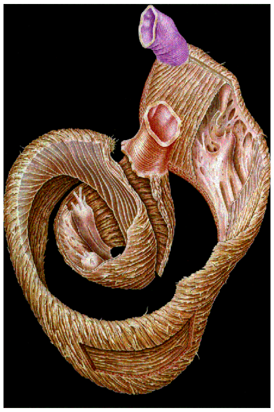
# Cardiac Muscle Properties

- Structural Properties
  - tissue
  - cellular
  - molecular
- Functional Properties
  - electrophysiological
  - mechanical
- Experimental Considerations

# Cardiac Muscle Fiber Architecture

**Classical Muscle Band Model**



Torrent-Guasp, 1995

**Modern Continuum Model**

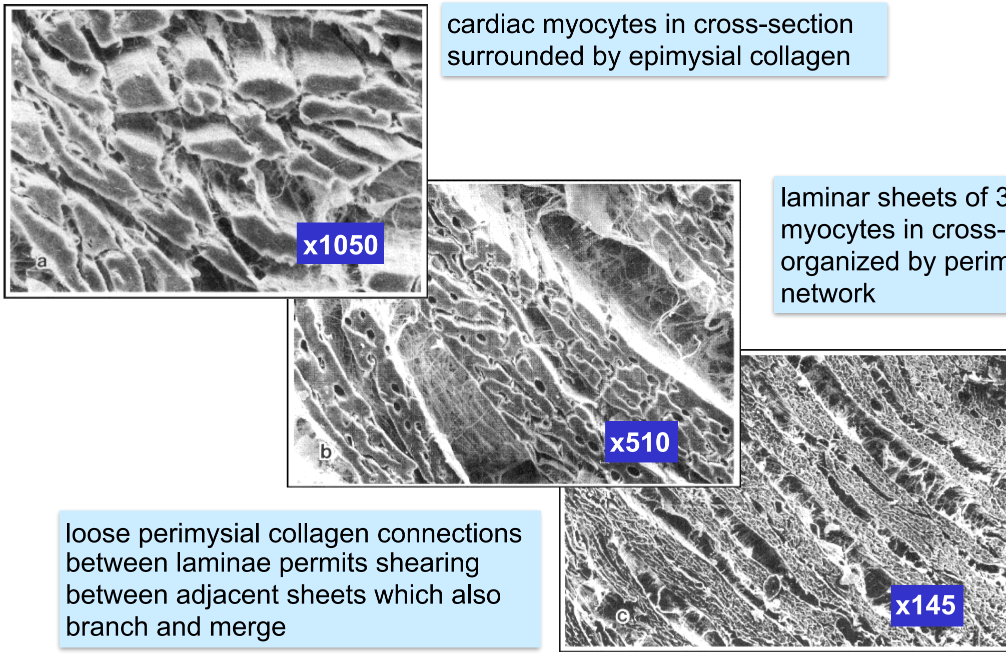
endocardium

midwall

epicardium

Streeter et al. (1969)

## Laminar Myofiber Sheet Structure

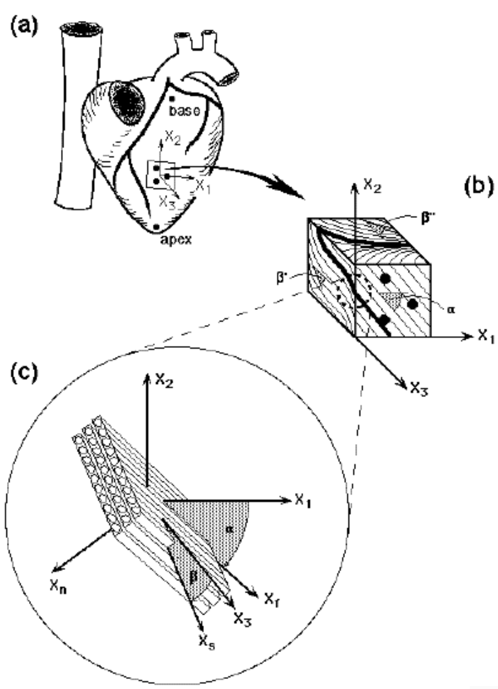


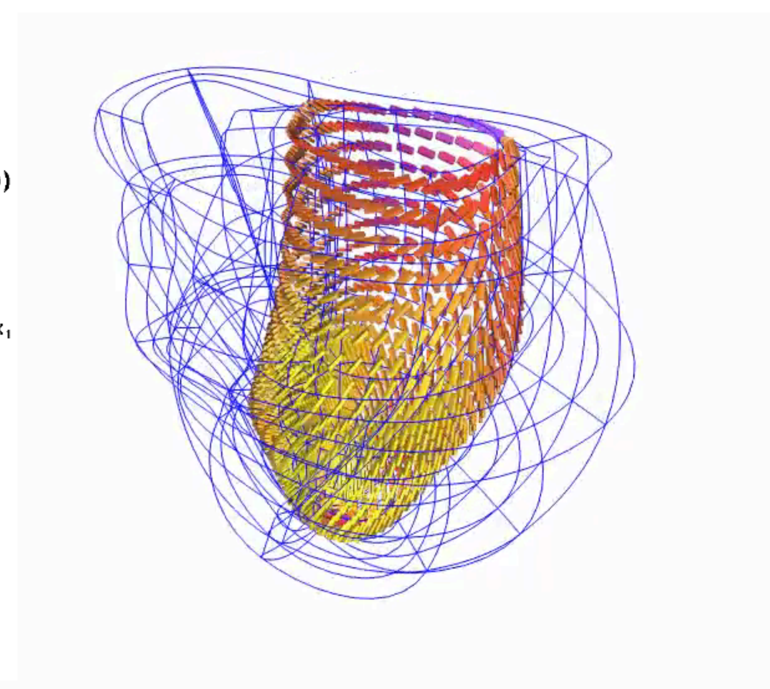

cardiac myocytes in cross-section surrounded by epimysial collagen

laminar sheets of 3-5 cardiac myocytes in cross-section organized by perimysial collagen network

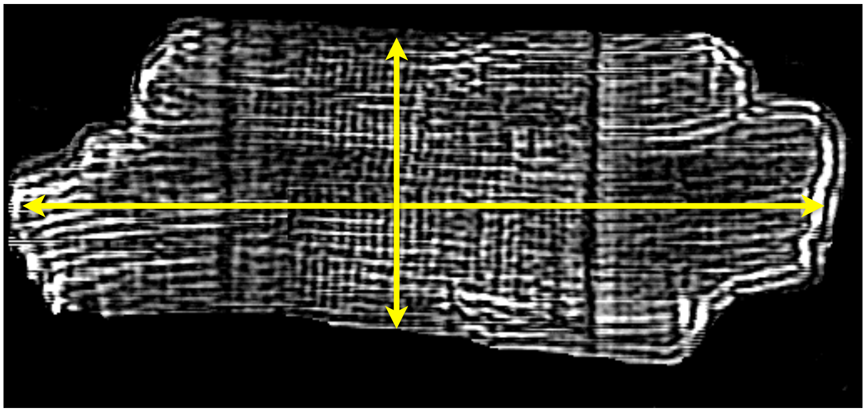
loose perimysial collagen connections between laminae permits shearing between adjacent sheets which also branch and merge

## Laminar Fiber Sheet Model



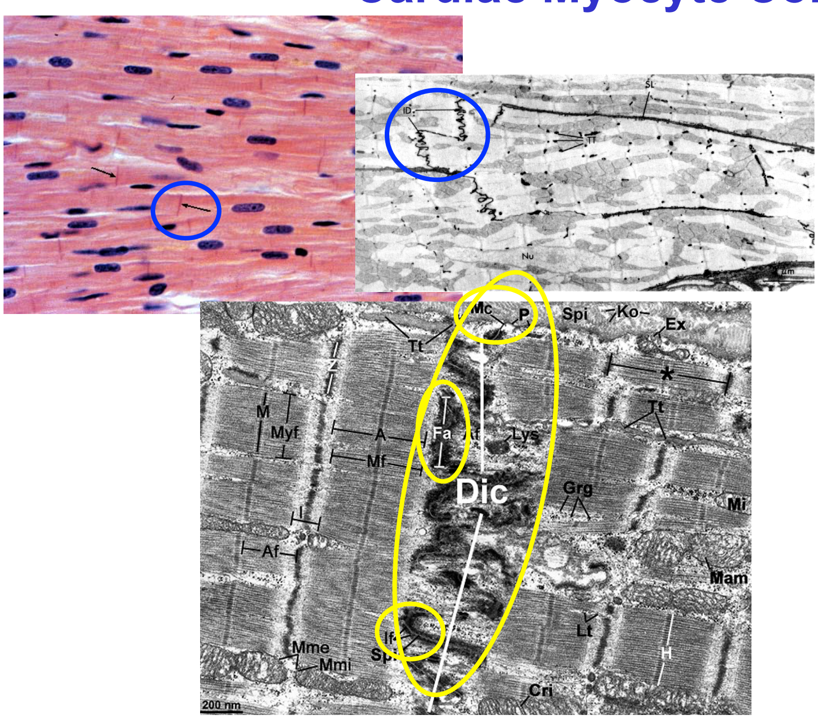



## Cardiac Myocytes



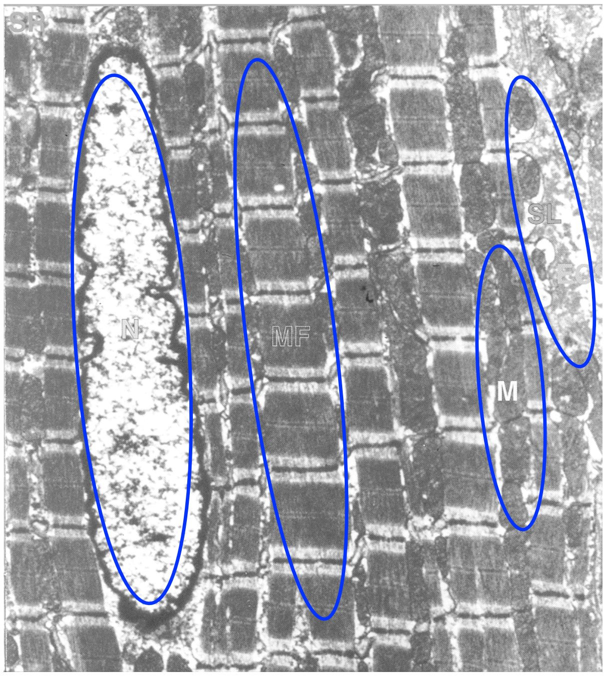
- Rod-shaped
- Striated
- 80-100 μm long
- 15-25 μm diameter

## Cardiac Myocyte Connections




- Myocytes connect to an average of 11 other cells (half end-to-end and half side-to-side)
- Functional *syncytium*
- Myofiber angle *dispersion* (about 12-15º)
- Intercalated discs
  - gap junctions
    - connexons
      - connexins
  - adherens junctions (fascia adherens)
    - anchoring sites for sarcomeres (actin)
  - desmosomes (macula adherens)
    - connect cytoskeleton (intermediate filaments) between cells.

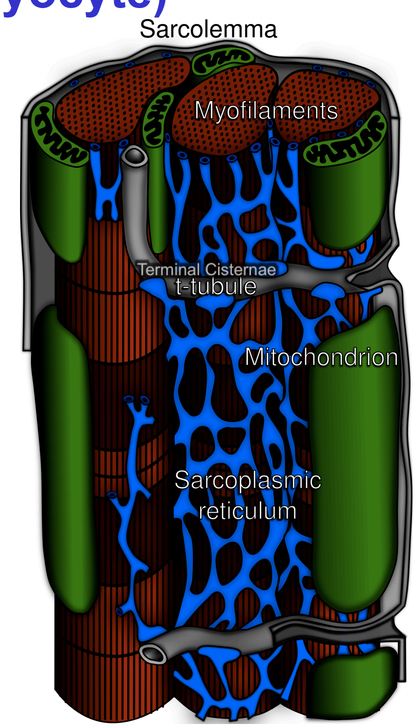
## Myocyte Ultrastructure




- Sarcolemma (SL) - cardiac specific ion channels, e.g. L-type Ca channels
- Mitochondria (M) ~30% of cell mass
- Nucleus (N) - occasionally bi-nucleated
- Sarcoplasmic reticulum and T-tubule network
- Myofibrils (MF)
    - A, I, H bands, M-line and Z-disks
    - Slow cardiac myosin isoforms
    - Cardiac troponin isoforms
    - Cardiac titin isoforms

## The Muscle Fiber (Myocyte)

- Sarcolemma (plasma membrane)
- Sarcoplasm (cytosol)
- Sarcoplasmic Reticulum (ER)
- Mitochrondria (ATP powerhouse)
- Transverse (T) Tubule
- Terminal Cisternae



## The Muscle Fiber (Myocyte)

What functional purpose does the transverse tubule system serve?

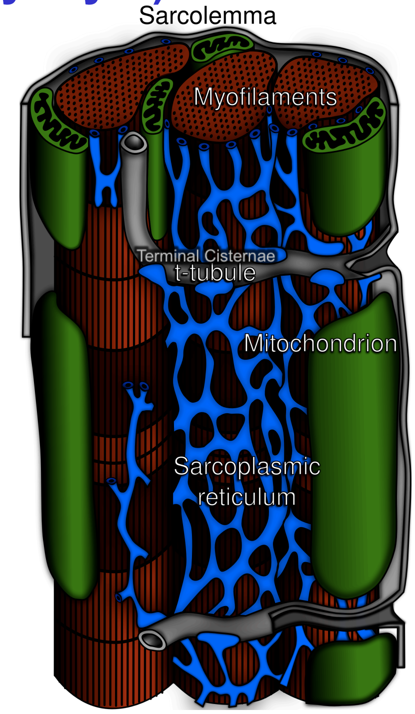
(A) It helps deliver extracellular nutrients into the cell?

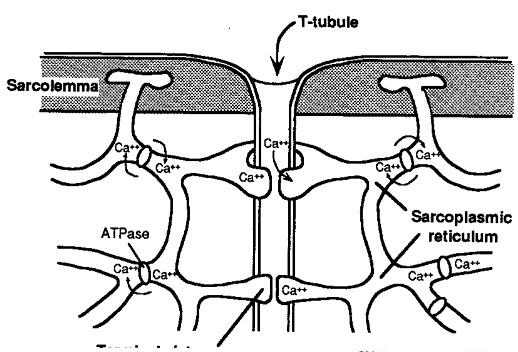
(B) It allows the myocyte membrane to stretch and shorten more easily

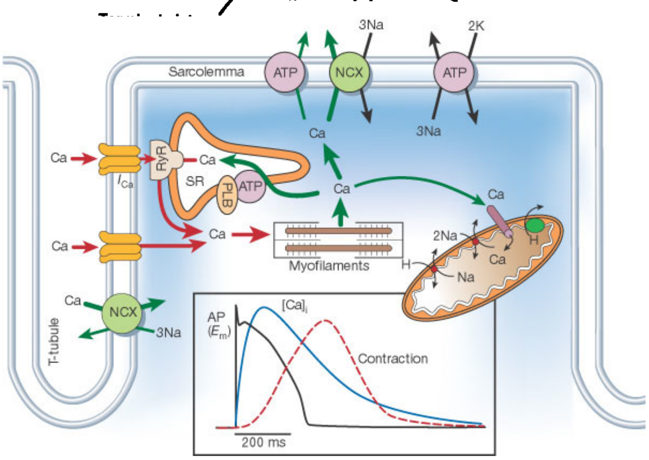
(C) It allows the calcium ions that cross the membrane during the action potential to reach intracellular receptors deep in the cell at the same time they reach receptors near the outside

(D) A, B and C

(E) None of the above



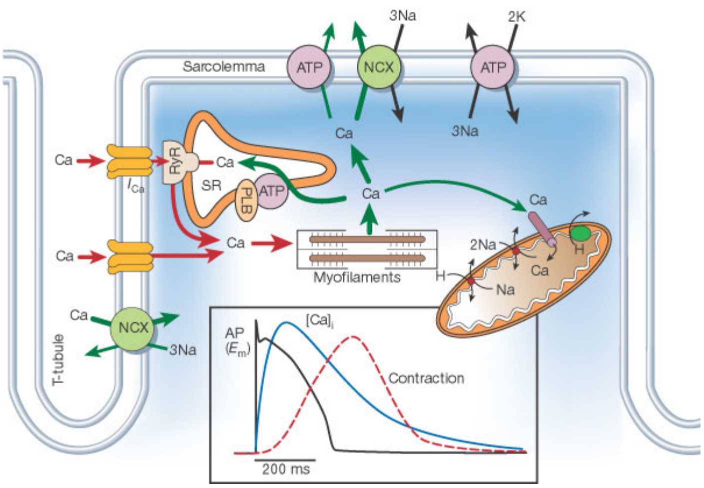




## Excitation-Contraction Coupling

- L-type Calcium current
- Calcium-induced calcium release from the sarcoplasmic reticulum *via* ryanodine receptors
- $Na^+/Ca^{2+}$ exchange
- Sarcolemmal $Ca^{2+}$ pump
- SR $Ca^{2+}$ ATP-dependent pump

## Excitation-Contraction Coupling




Given how the myocyte calcium cycling works, what do you expect to happen if the frequency of stimulation (i.e. the heart rate) was increased

(A) The amount of calcium in the cell would increase immediately and so the twitch force would increase instantaneously.

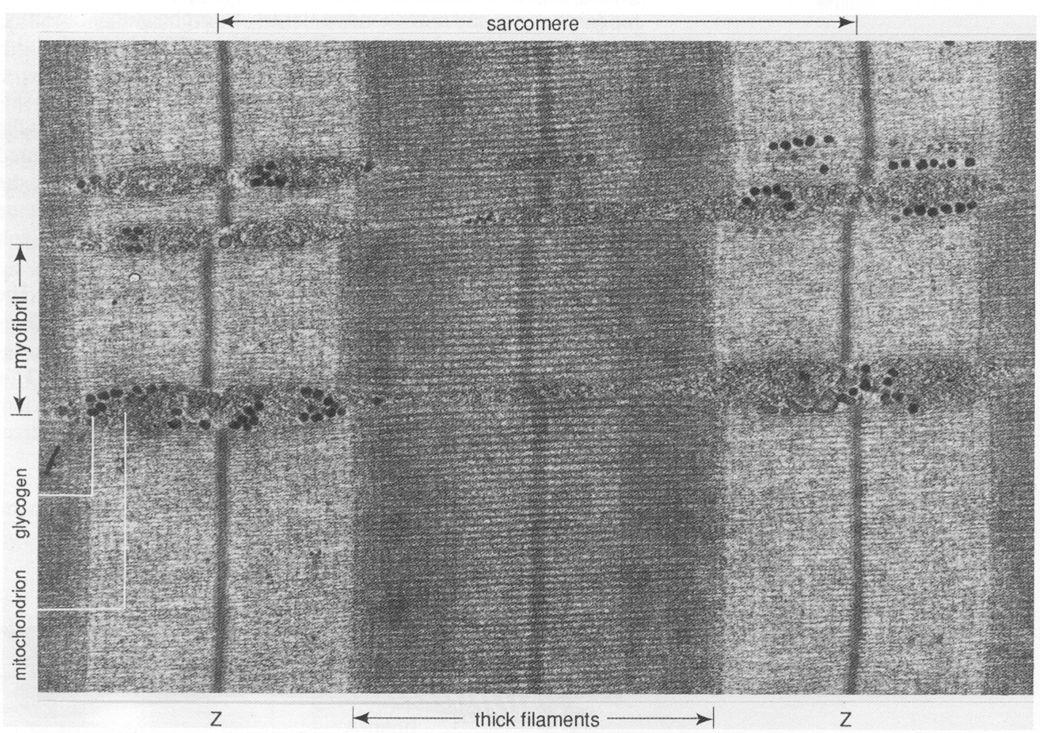
(B) The amount of calcium in the cell would increase gradually and so the twitch force would increase progressively.

(C) The amount of calcium in the cell would decrease immediately so the twitch force would decrease instantaneously.

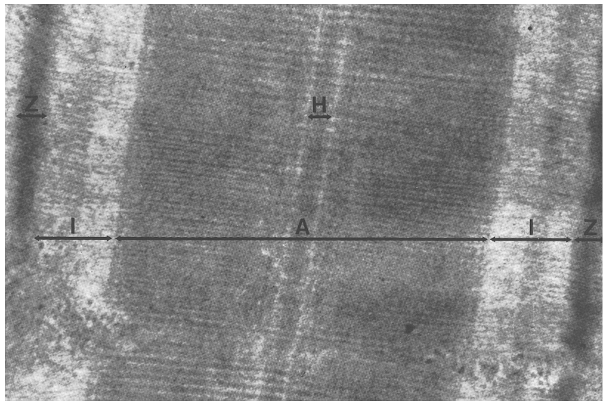
(D) The amount of calcium in the cell would decrease gradually so the twitch force would decrease progressively.

(E) There would be no change in the amount off calcium in the cell or force production during the twitch.

## The Sarcomere



## Cardiac Myofilaments

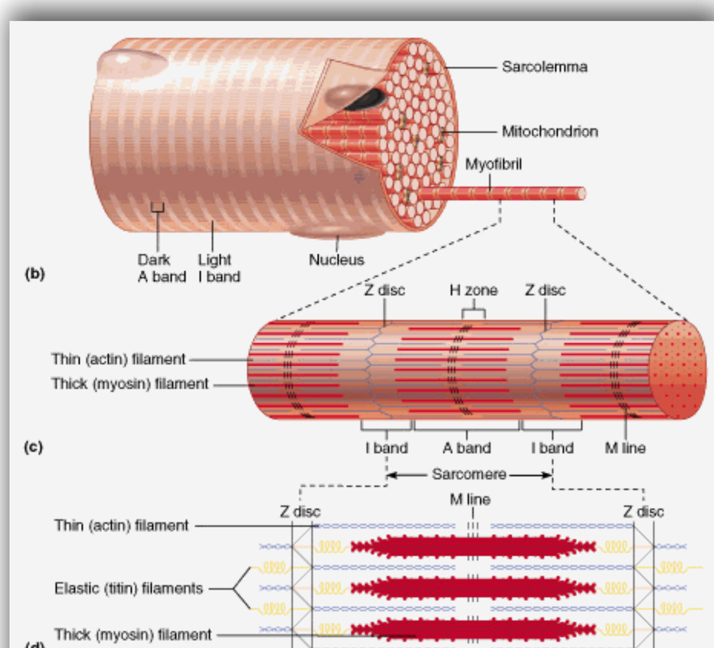



- Slow cardiac myosin isoforms
- Cardiac troponin
- Titin

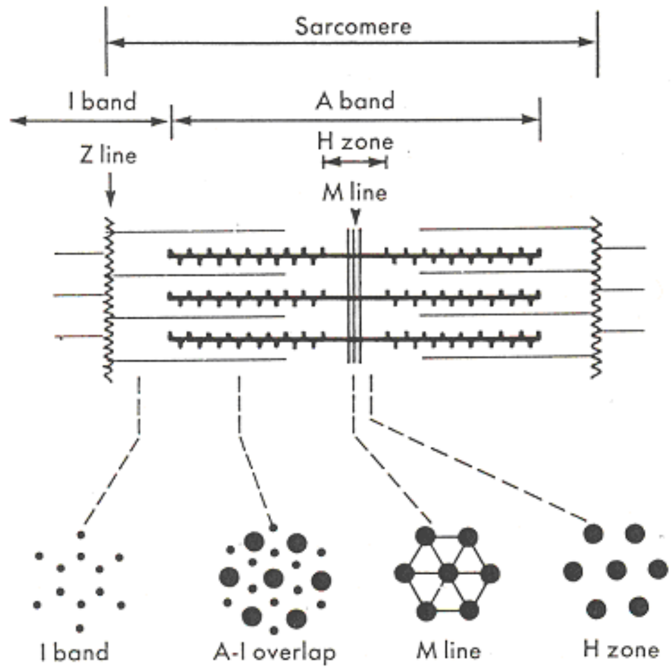
## The Sarcomere

**Contractile components**
- Thick Myosin Filaments
- Thin Actin Filaments
- Z-line to Z-line
- A and I bands
- Sliding Filament Theory

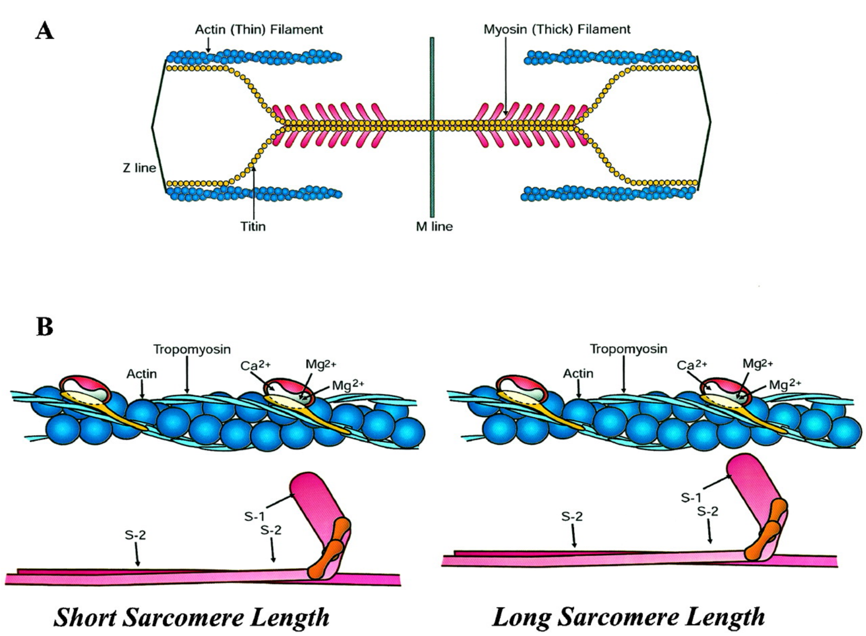
**Titin**
- Entropic molecular spring
- Connects Z-line to M-line, allows for force transmission
- Limits range of sarcomere length in tension, primary contributor to passive stiffness



## Hexagonal Arrangement of Myofilaments in Cross-Section

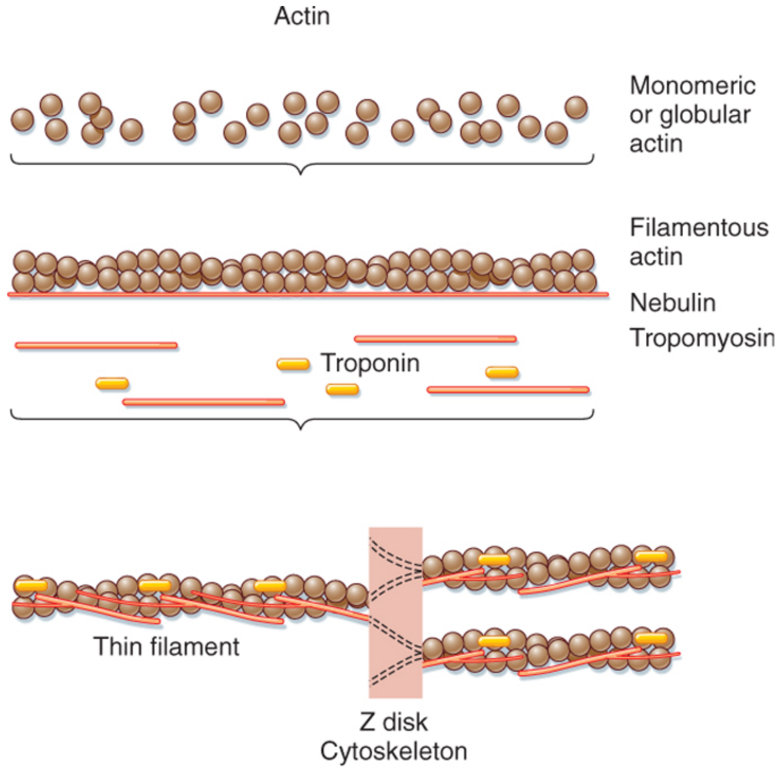



## The Sarcomere



# Actin

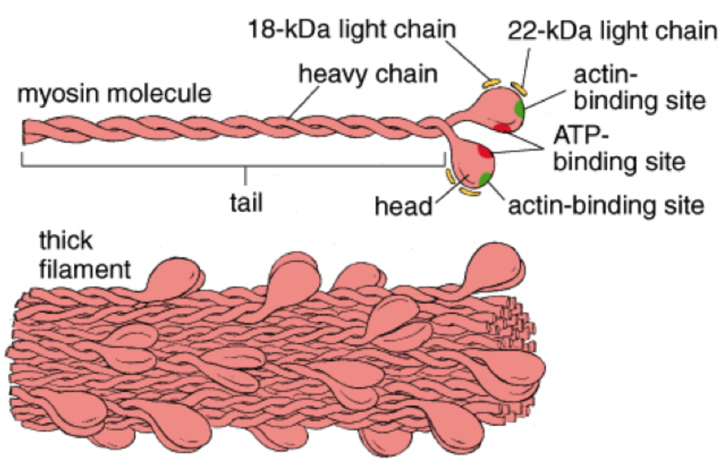
- F-actin molecule (7 nm diameter) formed from G-actin
- Twisted with a period ~37 nm into a double helix
- Nebulin is a large protein (600–900 kDa) that binds as many as 200 actin monomers and may control thin filament length
- One strand of tropomyosin each ~40 nm long per actin period
- Globular troponin T, I and C molecules (with a strong affinity for $Ca^{2+}$) attach to tropomyosin strands, every 7 G-actin monomers
- Actin filaments anchor at Z- disks




Koeppen & Stanton: Berne and Levy Physiology, 6th Edition.

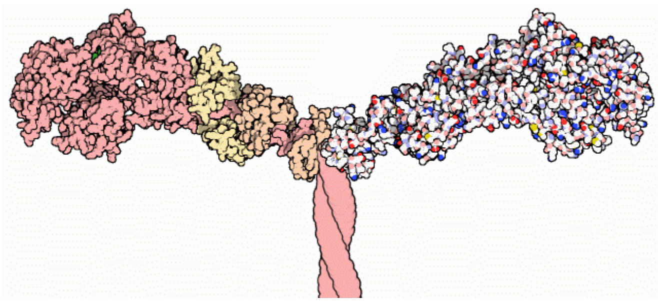

# Myosin II

- Thick filament contains oligamerized myosin molecules
- Myosin subdomains:
  - Two myosin heavy chains
  - Two myosin light chains per heavy chain
  - One S1-head molecular motor domains per heavy chain
- Myosin heads protrude in pairs at 180º
- There are about 50 *pairs* of cross-bridges at each end of the thick filament
- The filament makes a complete twist 310º every 3 pairs
- 14.3 nm interval between pairs

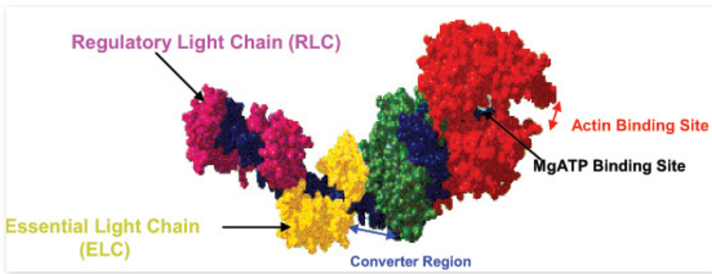



Unfig. 10.2. **Thick filament.**

## Myosin Light Chains

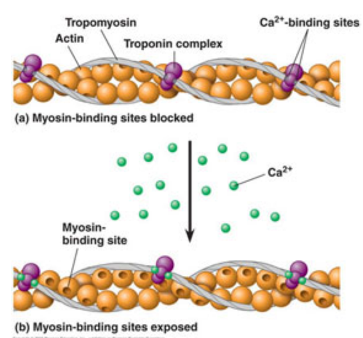


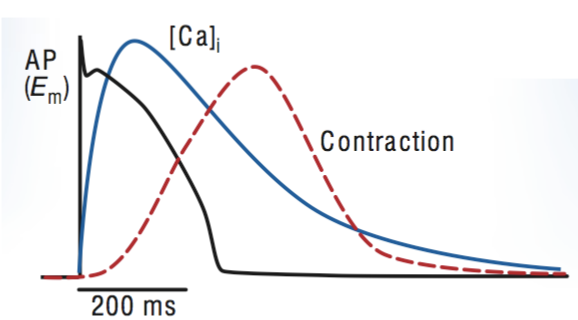
**Description**: Part of the myosin structure, atoms in the heavy chain are colored red on the left-hand side, and atoms in the light chains are colored orange and yellow. (image PDB)
**Author**: David S. Goodsell of The Scripps Research Institute

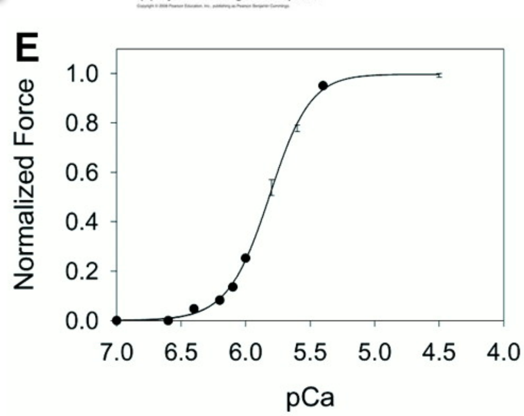



## Myofilament Activation

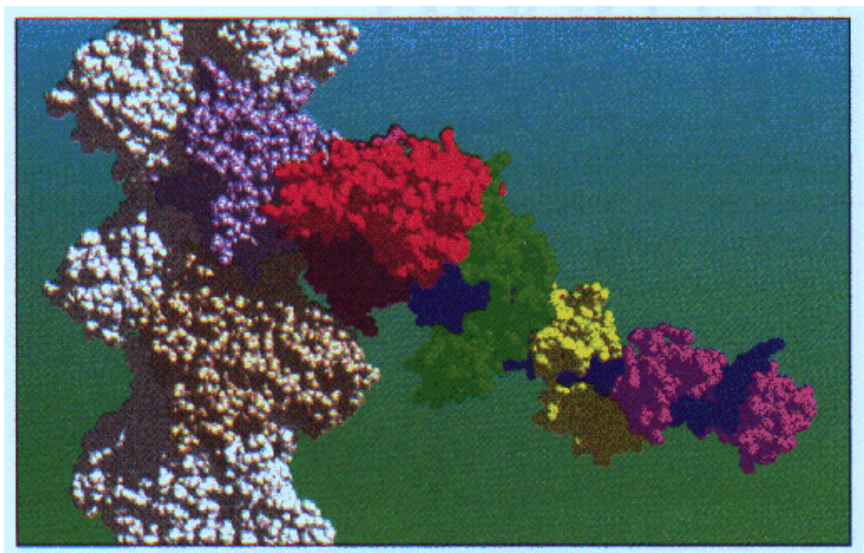
- Force-development is triggered by the intracellular [$Ca^{2+}$] transient.
- $Ca^{2+}$ binds to troponin-C sites, which are separated by neighboring troponin-C sites every 7 actin monomers
- Nearest neighbor cooperative activation



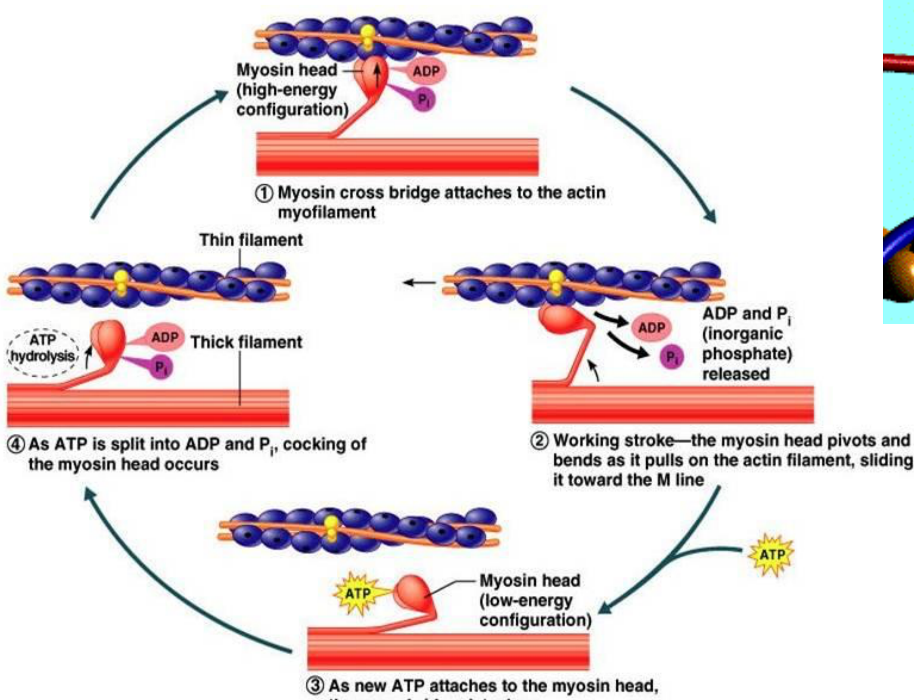



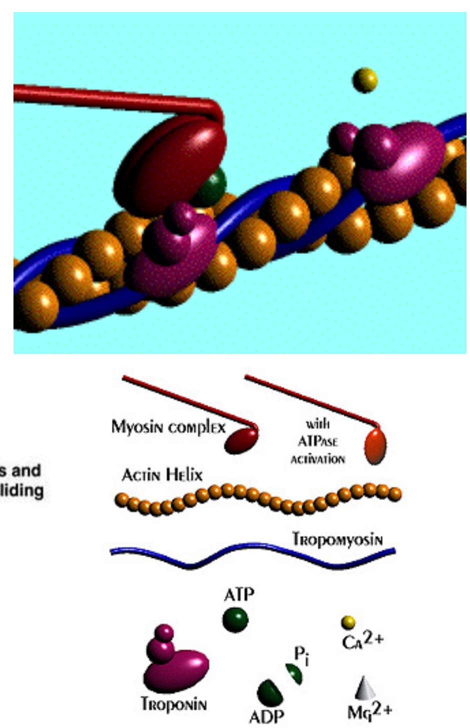
## Myosin binding to actin
## (from Rayment *et al.*,1993)



The actomyosin complex, a molecular motor (1993, Rayment et al.)

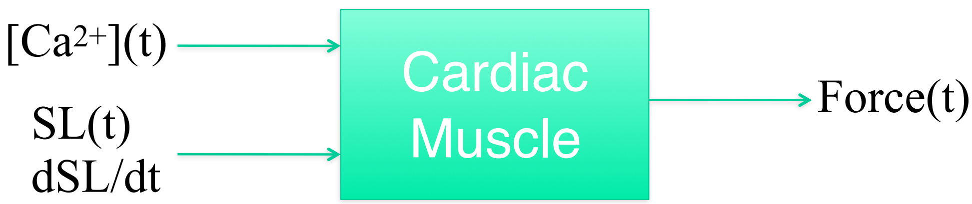
## Crossbridge Cycle



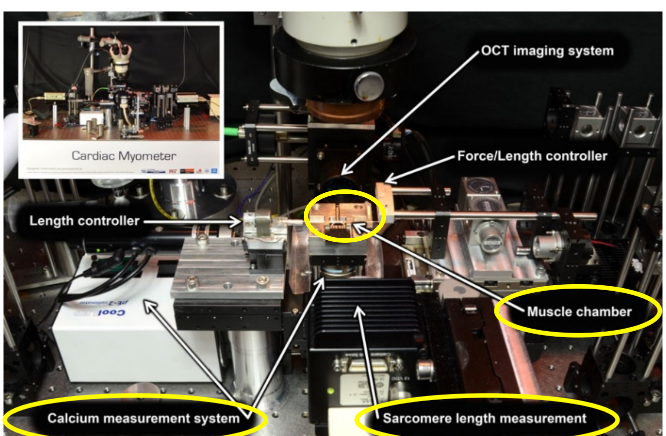



## Primary Determinants of Force Production in Cardiac Muscle

- Intracellular calcium concentration
- Sarcomere length (SL)
- Rate of change in SL (velocity)




## Cardiac Muscle Testing




Tissue chamber and preparation

- isolated right ventricular trabeculae
- 4000x200x90 $\mu$m

Force transducers

- cantilever - laser interferometry
- piezoresistive 1 *V/mN,* capacitive 0.1 *V/mN*

Motor

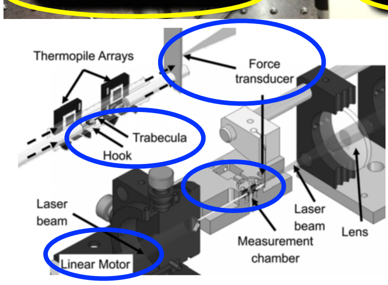
- linear servomotor

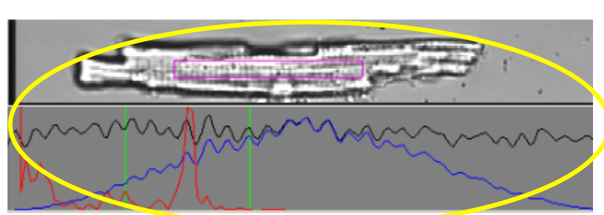
Sarcomere length

- laser diffraction or Fourier imaging

Calcium

- ratiometric fluorescence imaging



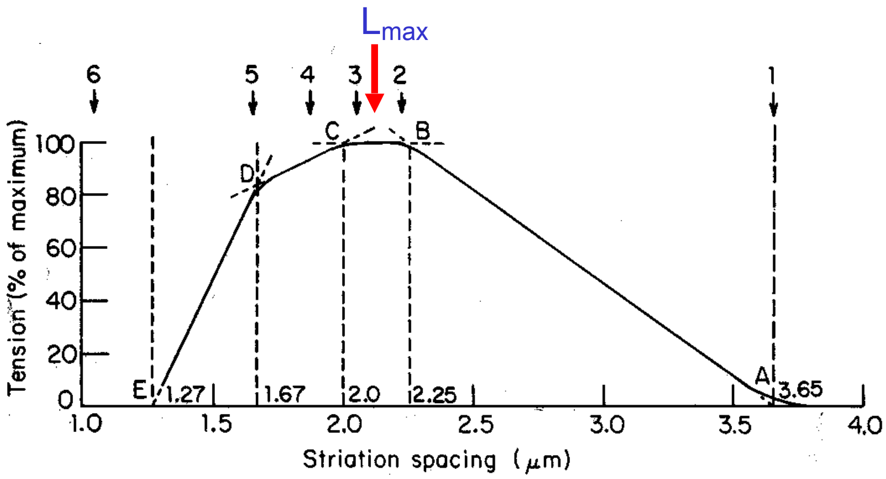



# Cardiac Muscle Testing

Cardiac muscle is much more difficult to test than skeletal muscle:

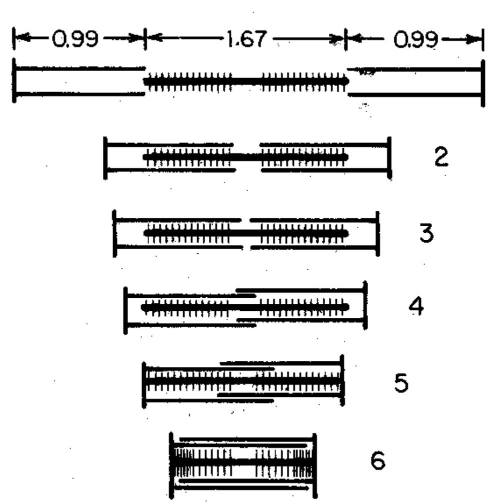
- tissue structure is complex and 3-D
- long uniform preparations with tendons attached are not available
- the best preparations are isolated *papillary muscles* (which hold atrioventricular valves closed during systole) and isolated *trabeculae*, which are more uniform but very small
- cardiac muscle branching scatters light making laser diffraction more difficult
- intact cardiac muscle can not be tetanized so it must be tested dynamically or artificially tetanized

# Isometric Tension: Sliding Filament Theory




Developed tension versus length for a single isometric fiber of frog semitendinosus muscle

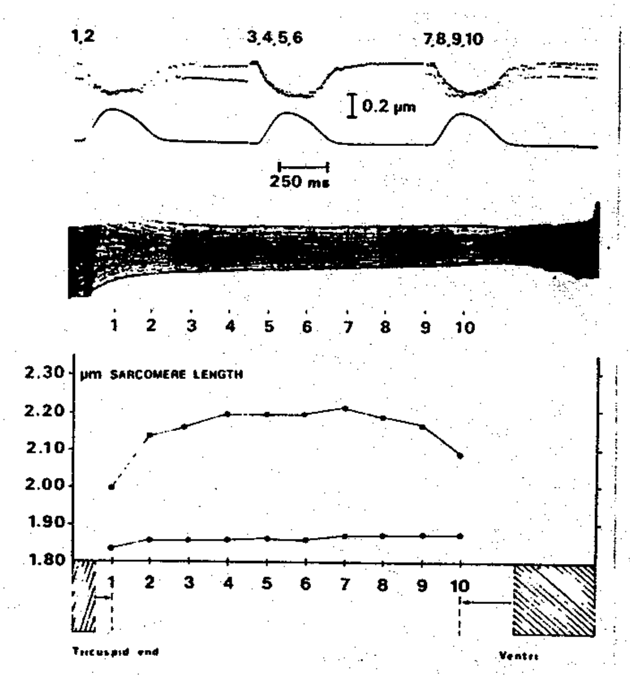
Sliding filament theory

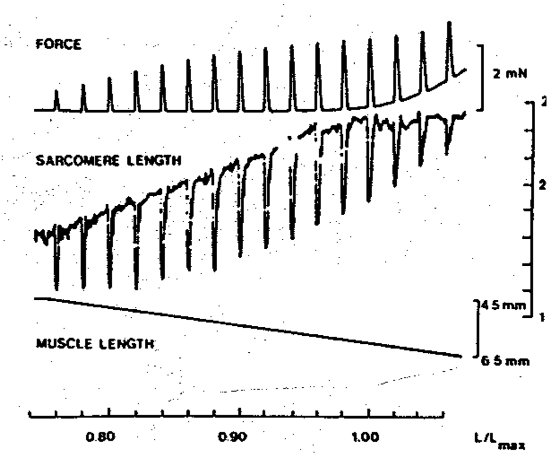


## Evidence for sliding filaments and cycling force generators

- The contractile unit is the sarcomere
- The sarcomere is composed of actin & myosin
- Filaments slide but do not change length
- Force proportional to degree of overlap
- The maximum sarcomere length for contractile force development is ~3.6 $\mu$m - above this length no active force is generated
- <u>Maximum unloaded velocity is independent of SL</u>

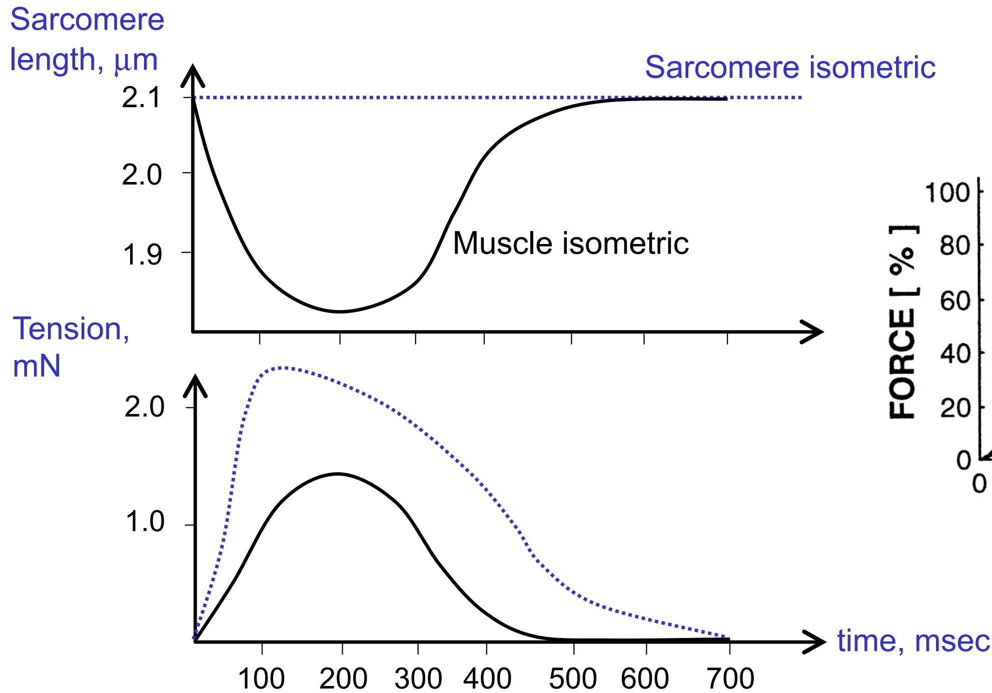
## Isometric testing

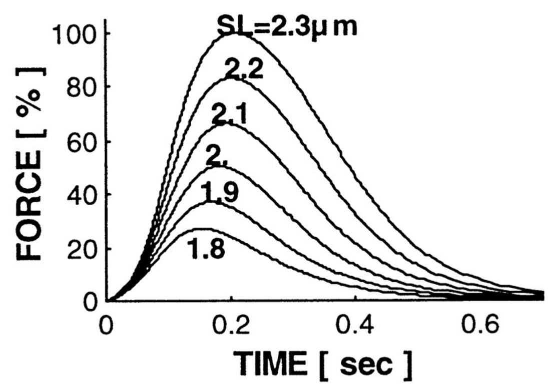



At constant muscle length, muscle preparation shortens in the middle at the expense of lengthening at the damaged ends

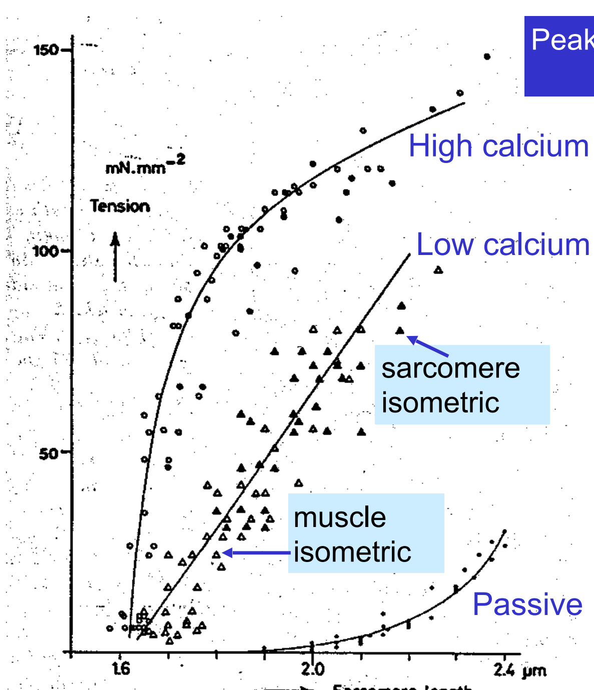
# Isometric Twitch Testing

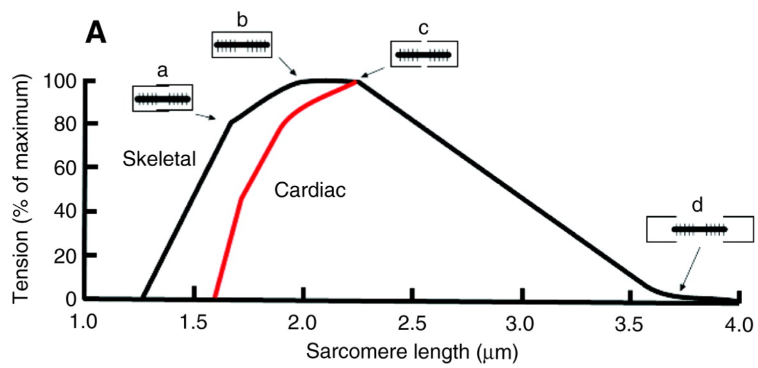



# Isometric Length-Peak Tension Curve

Peak developed isometric twitch tension (total-passive)

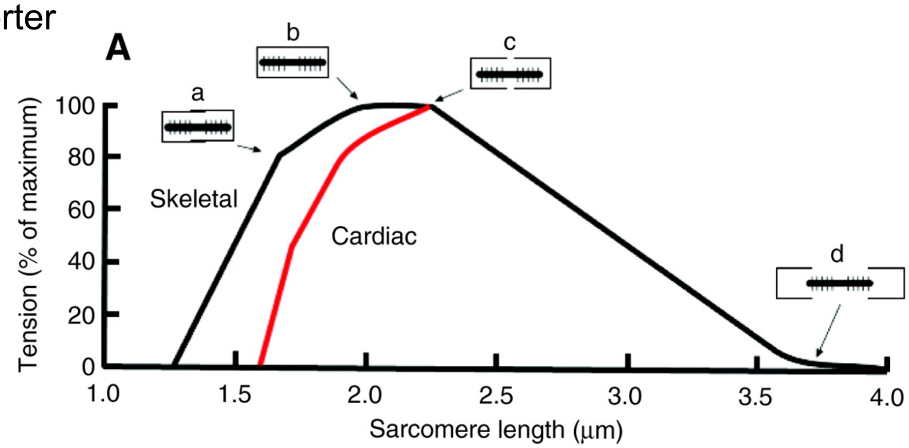


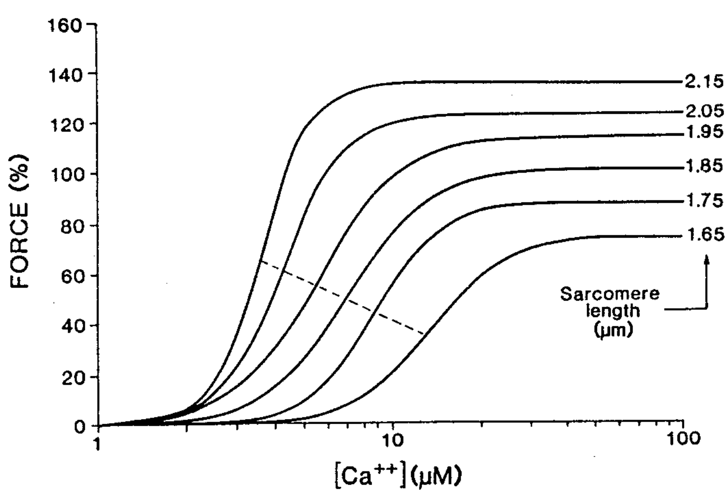
## Isometric Length-Peak Tension Curve

What best explains the difference in the cardiac muscle length-tension curve?

(A) cardiac I bands are longer
(B) cardiac I bands are shorter
(C) cardiac titin is shorter
(D) A and C
(E) B and C



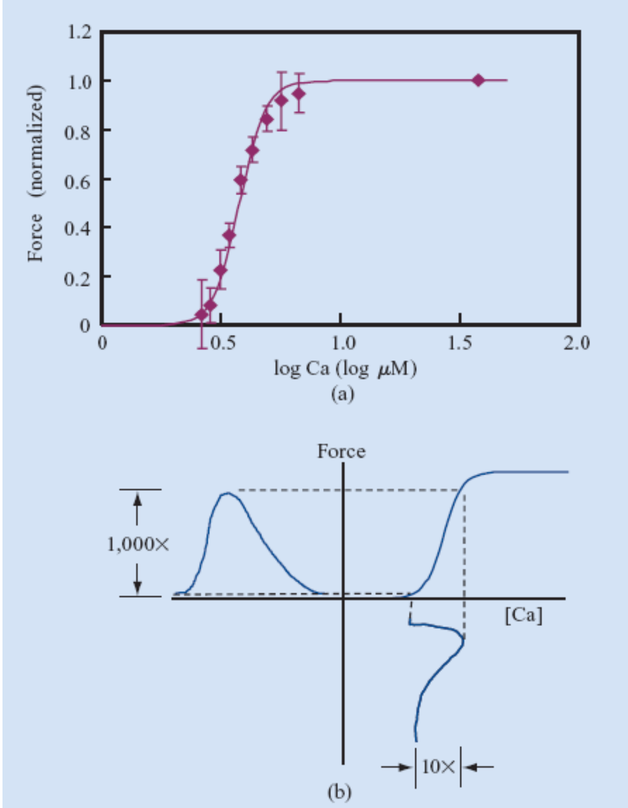

## Length-Dependent Activation




Isometric peak twitch tension in cardiac muscle continues to rise at sarcomere lengths >2 µm due to sarcomere-length dependent increase in myofilament calcium sensitivity

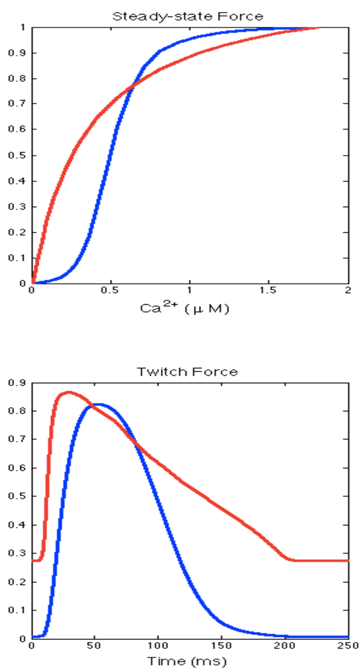
## Cooperative Myofilament Activation

- Developed force is a steep nonlinear function of activator calcium (Ca) concentration.
- (a) is experimental data from rat cardiac muscle collected by de Tombe's lab.
- The steep calcium sensitivity produces a much larger change in force than the relative change in intracellular calcium
- (b) Twitch forces rises slower than the calcium transient and falls faster

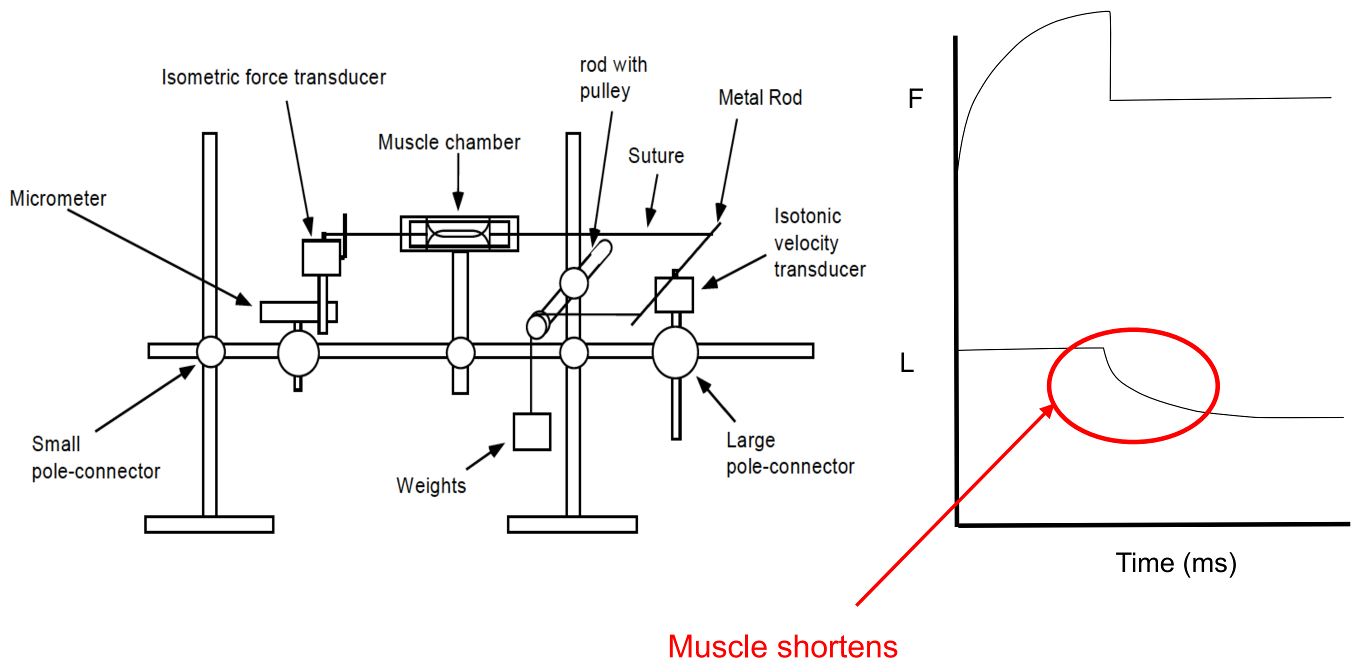



## Cooperative Activation of Myofilaments by $Ca^{2+}$

- Thought to be mediated by overlap of adjacent tropomyosin molecules
- Inhibits force at diastolic $[Ca^{2+}]$
- Enhances force during *late* twitch and greatly impacts twitch dynamics



# Isotonic (constant force) Test




# Isotonic (constant force) Test

Consider two isometric tests. In test 1, the isotonic force is about 50% of the isometric value as seen in the diagram. In test 2, the isotonic force is lower, only about 20% of the the isometric value. Which is true?

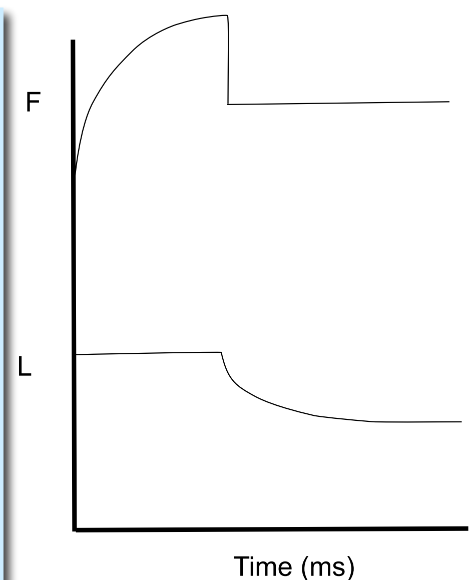
(A)The shortening velocity is higher in test 1

(B)The shortening velocity is higher in test 2

(C)The shortening velocity is the same in test 1 and test 2 but the total amount of shortening is greater in test 1

(D) The shortening velocity is the same in test 1 and test 2, but the total amount of shortening is greater in test 2

(E)The shortening velocity and the total amount of shortening are the same in test 1 and test 2



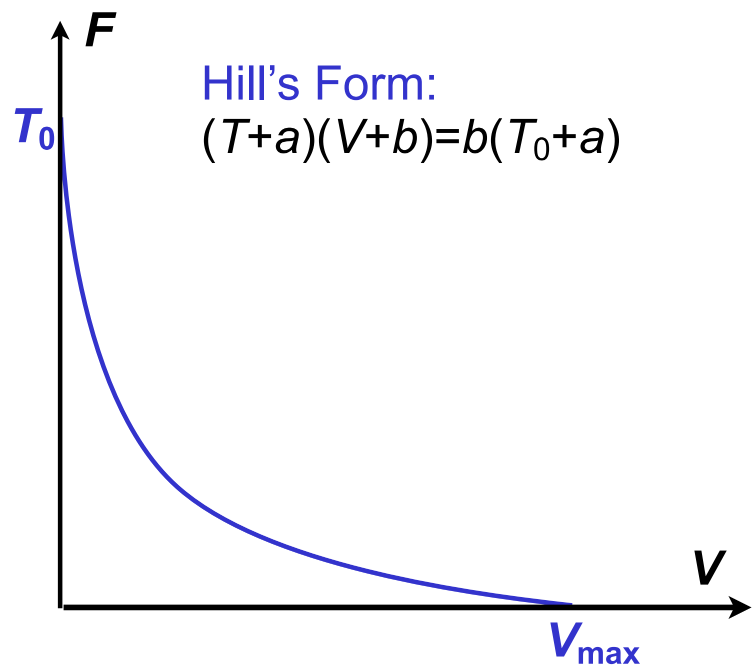
## Hill's Force-Velocity Relation

Dimensionless forms:

$$\frac{V}{V_{max}} = \frac{1 - T/T_0}{1 + c\left(T/T_0\right)}$$

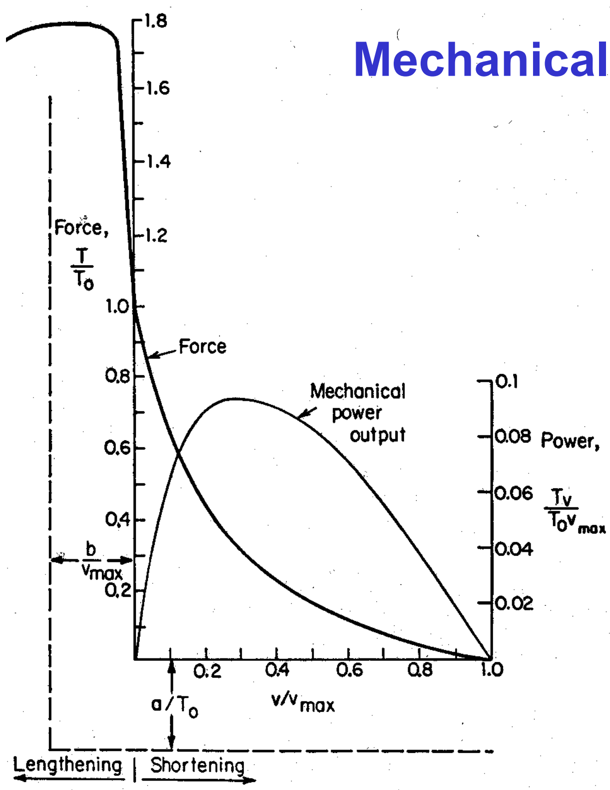
$$\frac{T}{T_0} = \frac{1 - V/V_{max}}{1 + c\left(V/V_{max}\right)}$$

- -a, -b = asymptotes
- $T_0$ = Isometric tension
- $V_{max}$= unloaded shortening velocity
- c = $T_0$/a (ranges from 1.2-4.0)




Hill's Form:
$(T+a)(V+b)=b(T_0+a)$

## Mechanical Power Output

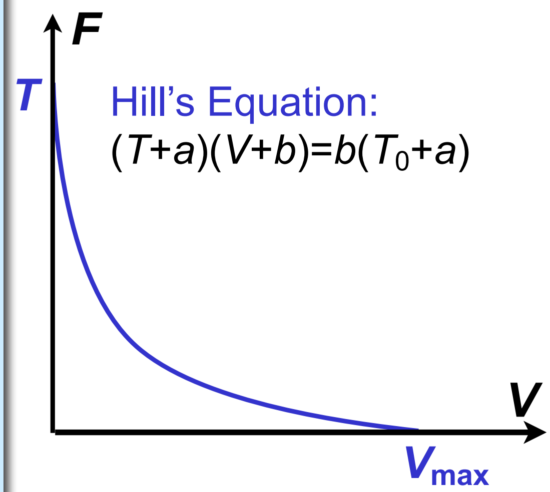



- Mechanical power output is the product of T and V
- The shortening part ($V$>0) of the curve was computed from Hill's equation with $c$ = 4
- The asymptotes for Hill's hyperbola (broken lines) are parallel to the $T/T_0$ and $V/V_{max}$ axes

## Hill's Force-Velocity Relation

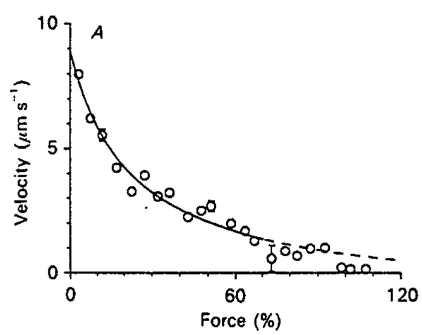
The force-velocity relation is generally interpreted as being a property of the contractile apparatus of the sarcomere, but it is also affected by the passive non-contractile components of the tissue. Imagine that the effects of passive non-contractile structures were represented by a dashpot in parallel with the contractile element. How would this alter the F-V relation?

(A) It would increase $T_0$
(B) It would decrease $T_0$
(C) It would increase $V_{max}$
(D) It would decrease $V_{max}$
(E) B and D

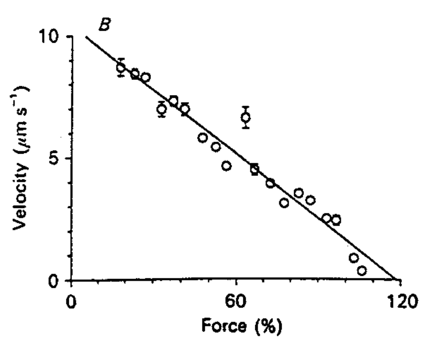



## Isotonic Force-Velocity Relation

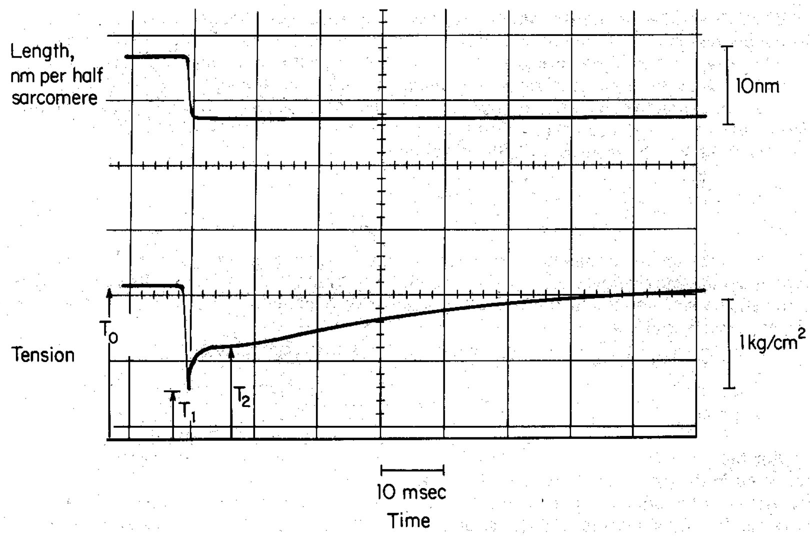
Isovelocity release experiment conducted during a twitch




Cardiac muscle force-velocity relation corrected for viscous forces of passive cardiac muscle which reduce shortening velocity



## Small Length Step Response

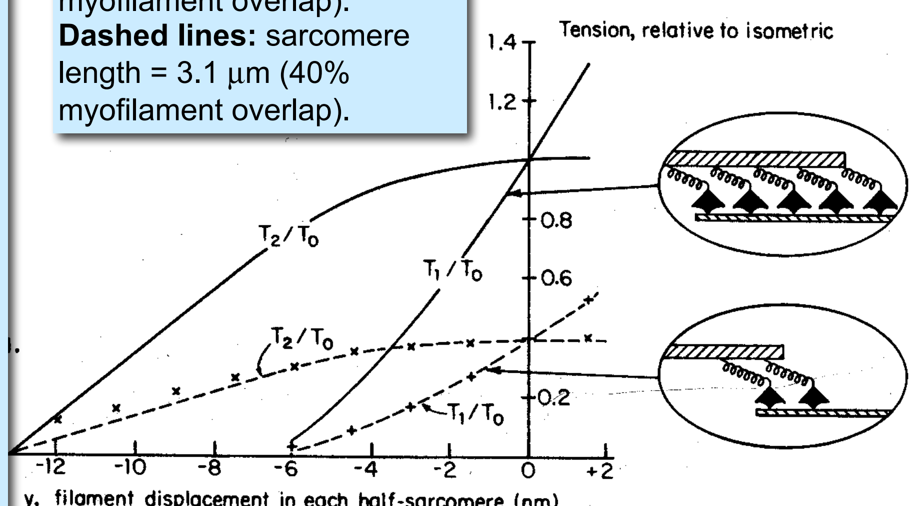



Tetanized single frog muscle fiber at 0ºC during a 1% shortening step lasting 1 ms

## Instantaneous and Plateau Tension

(A) The instantaneous elasticity represents the contribution of titin in the sarcomere.
(B) The slope of the curve is the parallel elastic element
(C) The slope of the curve is the series elasticity in this experiment
(D) The slope of the curve is a reflection of cross-bridge stiffness
(E) C and D

**Solid lines:** sarcomere length = 2.2 μm (maximal myofilament overlap).
**Dashed lines:** sarcomere length = 3.1 μm (40% myofilament overlap).



## Cardiac Muscle: Summary

- *Cardiac muscle fibers* (cells) are short and rod-shaped but are connected by *intercalated disks* and collagen matrix into a spiral-wound *laminar fibrous architecture*
- The *cardiac sarcomere* is similar to the skeletal muscle sarcomere
- Cardiac muscle has a very slow twitch but it can not be tetanized because the *cardiac action potential* has a refractory period
- *Calcium* is the intracellular trigger for cardiac muscle contraction
- *Cardiac muscle testing* is much *more difficult* than skeletal muscle: *laser diffraction* has been used in trabeculae
- Cardiac muscle has relatively *high resting stiffness* due to more collagen and shorter titin isoforms.
- The *cardiac muscle isometric length-tension curve* has no real descending limb