Following the Question: From Bedrest to Outer Space and Beyond

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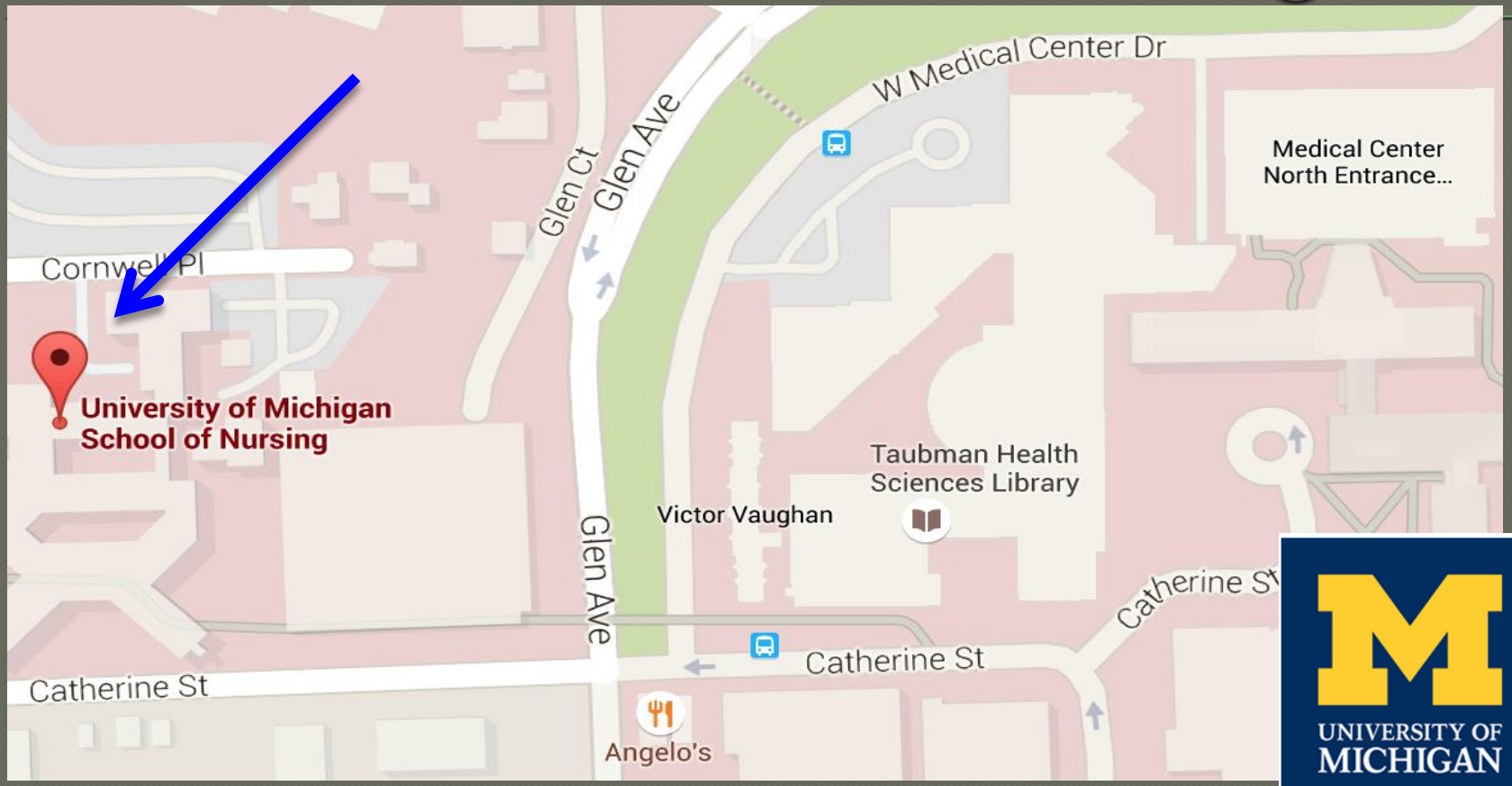
Where it began



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UNIVERSITY OF MICHIGAN

The Question

Why are patients so fatigued after a period of bedrest or immobilization?

And how can the rate of recovery be shortened?

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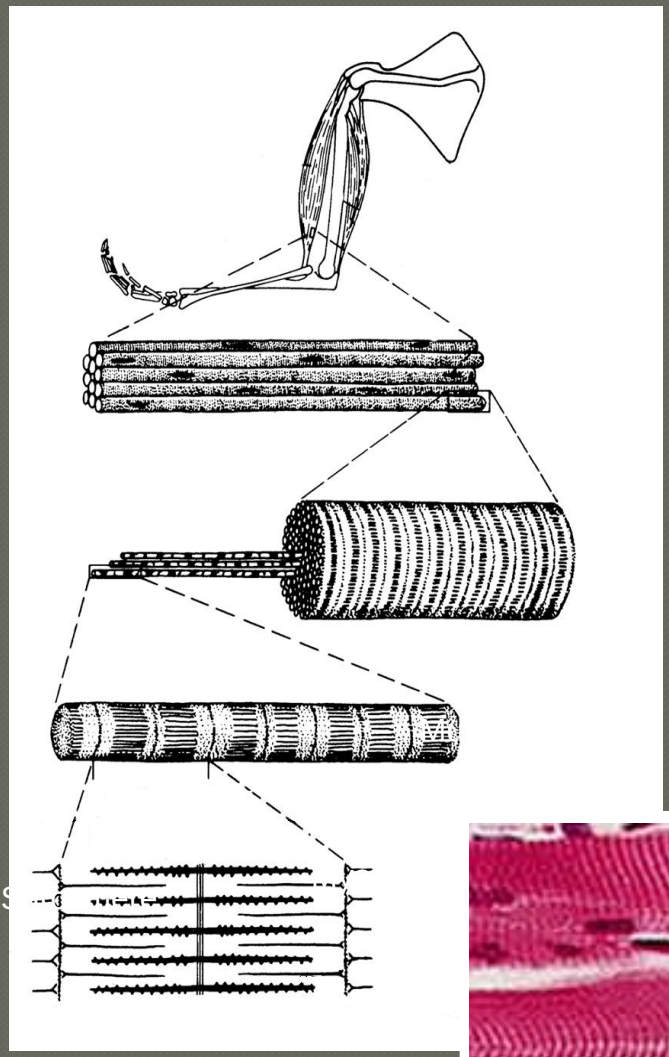
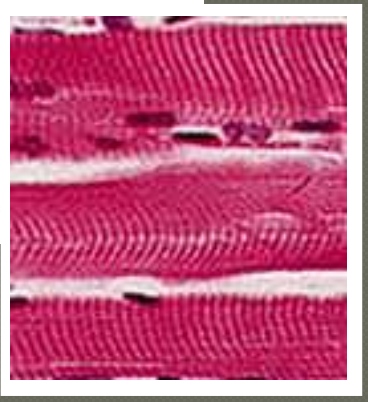
Causes of Skeletal Muscle Atrophy

- Disuse
  - Activities of Daily Living
  - Weight-bearing motion
  - Bed rest
  - Mechanical ventilation
  - Orthopedic immobility
  - Paralysis
  - Muscular Dystrophies
  - Glucocorticoids & Chemotheraputic agents

Structural hierarchy of skeletal muscle

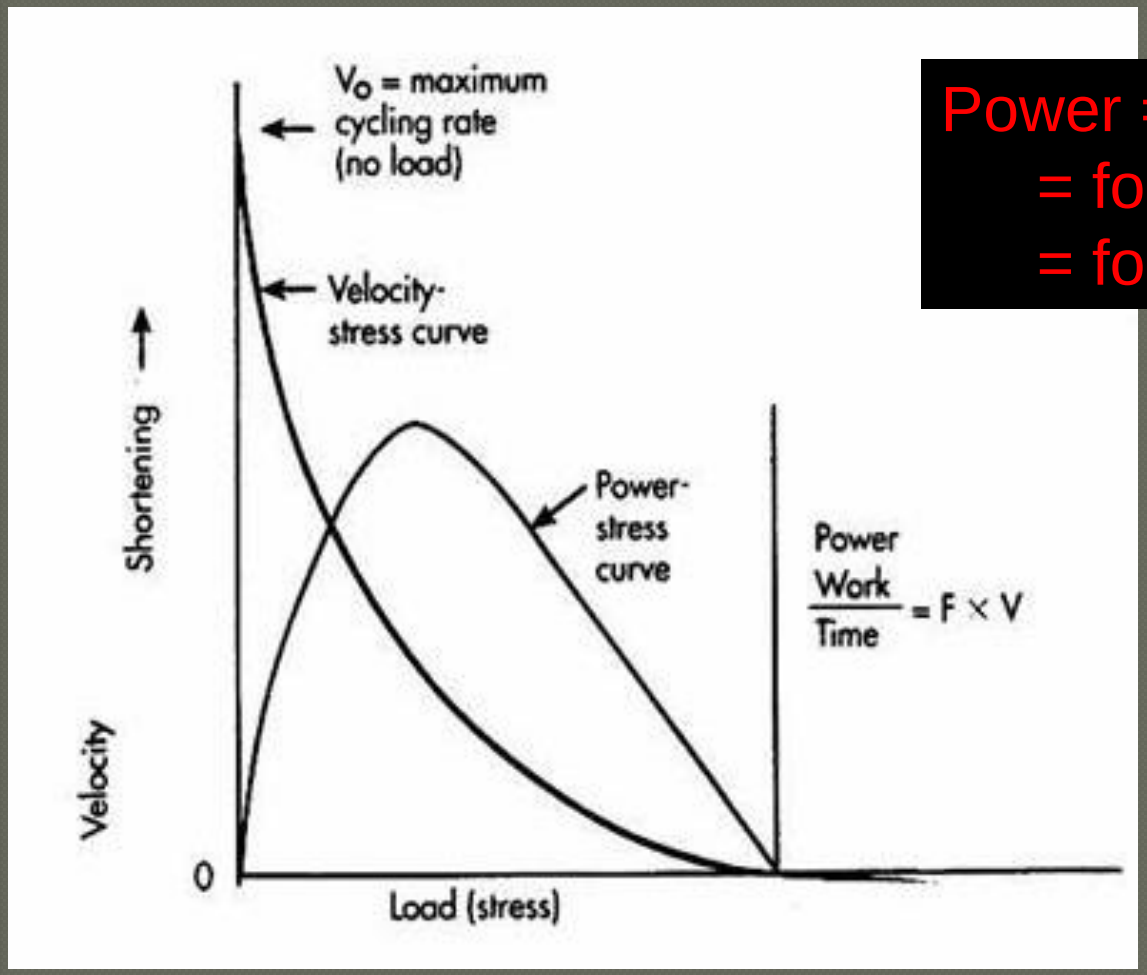




A little less than half of the body's mass is composed of skeletal muscle, with most muscles linked to bones by tendons through which the forces and movements developed during contractions are transmitted to the skeleton.

Modified from McMahon, *Muscles, Reflexes and Locomotion* Princeton University Press, 1984.

Power output: the most physiologically relevant marker of performance



Power = work / time
= force x distance / time
= force x velocity

Peak power obtained at intermediate loads and intermediate velocities.

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Figure from Berne and Levy, *Physiology* Mosby—Year Book, Inc., 1993.

Aims

- What was the morphological response of skeletal muscle to 4 weeks of hypokinesia?
- What was the influence of physical conditioning on the recovery of structure and function of skeletal muscle from hypokinesia?

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How Do You Mimic Bedrest in an Animal Model ?

- Criteria
  - Removal of Loading/weightbearing
  - Preserve movement
  - Preserve ability to isometrically contract

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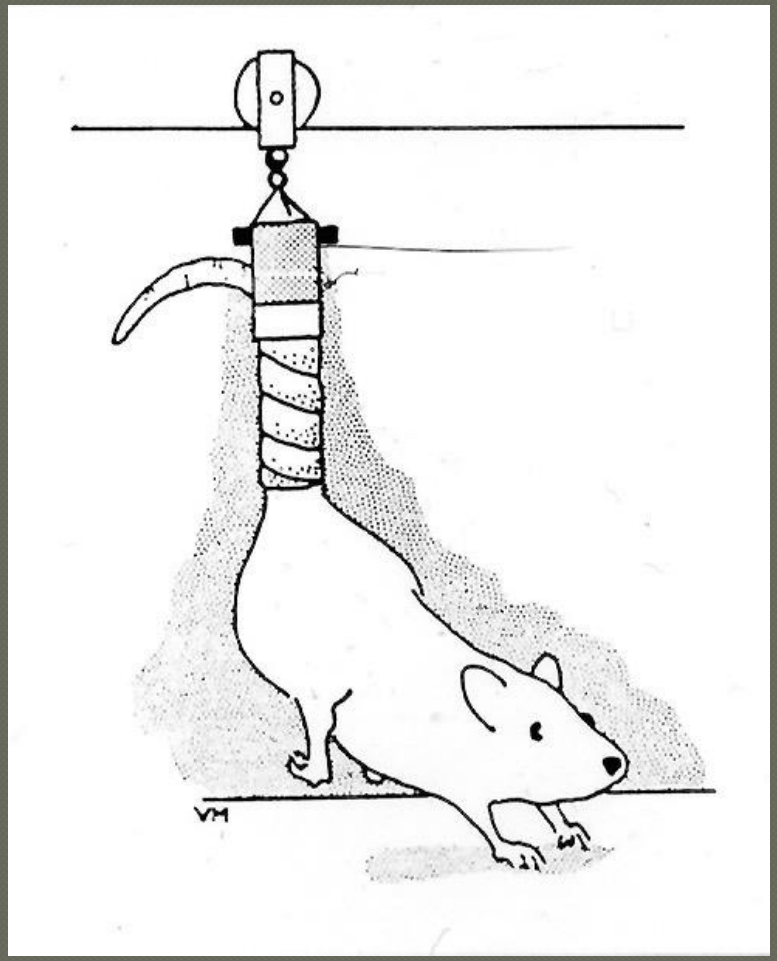
Tail Hindlimb Suspension



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How Do You Mimic Exercise in an Experimental Animal Model ?

- Criteria
  - Graded levels of speed
  - Enforcement of movement
  - Ability to quantify exercise

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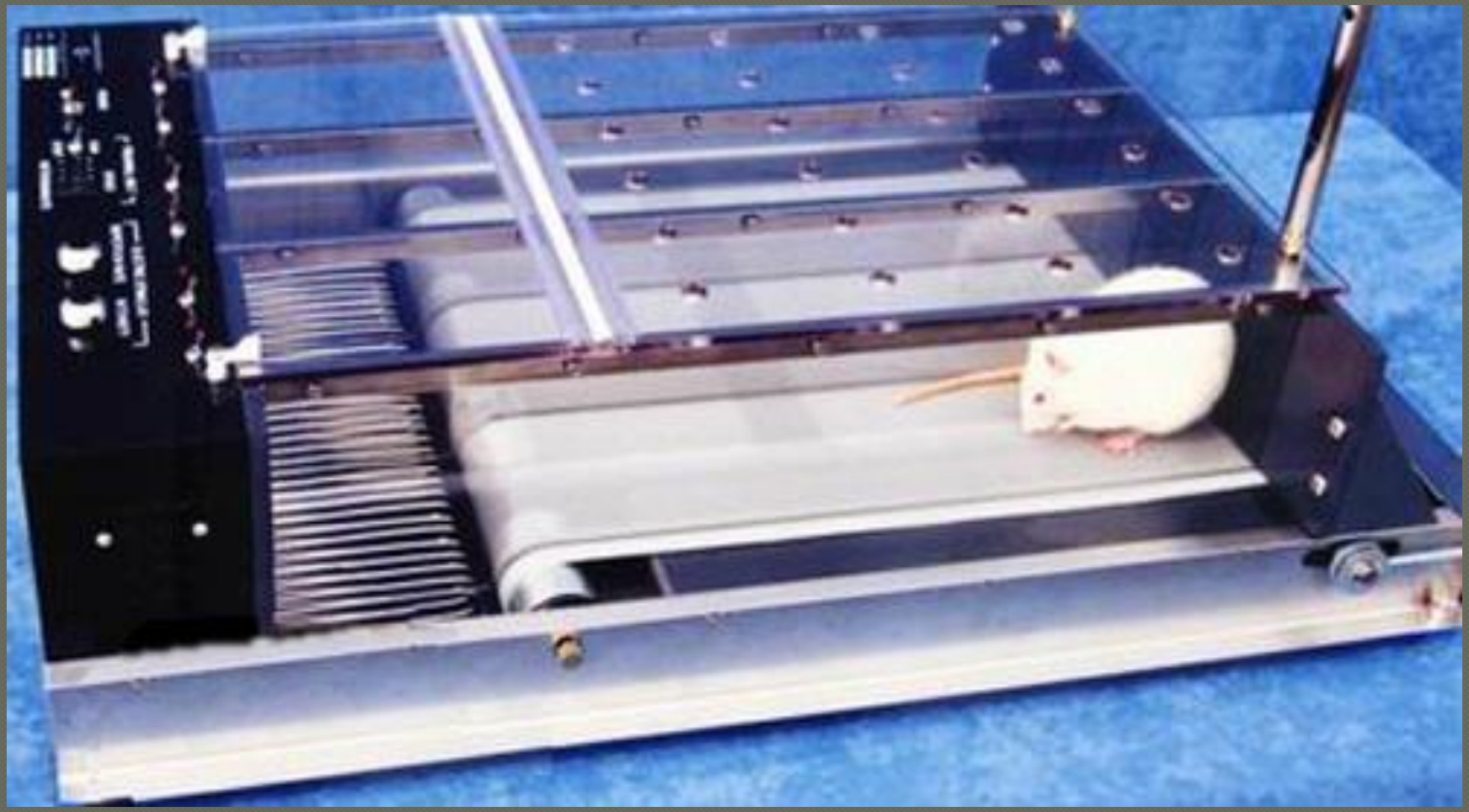
4 Stall Rodent Treadmill



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Running during recovery from hindlimb suspension induces transient muscle injury

CHRISTINE E. KASPER, TIMOTHY P. WHITE, AND LEO C. MAXWELL
Department of Kinesiology, The University of Michigan, Ann Arbor, Michigan 48109-2214;
and Department of Physiology, University of Texas Health Science Center, San Antonio, Texas 78284

KASPER, CHRISTINE E., TIMOTHY P. WHITE, AND LEO C. MAXWELL. *Running during recovery from hindlimb suspension induces transient muscle injury.* J. Appl. Physiol. 68(2): 533–539, 1990.—The objectives were to study morphological adap-

and 28 days of hindlimb suspension and at 7, 14, and 28 days of recovery. The objective was to determine the time course and magnitude of recovery of soleus muscle fiber characteristics when muscle loading was resumed after 1

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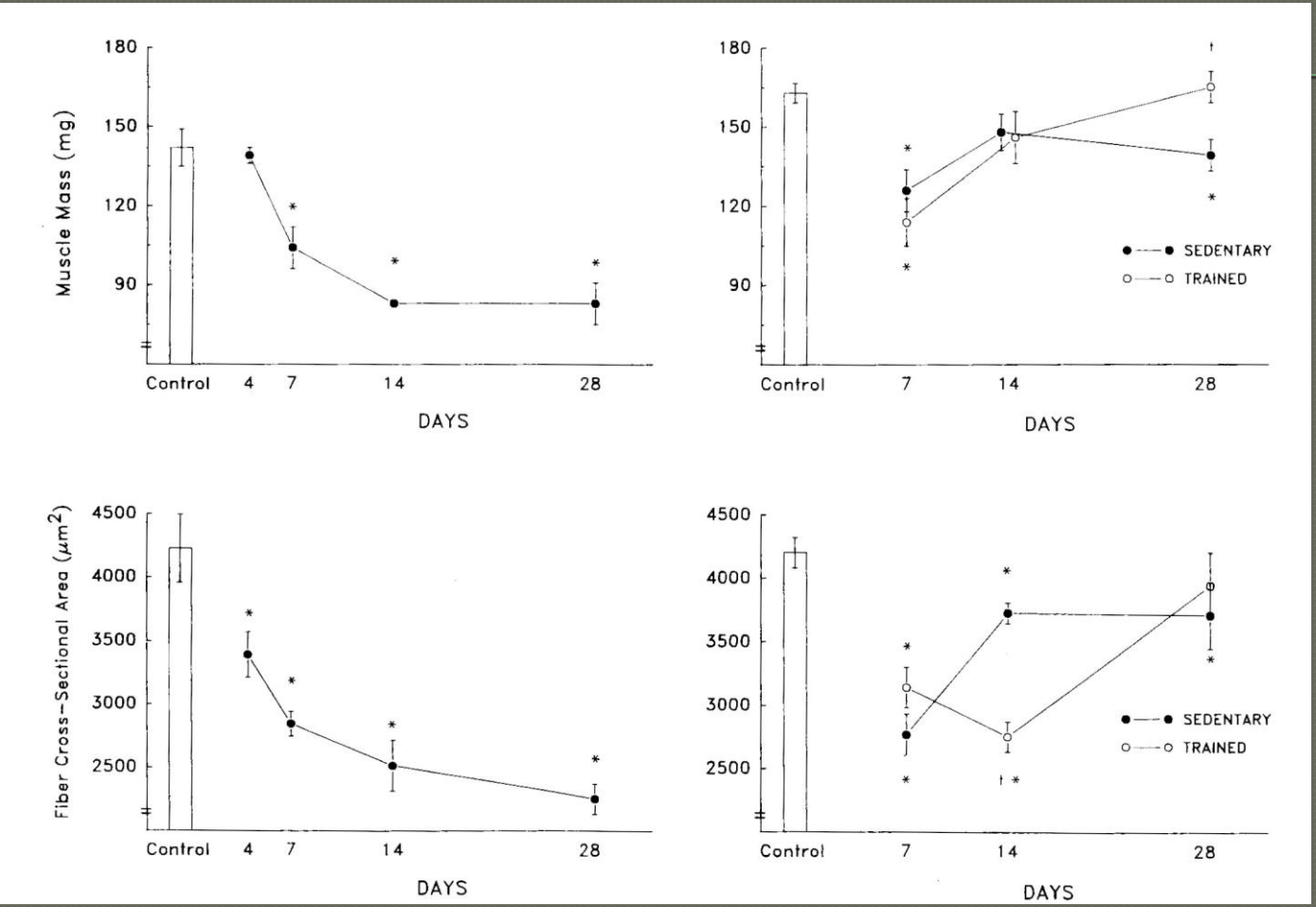
HS

Recovery



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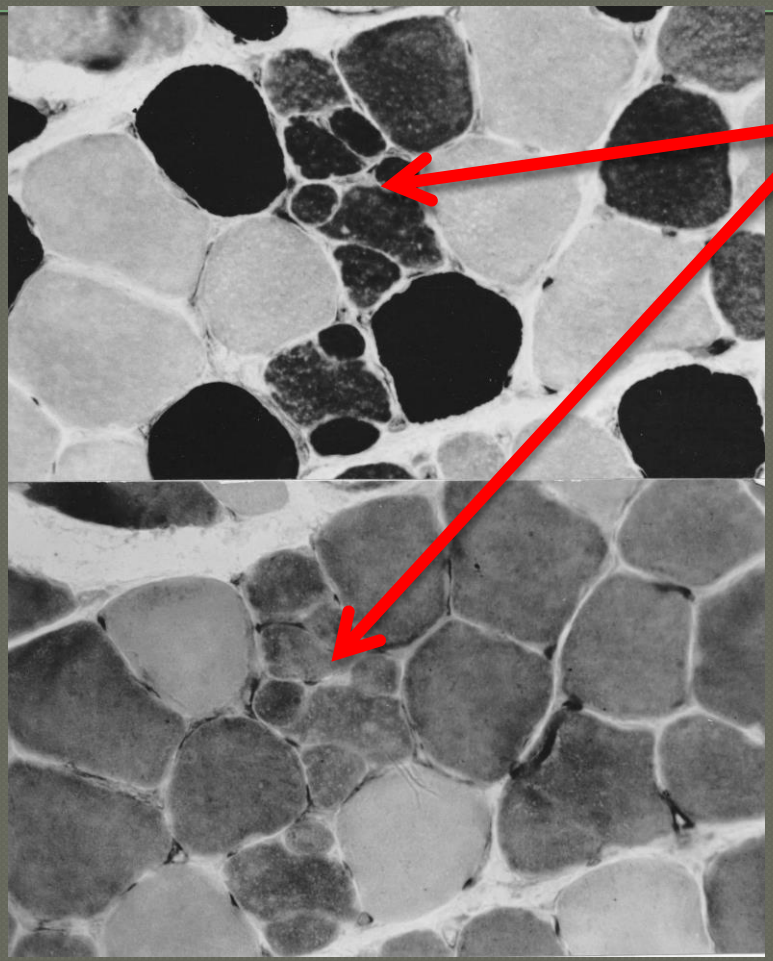
Soleus 14th day of Recovery from 28 days of Hypokinesia

Top: myofibrillar ATPase, pH 10.3 at 840x mag



Type IIC fibers/ new

Bottom: Top: myofibrillar ATPase, pH 4.3 at 840x mag

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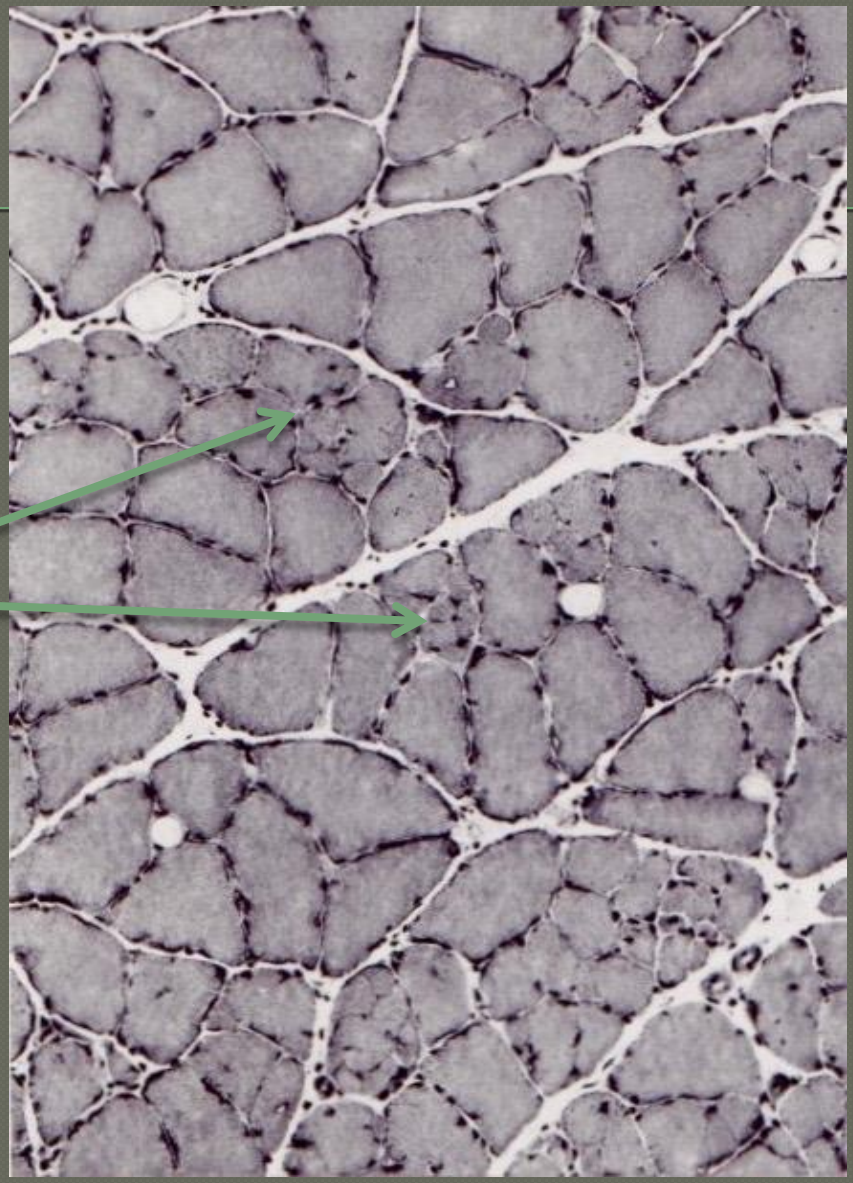
Soleus: H&E Stain. 7 days of Conditioning after 28 days of HS



Areas of necrosis & regeneration

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Question

- Why are individual atrophic myofibers are necrosed during recovery?
- Are the contractile proteins changing?
- Is the speed of myofiber (muscle cell) shortening changing?

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Myosin subunits and contractile properties of single fibers from hypokinetic rat muscles

PETER J. REISER, CHRISTINE E. KASPER, AND RICHARD L. MOSS
Department of Physiology, School of Medicine and School of Nursing, University of Wisconsin, Madison, Wisconsin 53706

REISER, PETER J., CHRISTINE E. KASPER, AND RICHARD L. MOSS. *Myosin subunits and contractile properties of single fibers from hypokinetic rat muscles.* J. Appl. Physiol. 63(6): 2293–2300, 1987.—The effects of prolonged hypokinesia on the contractile properties and myosin isozymes of single fibers from

and a prolongation of these times for the EDL have been reported (33, 35, 36).

The effects of hypokinesia have also been examined using methods of suspension with a harness wrapped either around the tail or around the trunk of the body

- 31% increase in Vmax SOL fibers
- Marked increase f-MHC proteins in SOL
- Changes in myosin isozymes corr with changes in Vmax

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Question

- Is bedrest and hypokinesia the same as in a microgravity (μg) environment?
  - Microgravity = weightless environment = Spaceflight
  - Severe atrophy known to occur following spaceflight in humans

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Launch of STS-48
or Rats in Space

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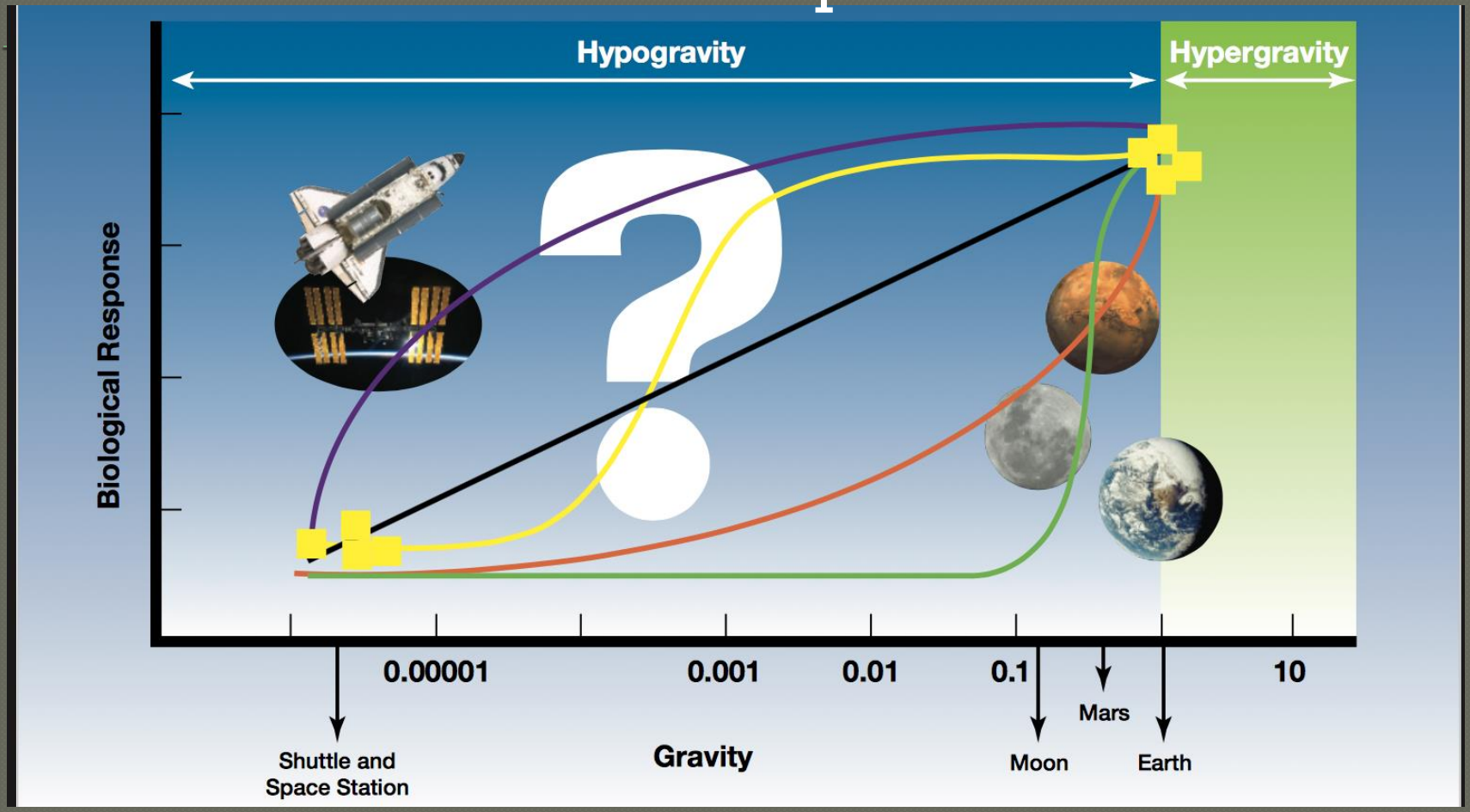
Long-term survival in space is dependent on muscle adaptation



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Physiological and Anatomical Rodent Experiment 1

- PARE 1
  - Species: *Rattus norvegicus* (Rat)
    - 26 days old, juvenile
  - Microgravity vs hindlimb suspension (HS)
- Microgravity & HS for 5.4 days
- Muscles were removed < 3 hours of landing.
- Determine response of myonuclei to µg

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PARE

Middeck with the Animal Enclosure Module

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PARE *Animal Enclosure Modules*

Animal Access Unit (AAU)

Rodent Habitat

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Muscle adapts at the level of the myonucleus during short-term μg

Journal of Muscle Research and Cell Motility **17**, 595–602 (1996)

Cytoplasm-to-myonucleus ratios following microgravity

CHRISTINE E. KASPER and LIN XUN

Jerry Lewis Neuromuscular Research Center, University of California, Los Angeles, 700 Tiverton Ave, Box 956918, Los Angeles, CA 90095-6918, USA

Received 6 November 1995; revised 16 February 1996; accepted 1 April 1996

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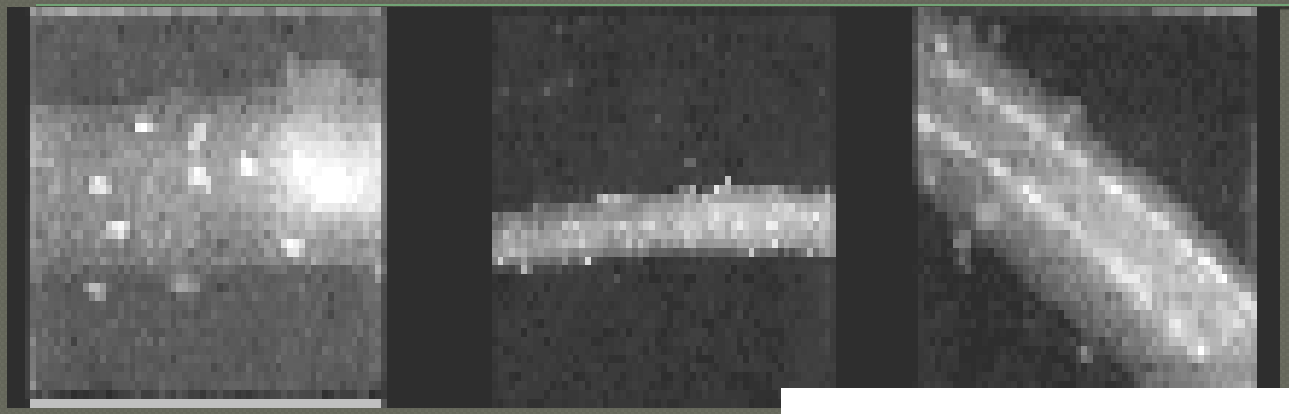
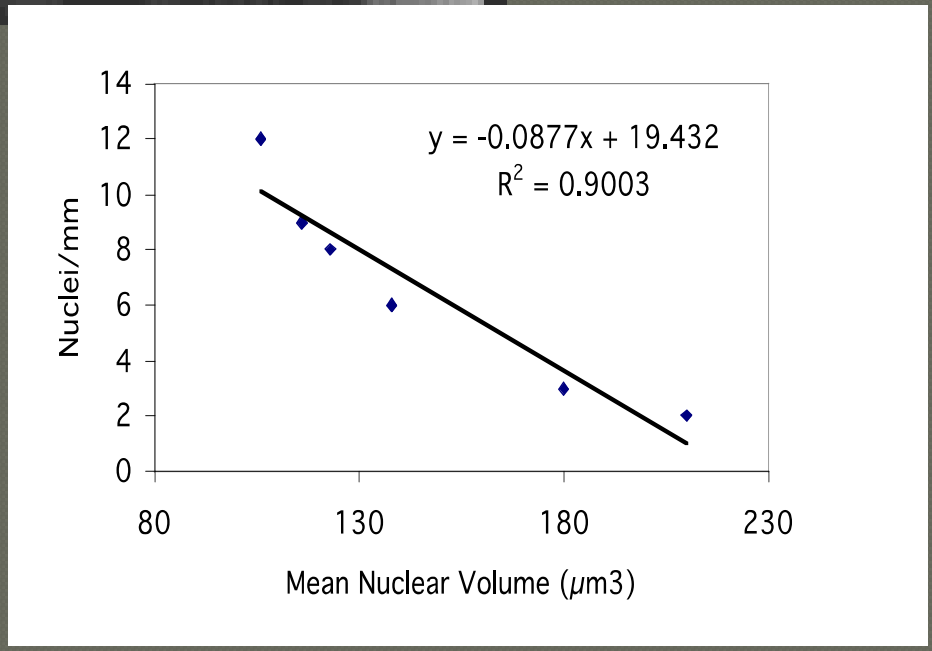
Myonuclei realign during adaptation



Mean nuclear volume vs. number of nuclei per mm in neonatal SOL. From L: 4, 7, 14, 21, 28, 32, and 42 days . R^2= 0.9003.



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Question

Does skeletal muscle structure and function change in the presence of heavy metals?

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Muscle and Military Relevant Heavy Metals

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Wounded Warriors: By the Numbers

- Total WIA in GWOT: 52,336
- Total number of shrapnel injuries unknown
- Estimates: 35-50% of injured military personnel retain embedded fragments

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Source: www.defense.gov/news/casualty.pdf, accessed May 8, 2015, 10am EST.

Early Embedded Metals

- ….."By the late seventeenth century, when it was realized that the ball was not poisonous, surgeons were urged not to probe deeply but rather to let the ball remain if it could not be located easily."

  - Mary Gillette, "The Army Medical Department, 1775-1818," p. 18.

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Six Ounces of Shrapnel Found in WWII Vet's Remains

abc NEWS

Ronald Brown Lived 68 Years Believing He Had a Single Bullet in His Leg

Rex Features via AP Images

The family of a World War II veteran was surprised to find about 6 ounces of shrapnel in his remains following his cremation.

By JOSHUA GARDNER
10/20/12, 6:38 AM EDT

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Gulf War I and OEF/OIF Exposures to DU

- Friendly-fire incidents exposed US soldiers to:
  - DU shrapnel
  - Aerosolized DU oxides
    - Inhalation, ingestion, wound contamination
- Burning of munitions storage facility
- Decontamination of military equipment

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Potential Toxicity of DU

- **Systemic effects due to release of U ions to circulation and uptake by organs**
  - Specific target organs of U
    - Kidney
    - Bone
  - Primarily chemical toxicity of U
- **Local effects on tissue surrounding DU embedded fragments**
  - May be combination of chemical toxicity and radioactivity of U

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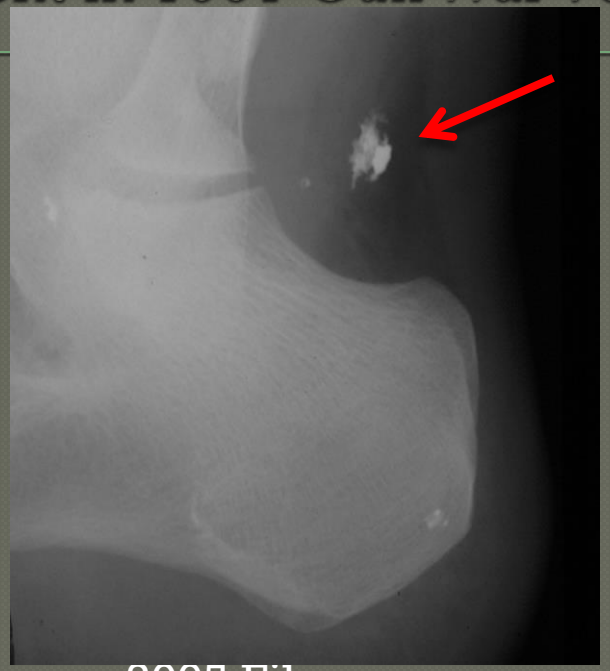
Embedded DU Fragment in 1991 Gulf War Veteran

1995 Film



2005 Film

Change in DU fragment appearance over time suggests oxidation in situ and removal of the fragment is warranted.

Source: McDiarmid, M., Engelhardt, S., & Squibb, K. S. (2006). Indications for surgical removal depleted uranium (DU) shrapnel in Gulf War I veterans. *Toxicological Science, 90*(S-1), 2072.

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DU pellet implants: new and 12 weeks

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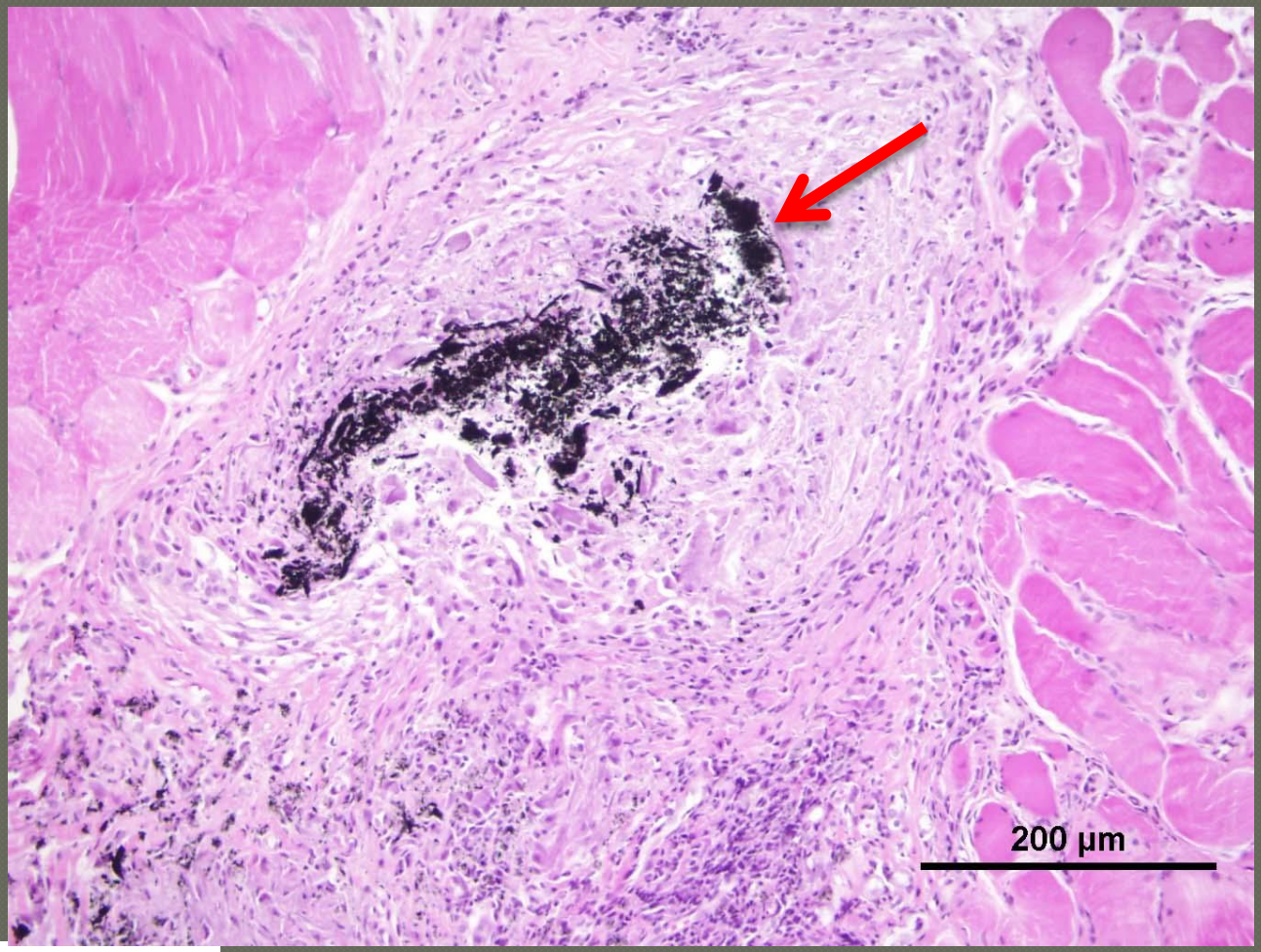
Pellet Implantation Site – DU / 3 Month Group



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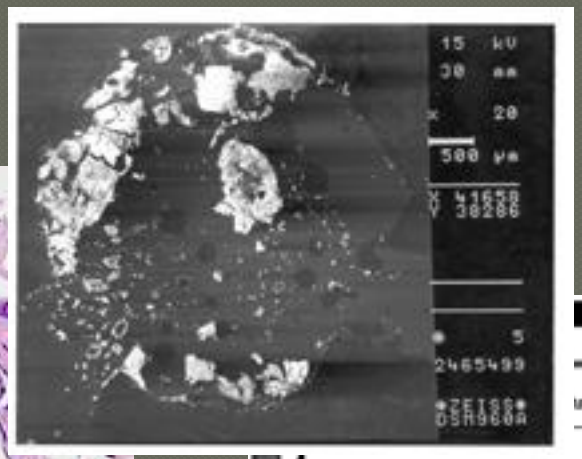
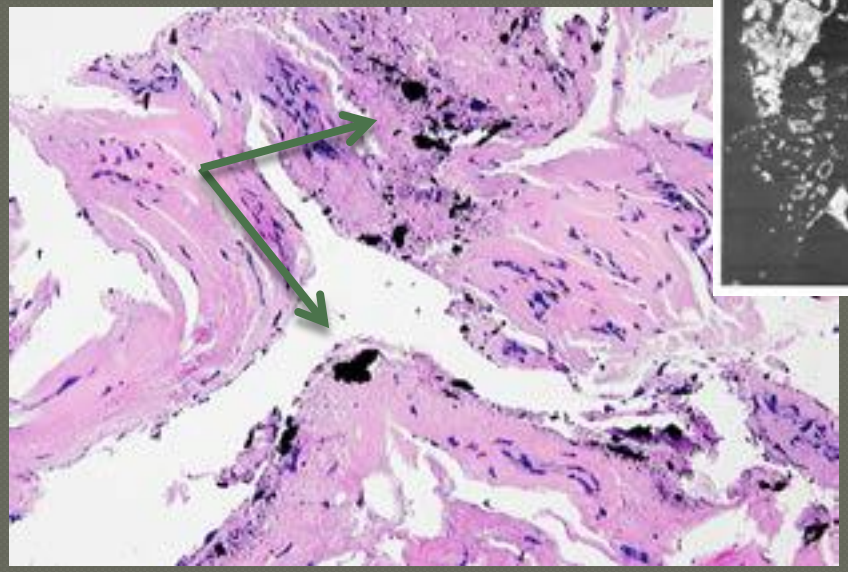
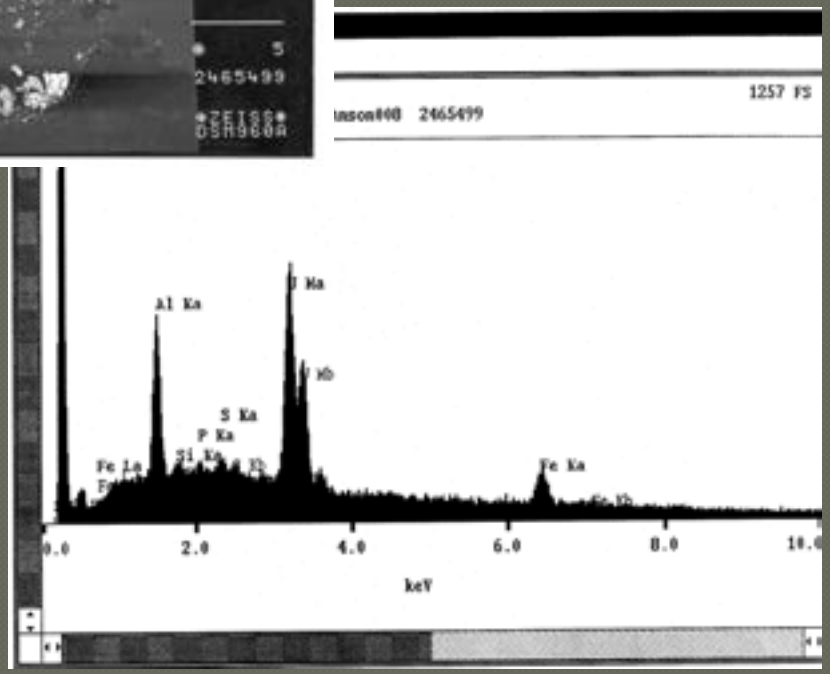
Raman Spectroscopy for Metals Identification

Scanning EM of DU inclusion



DU





Energy dispersive x-ray microanalysis of DU inclusion

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Alternatives to DU

- DU is considered the “gold standard” for armor penetrating munitions.

- However, continuing uncertainty about long-term DU health effects and widespread public efforts to ban use of DU in munitions have led to a search for substitutes for DU in munitions.

- Tungsten alloys WaNiCo or WaNiFe were selected

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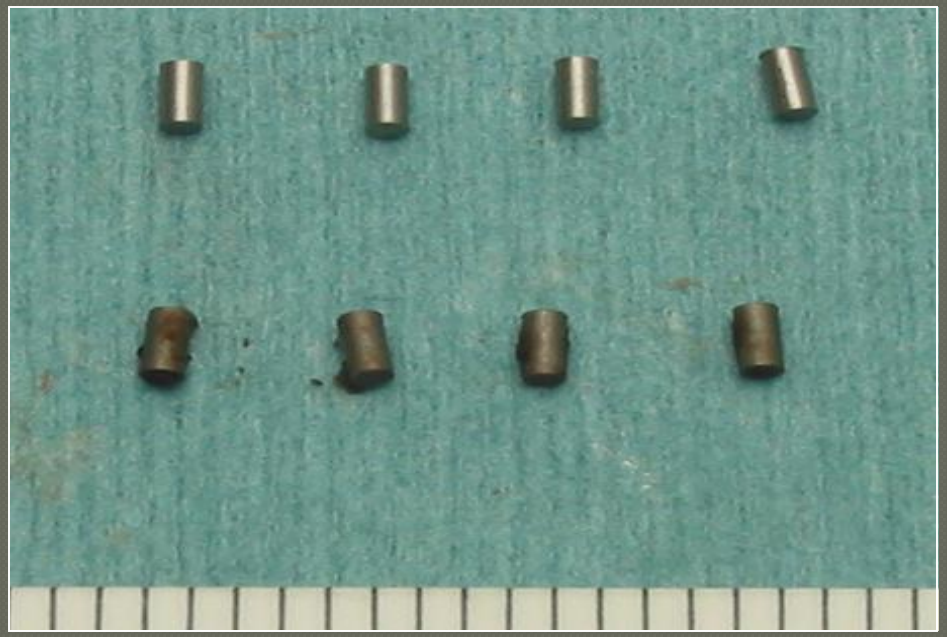
Tungsten Alloy Pellets

Prior to implantation

After 22 weeks of implantation



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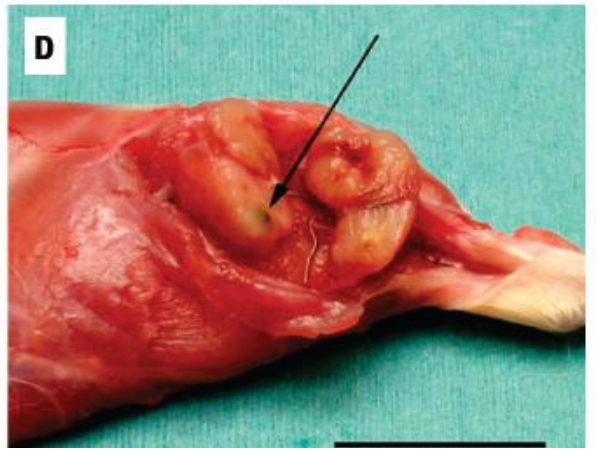
New Alloy Used in Munitions: W/Ni/Co

- **Metastatic tumors caused by implanted tungsten alloy (W/Ni/Co) fragments in rats**
  - Rhabdomyosarcomas rapidly metastasize to lung
  - Survival time is significantly reduced

Kalinich et al. Env. Hlth Perspec. 113: 729-734, 2005



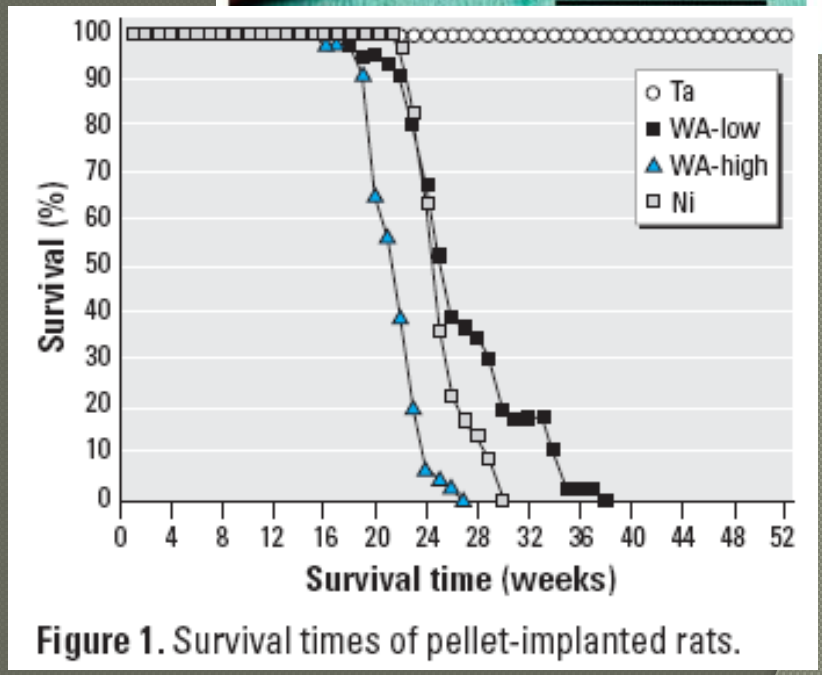


Figure 1. Survival times of pellet-implanted rats.

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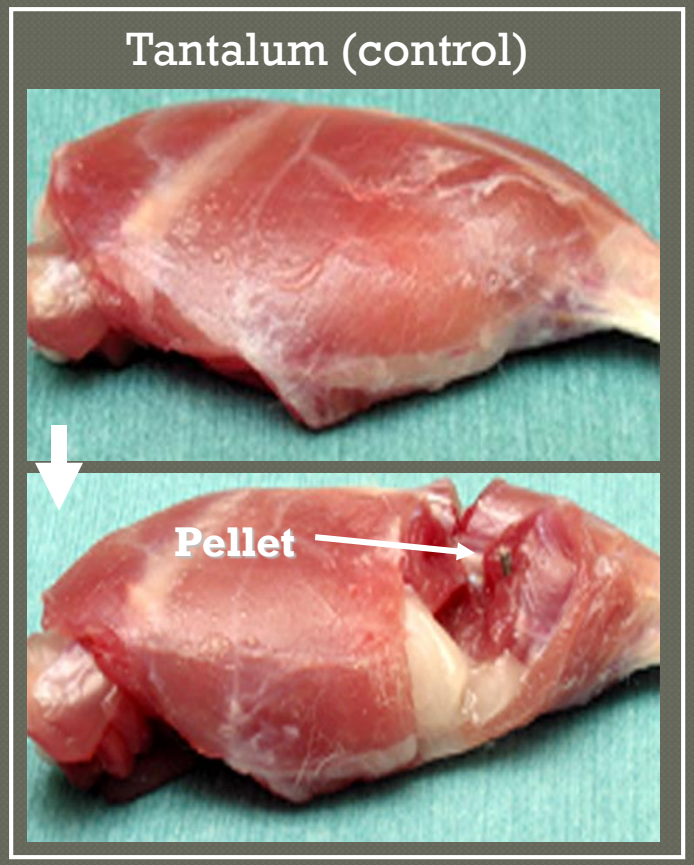
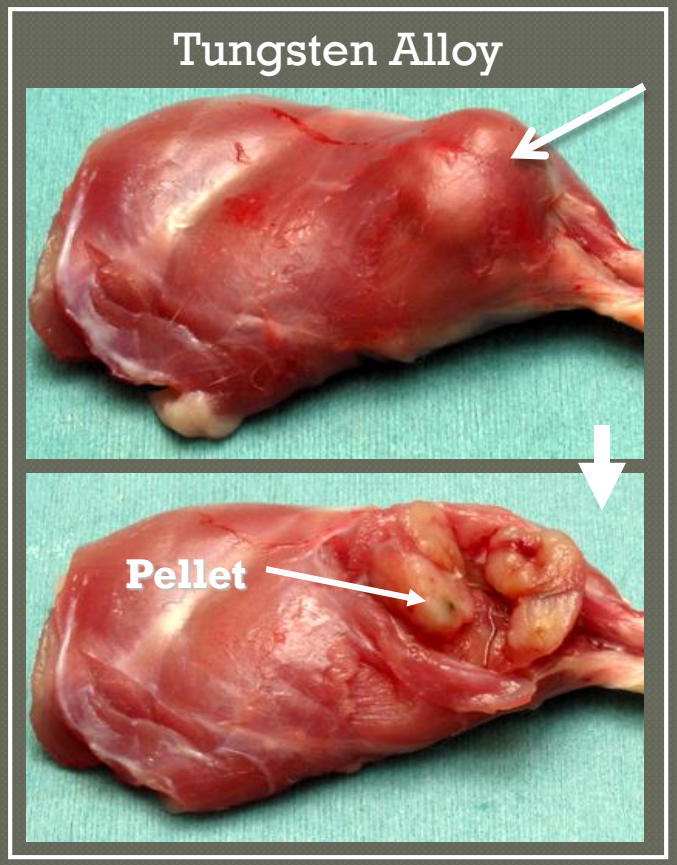
Tungsten Alloy-Associated Tumors





- No tumors in Ta controls
- Rapidly growing tumors up to 5 cm in diameter

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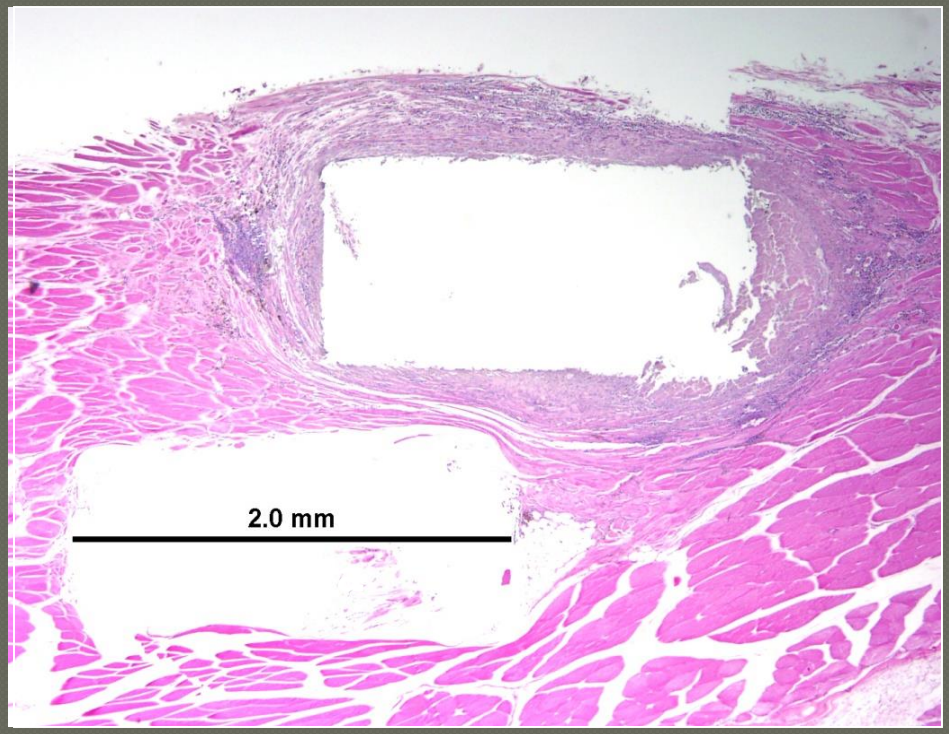
Tumor Development

WA versus Ta Pellet

3 Month WA (low-dose) Group



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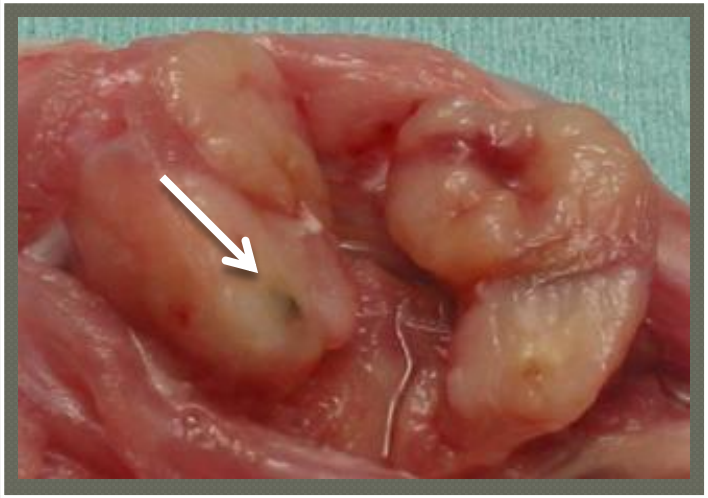
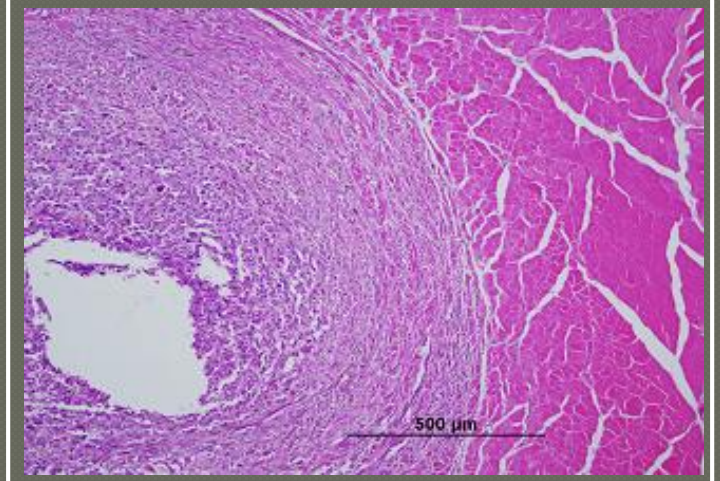
WA Tumor Characterization





- Tumor surrounds pellet
- Tumor displaces and replaces skeletal muscle around pellet
- Tumor identified as rhabdomyosarcoma

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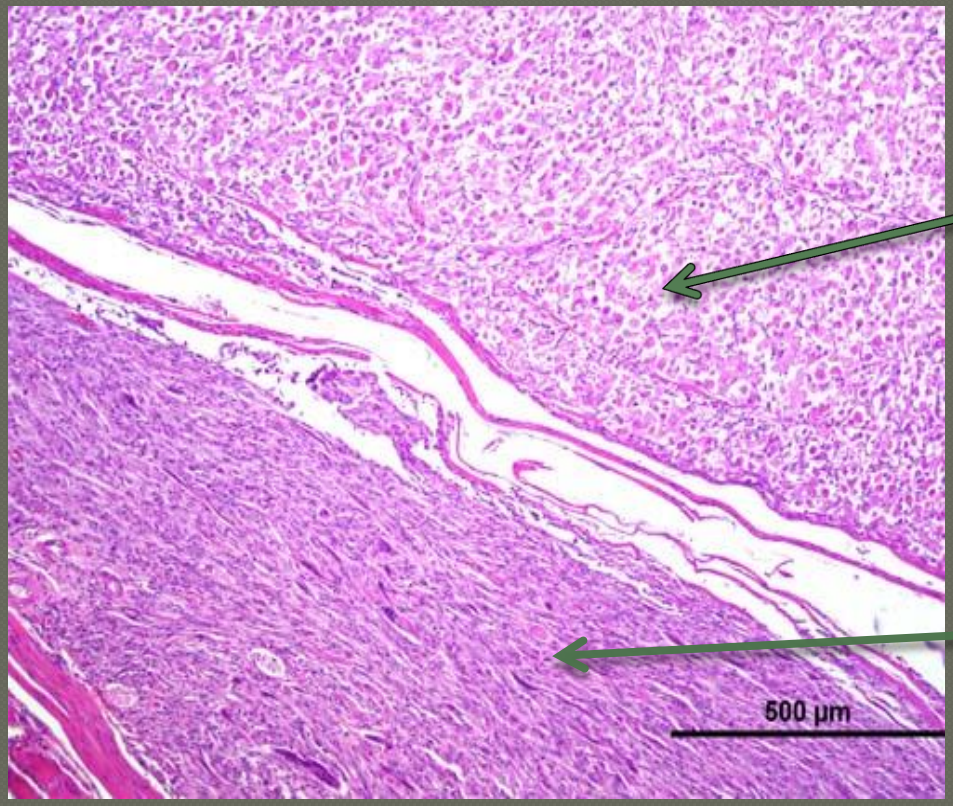
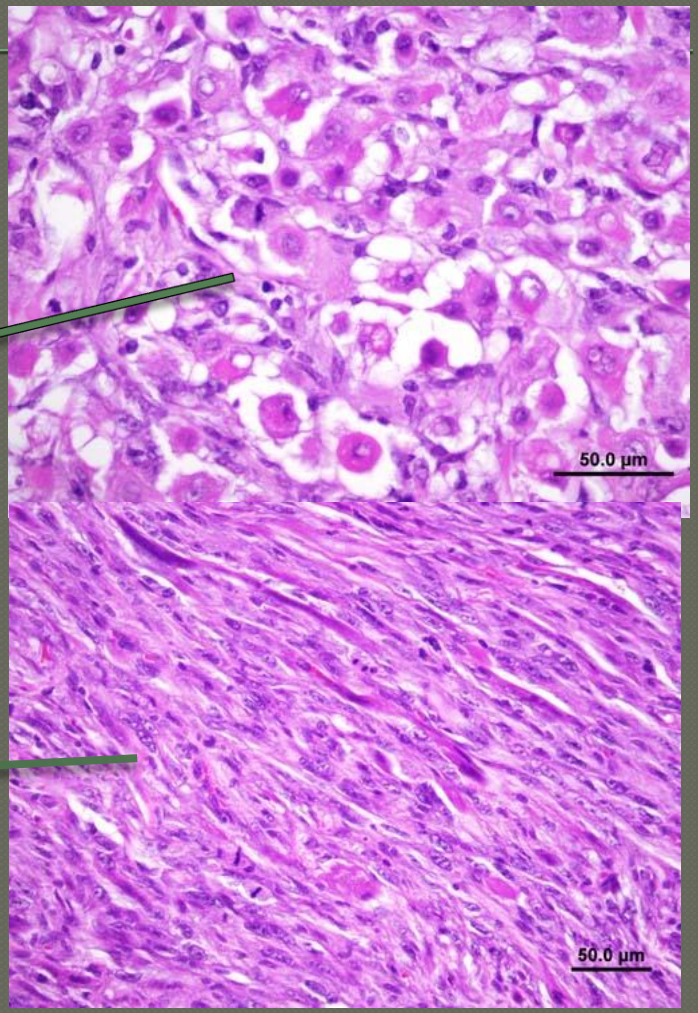
Variable Rhabdomyosarcoma Patterns

Embyonal + Pleomorphic RMS
Co-located





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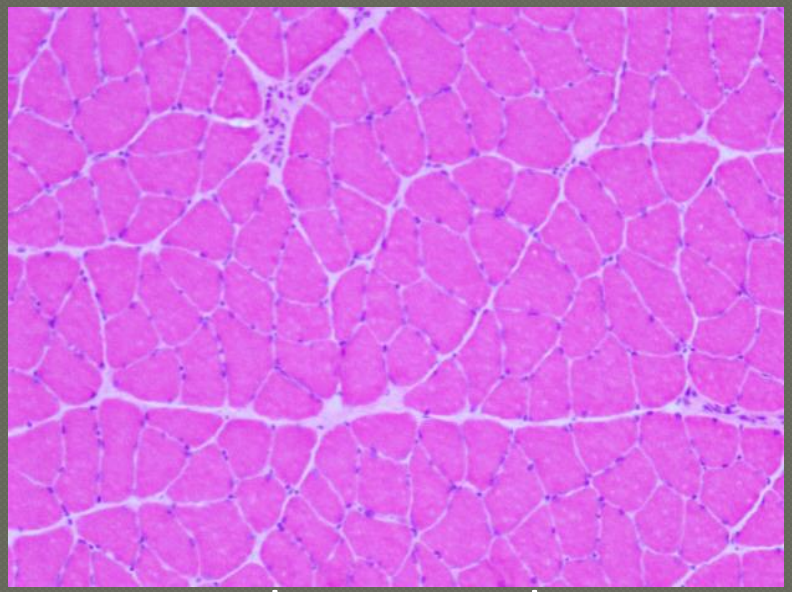
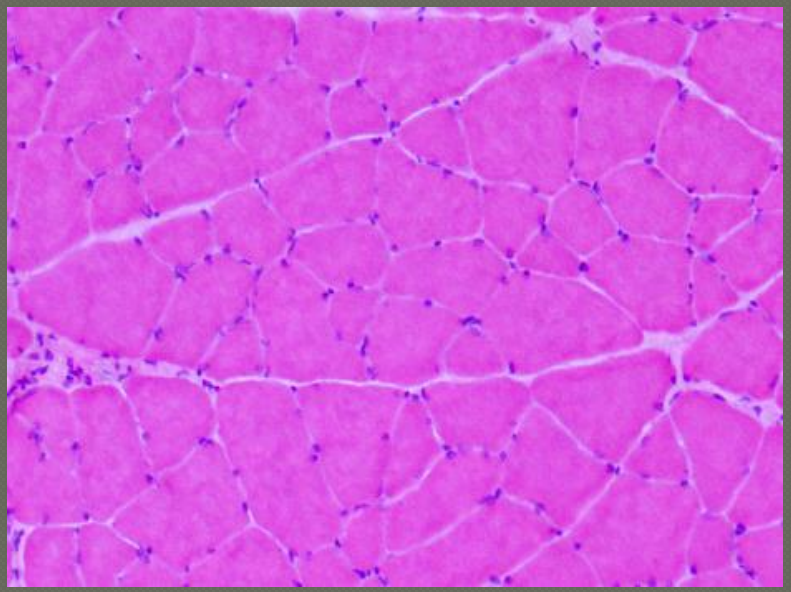
Histology – H & E
Sham Control Week 1



ID# 1 Soleus Muscle



ID# 1 Plantaris Muscle

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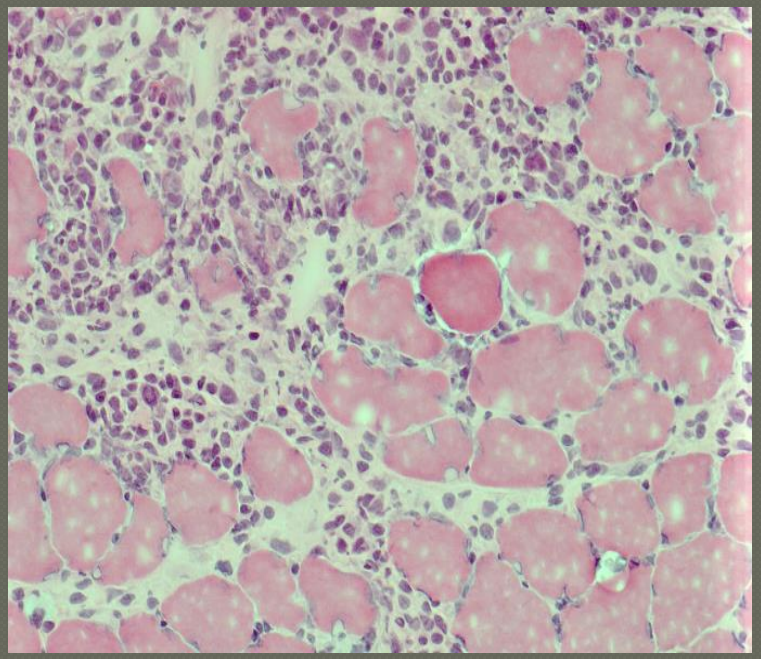
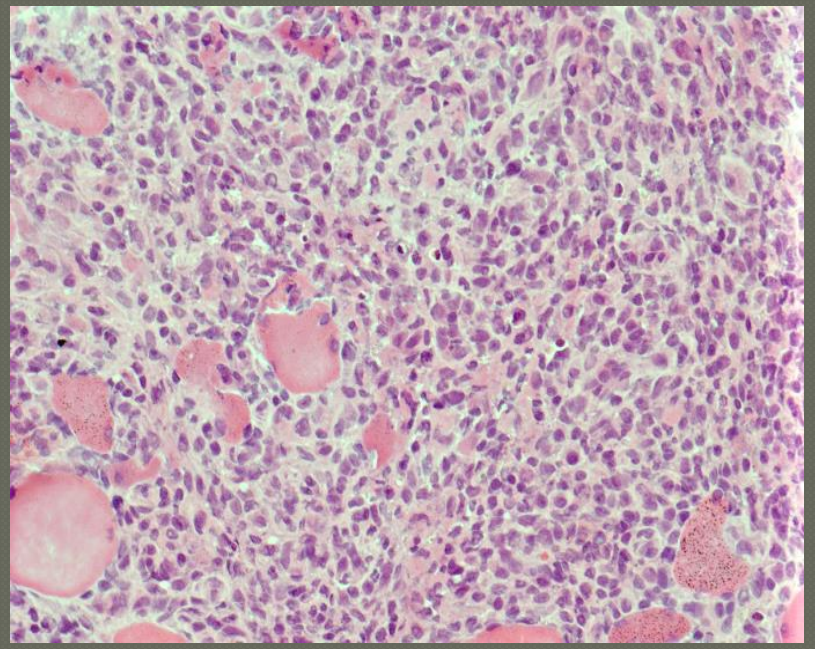
Histology - H & E
WA Week 1



ID# 25 Soleus Muscle



ID# 26 Plantaris Muscle

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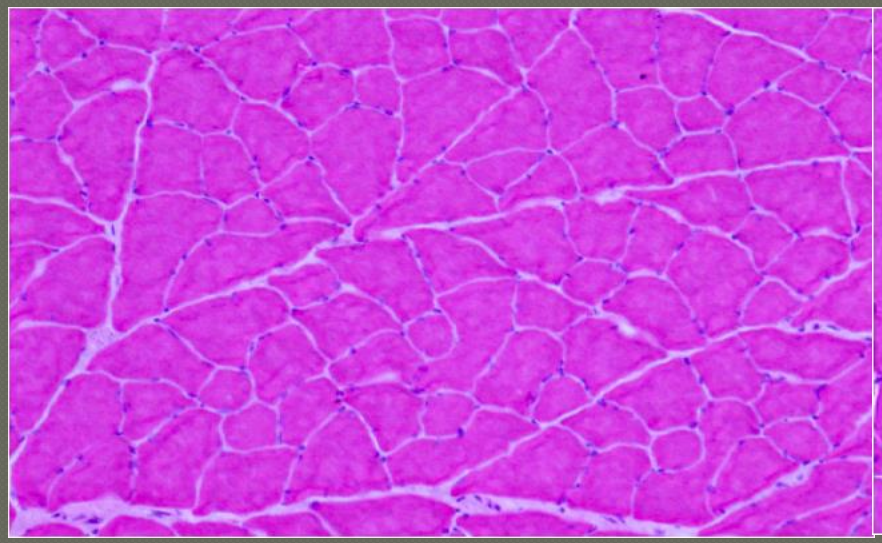
Controls H & E Week 16

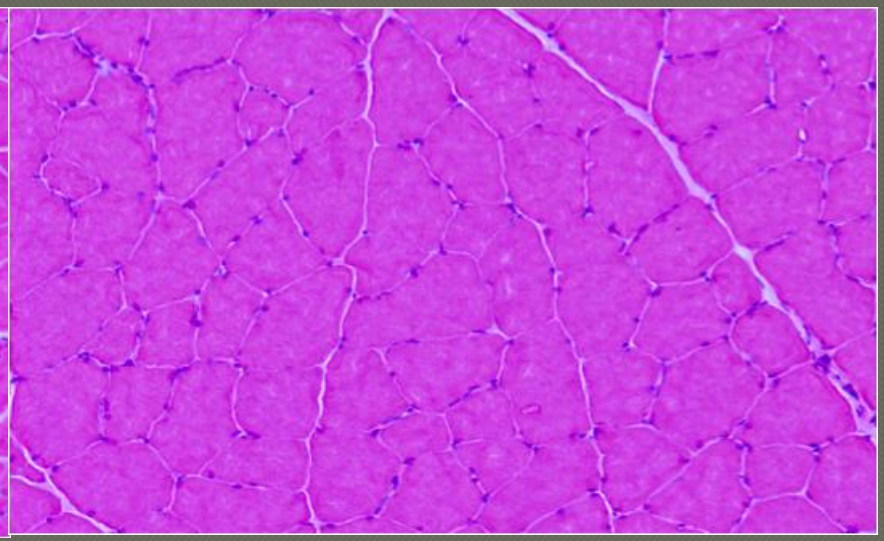
SHAM

- GASTROC Rat #1



TA

- GASTROC Rat #8



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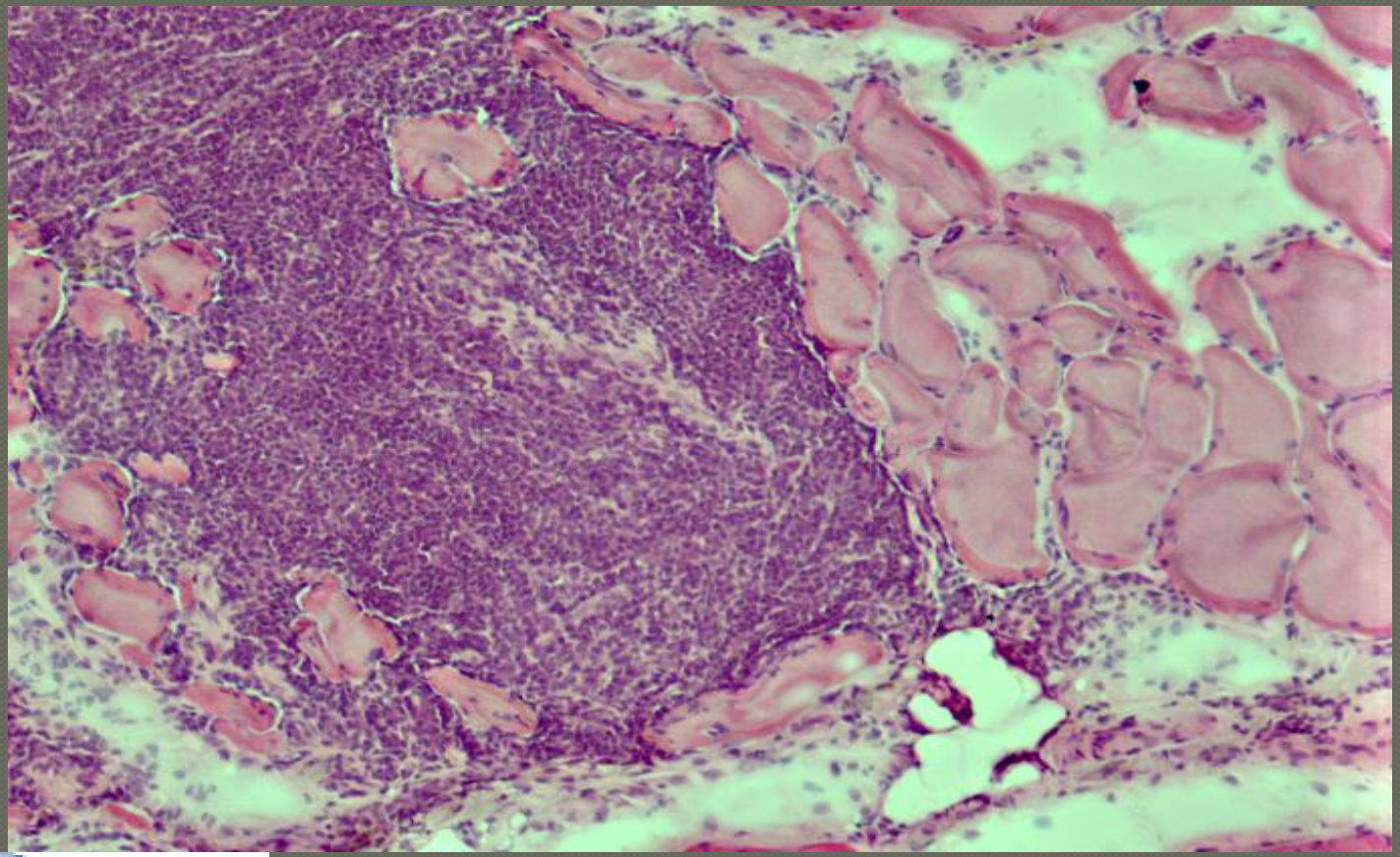
WA H&E Week 16

GASTROC Rat #19



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Public Health
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www.mdpi.com/journal/ijerph

Article

Genotoxic Changes to Rodent Cells Exposed *in Vitro* to Tungsten, Nickel, Cobalt and Iron

Stephanie Bardack [1], Clifton L. Dalgard [2], John F. Kalinich [3] and Christine E. Kasper [4,5,*]

Table 6. Validated genes found to be associated with genotoxic pathways.

Gene	Name	Accession #
CHRND	Chrnd cholinergic receptor, nicotinic, delta	NM_019298.1
EGR1	Egr1 early growth response 1	NM_012551.1
FN3K	Fructosamine 3 kinase	NM_001109051.1
FOS	Fos FBJ murine osteosarcoma viral oncogene homolog	NM_022197.2
NMT2	N-myristoyltransferase 2	NM_207590.1

Health Affairs Policy HA-07-029:
Policy on Analysis of Metal Fragments Removed from Department of Defense Personnel

- "…*requires* the Services to conduct a laboratory analysis of metal fragments, resulting from enemy or friendly fire, that are removed from surviving DoD personnel in DoD military treatment facilities (MTFs)."

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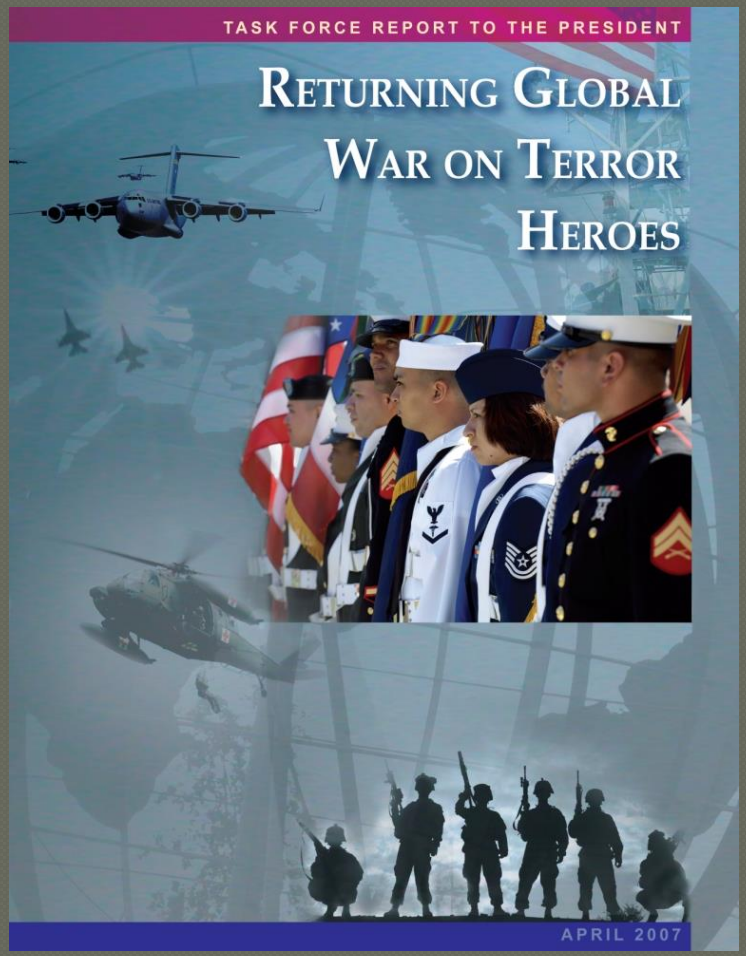
VHA directed to address the issue of embedded fragments

- Presidential Task Force Recommendation P-7 directed the VHA to establish a registry and medical surveillance program for Veterans with embedded fragments
- VA Toxic Embedded Metals Center



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The Questions Continue

- Do metals move to the brain with blast TBI?
- Can Reactive Oxygen Species (ROS) be blocked in metal embedded muscle to prevent cancer?
- What is the effect of HM on bone growth?
- And the question will continue…….

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