

INTRODUCTION A L'HISTOLOGIE MUSCULAIRE

Dr Nathalie Streichenberger

Neuropathologie – CHU LYON

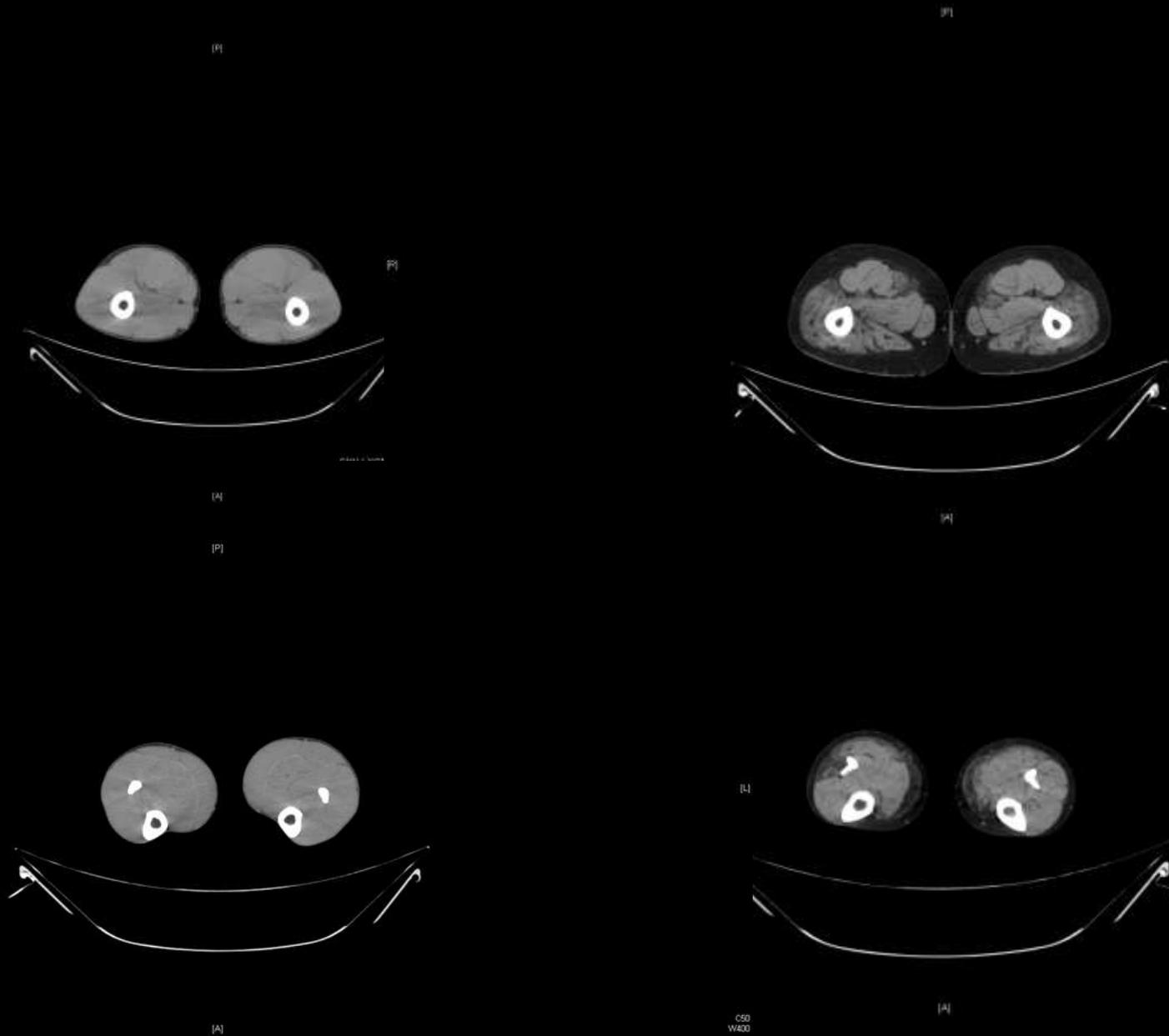
DIU pathologie neuromusculaire

PARIS 2015

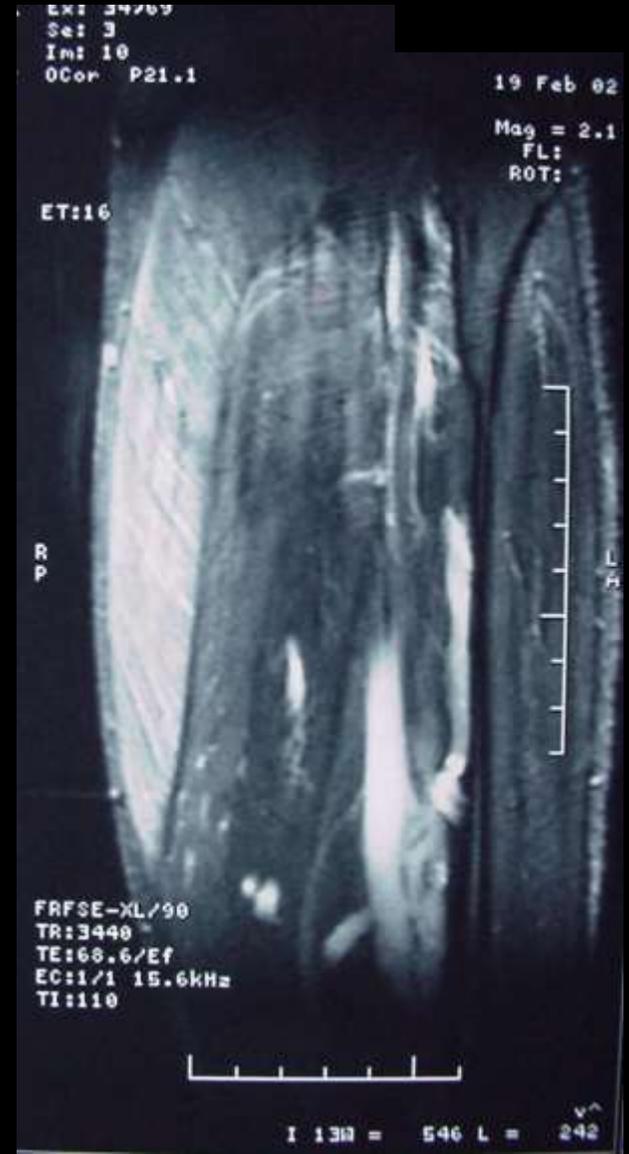
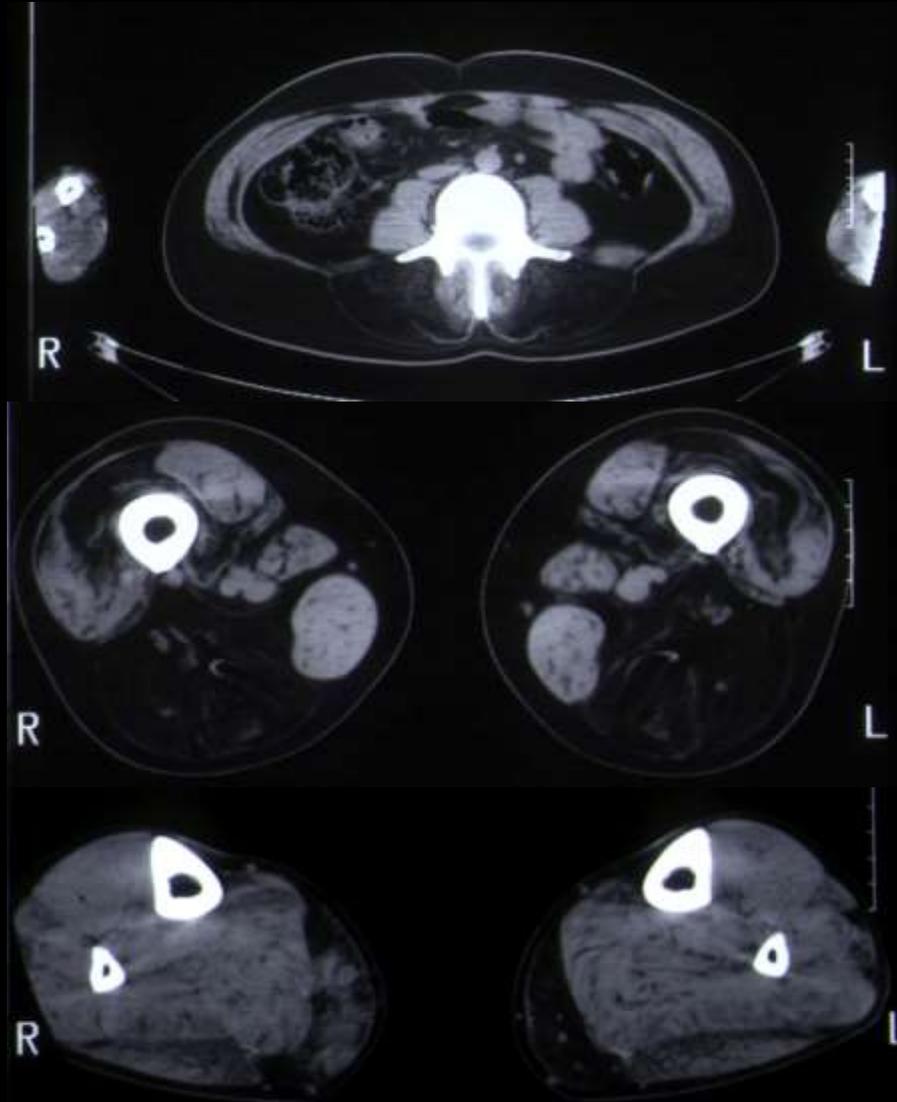
BILAN MINIMUM AVANT LA BIOPSIE

- **1- EVALUATION CLINIQUE**
 - Déficit +++
- **2- EVALUATION BIOLOGIQUE**
 - Bilan de coagulation
 - enzymes musculaires : CPK
 - Myosites : Bio inflammatoire – AC spécifiques
- **3- EXPLORATION FONCTIONNELLE**
 - EMG hémi corporel +++
- **4- IMAGERIE**
 - Si atrophie : SCANNER ou IRM
 - Si inflammation : IRM-STIR

SCANNER MUSCULAIRE



IRM MUSCULAIRE



DECONSEILLES AVANT LA BIOPSIE

- 1- mauvais bilan de coagulation
- 2- Prise d'aspirine ou dérivés dans les 10 jours précédents

- ACTRON
- AFEBRYL
- ALGO-NEVRITON
- ALKA-SELTZER
- ANTIGRIPPINE à l'aspirine
- ASASANTINE LP
- ASPEGIC (LYSINE)
- ASPIRINE 500 mg Vit C (Oberlin)
- ASPIRINE UPSA Vit C
- ASPIRINE Vit B1 C (Derol)
- ASPIRINE à croquer (Monot)
- ASPIRINE (Bayer)
- ASPIRINE DU RHONE 500
- ASPIRINE ENTERIQUE (Sarein)
- ASPIRINE PH 8
- ASPIRINE soluble (Evans)
- ASPIRINE UPSA effervescent 1000
- ASPRO
- ASPRO Vit C
- ASPRO 500 effervescent
- BRONCHO-TULISAN Eucalyptol
- CATALGINE
- CEFAPYRINE
- CEPHALGAN
- CEPHYL
- CLARAGINE
- COMPRALGYL
- DETOXALGINE
- DOLODERM
- FINIDOL
- HEMAGENE-TAILLEUR
- JUVEPIRINE
- KARDEGIC
- LONGALFIC
- METASPIRINE
- MIGPRIV (Lysine)
- NOVACETOL
- PLAVIX (Clopidogrel)
- PRENOXAN
- RHONAL
- RUMICINE
- SALIPRAN
- SARGEPIRINE
- SEDASPIR
- SOLUPSAN
- THEINOL
- TICLID
- TRANSCOGESIC
- VEGADEINE

- **SONT AUTORISES LES MEDICAMENTS A BASE DE PARACETAMOL : Exemple : DOLIPRANE**

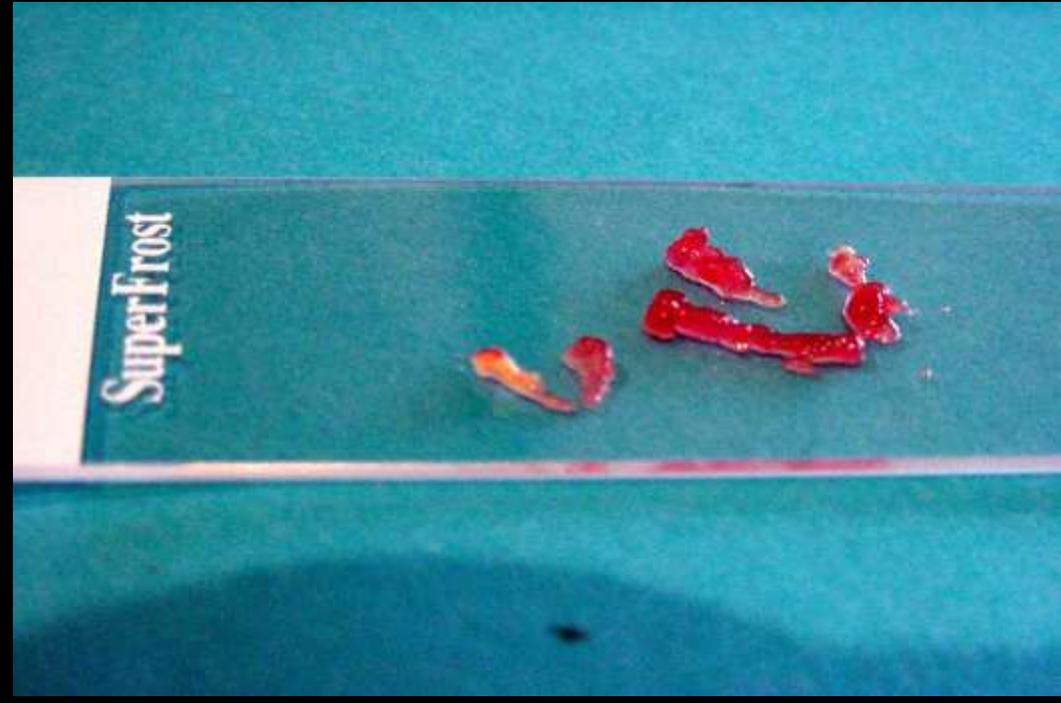
1. BIOPSIE MUSCULAIRE A L'AIGUILLE

En salle de consultation

50 mg de tissu.

Suites simples : pas de fil, pas d'arrêt de travail

Intérêt : contrôle de polymyosite



2. BIOPSIE MUSCULAIRE A LA PINCE



Une méthode miniaturisée : anesthésie locale, incision de 7mm, 100mg de muscle, suture sans point

3. BIOPSIE MUSCULAIRE CHIRURGICALE

Au bloc opératoire / salle de consultation :

AL xylocaïne 1%

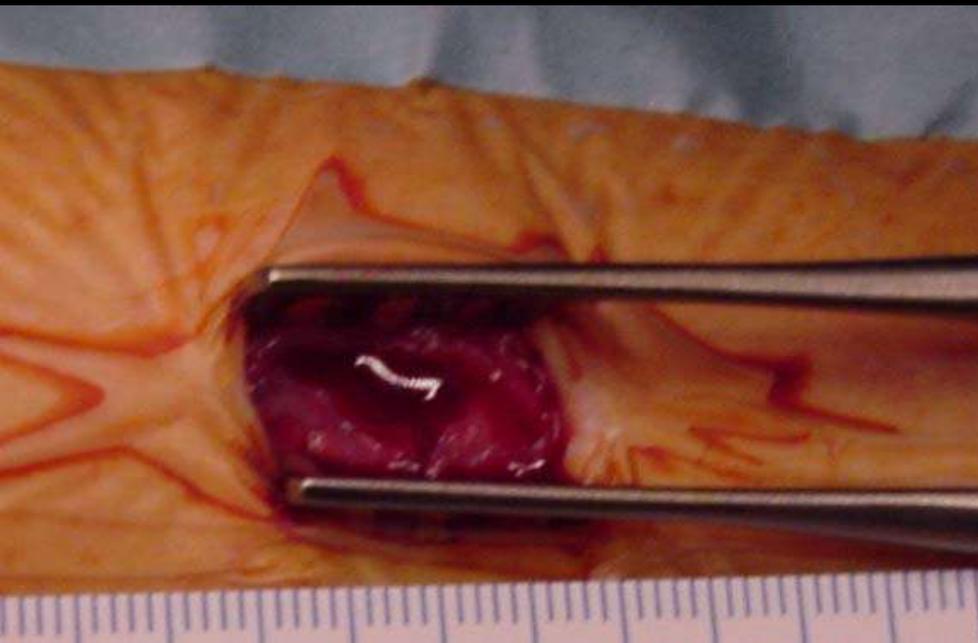
AG : enfant (bloc opératoire)

Incision de 0,5 à 1 cm : 300-400 mg de muscle bien orienté

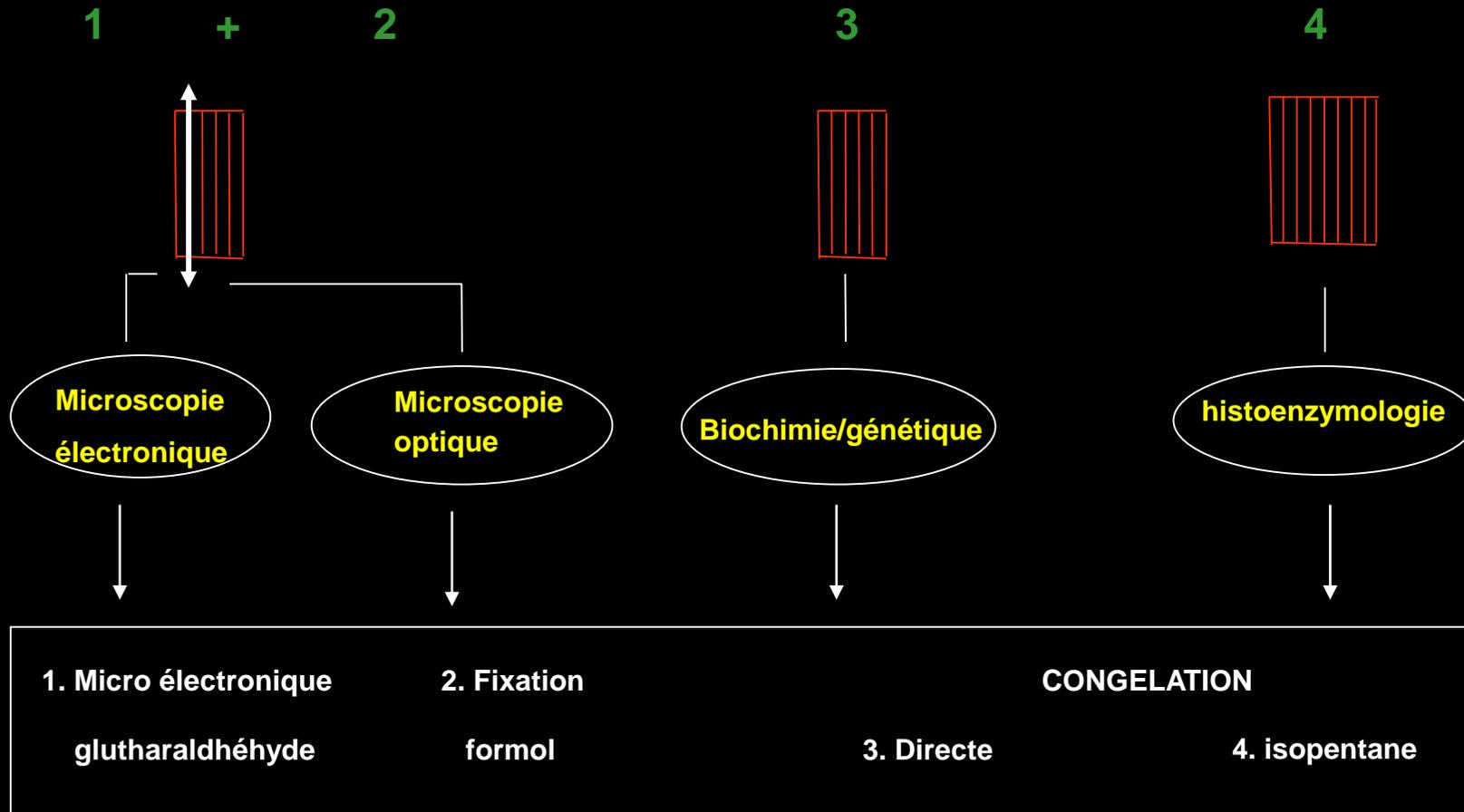
Durée de la biopsie : 15 à 20 minutes (asepsie incluse)

Surjet sous cutané fil résorbable + colle

Arrêt de travail si profession « physique »



TECHNIQUES DE CONDITIONNEMENT



Biopsie Musculaire

I – Coupes en paraffine

Coupes en paraffine (1)



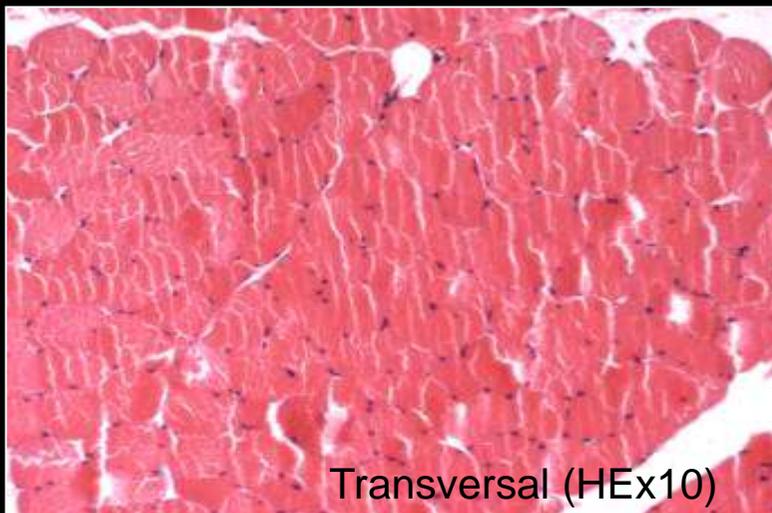
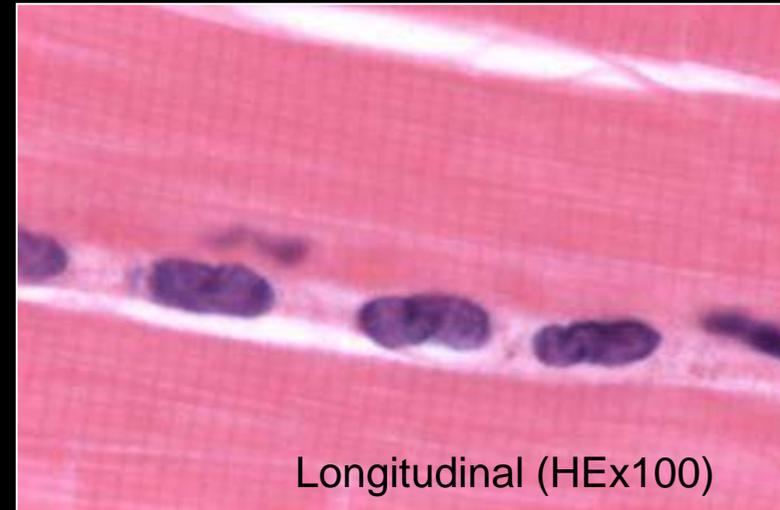
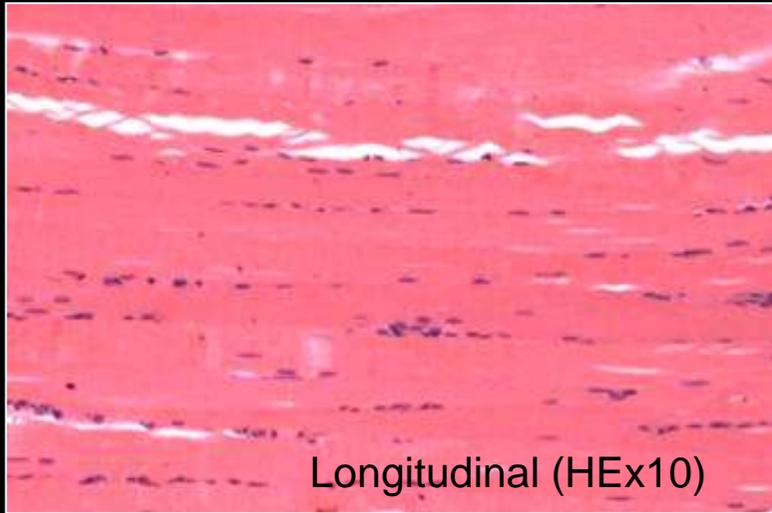
- **Après fixation au formol**
- **Inclusion dans la paraffine** (automate à inclusion)
- **Coupes de 6 μm**
- **Déposées sur lames de verre**
- **Colorées** ou utilisées pour **immunohistochimie**
- **Bloc et lames sont stockés** après observation en MO

Coupes en paraffine (2)

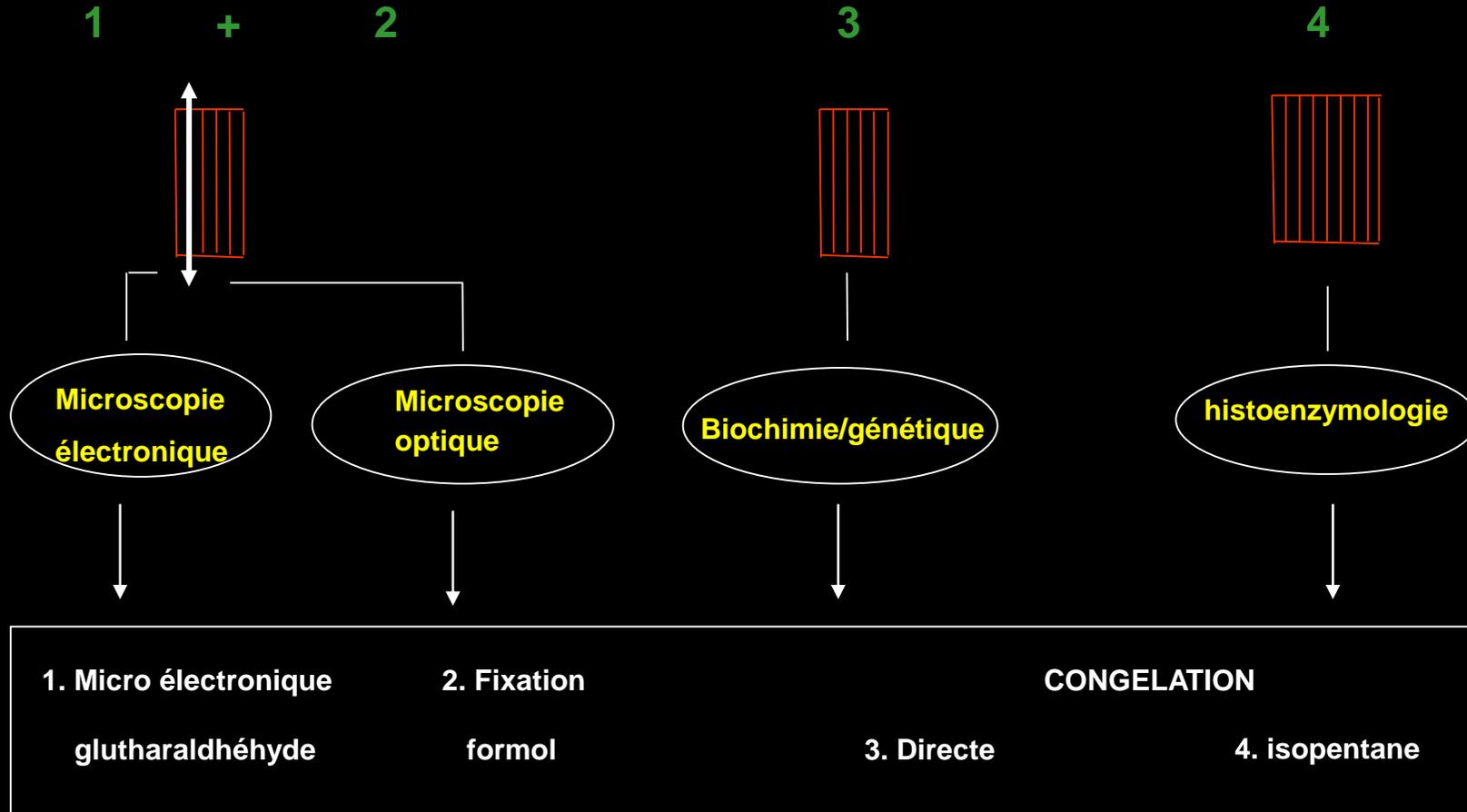
- ❖ indispensable pour l'étude du tissu interstitiel et des vaisseaux
- ❖ en cas de suspicion de myopathie inflammatoire, au moins 8 niveaux de coupe
- ❖ études immunohistochimiques avec anticorps dirigés contre les macrophages (CD68), les lymphocytes T (CD3) et B (CD20) (infiltrats inflammatoires)

Coupes en paraffine (3)

Muscle normal



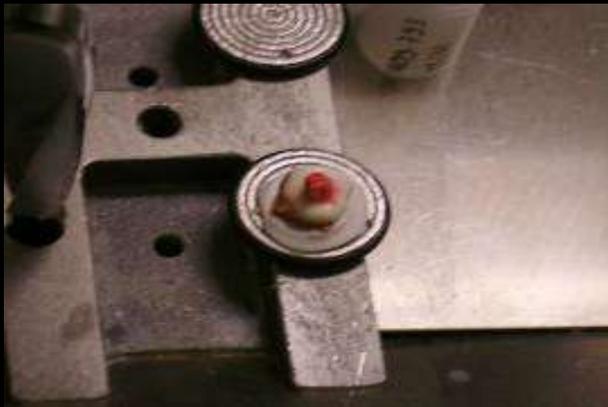
TECHNIQUES DE CONDITIONNEMENT



Biopsie Musculaire

II – Coupes au cryostat

Congélation



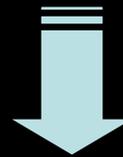
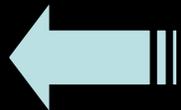
FRAGMENT CONGELE MONTE
PRES A ETRE COUPE



ISOPENTANE

AZOTE (-160°C)

CONGELATION DU FRAGMENT DANS
L'ISOPENTANE REFROIDI A L'AZOTE

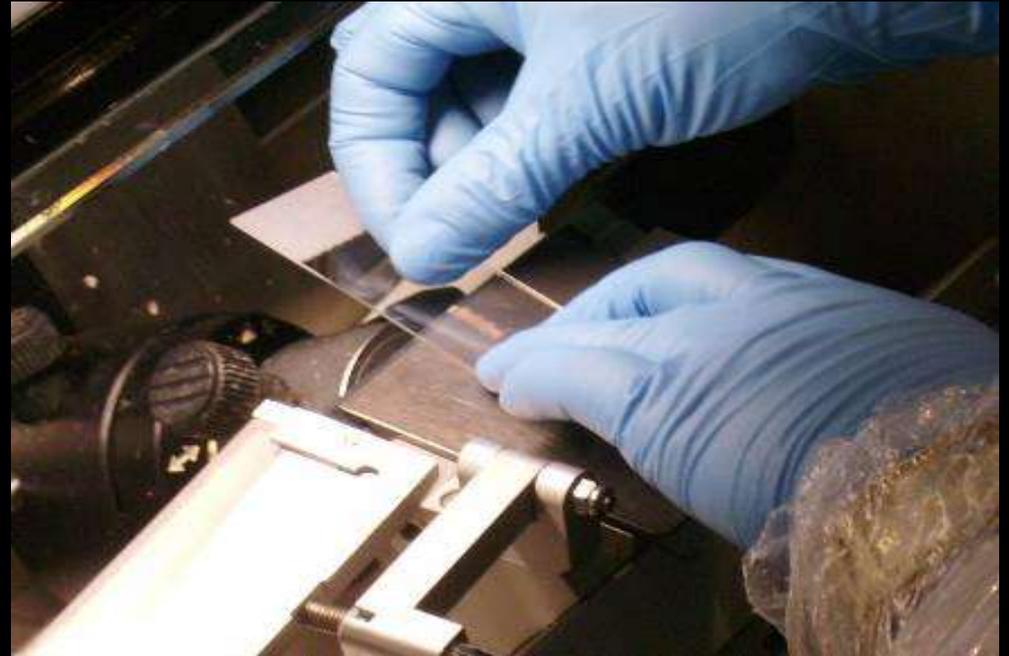


Coupes au cryostat (1)



ENCEINTE REFRIGEREE A - 18/ - 20°C

Coupes au cryostat (2)



Colorations



HISTOCHEMICAL TECHNIQUES PERFORMED ON MUSCULAR BIOPSIES

- **HES**

- **modified trichrome stain**

mitochondriopathies : *ragged red fibres*

inclusion bodies : *ex : rimmed vacuole*

- **PAS**

Glycogenesis accumulation :

- **Sudan black – red oil**

lipid accumulation

- **Mitochondrial enzymes complex :**

– DPNH (I), SDH (II), COX (IV)

- **ATP Enzymes : fibre types**

– Ph 4.35

– Ph 4.63

– Ph 9.4

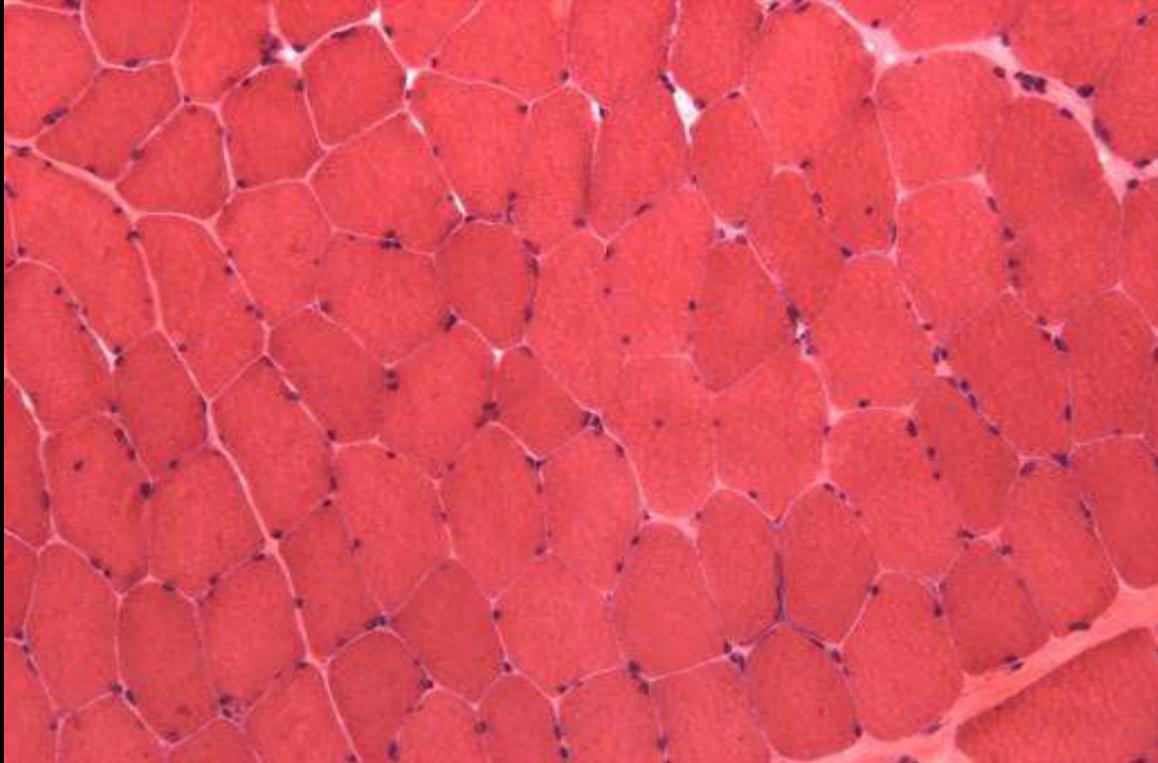
black *white* *intermediary*

I II

I IIa IIb

II I

Colorations usuelles (1)



Hématoxyline-éosine

- fibres musculaires : rose
- noyau : brun
- tissu interstitiel : rose clair
- fibres basophiles de régénération : rose/violet

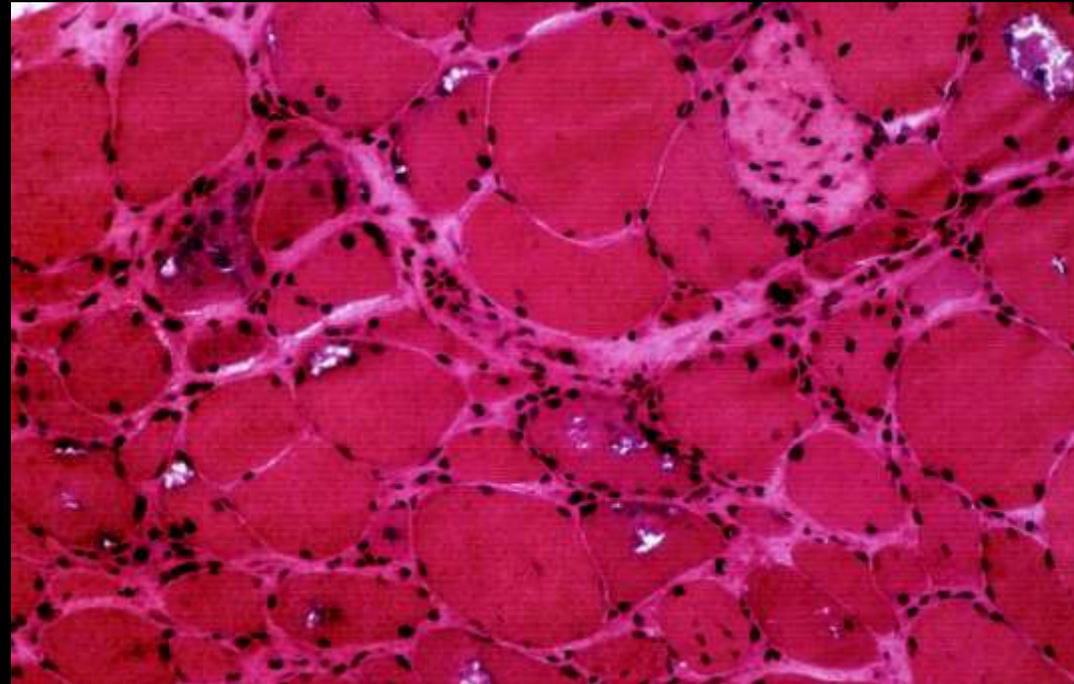
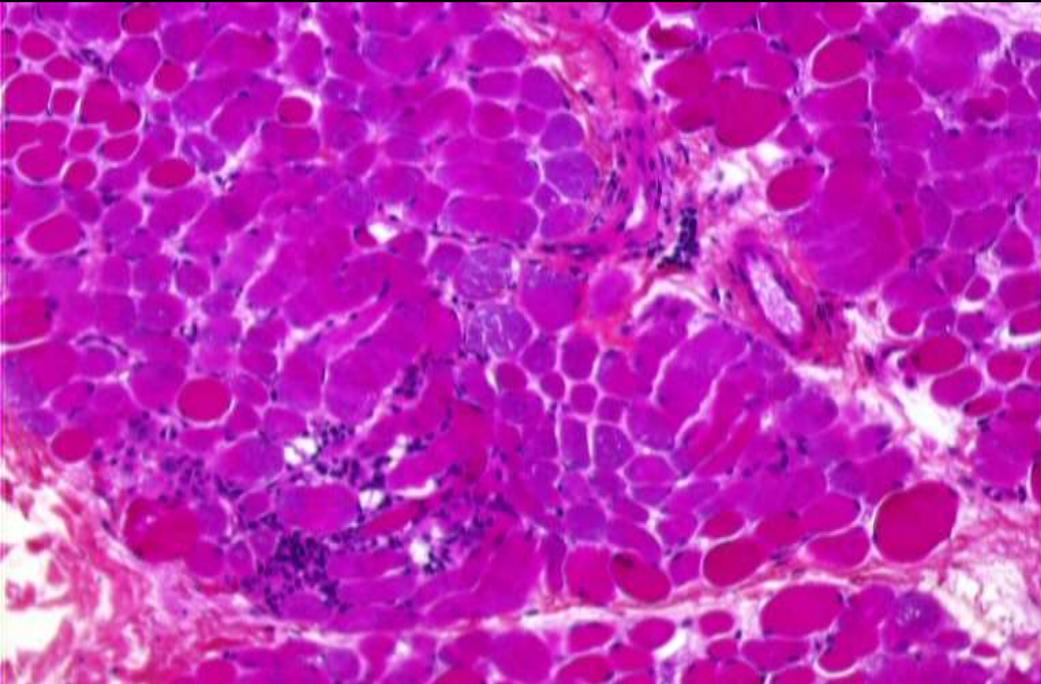


FIXATION - ISOPENTAN FREEZING

inclusion bodies detection only on frozen slides :



Treatments in IBM and polymyositis are différent.



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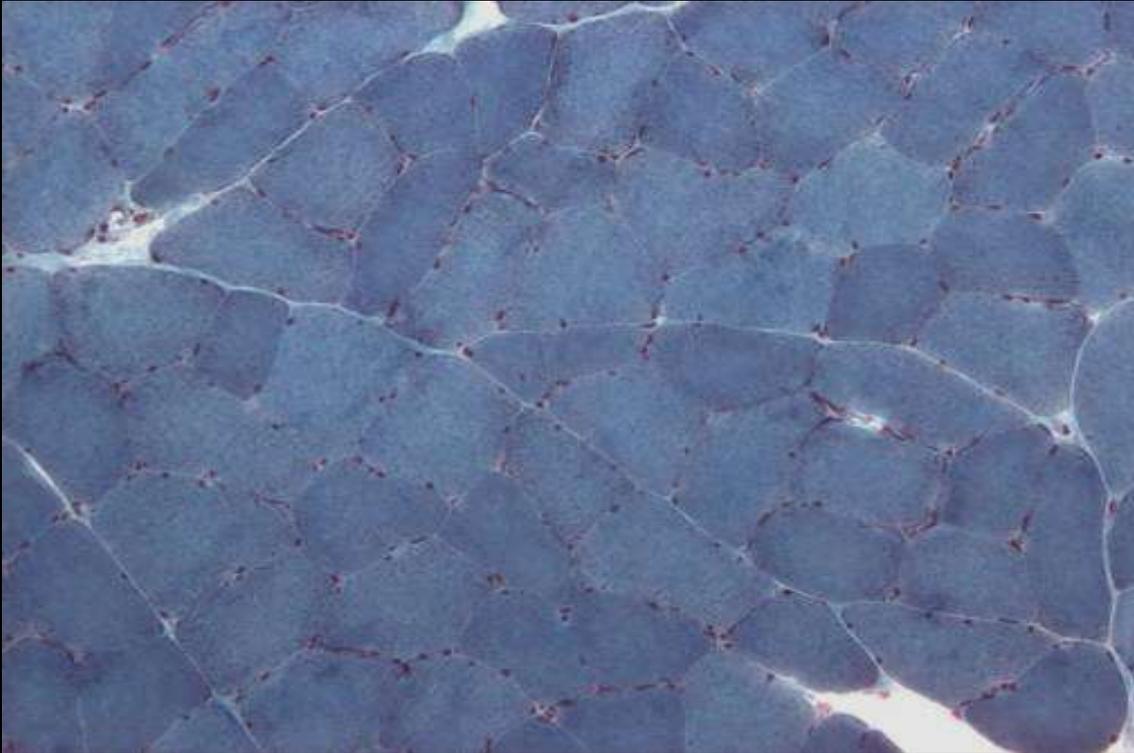
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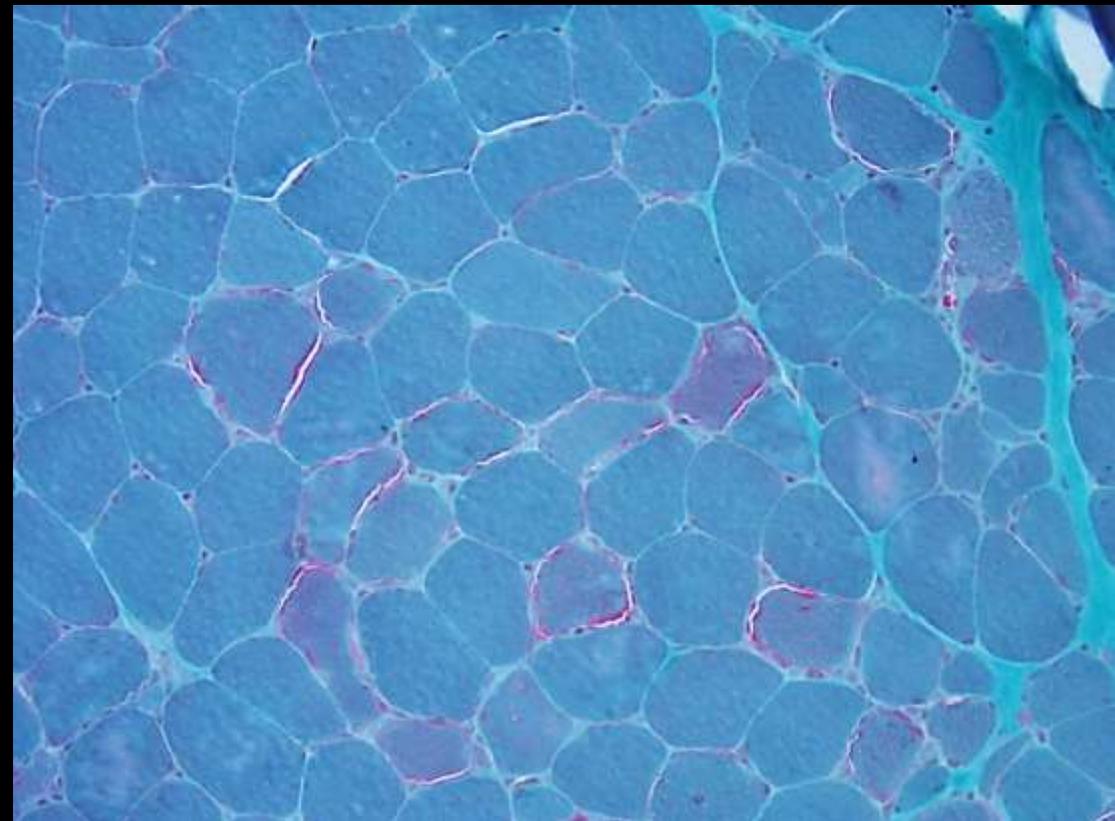
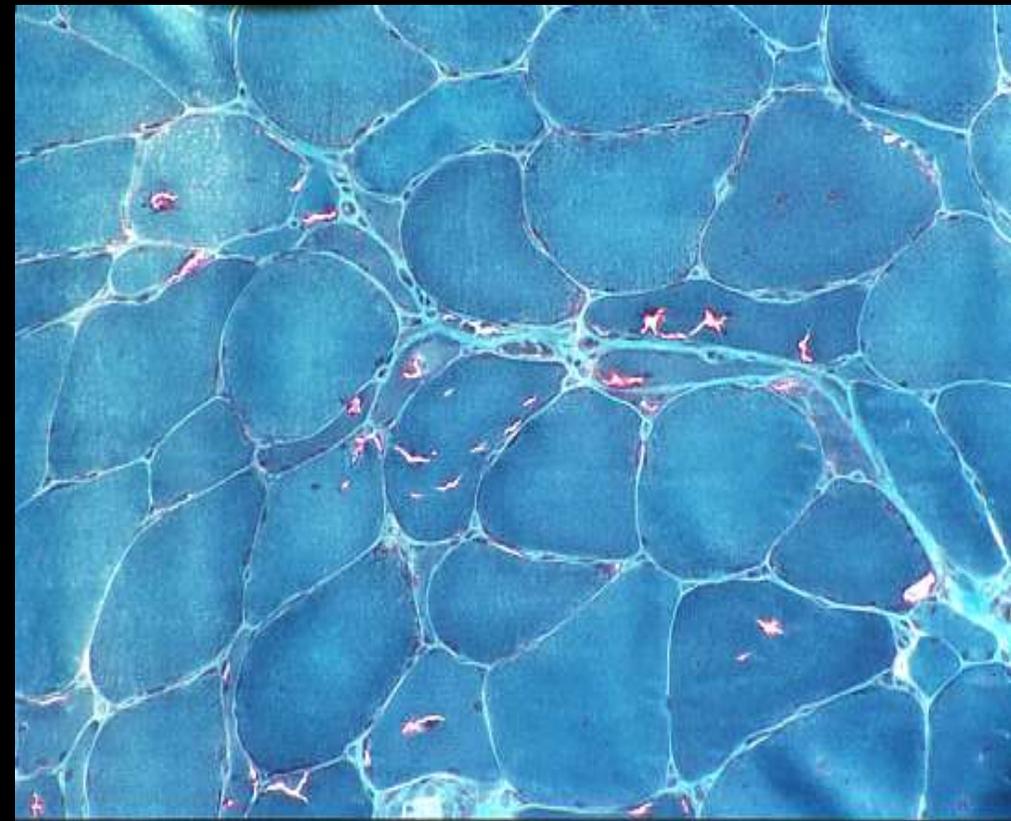
Colorations usuelles (2)

Trichrome de Gomori



- fibres musculaires : vert/bleu
- noyau : brun
- tissu interstitiel : vert/bleu clair
- mitochondries/corps cytoplasmiques : rouge
- bâtonnets : noir

TRICHOME DE GOMORI



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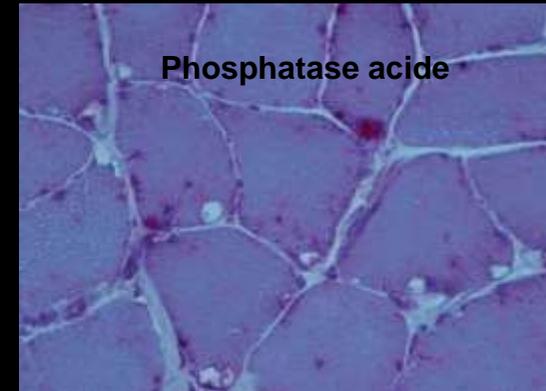
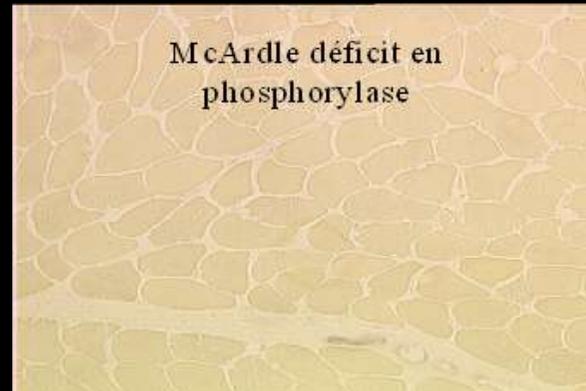
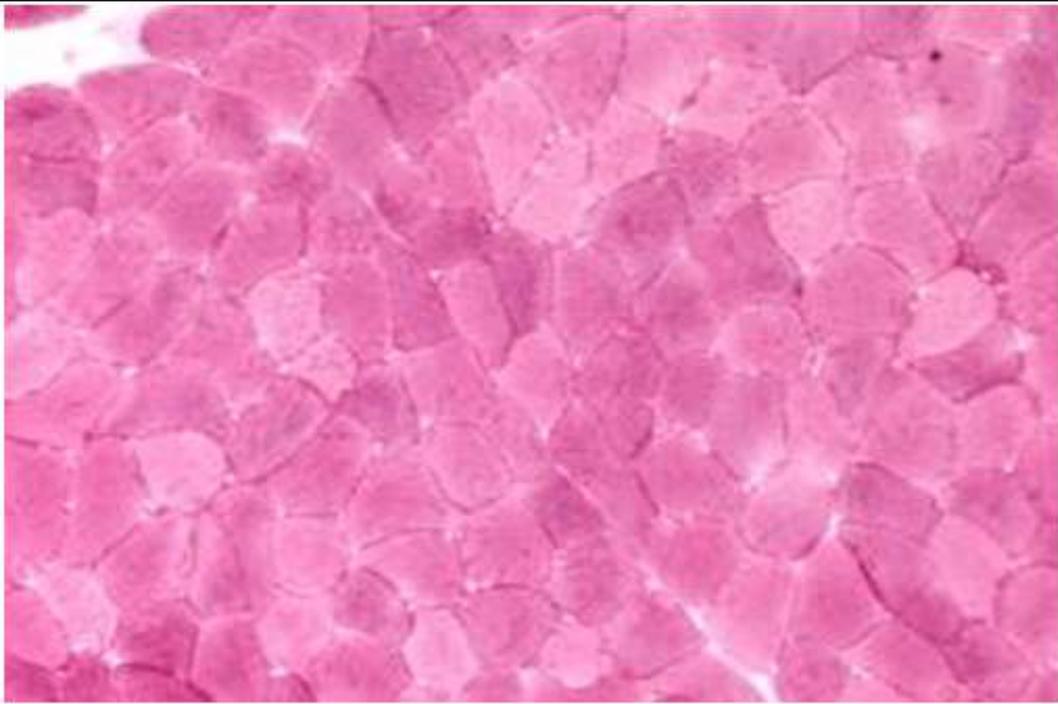
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II I

PAS



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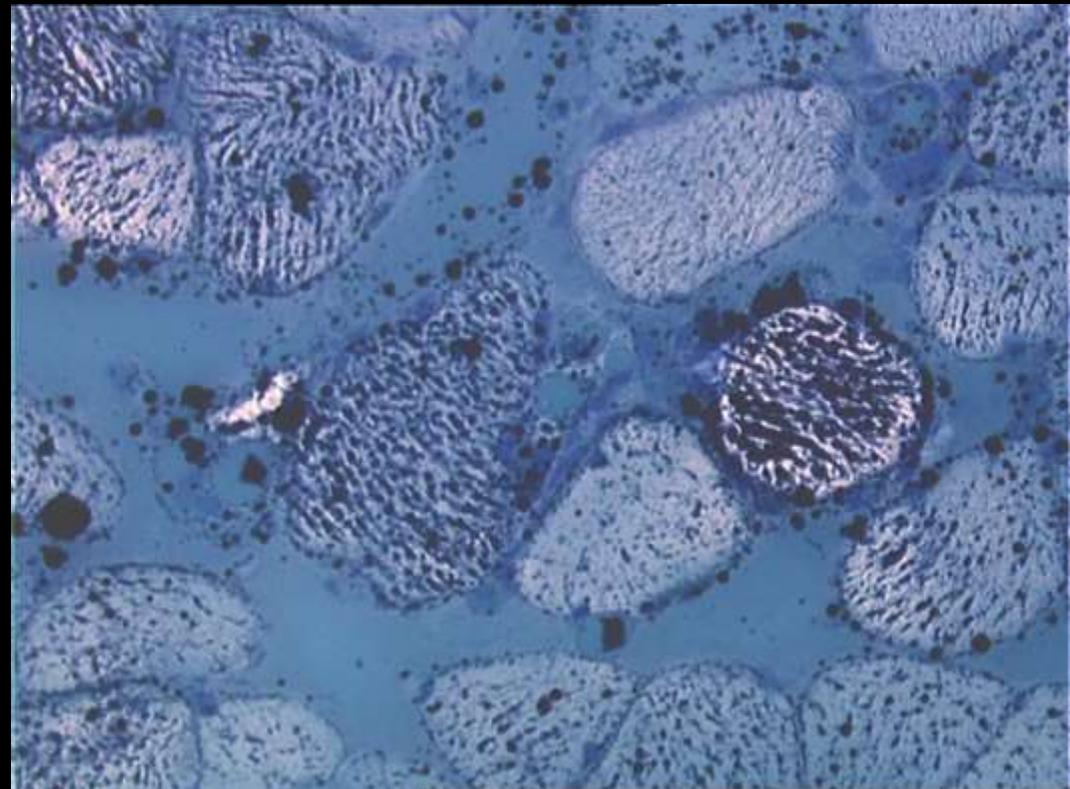
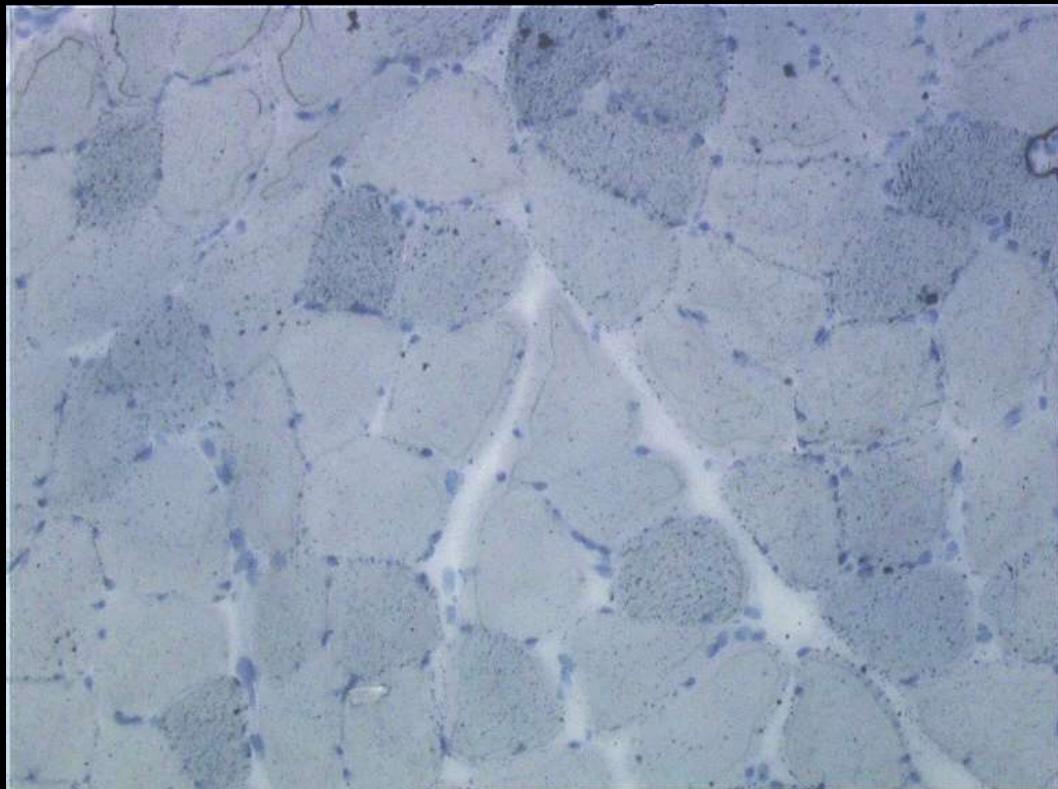
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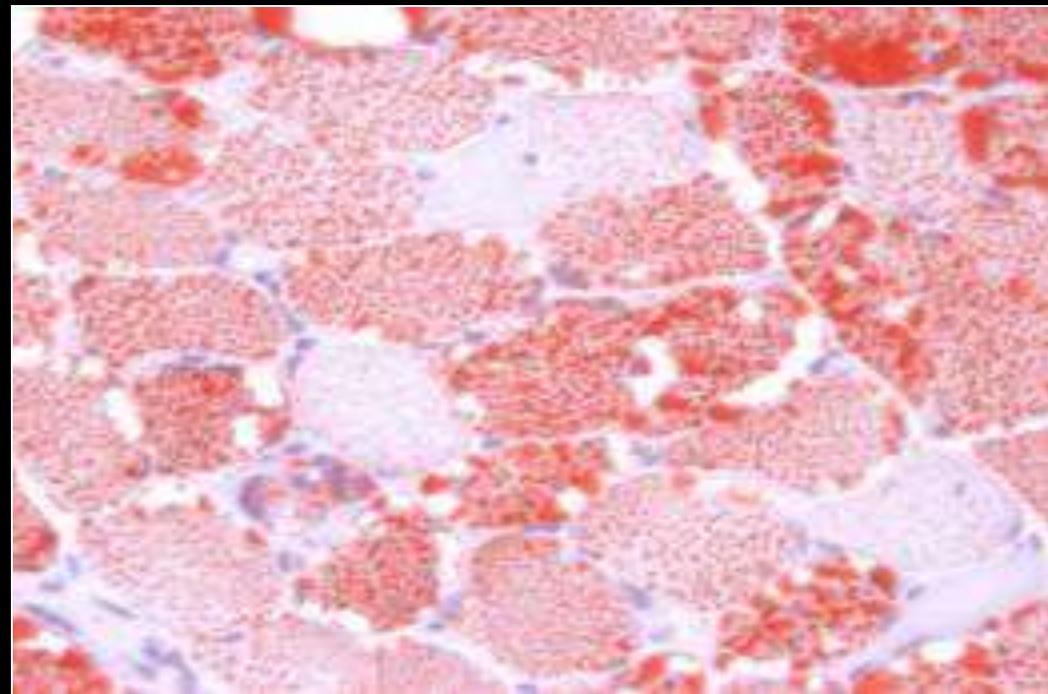
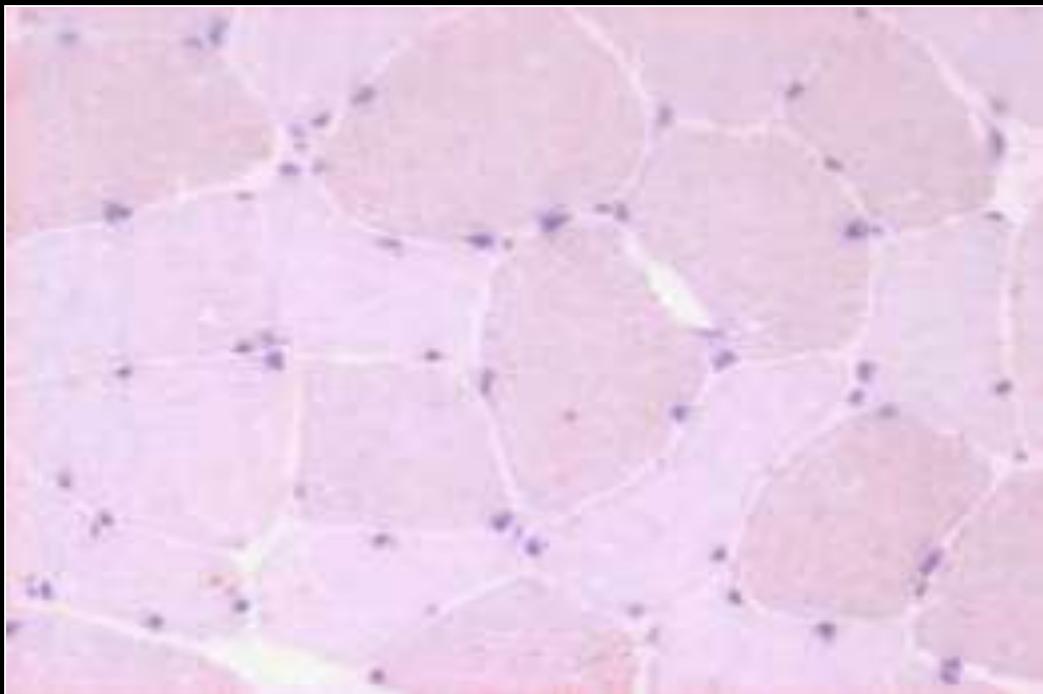
II I

NOIR SOUDAN



Rouge Soudan

surcharge lipidique



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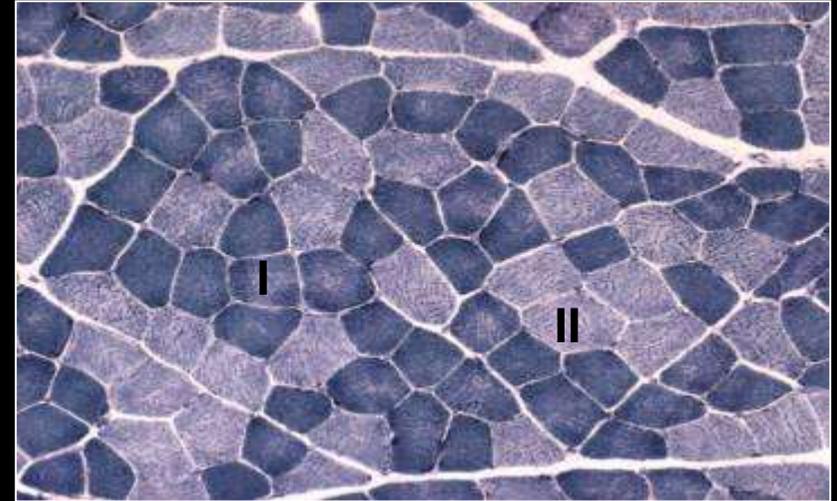
II I

Techniques histoenzymologiques

1 - Mise en évidence des différents types de fibres

NADH (Nicotinamide Adénine Dinucléotide)

- pH = 7,4
- Activité enzymatique présente dans les mitochondries et le réticulum sarcoplasmique
- coloration du réseau intermyofibrillaire, myofibrilles non colorées
- Très utile pour :

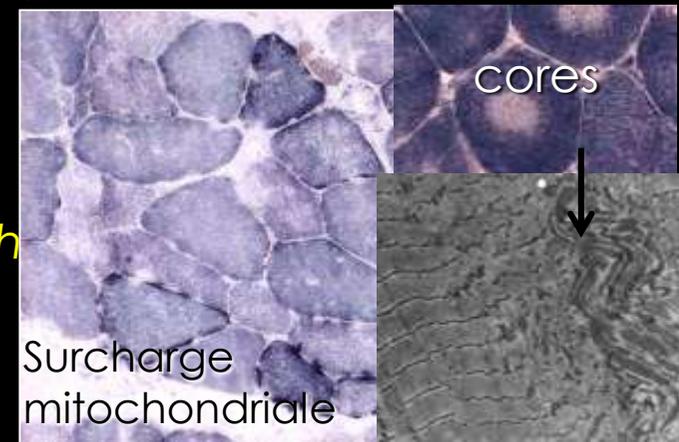


(1) Distinguer en routine les fibres de type I et II (pas les sous types II)

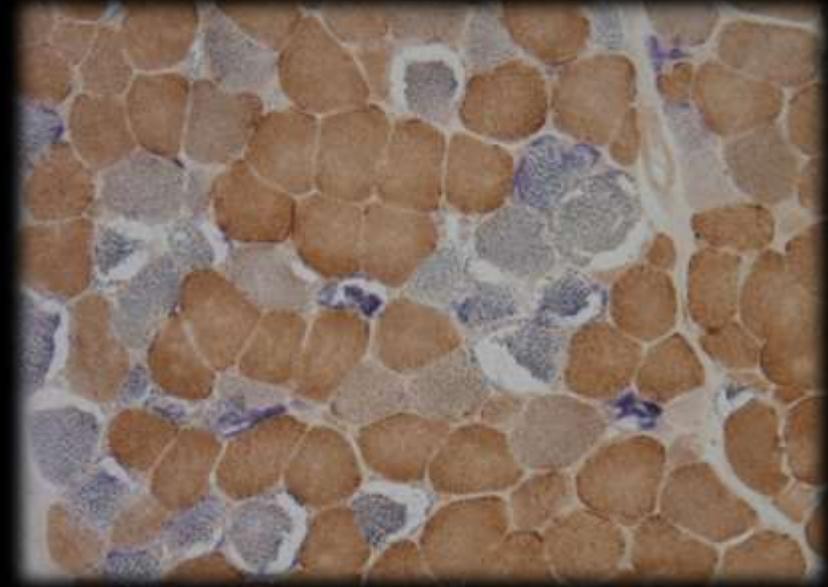
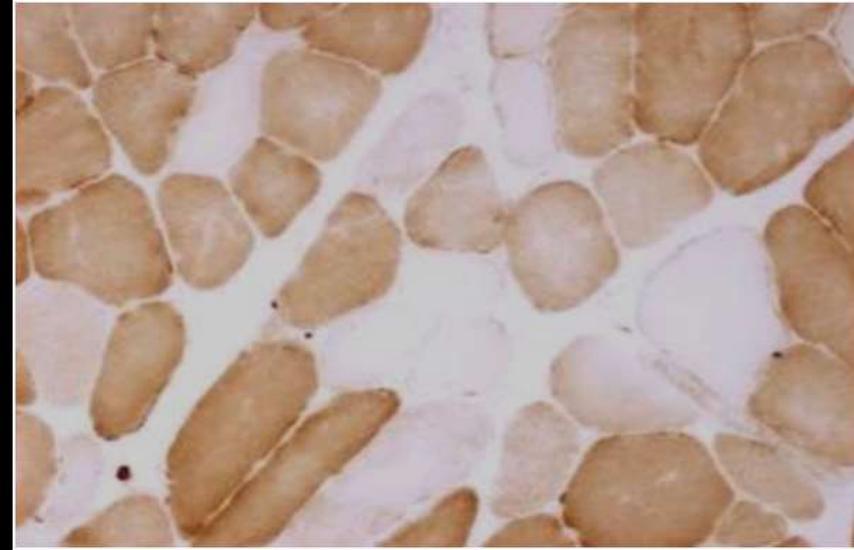
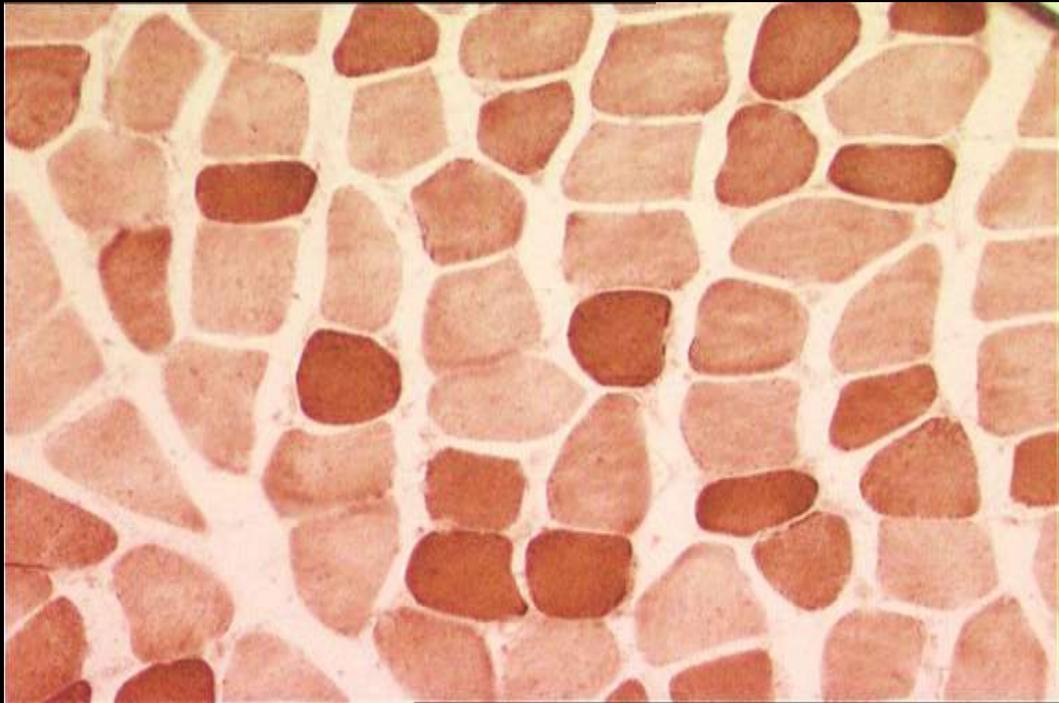
(2) Mettre en évidence les remaniements du réseau intermyofibrillaire

SDH

- pH = 7,4
- résultat identique à la NADH mais **spécifique des mitochondries**
- fibres I plus foncées que fibres II



COMPLEXE DE LA CHAÎNE RESPIRATOIRE MITOCHONDRIALE



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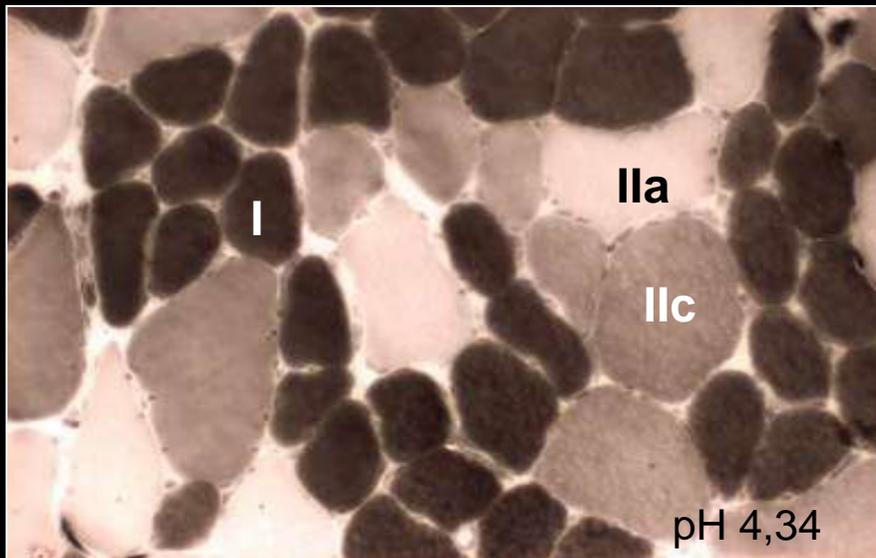
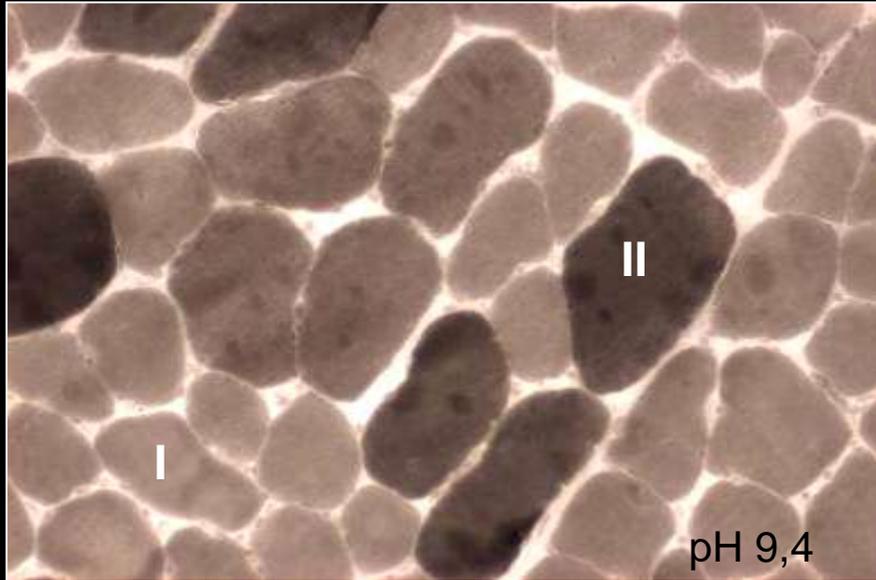
black *white* *intermediary*

I II

I IIa IIb

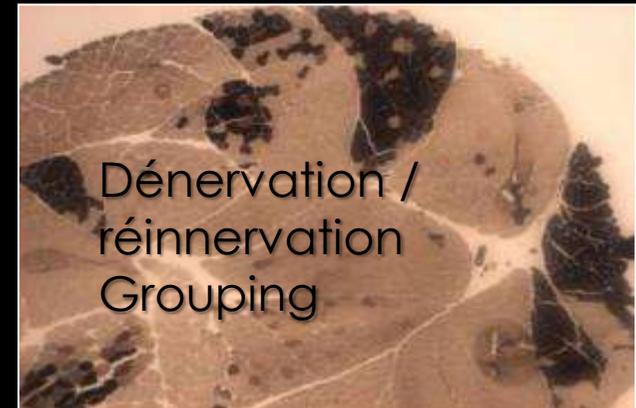
II I

ATPase



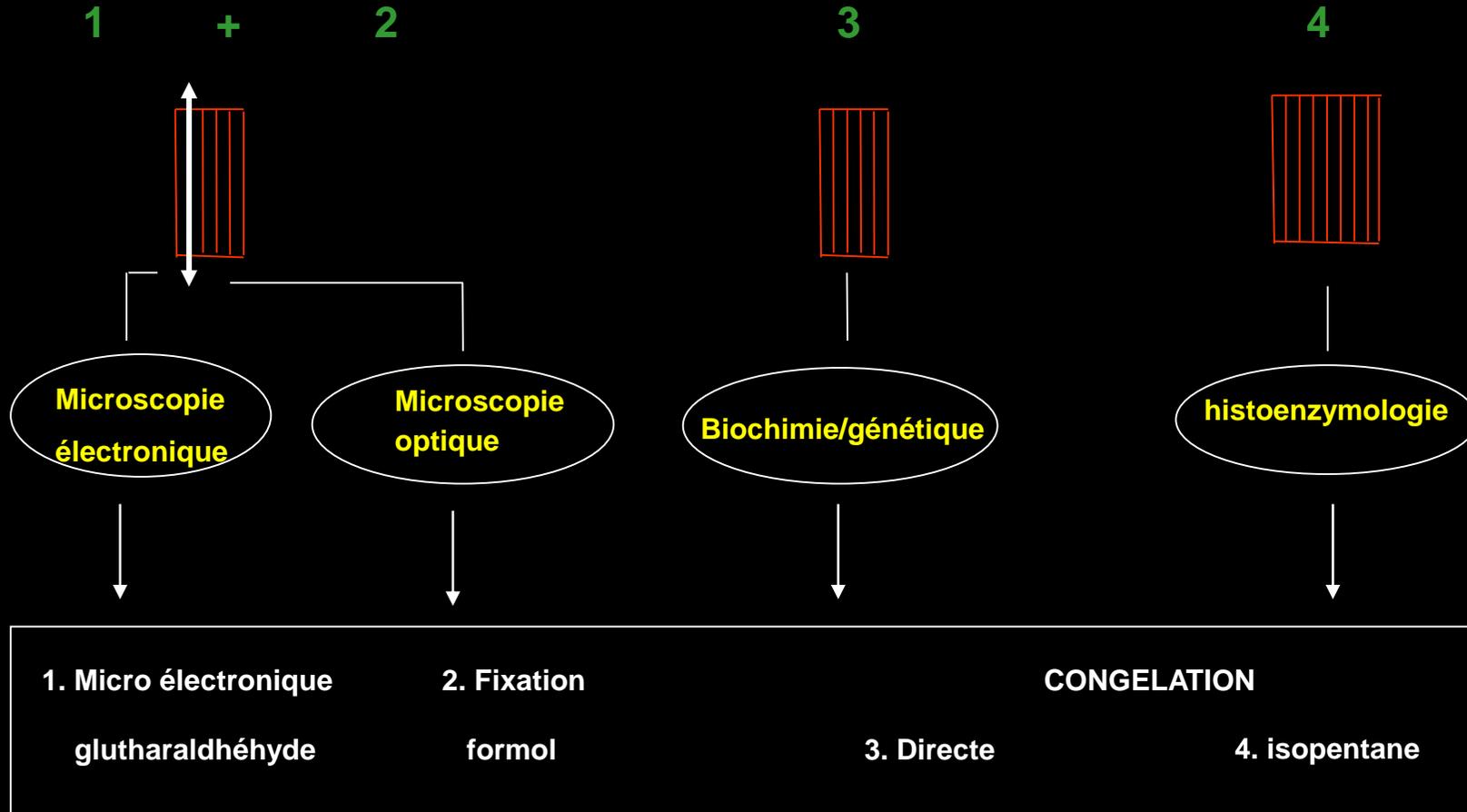
Les myofibrilles sont colorées dans des tons différents en fonction du pH :

- à pH 9,4 : type II foncées, type I claires



- à pH 4,34 : type I foncées, type IIa et IIb, claires ou blanches, type IIc intermédiaires

TECHNIQUES DE CONDITIONNEMENT



Biopsie Musculaire

III – Microscopie électronique

Indications

- préciser la nature d'anomalies particulières observées en microscopie optique : **myopathies structurales**
 - bâtonnets
 - corps cytoplasmiques
 - inclusions
 - anomalies de structure (cores/multiminicores)
 - accumulations de matériel (myopathies myofibrillaires)
- identifier des structures uniquement visibles en ME :

Coupes semi-fines et ultra-fines

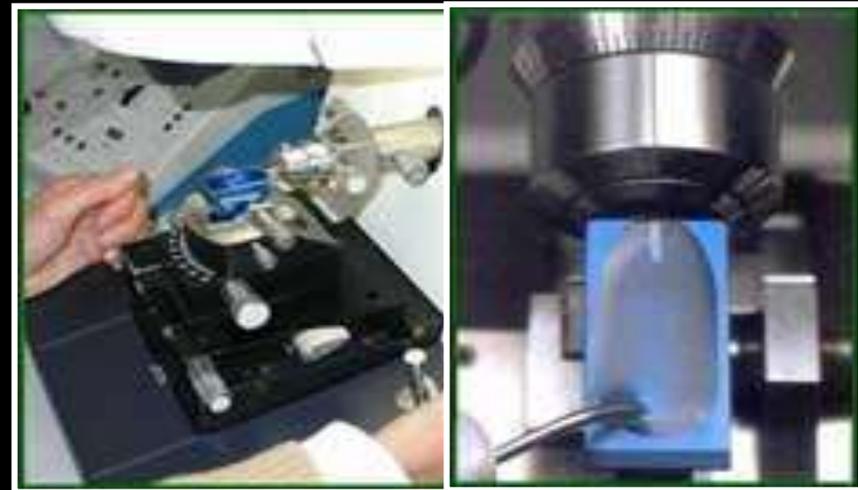


- **Après fixation** dans la glutaraldéhyde et post-fixation à l'acide osmique

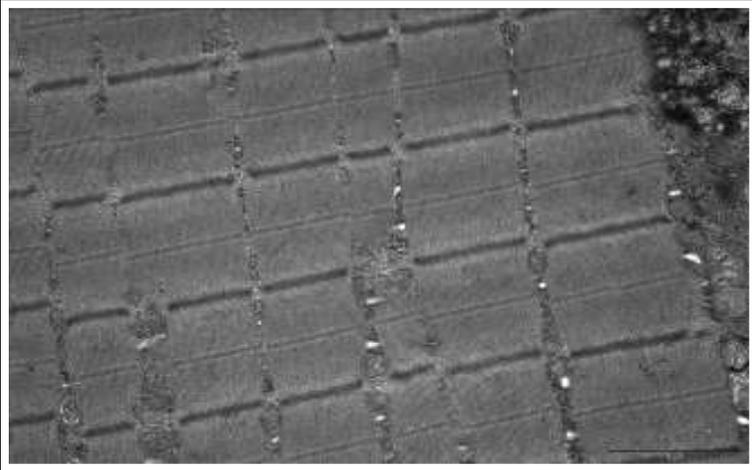
- **Inclusion** dans une résine (Epon)

- **Coupes semi-fines de 0,5 μm** , recueillies sur des lames de verre, observables en MO, colorées par le **bleu de Toluidine**

- **Coupes ultrafines de 80 nm** à partir des mêmes blocs, recueillies sur des grilles de cuivre, **contrastées par** l'acétate d'uranyle et des sels de plomb (citrate de plomb), observables en ME



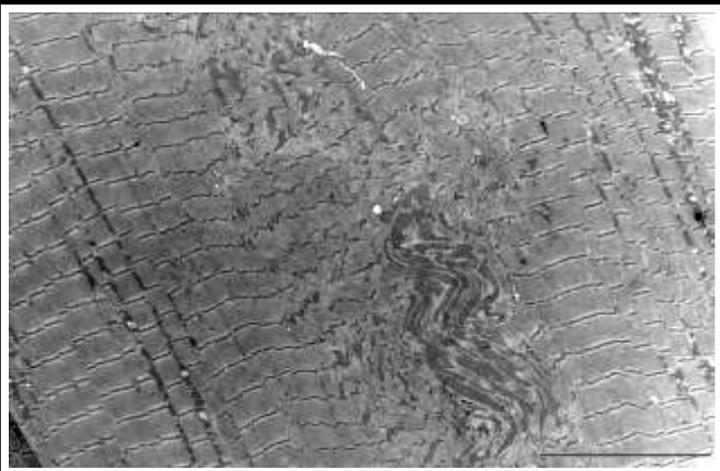
Quelques exemples...



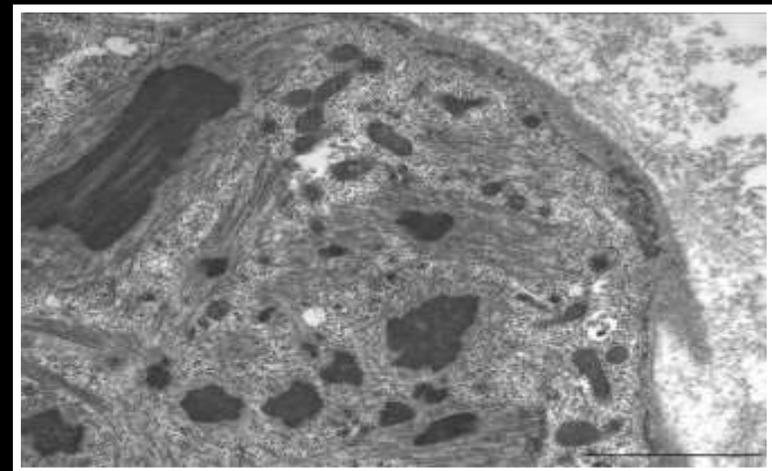
Muscle normal



Inclusions tubulo-filamentaire



Mini-core



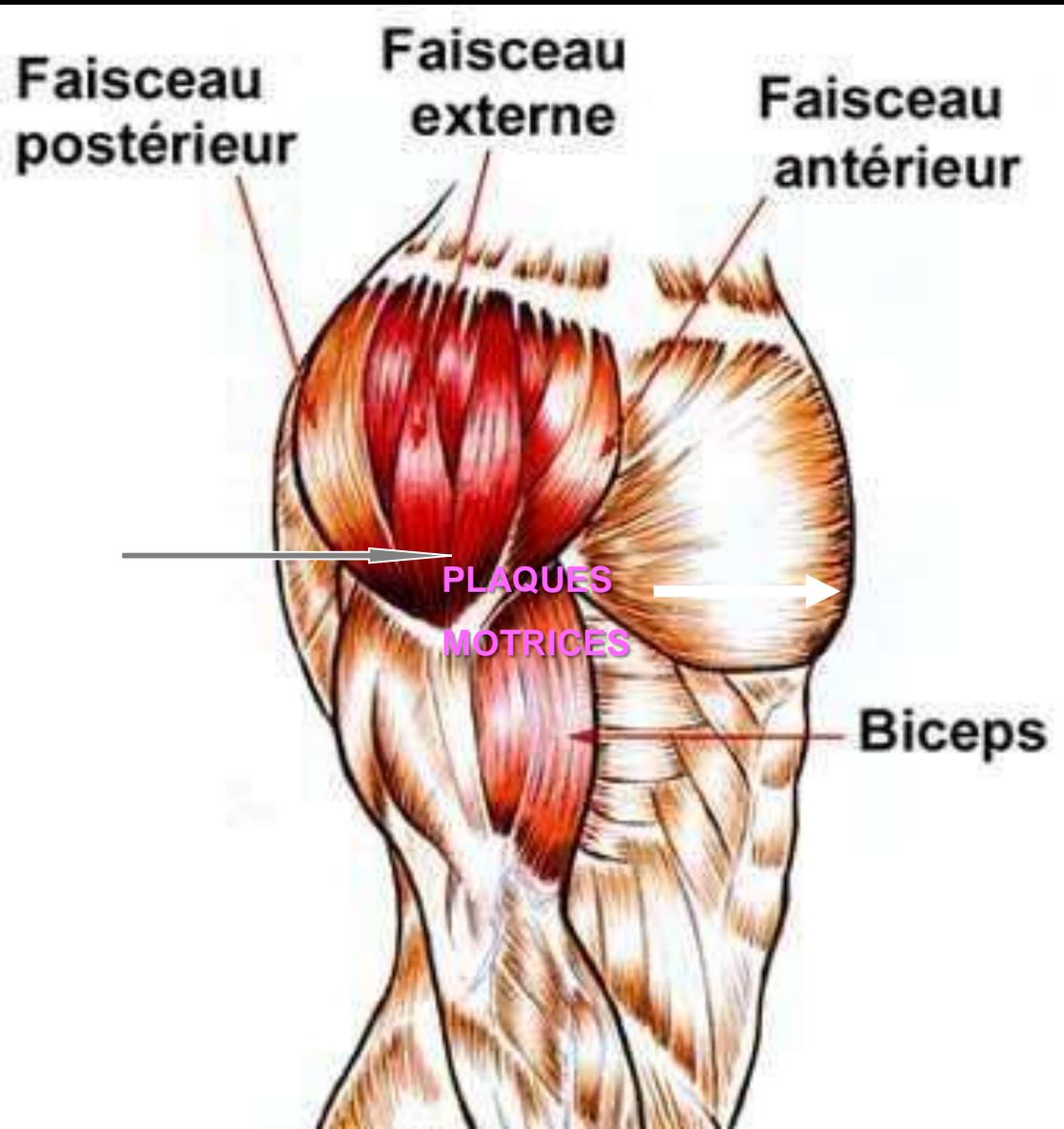
Bâtonnets

Biopsie Musculaire

IV – Jonctions neuromusculaires

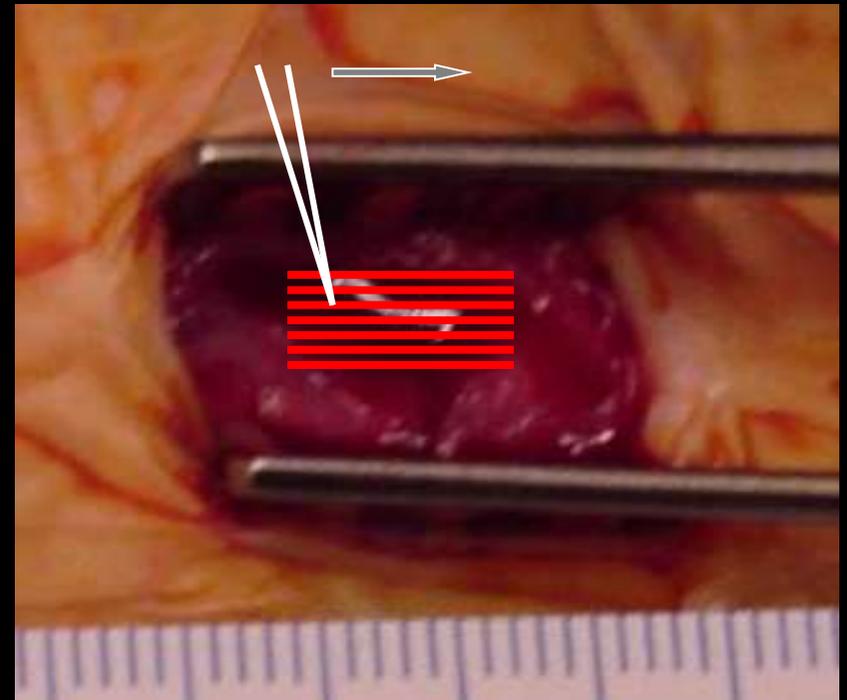
JONCTIONS NEUROMUSCULAIRES

SITE DE PRELEVEMENT



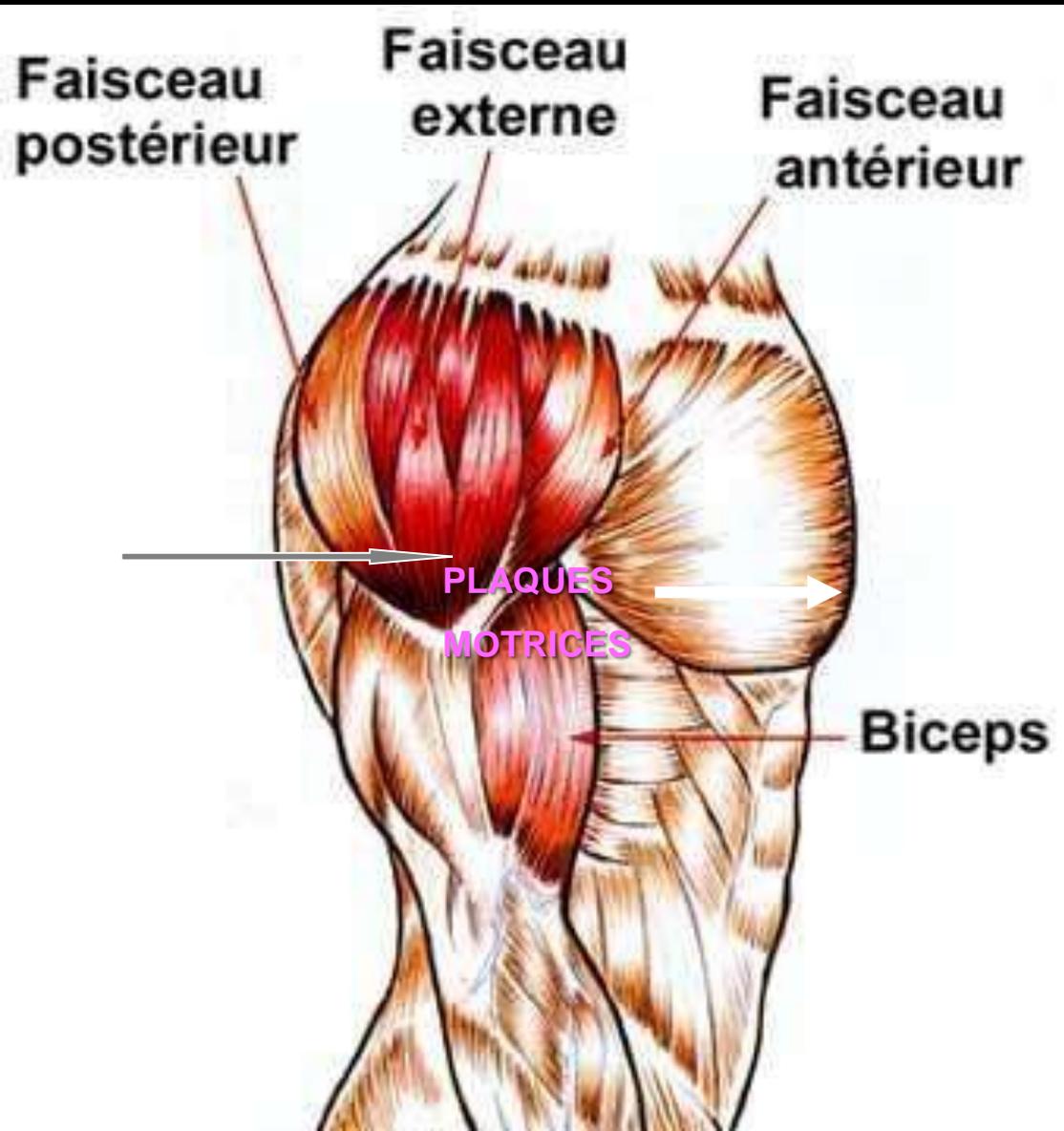
Stimulation des fibres musculaires

- à la pince
- électriquement



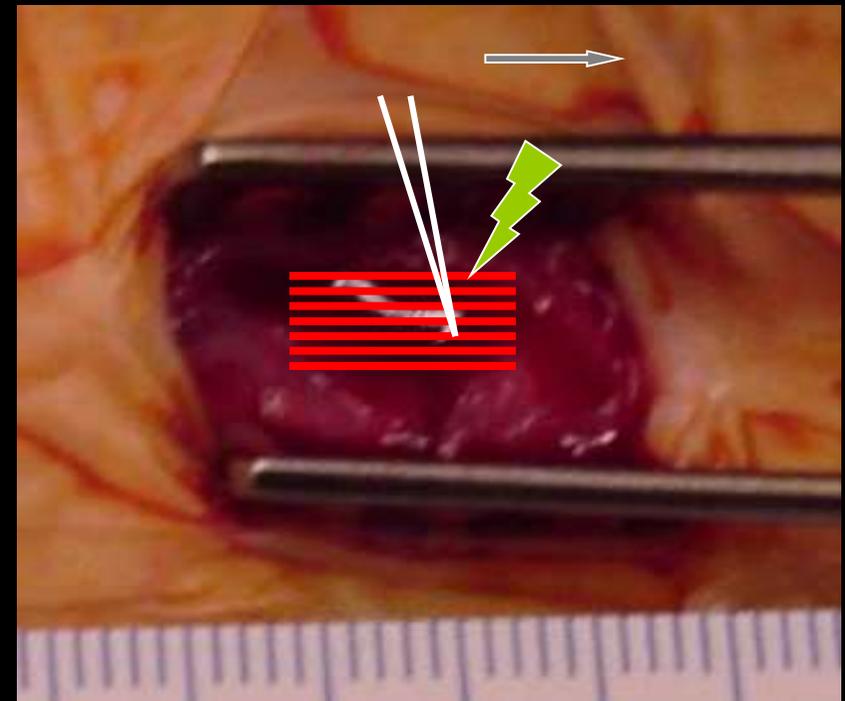
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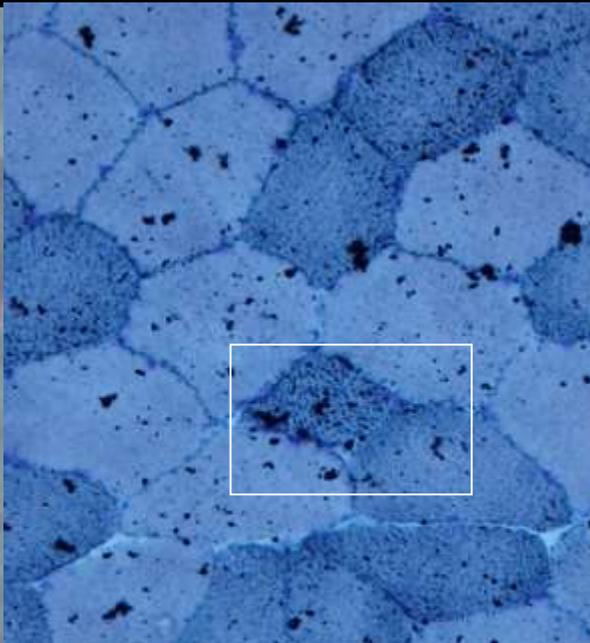
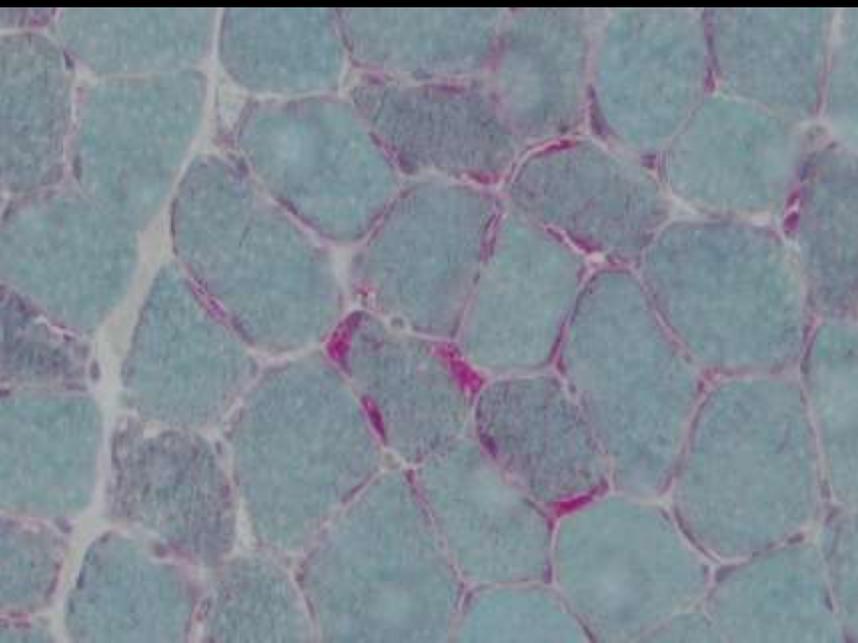
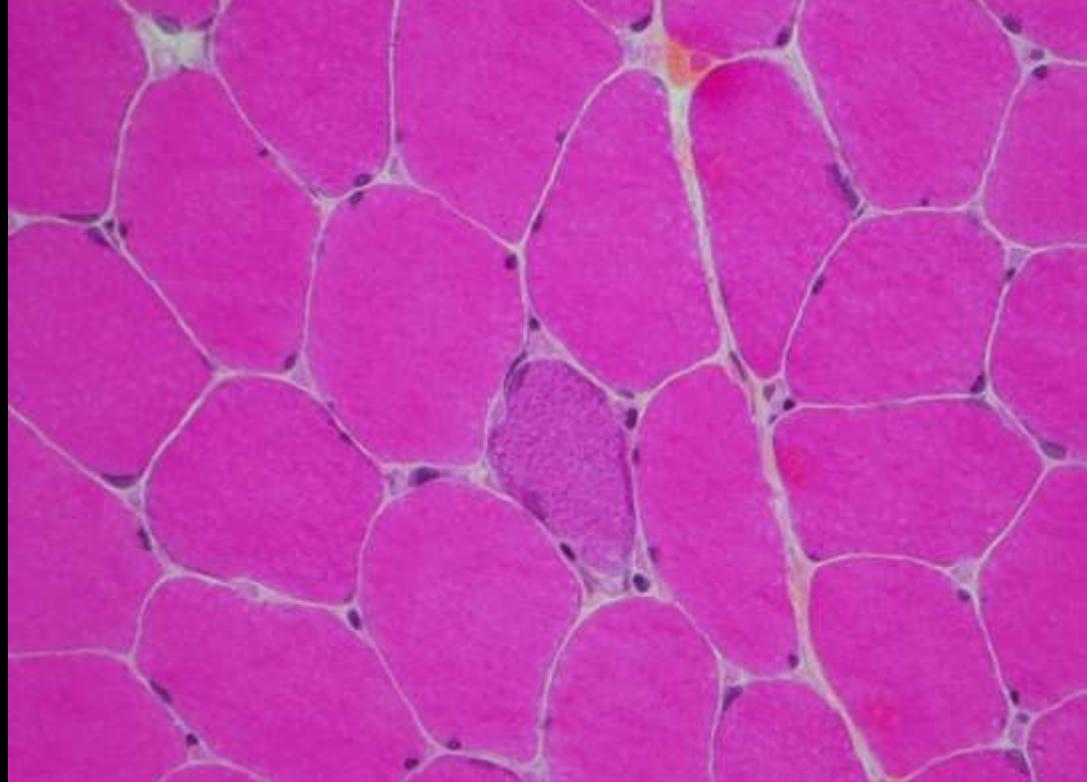
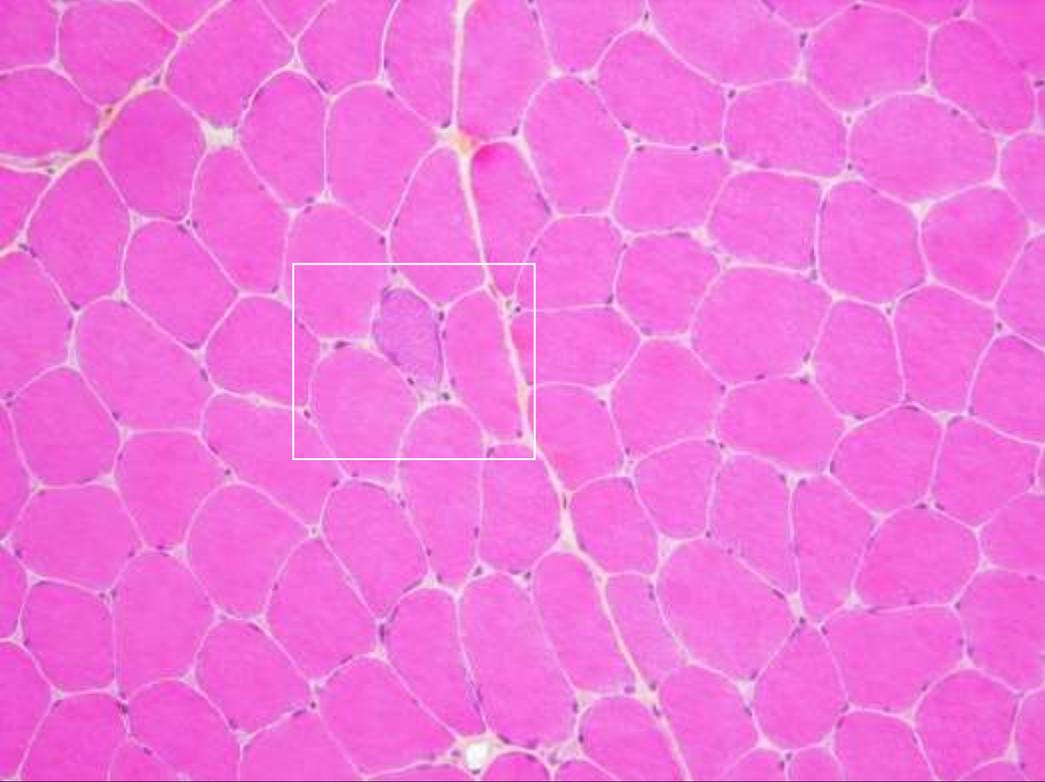
SITE DE PRELEVEMENT



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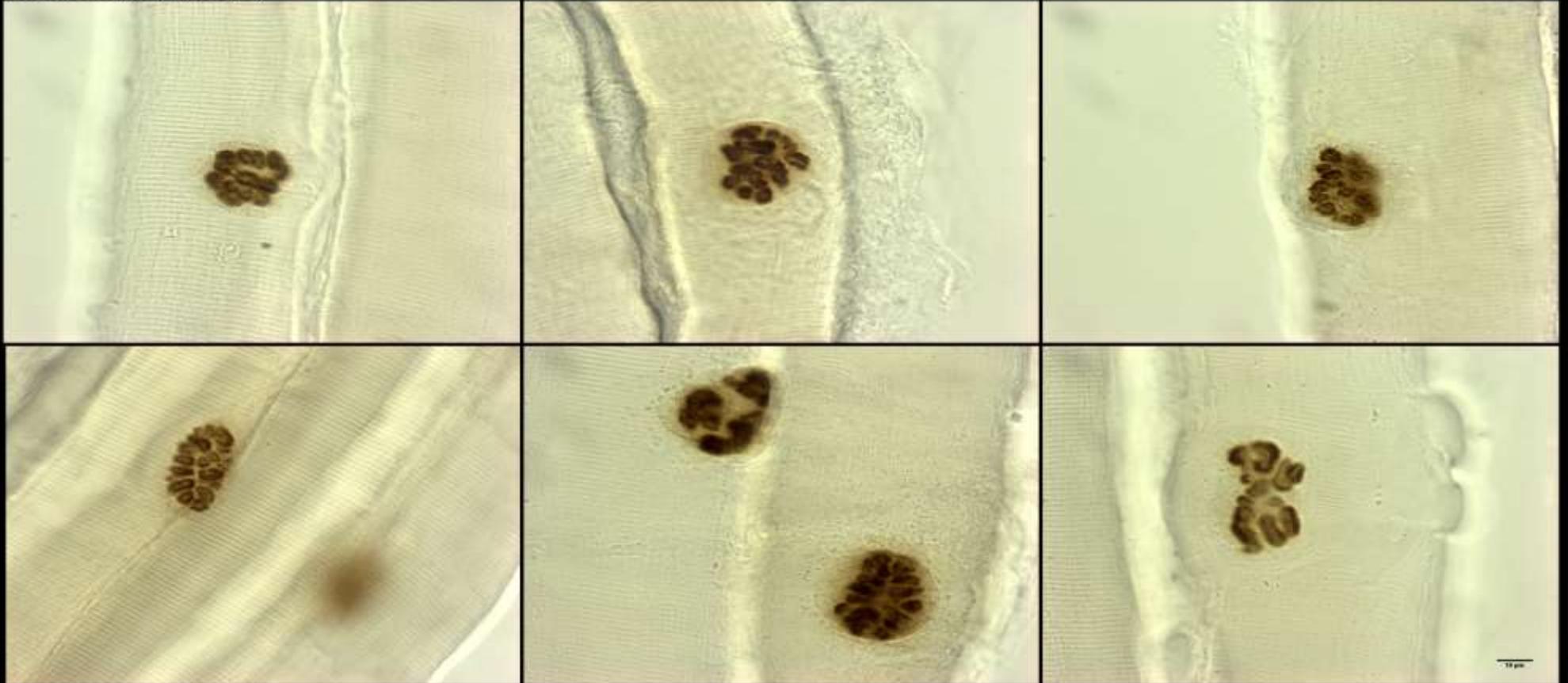
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TISSU FRAIS

AchE activity (Koelle)

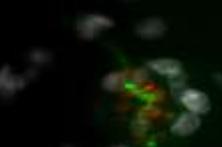
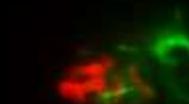
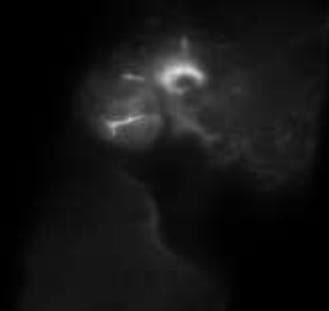
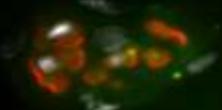
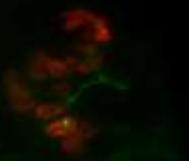
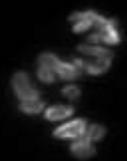


Gut JM Lyon 051110

AchR

NF (160 KDa)

FIXATION PFA



PATHOLOGIES

DENERVATION

ANOMALIE DE LA JONCTION NEURO MUSCULAIRE

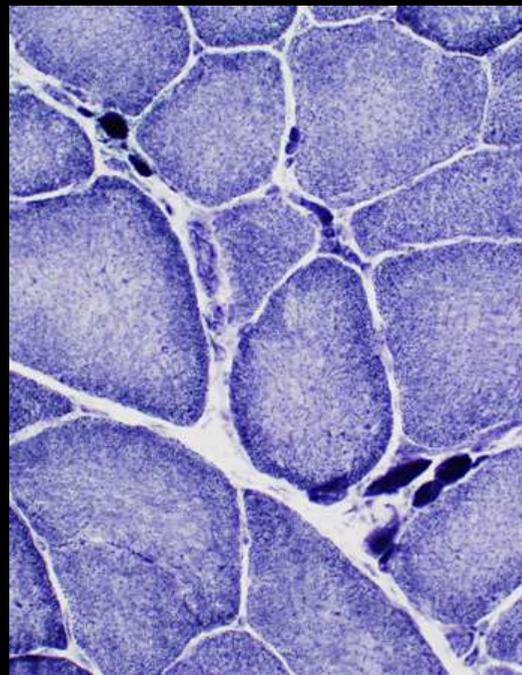
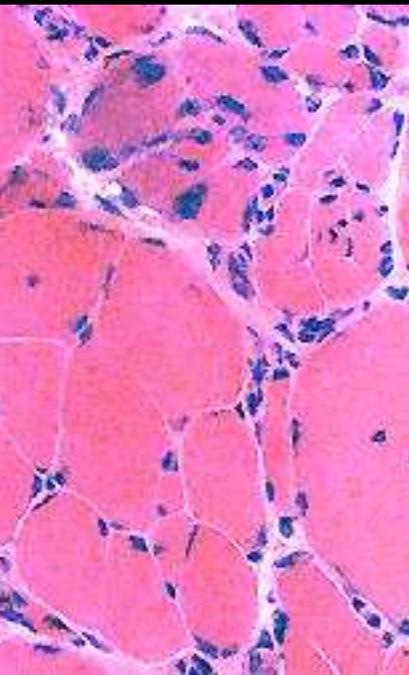
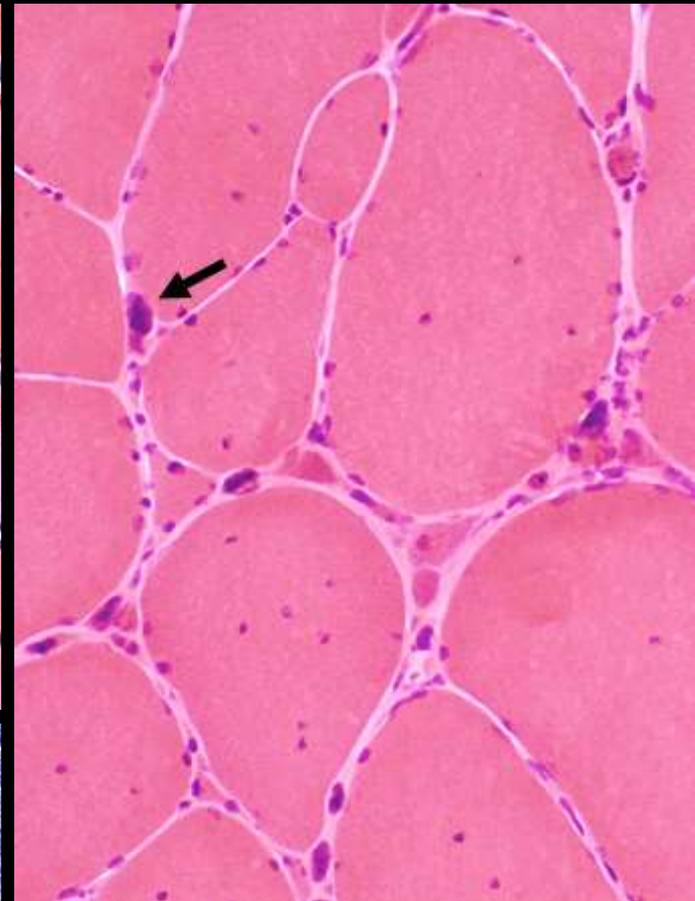
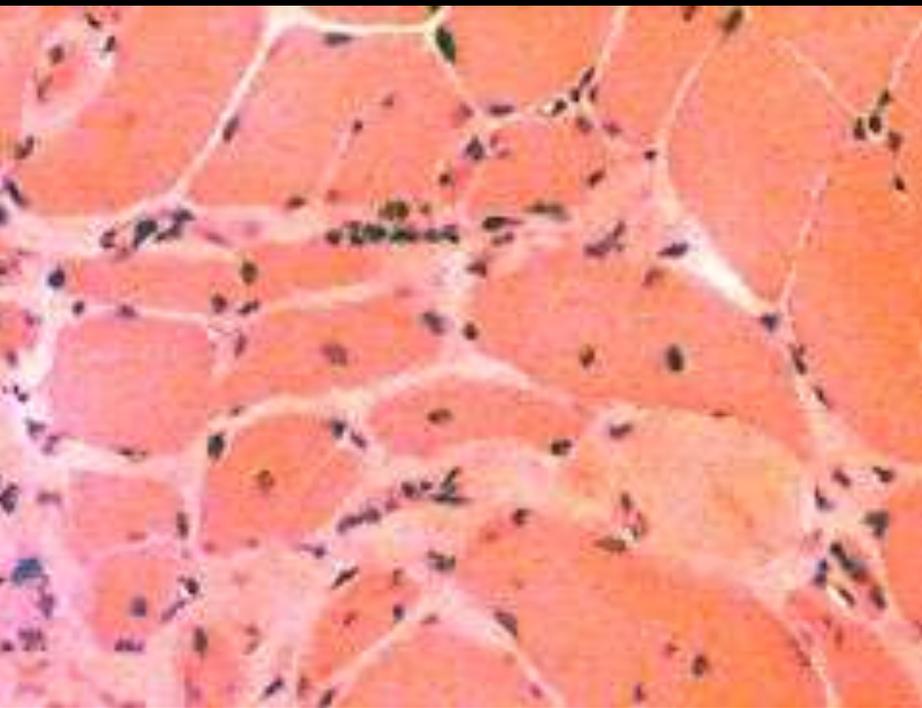
MYOSITE

DYSTROPHIE MUSCULAIRE

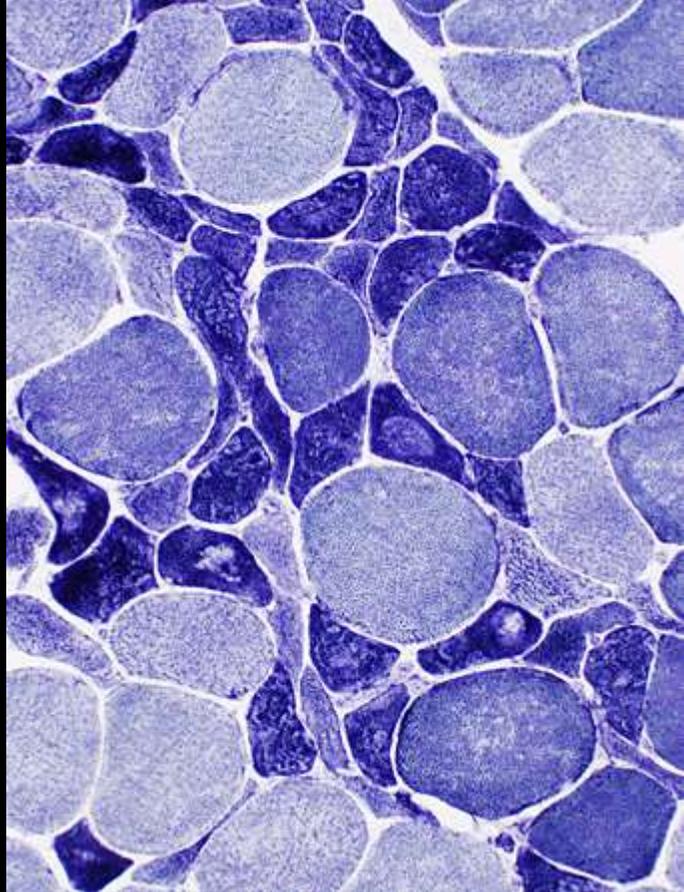
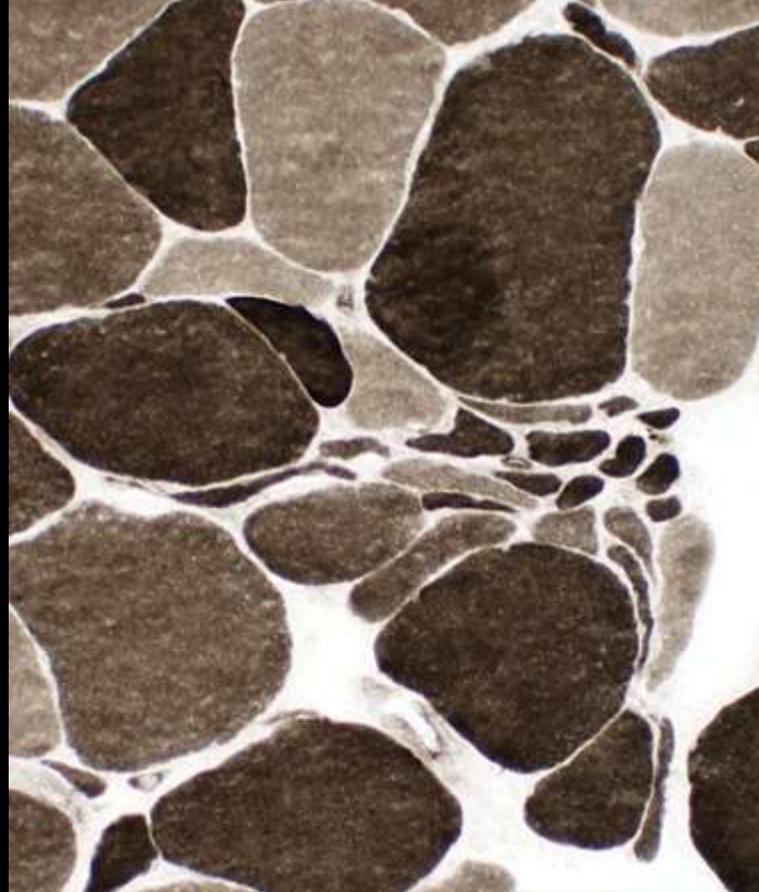
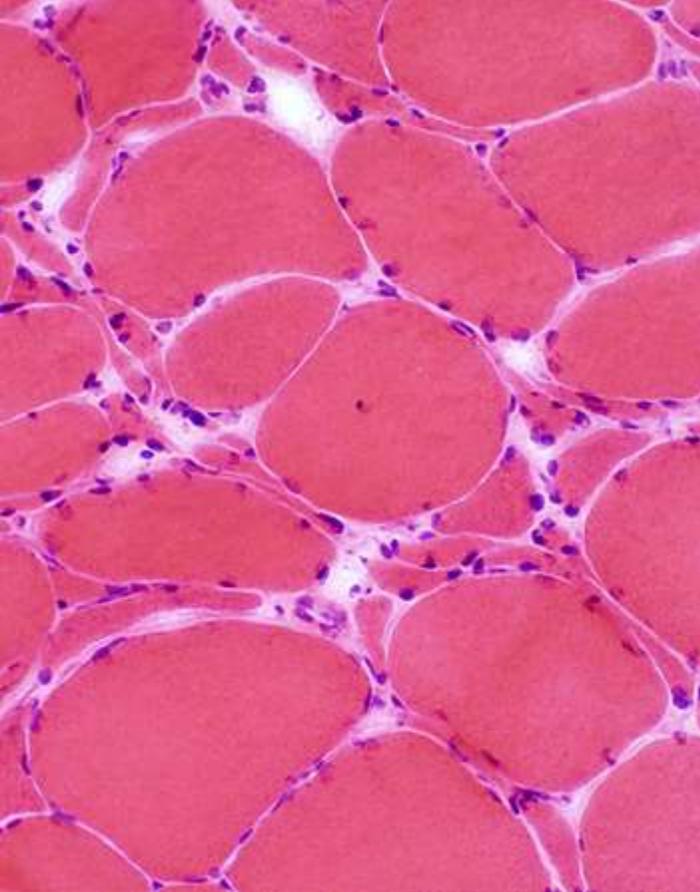
MYOPATHIE

DENERVATION

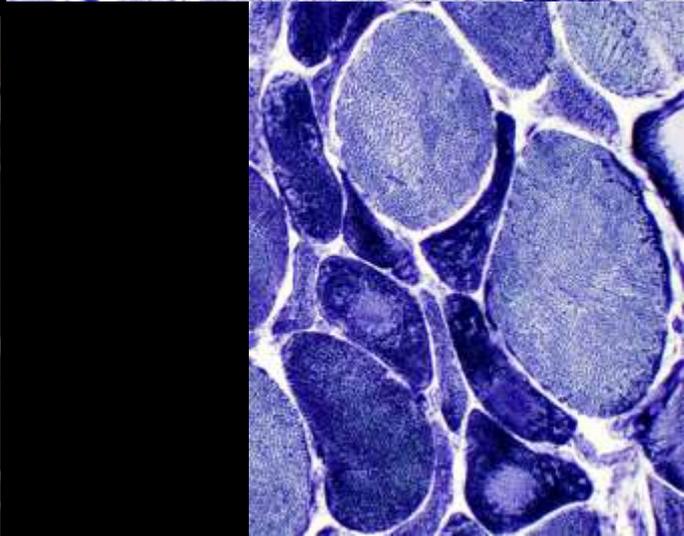
- **ATROPHY**
 - LARGE GROUP
 - SMALL GROUP
- **RARE OR NO CENTRAL NUCLEI**
- **TARGET FIBERS**

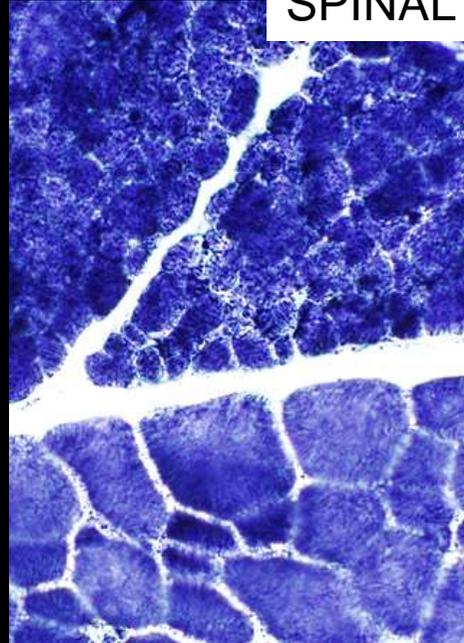
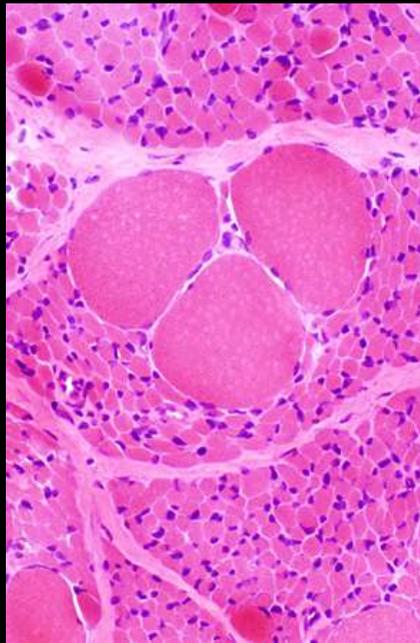
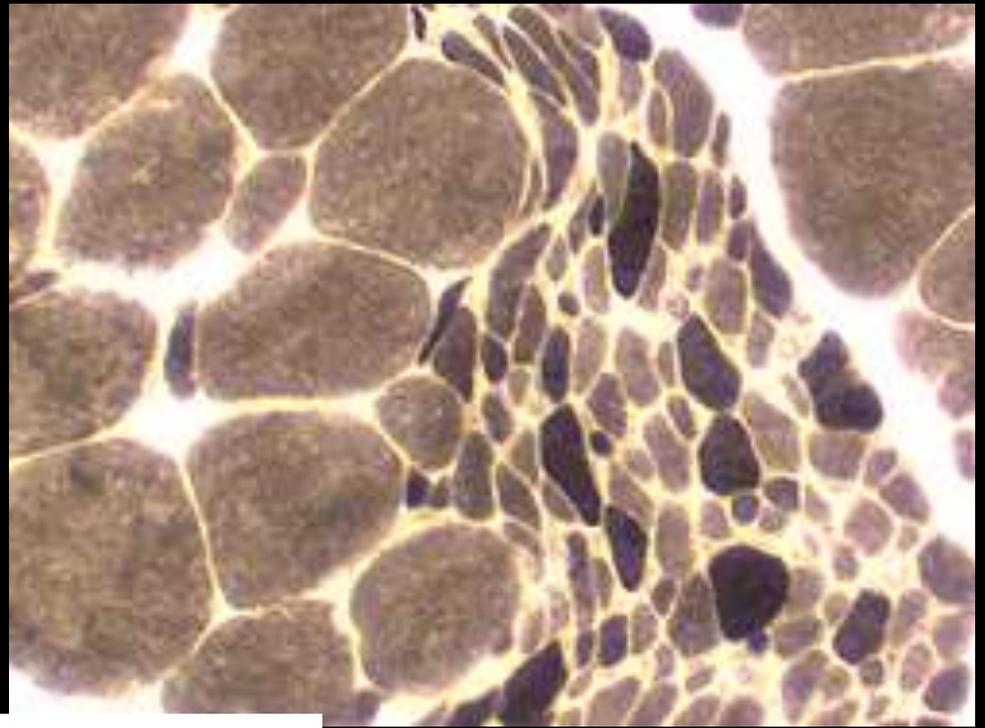
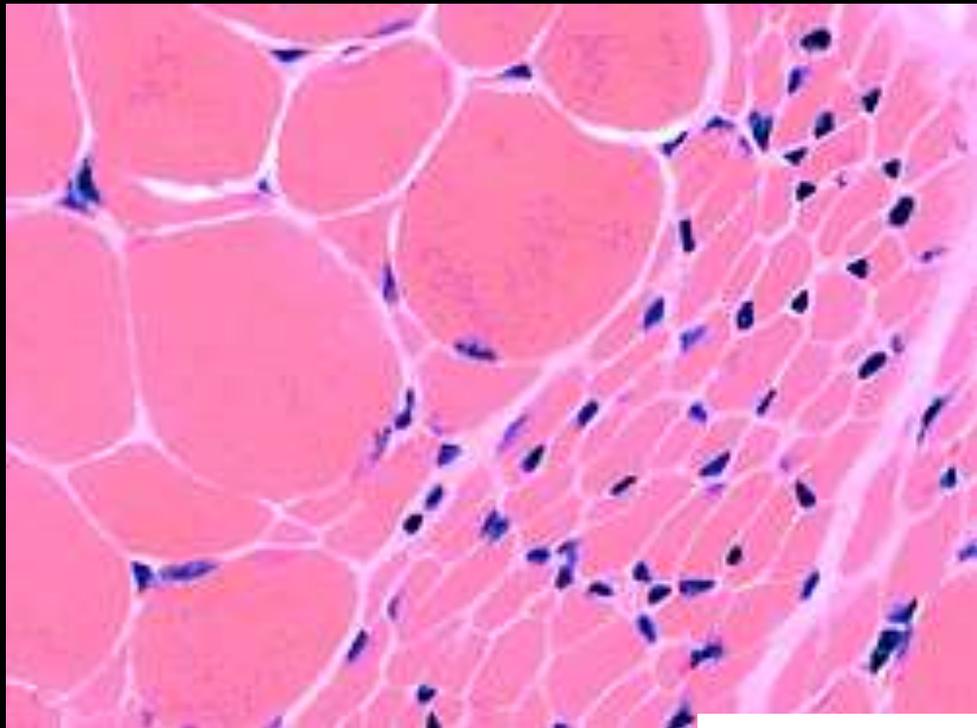


CHRONIC
PARTIAL
DENERVATION

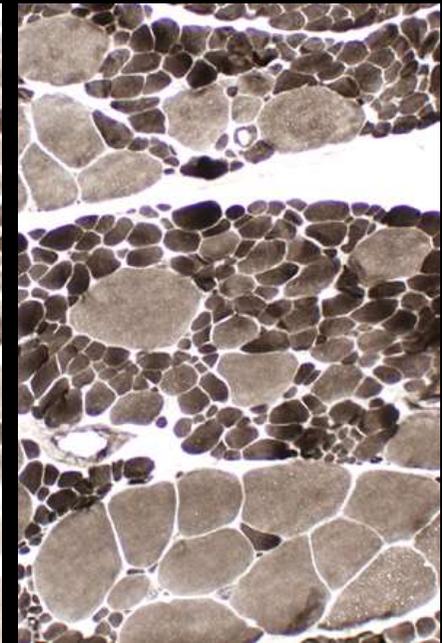
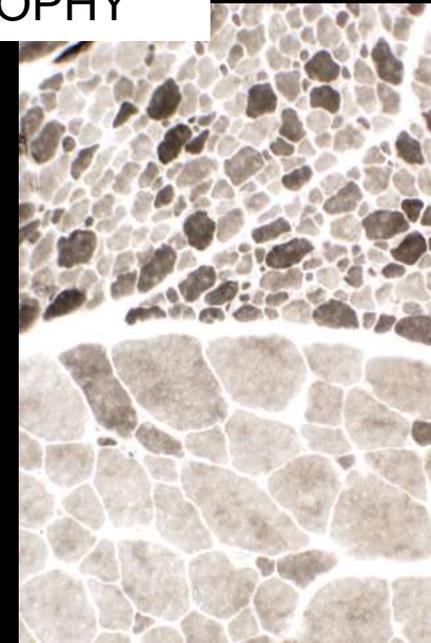


AMYOTROPHIC
LATERAL
SCLEROSIS





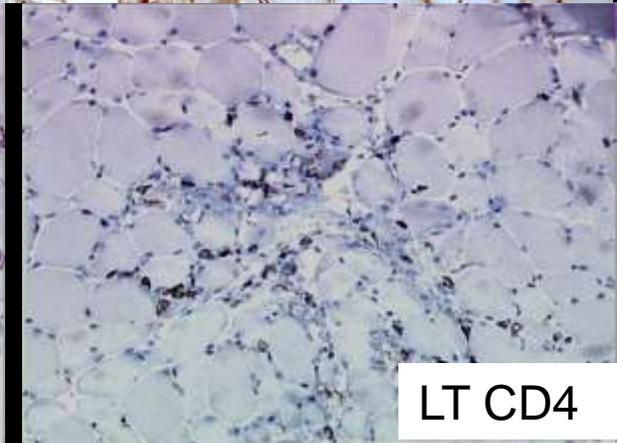
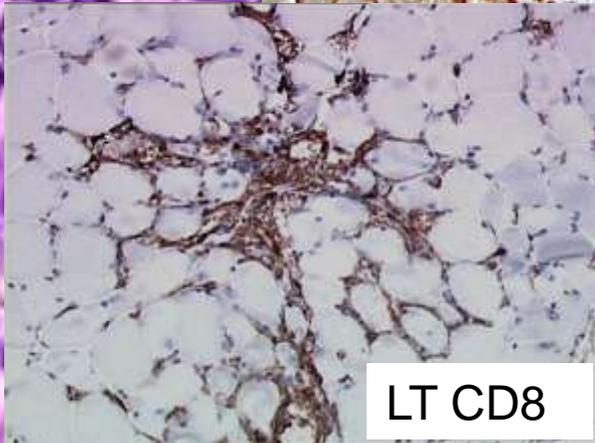
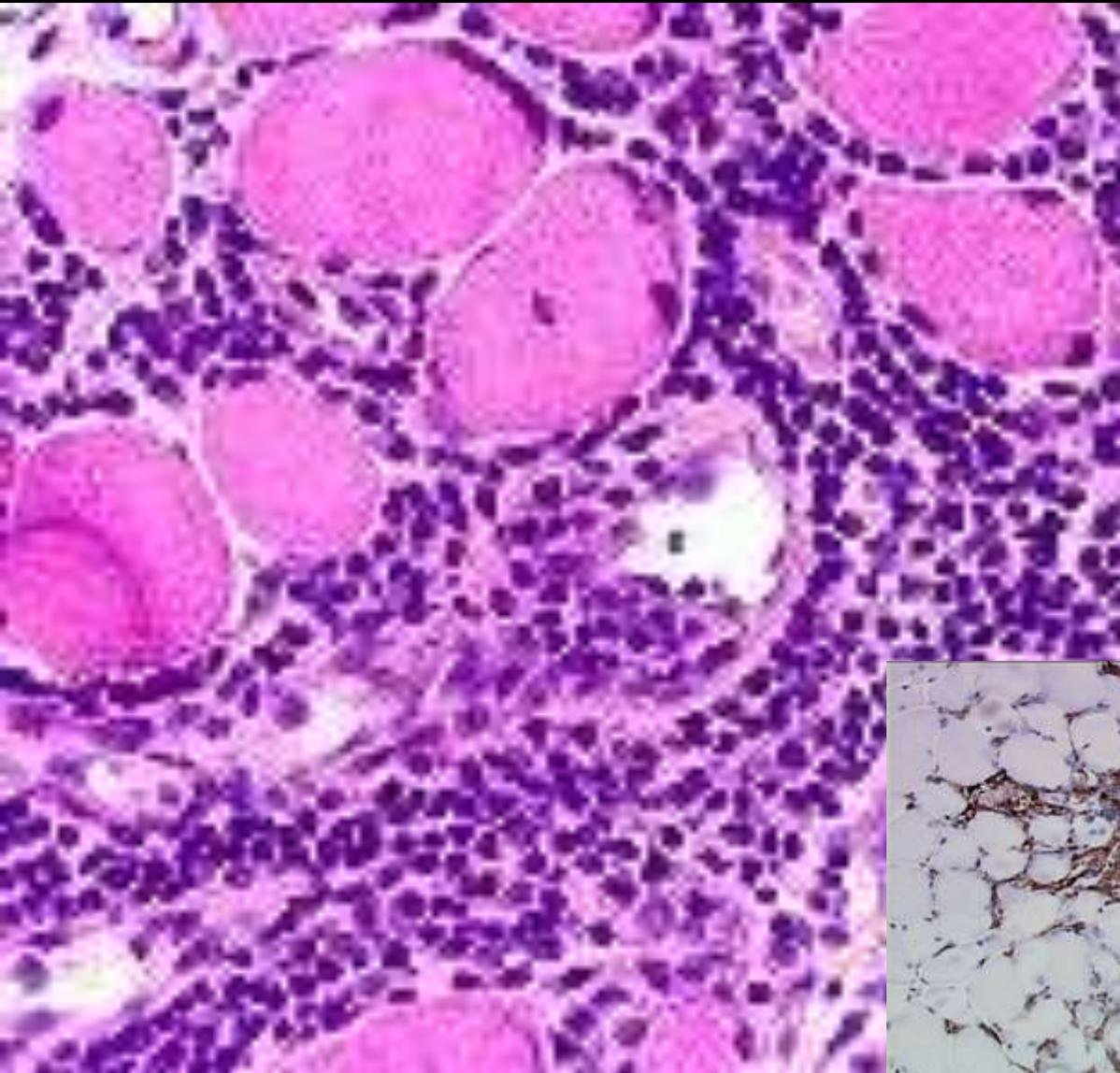
SPINAL AMYOTROPHY



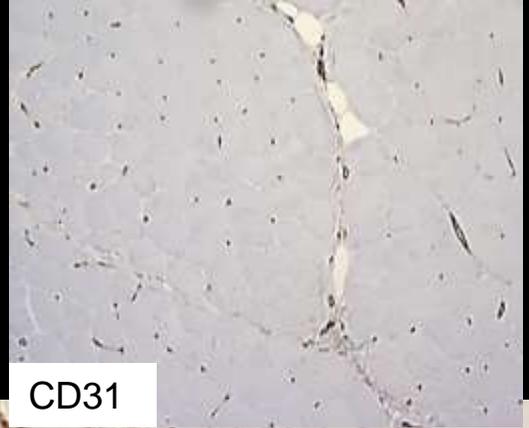
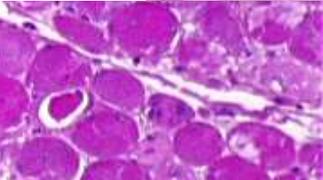
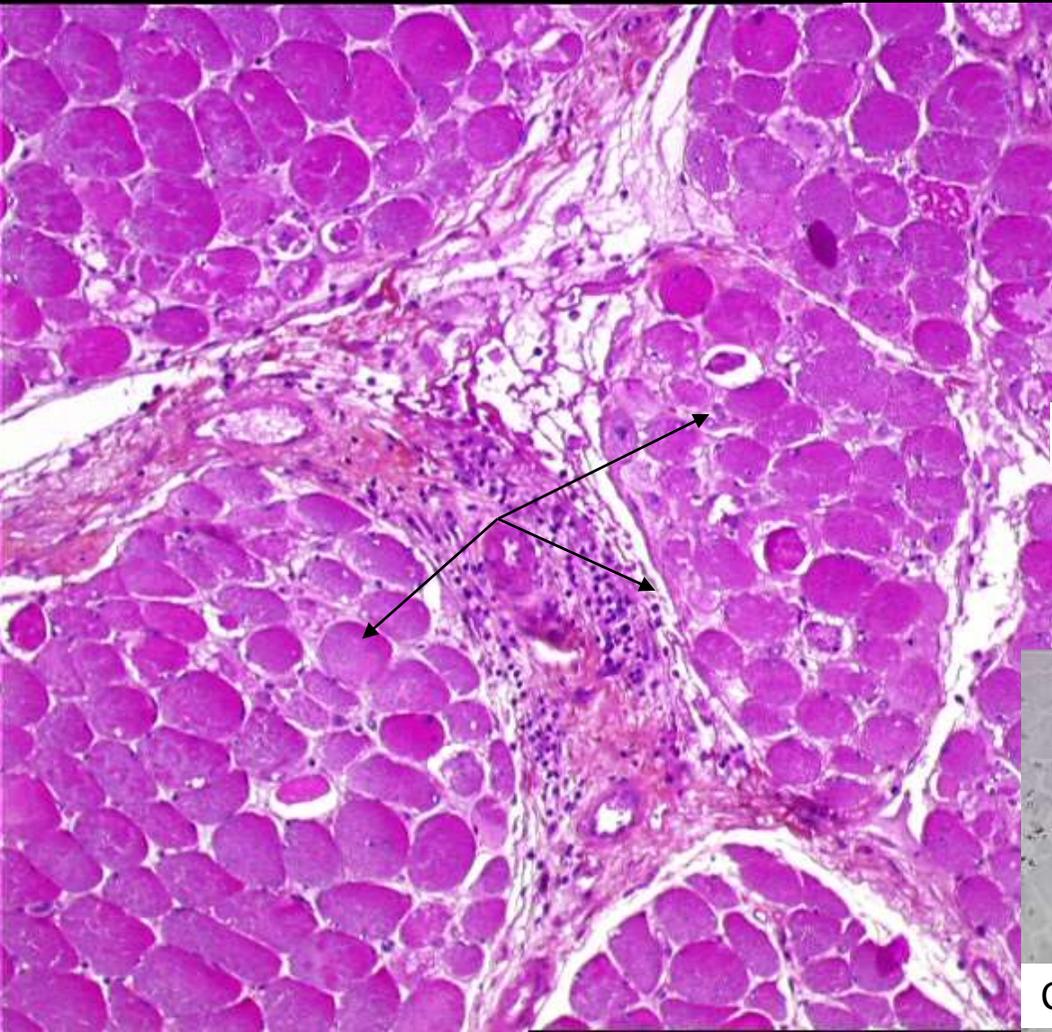
PATHOLOGIES MUSCULAIRES

- **Myopathies inflammatoire :**
 - Polymyosites
 - Dermatomyosites
 - Myosite à inclusions
 - Myosite nécrosante
- **Dystrophies musculaires :** dégénérescence de fibres matures
 - dystrophie musculaire congénitale
 - Dystrophinopathie, dystrophie musculaire des ceintures
 - dystrophie fascio-scapulo-humérale
 - dystrophie oculopharyngée
- **Myopathies congénitales :** anomalies du développement : myopathies structurales
- **Myopathies métabolique :** dysfonction énergétique de la cellule
 - Glycogénoses
 - myopathies mitochondriales et autres anomalies du métabolisme des lipides

POLYMYOSITE

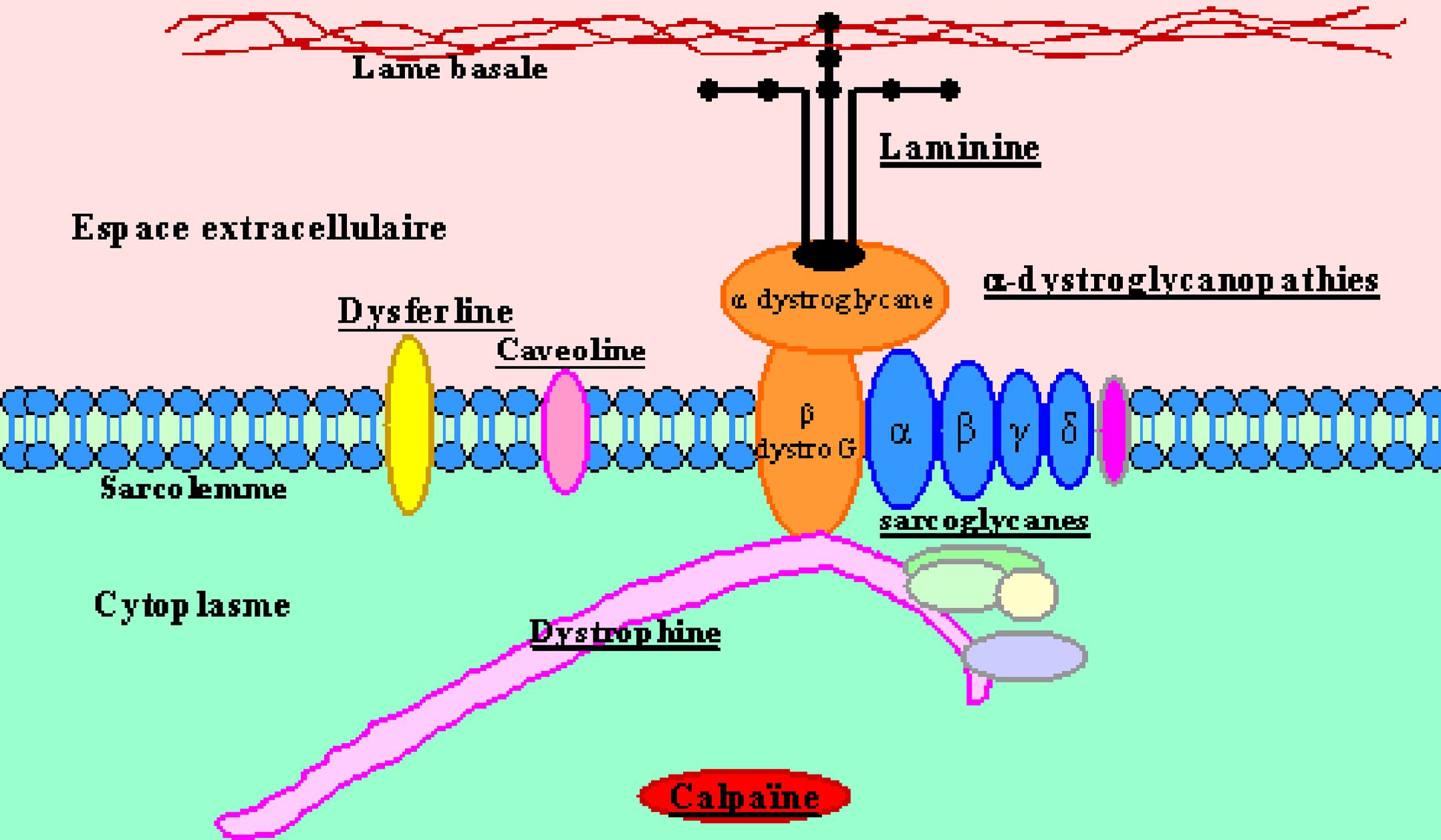


DERMATOMYOSITE



PATHOLOGIES MUSCULAIRES

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Lame basale

Laminine

Espace extracellulaire

α -dystroglycanopathies

Dysferline

Caveoline

α dystroglycane

β dystro G

α

β

γ

δ

sarcoglycans

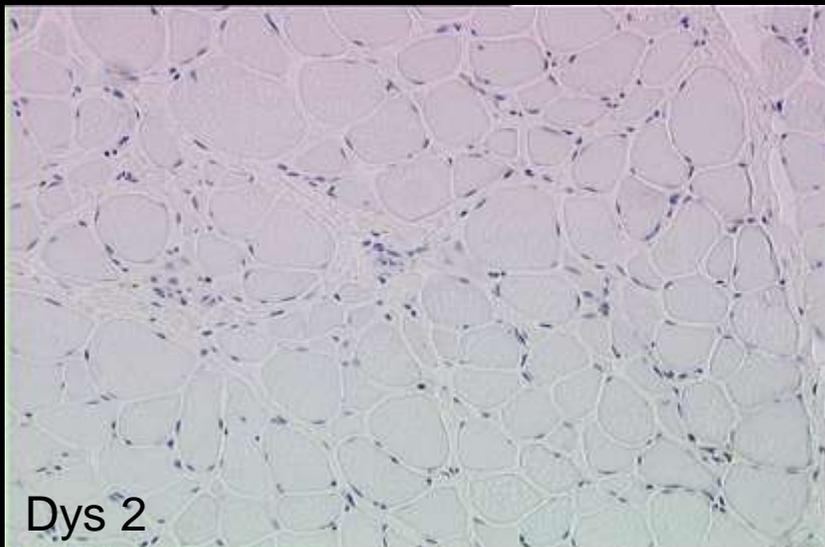
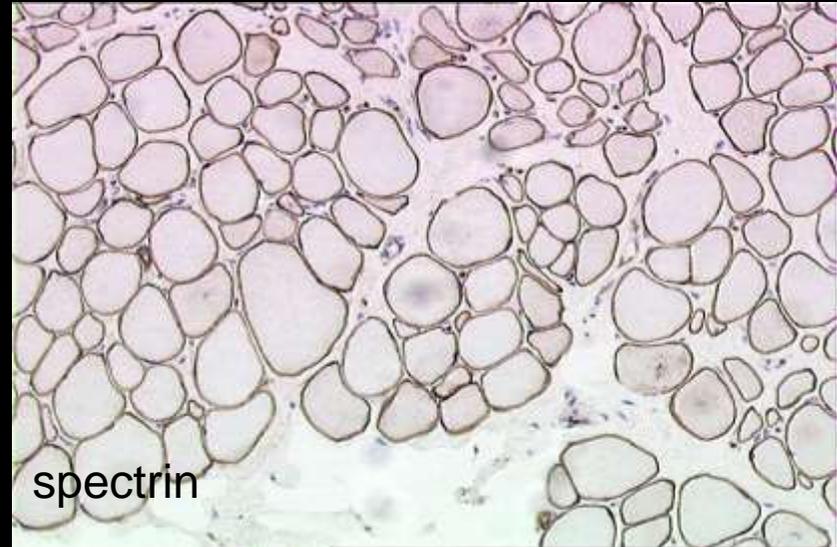
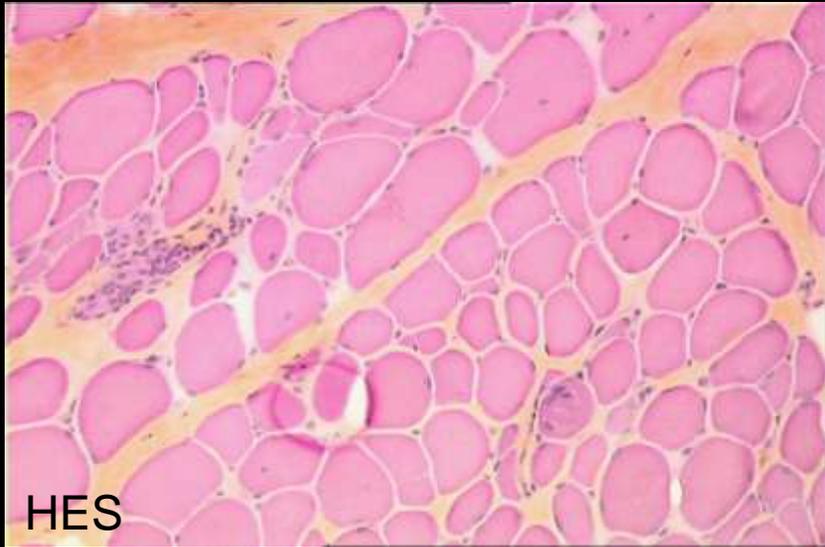
Sarcolemme

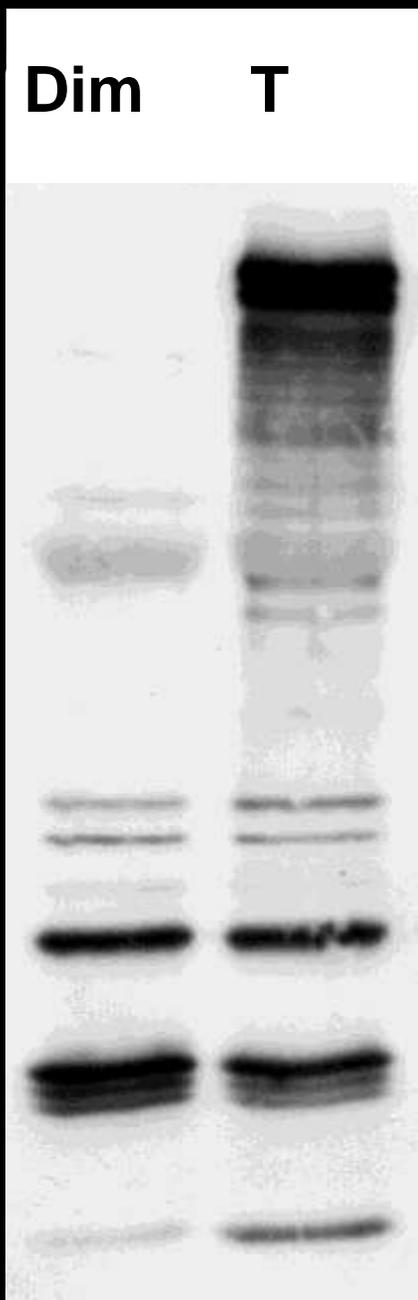
Cytoplasme

Dystrophine

Capaine

EXEMPLE DE DYSTROPHIE MUSCULAIRE 1





Dim

T

DYS 1

Dysferline

Calpaïne 94

Calpaïne 60

α -sarcoglycane



Dim

T

DYS 2

Calpaïne 94

γ -sarcoglycane

Calpaïne 30

MOLECULAR BIOLOGY

- DELETION of the exon 52 of the dystrophin gene
- Shift of the amino acid reading frame

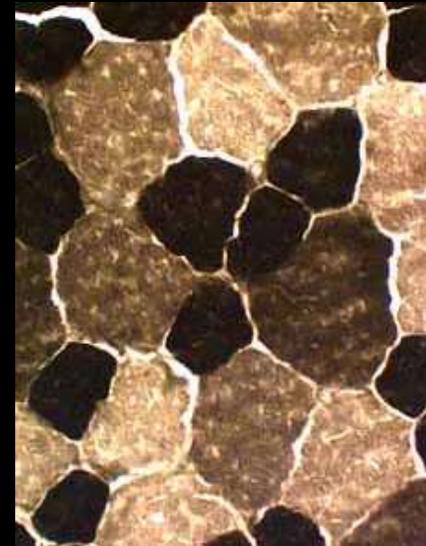
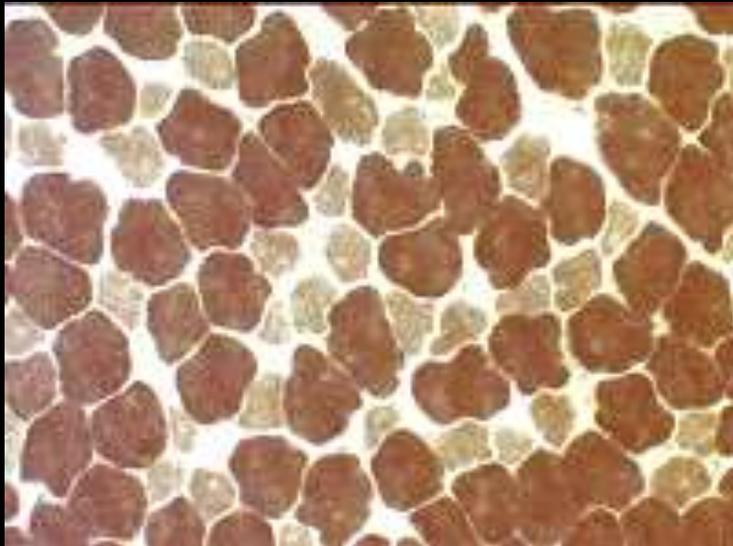
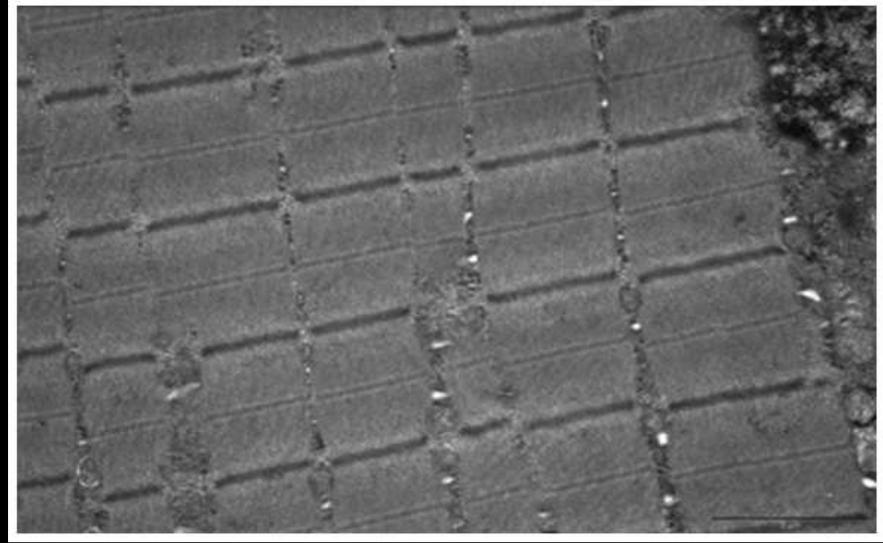
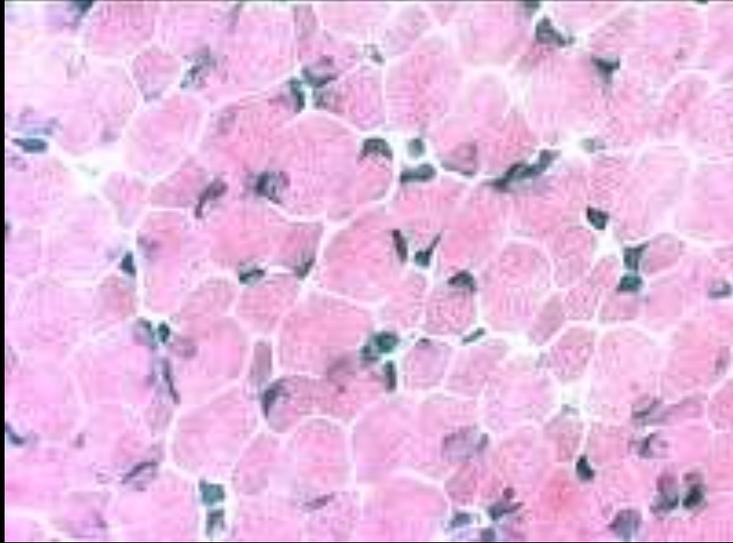


Duchenne muscular Dystrophy

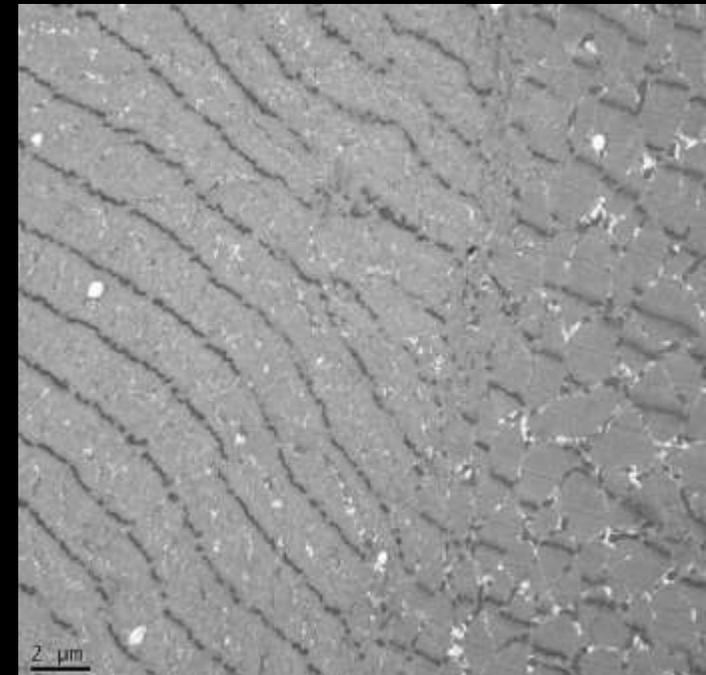
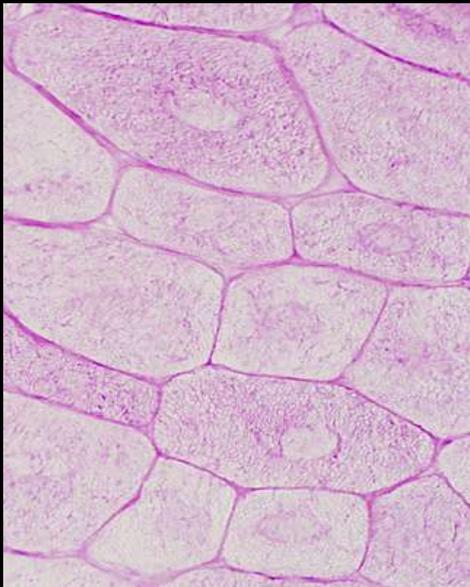
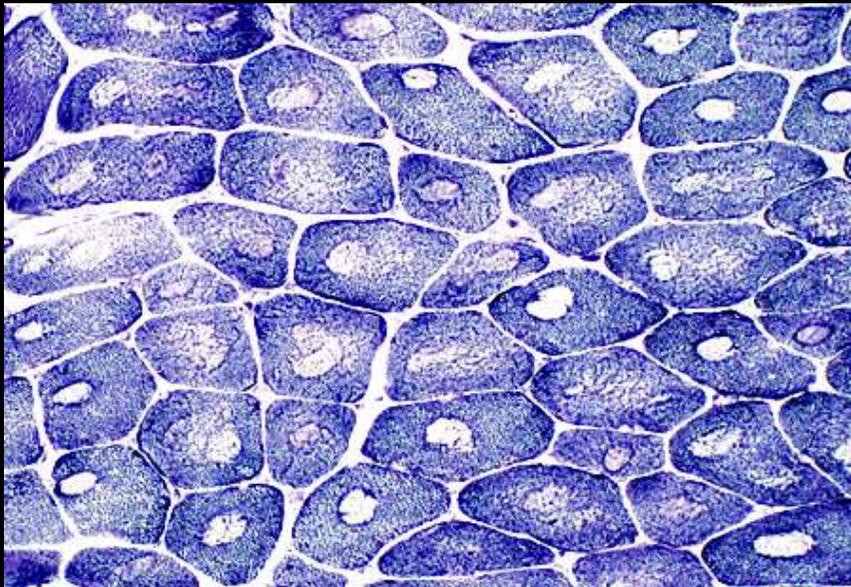
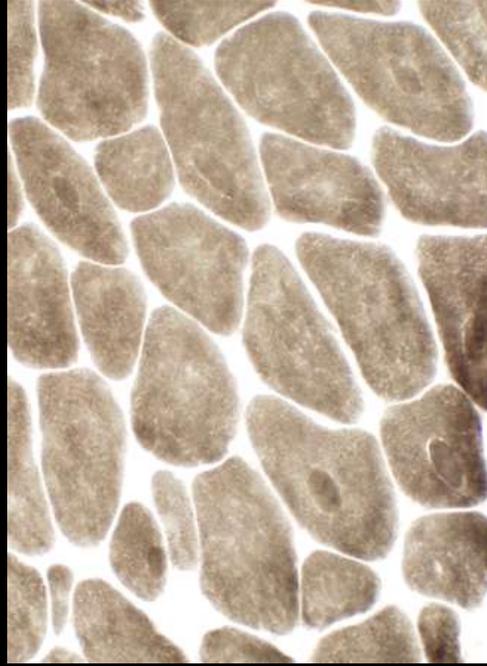
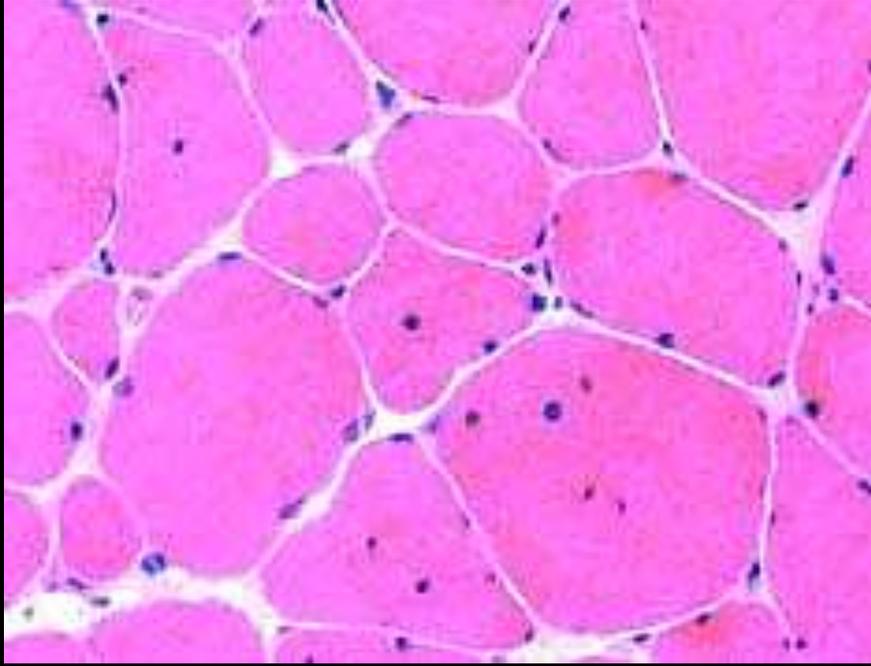
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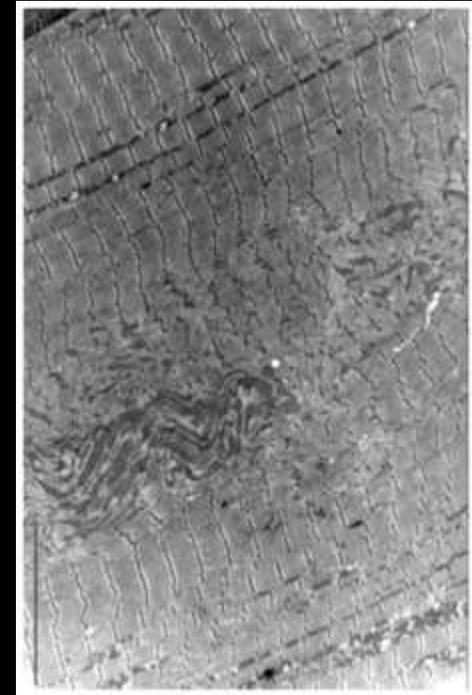
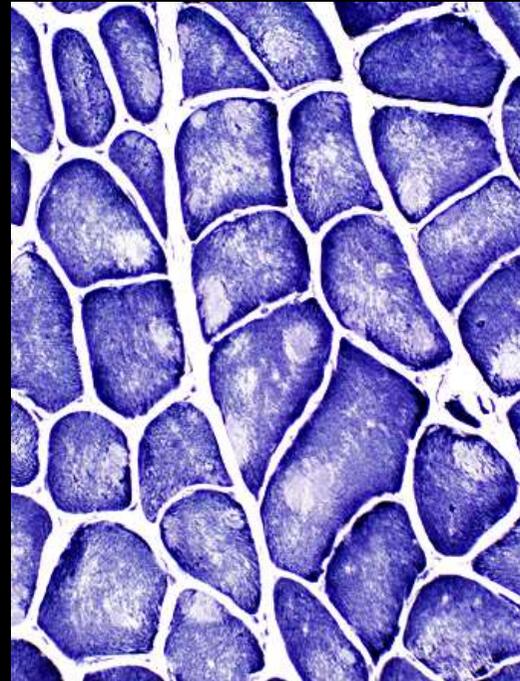
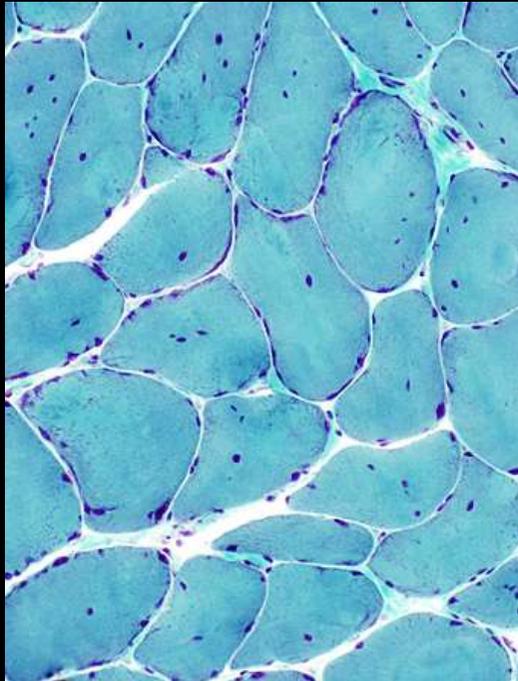
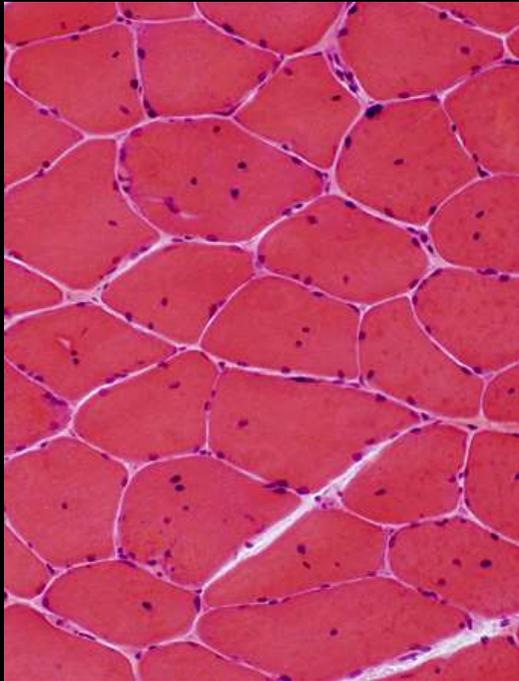
CONGENITAL FIBER TYPE SIZE DISPROPORTION



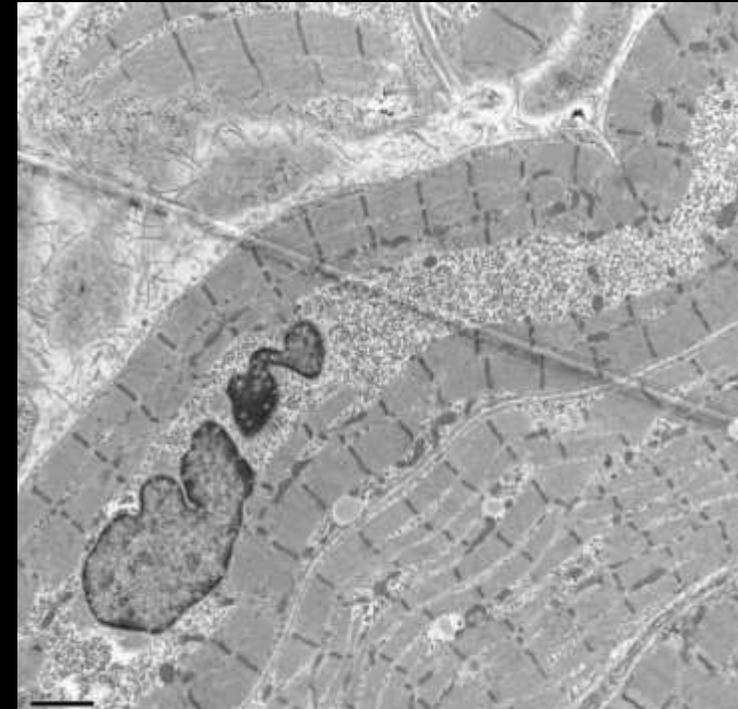
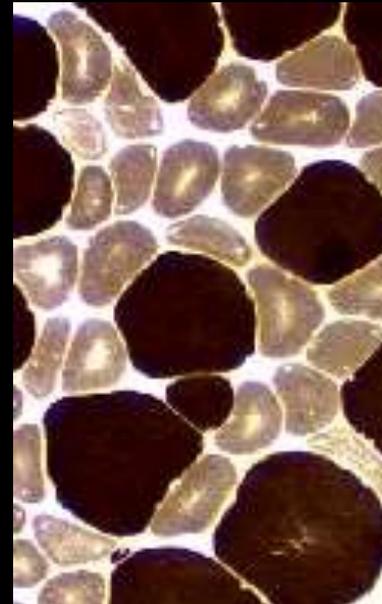
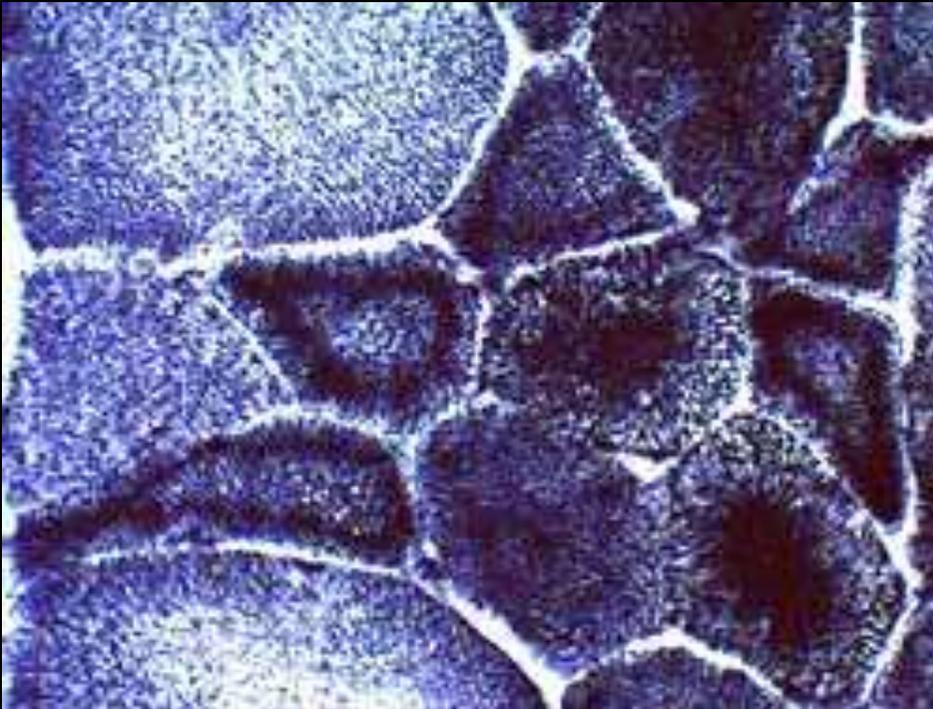
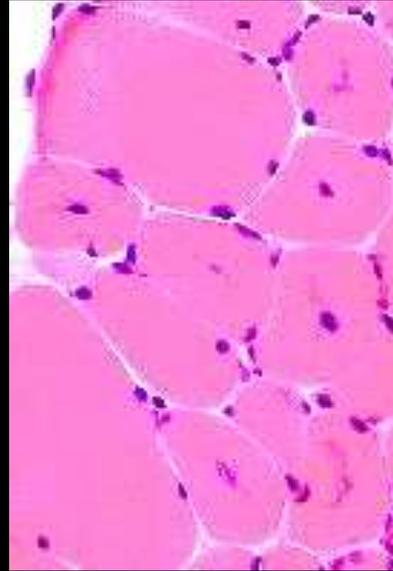
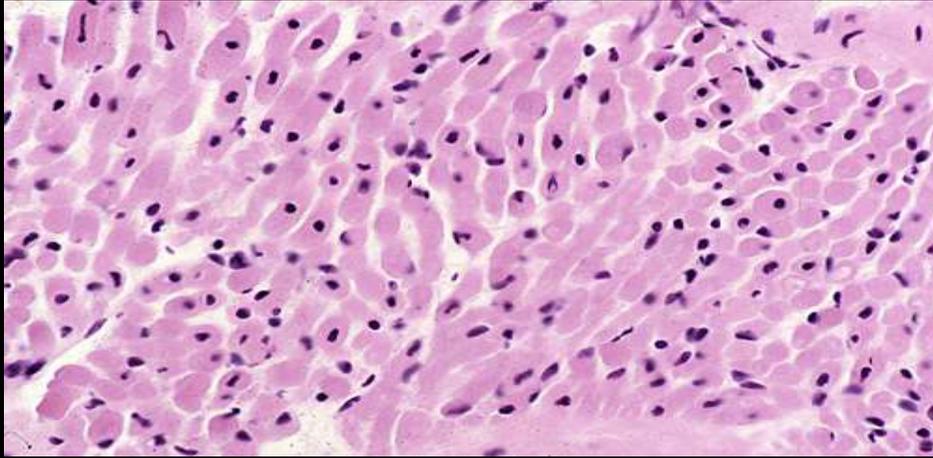
CENTRAL CORE MYOPATHY



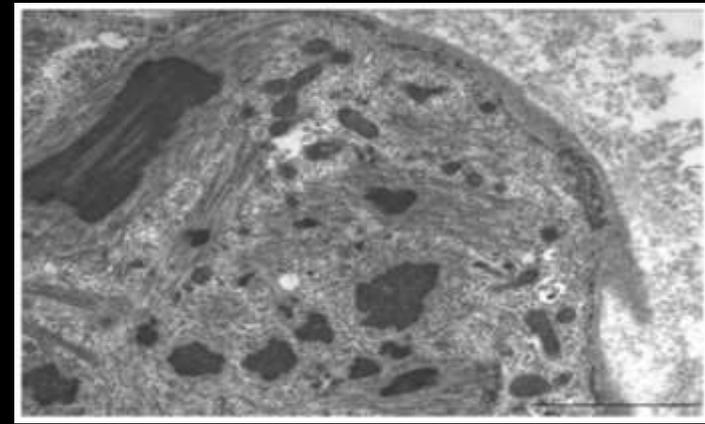
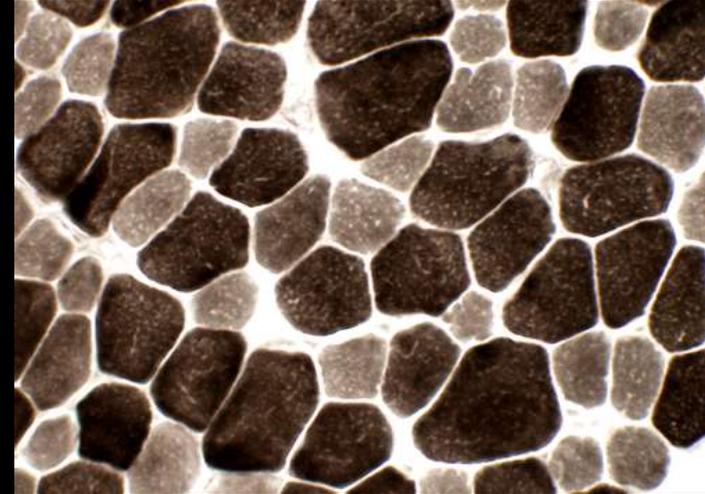
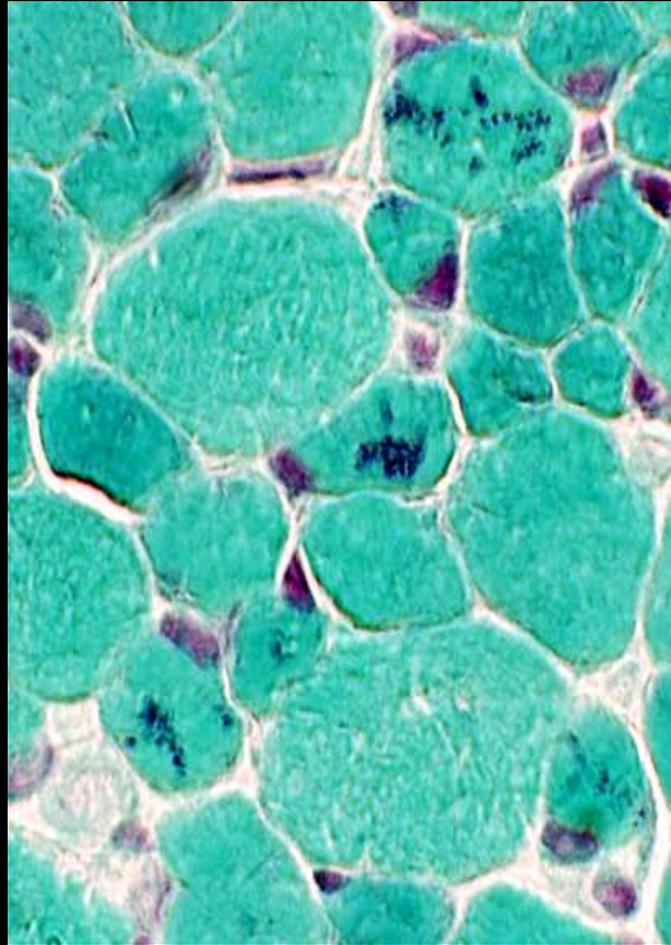
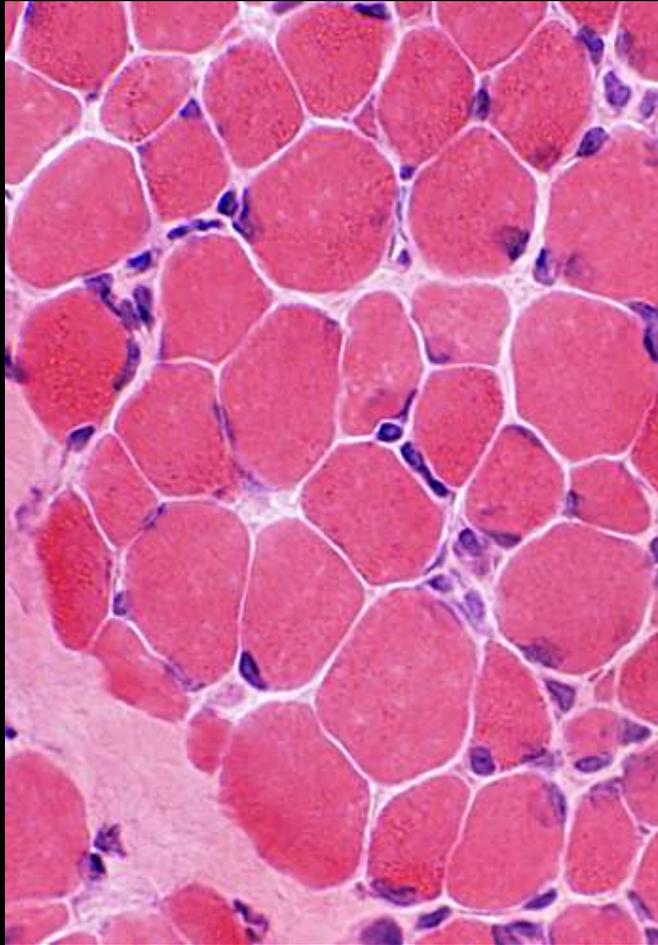
MULTICORE MYOPATHY



CENTRONUCLEAR MYOPATHY



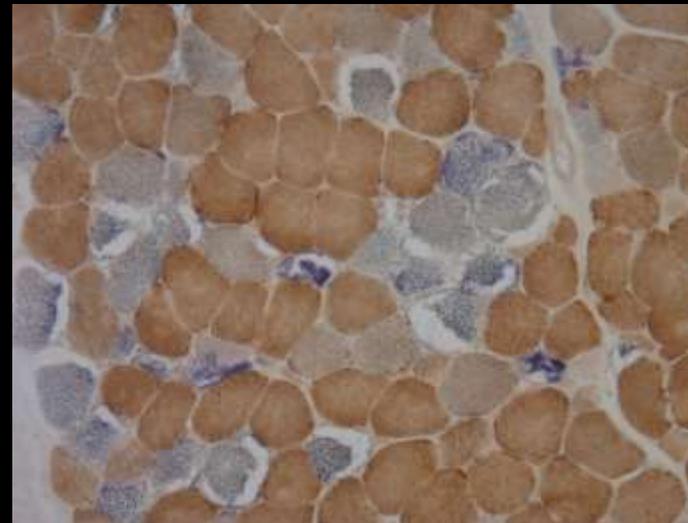
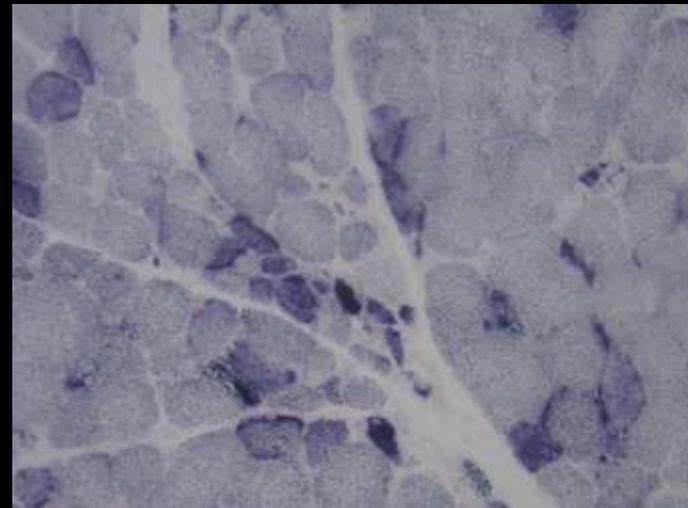
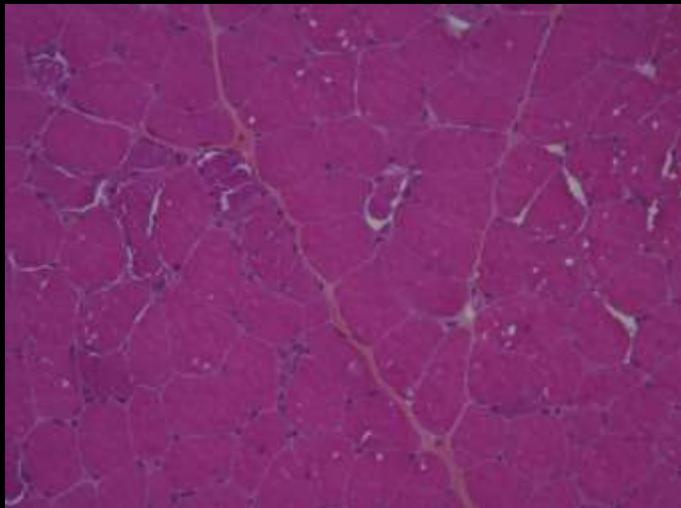
NEMALINE MYOPATHY



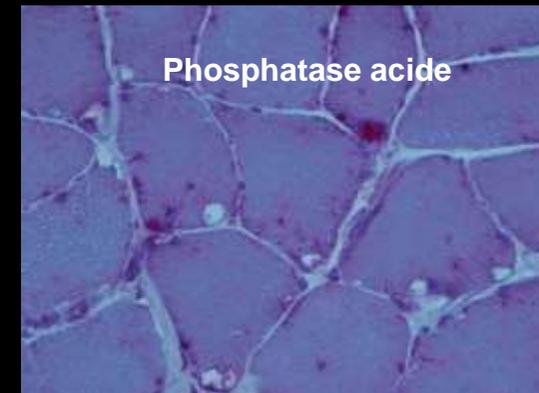
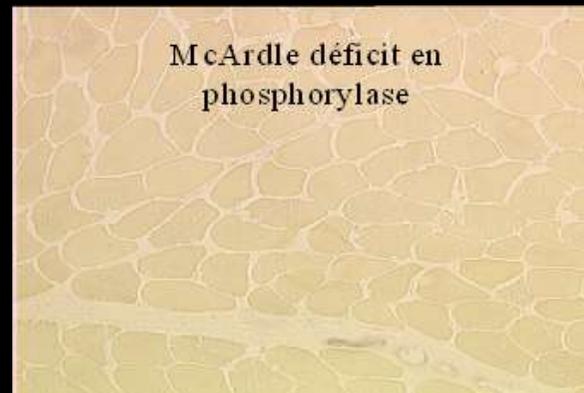
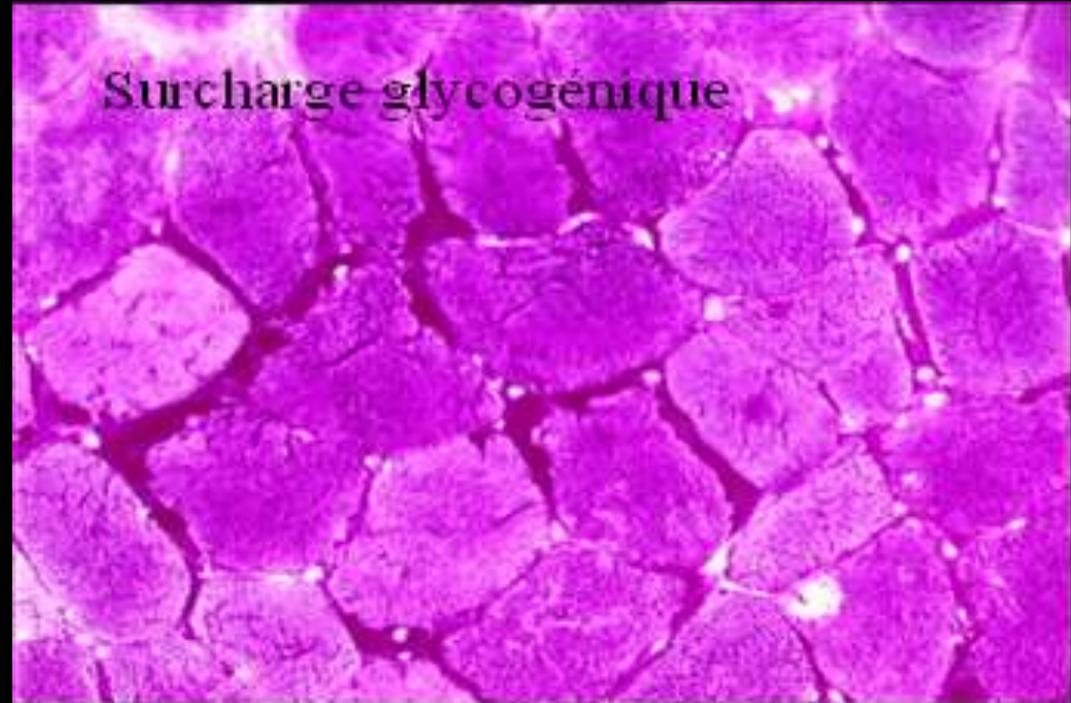
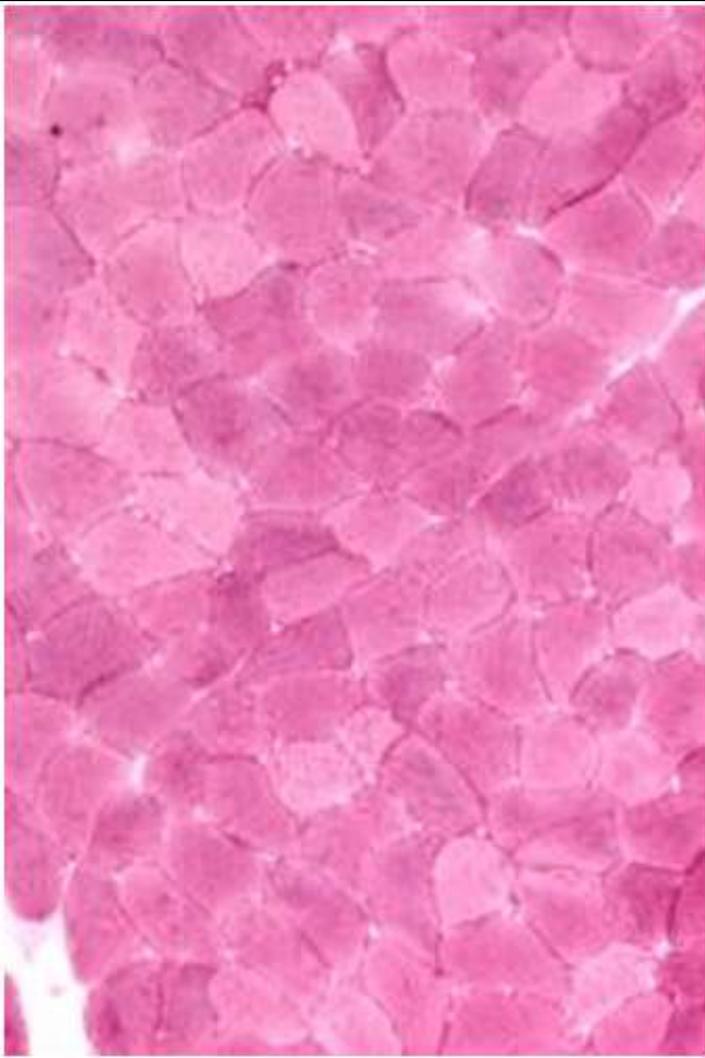
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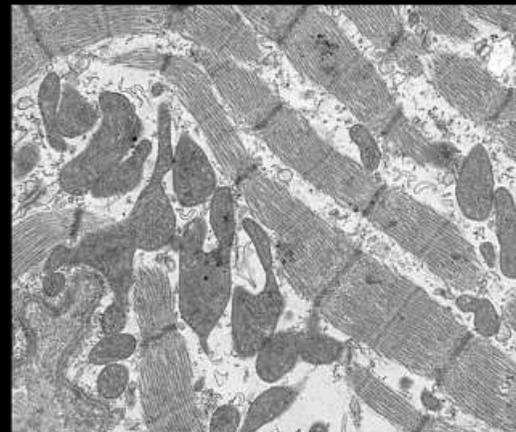
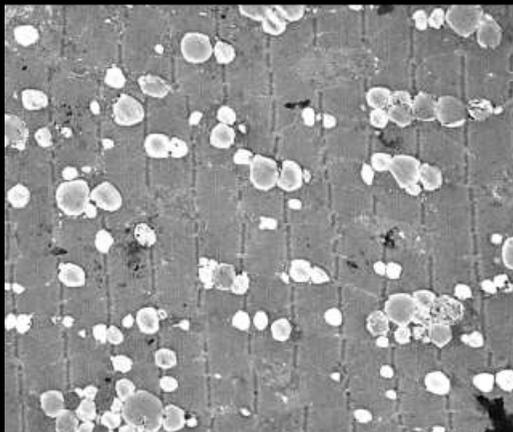
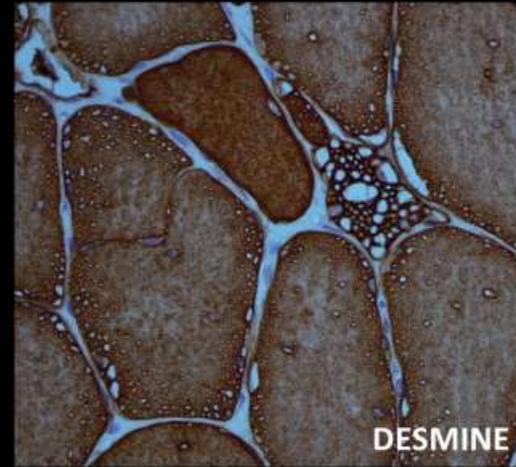
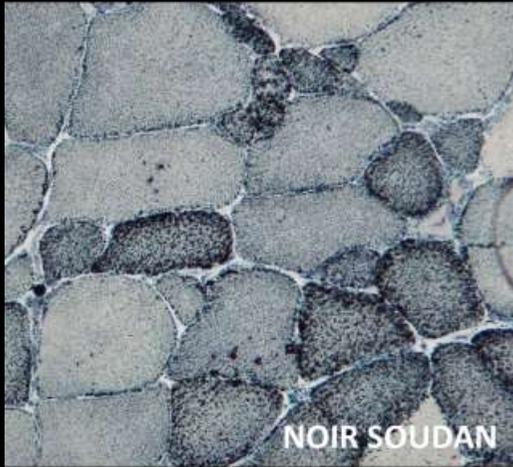
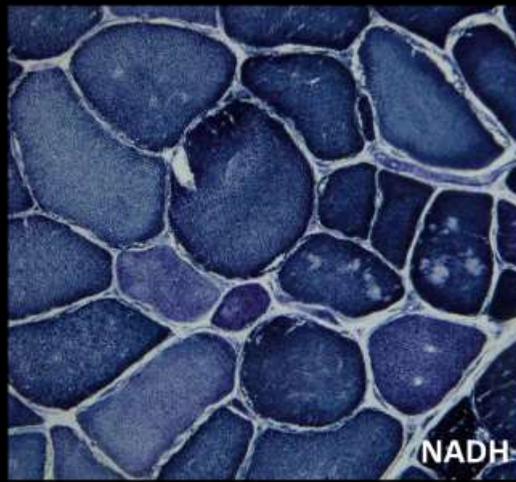
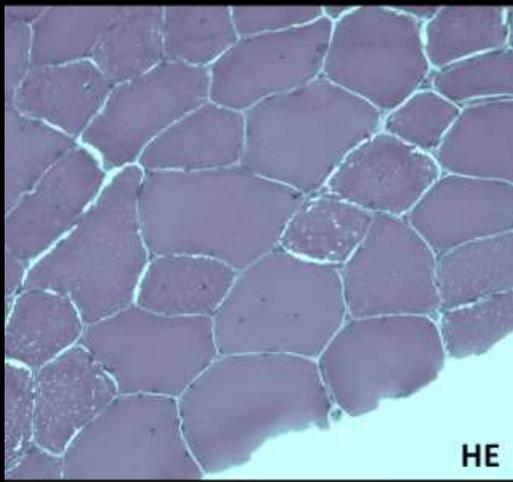
CYTOPATHIE MITOCHONDRIALE



GLYCOGENOSES



LIPIDOSES



CONCLUSION

□ Aboutissement d'une chaîne d'examens diagnostiques



□ Sélection du muscle prélevé = rentabilité

□ Geste rapide et peu douloureux

□ Réponse :

- Au médecin prescripteur de la biopsie

- Délai : 3 jours fixation, 15 jours congélation,

jusqu'à 2 mois ou plus biochimie - génétique