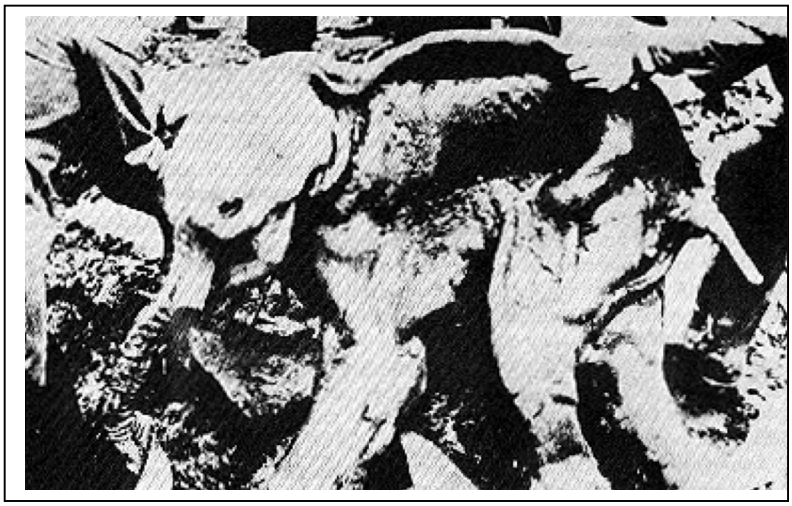
ANSC/FSTC 607
Physiology and Biochemistry of Muscle as a Food
POSTMORTEM PROTEOLYSIS, IONIC STRENGTH, AND THE RESOLUTION OF RIGOR



I. Rigor and its resolution

A. The rigor bond

1. ATP consumed by non-contractile activity of A-M-ATPase and ion pumps.
2. Irreversible crosslinking of actin and myosin occurs.
3. Muscle becomes inextensible (rigor).

B. Ultrastructural changes

1. Z-Line (most obvious change).
    a. Extensively degraded in type II.
    b. Relatively unchanged in type I.
    c. Both fiber types fragment at Z-disc after aging.
    d. Loss of Z-line structure due to degradation of desmin.
2. Effects of intracellular calcium.
    a. Increases activities of proteases.
    b. May increase rate of glycogenolysis and thereby increase glycolysis.

II. Changes in specific myofibrillar proteins during the aging process

A. Myofilaments

1. No visible degradation of thick and thin filaments.
2. No detectable breakdown products of thick and thin filaments.

B. Desmin

1. Degraded during aging.

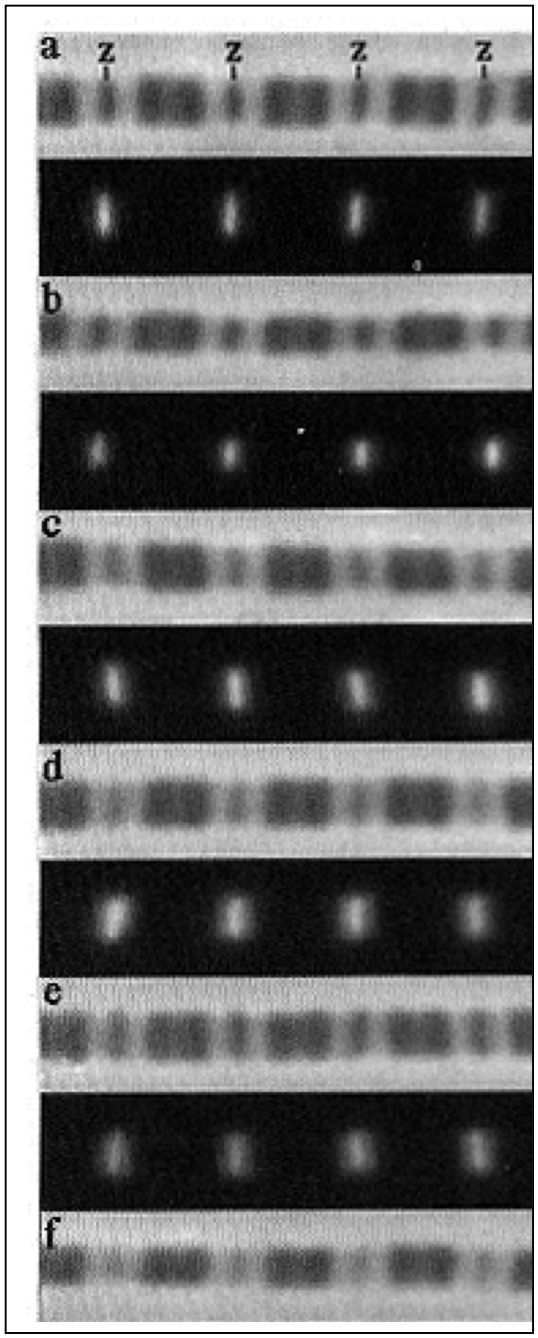
2. Probably causes fracture at Z-line.

C. Titin

1. Thought to contribute to tenderness.
2. Extensively disrupted at pH 5.5.
3. Disruption occurs early postmortem.
4. Degradation of titin at specific sites leads to a broadening of the Z-line.

D. Troponin-T

1. Degrades to "30 K subunit".
2. Extent of degradation is correlated loosely with degree of tenderness



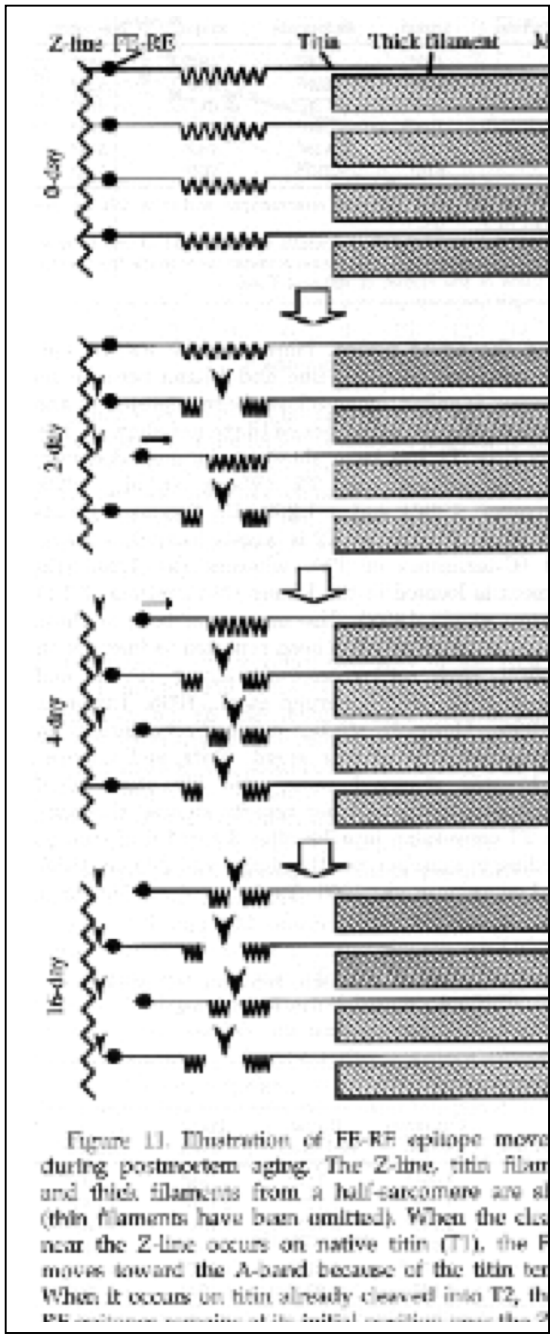


Figure 11. Illustration of FF-RF epitope move
during postmortem aging. The Z-line, titin filam
and thick filaments from a half-sarcomere are sl
(thin filaments have been omitted). When the cle
near the Z-line occurs on native titin (T1), the F
moves toward the A-band because of the titin ter
When it occurs on titin already cleaved into T2, th

III. Contribution of endogenous proteases to the resolution of rigor

A. Cathepsins

1. Located primarily in lysosomes, normally responsible for turnover of proteins.

3. Active at acidic pH.

4. *In vitro,* digest many proteins and structures that are not affected during normal aging.

5. Primarily involved in the degradation of sarcoplasmic proteins.

B. Calpains (calcium-activated proteases)

1. Located primarily at the Z-disc.

2. Normal function is inhibited by calpastatin.

3. Most active at alkaline pH.

4. Require calcium for activity.

a. Calpain system probably is involved in myofibril assembly and myogenesis.

b μ-calpain requires micromolar concentrations of calcium (as seen in postmortem muscle).

c. m-calpain requires millimolar concentrations of calcium.

d. Autolysis may reduce their requirements for calcium.

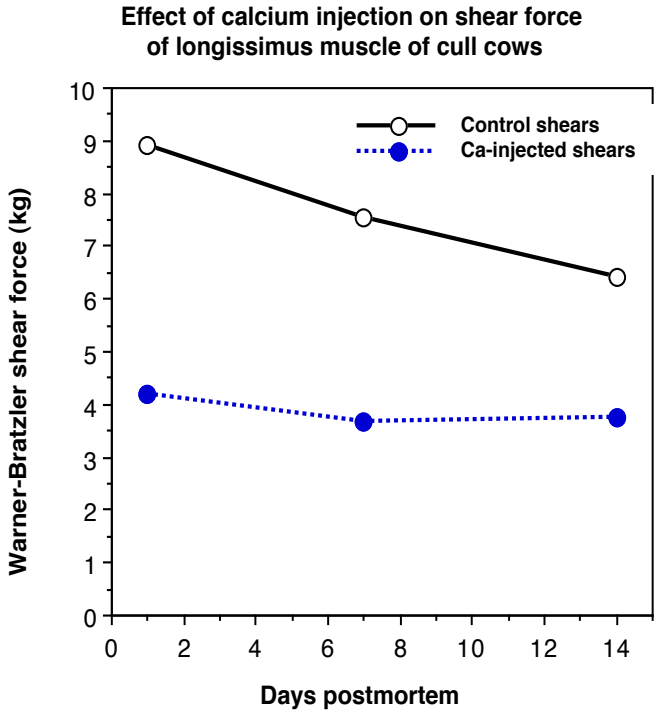
5. Activity is strongly depressed by increasing ionic strength.

Protease	In muscle cells?	pH optimum	Muscle protein digested
Cathepsin A	Yes	5.0-6.0	Myosin, myoglobin
Cathepsin B	Yes	3.5-6.0	Actin, myosin, intact myofibrils, collagen
Cathepsin C	Yes	5.0-6.0	Not determined
Cathepsin D	Yes	2.5-5.0	Actin, myosin, intact myofibrils
Cathepsin H	Yes	5.5-6.5	Actin, myosin, α-actinin, troponin-T, troponin-I, collagen
Cathepsin J	Yes	5.5-7.5	Unknown
Cathepsin L	Yes	3.0-6.5	Actin, myosin, α -actinin, troponin-T, troponin-I
Calpains	Yes	7.2-8.0	Tropomyosin, troponin-T (to 30 kd protein), troponin-I, C-protein, desmin, titin (?)
Multicatalytic Proteinase	Yes	9.0-10.0	Unknown

C. Evidence for calpains

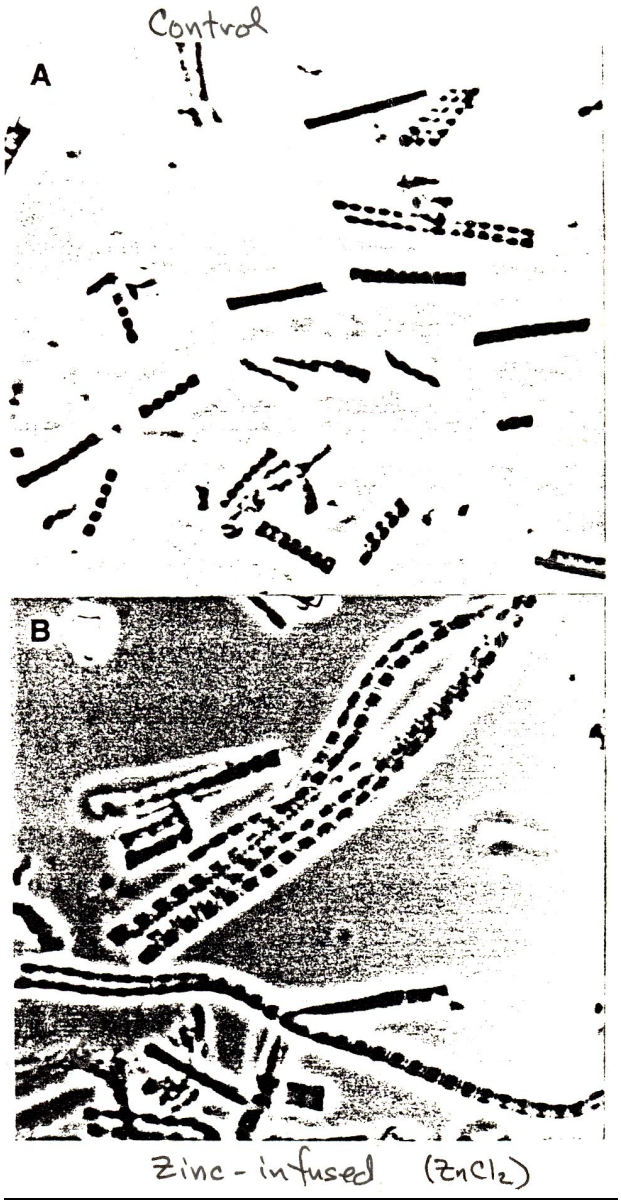
1. Calcium infusion accelerates rate of resolution of rigor.

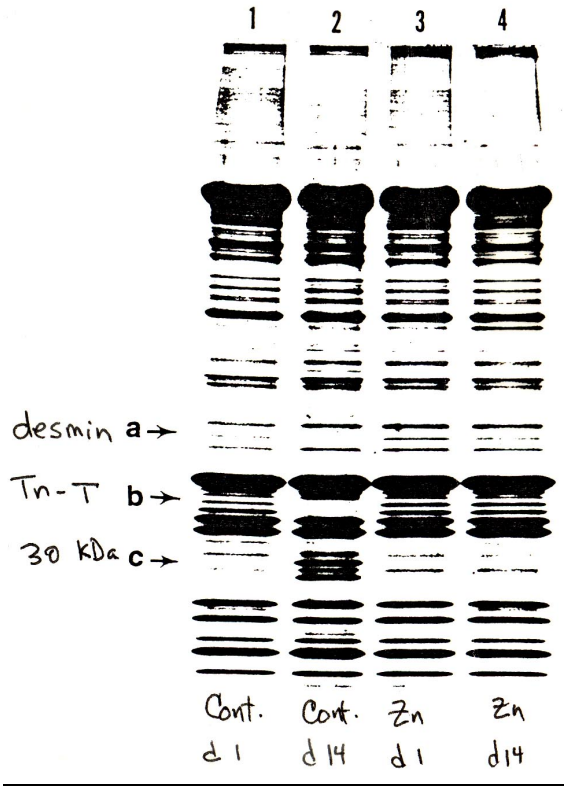
2. Zinc infusion (which inhibits calpain activity) stops the aging process.



3. Muscle from zinc infused carcasses does not become more tender over time.
   a. Myofibrills from zinc-infused carcasses do not fragment.
   b. CDP-inhibitor (calpastatin) activity does not decline over time.
   c. Desmin and troponin-T do not fragment postmortem.

	Control		$ZnCl_2$-infused	
	d_1	d_{14}	d_1	d_{14}
Zn (μg/g)	8.25		62.48	
Shear Force[a]	11.47	5.72	11.25	10.13
MFI	43.63	75.92	39.07	39.67
CDP-I[b]	76.93	17.26	115.13	22.78
CDP-II[c]	115.19	109.30	126.01	92.88
CDP-inhibitor[d]	125.53	9.96	142.18	187.47
Cathepsin B[e]	85.17	105.82	93.88	97.57
Cathepsin B+L[e]	77.62	90.72	92.63	84.09


Control
A
B
Zinc-infused ($ZnCl_2$)


1
2
3
4
desmin a
Tn-T b
30 kDa c
Cont. d1
Cont. d14
Zn d1
Zn d14