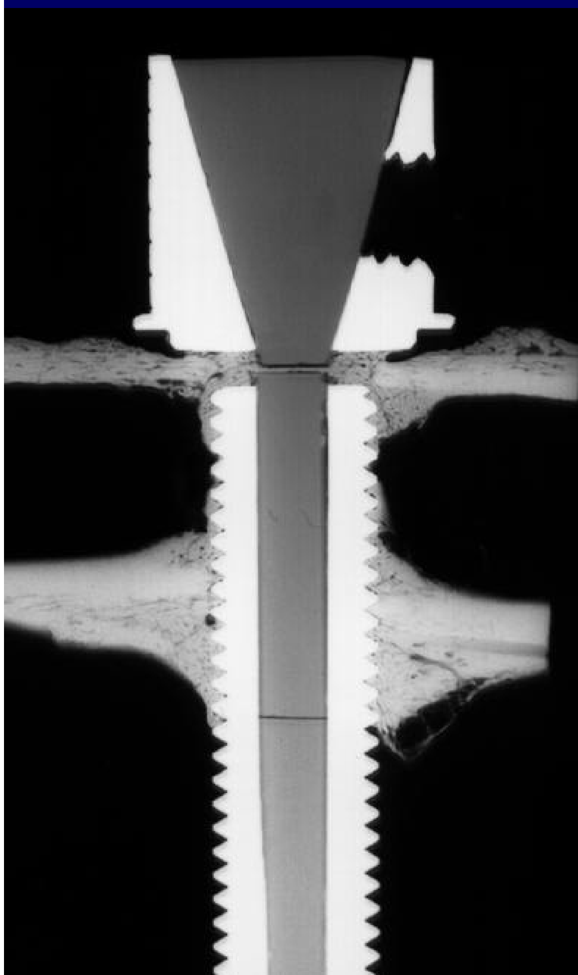
MUSCLE PUMP-ENHANCED MICROFLUIDIC PERCOLATION OF BONE AS AN ADJUNCT TO FLUID SHEAR STRESS MODULATION OF BONE CELLS *IN VIVO*



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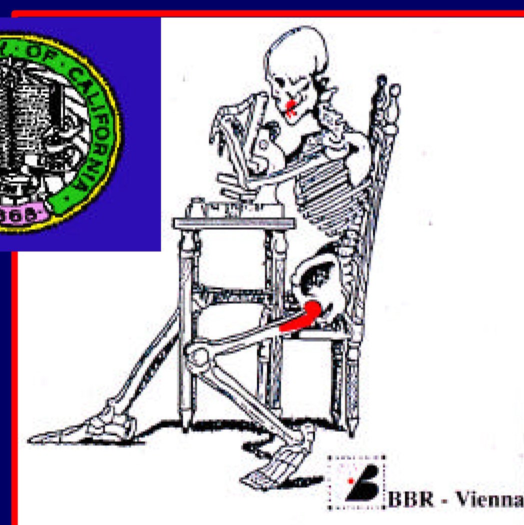
MICROFLUIDIC FLOWS IN NATURE AND MICROFLUIDIC TECHNOLOGIES

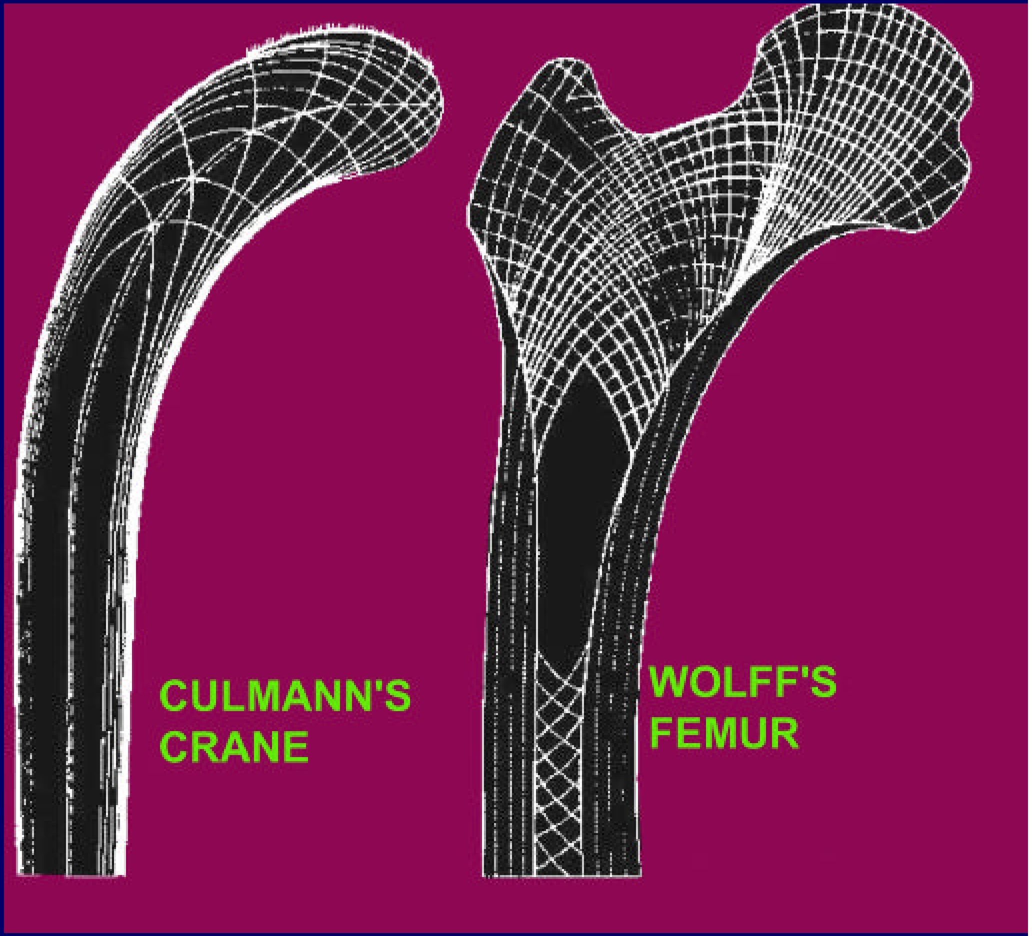


HOW IS THE STRUCTURE OF THE BONE SCAFFOLD DETERMINED?

A. WHAT IS THE CONTRIBUTION OF GENETICS (NATURE)?

B. WHAT IS THE CONTRIBUTION OF ENVIRONMENT (NURTURE)?

WOLFF'S SOLID MECHANICAL MODEL



WOLFF'S SOLID MECHANICAL MODEL
"Wolff's Law"

STRAINS IN BONE TISSUE RESULTING FROM ENVIRONMENTAL STRESSES ARE THE SIGNALS WHICH DIRECT REMODELING PATTERNS

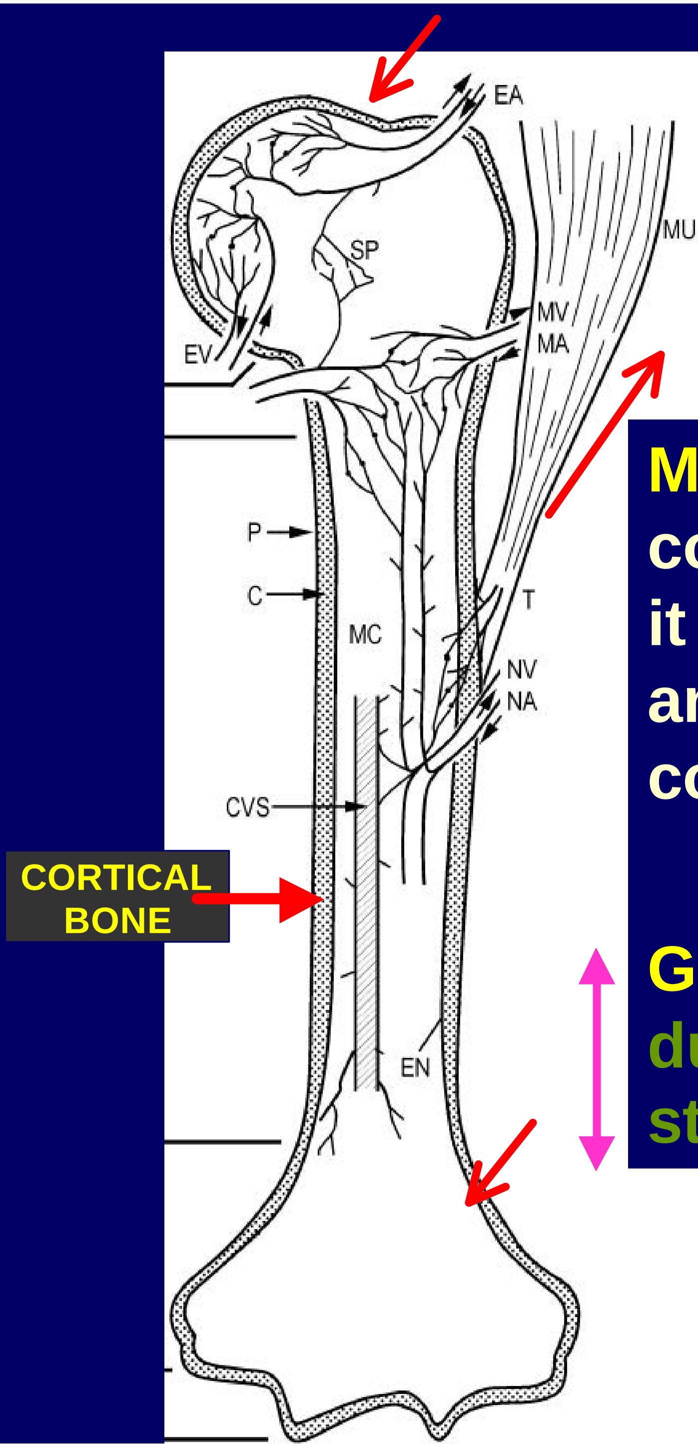
Subsequent experimental and clinical observations indicated that

TENSION? OSTEOGENESIS

COMPRESSION? RESORPTION

HEUTER-VOLKMANN "LAW"

Two sources of load which make bone deform



MUSCLE CONTRACTION: Inserts in cortical bone and pulls on cortex when it contracts, causing tension in distal and compression in proximal level of cortex

FOCUS OF THIS TALK

GRAVITY: Weight of bone stretches it during lift and compresses it during step

HOW IS STRESS ON BONE TRANSDUCED INTO BONE SCAFFOLD STRUCTURE?

A. In the 1940's cell electrophysiology advanced to indicate that all cells conducted currents to some degree.

B. Proteins, such as collagen, are zwitterions and could act as conductors.

HOW IS STRESS ON BONE TRANSDUCED INTO BONE SCAFFOLD STRUCTURE?

C. Fukada showed that all proteins tested were piezoelectric crystals.

D. Yasuda tested* hypothesis that bone collagen piezoelectrically converted bending stress into currents which activated bone cells =The mechanism for Wolff's Law

*ON DRY BONE

HOW DIFFERENT IS WET (PHYSIOLOGICAL) BONE?

It has been known since 1970 *[Andersen & Erickson, Nature]* that the mechanical signal which modulates bone cell behavior *in vivo* comes directly (>97%) from bone's fluid (>97%) not its solid (<3%) phase.

RECENT HISTORY OF MECHANICAL MODULATION OF BONE REMODELING

NAME	CONCEPT	DATE
Julius Wolff SOLID CRANE	Living bones change in accord ance with the stress and strain acting on them. BUT HOW?	1884
Iwao Yasuda ELECTRIC	Bone strain generates piezoelectric currents which modulate bone metabolism. DRY BONE	1953
Eiichi Fukada	Proteins are piezoelectric crystals	1957
Anderson & Ericksson FLUID	Modulation of bone metabolism requires wet bone and streaming potentials. Where is Wolff?	1970
Piekarski & Munro	Bone strain generates fluid flow which modulates bone metabolism and always moves centrifugally	1977

II. Bone Fluid Flow Regimes

WHERE DOES FLUID FLOW IN BONE?

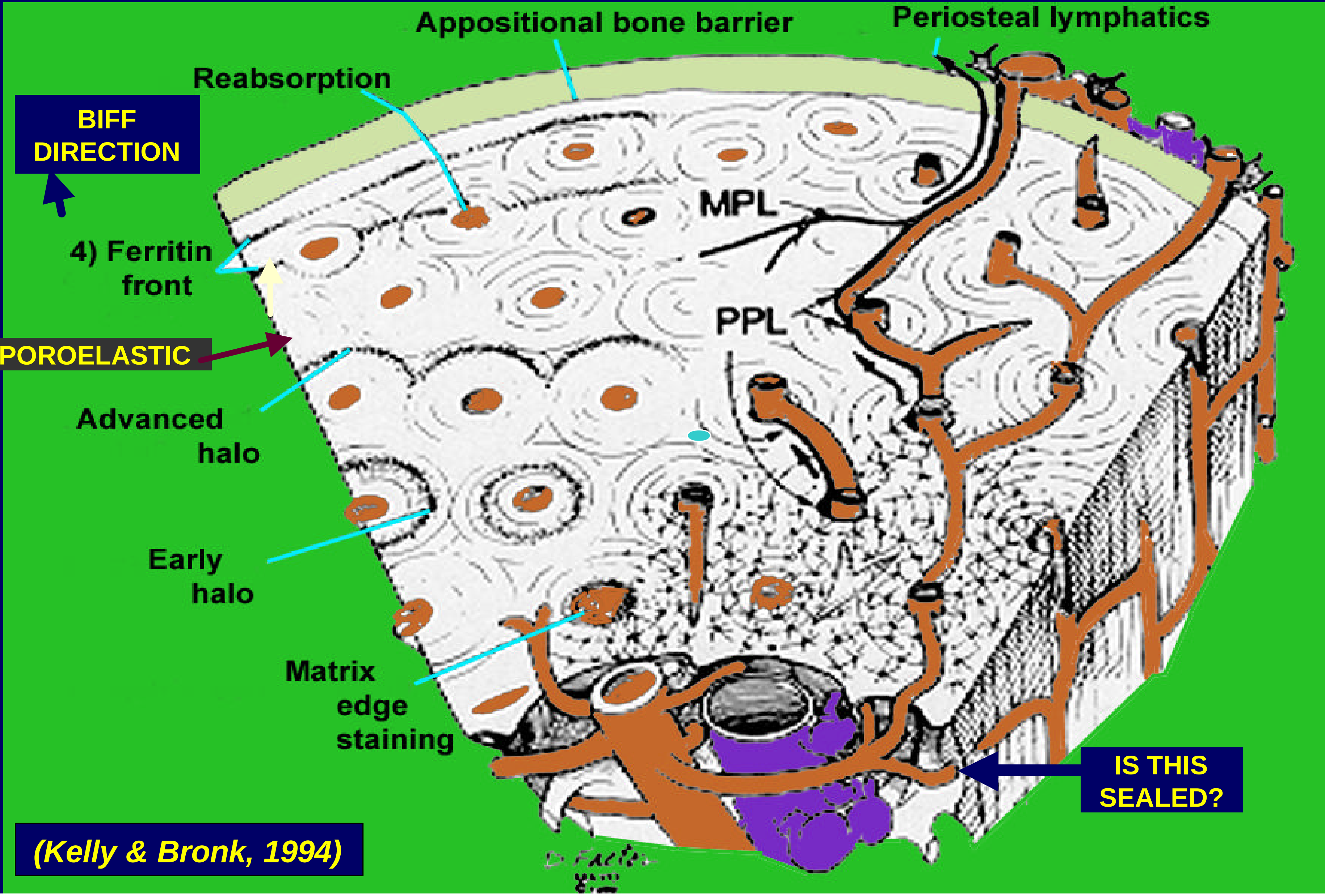
- IN BLOOD VESSELS (BLOODFLOW)
- FROM BLOOD VESSELS (CAPILLARY FILTRATION)
- BONE INTERSITIAL FLUID FLOW (BIFF)

  CANALS (PERIVASCULAR)

  CANALICULI (PERIOSTEOCYTE)

  MINERALIZED COLLAGEN-PROTEO-GLYCAN MATRIX (MICRO- POROUS/FLUIDIC)

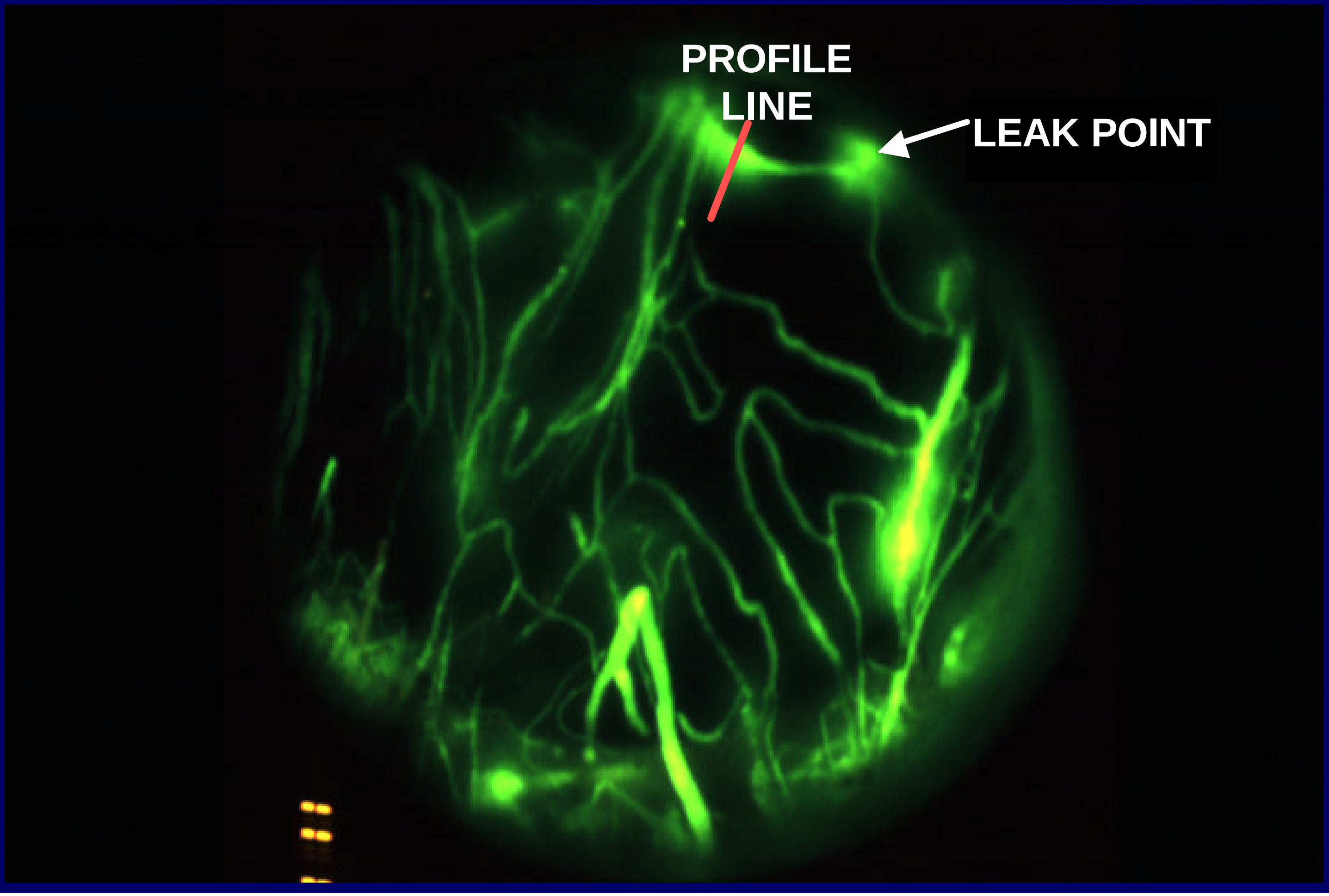
TRANSPORT TO AND PERCOLATION OF FLUID THROUGH THE MATRIX OF CORTICAL BONE



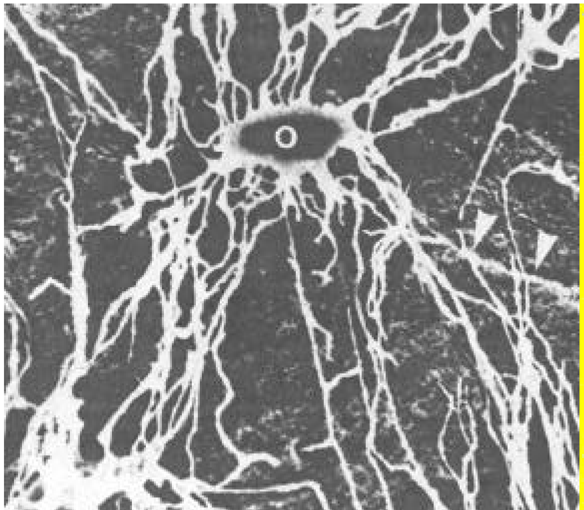
(Kelly & Bronk, 1994)

BLOOD's LIQUID PHASE BECOMES BIFF FLUID, WHEN DELIVERED TO CORTICAL BONE THROUGH CAPILLARY WALLS BY FILTRATION

HERE IS CONVECTIVE CAPILLARY FILTRATION in the **absence** of loading



In vitro evidence accumulated since 1990 *[Reich et al, J. Cell. Physiol.]* indicates that the cell-stimulating fluid mechanical signal is **strain** generated by fluid shear stress at the bone cell-fluid interface.



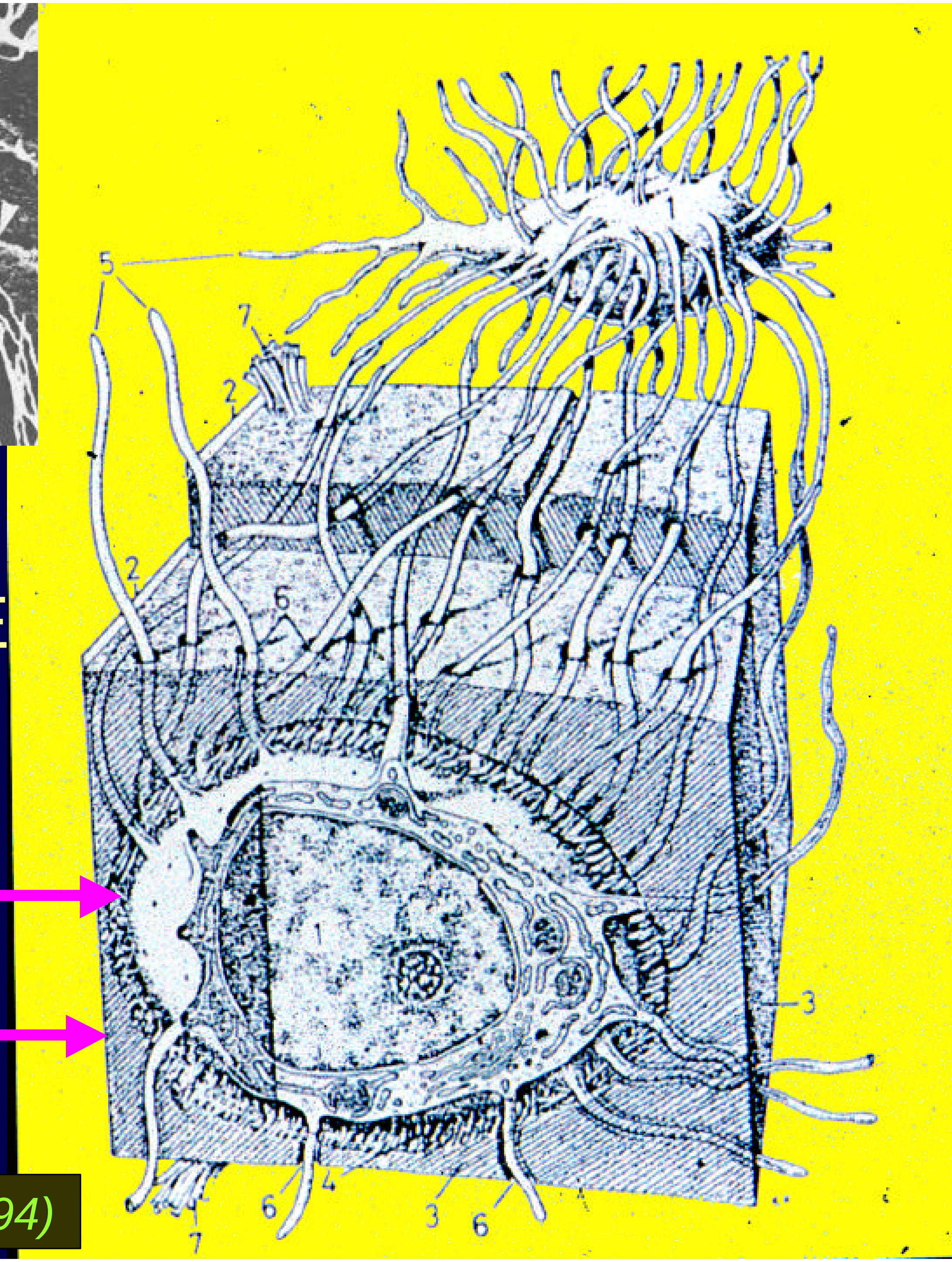
THE OSTEOCYTE

IN SITU

MACROPOROUS (PERIOSTEOCYTE)

FLUID FLOW

MICROPOROUS

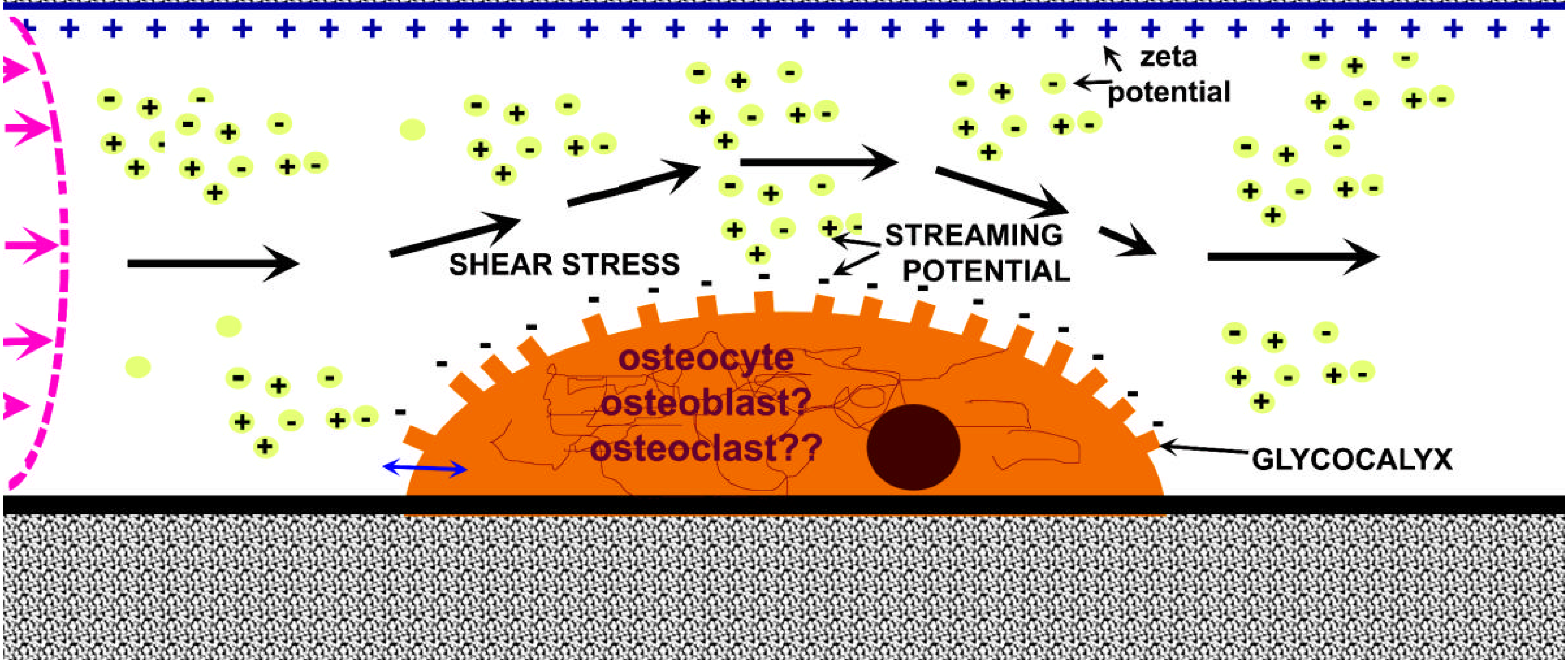


(Weinbaum et al., 1994)

BIFF Over Bone Cells

WHICH STIMULUS MODULATES BONE REMODELING MORE,

FLUID SHEAR STRESS OR STREAMING POTENTIAL?



RECENT HISTORY OF MECHANICAL MODULATION OF BONE REMODELING

NAME	CONCEPT	DATE
Piekarski & Munro	Bone operates as a sponge with a dense, fluid-filled interstitial matrix	1977
Frangos et al.	Bone cells have mechanotransducers for fluid flow shear stress like those of endothelial cells	1989
Weinbaum et al.	BONE IS A POROELASTIC MATRIX Shear stress of bone fluid flowing over osteocytes causes surface strains which activate them	1994
Pollack et al.	Streaming potentials are still a valid mechanism for modulating bone cell activity	1984, 2001

By assuming that bone is a POROELASTIC solid which propels its fluid phase when deformed, Weinbaum et al *[J. Biomech. 1994]* developed a model which predicts BIFF shear values sufficient for cell-stimulating strains under normal bending loads.

HOWEVER, Recent analysis of poroelastic model behavior at higher frequencies (e.g. exercise rates) predicts a damping effect which would limit BIFF-caused stimulation *[Buechner et al, Ann. Biomed. Engin. 2001]* .

Insufficiency of the POROELASTIC model, plus evidence that increased capillary filtration enhances regenerative bone growth, *[Kelly & Bronk, Vasc. Res. 1990],* led us to predict that forces driving vessel hydraulic pressure supplement poroelastic BIFF by increasing capillary filtration during exercise.

The main force which drives vascular hydraulic pressure during exercise is

SKELETAL MUSCLE PUMPING

COMPARTMENTS OF A LIMB

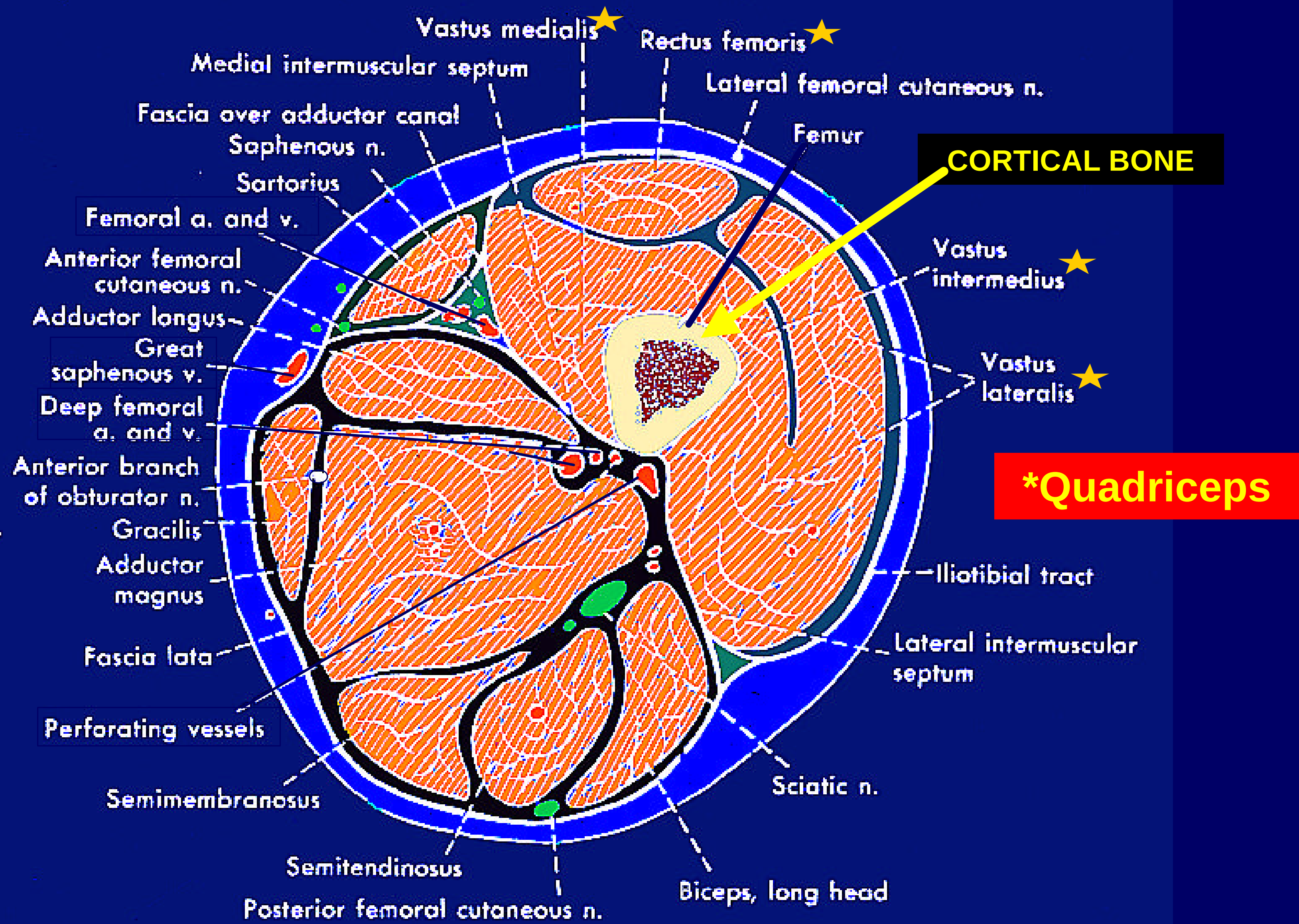
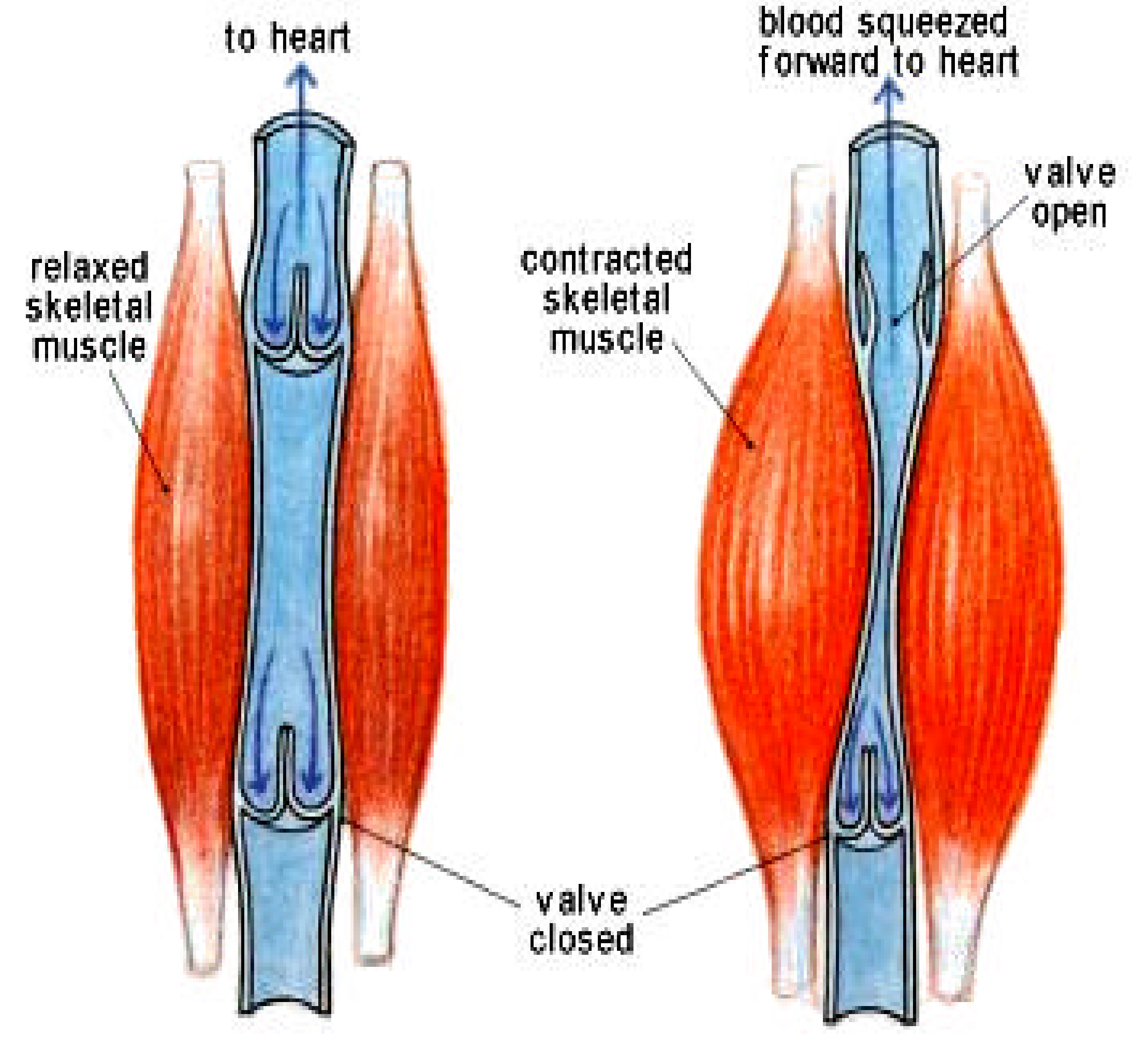


Fig. 8-45. Cross section through the middle third of the thigh. (Redrawn from Eycleshymer AC, Schoemaker DM: A Cross-section Anatomy. New York, Appleton, 1923)

MUSCLE PUMP

- Gravity makes blood tend to accumulate in the lower extremities

- Skeletal muscle contraction and venous valves drive blood toward the heart



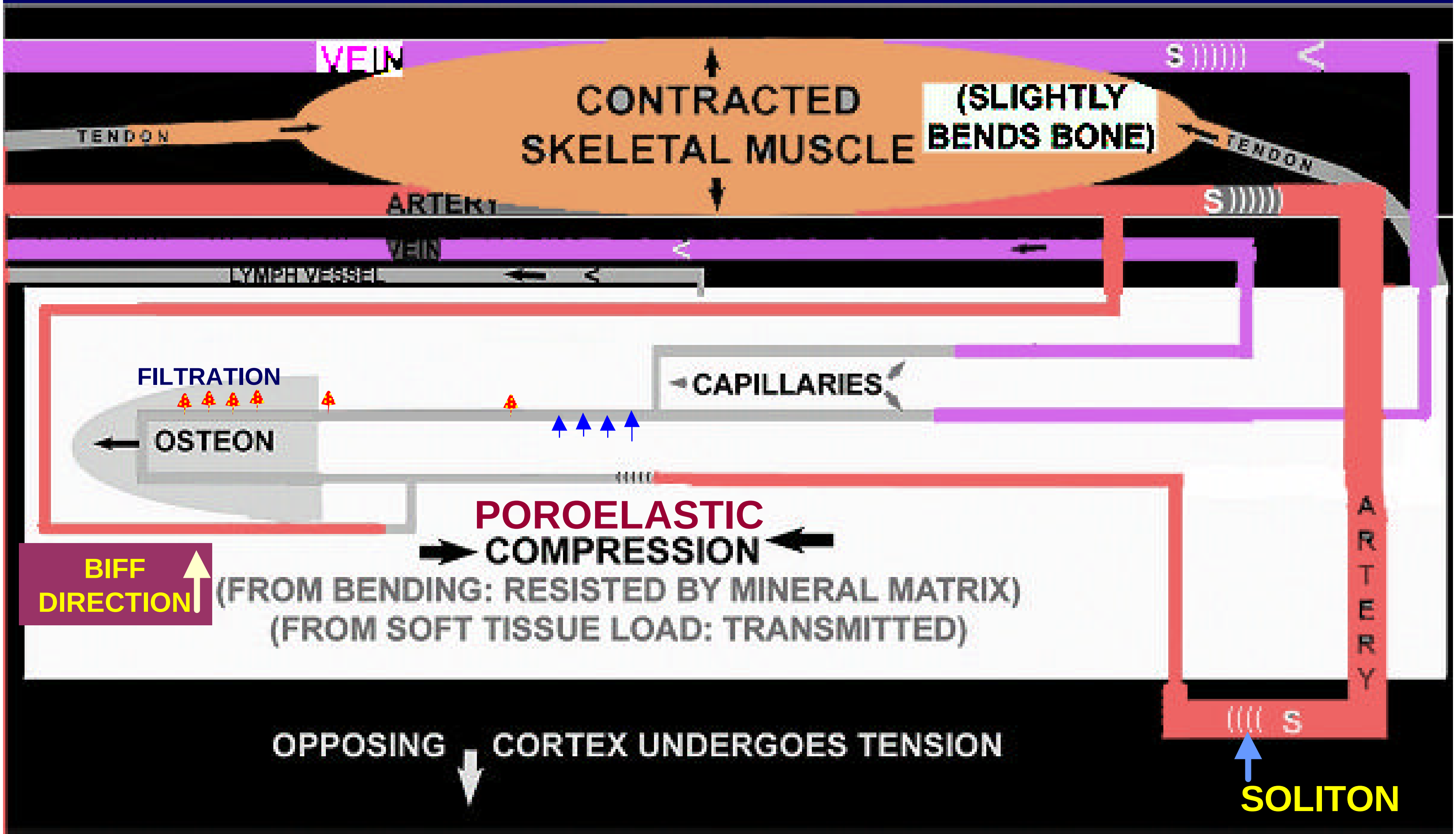
How does the muscle pump generate BIFF-driving pressure in bone?

✍THE FLUID-SATURATED BONE IS BENT BY MUSCLE CONTRACTIONS (POROELASTIC MODEL).

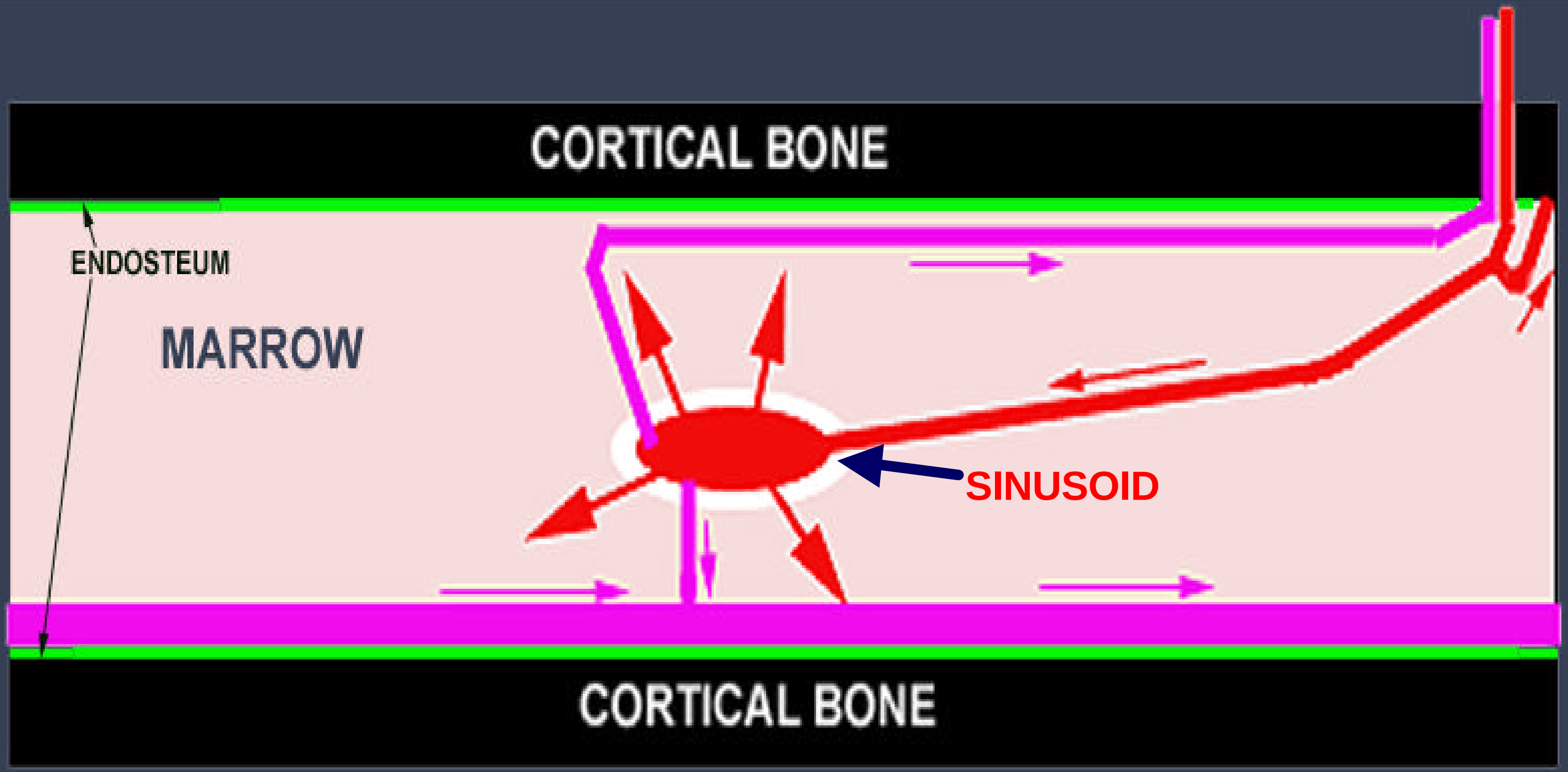
✍ARTERIES AND VEINS NEXT TO BONE ARE COMPRESSED BY MUSCLE CONTRACTIONS --generating pressures as high as 570 mmHg *[Semester et al., 1984].*

(ALSO, DURING EXERCISE BLOOD PRESSURE CAN RISE ABOVE 180 mmHg.)

MUSCLE PUMP-GENERATED PULSE WAVE PROPAGATION IN VESSELS



INCREASE IN MARROW PRESSURE DUE TO SINUSOIDAL CAPILLARY FILTRATION



HOW MUCH FILTRATION PRESSURE IS BUILT UP IN MARROW DURING EXERCISE?

IS THERE EVIDENCE THAT THE MUSCLE PUMP ENHANCES CAPILLARY FILTRATION IN CORTICAL BONE?

OUR PRELIMIARY DATA SUGGEST THAT MUSCLE PUMP-GENERATED PRESSURE WAVES IN VESSELS ARE PROPAGATED TO BONE CAPILLARIES.

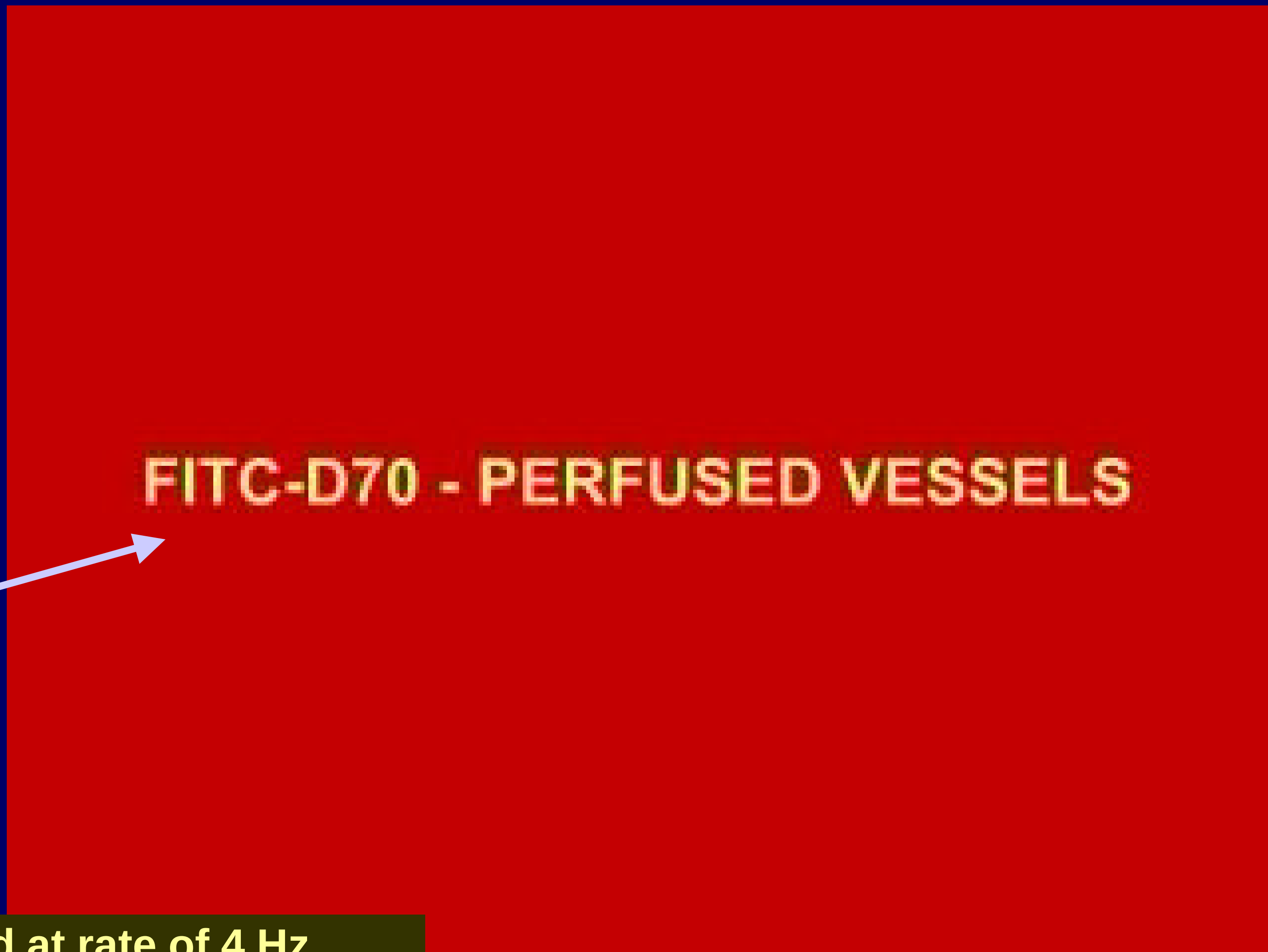
INTRAVITAL MICROSCOPY REVEALS THAT THEY

- ✍ ALTER VASCULAR PRESSURE (MICROSPHERE MOTION)
- ✍ DRIVE CONVECTIVE TRANSPORT ACROSS THE VESSEL WALL (DYE LEAKAGE) [FAST ENOUGH TO GENERATE CELL-STIMULATING BIFF???]

DIRECT* EFFECT OF MUSCLE PUMP ON FLOW IN CORTICAL CAPILLARIES

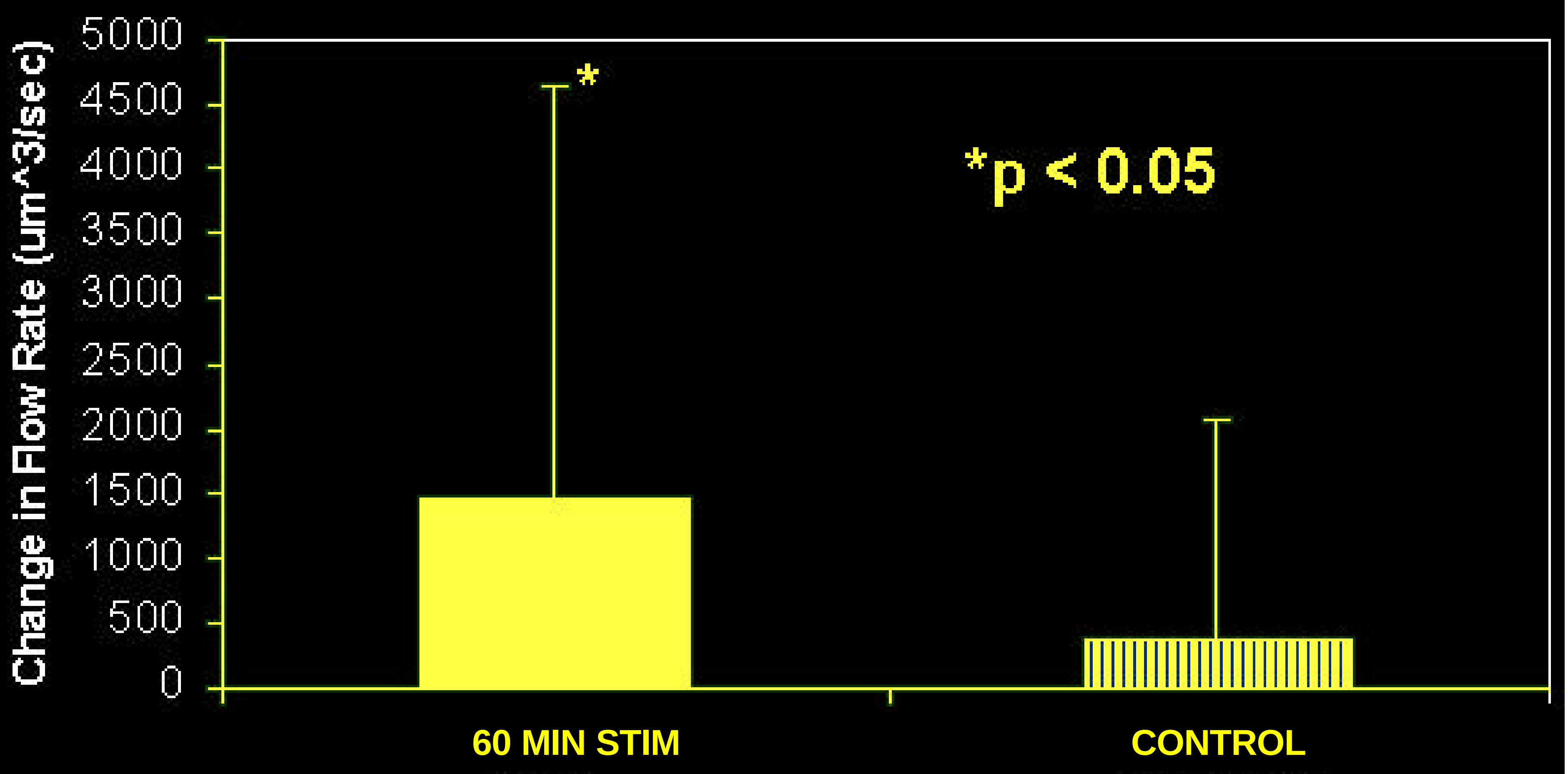
***MUSCLE INSERTION HAS BEEN DETACHED/RELEASED**

WATCH THESE VESSELS FOR PRESSURE PULSES

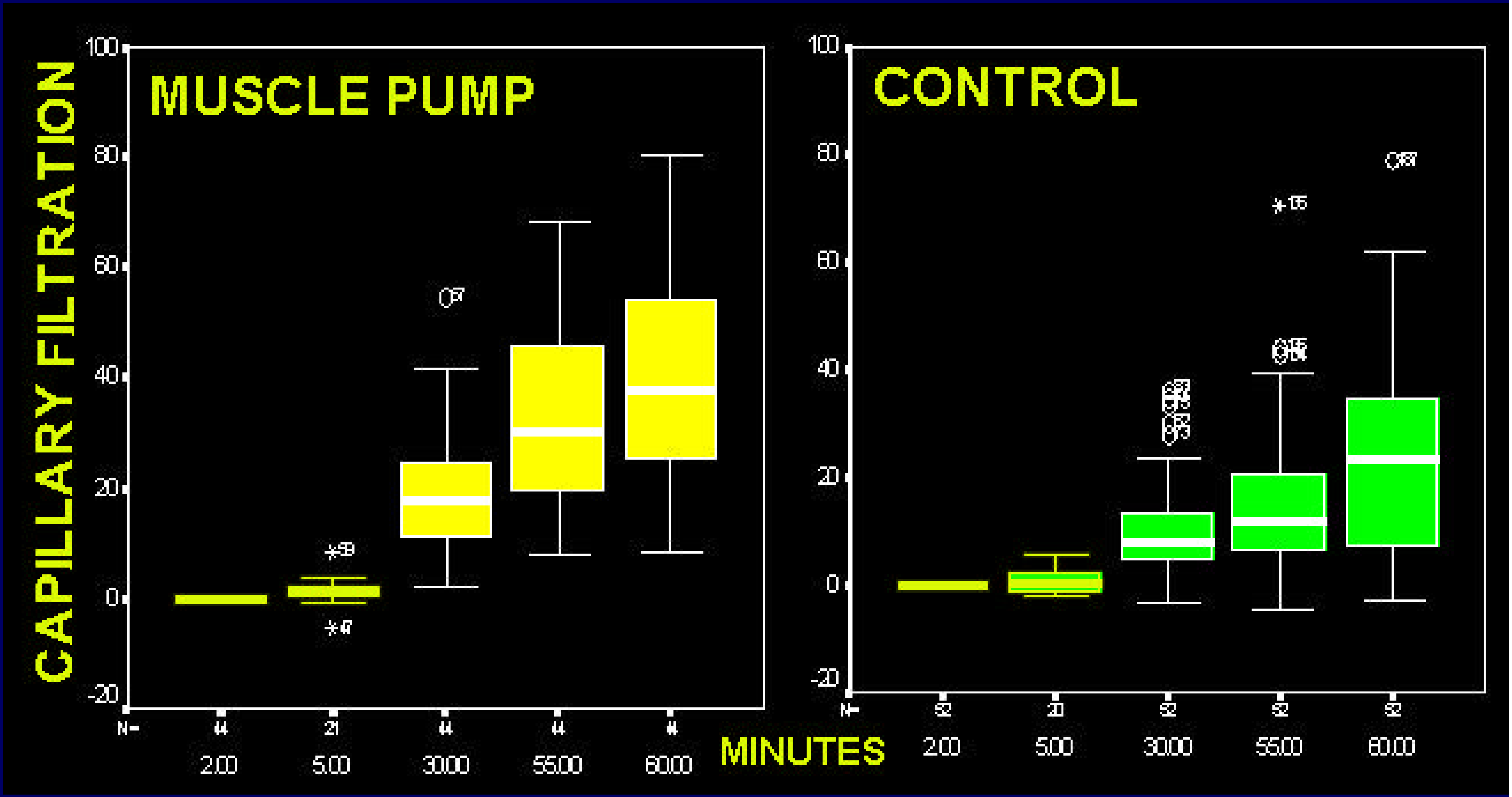


TENS applied at rate of 4 Hz
Biphasic, symmetrical waveform
0.04-4V output

EFFECT OF MUSCLE PUMP ON BLOOD FLOW

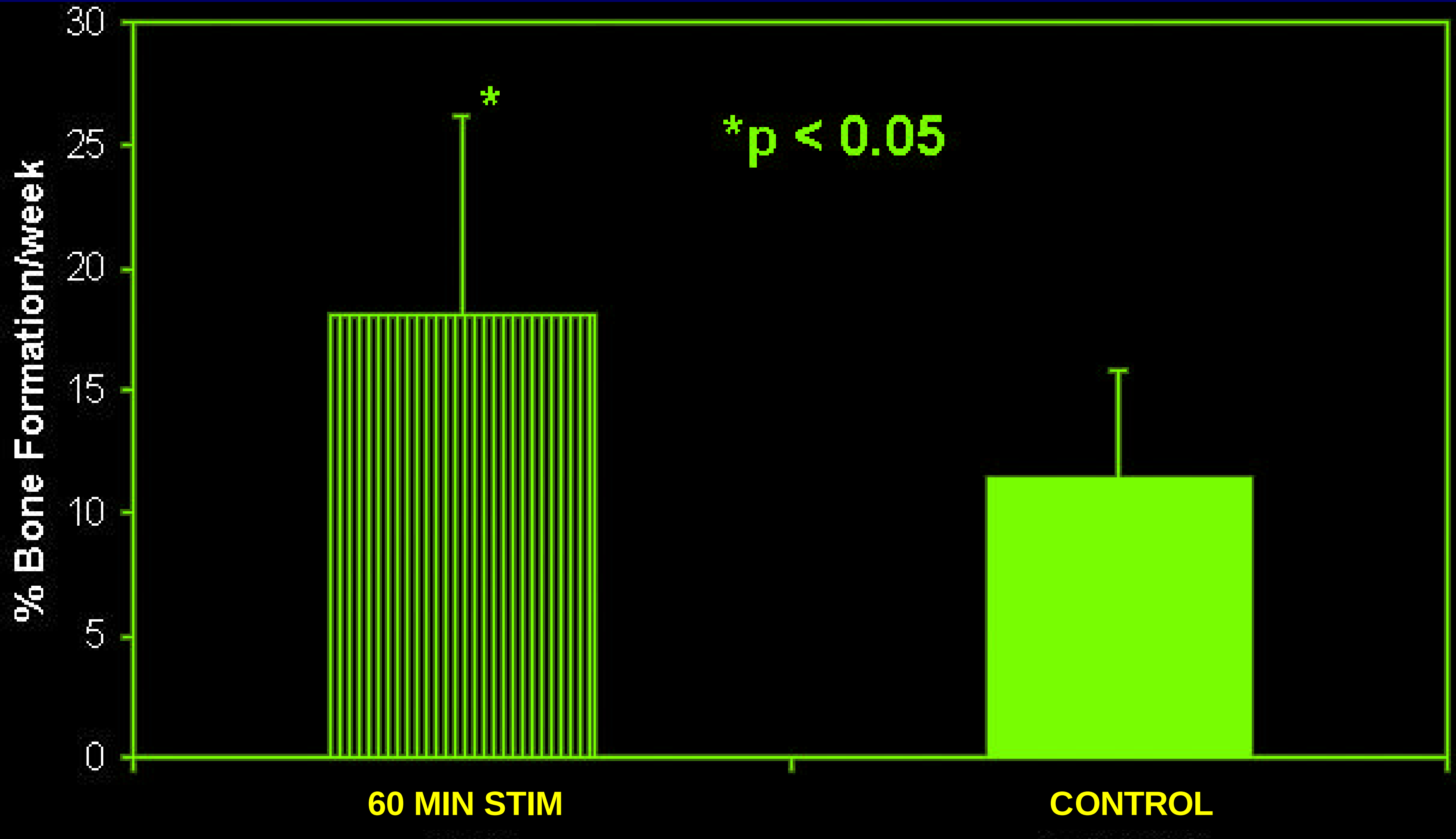


EFFECT OF MUSCLE PUMP ON CAPILLARY FILTRATION* OVER 60 MINUTES



*OF FITC-D70, A FLUORESCENT DYE

EFFECT OF MUSCLE PUMP ON NET BONE FORMATION RATE



CAVEATS, CONCLUSIONS & DISCUSSION

EXCEPT FOR THE VIDEO SEQUENCE, ALL DATA PRESENTED WERE OBTAINED FROM RABBITS IN WHICH ALL TENDONS WERE INTACT.

MOREOVER, CONTRACTION AMPLITUDES WERE WELL BELOW THAT EXHIBITED DURING EXERCISE.

NEVERTHELESS, SIGNIFICANT ENHANCEMENT OF CAPILLARY FILTRATION IS ASSOCIATED WITH MUSCLE PUMP ACTIVITY.

FUTURE INVESTIGATIONS WILL HAVE TO DETERMINE

1. THE VELOCITY OF AN ELEMENT OF FILTRATING FLUID
2. EFFECT OF RELEASED MUSCLE PUMPS (TENDON RESECT) ON CAPILLARY FILTRATION

3. BOTH OF THE ABOVE AT EXERCISE-LEVEL PUMPING

4. ALL OF THE ABOVE NEAR A T-E SCAFFOLD IMPANT

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THANK YOU

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BIOMEDICAL ENGINEERING DEPARTMENT