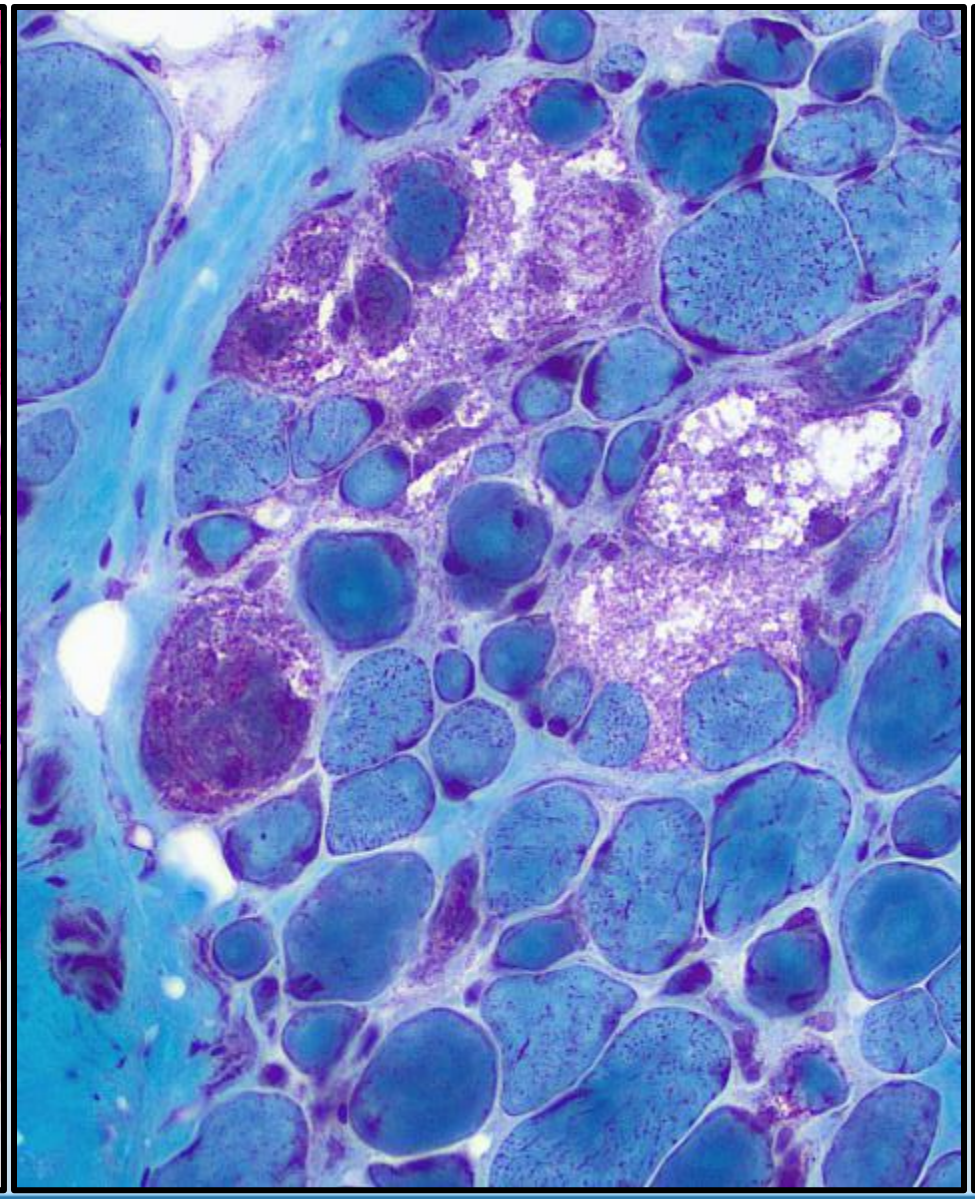
Glycogen on epon sections

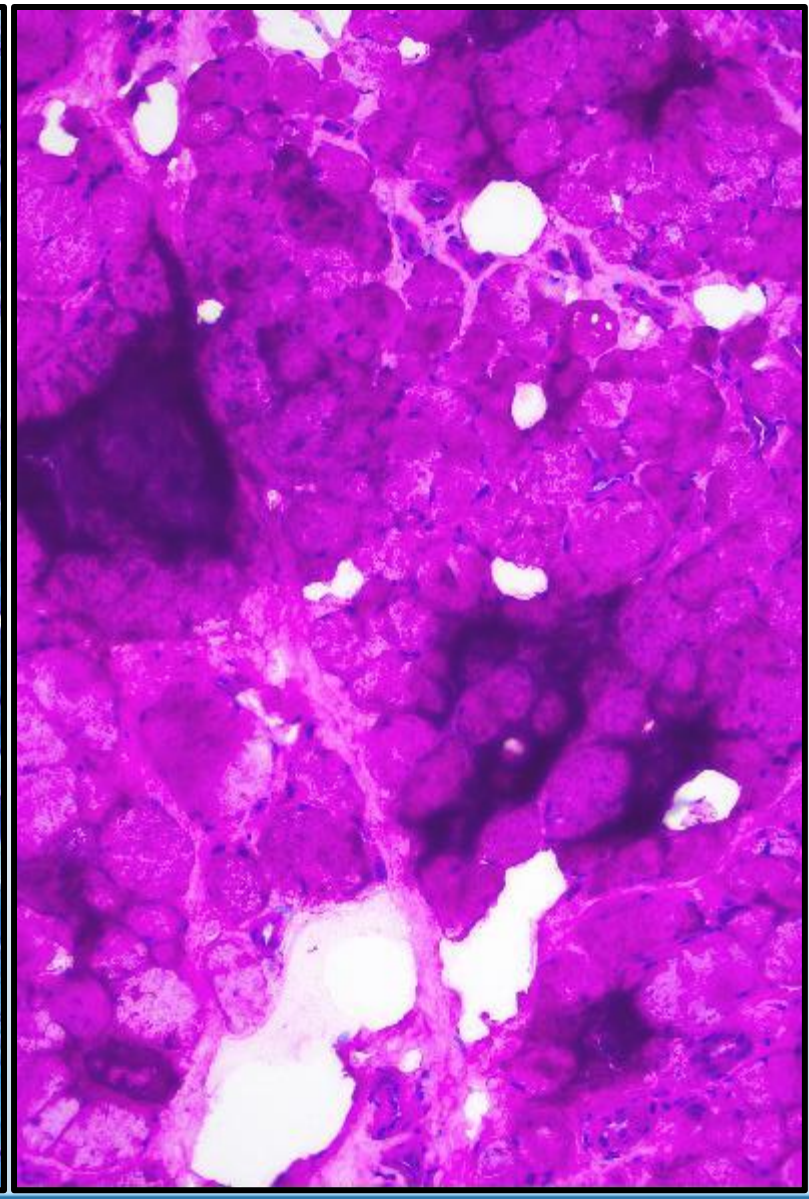




Glycogen on frozen sections







LYSOSOMES AND RELATED STRUCTURES

1. Myofibrils and the contractile apparatus
Are the myofibrils organized? Sarcomeric structure intact? Z-lines normal?

2. Membrane systems
Any obvious abnormalities in the T-tubules or SR?

3. Organelles
Are mitochondria, lysosomes, glycogen and lipid normal?

4. Nucleus
Is the nucleus appropriately positioned and structurally intact? Any inclusions?

5. Subsarcolemmal space and basal lamina
Any abnormal material accumulating or inclusions? Is the basal lamina abnormal?

6. Extracellular compartment
Is the interstitum and vasculature normal? Any abnormal material, inclusions, or cellular infiltrates?

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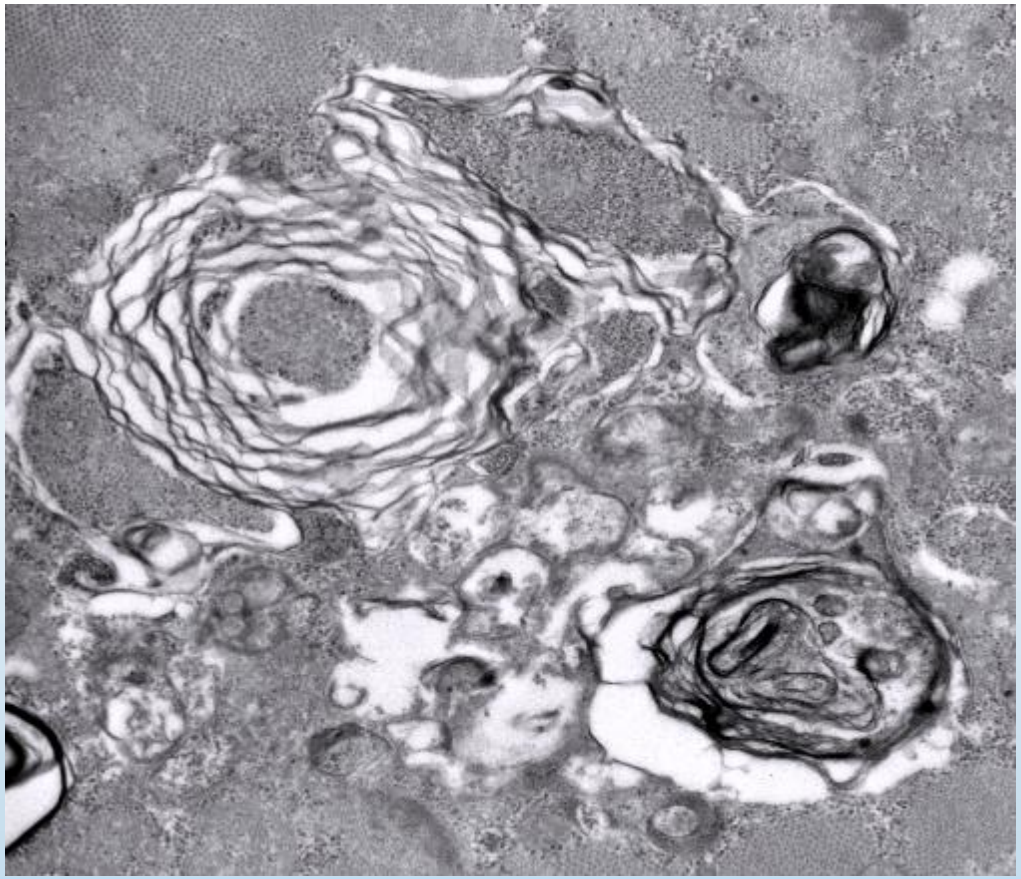
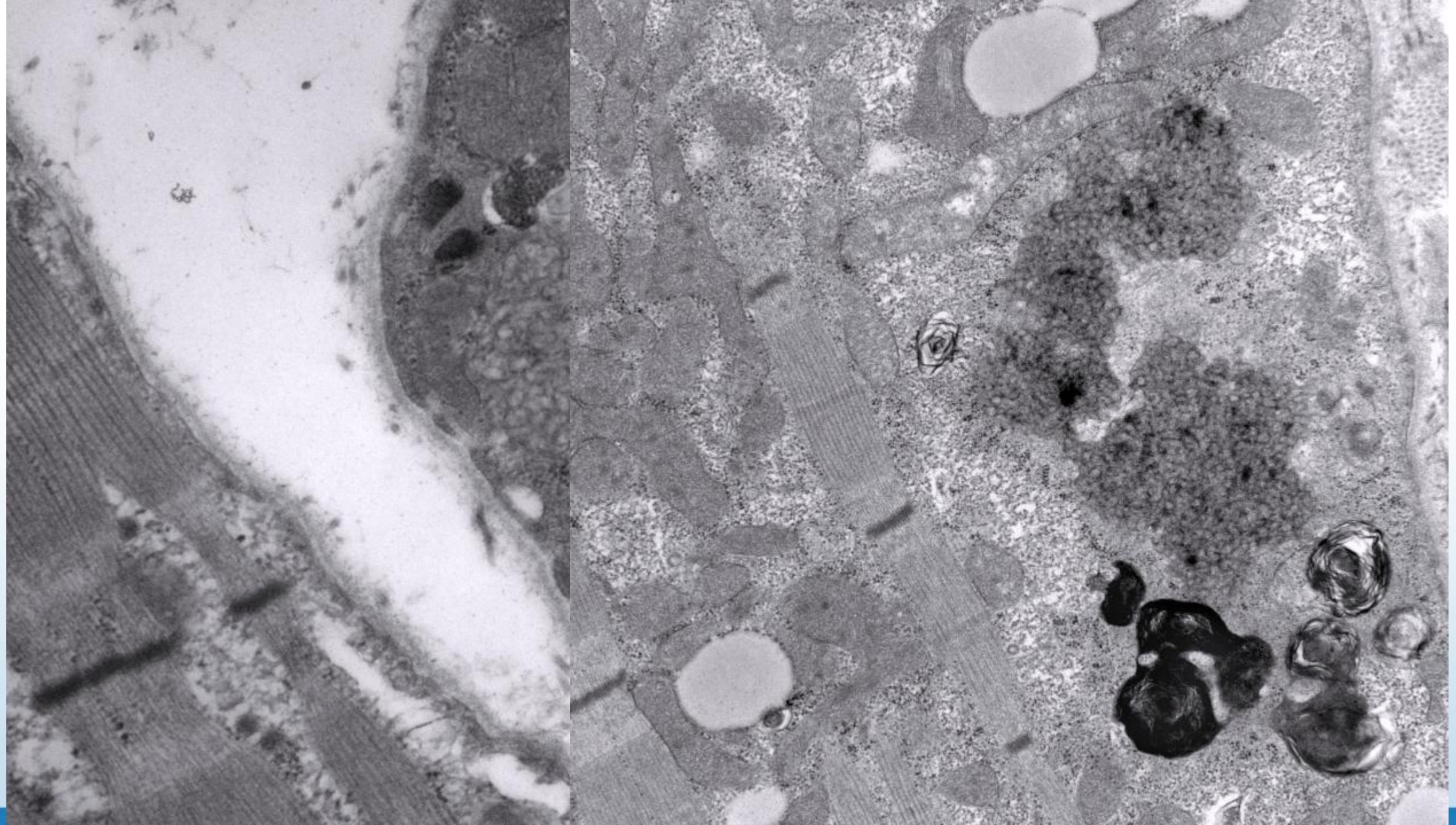
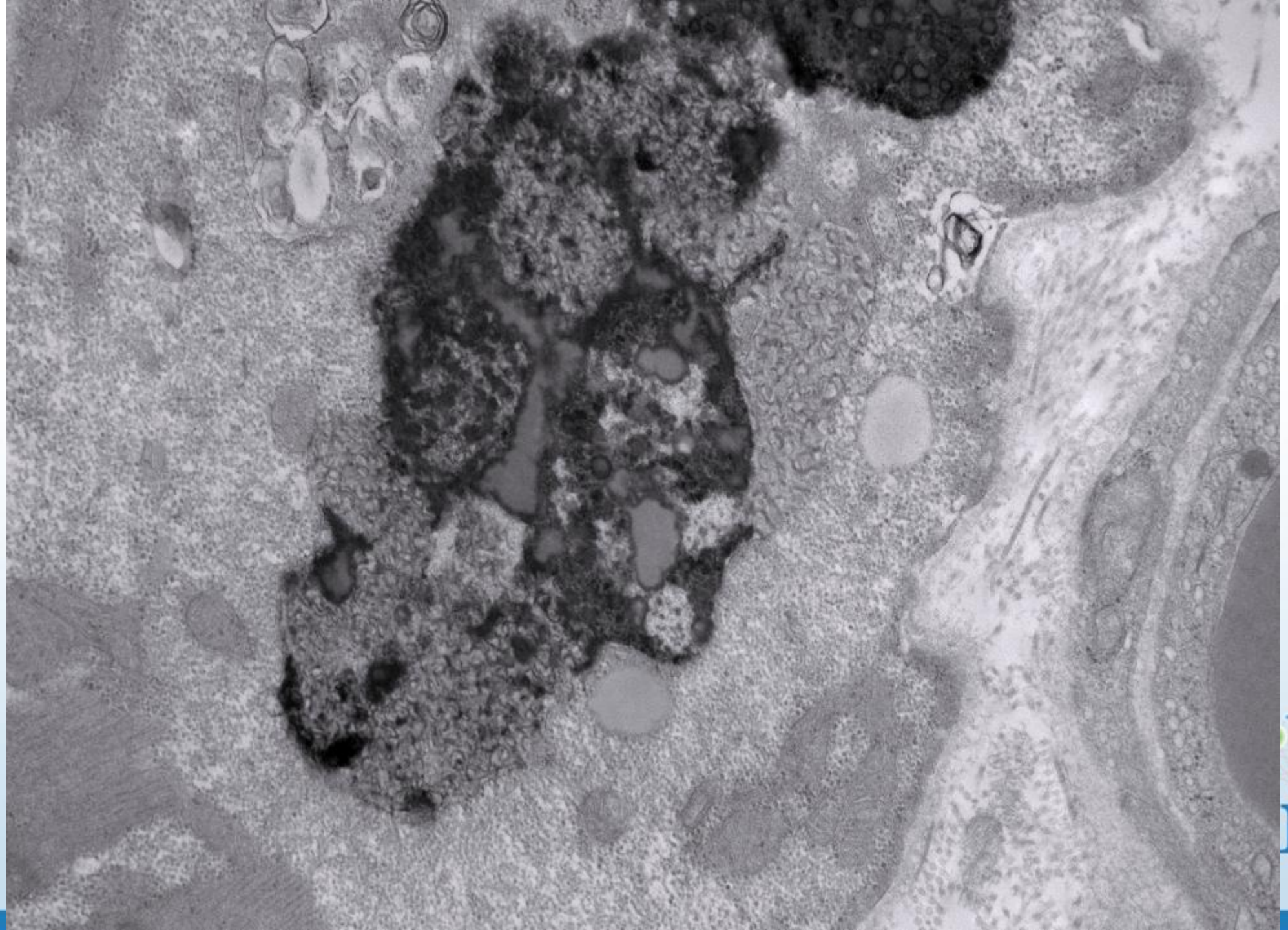
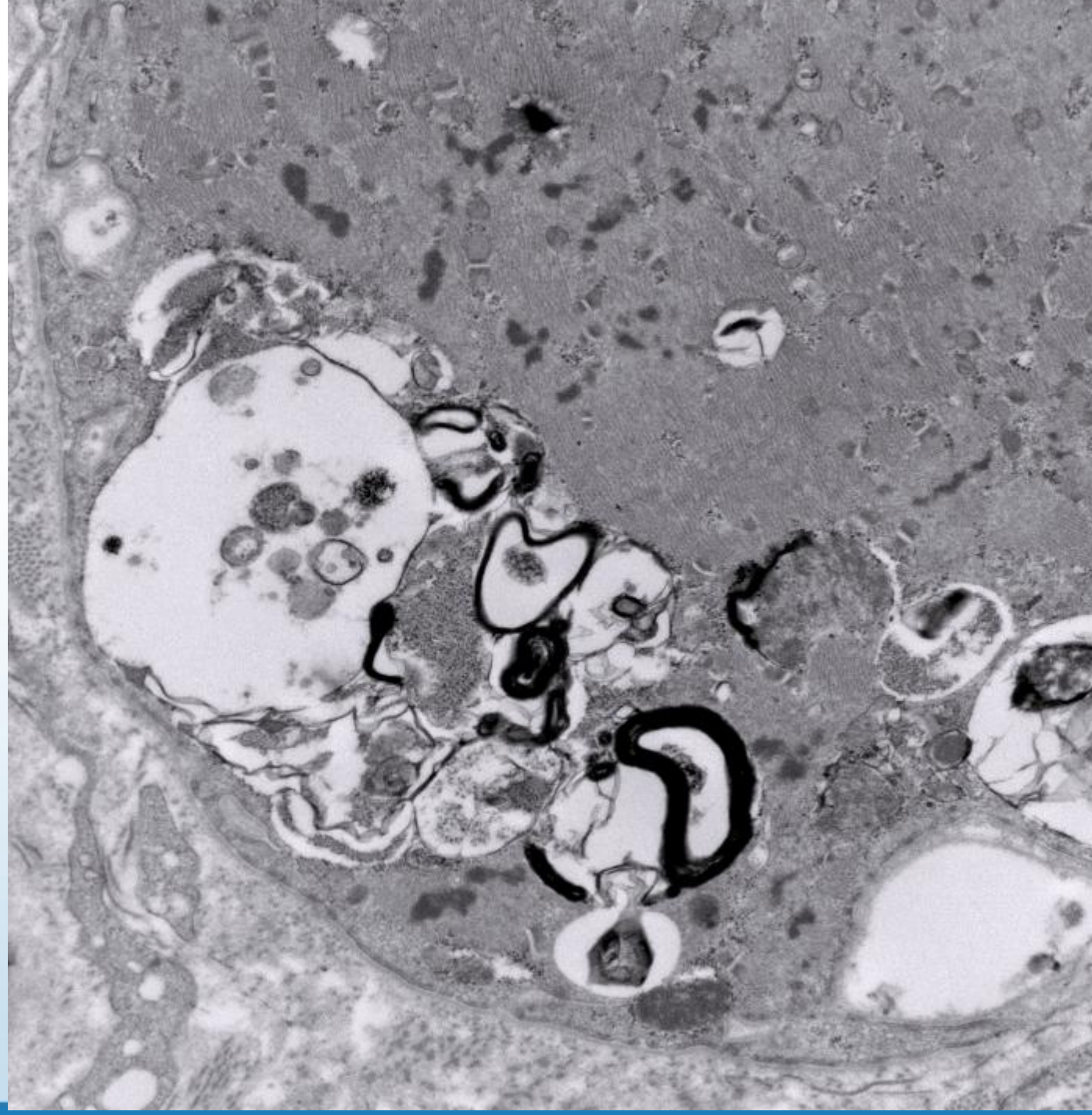
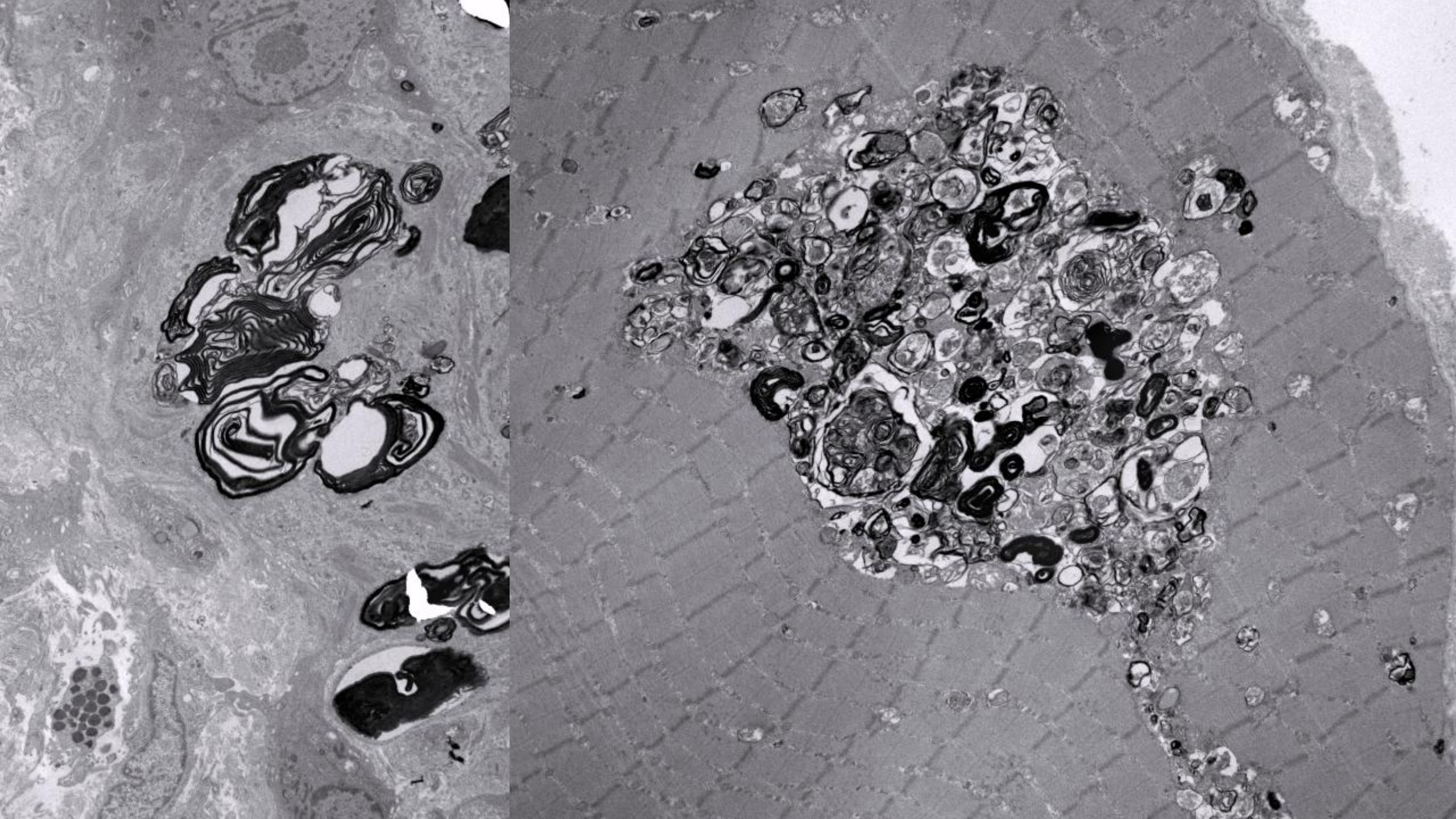
Abnormal lysosomes: autophagic vacuoles











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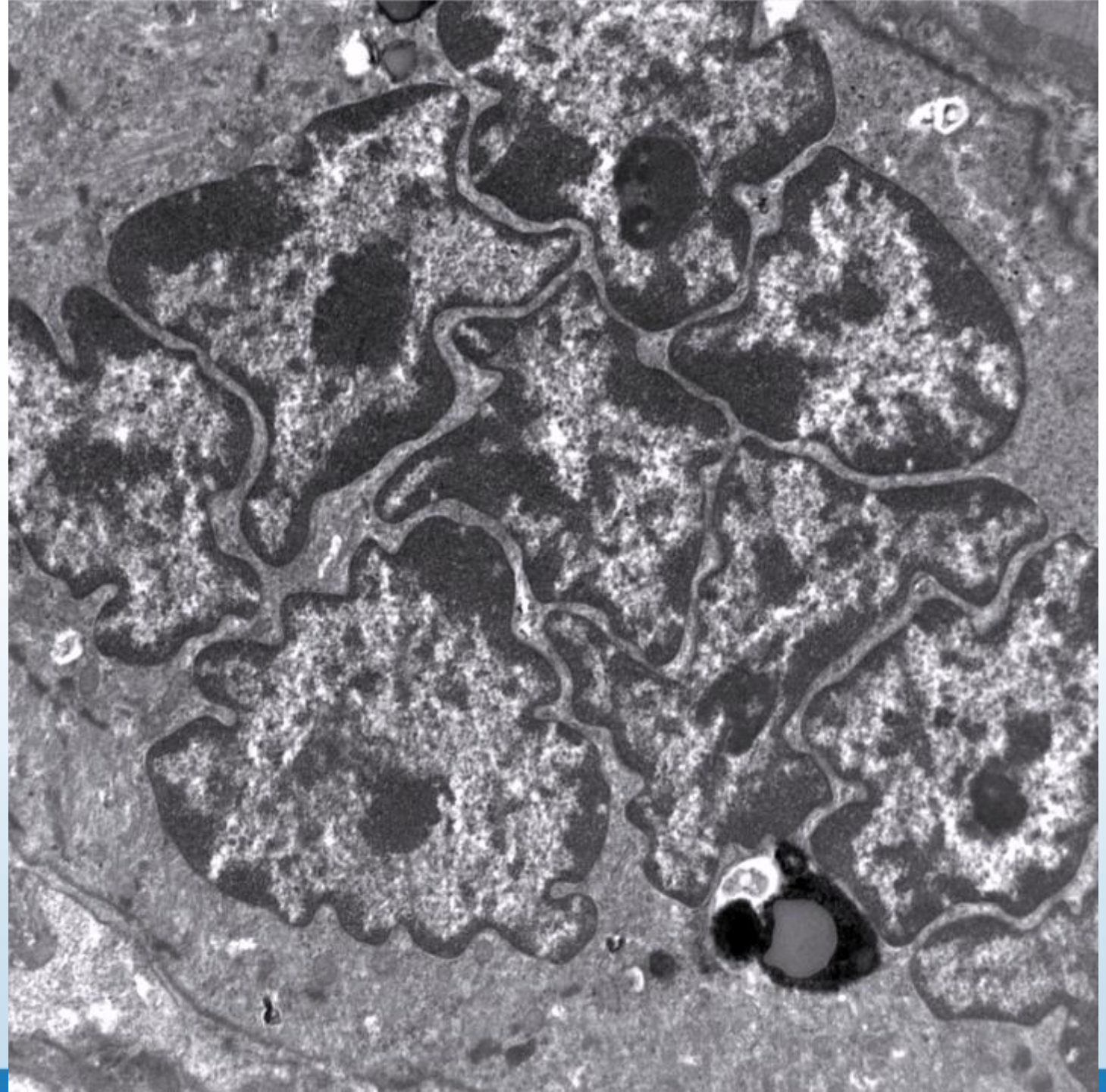
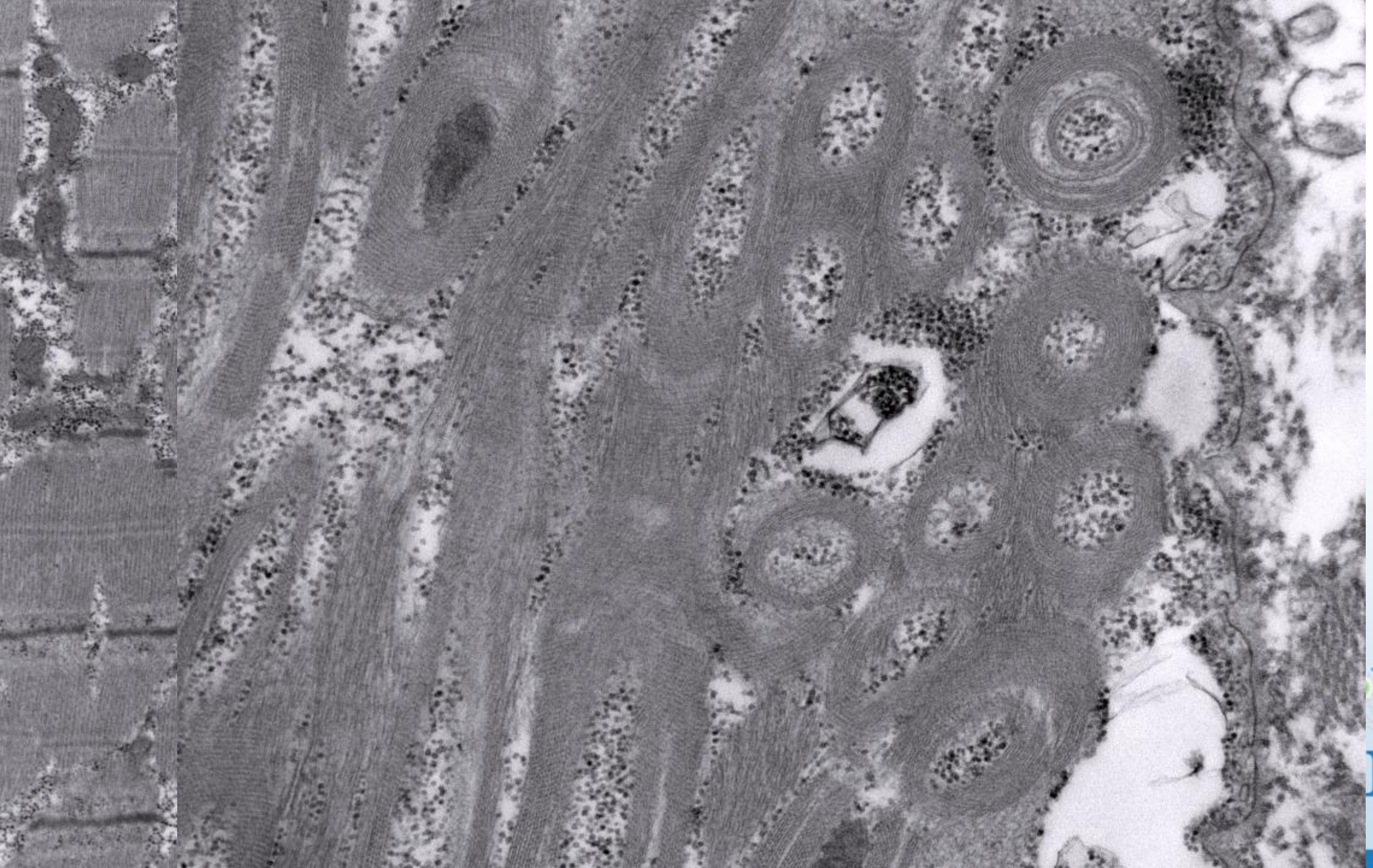
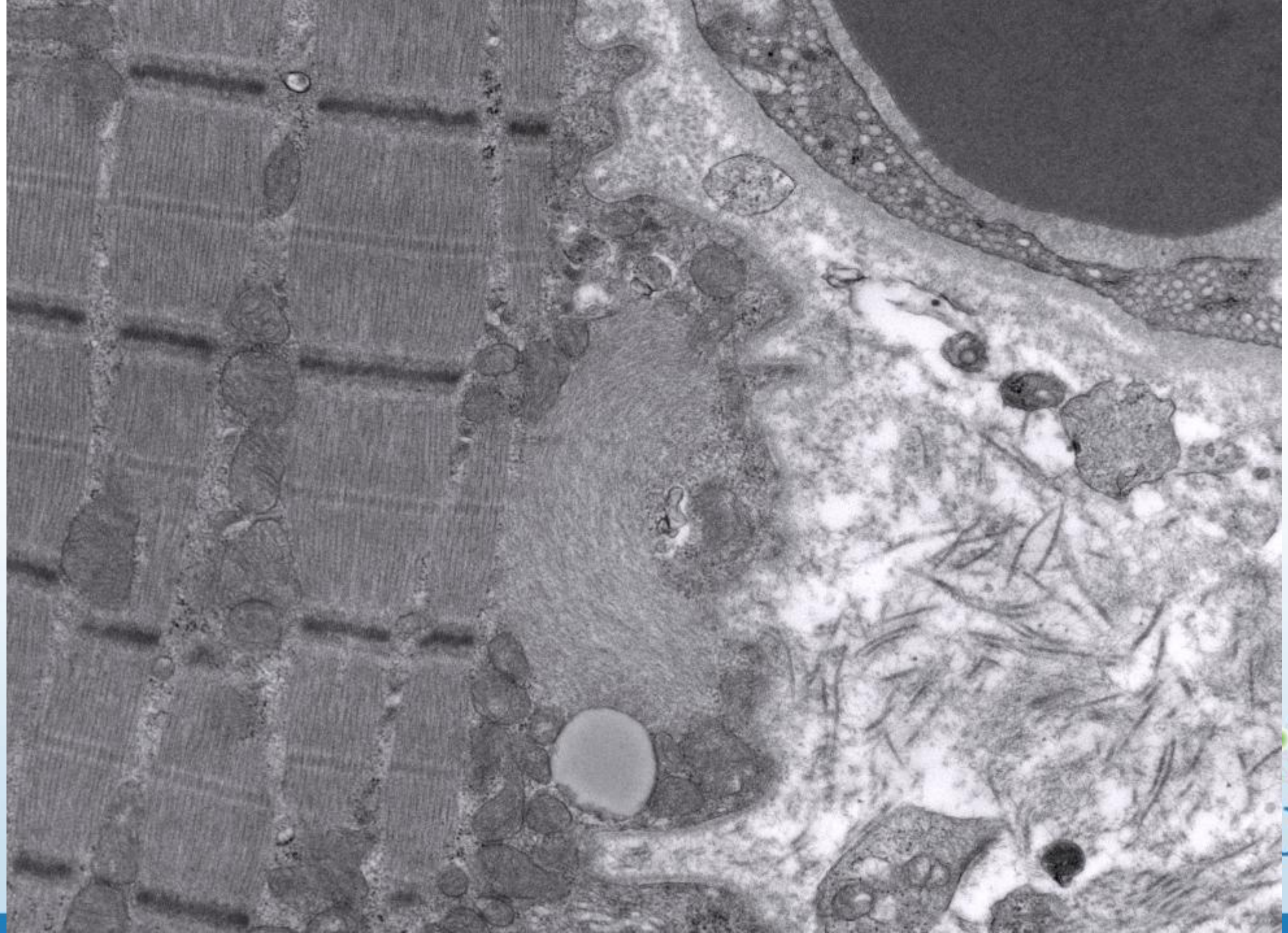
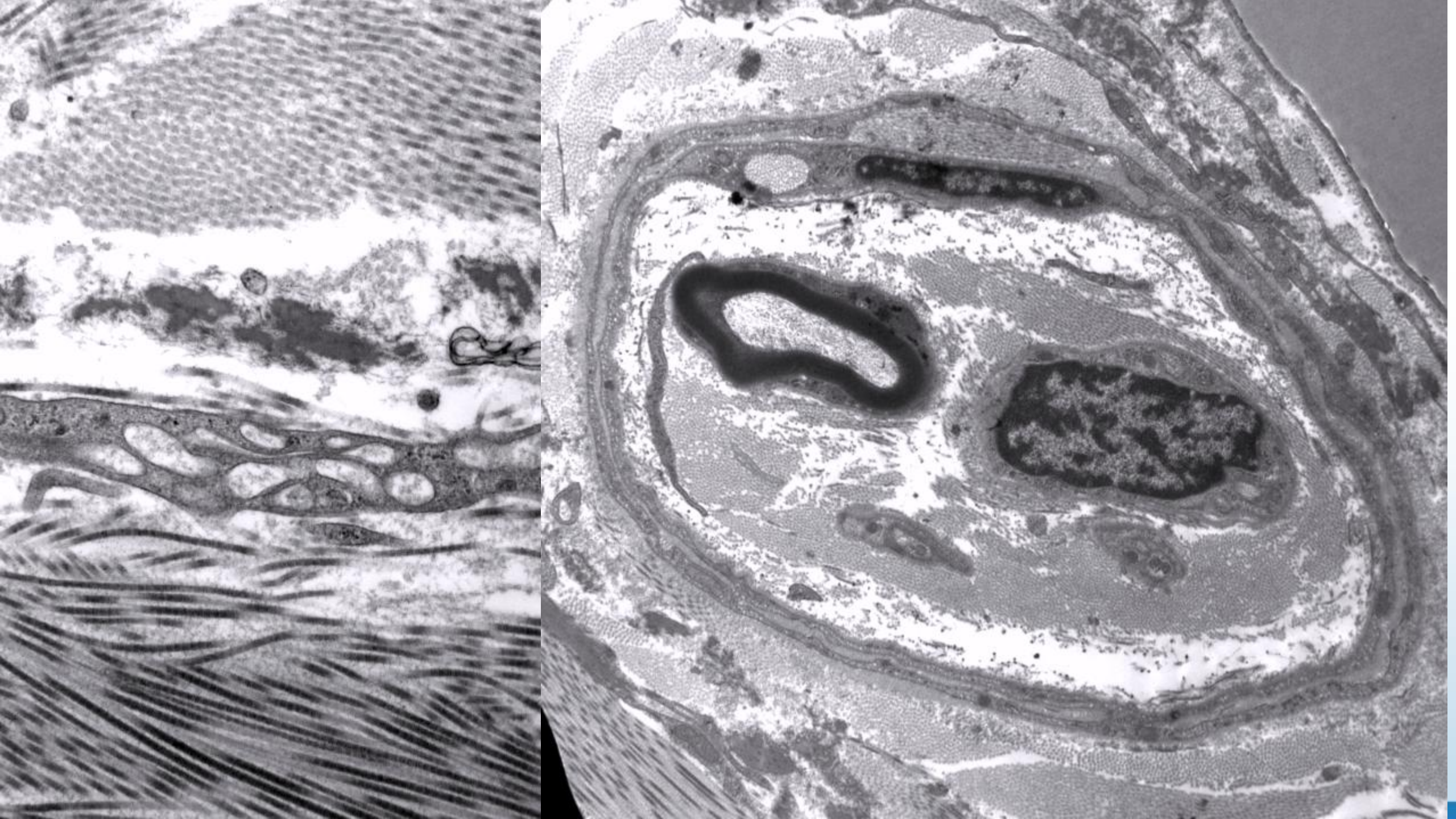


BASAL LAMINA AND SUBSARCOLEMMAL SPACE





INTERSTITUM



Questions?