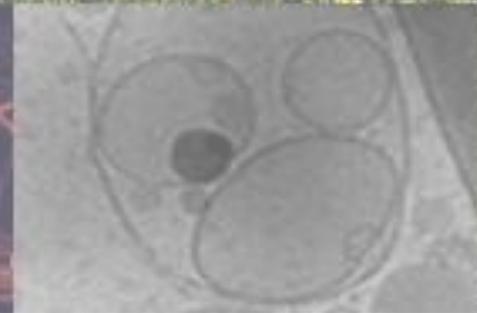
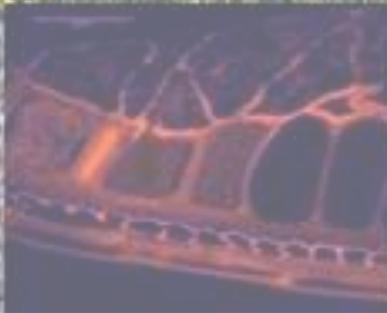
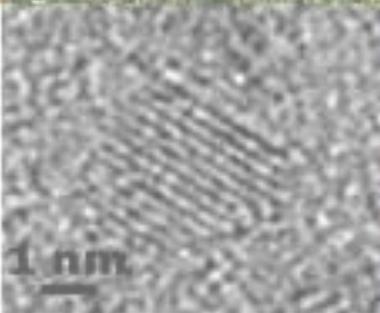
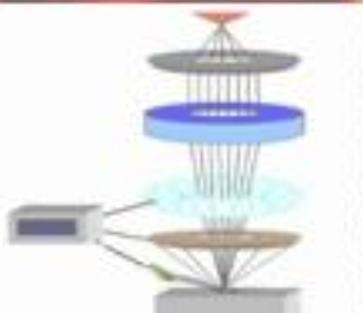
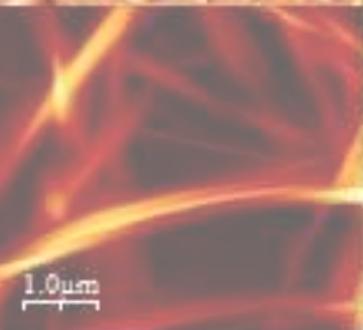


Réseau national des Microscopistes de l' INRA

MEB

**Applications dans le domaine animal :
vers une complémentarité des techniques**



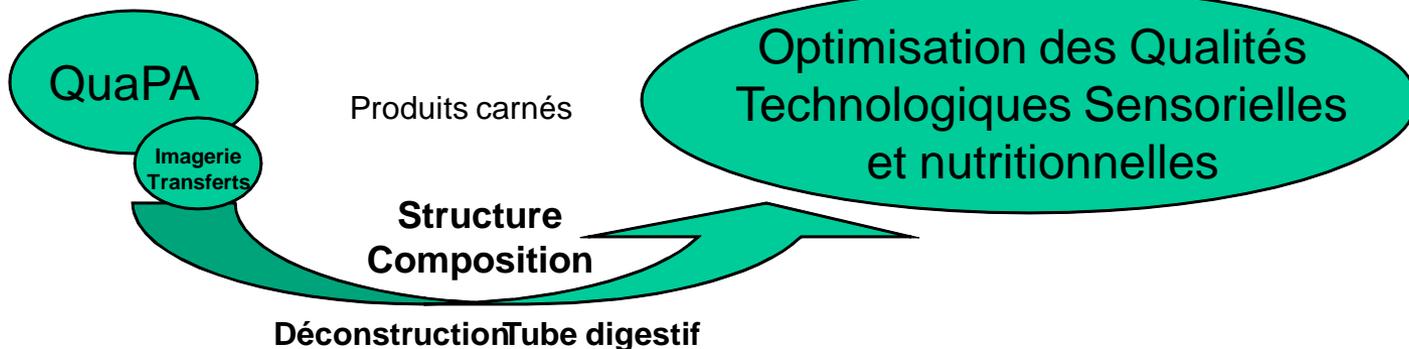
Thierry Astruc (INRA de Clermont-Ferrand-Theix)

Matière première
d'origine animale

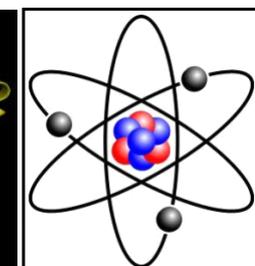
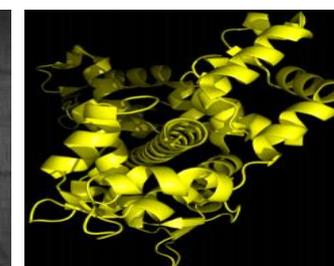
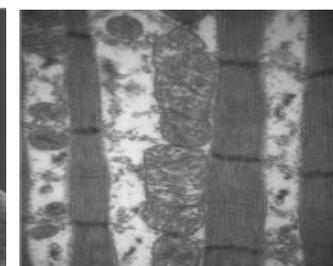
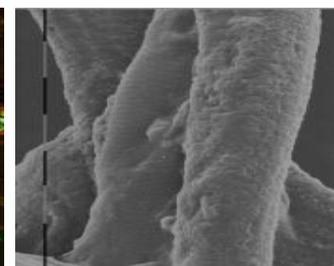
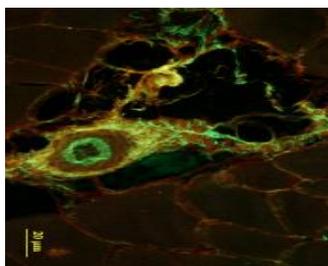
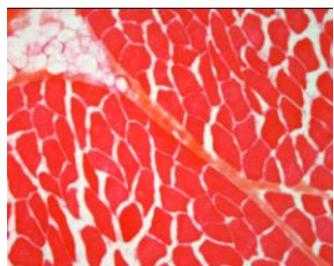
Procédés de transformation

(Réfrigération, Cuisson, Salage, Affinage ...)

Aliment



tissus → cellules → Organites → molécules → Elément



Macroscopie
Microscopie photonique
(fond clair, fluorescence, confocale...)

Microscopie électronique
(Balayage, Transmission)

Microspectroscopies
(IR, UV, Raman, TOF SIMS) Fluorescence X
Microanalyse X

Quantification par analyse d'images

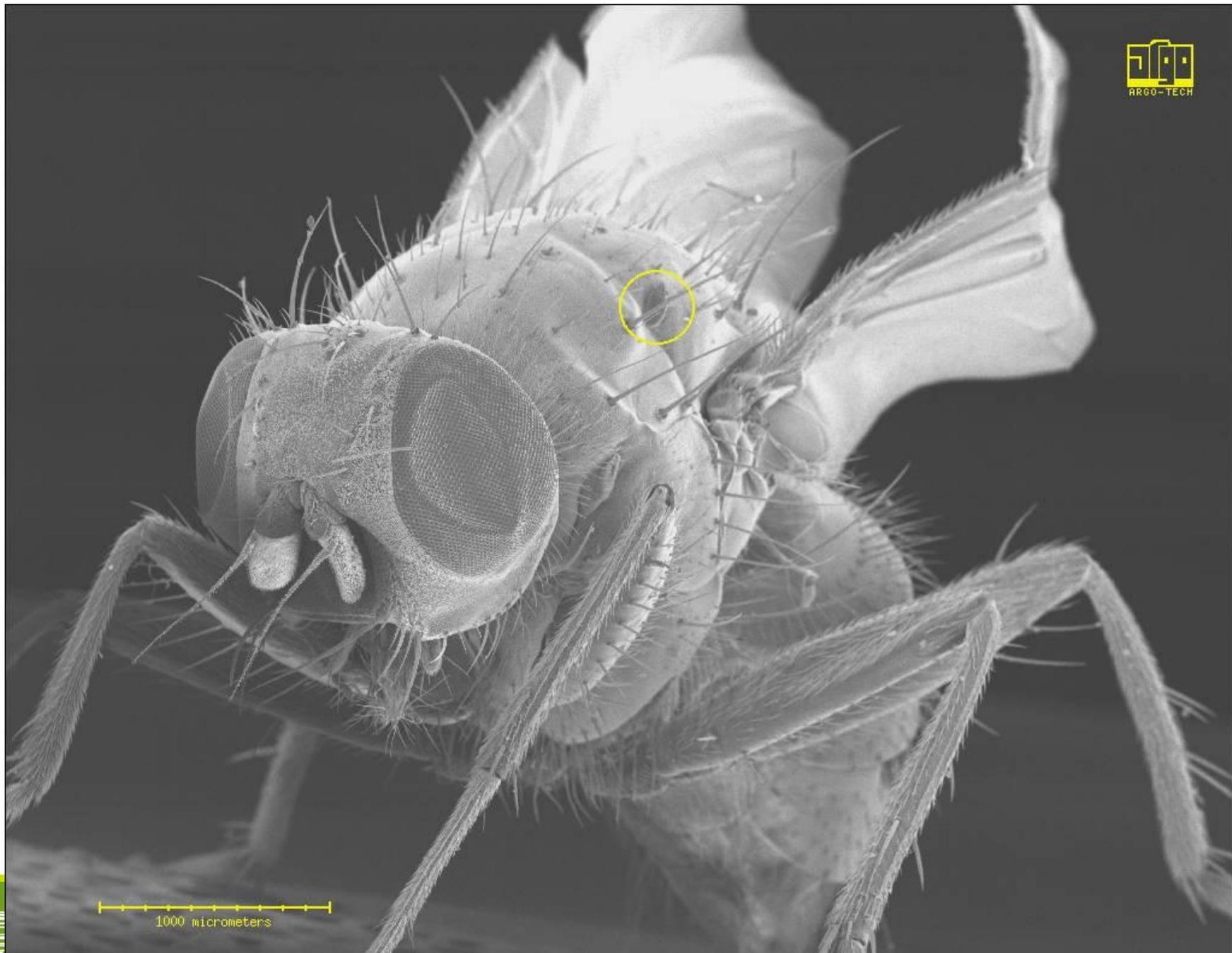
Plan

Cellules et tissus

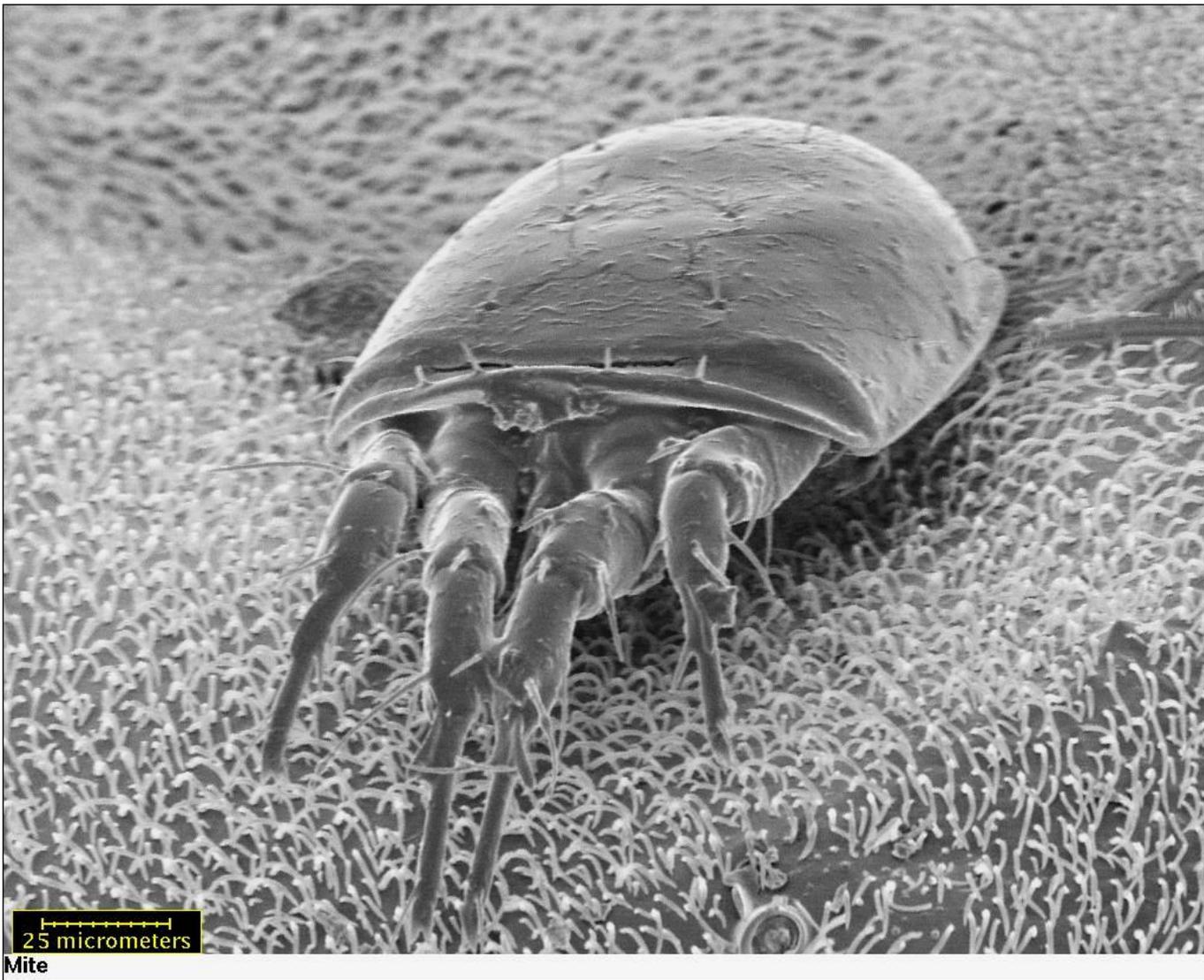
Impact du chauffage sur l'évolution des viandes

Etude du salage sur l'évolution des viandes

Perspectives d'applications de nouveaux appareillages (MEB, Options)

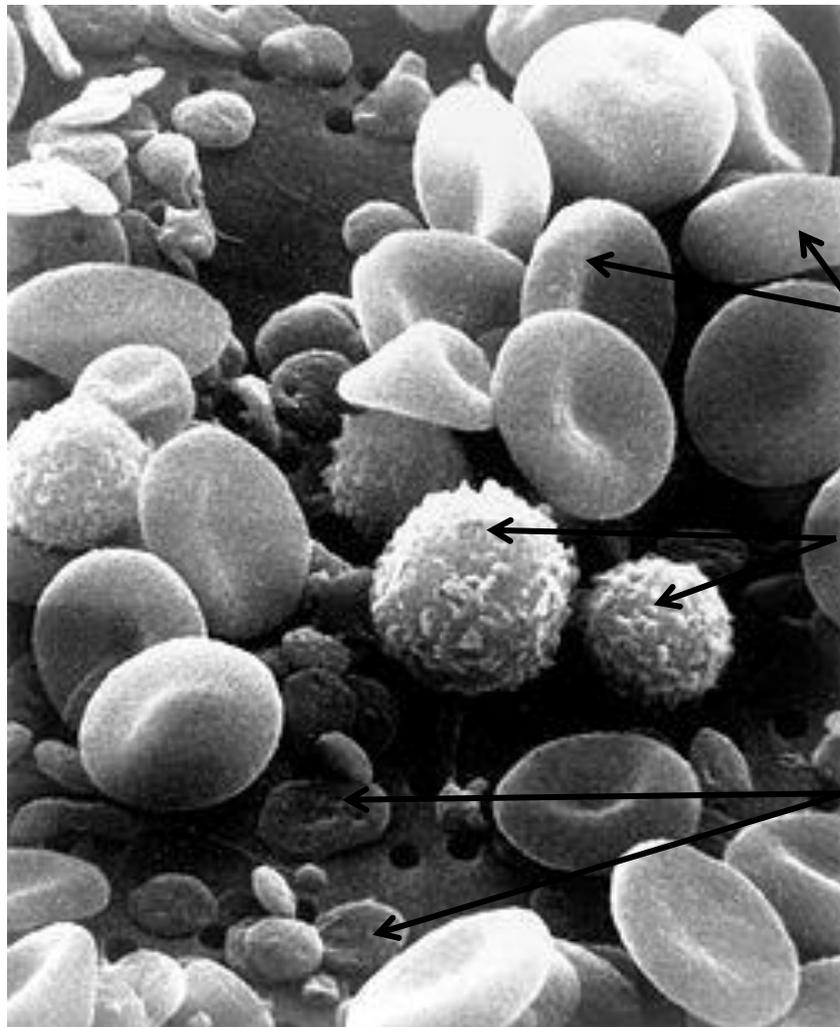


1000 micrometers



<http://www.atclabs.com/Photos/mite.jpg>

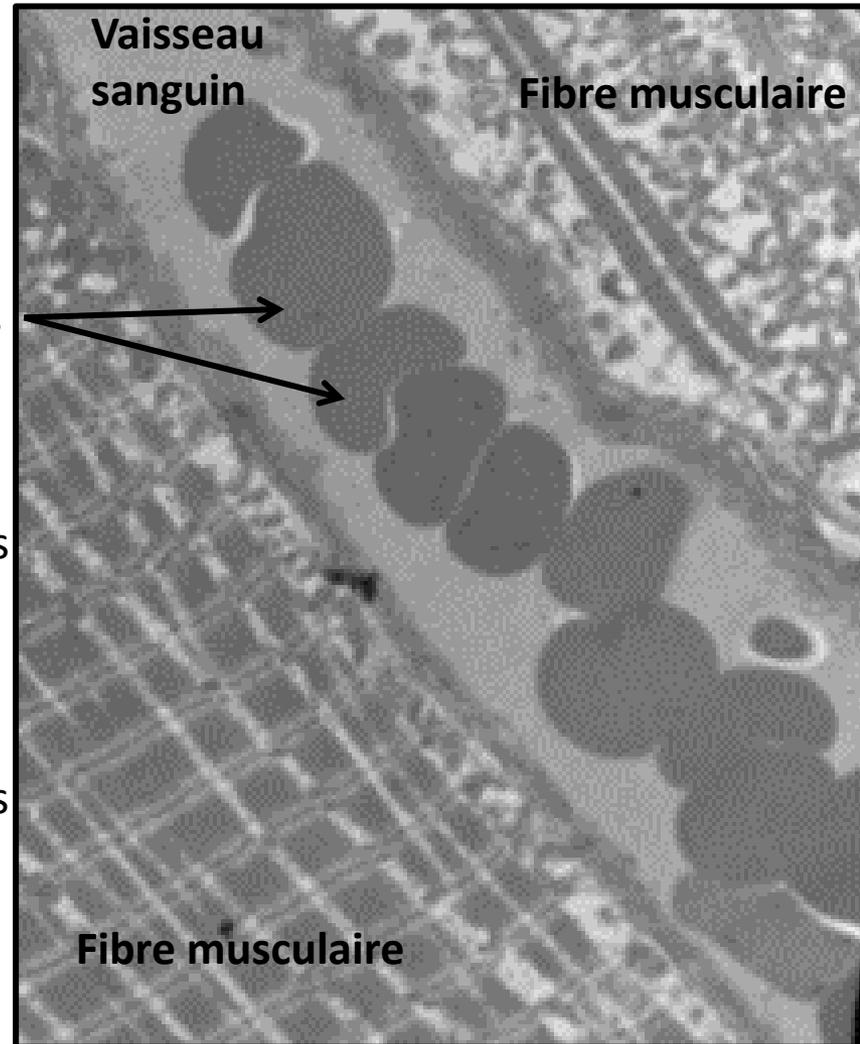
Application aux tissus animaux. 1-) le sang



Hématies

Leucocytes

plaquettes



Vaisseau sanguin

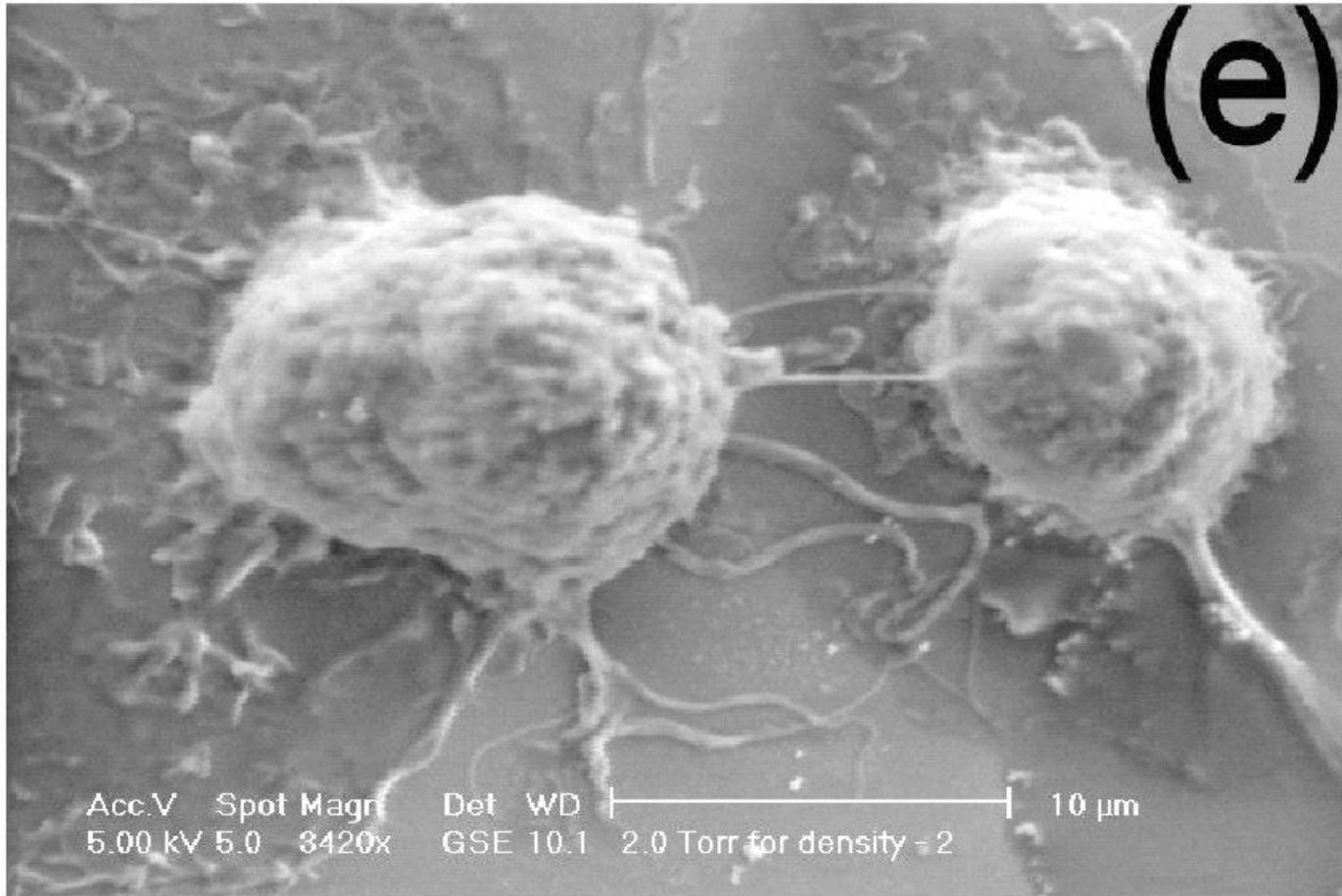
Fibre musculaire

Hématies

Fibre musculaire

SEM : http://en.wikipedia.org/wiki/White_blood_cell

Globules rouges TEM: T. Astruc

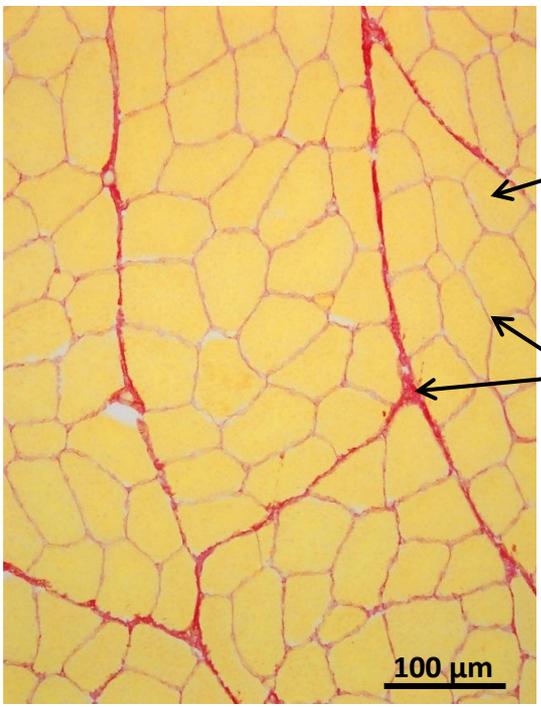


ESEM image of human macrophages

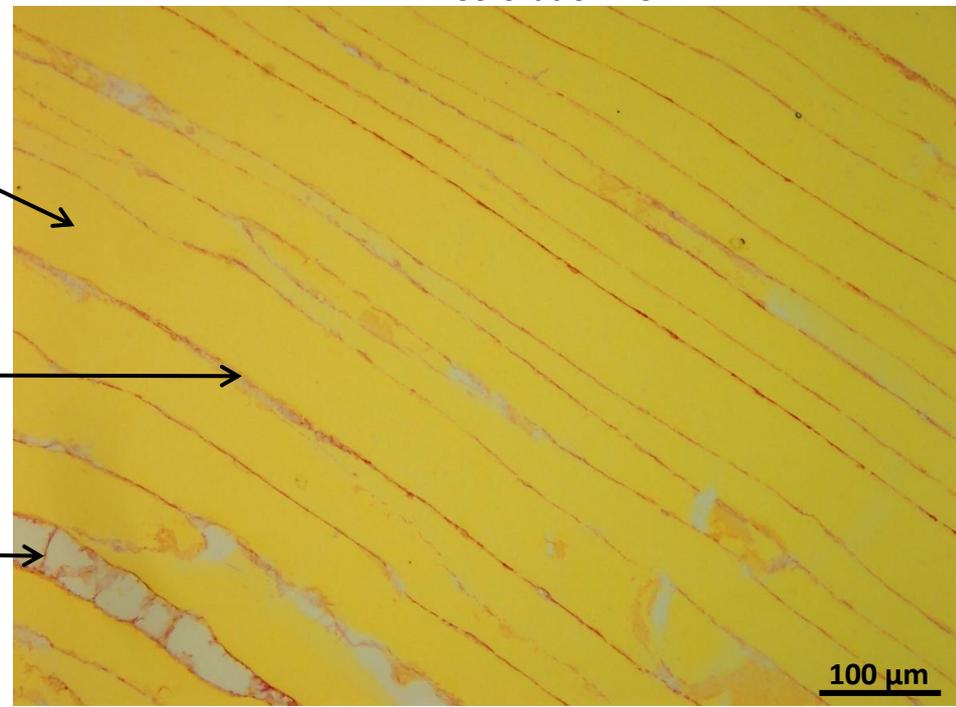
<http://www.phy.cam.ac.uk/research/bss/esem.php>

2-) Tissu et fibres musculaires, microscopie optique

Histologie (coupe longitudinale)
Coloration RS

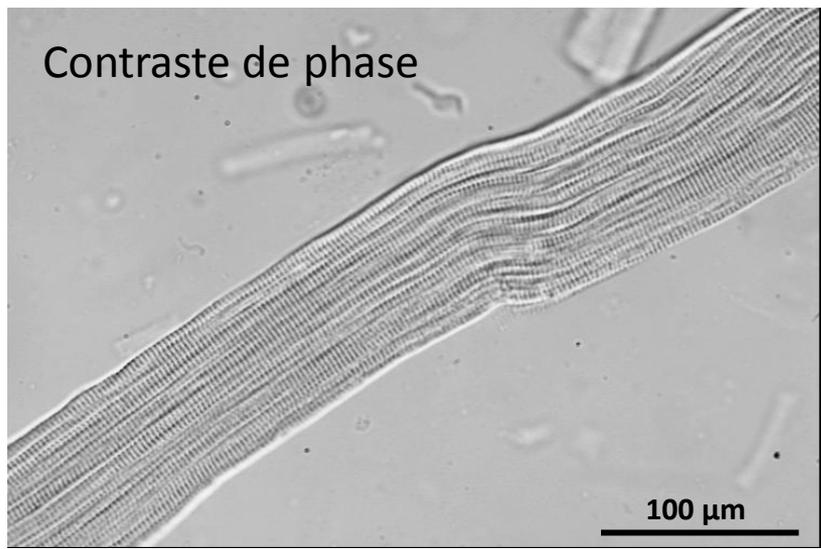
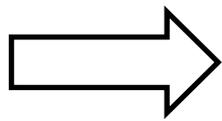


Fibre musculaires
Tissu conjonctif (90 % collagène)
Adipocytes



Histologie (coupe transversale)
Coloration RS

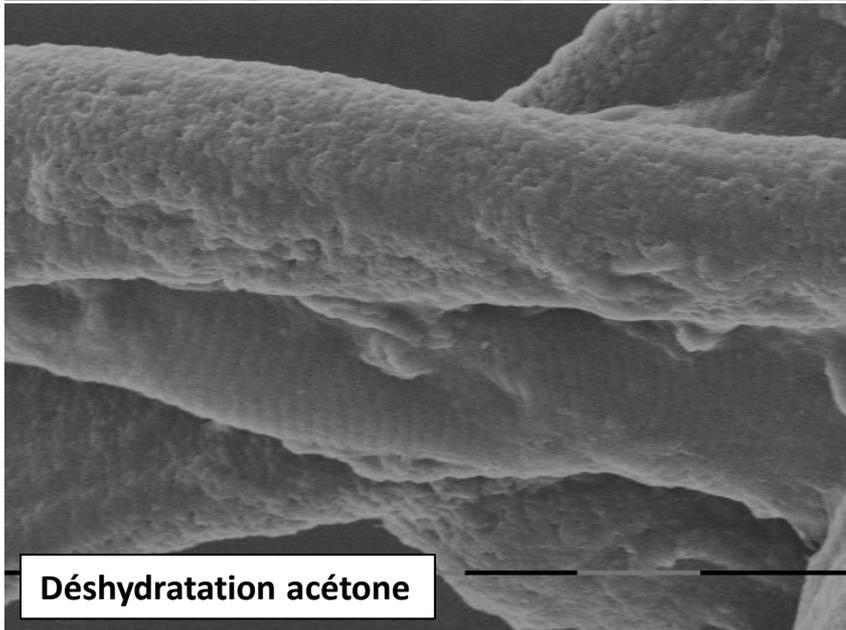
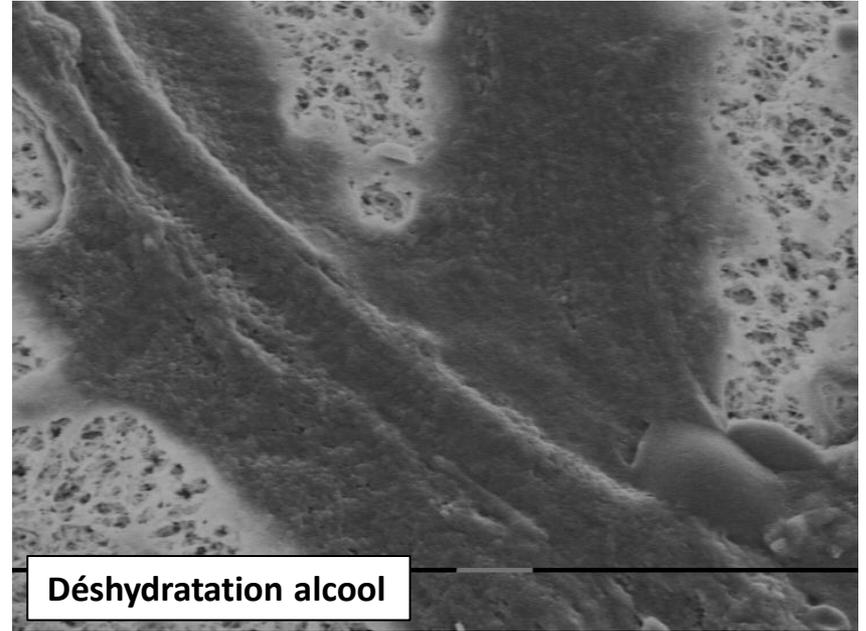
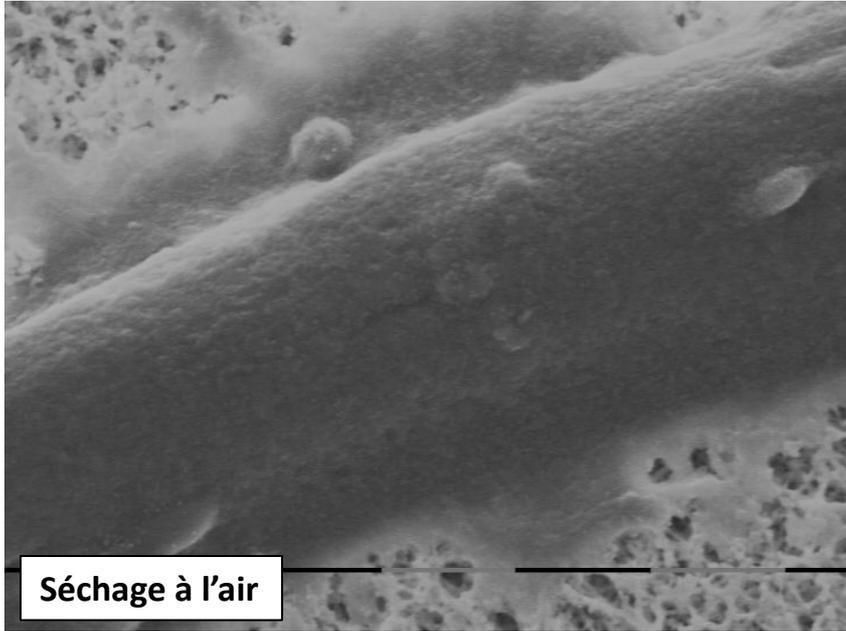
Isolement des fibres individuelles
par action de la collagénase.



2-) Tissu et fibres musculaires, MEB

Impact de la méthode de déshydratation sur la préservation de la morphologie

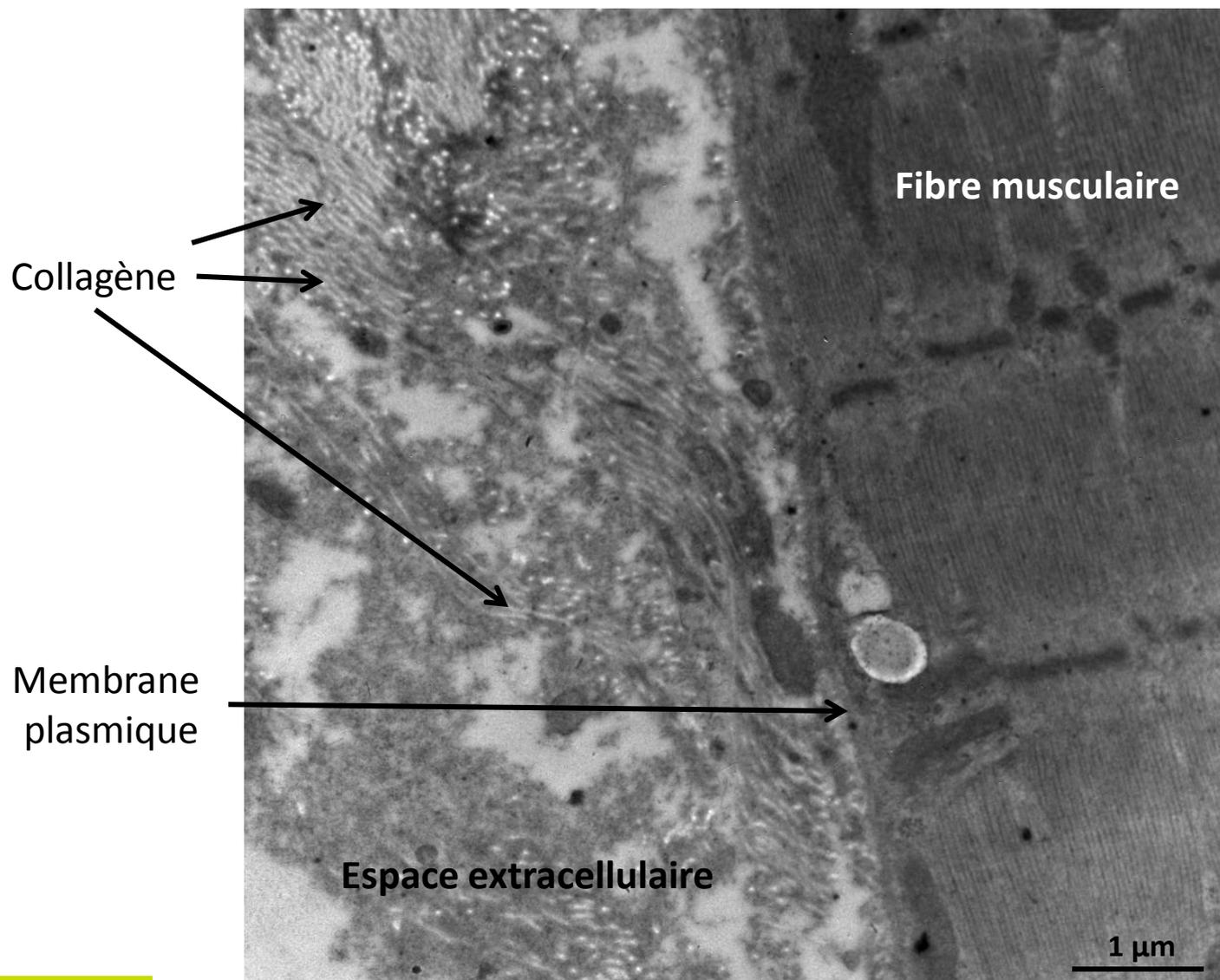
Fibres musculaires isolées, fixées au glutaraldéhyde 2.5 % déshydratées et métallisées. Observation MEB 505 Philips



Contraintes pour déshydrater les fibres par point critique (matériel d'étude, centrifugation, déformation des fibres...)

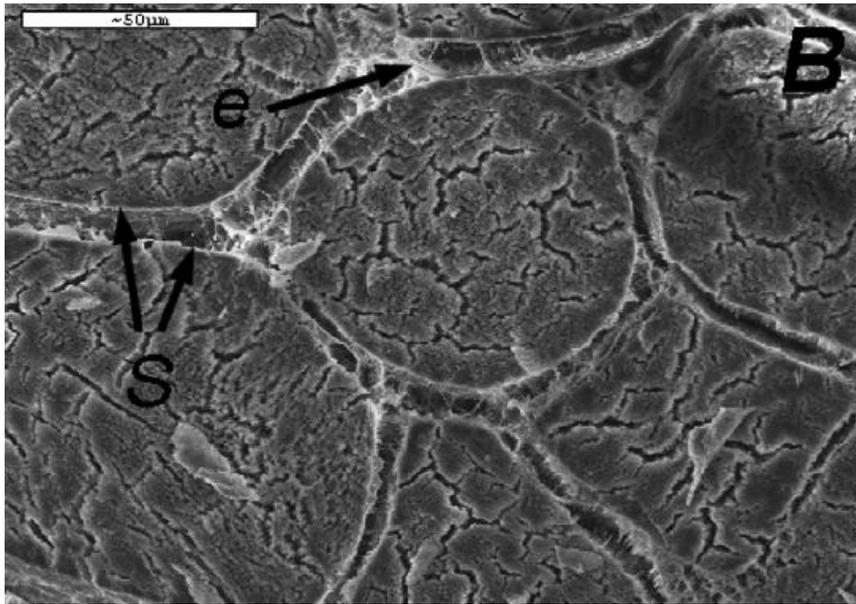
Acétone = meilleure alternative

Tissu conjonctif intramusculaire : Observation en MET

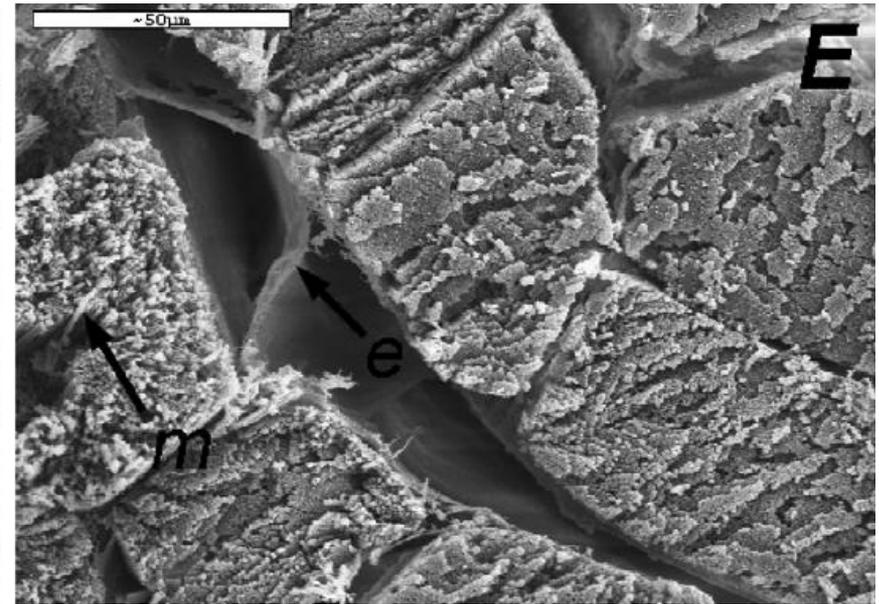


Muscle semimembranosus de porc

Cryo-SEM



SEM



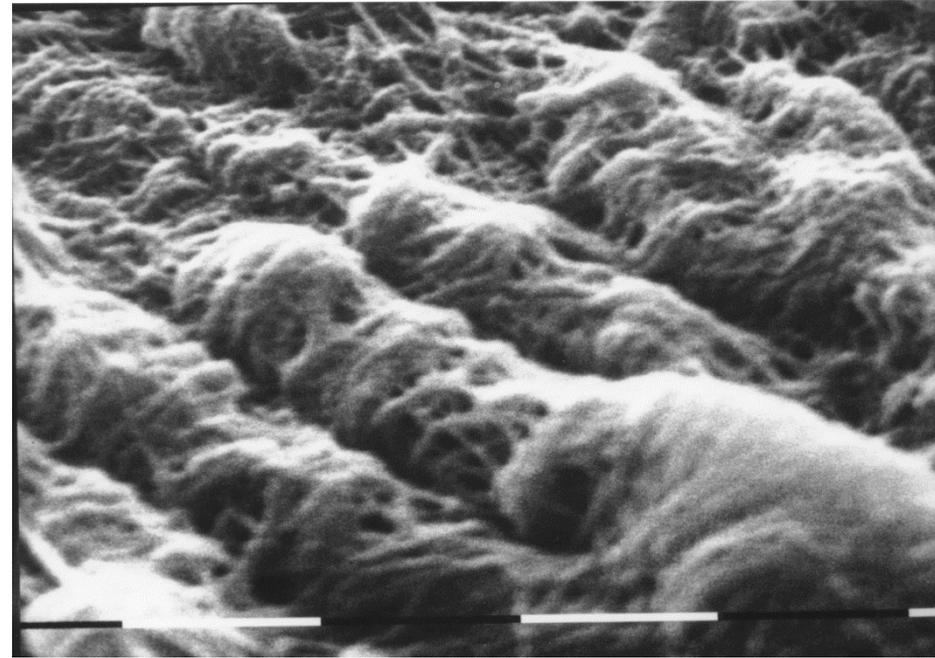
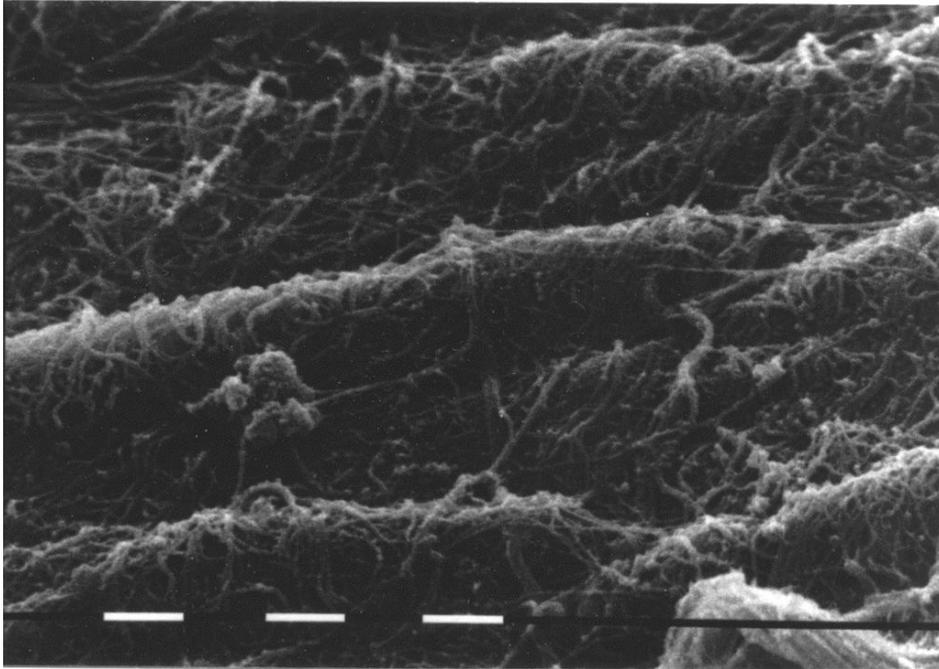
e: endomysial connective tissue, S: sarcolemma, m: myofibrils

Cryo-SEM : Cubes were immersed in slush Nitrogen (210°C) and transferred to a cryo-trans (CT 15000 C from Oxford Instruments, Oxford, England) linked to a scanning electron microscope JEOL JSM 5410 (Jeol Tokyo, Japan), operating at a temperature below 130° C. Samples were cryo-fractured at 180° C and etched at 90° C. The observations were carried out at 15 kV and at a working distance of 15 mm.

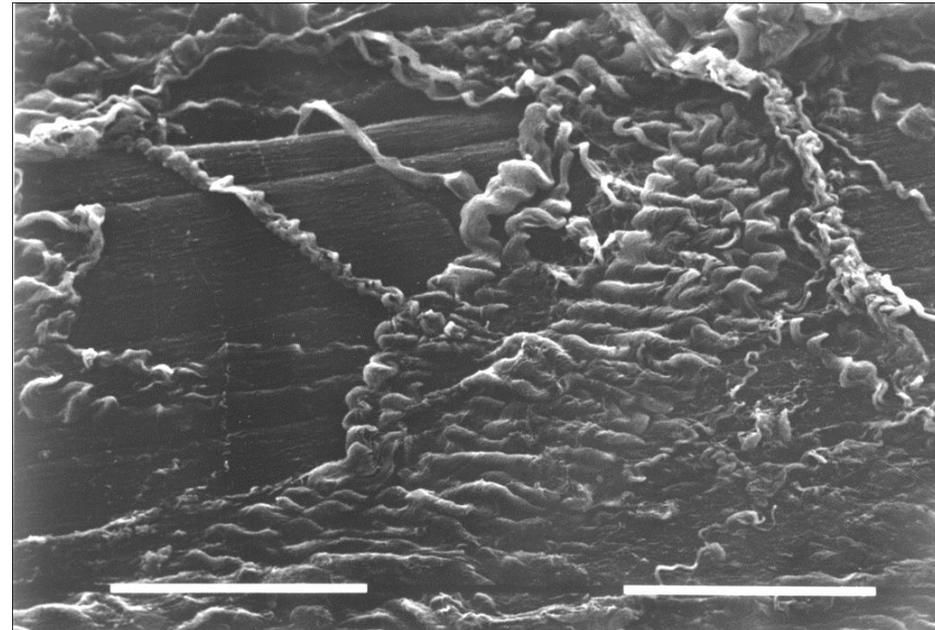
SEM : Fixation glutaraldéhyde, postfixation tetroxyde d'osmium, déshydratation éthanol 10, 20, 40, 60, 80, 100%, acétone point critique. Or, observation.

Larrea et al. 2007

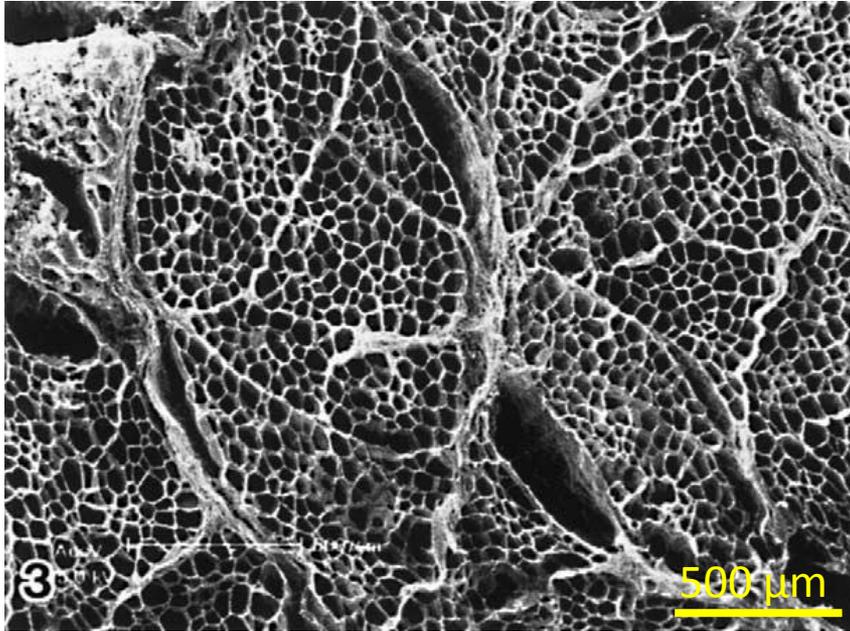
Tissu conjonctif intramusculaire : Observation en MEB



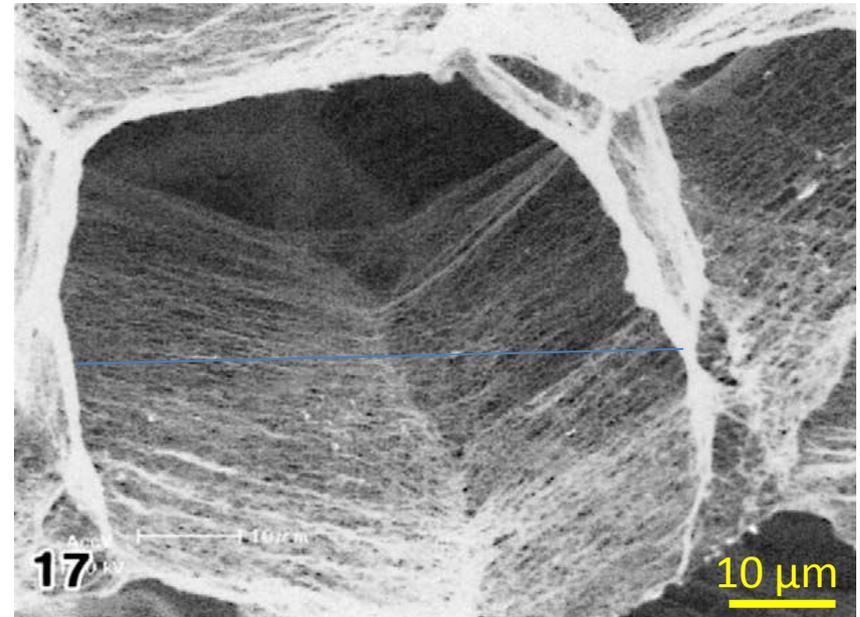
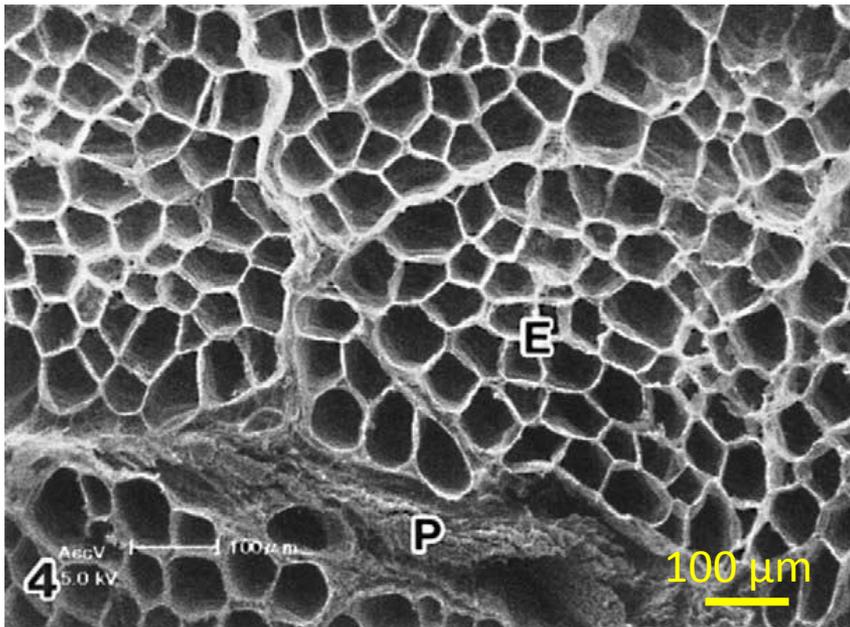
Fixation glutaraldéhyde 2.5 %
Déshydratation alcool
Point critique
Métallisation
Observation MEB Philips 505



Tissu conjonctif intramusculaire : Observation en MEB

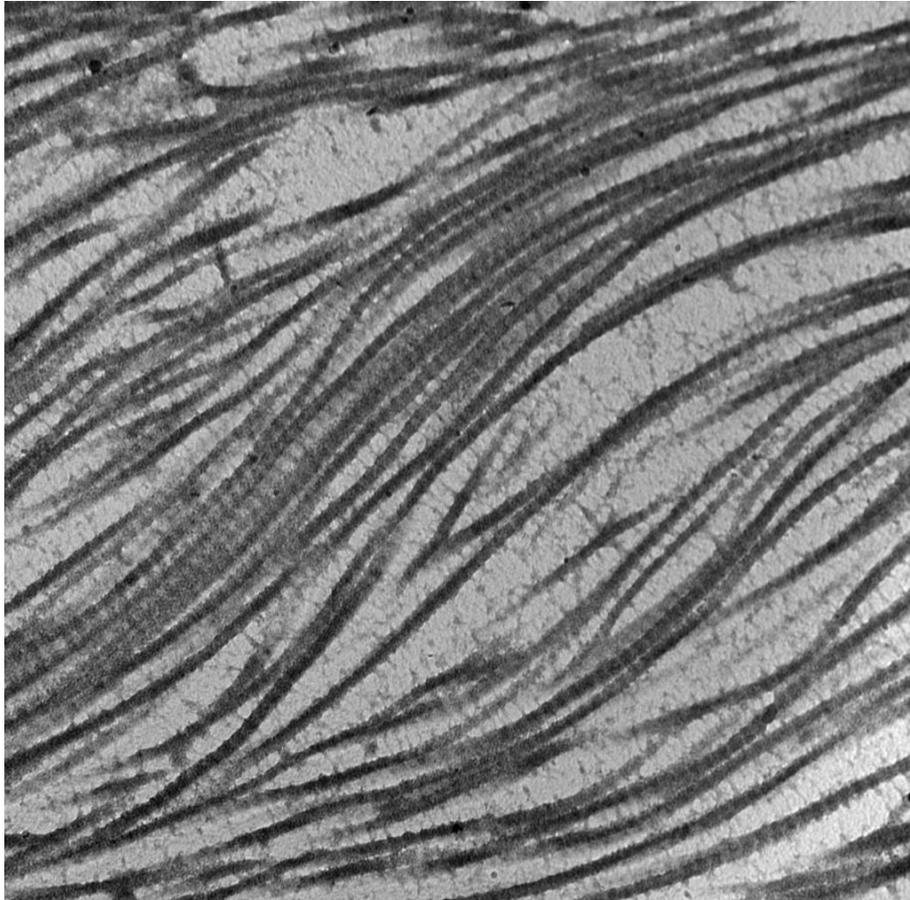


Fixation chimique (PAF-Glut)
Coupe transversale aux fibres
Incubation dans NaOH 2M pendant 5j
Post-fixation osmique
Déshydratation
Lyophilisation
Observation SEM



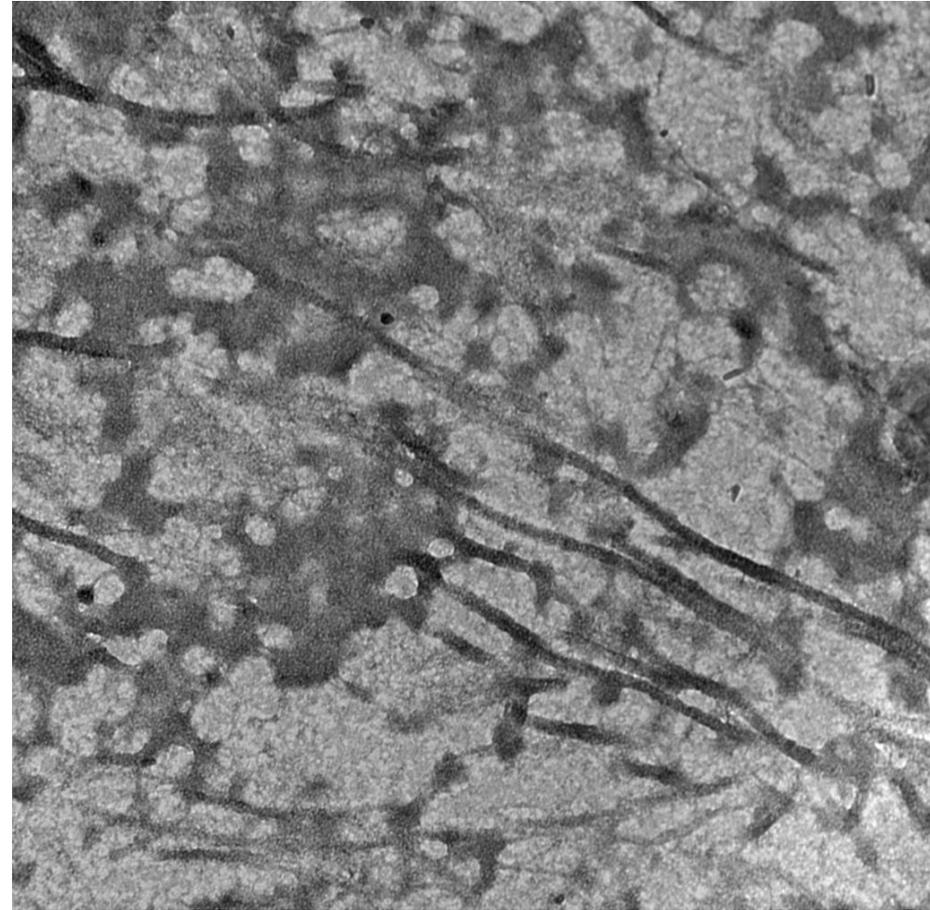
Purslow & Trotter, 1994
Nakamura et al. 2003
Oshima et al. 2009

Effet du chauffage sur le tissu conjonctif



Astruc T MET 23-11-12.039.tif
552-J2-L
conjonctif
Print Mag: 42900x @ 150 mm
11:20 11/23/12
TEM Mode: Imaging

500 nm
HV=80.0kV
Direct Mag: 40000x
CICS CLERMONT-FERRAND

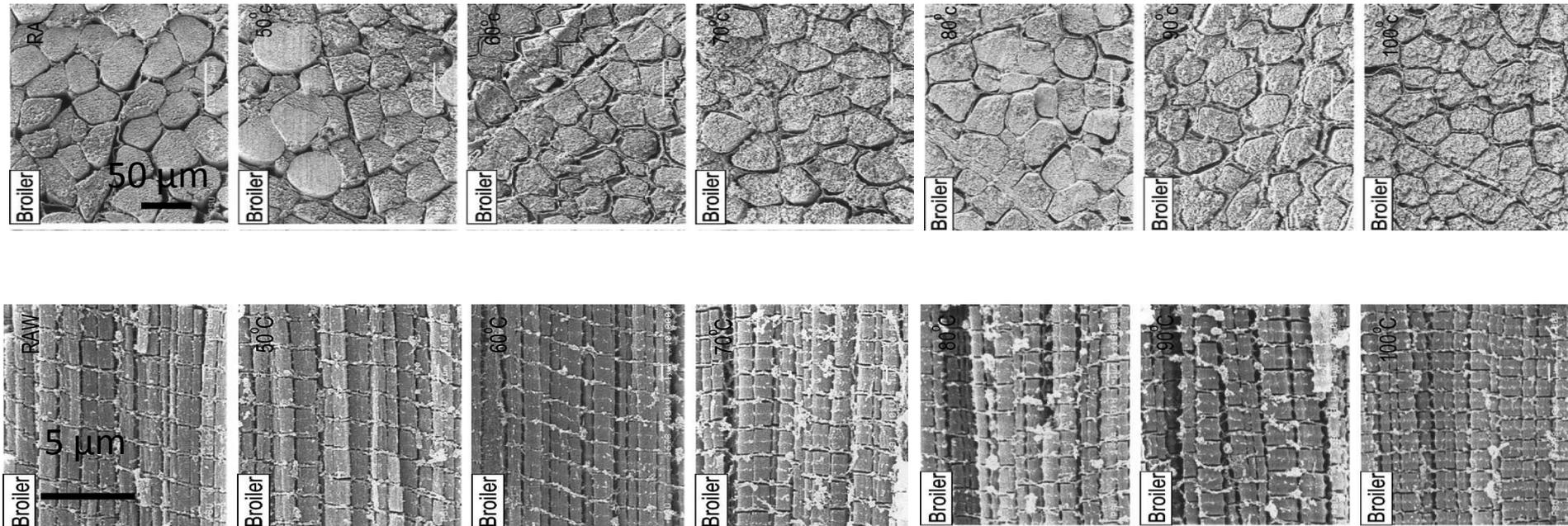


Astruc T MET 07-12-12.151.tif
C2L
Print Mag: 64300x @ 150 mm
14:17 12/07/12
TEM Mode: Imaging

500 nm
HV=80.0kV
Direct Mag: 60000x
CICS CLERMONT-FERRAND

Evolution du muscle *pectoralis* de poulet au cours du chauffage

cru 50° C 60° C 70° C 80° C 90° C 100° C



Fixation Glutaraldéhyde
Déshydratation éthanol 25,50,70,95, 100
Lame de rasoir dans N2 Liquide
Or, observation 5-10 KV

Contraction latérale des cellules
Contraction des sarcomères au-delà de 80 °C

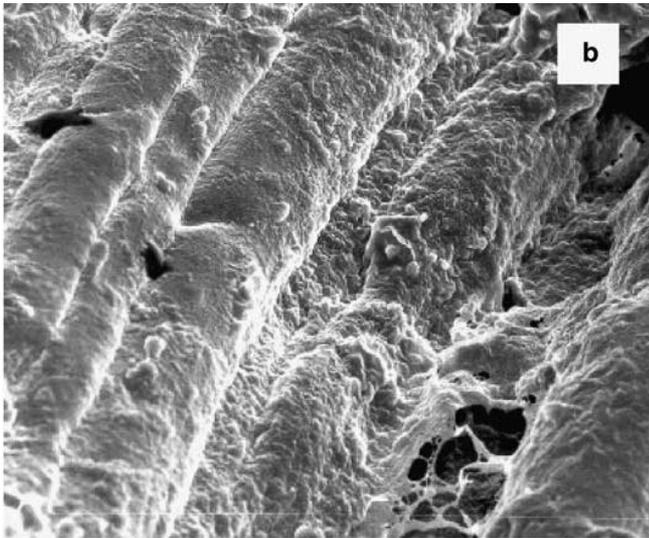
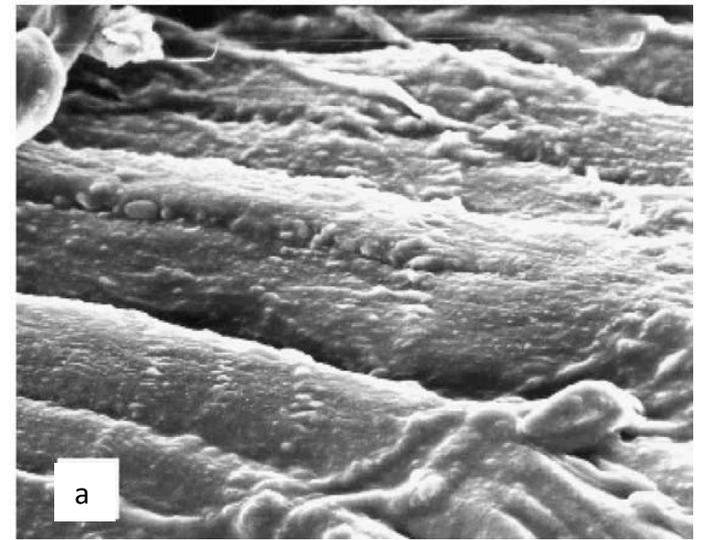
S. Wattanachant et al. / Food Chemistry 93 (2005) 337–348

Effet du sel sur les fibres et le tissu musculaire

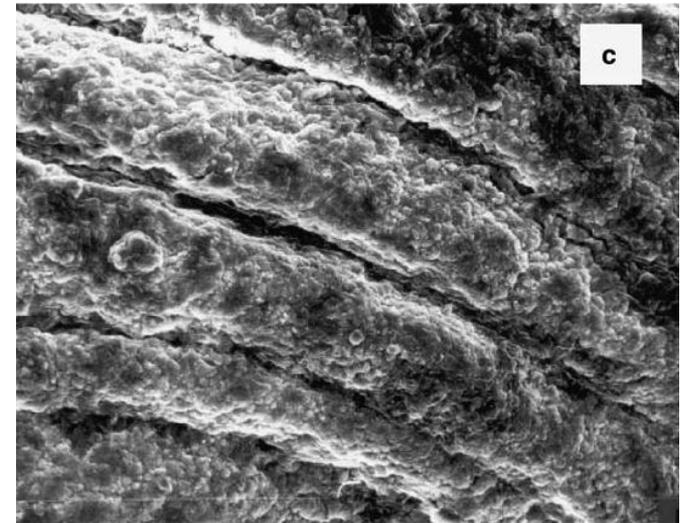
Fixation,
Déshydratation gradient d'alcool ,
Or et observation

(Graiver et al. 2006)

NaCl 5 g/l

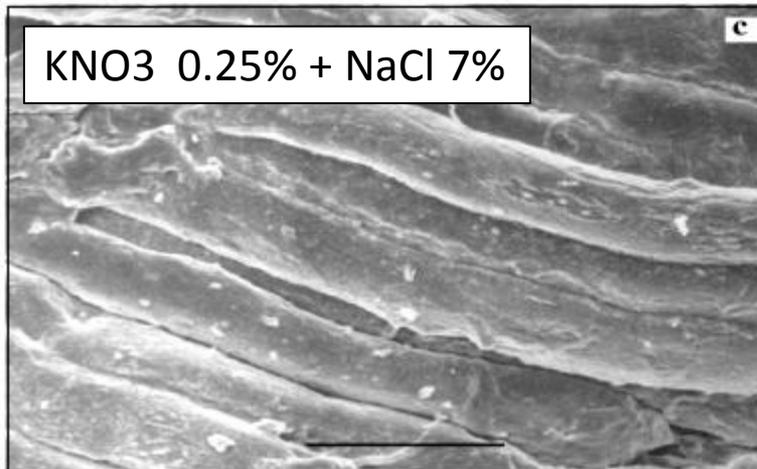
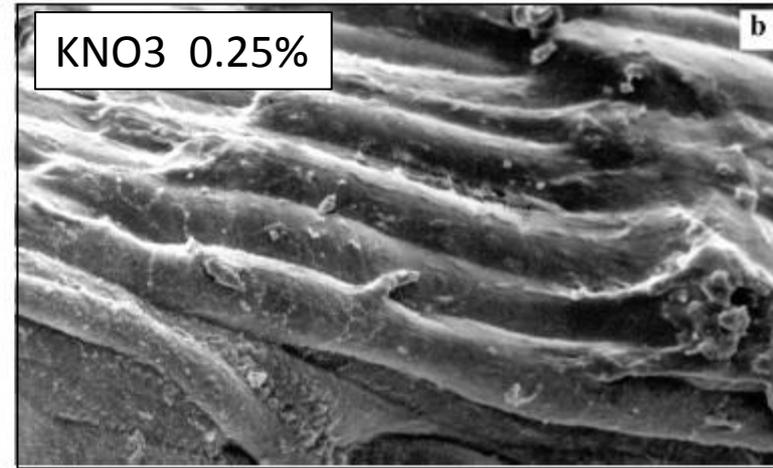
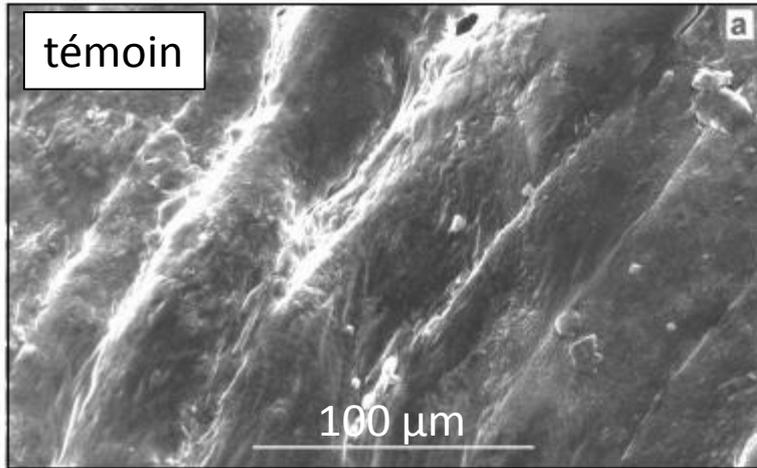


“Fibers in Fig. b submitted to NaCl 140 g/l show swelling “ !!!

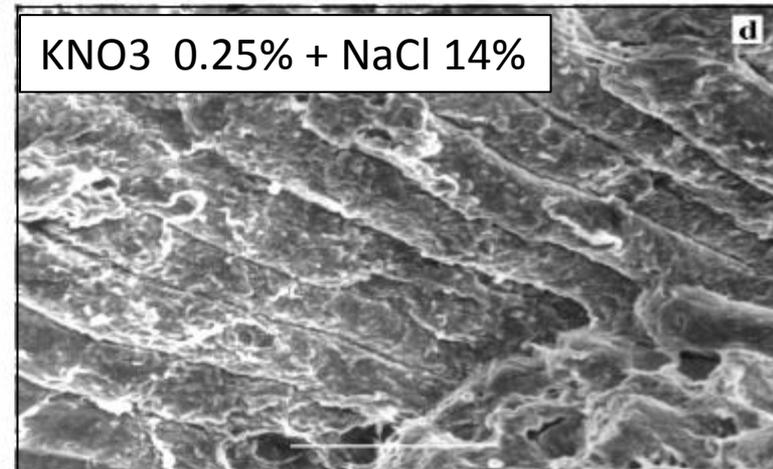


“NaCl 330 g/l (Fig. c) produced fragmented and dehydrated fibers, with a granular appearance”

Viande de porc salée (+ nitrate)



Fibres Gonflées

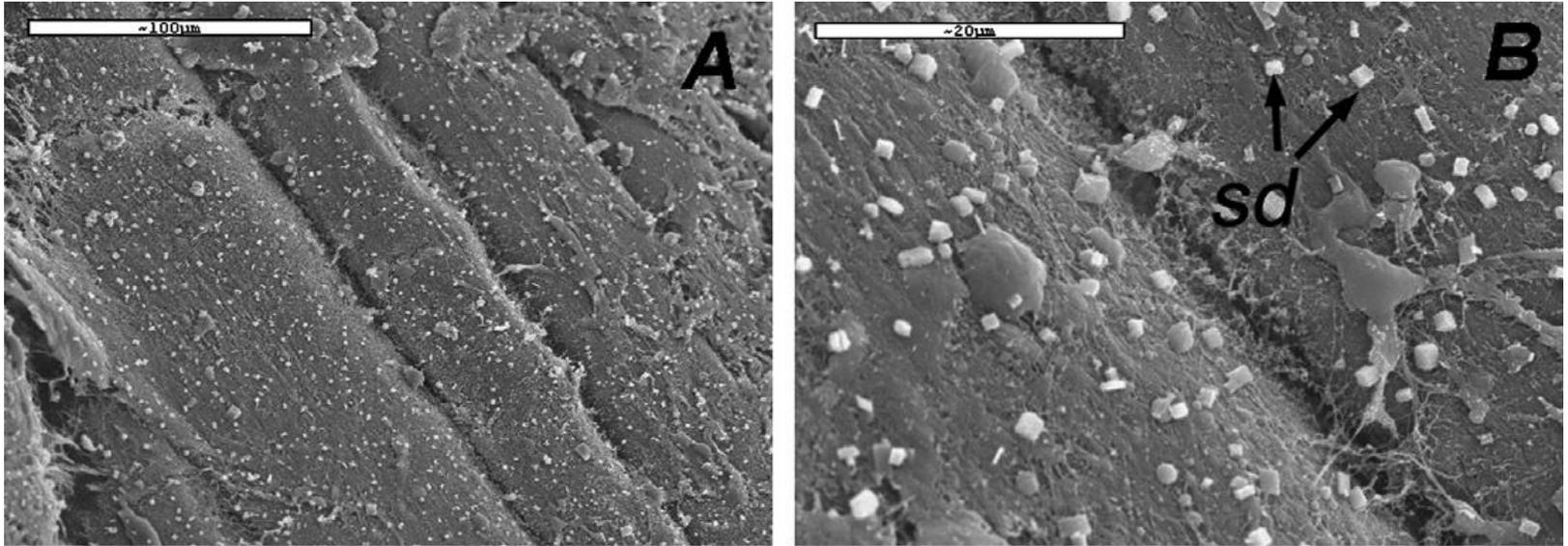


Fibres fragmentées et déshydratées

Fixation Carnoy (60% éthanol, 30% chloroforme, 10% acide acétique glacial, v/v) 24 h à 4 °C.

Déshydratation gradient d'éthanol : 70 % (12 h), 95 % (2 h) et 100% (2 h)

Jambon sec



Fixation glutaraldéhyde,
Postfixation tetroxyde d'osmium,
Deshydratation ethanol 10, 20, 40, 60, 80, 100%,
Acétone point critique,
Or,
Observation.

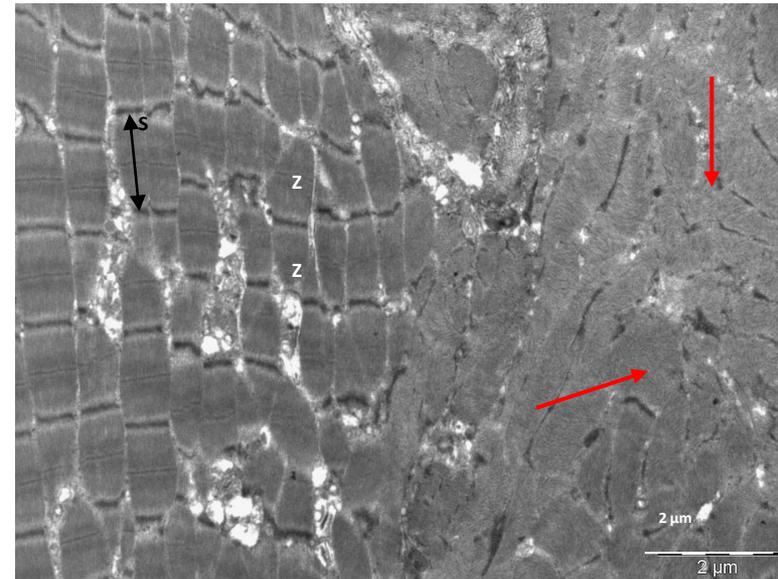
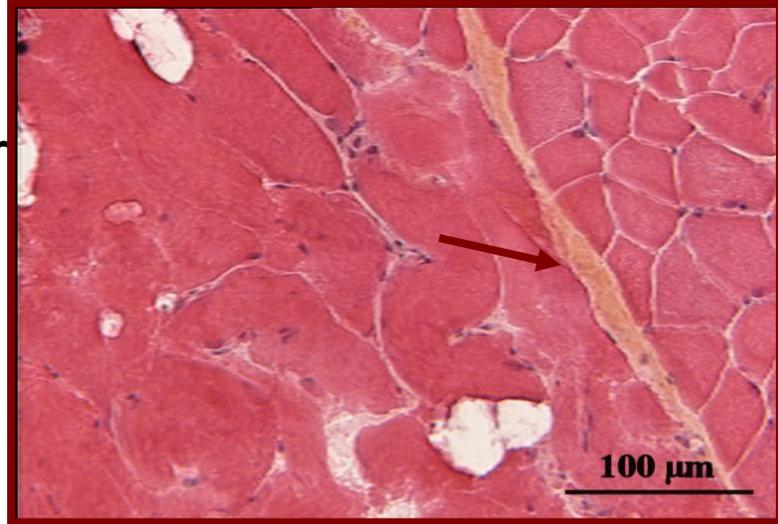
Larrea et al. 2007

Mécanismes – Microscopie/structure

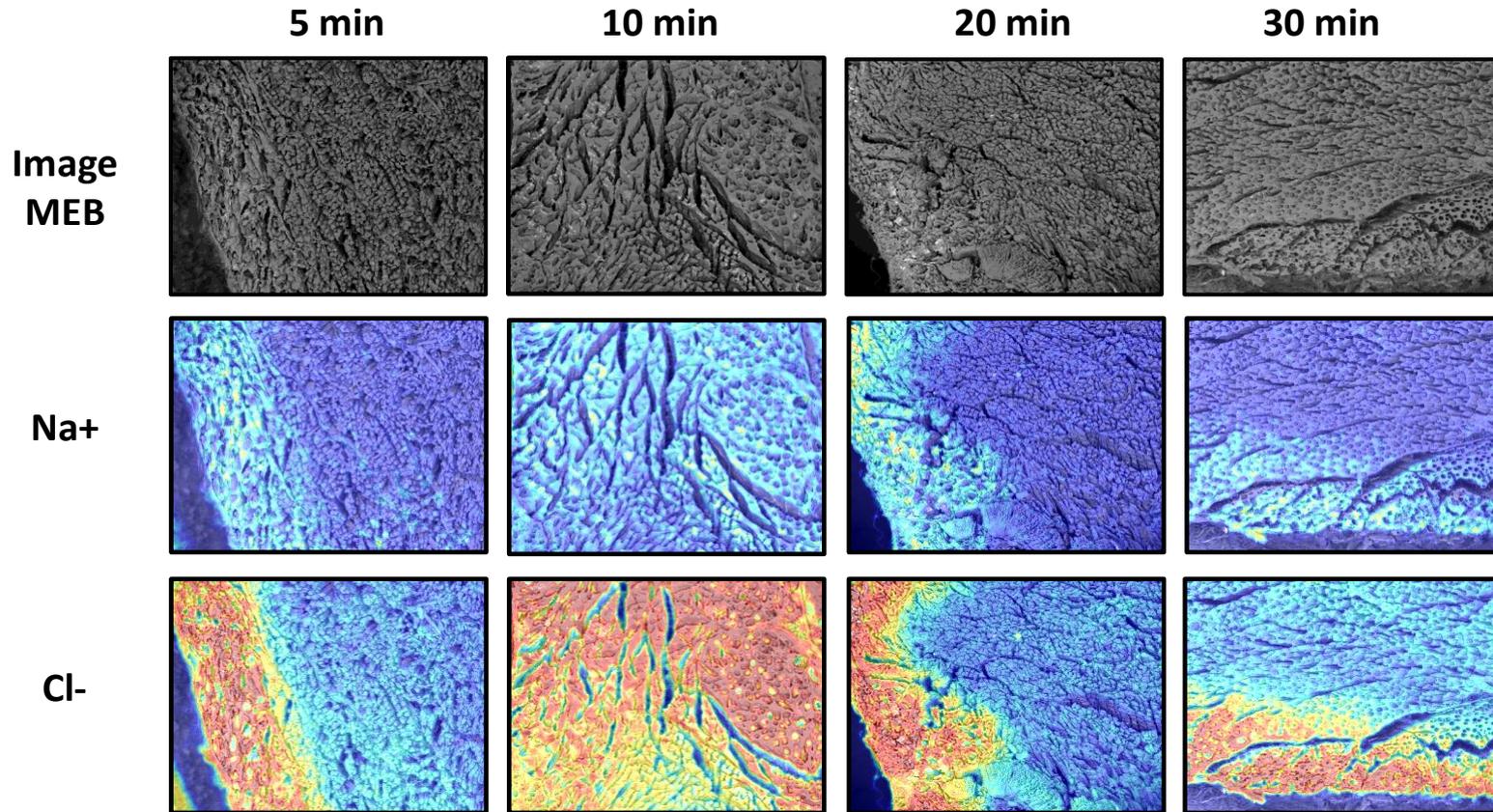
Incidence du taux de NaCl sur

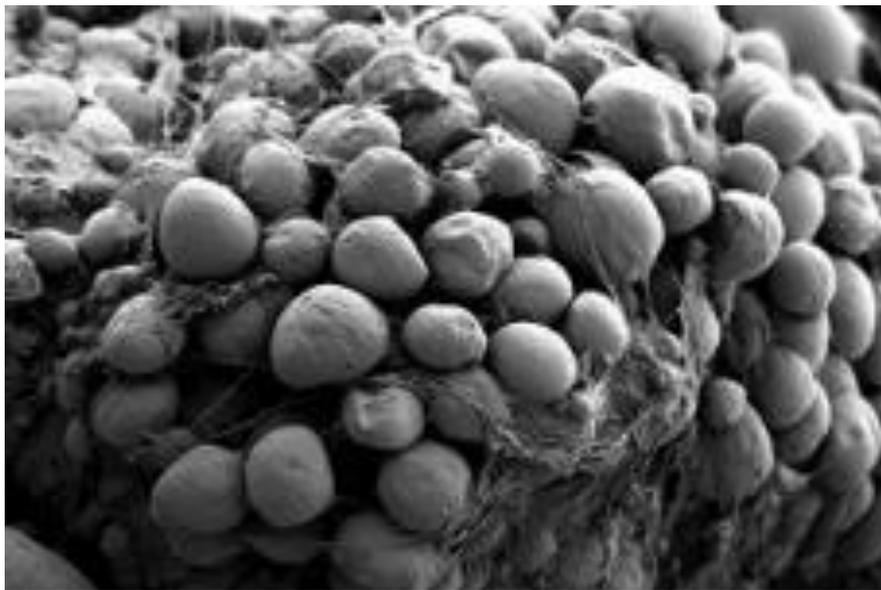
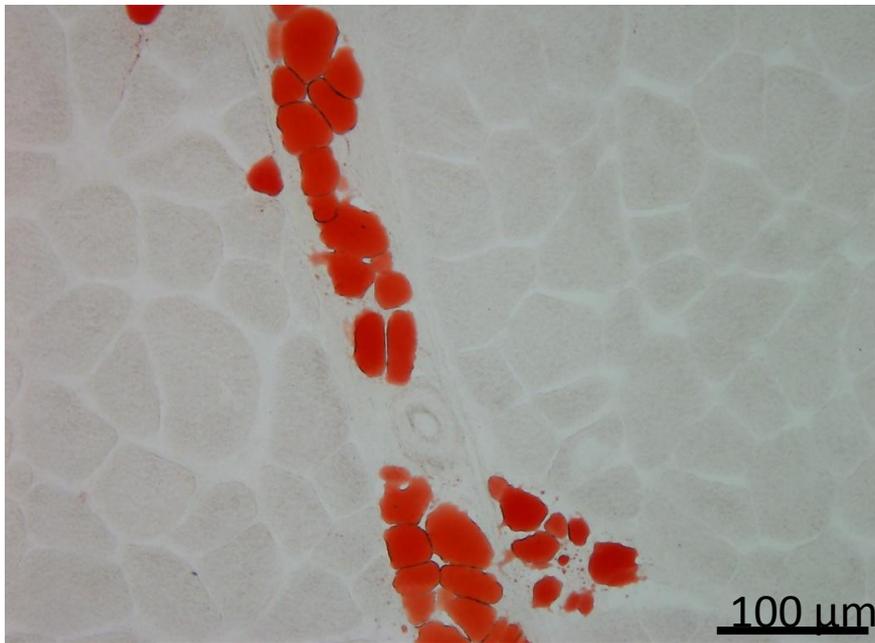
– coupes histologiques

– ultrastructure

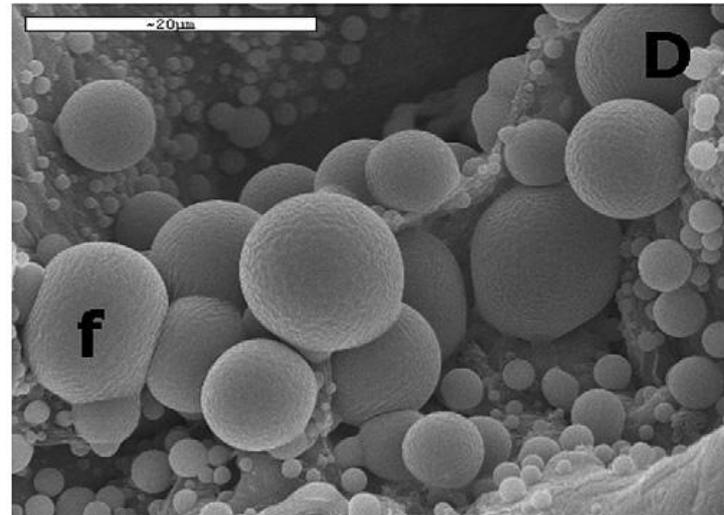
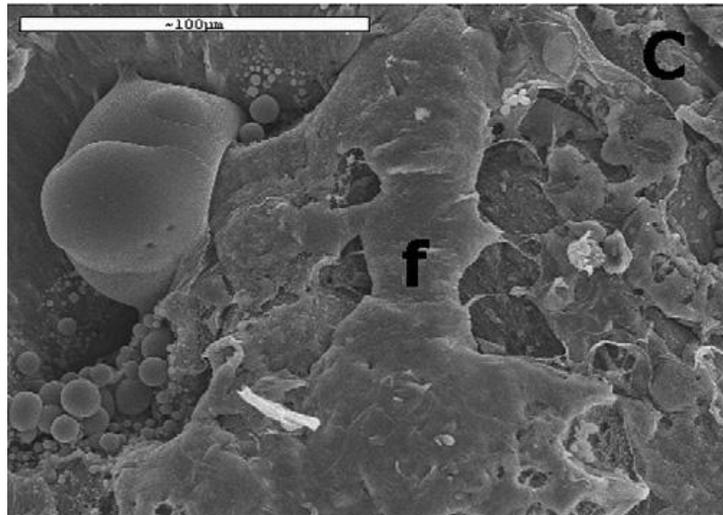
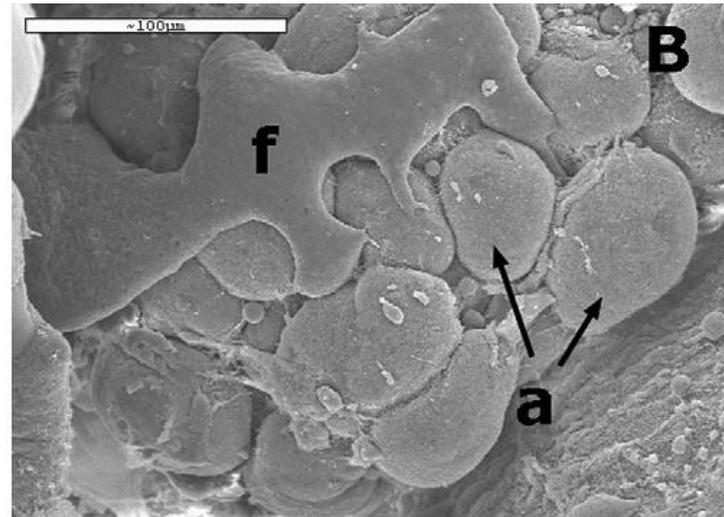
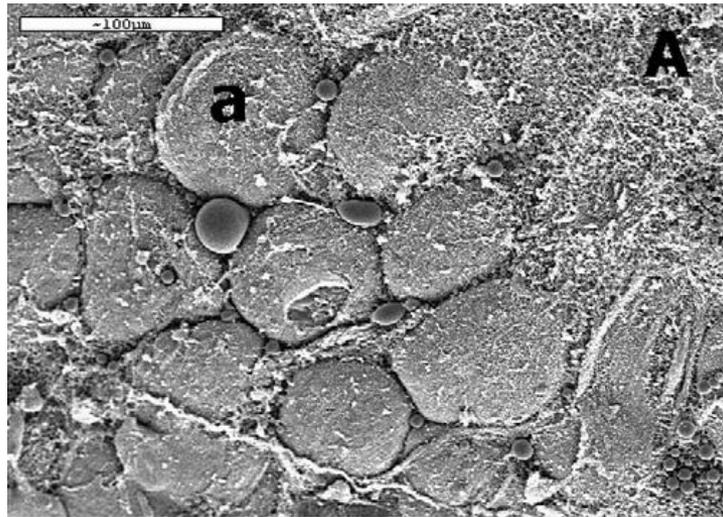


Incubation dans le sel sec

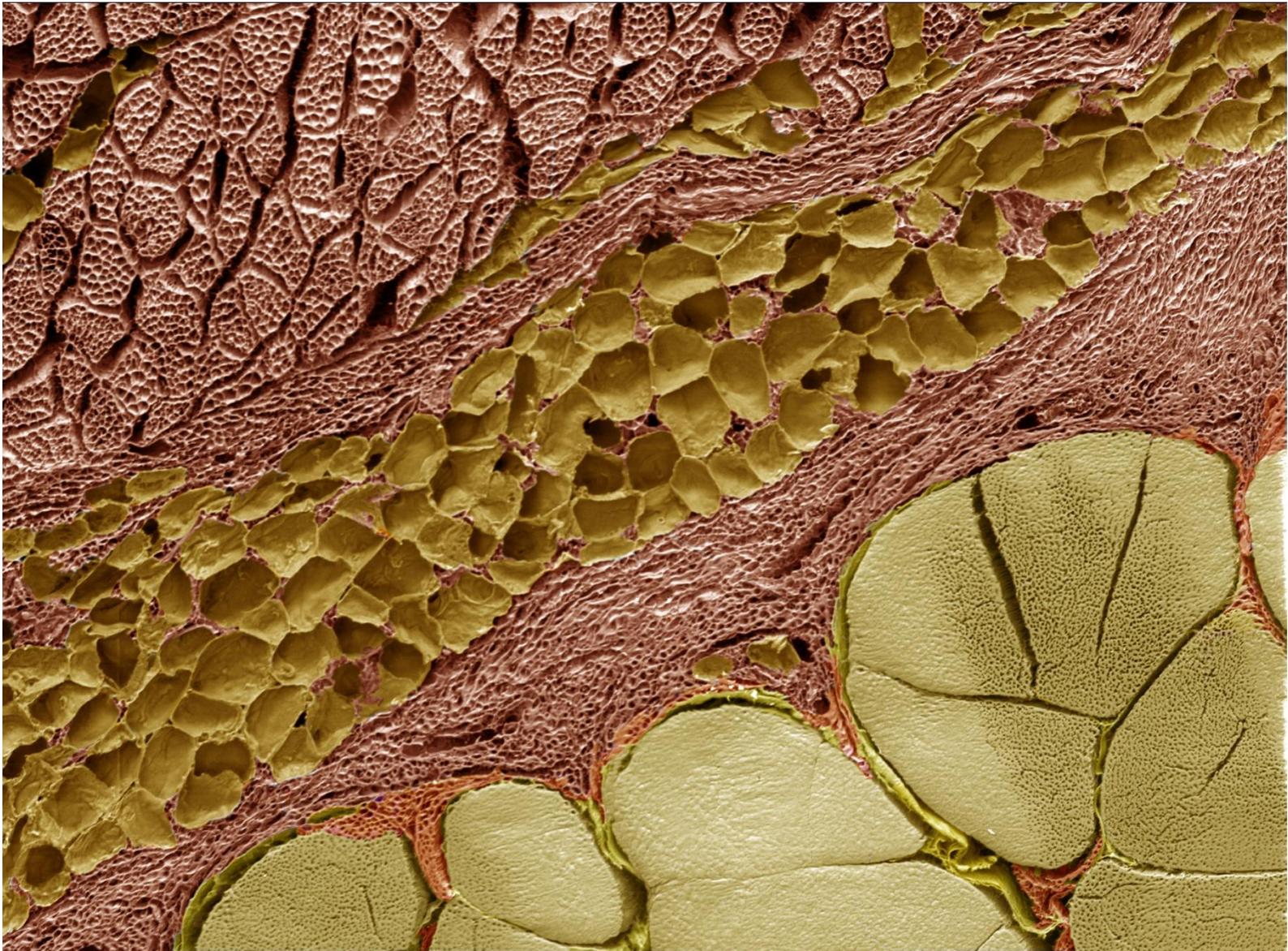




Adipocytes dans le jambon sec

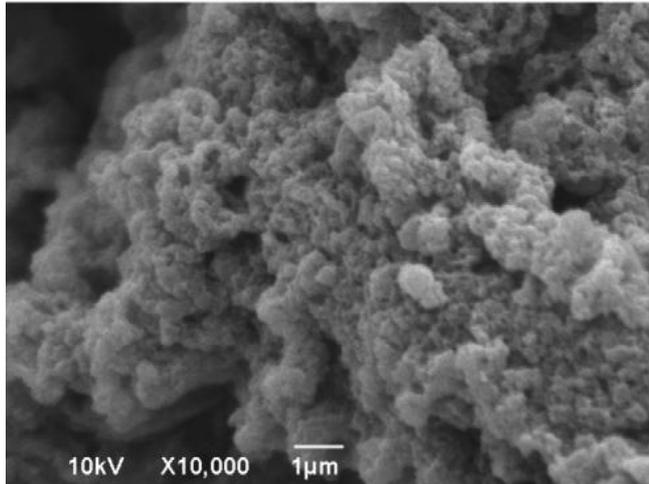


Transversal section of intramuscular fat in *biceps femoris* of raw ham (A), salted ham (B) and “PDO Teruel” dry-cured ham (C and D) observed by SEM. (f, free fat released from the adipocytes; a, adipocytes). (Larrea et al. 2007)

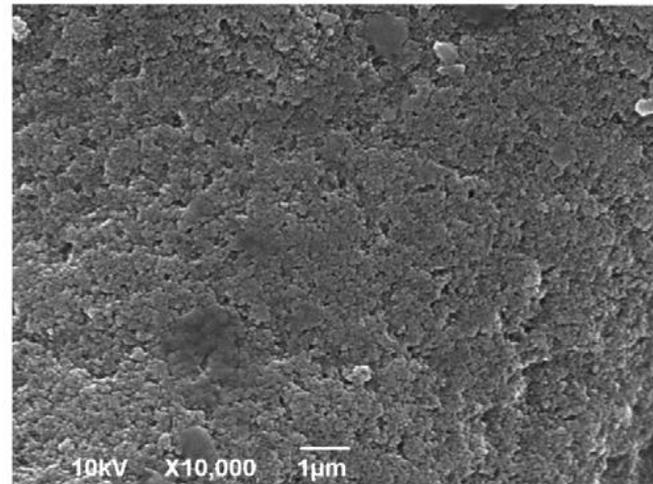


Scanning electron micrographs of myosin gel at various pH

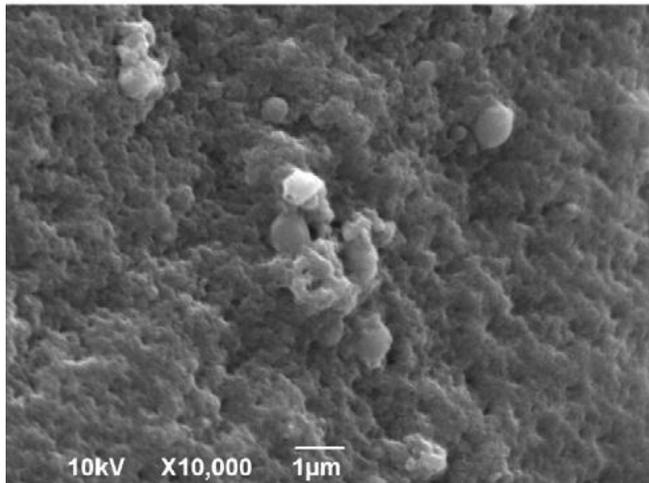
pH 5,5



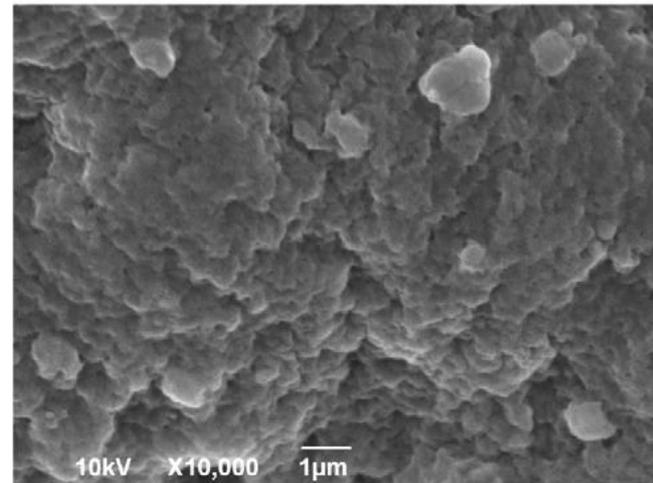
pH 6,5



pH 7,0

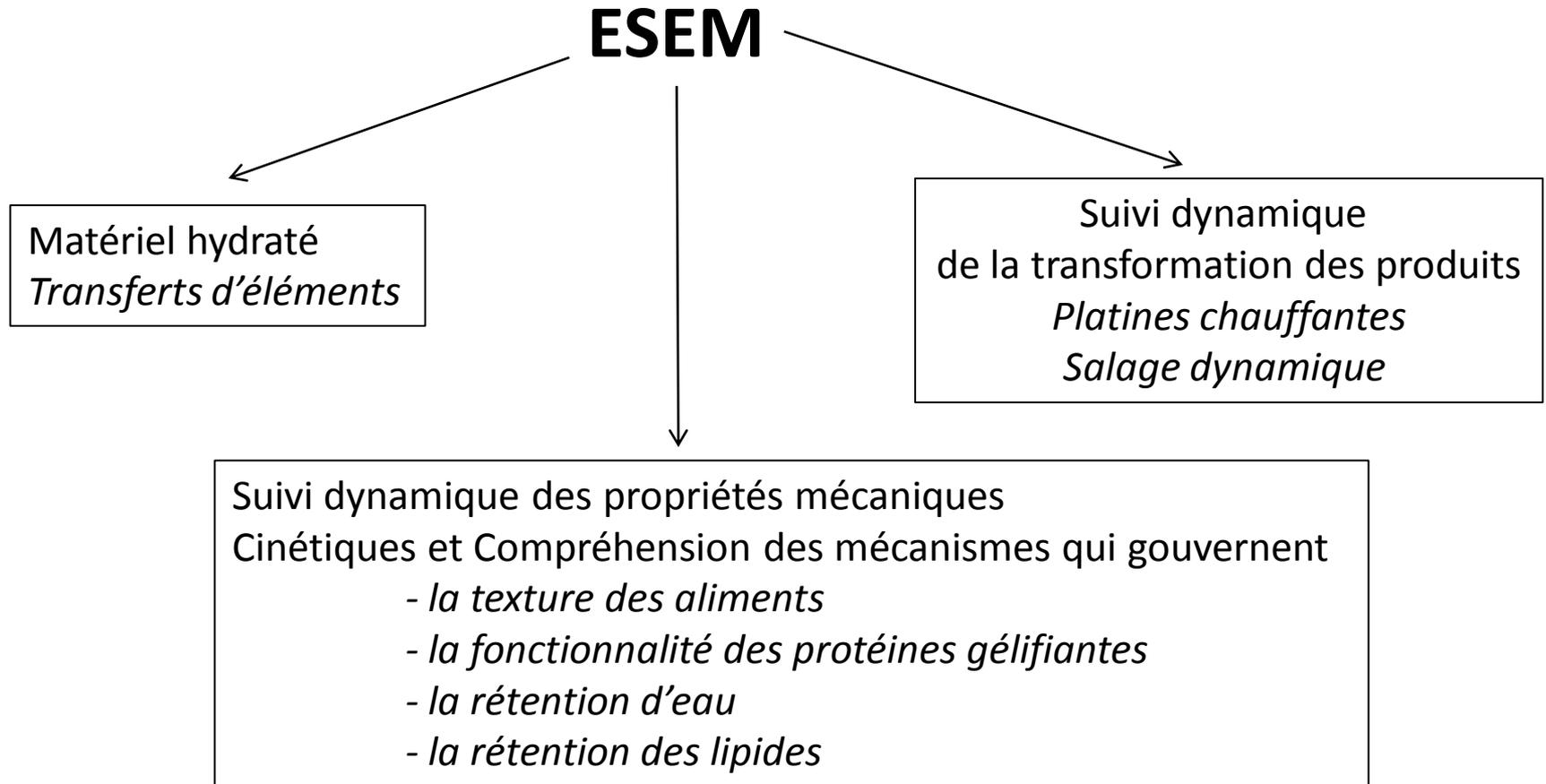


pH 9,0

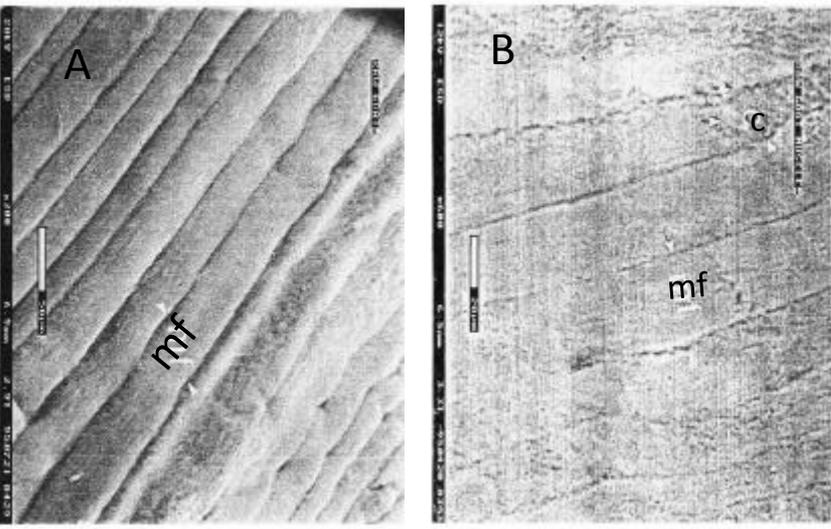


(Liu et al. 2008)

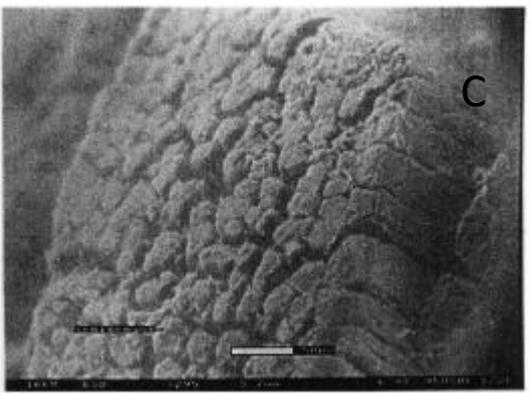
Perspectives



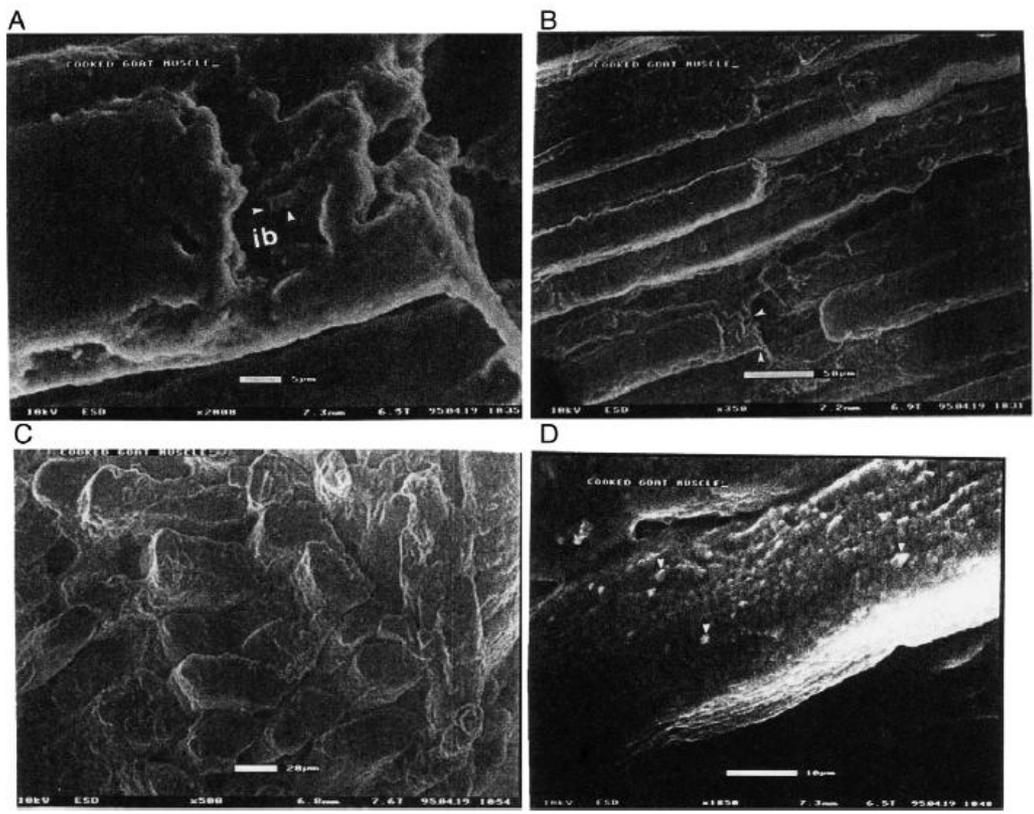
A-B) ESEM of structure of raw goat SM muscle



C) Structure of SM muscle using Miller stain showing bright collagen network



mf : muscle fiber; c : collagen

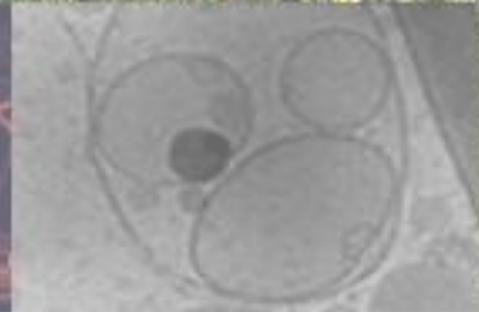
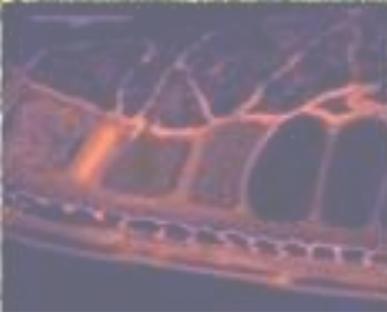
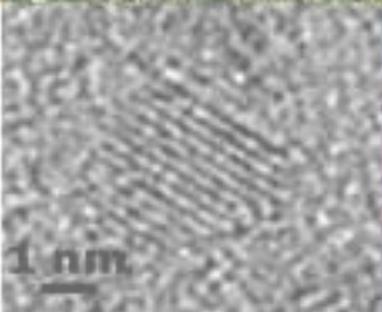
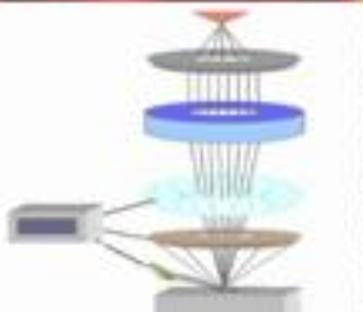
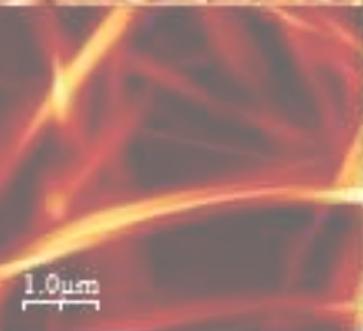


- A) ESEM of effect of roasted goat SM muscle with arrows indicating transverse breakage
- B) ESEM of transverse and internal breakage (ib) of individual muscle fiber,
- C) cross section of roasted goat SM muscle,
- D) individual muscle fiber in roasted goat SM muscle showing granulation at surface of muscle.

M.S. Yarmand, A. Homayouni / Meat Science 86 (2010) 451–455

Réseau national des Microscopistes de l' INRA

Merci de votre attention



Des questions ???