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EFFECT OF TEMPERATURE ON POST-MORTEM STRUCTURAL CHANGES

IN RABBIT, BOVINE AND PORCINE SKELETAL MUSCLE

by

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INTRODUCTION

Almost all the palatability attributes of meat are estab-
lished during rigor mortis and post-rigor softening, so from
the standpoint of meat quality, it is important to characterize
the chemical and ultrastructural changes accompanying or
resulting from rigor mortis. In spite of much recent effort in
this area, the molecular events responsible for the observed
physical changes occurring during rigor mortis remain an enigma.
It has been suggested that state of contraction in post-mortem
muscle has an important influence on meat quality, especially
tenderness. Moreover, it has been shown that state of contrac-
tion varies in an unexplained way among different species of
animals, with different times and temperatures of post-mortem
storage and with physiological state of the animal just prior
to exsanguination. Although many of the gross chemical changes
occurring in post-mortem muscle have been well characterized,
this information has not led to an understanding of the molecular
events contributing to changes in meat quality. For example,
it is well-known that to varying extents lactic acid increases
and pH, adenosine triphosphate, and phosphocreatine decrease
soon after death. Yet none of these changes, either singly or
in combination, appears to be simply related to meat quality and
to improvements which occur in meat quality during post-mortem
aging. The myofibrillar proteins are responsible for the

contractile process in muscle, and a study of the changes which
occur in subcellular properties of the contractile proteins
themselves during rigor mortis may be expected to contribute
significantly to our understanding of variations in meat
quality. A first step in such a study should include a thorough
ultrastructural characterization of rigor mortis in myofibrils
from different species and after different times and tempera-
tures of post-mortem storage.

The study of rigor mortis and post-mortem shortening may
also provide valuable insights into the nature of the actin-
myosin interaction and the mechanism of contraction in living
muscle. Post-mortem shortening resembles in vivo contraction
in several ways, since both of these processes are triggered
by an efflux of calcium, use ATP as an energy source, and have
similar active state properties. Post-mortem shortening has
the inherent advantage of occurring over a period of several
hours instead of the few milliseconds required for in vivo
contraction. This makes it easier to study the shortening
process at different stages. The fact that it is temperature-
dependent also makes it possible to exert some control over
post-mortem shortening by merely regulating storage temperatures.

The purpose of this study was threefold. One objective
was to determine, both at the light and electron microscopic
levels, the structural changes that take place in muscle during
post-mortem storage at various temperatures. It was also the

intention of this investigation to compare post-mortem structural changes in bovine, porcine and rabbit skeletal muscle, thereby indicating whether studies on one species can be related to those on the other species. This information may also afford some explanation for the widely differing responses of muscle from these three species to different post-mortem storage temperatures. Objective three of this study was to compare the structure of isolated myofibrils with the structure of fixed, sectioned muscle. The reason for this interest in myofibrillar structure is that the outcome of biochemical and physiological studies conducted on post-mortem muscle will depend, in many cases, on the structural state of the contractile elements. It is often possible to reach more definite or more encompassing conclusions in experiments on post-mortem muscle or meat if the preparations have been morphologically monitored. Myofibrils lend themselves to this type of monitoring because they are relatively easy to prepare and observe in a short time using phase microscopy. If preparations of myofibrils do not in fact accurately represent the in situ structure of muscle, it is fruitless to apply this monitoring technique to studies on myofibrils. The method of myofibril isolation was also studied to ascertain the most appropriate method for preservation of the structural integrity of myofibril preparations.

Due to the intensive nature of this study and the large number of samples involved for each animal, only a small number

of animals of each species could be incorporated into this investigation. Furthermore, the genetic background and physiological state (nutritional and environmental background) were not known on the animals used in this study. Although the genetic background could exert some influence on the post-mortem response of muscle, it is not anticipated that this should be a major effect. Also, only the physiological state of the animal just prior to death would be expected to exert any major effect on the post-mortem response of muscle. This physiological state was controlled, insofar as was possible under our conditions. It is therefore felt that the results of this study should provide a reasonably accurate account of ultrastructural changes in post-mortem muscle, although the exact time post-mortem at which these changes occur will vary under different conditions.

REVIEW OF LITERATURE

Early studies of the structure of voluntary muscle (Jordan, 1933) showed that the muscle cell or fiber contains longitudinal threads called myofibrils and that living muscle is cross-striated, consisting of alternating light and dark bands. It was later learned that myofibrils themselves are cross-striated and that in living muscle, adjacent myofibrils lie in register to confer a cross-striated appearance on the entire cell. The dark band is called the A-band and the light band is called the I-band. Under polarized light, the A-band is birefringent or anisotropic whereas the I-band is only weakly birefringent or isotropic. Thus, in the polarizing microscope, the appearance of the two bands is reversed from their appearance in the light microscope, and the A-band is light and the I-band dark. Other structural features of the myofibril as seen in the phase microscope, include the Z-disk which is seen as a dark line bisecting the I-band. In the middle of the A-band of resting muscle is a lighter area called the H-zone, after the German word "heller" which can be trans-lated "lighter or brighter." In the middle of the H-zone is a dark line called the M-line, which is observed only occasionally in the light microscope but is easily seen in electron micro-graphs. The area from one Z-line to the next is known as a sarcomere and is regarded as the structural and functional unit of muscle.

The work of many early histologists on muscle structure
has now been confirmed and extended by the use of phase micros-
copy (Huxley, 1953b), interference microscopy (Huxley and
Niedergeke, 1954), and electron microscopy (Huxley; 1953a;
1953b; 1963; 1964a). Use of these techniques has shown that
the striated appearance of myofibrils originates from a double
array of interdigitating thick and thin filaments. The thin
filaments are attached to the Z-disk and extend into the
sarcomere for a length of approximately 1 μ on either side of
the Z-disk. The thick filaments, about 1.5 μ long in rabbit
muscle, lie in the middle of the sarcomere. The A-band appears
dark under the phase microscope because this is the region that
contains the thick filaments. The I-band does not contain
thick filaments and appears light in the phase microscope.
Since the thin filaments do not extend all the way to the center
of the sarcomere, there is a small region (about 0.5 μ wide if
the sarcomere length is 2.5 μ) in the center of the sarcomere
which does not contain any thin filaments. This area is the
H-zone. The M-line, seen in the electron microscope as a dark
line in the center of the A-band, has been suggested (Huxley,
1966) to originate from the presence of three to five sets of
cross-bridges between adjacent thick filaments, these cross-
bridges holding the thick filaments in register in each A-band.
Adjacent sets of thick and thin filaments lie in register to
cause the striated appearance of myofibrils. Using selective

extraction techniques, Hanson and Huxley (1953) and Szent-
Györgyi et al. (1955) have found that the A-band is removed
when myosin is extracted from myofibrils. Selective extraction
for actin removes materials from the I-band leaving only the
Z-line. It had earlier been shown by Szent-Györgyi and co-
workers, (Banga and Szent-Györgyi, 1942; Straub, 1942) that
myosin and actin were the two major proteins making up the
myofibril. Recent studies (Pepe, 1966; Szent-Györgyi and
Holtzer, 1963a; 1963b) using the fluorescent antibody technique
also indicate that myosin is located in the A-band and that
actin is situated in both the I-band and the A-band. It is
concluded from these and other studies that thick filaments
are composed almost entirely of myosin whereas thin filaments
contain principally actin. Corsi and Perry (1958) have reported
evidence that tropomyosin, a third fibrous protein found in
myofibrils, is associated with the thin filament and is extracted
with actin.

The molecular properties and interactions among the three
major myofibrillar proteins, myosin, actin and tropomyosin,
have received extensive study. Rice (1961a; 1961b) has observed
individual myosin molecules in the electron microscope and
found that they are long, rod-shaped molecules with a globular
"head." These molecules were estimated to be about 1550±150Å
long with the tail being 15-20Å wide and the globular head

being 40-50Å in width. In other electron microscopic investigations, Zobel and Carlsen (1963) reported a length of 1600Å for myosin with the length of the globular head being 400Å. Recent evidence suggests that the tail portion of myosin consists almost entirely of two or more peptide chains, each in the form of an α-helix, coiled around one another to produce a coiled-coil structure (Cohen and Holmes, 1963). In the head portion of the myosin molecule, these peptide chains separate to produce a globular structure.

Gergely (1950) first reported that short trypsin treatment of myosin results in a marked loss of viscosity and subsequent studies (Mihalyi, 1953; Mihalyi and Szent-Györgyi, 1953a; 1953b) showed that trypsin as well as chymotrypsin (Gergely et al., 1955) and subtilisin (Middlebrook, 1959) split the myosin molecule near its center into two different components, each about 800Å in length. These two components were named light meromyosin (LMM) and heavy meromyosin (HMM) because of their relative rates of sedimentation in the analytical ultracentrifuge. All three enzymes cleave the myosin molecule at approximately the same location, ostensibly because the normal α-helical structure of the myosin "tail" does not exist at this point, thereby rendering this area more vulnerable to enzymatic attack. Segal et al. (1967) have shown that there is a very high proline content at this proteolytically-sensitive region of myosin, and since proline cannot be sterically-accommodated

in an α-helical structure, this may account for the absence of the α-helical structure at this site. It appears that this proteolytically-sensitive region is about 200-300Å long, and that after proteolysis, a LMM molecule, 600-700Å in length, and a HMM molecule, 400-600Å in length, are obtained (Young et al., 1965). The LMM molecule is rod-shaped and almost 100% α-helical in nature, indicating that it comes from the tail portion of myosin, whereas the HMM molecule has a globular head with a short "tail" and is only about 45% α-helical indicating that it comes from the head portion of myosin (Lowey et al., 1966; Szent-Györgyi et al., 1960). Both the ATPase and actin-combining activities of myosin are associated entirely with the HMM fragment which now differs from the parent myosin molecule by being soluble in water (Perry, 1960). The LMM fragment, however, retains the aggregating abilities of the parent myosin molecule.

The subunit structure and molecular weight of myosin remain controversial. Kielley and Harrington (1960) indicated that in 5M guanidine hydrochloride, myosin is separated into subunits of 200,000 molecular weight. Sedimentation equilibrium studies found a molecular weight of 600,000 for the parent myosin molecule suggesting that myosin is composed of three subunit polypeptide chains. Lowey and Cohen (1962) on the other hand found a molecular weight of 420,000 for myosin and suggested that two subunit polypeptide chains are present in myosin.

Huxley (1963) using purified preparations of actin and HMM found that HMM combines with actin to form "arrowhead" structures, and concluded that the cross bridges extending from the surface of thick filaments represent the HMM portion of the myosin molecule. When interacted with actin filaments, the HMM is oriented in the same direction all along the surface of the actin filament. If thin filaments still attached to the Z-disk are isolated it is found that HMM is oriented in one direction on one side of the Z-disk and in the opposite direction on the other side of the Z-disk. The arrowhead-like structures that form from the HMM attaching to the actin filaments point away from the Z-line (Huxley, 1963; 1965).

Huxley (1963) suggested that thick filaments are formed from myosin molecules such that the HMM portion of the myosin extends outward from the surface of the thick filament to form the cross-bridges that interact with actin in the thin filaments. This outward bending of the HMM portion of myosin may be facilitated by the absence of the α-helical conformation in the proteolytically-sensitive region of the molecule. In agreement with Huxley (1963), Pepe (1967b) also found that the HMM head of the myosin molecule bends and forms the cross-bridges. Pepe suggested that cross-bridges near the tapering ends of the thick filaments are longer than near the center because, near the end of the thick filament, the bend occurs at the proteolytically-sensitive region of myosin whereas, near the

center of the thick filament, the bend occurs at the junction
of the globular head with the fibrous tail. The LMM fragments
which possess the aggregating properties of myosin, interact
with each other to form the shaft of the thick filaments.
Myosin molecules on opposite sides of the center of the thick
filament are oriented in opposite directions so that, at the
center of the thick filament shaft, myosin molecules aggregate
tail-to-tail, with the heads of the molecules pointed in
opposite directions. This means that the cross-bridges on the
surface of the thick filament are separated at the middle of
the thick filament by a bare smooth area devoid of cross-
bridges, since this area contains only the LMM portion of
myosin (Huxley, 1963). The bare center portion of the thick
filaments has been named the pseudo-H-zone. Pepe (1967a) has
presented a detailed model for the structure of the thick
filament based on antibody staining of myofibrils and observa-
tions from electron microscopy. In this model, the myosin
molecules are aggregated in parallel rows with a tail-to-tail
aggregation in the pseudo-H-zone and M-line regions. There are
12 rows of myosin molecules in the cross-section of the thick
filament at the M-line region and 18 rows from the pseudo-H-
zone to the tapered ends of the thick filament. The myosin
molecules are so arranged that the 430$\overset{\circ}{A}$ repeat of the thick
filament can be accounted for by the position of the cross-
bridges.

Actin the second major protein of the myofibril makes up 25-30% of the total myofibrillar protein (Huxley and Hanson, 1957). Actin exists in two forms, globular or G-actin and fibrous or F-actin. Using negative staining techniques, Hanson and Lowy (1963, 1964) found that F-actin either in solution or in vivo before extraction consists of strands of globular subunits, with two such strands wound around one another to form a double helix. Selby and Bear (1956) and Cohen and Hanson (1956) using x-ray diffraction concluded that the length between cross-over areas was either 350 or 406Å. Electron microscopic evidence now suggests that the cross-over points of the actin helix are 350Å apart. Hanson and Lowy (1962, 1963) have been able to count the number of subunits in the actin helix and find thirteen per turn (six in one strand and seven in the other). These subunits are spherically shaped with a diameter near 55Å. Depue and Rice (1965) using the mica replication technique have reported that the F-actin helix is right-handed.

The third major fibrous protein of the myofibril, tropomyosin, was discovered by Bailey (1946). Tropomyosin makes up about 10-15% of total myofibrillar protein and is therefore present in quantitatively lower amounts than either myosin or actin. It has been shown (Hodge, 1959; Huxley, 1963) that tropomyosin will crystallize, being the only myofibrillar protein with this property. The exact location of tropomyosin

in the myofibril is not known; however, it is thought to be
present in the Z-disk and along at least part of the length of
the thin filament (Corsi and Perry, 1958; Endo et al., 1966;
Huxley, 1963). The role of tropomyosin is also not completely
understood at this time; it appears to be associated with the
troponin-actin interaction and is presently considered one of
the regulatory proteins.

Ebashi and coworkers (Ebashi and Ebashi, 1965; Ebashi
and Kodama, 1965; Maruyama, 1965) have recently discovered
three new proteins that (along with tropomyosin) appear to
regulate the contractile activity of muscle. These proteins
have been named α-actinin, β-actinin, and troponin. The
available evidence suggests that α-actinin is bound to only
actin (Briskey et al., 1967a, 1967b; Seraydarian et al., 1967)
and that it has the function either of promoting and strenth-
ening the actin-myosin interaction, or simply of cross-linking
actin filaments together, or both (Goll et al., 1968). There
is good evidence t_ , α-actinin is located in or very close to
the Z-line (Goll et al., 1968).

β-actinin was discovered by Maruyama (1965) as an impurity
in certain actin preparations. This protein appears to regulate
the length of the F-actin filaments (Maruyama, 1966a; 1966b).
In the presence of β-actinin, F-actin filaments are restricted
to lengths of approximately 1 μ, their length in vivo, whereas
in the absence of β-actinin, F-actin filaments may grow to

lengths of 7-8µ.

Troponin has been reported to sensitize actomyosin to calcium, (Ebashi and Ebashi, 1964; Ebashi and Kodama, 1965; Seraydarian et al., 1967). In the presence of troponin, small amounts of calcium (10^{-6} to 10^{-5} M) are necessary for contraction of actomyosin systems, but in the absence of troponin, actomyosin will contract in the absence of calcium. Ebashi and Kodama (1966) report that troponin binds to tropomyosin which in turn binds to actin. Thus, the presence of tropomyosin appears to be a necessary prerequisite to the binding and subsequent biological activity of troponin. Troponin is located along the length of the thin filament, where it may be distributed in a periodic fashion (Ohtsuki; et al., 1967), but is not found in the Z-line.

The ultrastructure of the Z-disk was first described by Knappeis and Carlsen (1962). They found that the I- filaments do not pass intact through the Z-line but instead divide into four smaller filaments which then pass diagonally through the Z-line to form parts of four thin filaments on the opposite side. In cross-section, arrays of four actin filaments on one side of the Z-disk are arranged to form the corners of a square, and the original actin filament donating parts to these four filaments is found in the center of this square on the other side of the Z-disk. Since the single strands pass diagonally through the Z-disk a zigzag configuration is observed in the Z-disk. Several other studies (Franzini-

Armstrong and Porter, 1964b, Reedy, 1964) have confirmed the
Z-line structure suggested by Knappeis and Carlsen. Tropomyosin
and/or actin are thought to be the primary proteins making up
the Z-disk. Corsi and Perry (1958) noticed that upon extraction
of these two proteins, the Z-line and the thin filaments
disappear. In sections through the Z-line, Huxley (1963) found
a lattice structure very similar to the lattice structure of
crystallized tropomyosin (Hodge, 1959). Ashley et al. (1951)
and Stromer et al. (1967a) reported that trypsin rapidly removes
the Z-line. Since tropomyosin is very labile to the proteolytic
action of trypsin, this finding provides additional presumptive
evidence for the existence of tropomyosin in the Z-disk. It
has now been reported (Stromer et al., 1967b) that selective
extraction procedures will remove both the Z-line and M-line
from glycerinated rabbit muscle, leaving the rest of the
myofibrillar structure intact, and furthermore, that by ad-
justing the ionic conditions in the presence of certain protein
fractions, the Z-line can be replaced in these extracted
myofibrils. The characterization of these protein fractions
has not been completed at the present time.

The sarcolemma or cell membrane of the muscle fiber is
another structural component important in muscle contraction.
Kono and Colowick (1961) and Rosenthal et al. (1965) have
published methods for isolating intact sarcolemma from skeletal
muscle. Kono and Colowick (1961) found that the sarcolemma is

65% protein, 15% lipid and 3% carbohydrate. The major protein component of the sarcolemma appears to be collagen since collagenase will dissolve the sarcolemmal membrane, and a thermal shrinkage temperature of 57-59° was obtained on isolated sarcolemma. The lipid component was mainly phospholipid. An electron microscope study of the isolated sarcolemma showed that sarcolemmal membranes are composed of three layers, an outer layer of collagen fibers, a middle basement membrane layer and an inner plasma membrane (Kono et al., 1964). Abood et al. (1966) found that bull frog sarcolemma contains 16% lipid, 67% protein and 0.9% polysaccharide. Japanese workers (Hotta and Usami, 1967) found that the sarcolemma exhibits an ATPase activity that is activated by Na^+, K^+, and Mg^{++}. Calcium ions alone inhibit this ATPase activity, but when added in combination with the above three ions, Ca^{++} causes a slight activation. Sulfhydryl groups also appear to be involved in the sarcolemmal ATPase activity. Studies on calcium binding suggested that calcium is not bound by the sarcolemma, but that Ca^{++}-binding ability of the muscle cell is localized in the sarcoplasmic reticulum.

Structurally the sarcoplasmic reticulum of muscle is in some ways analogous to the endoplasmic reticulum of other cells, but functionally, the sarcoplasmic reticulum has a specialized role of Ca^{++} binding and release in muscle contraction. Although the sarcoplasmic reticulum morphologically

resembles endoplasmic reticulum by being composed of a series

of intracellular membranous tubules, its structure differs

from normal endoplasmic reticulum in that it can be divided

into a transverse or T-system and a longitudinal or L-system

(Franzini-Armstrong and Porter, 1964a). The tubules of these

two systems do not directly communicate with one another and

the two systems have distinctly different functions. The

transverse or T-system consists of a number of tubules extending

perpendicularly from the sarcolemma into the body of the fiber

at regular intervals along the length of the fiber. Depending

on the type of muscle and the species of animal, these in-

vaginations may occur either at the level of each Z-disk (one

per sarcomere) or at each A-I junction (two per sarcomere).

The tubules open directly to the extracellular space and

represent long invaginations of the sarcolemma into the muscle

cell. Huxley and Taylor (1958) have suggested that the T-

system is responsible for the transmission of nerve impulses

into the myofibrils and this suggestion has received much recent

support (Costantin and Podolsky, 1967). Porter and Palade

(1957) have reported the existence of small finger-like pro-

jections extending from the T-tubules down into the myofibrils

for a depth of several filaments. These projections could

possibly aid in rapid conduction of impulses into the center

of the myofibril.

The longitudinal or L-system of the sarcoplasmic reticulum

consists of a series of fine longitudinally-oriented tubules

extending in both directions from cisternae associated with

the T-system. These longitudinal tubules anastomose with

tubules extending from adjacent T-tubules at a point approxi-

mately halfway between the T-tubules. The longitudinal or

L-system is apparently the site of the ATPase-dependent,

calcium-binding activity of muscle (Costantin et al., 1965),

and is postulated to be the site of the relaxing factor of

muscle.

The relaxing factor system in muscle was first described

by Marsh (1951). Briggs et al. (1959) and Briggs and Portzehl

(1957) suggested that the relaxing factor system consists of

two components, one composed of microsomal granules and the

other being a dialyzable co-factor. The existence of the

dialyzable co-factor has remained controversial, however, and

it is now widely believed that the microsomal system itself

represents the complete relaxing factor system. The myofibril-

lar system in vivo contains troponin and tropomyosin and there-

fore requires Ca^{++} for contraction and maximal ATPase activity.

Thus, contractile activity can be turned on and off by simply

regulating intracellular free Ca^{++} levels. With this in mind,

a number of investigators have studied the location of calcium

during excitation, contraction, and relaxation of muscle.

Winegrad (1965) using the technique of autoradiography showed
that calcium in resting or relaxed muscle of frog skeletal
muscle is most concentrated at the A-I junction, which is the
location of the T-tubules in this muscle. The greater the
tension produced by muscle the greater the accumulation of
calcium in the A-band. Bianchi and Shanes (1959) and Bianchi
(1965) reported that calcium entry into the muscles increases
upon contraction, and Frank (1960) reported that muscle will
not contract in a calcium-free solution. Also, Weber and
associates have shown that contractions of either glycerinated
muscle fibers, myofibrils or "natural actomyosin" require
calcium (1961a, 1961b, 1964). The sarcoplasmic reticulum has
been shown to possess the ability to accumulate calcium against
a concentration gradient until the calcium level inside the
sarcoplasmic reticulum is about 5000 times higher than outside
(Hasselbach, 1964). The increase of calcium in the sarco-
plasmic reticulum is accomplished by a calcium pump and the up-
take of calcium is coupled to the splitting of ATP which
provides energy for the reaction. This reaction (the uptake
of calcium) can proceed until the level of free calcium ions
has been reduced to about 10^{-8} to 10^{-9} M. It is not clear at
the present time, however, how the activity of the Ca^{++} pump
is turned on and off.

The molecular events contributing to muscle contraction
remain a mystery, but much information has accumulated on the

structural framework in which contraction occurs. In the early
1950's, Huxley and Hanson (1954) and Huxley and Niedergerke
(1954) independently observed that the H-zone disappears and
the I-band becomes shorter during contraction. This discovery
coupled with the finding that myofibrils are constructed of a
double array of interdigitating thick and thin filaments
(Hanson and Huxley, 1953) led to the conclusion that during
contraction the thin filaments slide past the thick filaments
causing the H-zone to disappear and the I-band to shorten.
The important feature distinguishing the sliding filament
theory of contraction from most of the earlier theories in
this area is that in the sliding filament theory, neither the
thick nor the thin filaments change in length during shortening;
rather shortening is accomplished by a sliding of filaments
past one another. The force behind this sliding is apparently
generated by the interaction of the HMM cross-bridges extending
from the surface of the thick filament with actin in the thin
filament. It has, however, been difficult to obtain detailed
information on the nature of the interaction of the cross-
bridges with actin during contraction. Recently Reedy (Reedy,
1967; Reedy et al., 1965) reported that in resting insect
muscle the cross-bridges are horizontal to the filaments but
become slanted during rigor and may push the actin filaments
toward the M-line. This suggests that cross-bridges do move
during contraction, as is predicted by the sliding filament

model for contraction.

In spite of the considerable weight of evidence in its favor, the sliding filament hypothesis has not been accepted by all investigators in the field of muscle research. Sjöstrand (1964) and Sjöstrand and Jagendorf-Elfvin (1967) present evidence that glycerinated rabbit psoas muscle contracted with ATP does not shorten by a sliding of filaments; instead a slight shortening of the A and I bands occurs and ATP=contracted muscle is characterized by contraction bands forming at the A-I junction. Sonnenblick et al. (1963) reports that the width of the H-band in heart muscle does not change even after the I band has disappeared. This would suggest that there is a folding of the thin filaments during contraction.

A few years ago, several workers (Carlsen et al., 1961; deVillafranca, 1961; deVillafranca and Marschhaus, 1963; Knappeis and Carlsen, 1956) reported that the A-band and there-fore, the thick filaments, shorten during contraction. Huxley and coworkers (Page, 1964; Page and Huxley, 1963) conducted an extensive examination of filament lengths at different stages of contraction and found that filaments do not change in length during the contraction process. These workers suggested that the reported changes in A-band length can be attributed in part to alterations caused by the dehydration and staining procedures used to prepare specimens for electron microscopic examination. However, reports of A-band shortening, particularly in cases of

extreme contraction, persisted. A very likely explanation for these reports has now been suggested by the work of Hoyle et al. (1965) on supercontracted muscle. Supercontracted muscle is muscle shortened to sarcomere lengths of 1.5 μ or less (or to less than 40% of rest length). Hoyle's report suggests that upon supercontraction the thick filaments pass through the Z-disk and overlap with thick filaments from the adjacent sarcomere. This causes substantially increased density around the Z-line. Huxley (1965) indicated that the thick filaments may not pass through the Z-line but instead crumple against it and may in fact fold back on themselves. Stromer et al. (1967a) observed that bovine muscle stored at 2° for 24 hours post-mortem was supercontracted and that the thick filaments in this muscle appeared in some cases to pass through the Z-disk. Osborne (1967) studying the supercontraction of muscle in the blowfly larva showed that the thick filaments pass through the Z-disk which has circular holes in it. In all these cases, overlapping or crumpling of the thick filaments at the Z-line may cause changes in the phase microscopic image which could be easily interpreted as a shortening of the A-band. Furthermore in some cases of severe contraction, the M-line filaments may rupture and thick filaments may slide out of register with one another. In this case, only the region of thick filament overlap, which will be shorter than the total length of the thick filaments, will appear as an A-band in the phase microscope.

There have been several reports that muscle stretched to the point that the thin filaments no longer overlap the thick filaments will still develop tension and shorten to a sarcomere length of 0.7 μ. If tension is developed by an interaction of the cross-bridges on the thick filaments with specific sites on the thin filaments, as is indicated by the sliding filament theory, the ability to develop tension should cease when the thick and thin filaments no longer overlap (Carlsen et al., 1965a; Setlow and Pollard, 1962). Carlsen et al. (1965b) suggest that shortening takes place by a sliding of filaments only if the filaments are overlapped at the onset of contraction. If, however, the muscle before stimulation is stretched until a gap exists between the thick and thin filaments, the I-band becomes shorter by a curling of the thin filaments at the A-I boundary, and no overlap of thick and thin filaments is observed. Recently Hoyle (1967) and McNeill and Hoyle (1967) have reported evidence for an elastic "thin-thin" filament which could account for the tension development and shortening of muscle stretched to the point of no overlap. Huxley and coworkers maintain however, that muscle stretched to the point of no overlap will not develop any tension and that results indicating otherwise are due to failure to get all the sarcomeres in a fiber stretched to this point.

In a very recent report Huxley and Brown (1967) have presented x-ray diffraction evidence on living muscle showing

that the cross-bridges do indeed undergo a cyclic and repetitive movement during the contraction process. Using a special low angle x-ray diffraction device, Huxley found that during contraction, reflections originating from the cross-bridges change in intensity in their azimathal and radial positions. On the other hand, reflections from the actin filaments and the body of the myosin filaments do not change significantly during contraction. This evidence provides strong support for the sliding filament model of muscle contraction and for the involvement of cross-bridges in tension generation, and it must be concluded at this stage, that muscle probably contracts in a manner very similar to that proposed by the sliding filament theory.

Davies (1963) has proposed a detailed theory outlining the molecular events leading to tension development and shortening in muscle. Davies theory is phrased in terms of the sliding filament theory and may be described as follows: A nerve impulse activates the muscle by depolarizing the sarcolemma and the sarcoplasmic reticulum. Due to this depolarization, calcium is liberated from the sarcoplasmic reticulum and forms a chelate link between an ATP bound to the extended HMM cross-bridge and the bound ADP which is known to exist in the F-actin filament. In resting muscle, negative charges of the bound ATP on HMM and of the ADP on actin repel one another, thereby preventing the actin-myosin interaction and keeping the muscle relaxed. Because of neutralization of the negative

charge on the terminal phosphate of the ATP bound to HMM, the coulombic repulsion within the extended HMM polypeptide is eliminated, and the extended cross-bridge therefore contracts spontaneously once the bridge with actin is formed (calcium linkage). This shortening results in the thin filaments being pulled past the thick filaments, causing tension development. Davies suggests that active site of the HMM ATPase activity is located inside the cross-bridge, where it does not have easy access to either free cytoplasmic ATP or the ATP bound to the extended HMM polypeptide chain. When the polypeptide chain contracts, however, it makes the bound ATP of HMM accessible to the ATPase site which then hydrolyzes the ATP and breaks the bridge between the thick and thin filaments. The resulting ADP is then phosphorylated by cytoplasmic phosphocreatine and creatine kinase, restoring the negative charge on the end of HMM. This in turn renews the coulombic repulsion between the end of the HMM cross-bridge and the body of the thick filament. The HMM polypeptide chain extends and, pending the availability of sufficient Ca^{++}, the whole process can repeat itself again and again until the muscle is contracted or the nerve impulse ceases. When calcium is removed from the system, the cycle is broken and the muscle relaxes.

The phenomenon of rigor mortis has for many years been an active and intriguing area of research. The stiffening of the

cadaver after death is mentioned in accounts written hundreds of years ago. In spite of the efforts of pathologists, meats researchers, and others, the events causing rigor mortis are still poorly understood. Hoet and Marks (1926) presented data suggesting that loss of one of the sugar phosphates is in part responsible for the development of both acid and alkaline rigor. Erdös (1943) found that the onset of rigor is closely correlated with the post-mortem loss of ATP and suggested that stiffening is due to the formation of actomyosin chains. Because of the lack of ATP in post-mortem muscle, these chains are not able to dissociate to actin and myosin as they can in living muscle, where an active ATP-generating system is available.

Between 1935 and 1955, most of the work on rigor mortis emanated from the Low Temperature Research Station at Cambridge, England. During this period the efforts of first E. C. Bate-Smith and later J. R. Bendall and B. B. Marsh succeeded in characterizing most of the gross chemical and physical changes occurring in muscle during the onset of rigor mortis and sub-sequent post-rigor softening. It was shown that in rabbit muscle, the modulus of elasticity increases about tenfold as rigor mortis develops and that muscle connective tissue is not involved in the loss of elasticity or in the stiffening ob-served during the onset of rigor (Bate-Smith, 1939). The

Cambridge group also found that the onset of rigor closely
coincides with depletion of muscle ATP (Bate-Smith and Bendall,
1949) and suggested that because of its potential for regener-
ation of ATP via anaerobic glycolysis, muscle glycogen concen-
tration is the one factor most highly related to the rate with
which rigor mortis developed after death. In their studies,
the English workers (Bate-Smith and Bendall, 1949; Marsh, 1952;
Marsh, 1951) followed the time-course of rigor development by
measuring elasticity of muscle strips. So long as the strips
are elastic, the muscle is said to be in the pre-rigor or delay
phase. That period in which elasticity rapidly decreases is
called the rapid phase and corresponds to the onset of rigor
mortis.

Depletion of muscle glycogen reserves by starvation, in-
sulin shock, or a violent death drastically shortens the delay
phase and also increases the ultimate pH of the muscle, since
less lactic acid is produced by anaerobic glycolysis. It was
shown in this work that initial pH is determined by the severity
of struggle at death and ultimate pH is determined by the level
of feeding and the degree of fatigue before death. Onset of
rigor in rabbit muscle appears to be dependent on ATP, and not
on the ultimate pH, but lactic acid production parallels ATP
loss. After breakdown to ADP, ATP can be regenerated by phos-
phocreatine and creatine kinase. Bendall (1951) showed that
phosphocreatine is the first high energy phosphate compound to

be broken down in muscles stored at post mortem temperatures of 37° and 17° and that net ATP degradation commences after 70% of creatine phosphate is gone. As a result of this work at Cambridge, Bendall concluded that rigor mortis can best be explained as a very slow irreversible contraction, and that ATP is a necessity for both rigor shortening and physiological contraction.

The most recent work on rigor shortening has emphasized the role of Ca^{++} in post-mortem shortening. Nauss and Davies (1966) induced rigor mortis in frog sartorius muscle by using 2.4-dinitro-fluorobenzene to inhibit creatine kinase, thereby preventing rephosphorylation of ADP to ATP by phosphocreatine. Extent of shortening and total ATP and free Ca^{++} concentrations were measured on muscle strips after exposure to this treatment. It was shown that an efflux of Ca^{++}, originating from the sarcoplasmic reticulum, occurs almost simultaneously with the initiation of rigor shortening. Both the Ca^{++} efflux and rigor shortening commences before ATP is completely depleted. Nauss and Davies suggested that the Ca^{++} efflux is the event which triggers rigor shortening. The calcium efflux may originate from ATP concentration falling to a level to low to maintain the activity of the Ca^{++} pump in the sarcoplasmic reticulum, or it may simply reflect a degeneration of membrane integrity, destroying its ability to accumulate Ca^{++} against a

concentration gradient. Greaser et al. (1967) studied the effects of in situ post-mortem storage of porcine muscle on the ability of microsomal fractions, isolated from the muscle after 1, 2, 3, or 24 hours post-mortem, to accumulate Ca^{++}. The ability of the "heavy sarcoplasmic reticulum" fraction (between 8,000 xg and 30,000 xg) to accumulate Ca^{++} is decreased by 40% after three hours post-mortem; after 24 hours post-mortem, the Ca^{++} accumulating ability of all the subcellular fractions (myofibrils, 1000 xg; mitochondria, 5000 xg; heavy sarcoplasmic reticulum, 30,000 xg; light sarcoplasmic reticulum, 60,000 xg) had fallen to 10% or less of their initial values. Electron microscopic observations showed that structure of the heavy sarcoplasmic reticulum fraction changed very little during post-mortem aging, thus failed to provide a structural basis for the post-mortem loss of Ca^{++} -binding activity. Other evidence implicating the release of Ca^{++} as the initial event causing rigor shortening and the onset of rigor mortis has been provided by the finding that anti-mortem injection of EDTA (Weiner and Pearson, 1966) or soaking of muscle strips in EDTA or EGTA solutions (Feinstein, 1966) inhibits post-mortem shortening of rabbit muscle, and also prevents the development of inextensibility during rigor, even though such injection does not affect the rate of ATP breakdown. Together the preceding studies provide strong evidence that rigor shortening resembles muscle contraction in two ways: 1) both require

ATP, and 2) both are probably triggered by an efflux of Ca^{++} from the sarcoplasmic reticulum.

Recently Huxley and Brown (1967) reported that rigor shortening differs from physiological contraction in that the cross-bridges in rigor change in orientation so that all cross-bridges will be in a position to attach to an actin molecule. This finding is in agreement with the study of Robson et al. (1967) who, based on the changes of nucleoside triphosphatase activities of myosin B, suggested that a change occurs in the conformation of myosin or the actin-myosin complex during the onset of rigor mortis.

Temperature has a profound effect on post-mortem shortening. Early studies by Bendall (1951) provided evidence that rabbit muscle shortens considerably at post-mortem temperatures of 37° but shortens very little at either 17° or 2°. On the other hand, Locker and Hagyard (1963) found that bovine muscle shortens more at 2° than at 37° with minimal shortening occurring in the range of 14° - 19°. The shortening at 2° was termed "cold shortening." Rabbit muscle did not show this biphasic pattern of post-mortem shortening but rather exhibited increased rigor shortening with increasing temperature in the range 0 - 40°. Other experiments showed that, like bovine muscle (Locker and Hagyard, 1963), extent of shortening in porcine (Galloway and Goll, 1967) or ovine (Cook and Langsworth,

1967) muscle strips was a maximum at post-mortem storage temperatures of 2°, passed through a minimum near 16° and increased with increasing temperatures up to 37°, although degree of shortening at 37° was not as great as that at 2°.

In contrast to the earlier studies, which had measured only isotonic shortening and elasticity of post-mortem muscle strips, Jungk et al. (1966) studied the pattern of isometric tension development of post-mortem muscle strips. These isometric tension studies showed that development of isometric tension coincided with the onset of rigor shortening and that presumably, the same phenomena are responsible for both isometric tension development and post-mortem shortening. It is not clear at the present time, however, that amount of isometric tension developed corresponds directly to the extent of shortening.

Bovine muscle at 2° begins to develop isometric tension 1 to 3 hours post-mortem and reaches maximum tension development between 3 to 24 hours post-mortem. The exact time course of isometric tension development depends on the muscle, with the psoas muscle developing isometric tension sooner post-mortem and reaching maximum tension development before the semitendinosus muscle (Busch et al., 1967). Also, muscle from animals which have been stressed begins to develop isometric tension sooner post-mortem than muscle from rested, well-fed animals. After 12 - 36 hours post-mortem, isometric tension

gradually begins to decline and in some cases after 72 hours falls to less than 40% of maximum tension development. This decrease in isometric tension very likely corresponds to a "resolution of rigor" (Goll and Robson, 1967) and is the first discovery in post-mortem muscle of a measurable property which indicates that such a resolution may indeed occur. Earlier studies on rigor-mortis had all used extensibility to objectively measure rigor, and once post-mortem muscle becomes inextensible, it does not regain its extensibility until chemical or bacterial degradation has reached an advanced stage. For this reason, Bendall (1960) has indicated that there is no resolution of rigor mortis independent of bacterial action. It now appears, however, that if rigor mortis is defined in terms of cadaver stiffness, which could result from tension development by muscles on opposite sides of a bone, loss of this tension would cause loss of cadaver stiffness and a "resolution of rigor."

Further studies on isometric tension development showed that porcine muscle exhibits the same pattern of isometric tension development and decline at post-mortem storage temperatures of 2° as does bovine muscle (Galloway and Goll, 1967), and furthermore, that bovine, porcine, and rabbit muscle all develop considerable amounts of isometric tension at post-mortem storage temperatures of 37°, but that decline in

isometric tension development is not nearly so remarkable at 37° as at 2° (Busch, 1966; Busch et al., 1967; Galloway and Goll, 1967). Post-mortem shortening of bovine muscle at 37° also differs from that at 2° since shortening at 37° begins later post-mortem, when muscle ATP levels are less than 1mM and the pH is below 6.0, whereas shortening at 2° commences in the presence of 5-6 mM ATP and at pH values above 6.0 (Busch et al., 1967). Moreover, it was shown that post-mortem shortening of bovine and porcine muscle at 2° develops ten times more isometric tension than at 16°, and two to three times more isometric tension than at 37° (Busch et al., 1967; Galloway and Goll, 1967). Thus, the effect of post-mortem storage temperature on amount of isometric tension development closely parallels its effect on extent of shortening. On the basis of these findings, Busch et al. (1967) suggested that post-mortem shortening at 37° may be due in part to the thermal shrinkage of collagen fibers, whereas shortening at 2° was caused by a sliding of filaments in a manner resembling in vivo contraction.

The morphology of muscle during the onset of rigor mortis and during post-rigor aging was examined by light microscopy many years ago, but ultrastructural observations have been made only recently. The most complete characterization of post-mortem morphological changes in muscle appears to be that of Stromer and coworkers (Stromer and Goll, 1967a; 1967b; Stromer

et al., 1967a) on bovine muscle. Using phase and electron microscopic observations on either isolated myofibrils or on intact sectioned muscle, this investigation showed that bovine muscle is relaxed at death, but after 24 hours post-mortem at 2°, it is markedly shortened and resembles supercontracted muscle as described by Hoyle et al. (1965). On the other hand, after 24 hours at 16°, post-mortem myofibrils are only slightly contracted. These results correspond directly to the results of the isometric tension development and isotonic shortening experiments, and show that during the first 24 hours post-mortem, bovine muscle shortens markedly at 2° but only slightly at 16°. Phase micrographs suggest that post-mortem porcine muscle also exhibits these same morphological changes during the first 8 hours post-mortem at 2° and 16° (Galloway and Goll, 1967). After 24 hours post-mortem at 37°, bovine muscle (Stromer et al., 1967a) is markedly shortened, although not to the extent that the 2° - 24 hour muscle is. Porcine myofibrils from muscle stored at 37° for 8 hours, however, exhibit several unusual and unexplained structural features (Galloway and Goll, 1967), and it cannot be clearly decided whether post-mortem shortening at 37° proceeds via a different mechanism than post-mortem shortening at 2°.

At the present time, there appear to be several points of controversy concerning ultrastructural changes in muscle during

the first 24 hours post-mortem. Both Locker (1959) and Stromer
et al. (1967a) have presented ultrastructural evidence clearly
indicating that rigor-shortening of bovine muscle stored at 2°
occurs by a sliding of filaments in a manner analogous to that
observed for contraction. This work provides another point of
similarity between post-mortem shortening and muscle con-
traction. Neither Locker nor Stromer et al. report any excep-
tions to these general observations concerning post-mortem
contraction; yet Cook and Wright (1966) report that both
relaxed and contracted myofibrils were observed side-by-side in
ovine muscle stored at 0° for 24 hours. Paul et al. (1944)
presented light microscopic observations showing that during
post-mortem shortening of bovine muscle at 2°, the myofibrils
became kinked and crinkled. Bendall (1960) and more recently
Newbold (1966) have suggested that this indicates that only
some of the sarcomeres in a myofibril actually shorten during
post-mortem shortening, while the rest of the sarcomeres be-
come locked at approximately their rest lengths and are then
bent or kinked as a result of the force developed by adjacent
actively shortening sarcomeres. If so, it should be possible
to observe a substantial number of sarcomeres in 2° - 24 hour
bovine muscle having sarcomere lengths above 2 μ (rest length
is approximately 2.5 μ), Stromer et al. (1967a) did not
observe any such sarcomeres in their study. Another morphol-
ogical feature in post-mortem muscle is the appearance of

contraction bands, irregular transverse bands that occur during thaw rigor (Marsh and Thompson, 1958) and other violent contractions. Contraction bands were suggested to originate from precipitation of sarcoplasmic proteins on the myofibril (Bendall and Wismer-Pederson, 1962); however, Cassens et al. (1963c) reported that contraction bands possess a fibrillar structure in the electron microscope and originate from a disturbance of the fibrillar system. In a more recent study, Cook and Wright (1966) report that all transverse striations in post-mortem muscle can be accounted for either as definable components of the sarcomere or by degree of contraction. Ultrastructural studies of post-mortem changes in porcine muscle (Cassens et al., 1963a; 1963b) showed that after storage at 2° for 24 hours, the sarcoplasmic components of the muscle cell are disrupted. The myofibrils are contracted but appear well-preserved structurally. Porcine animals treated to induce the pale, soft, and exudative condition were found to be contracted with the I-band disrupted.

Although the morphological evidence just described agrees closely with the results of the isometric tension and isotonic shortening experiments and suggests that muscle shortens to varying degrees during the first 24 hours post-mortem, these findings do not provide any explanation for post-mortem tenderization of muscle which is usually observed only after 24 to 48 hours post-mortem in excised muscles (Goll et al., 1964).

In an attempt to obtain some ultrastructural information on the
cause of post-mortem tenderization, Stromer and coworkers
(Stromer and Goll, 1967a; 1967b; Stromer et al., 1967a) ex-
amined bovine muscle after 312 hours of post-mortem storage
at either 2° or 16°. Structure of muscle stored for 312 hours
at 16° did not differ greatly from that of muscle stored for
only 24 hours at 16° although the Z-line was partially disrupted
in the 16° - 312 hour muscle. However, it should also be noted
that tenderness of muscle stored at 16° does not change dras-
tically between 24 and 312 hours post-mortem. On the other hand,
tenderness of muscle stored at 2° improved markedly between 24
and 312 hours post-mortem, and ultrastructural examination of
the 2° - 312 hour muscle revealed that, remarkably, the ex-
tremely shortened sarcomeres observed after 24 hours at 2° had
lengthened back to nearly 70% of their at-death length. This
lengthening apparently occurred because of a weakening of the
actin-myosin interaction after 24 to 48 hours post-mortem and
very likely corresponds to the loss of isometric tension devel-
opment in muscle strips and to "resolution" of rigor in cadavers.
Gothard et al. (1966) had previously noted that within three
hours after maximum post-mortem shortening, sarcomeres in bovine
muscle began to lengthen and eventually reached 70 - 90% of
their rest length. Stromer et al. (1967a) also noted some
disruption of the Z-line in muscle stored for 312 hours post-
mortem at 2° and found (Stromer and Goll, 1967a) that myofibrils

prepared after 312 hours of post-mortem storage at either 2° or 16° were only three to four sarcomeres in length compared to myofibrils ten to fifteen sarcomeres in length from either at-death, 2° - 24 hour, or 16° - 24 hour muscle. This fragmentation ostensibly occurred because of some weakening of the myofibril at the junction of the thin filaments with the Z-line, this weakening resulting in shearing of the myofibril at this point during homogenization. Stromer's results on bovine muscle have been confirmed in chicken muscle by Takahashi et al. (1967) who observed both a fragmentation of myofibrils and, under some conditions, an increase in sarcomere length after 48 hours at 2°. Fukazawa and Yasui (1967) found that the zig-zag configuration of Z-line in chicken muscle was lost after storage at 0° for 24 hours and also suggested that weakening at or near the Z-line caused fragmentation of post-mortem myofibrils. Since Stromer et al. (1967a) have shown that the Z-line is one of the most proteolytically-sensitive areas of the myofibril, these results suggest that proteolysis by cathepsins may be responsible for both fragmentation and length-ening of rigor-shortened muscle. However, many reports have failed to find evidence in support of this idea. Using partially purified cathepsins, Bodwell and Pearson (1964) found no enzymatic action on preparations of actin, myosin and actomyosin. Very recently Martins and Whitaker (1968) using purified cathepsin D found no effect on the conformation of actomyosin

as measured by gel-filtration and viscosity.

It has been known for many years that muscle becomes more tender with increasing time of post-mortem storage (Deatherage and Harsham, 1947; Deatherage and Reiman, 1946; Goll et al., 1964; Moran and Smith, 1929) and bovine carcasses are often left to "age" for several weeks after death to insure tenderness. The tenderization of muscle during this aging was thought by early workers (Bate-Smith; 1948) to be the result of proteolysis probably caused by indigenous cathepsins in the muscle tissue. Subsequent work (Bodwell and Pearson, 1964; Husaini et al., 1950a; 1950b; Locker, 1960b; Martins and Whitaker, 1968), however, has provided a substantial amount of evidence against the view that proteolysis is primarily responsible for post-mortem tenderization of meat. Partmann (1963) suggests that post-mortem tenderization of muscle probably occurs as a result of dissociation of the actomyosin complex. On the basis of some ultrastructural evidence, Weidemann et al. (1967) suggest that the breaking of linkages between actin and myosin filaments and disruption of actin filaments at the Z-line are responsible for increased tenderness in post-mortem bovine muscle. This corresponds to the findings of Stromer et al. (1967a) on bovine muscle after 312 hour post-mortem and suggests that post-mortem tenderization is caused to a large extent by fragmentation of the myofibril at or near the Z-line and by

dissociation of the actin-myosin complex.

The preceding hypothesis supposes that degree of contraction is an important factor in meat tenderness and indeed evidence has been presented that degree of contraction of skeletal muscle is related to tenderness with more highly contracted muscle being less tender (Herring et al., 1965; Locker, 1960a). Marsh and Leet (1966) studied the effect of cold shortening on tenderness and showed that a 20% decrease in length of excised muscle caused little difference in tenderness. However, shortening between 20 and 40% of initial length caused a large decrease in tenderness. Muscle shortened to 50 - 60% of its initial length was more tender than muscle which was shortened only 40%. The post-mortem lengthening of rigor-shortened muscle, as described in the preceding paragraph, and the degree of muscular contraction appear to be important considerations in the study of post-mortem tenderization of muscle.

MATERIALS AND METHODS

Samples from bovine <u>semitendinosus</u> and porcine <u>longissimus</u> <u>dorsi</u> muscles were obtained from the Iowa State University meat laboratory as soon as possible after exsanguination. Rabbits were obtained from a certified supplier and held in small animal cages in the Food Processing Laboratory until use. Rabbits were stunned, immediately exsanguinated, and samples of the <u>longissimus</u> <u>dorsi</u> together with the leg muscles were used for preparation of myofibrils.

Samples for myofibril preparation and electron microscopy were taken immediately after death (15-30 minutes for porcine and bovine animals, 5-7 minutes for rabbit samples), and the remaining portions of the excised muscles were then divided and samples stored at each of four temperatures: 2°, 16°, 25°, and 37°. At 2° and 37°, samples for electron microscopy and myofibril preparation were taken after 4, 8, and 24 hours of post-mortem storage, whereas at 16° and 25°, samples were taken only after 24 hours of post-mortem storage.

Myofibrils were prepared according to a modified procedure of Stromer and Goll (1967a). Twenty to thirty grams of minced tissue were suspended in 5 volumes (v/w) of precooled 0.25 M sucrose containing 1 mM ethylenediaminetetraacetic acid (EDTA) and 0.05 M tris-(hydroxymethyl)-amino methane (Tris) buffer, pH 7.6, by use of a Waring blender or Lourdes homogenizer.

Three 15-second bursts with a 45-second cooling period between each burst were used for suspension. The homogenized suspension was then centrifuged at 2,500 $\underline{xg}$ for ten minutes, the supernatant discarded, and the sedimented myofibrils resuspended in 5 volumes of the sucrose solution. This suspension was centrifuged at 2,500 $\underline{xg}$ for 10 minutes, the supernatant discarded and connective tissue removal and washing of the myofibril sediment done according to the procedure of Stromer and Goll (1967a) with the exception that the 0.15 M KCl washes did not contain Tris buffer. The myofibril preparations were stored in 0.15 M KCl until use.

Experiments were also conducted to determine whether the presence of Tris in the 0.15 M KCl wash solutions had any effect on myofibril structure. These experiments involved two samples of muscle from the same animal, one of which was washed in 0.15 M KCl, 0.03 M Tris, while the other was washed in 0.15 M KCl alone.

The effects on myofibril structure of different levels of EDTA in the sucrose solutions used for the first two myofibrillar suspensions was investigated. The EDTA levels used in these experiments were: 0, 1, 2, and 5 mM. Four samples of minced tissue from the same animal were each suspended in sucrose solution containing one of these four levels of EDTA. This suspension was followed by a second suspension in the same sucrose solution after which the myofibrils were washed as

described in the preceding paragraph.

For phase microscopic observations a drop of myofibril suspension was placed on a clean glass slide and covered with a number 1 coverslip. Phase observations were obtained on a Zeiss Photomicroscope equipped with phase optics, a 100x planapochromatic objective, numerical aperature 1.3, and an achromatic-aplanatic condenser, numerical aperature 1.4. Micrographs were recorded on either Adox KB-14 or Kodak Plus-x-pan film with a green filter in the optical path.

Following Stromer's modification (1967a) of the technique of Price et al. (1965), samples of muscle for sectioning were taken by tying strips of muscle to a glass rod before freeing the ends of the strip from its attachments. After removal from the muscle, the strips were immersed in 2.5% gluteraldehyde containing 0.2 M Sorensons' phosphate buffer, pH 7.15, for 2 hours with fresh fixative added after the first hour. After the gluteraldehyde fixation, the fixative was removed by aspiration and the muscle strips washed with 0.2 M phosphate buffer, pH 7.15. The strips were then removed from the glass rod, placed on dental wax, flooded with Sorenson's phosphate buffer, and cut into approximately 1mm cubes. The cubes were placed in 1% osmium tetroxide buffered at pH 7.15 with 0.2 M Sorenson's phosphate buffer for a 2 hour post-fixation period. The fixative and buffer solutions were calculated at an osmotic strength of 480 milliosmoles according to the method of Powell

<u>et</u> <u>al</u>. (1964). Following fixation, the tissue was dehydrated in a graded series of acetone solutions, and the dehydrated tissue was infiltrated with an Epon-Araldite mixture as reported by Anderson and Ellis (1965). The infiltrated tissue was flat embedded in the Epon-Araldite mixture and polymerized at 60° for 24 hours. For phase microscopy, thick sections, 1.5 to 2 μ in thickness, were cut using glass knives and the Reichert OMU - 2 Ultramicrotome. For electron microscopy, thin sections, 60 mμ or less as judged by interference colors, were cut using a diamond knife. The thin sections were placed on uncoated 300 mesh copper grids, stained with 2% uranylacetate in methanol for 60 minutes, and then rinsed in two changes each of methanol, 50% methanol, and distilled water. After thorough drying, Reynolds lead citrate stain (Reynolds, 1965) was applied undiluted for 12 minutes. Electron micrographs were taken using the RCA EMU- 3F electron microscope at 100 KV.

RESULTS

The effect of Tris buffer in the KCl wash solutions used
in preparation of myofibrils was studied to determine whether
the absence of buffer in these solutions caused any structural
changes. The results of this study are shown in Fig. 1. Myo-
fibrils isolated from porcine muscle both immediately after
death (Fig. 1a,b) and after 8 hours of post-mortem storage at
2° (Fig. 1c,d) were relaxed and no difference in structure was
observed between myofibrils prepared using buffered or un-
buffered KCl wash solutions. Following storage at 2° for 24
hours (Fig. 1e,f), the myofibrils were contracted as evidenced
by their banding pattern, but again there was no detectable
difference between myofibrils washed in the presence or absence
of Tris.

The effect of EDTA on the structure of myofibrils was
studied to determine whether the variation observed in myofibril
preparations, especially immediately after death, could be
lessened. Muscle removed from the animal as soon as possible
after exsanguination is easily stimulated by cutting or grinding,
this stimulation causing the muscle to contract. Calcium,
which is believed to be involved in triggering contraction, is
chelated by EDTA. This makes Ca^{++} unavailable to the muscle
system, and therefore the presence of EDTA may be expected to
prevent muscle shortening. It was found that when EDTA was

Fig. 1a. Myofibrils from at-
death porcine muscle.
Washed in the absence
of Tris. X 2,000

Fig. 1b. Myofibrils from at-
death porcine muscle.
Washed in the pres-
ence of 0.03M Tris.
X 2,000

Fig. 1c. Myofibrils from 2°-8
hr. porcine muscle.
Washed in the absence
of Tris. X 2,000

Fig. 1d. Myofibrils from 2°-8
hr. porcine muscle.
Washed in the pres-
ence of 0.03M Tris.
X 2,000

Fig. 1e. Myofibrils from 2°-24
hr. porcine muscle.
Washed in the absence
of Tris. X 2,000

Fig. 1f. Myofibrils from 2°-24
hr. porcine muscle.
Washed in the pres-
ence of 0.03M Tris.
X 2,000
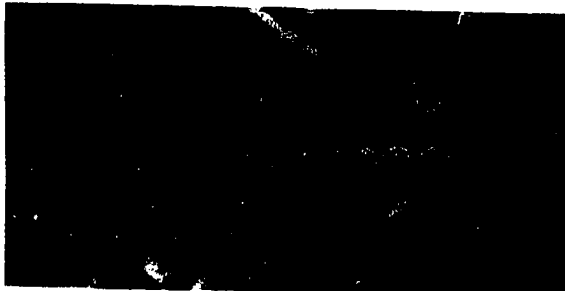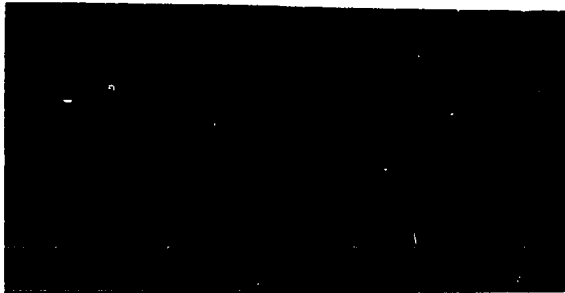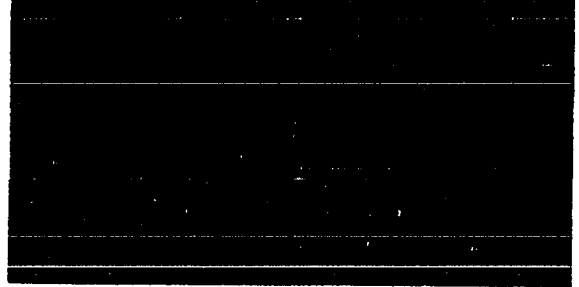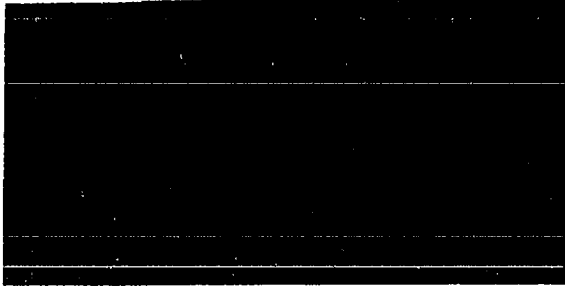
Fig. 2a.   At-death porcine myo-   Fig. 2b.   At-death porcine myo-
            fibrils isolated in   fibrils isolated
            the absence of EDTA.   using 1mM EDTA.
            X 2,000   X 2,000

Fig. 2c.   At-death porcine myo-   Fig. 2d.   At-death porcine myo-
            fibrils isolated   fibrils isolated
            using 2mM EDTA.   using 5mM EDTA.
            X 2,000   X 2,000

Fig. 2e.   Porcine myofibrils   Fig. 2f.   Porcine myofibrils
            after 4 hrs. at 2°   after 4 hrs. at 2°
            isolated in the ab-   isolated using 1mM
            sence of EDTA.   EDTA.   X 2,000
            X 2,000

Fig. 2g.   Porcine myofibrils   Fig. 2h.   Porcine myofibrils
            after 4 hrs. at 2°   after 4 hrs. at 2°
            isolated using 2mM   isolated using 5mM
            EDTA.   X 2,000   EDTA.   X 2,000

Fig. 2i.   Porcine myofibrils   Fig. 2j.   Porcine myofibrils
            after 8 hrs. at 2°   isolated after 8 hrs.
            isolated in the ab-   at 2° using 1mM
            sence of EDTA.   EDTA.   X 2,000
            X 2,000

Fig. 2k.   Porcine myofibrils   Fig. 2l.   Porcine myofibrils
            isolated after 8 hrs.   isolated after 8 hrs.
            at 2° using 2mM EDTA.   at 2° using 5mM EDTA.
            X 2,000   X 2,000

Fig. 2m.   Porcine myofibrils   Fig. 2n.   Porcine myofibrils
            after 24 hrs. at 2°   isolated after 24
            isolated in the ab-   hrs. at 2° using
            sence of EDTA.   1mM EDTA.   X 2,000
            X 2,000

Fig. 2o.   Porcine myofibrils   Fig. 2p.   Porcine myofibrils
            isolated after 24 hrs.   isolated after 24
            at 2° using 2mM EDTA.   hrs. at 2° using 5mM
            X 2,000   EDTA.   X 2,000
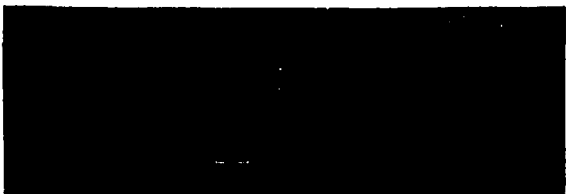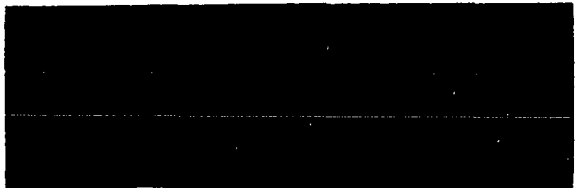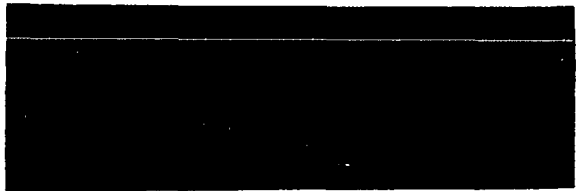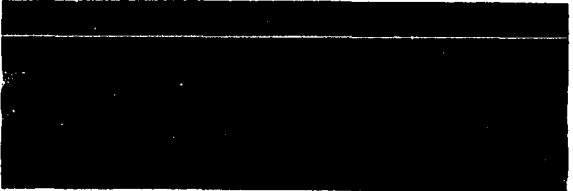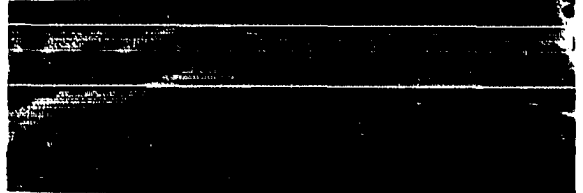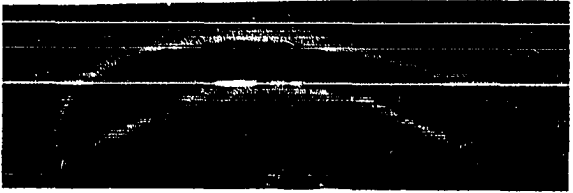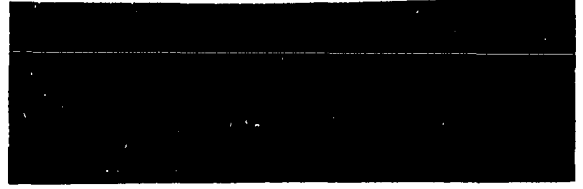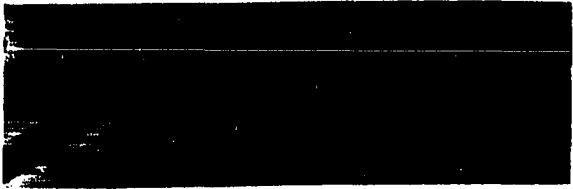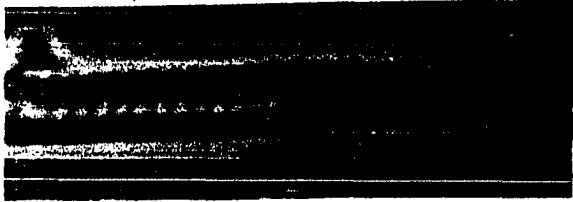
absent from the solutions used for initial suspensions, myo-
fibrils isolated at death (Fig. 2a), after 4 hours storage at
2° (Fig. 2e) and after 8 hours storage at 2° (Fig. 2i) were
supercontracted, as indicated by the presence of alternating
light and dark bands in the phase microscope. The presence of
1mM EDTA in the initial suspensions resulted in 80% or more
of the isolated myofibrils being relaxed after each of these
three post-mortem times (Fig. 2b,f,j). Increasing the level
of EDTA to 2mM (Fig. 2c,g,k) or 5mM (Fig. 2d,h,l) had no ad-
ditional structural effects over the 1mM EDTA level. After
24 hours storage at 2° (Fig. 2m,n,o,p), the presence or absence
of EDTA had no effect on structure of the myofibrils. EDTA
levels in the solutions used for initial myofibril suspension
also had no effect on the structure of myofibrils isolated
after storage at 37° for 4, 8 and 24 hours, and after storage
at 25° for 24 hours. Because of these results, myofibrils were
routinely prepared by using 1mM EDTA levels in the solutions
for initial suspensions. Also, since the presence of Tris in
the wash solutions had no demonstratable effects on myofibril
structure and since Tris interferes with the biuret test for
protein concentration, Tris buffer was not used in the KCl wash
solutions in this study.

Phase Microscopy Observations

This study investigated the structural changes that occur
in bovine, porcine and rabbit muscle during post-mortem aging at

different times (at-death, 4, 8 and 24 hours) and temperatures
(2°, 16°, 25° and 37°). The results of phase microscopic ob-
servations on myofibrils and on sectioned muscle are presented
first, starting with a discussion of the at-death samples and
continuing with the samples taken after increasing time of
post-mortem storage. All of the temperatures investigated are
discussed at each appropriate post-mortem sampling times.
Samples from bovine animals are discussed first, then the
samples from the porcine animals, and finally the rabbit samples.
After discussion of the phase microscopic observations, electron
microscopy results for all three species at each of the various
post-mortem times and temperatures are presented in the same
order described for the phase microscopy observations.

Bovine muscle

Myofibrils prepared from bovine semitendinosus muscle
immediately after death (Fig. 3a) were largely in the relaxed
state (90%), as evidenced by relatively wide I-bands and the
presence of distinct H-zones. There was, however, considerable
variability in sarcomere length, even among myofibrils with
similar banding patterns (Fig. 6a). All myofibrils with
sarcomere lengths 1.5 µ or greater exhibited a relaxed banding
pattern as shown by the presence of the H-zone, which is
reported to be the first structural component of the myofibril
lost during shortening or contraction. Since rest length of

bovine sarcomeres is normally about 2.2 - 2.6μ, this evidence suggests that isolation of myofibrils when the muscle is still excitable causes shortening of some sarcomeres in a manner entirely different from the ordinary shortening accompanied by a sliding of filaments and a loss of the H-zones. It was observed that the A-bands of these shortened myofibrils which still exhibit the relaxed banding pattern, are shorter (0.75 - 1.0μ) than the A-bands of sectioned muscle (1.25 - 1.5μ) sampled at the same time (cf. next paragraph). A few myofibrils isolated at death (10%) were in the supercontracted state with sarcomere lengths of less than 1.25μ. Slightly contracted myofibrils (H-zone gone with I-band still present) were not observed in at-death myofibril preparations of bovine muscle.

Sectioned muscle at-death (Fig. 4a) was always relaxed and exhibited the typical banding pattern described for relaxed muscle. The sarcomere lengths of at-death sectioned muscle (Fig. 6b) were longer than the sarcomere lengths of isolated myofibrils, again suggesting that some sarcomere shortening occurs during the isolation of myofibrils.

Myofibrils isolated from bovine muscle stored at 2° for 4 hours (Fig. 3b) exhibited the same relaxed banding pattern as that observed in myofibrils isolated at-death, with almost all myofibrils having the relaxed banding pattern. Sarcomere lengths in 2°-4 hour myofibrils (Fig. 6c) corresponded closely

Fig. 3a. Bovine myofibrils
          isolated at-death.
          X 2,000

Fig. 3b. Bovine myofibrils
          isolated after
          storage at 2° for
          4 hrs. X 2,000

Fig. 3c. Bovine myofibrils
          isolated after stor-
          age at 37° for 4 hrs.
          X 2,000

Fig. 3d. Bovine myofibrils
          isolated after stor-
          age at 37° for 4 hrs.
          X 2,000

Fig. 3e. Bovine myofibrils
          isolated after stor-
          age at 2° for 8 hrs.
          X 2,000

Fig. 3f. Bovine myofibrils
          isolated after stor-
          age at 37° for 8 hrs.
          X 2,000

Fig. 3g. Bovine myofibrils
          isolated after 24 hrs.
          of storage at 2°.
          X 2,000

Fig. 3h. Bovine myofibrils
          isolated after
          storage at 16° for
          24 hrs. X 2,000

Fig. 3i. Bovine myofibrils
          isolated after
          storage at 25° for
          24 hrs. X 2,000

Fig. 3j. Bovine myofibrils
          isolated after stor-
          age at 37° for 24
          hrs. X 2,000
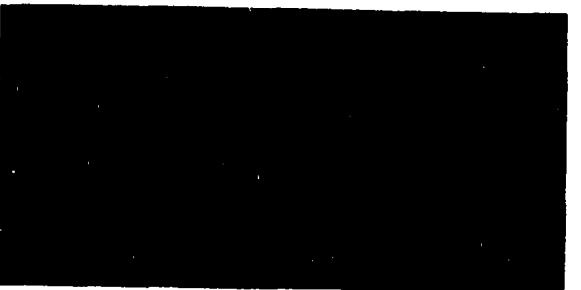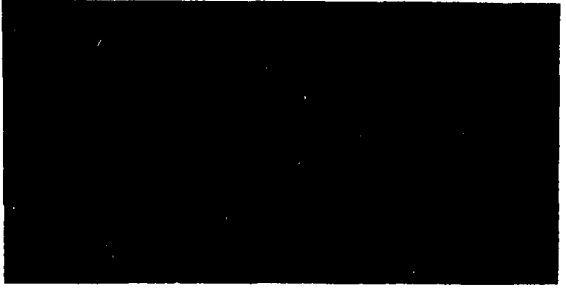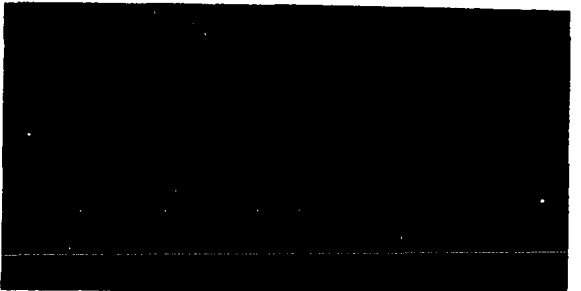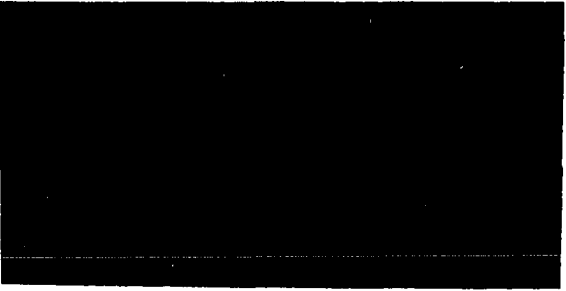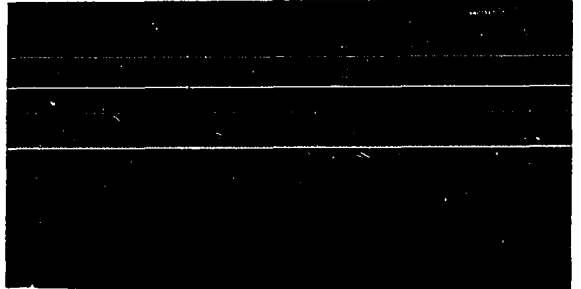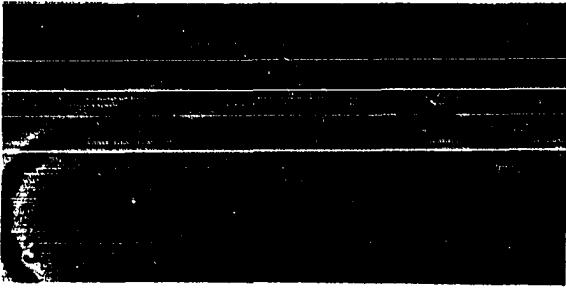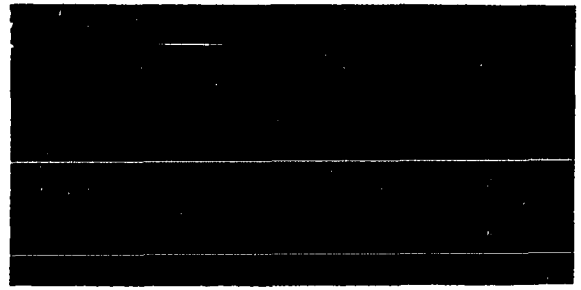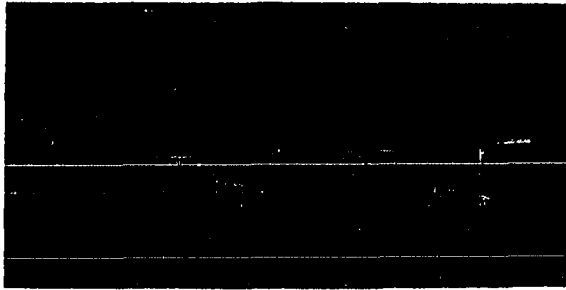
Fig. 4a.   Sectioned at-death   Fig. 4b.   Sectioned bovine
           bovine muscle.   muscle after stor-
           X 2,000   age at 2° for 4 hrs.
                                                X 2,000

Fig. 4c.   Sectioned bovine   Fig. 4d.   Sectioned bovine
           muscle after storage   muscle after stor-
           at 37° for 4 hrs.   age at 2° for 8 hrs.
           X 2,000   X 2,000

           Fig. 4e.   Sectioned bovine
                      muscle after stor-
                      age at 37° for 8
                      hrs.   X 2,000
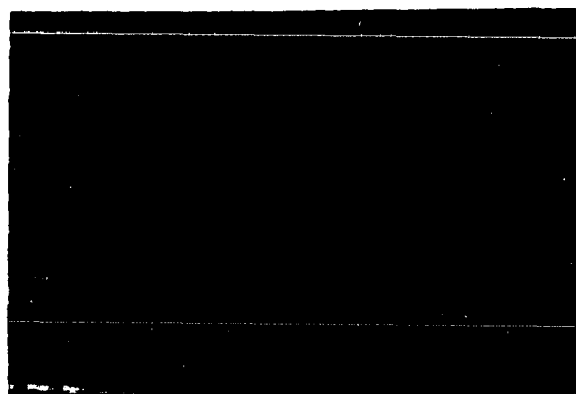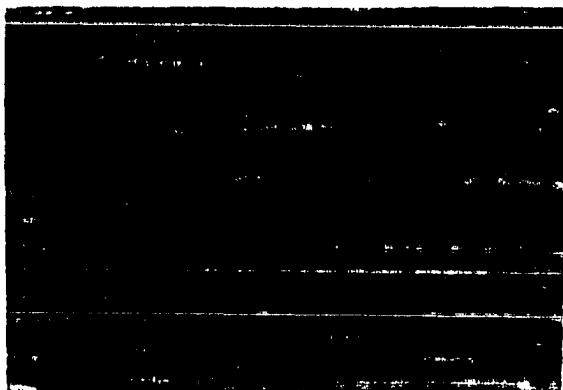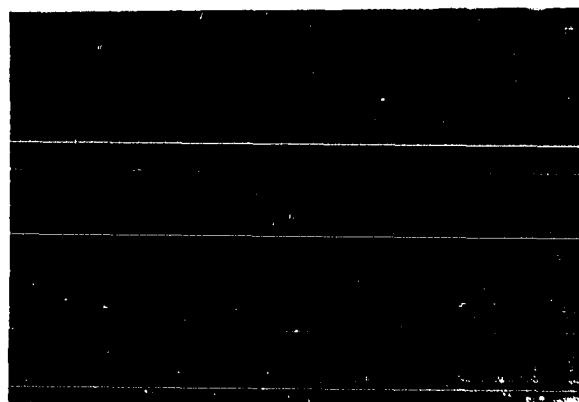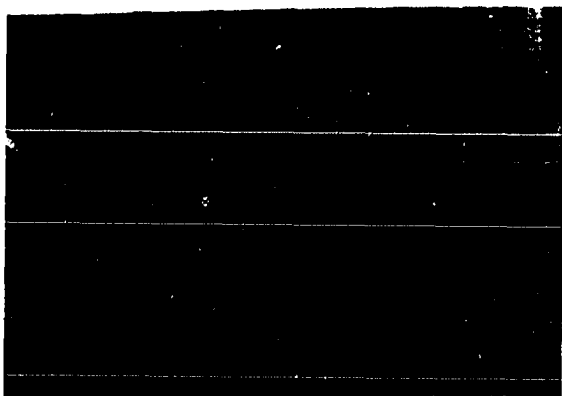
Fig. 5a. Sectioned bovine muscle after storage at 2° for 24 hrs. This muscle exhibits the typical super-contracted appearance. X 2,000

Fig. 5b. Sectioned bovine muscle after storage at 2° for 24 hrs. This micrograph shows a kinked area often seen in this muscle. X 2,000

Fig. 5c. Sectioned bovine muscle after storage at 16° for 24 hrs. X 2,000

Fig. 5d. Sectioned bovine muscle after storage at 25° for 24 hrs. X 2,000

Fig. 5e. Sectioned bovine muscle after storage at 37° for 24 hrs. Note the two dark bands on either side of an "H-zone". This banding pattern is typically observed in 37°-24 hr. bovine myofibril preparations. X 2,000

Fig. 5f. Sectioned bovine muscle after storage at 37° for 24 hrs. This muscle exhibits an extremely fragmented area. Arrows show absence of Z-line. X 2,000
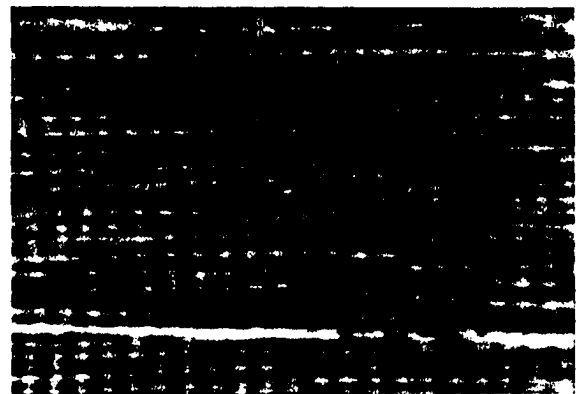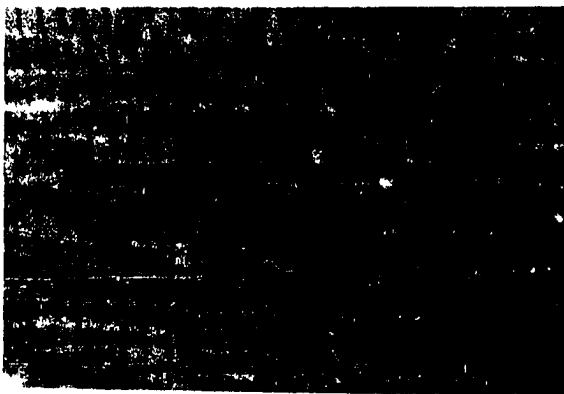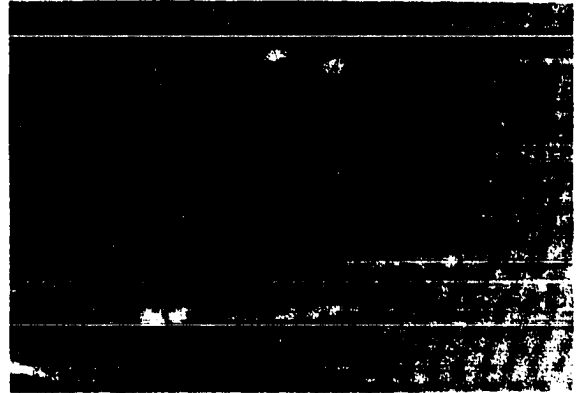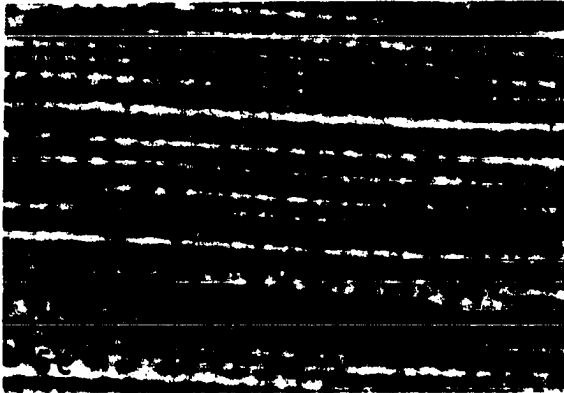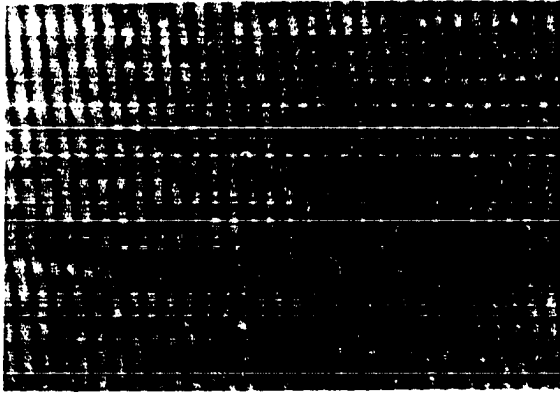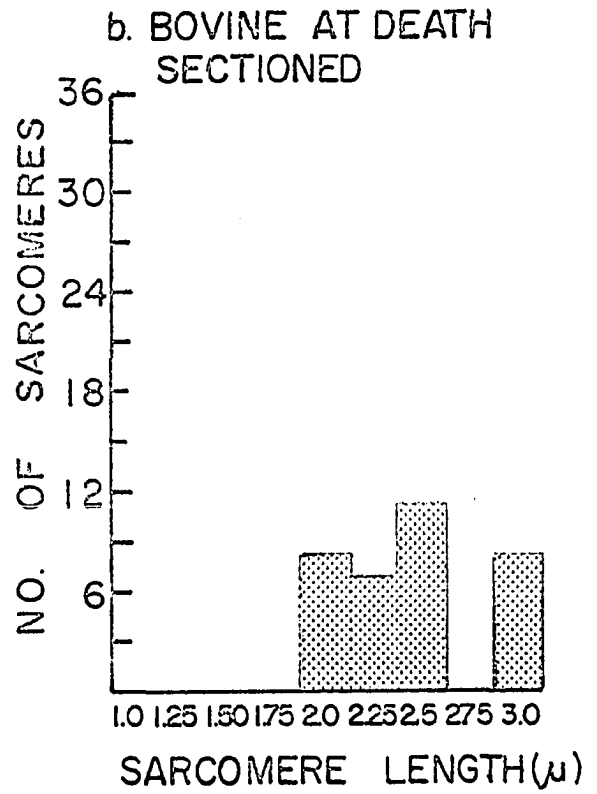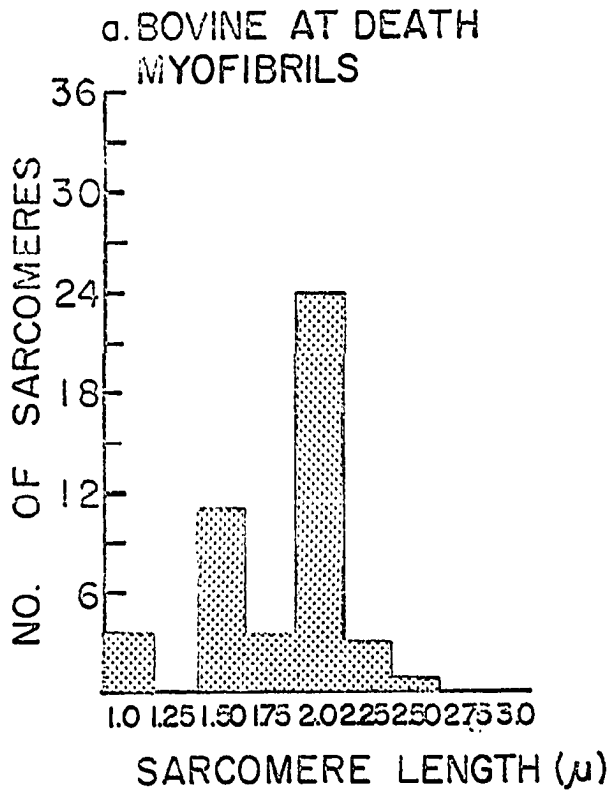
Fig. 6a. Distribution of sar-
comere lengths for
bovine myofibrils at-
death (47 measured).

Fig. 6b. Distribution of
sarcomere lengths
for sectioned
bovine muscle at-
death (34 measured).

Fig. 6c. Distribution of sar-
comere lengths for
bovine myofibrils
stored at 2° for 4
hours (47 measured).

Fig. 6d. Distribution of
sarcomere lengths
for sectioned
bovine muscle
stored at 2° for
4 hours (23
measured).

a. BOVINE AT DEATH MYOFIBRILS

b. BOVINE AT DEATH SECTIONED

c. BOVINE 2°- 4 hrs. MYOFIBRILS

d. BOVINE 2°- 4 hrs. SECTIONED
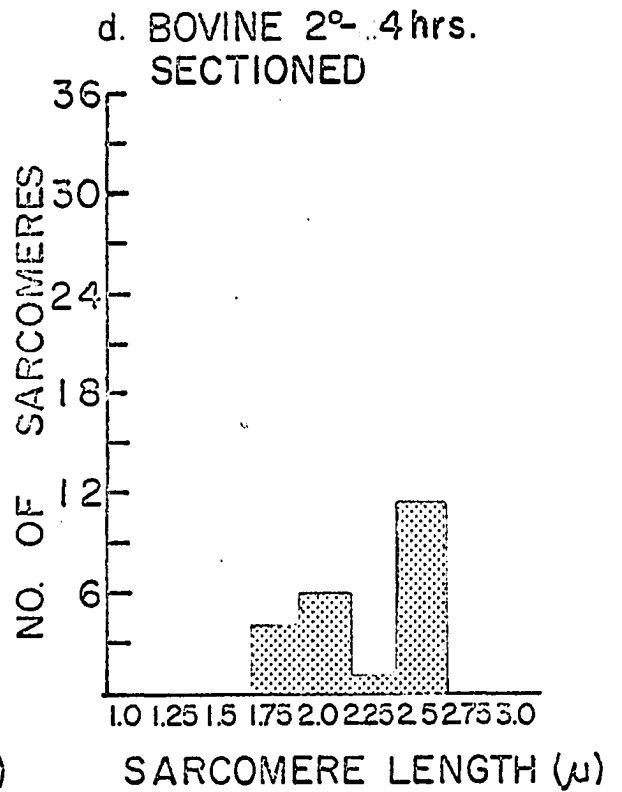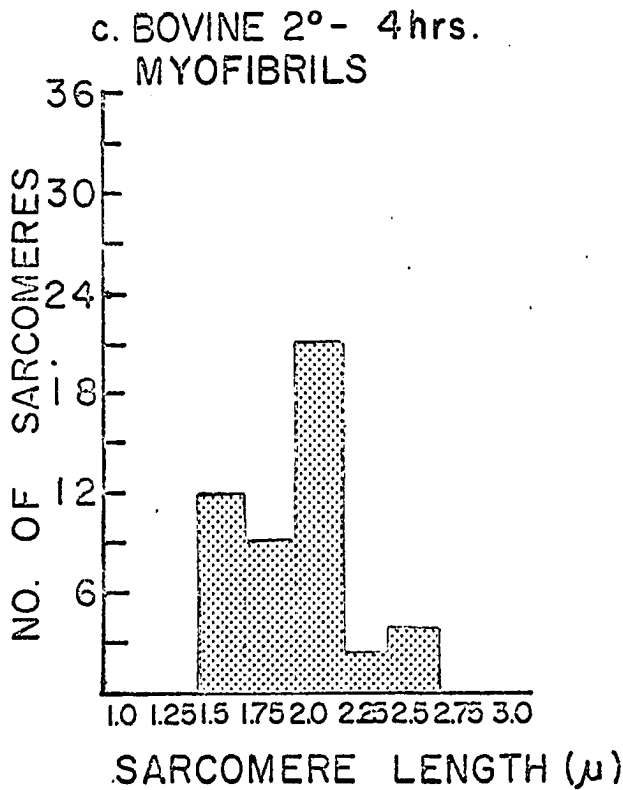
to those for at-death myofibrils, with the exception that no

supercontracted myofibrils were observed in muscle stored at

2° for 4 hours. It may also be noted that length of the A-

band in the 2°-4 hour myofibrils was shorter (0.75 - 1.0μ) than

A-band length in 2°-4 hour sectioned muscle. This suggests

that isolation of myofibrils, even after 4 hours at 2°, has

again caused some shortening of the A-band.

Sectioned muscle from 2°-4 hour samples (Fig. 4b) appeared

to be slightly shortened as evidenced by the loss of the H-

zone. Some sarcomere lengths (Fig. 6d) were still quite long

(up to 2.5μ), but on the average, sarcomere lengths of 2°-4

hour sectioned muscle were shorter than those of muscle sampled

at death (Table 1). Thus, both sarcomere lengths and banding

patterns of sectioned muscle suggest that some slight amount

of shortening is occurring in bovine muscle during the first 4

hours post-mortem at 2°.

Table 1. Average sarcomere lengths (μ) of myofibrils and
          sectioned bovine muscle

	At death	2°-4 hrs.	37°-4 hrs.	2°-8 hrs.	37°-8 hrs.	2°-24 hrs.	16°-24 hrs.	25°-24 hrs.	37°-24 hrs.
myo-fibrils	1.8	1.8	1.6	1.7	1.4	1.2	2.0	2.0	1.5
sec-tioned	2.6	2.2	2.2	2.0	1.5	1.3	1.7	1.8	1.7

Myofibrils from bovine muscle stored at 37° and sampled after 4 hours post-mortem exhibited two general classes of sarcomere lengths (Fig. 7a), one class having sarcomere lengths of about 2 - 2.25μ (Fig. 3c) and the other class exhibiting sarcomere lengths near 1.5μ (Fig. 3d). The shortened myofibrils showed a rather odd banding pattern having a wide dark band at the level of the Z-line and two narrow dark bands in the center of the A-band. These two dark lines appear to lie on either side of a lighter zone which may be the H-zone. The dark band at the level of the Z-line may result from extensive shortening causing the A-filaments to crumple against the Z-line whereas the two dark lines in the center of the A-band may originate from overlap of the thin filaments on either side of the H-zone in the center of the A-band.

Sectioned muscle stored at 37° for 4 hours appears to be shortened slightly, having lost its H-zones (Fig. 4c). Sarcomere lengths of this muscle (Fig. 7b) range from ·2.0 - 2.5μ, being somewhat shorter on the average than the sarcomere lengths from sectioned at-death muscle (Table 1).
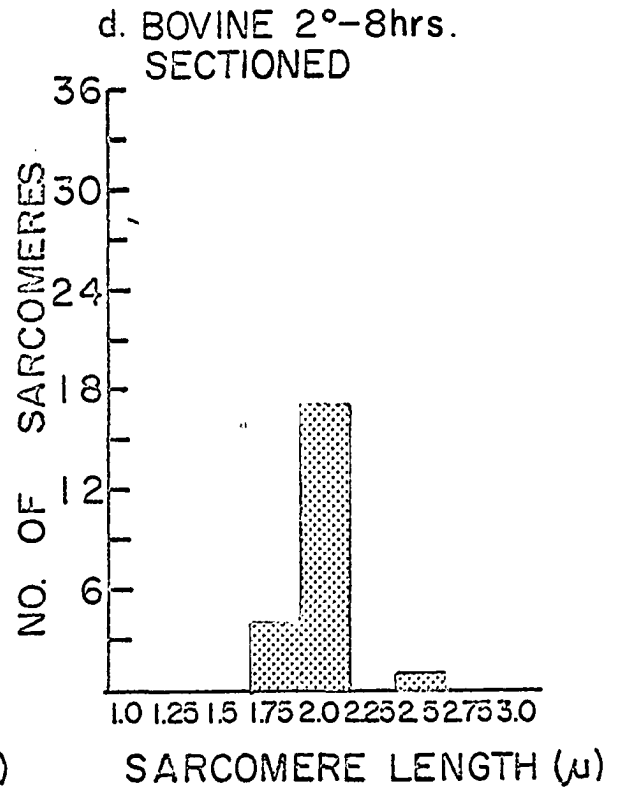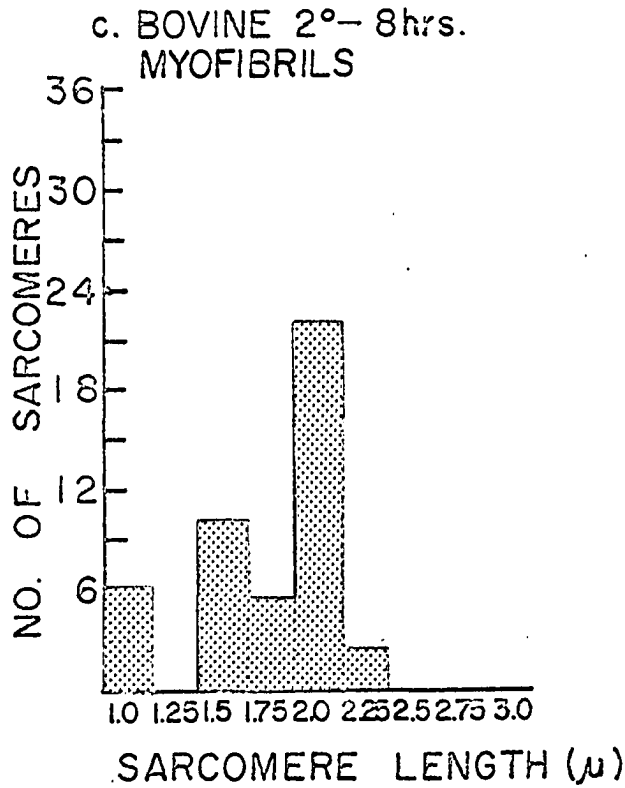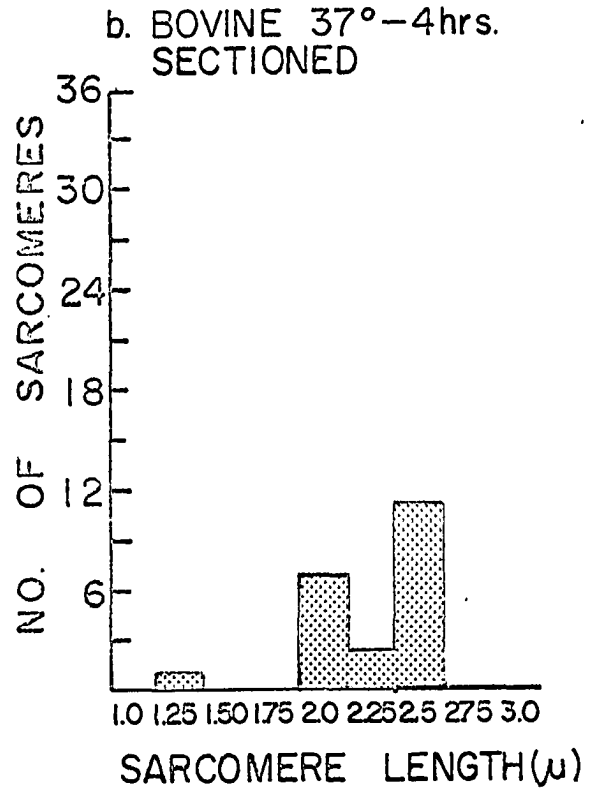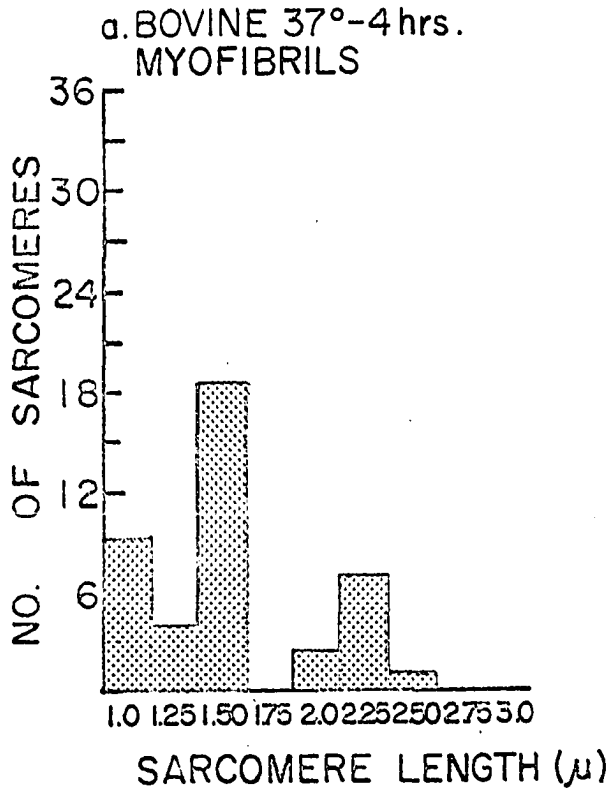
Myofibrils isolated from muscle stored at 2° for 8 hours (Fig. 3e) in most cases exhibit the relaxed banding pattern with only about 15% exhibiting the supercontracted banding pattern (Fig. 7c). The shortening of the A-band observed in the at-death and 2°-4 hour myofibrils does not occur after 8

Fig. 7a. Distribution of
          sarcomere lengths
          for bovine myo-
          fibrils stored at
          37° for 4 hours
          (42 measured).

Fig. 7b. Distribution of
          sarcomere lengths
          for sectioned
          bovine muscle
          stored at 37° for
          4 hours (20
          measured).

Fig. 7c. Distribution of
          sarcomere lengths
          for bovine myo-
          fibrils stored at
          2° for 8 hours
          (45 measured).

Fig. 7d. Distribution of
          sarcomere lengths
          for sectioned
          bovine muscle
          stored at 2° for
          8 hours (22
          measured).

a. BOVINE 37°-4hrs. MYOFIBRILS

b. BOVINE 37°-4hrs. SECTIONED

c. BOVINE 2°-8hrs. MYOFIBRILS

d. BOVINE 2°-8hrs. SECTIONED

hours of post-mortem storage at 2°. Sectioned muscle from these same samples (Fig. 4d) were contracted as evidenced by the absence of the H-zone. However, relatively wide I-bands were still present and the banding pattern of 2°-8 hour sectioned muscle resembled that of sectioned muscle stored at 2° for 4 hours, except that the average sarcomere lengths of 2°-8 hour muscle were shorter than those of 2°-4 hour muscle. This suggests a greater amount of shortening has occurred after 8 hours post-mortem at 2° than after 4 hours post-mortem at 2°.

Storage of muscle for 8 hours at 37° resulted in myofibrils (Fig. 3f) with the same banding pattern as that described for myofibrils isolated from 37°-4 hour muscle. Sectioned samples from muscle stored under these conditions (Fig. 4e) exhibited sarcomere lengths of approximately 1.5μ and were contracted to the point that the H-zone and the I-band were no longer visible. When compared to at-death sectioned muscle or sectioned muscle after storage at 37° for 4 hours (Table 1), it is evident that a much greater amount of shortening occurred between 4 and 8 hours post-mortem at 37° than between 0 and 4 hours post-mortem at 37°.
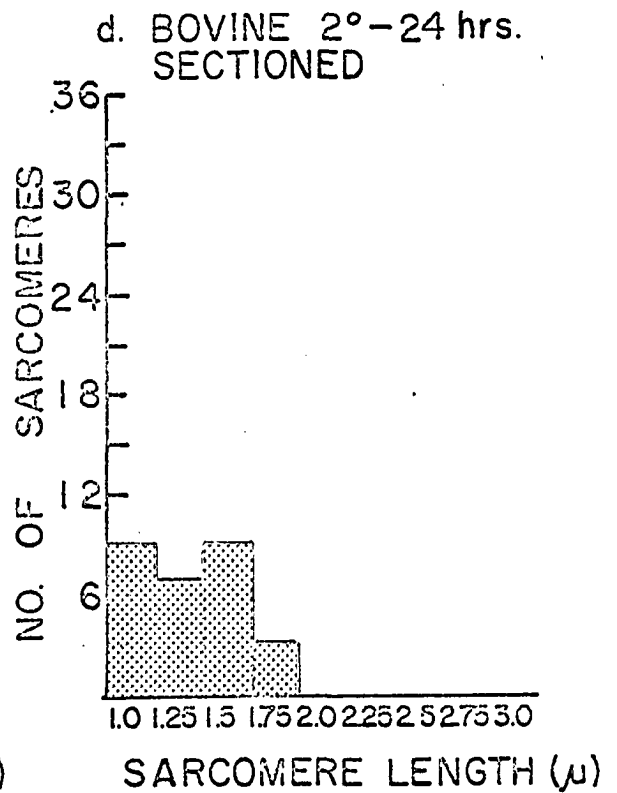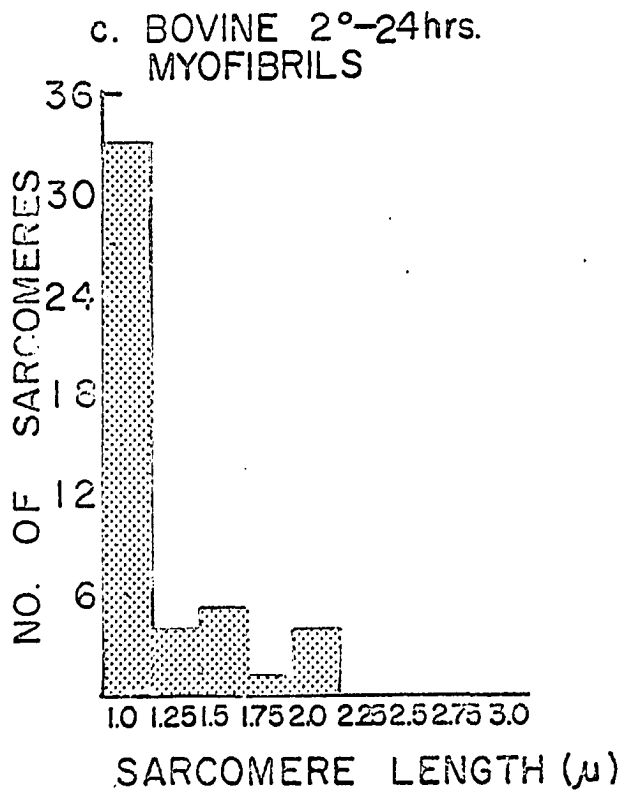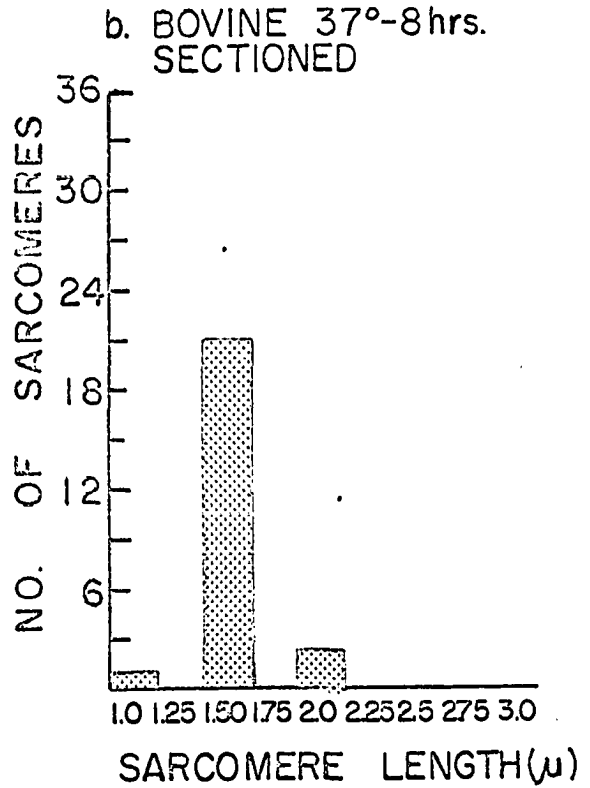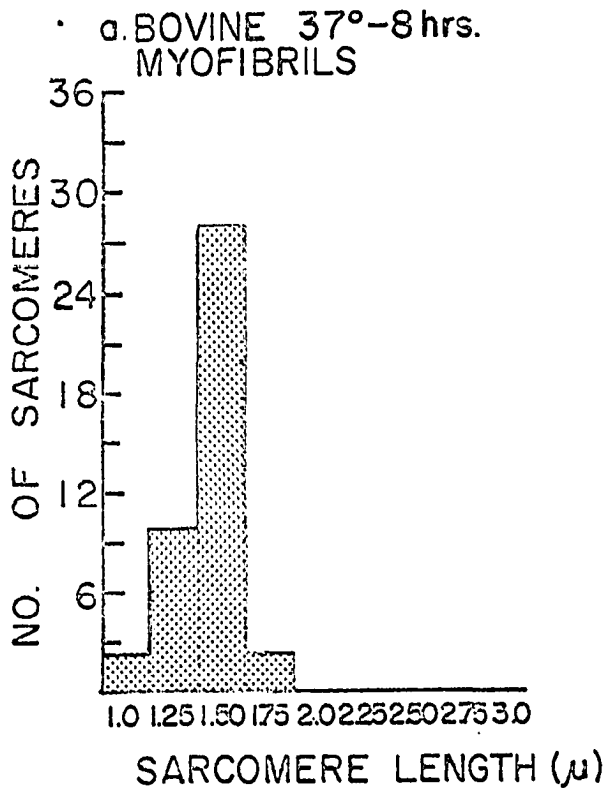
Bovine muscle stored at 2° for 24 hours was severely shortened (Table 1). Isolated myofibrils (Fig. 3g) were in general (70%) supercontracted (sarcomere lengths less than 1.5μ) with a few myofibrils (15%) being slightly contracted (sarcomere length of 1.5 to 1.75μ) and a very few (10%) relaxed

Fig. 8a. Distribution of
sarcomere lengths
for bovine myo-
fibrils stored at
37° for 8 hours
(42 measured).

Fig. 8b. Distribution of
sarcomere lengths
for sectioned
bovine muscle
stored at 37° for
8 hours (24
measured).

Fig. 8c. Distribution of
sarcomere lengths
for bovine myofibrils
stored at 2° for 24
hours (50 measured)

Fig. 8d. Distribution of
sarcomere lengths
for sectioned
bovine muscle
stored at 2° for
24 hours (28
measured).

a. BOVINE 37°-8 hrs. MYOFIBRILS

b. BOVINE 37°-8 hrs. SECTIONED

c. BOVINE 2°-24 hrs. MYOFIBRILS

d. BOVINE 2°-24 hrs. SECTIONED

with sarcomere lengths of 2.0μ (Fig. 8c). The alternating
light and dark bands observed in supercontracted muscle pre-
sumably result from a sliding of filaments to the point that
the ends of the thick filaments are crumpling against or
passing through the Z-line. The light band observed in the
supercontracted myofibril is the A-band and appears light as
a result of the increased density caused by the crumpling or
overlap of the thick filaments at the level of the Z-line.
The I-band and the H-zone have disappeared or have been masked
as a result of this extensive sliding of filaments. Sectioned
muscle (Fig. 5a) after 24 hours at 2° also was supercontracted
as indicated by the pattern of alternating light and dark
bands observed in the phase microscope and by sarcomere lengths
(Fig. 8d). Kinking or crumpling of fibers (Fig. 5b) was con-
sistently observed in 20 - 30% of the fibers in sectioned
muscle after 24 hours at 2°. Sarcomere lengths in this area
of kinking varied from 1.25 to 1.75μ, suggesting that the sar-
comeres in these kinked fibers were shortened but perhaps not
to the same extent as the sarcomeres in those fibers which were
not kinked.

Storage for 24 hours at 16° resulted in slightly contracted
myofibrils (Fig. 3h) as evidenced by a narrowed I-band and
disappearance of the H-zone. The dark line at the center of
the A-band suggests an overlap of the thin filaments in this
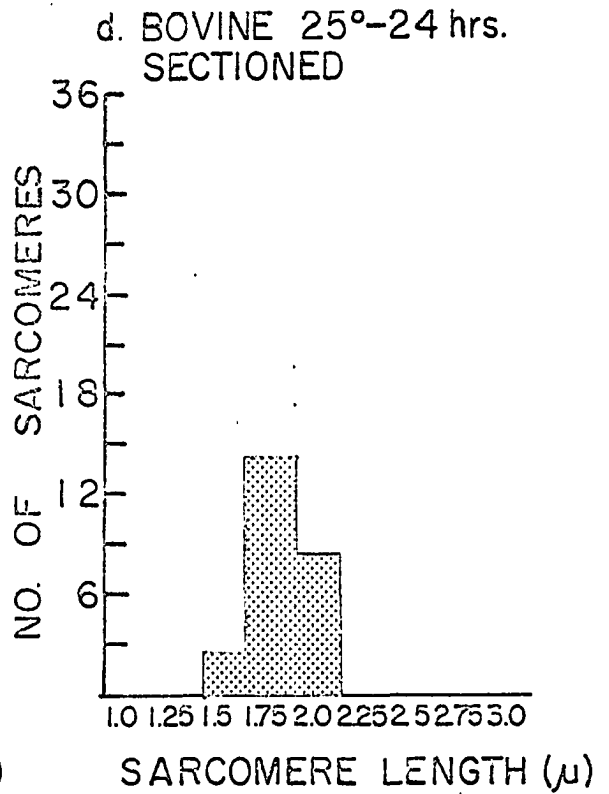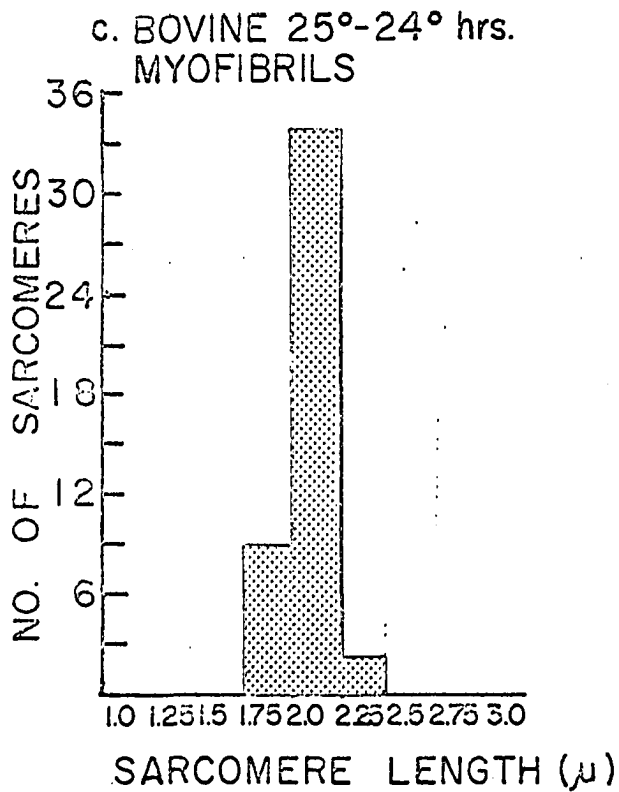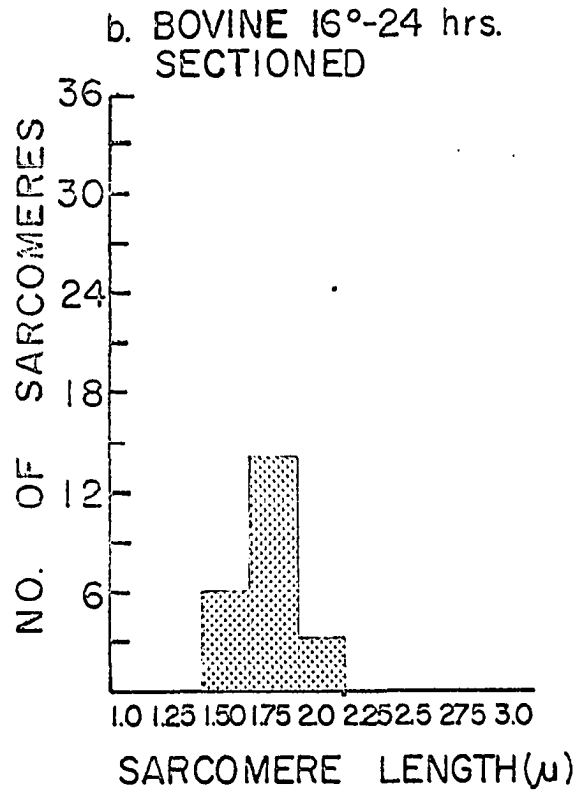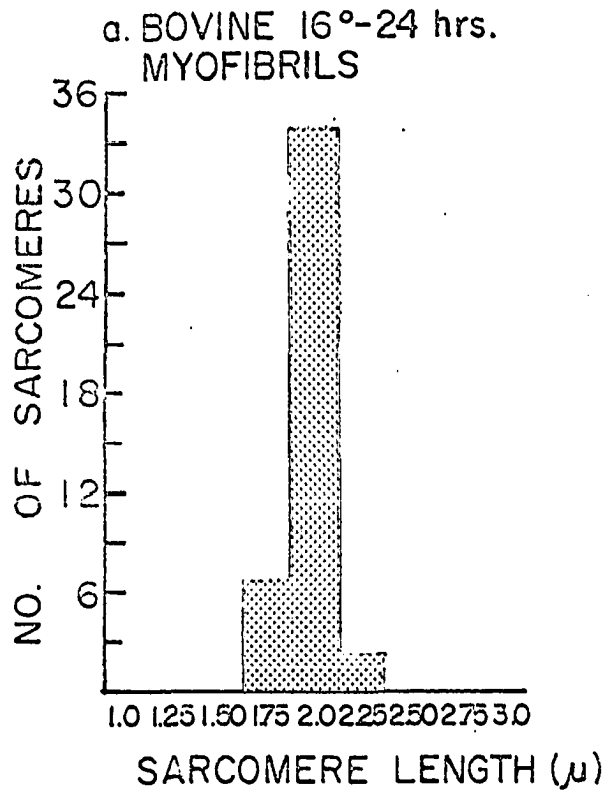area. Sarcomere lengths (Fig. 9a) of isolated myofibrils from

Fig. 9a. Distribution of
          sarcomere lengths
          for bovine myo-
          fibrils stored
          at 16° for 24
          hours (42
          measured).

Fig. 9b. Distribution of
          sarcomere lengths
          for sectioned
          bovine muscle
          stored at 16° for
          24 hours (23
          measured).

Fig. 9c. Distribution of
          sarcomere lengths
          for bovine myo-
          fibrils stored
          at 25° for 24
          hours (45
          measured).

Fig. 9d. Distribution of
          sarcomere lengths
          for sectioned
          bovine muscle
          stored at 25° for
          24 hours (24
          measured).

a. BOVINE 16°-24 hrs. MYOFIBRILS

b. BOVINE 16°-24 hrs. SECTIONED

c. BOVINE 25°-24° hrs. MYOFIBRILS

d. BOVINE 25°-24 hrs. SECTIONED

the 16°-24 hour muscle averaged slightly longer (Table 1) than

those of myofibrils isolated at-death. However, the myofibrils

isolated from the 16°-24 hour muscle all have A-bands about

1.25 - 1.5μ in length whereas 90% of the A-bands of at-death

myofibrils were between .75 - 1.0μ in length. These unusual

shortened A-band lengths observed in the at-death myofibrils

have already been discussed and are probably the reason that

average sarcomere length of the at-death myofibrils is shorter

than the average sarcomere length of the 16°-24 hour myofibrils,

particularly since the I-band lengths of 16°-24 hour myofibrils

are perhaps slightly shorter than those of at-death myofibrils.

This suggests that some sliding of filaments, resulting in

shortened sarcomere lengths, has occurred after 24 hours of

post-mortem storage at 16°. Sectioned muscle (Fig. 5c) after

24 hours of post-mortem storage at 16° also exhibits the same

slightly contracted appearance as that observed in the 16°-

24 hour myofibril preparations. Occasionally, a wavy appear-

ance is observed in the sectioned 16°-24 hour samples, with

sarcomeres in the wavy areas being contracted to the same

extent as those observed in the straight fibers. The wavy

appearance observed in the 16°-24 hour samples is less severe

than the kinked appearance observed in sectioned bovine muscle

stored at 2° for 24 hours. It is also interesting that the

sarcomere lengths (Fig. 9b) of sectioned 16°-24 hour muscle

are slightly shorter than those of isolated myofibrils from

this same muscle. Two possible explanations can be given for this small discrepency. One explanation is that the mechanical force applied in isolating myofibrils may have tended to stretch the sarcomeres very slightly. Another possible explanation is that fixation, dehydrating and embedding the muscle for sectioning may have caused a small amount of sarcomere shortening.

As evaluated by phase microscopy, the banding pattern of both myofibrils (Fig. 3i) and sectioned muscle (Fig. 5d) after storage at 25° for 24 hours appears the same as the 16°-24 hour samples described in the preceding paragraph. Sarcomere lengths (Fig. 9c,d) of muscle stored at 16° or 25° for 24 hours post-mortem also resembled one another very closely in both sectioned muscle and myofibrils.

Myofibrils isolated from muscle stored at 37° for 24 hours (Fig. 3j) had the same banding pattern as that described for myofibrils stored at 37° for 4 hours. However, after 24 hours at 37°, a large amount of fragmentation has occurred which results in myofibrils of only 2 to 4 sarcomeres in length. Sarcomere lengths of the 37°-24 hour myofibrils (Fig. 10a) are quite uniform with 75% of the sarcomeres being 1.5μ in length. The banding pattern of sectioned muscle stored at 37° for 24 hours (Fig. 5e) appears very similar to that described in the myofibril preparations made after this time and temperature of storage. It is evident that the myofibrils in sectioned muscle were also fragmented in many areas (Fig. 5f). The Z-line

Fig. 10a. Distribution of sarcomere
lengths for bovine myofibrils
stored at 37° for 24 hours
(43 measured).

Fig. 10b. Distribution of sarcomere
lengths for sectioned
bovine muscle stored at
37° for 24 hours
(22 measured).

a. BOVINE 37°-24 hrs. MYOFIBRILS

b. BOVINE 37°-24 hrs. SECTIONED
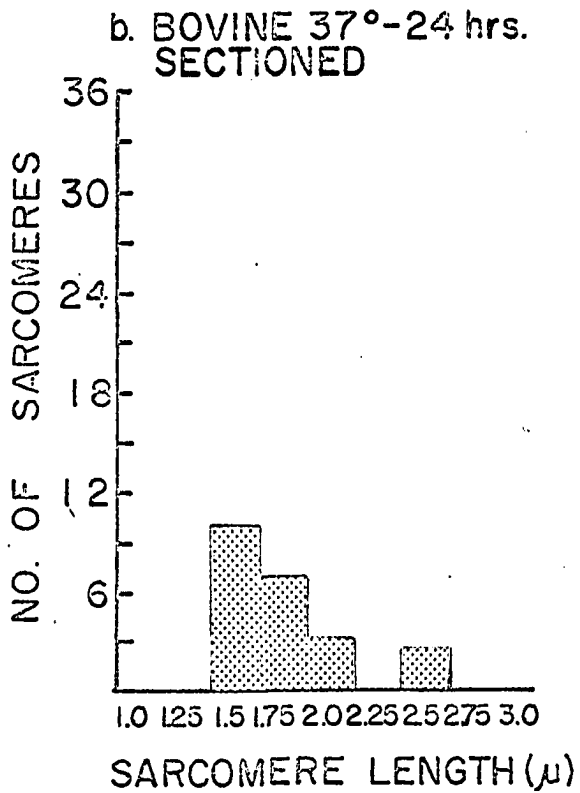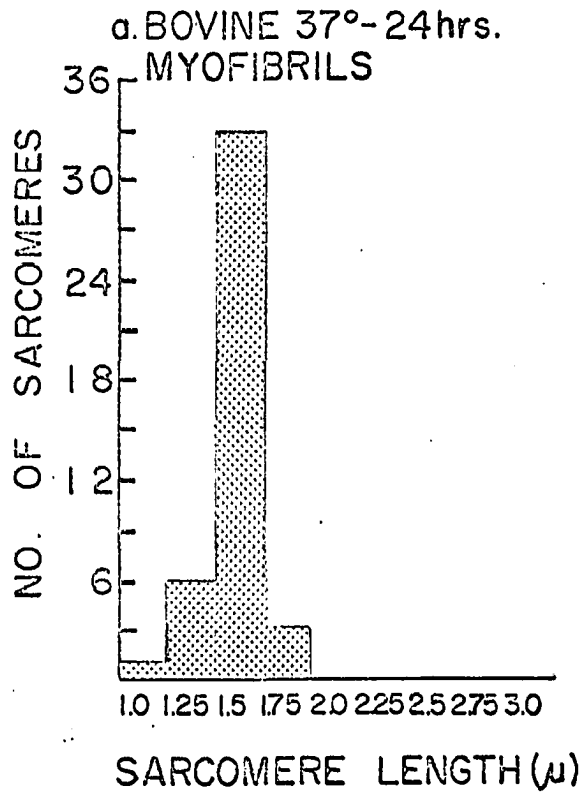
appears to be absent (arrows) in the 37°-24 hour muscle, thus giving the appearance of a wider I-band. Study of the sarcomere lengths of sectioned muscle from 37°-24 hour muscle (Fig. 10b) suggests that a lengthening has occurred between 8 and 24 hours; however, measurement of sarcomere lengths from the middle of one light zone to the middle of the next light zone resulted in slightly longer sarcomere lengths in fragmented areas than in the intact areas where sarcomere lengths were measured from one dark band to the next. Thus, it is likely that some of this apparent sarcomere lengthening between 8 and 24 hours post-mortem at 37° may be an artifact introduced by the measuring of sarcomeres without Z-lines.

As shown in Table 1, maximal shortening of bovine muscle occurred after storage at 2° for 24 hours, with the next largest amount of shortening occurring in muscle stored at 37° for 24 hours. Minimum shortening after 24 hours occurred in muscle stored in the 16° - 25° range.

Porcine muscle

Phase microscopic observations showed that with three exceptions isolated porcine myofibrils and sectioned muscle structurally resemble bovine muscle at the corresponding times and temperatures of post-mortem storage. Because of the similarity in post-mortem muscle structure between these two species, only photomicrographs of myofibrils and histograms of sarcomere

lengths of both myofibrils and sectioned muscle are presented
for porcine muscle.

The banding patterns of myofibrils from porcine muscle at-
death (Fig. 11a) and after 4 hours of post-mortem storage at-
2° (Fig. 11b) appear almost identical to the banding patterns
described for bovine myofibrils after the corresponding times
and temperatures of post-mortem storage. Also, the distri-
bution of sarcomere lengths in porcine myofibrils at-death
(Fig. 12a,b) and after 4 hours at 2° (Fig. 12c,d) are almost
identical to the corresponding distribution of sarcomere
lengths in bovine muscle. It is interesting to note that A-
band lengths in porcine myofibrils prepared from both at-death
and 2°-4 hour muscle are also shorter (.75 - 1.0μ) than sec-
tioned muscle (1.25 - 1.5μ).

Except for one distinctly different feature, myofibrils
from porcine muscle stored at 37° for 4 hours (Fig. 11c) have
the same banding pattern as that described for bovine myofibrils
stored at 37° for either 4, 8 or 24 hours. The dark band at
the level of the Z-line seen in bovine myofibrils was not
present in porcine myofibrils (arrows). Sectioned porcine
muscle from this same sample, however, appeared to possess this
dark line and was structurally identical to sectioned bovine
muscle stored at 37° for 8 hours. It is possible that the
washing steps used in myofibril preparation removed the Z-line
material from porcine myofibrils but did not affect the

Fig. 11a.   Porcine myofibrils
            isolated at-death.
            X 2,000

Fig. 11b.   Porcine myofibrils
            isolated after
            storage at 2° for
            4 hrs.   X 2,000

Fig. 11c.   Porcine myofibrils
            isolated after
            storage at 37° for
            4 hrs.   Arrows
            indicate absence
            of Z-lines.
            X 2,000

Fig. 11d.   Porcine myofibrils
            isolated after
            storage at 2° for
            8 hrs.   X 2,000

Fig. 11e.   Porcine myofibrils
            isolated after
            storage at 37° for
            8 hrs.   The banding
            pattern observed is
            typical for 37°
            muscle.   X 2,000

Fig. 11f.   Porcine myofibrils
            isolated after
            storage at 2° for
            24 hrs.   This muscle
            has contracted con-
            siderably as evi-
            denced by loss of H-
            zone and the very
            narrow I-bands.
            X 2,000

Fig. 11g.   Porcine myofibrils
            isolated after
            storage at 16° for
            24 hrs.   X 2,000

Fig. 11h.   Porcine myofibrils
            isolated after
            storage at 25° for
            24 hrs.   This mus-
            cle exhibits a con-
            tracted appearance
            with the presence of
            Z-lines.   X 2,000

Fig. 11i.   Another example of
            porcine myofibrils
            isolated after
            storage at 25° for
            24 hrs.   This sam-
            ple exhibits a con-
            tracted appearance
            with the Z-lines
            absent.   X 2,000

Fig. 11j.   Porcine myofibrils
            isolated after
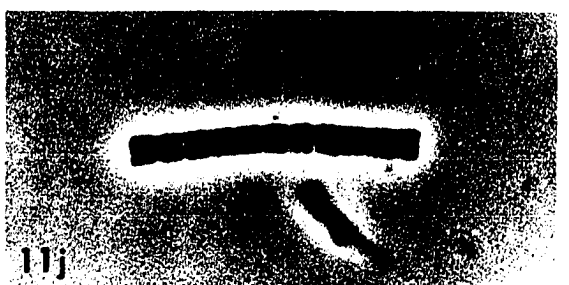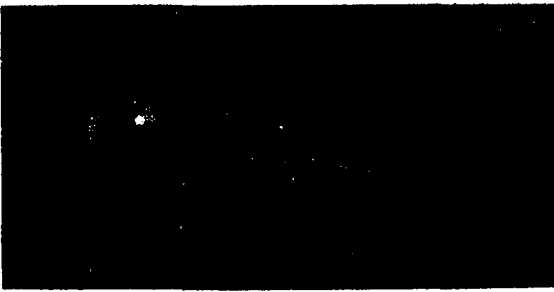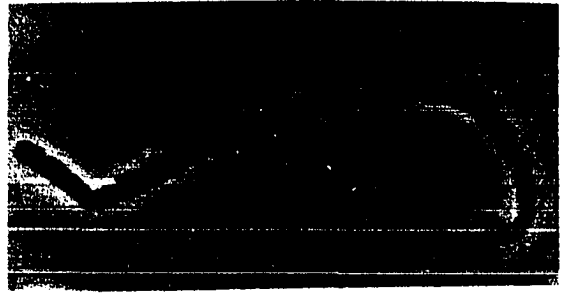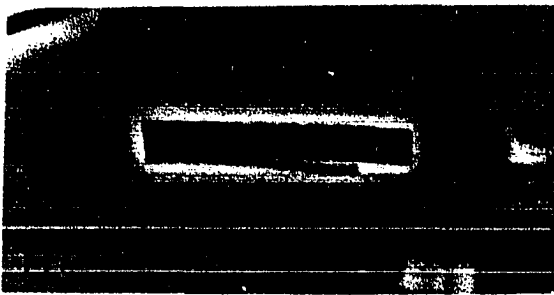   storage at 37° for
            24 hrs.   X 2,000

11i 11j
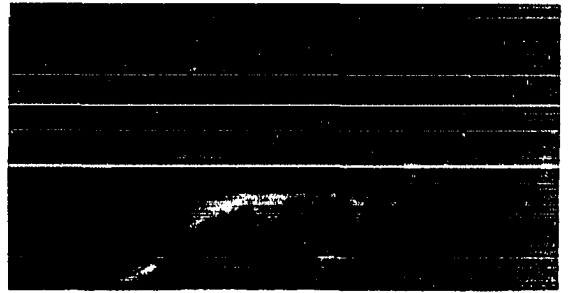
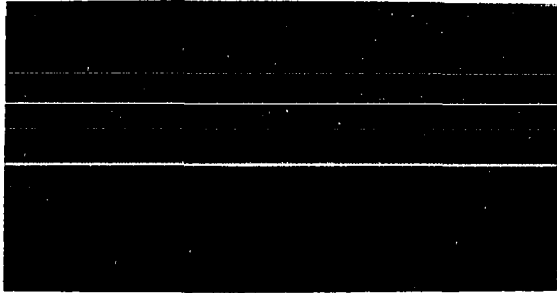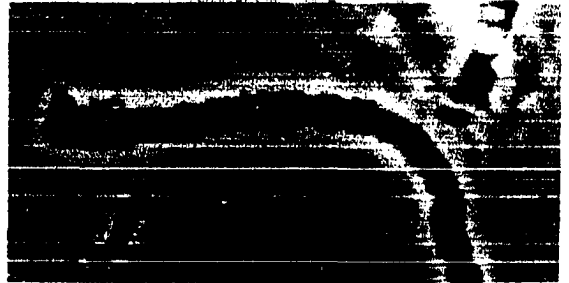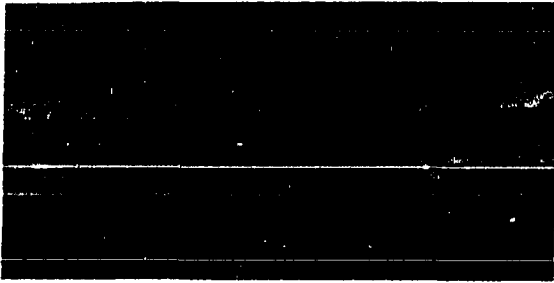Fig. 12a. Distribution of
           sarcomere lengths
           for porcine myo-
           fibrils at-death
           (36 measured).

Fig. 12b. Distribution of
           sarcomere lengths
           for sectioned
           porcine muscle at-
           death (24 measured).

Fig. 12c. Distribution of
           sarcomere lengths
           for porcine myo-
           fibrils stored
           at 2° for 4 hours
           (40 measured).

Fig. 12d. Distribution of
           sarcomere lengths
           for sectioned
           porcine muscle
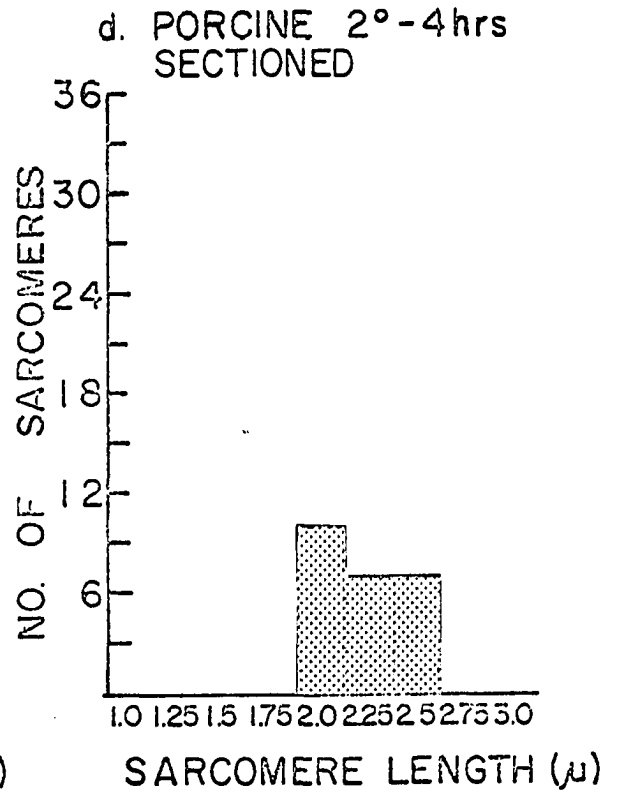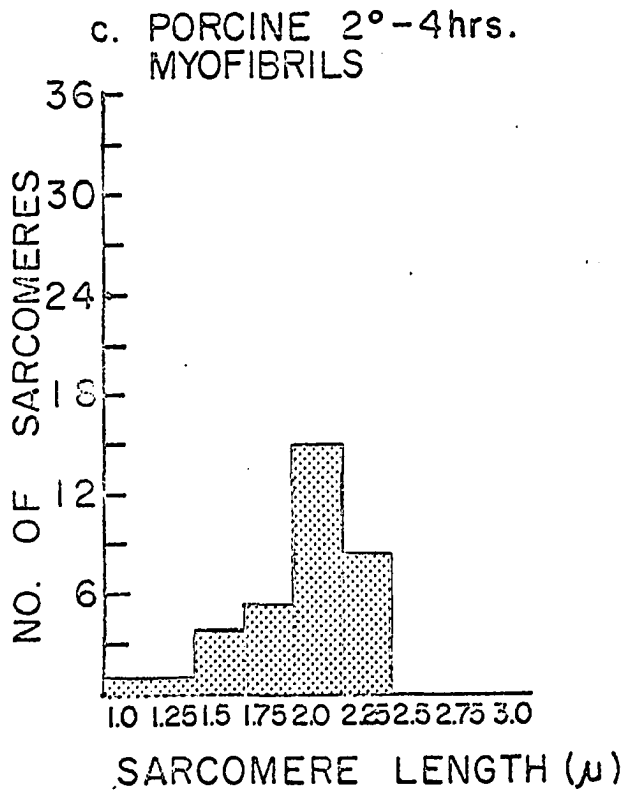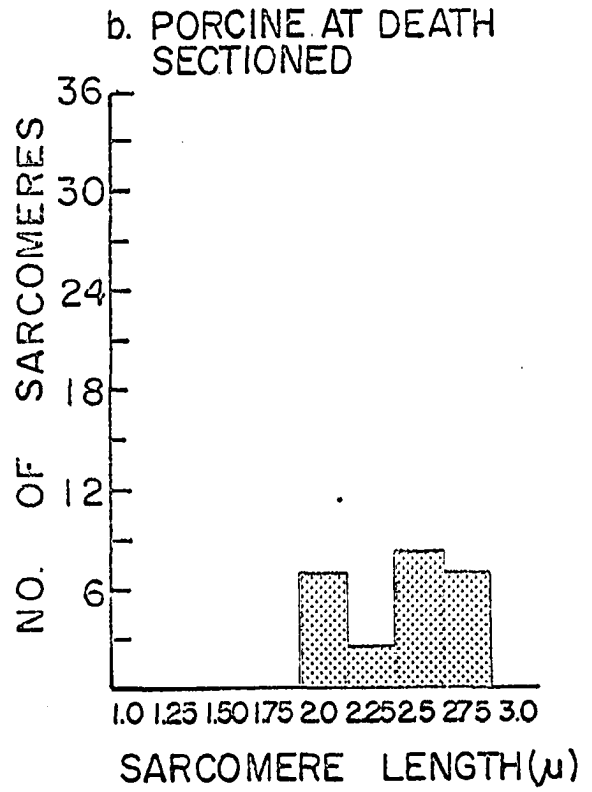   stored at 2° for
           4 hours (24
           measured).

a. PORCINE AT DEATH
MYOFIBRILS

NO. OF SARCOMERES

SARCOMERE LENGTH (μ)

b. PORCINE AT DEATH
SECTIONED

NO. OF SARCOMERES

SARCOMERE LENGTH (μ)

c. PORCINE 2°–4 hrs.
MYOFIBRILS

NO. OF SARCOMERES

SARCOMERE LENGTH (μ)

d. PORCINE 2°–4 hrs
SECTIONED

NO. OF SARCOMERES

SARCOMERE LENGTH (μ)

Fig. 13a. Distribution of
sarcomere lengths
for porcine myo-
fibrils stored
at 37° for 4
hours (36
measured).

Fig. 13b. Distribution of
sarcomere lengths
for sectioned por-
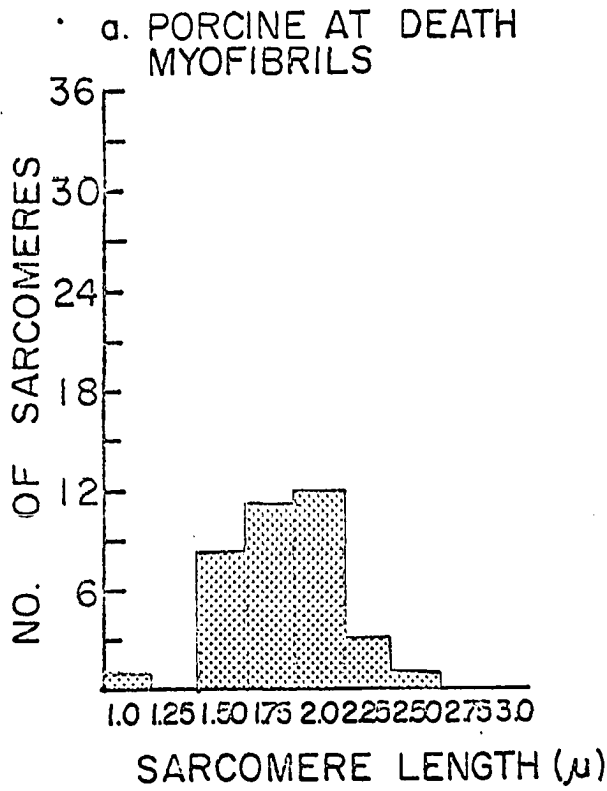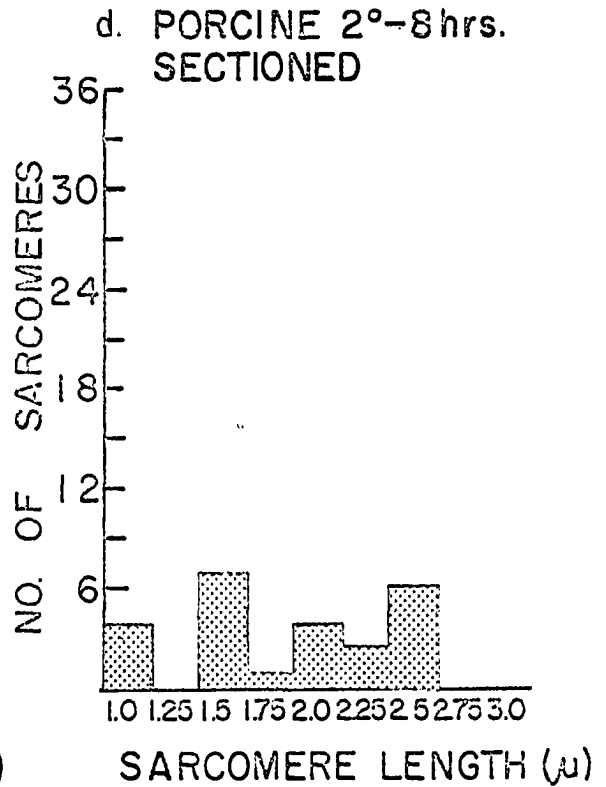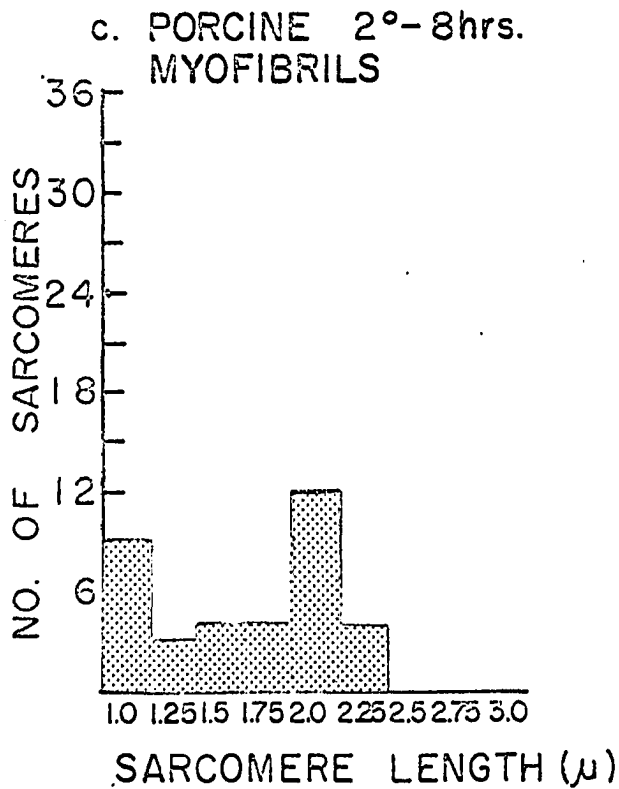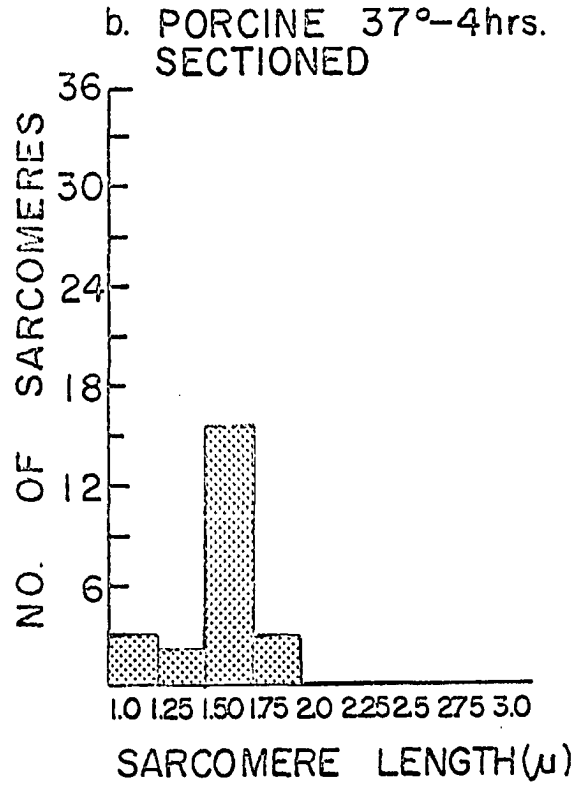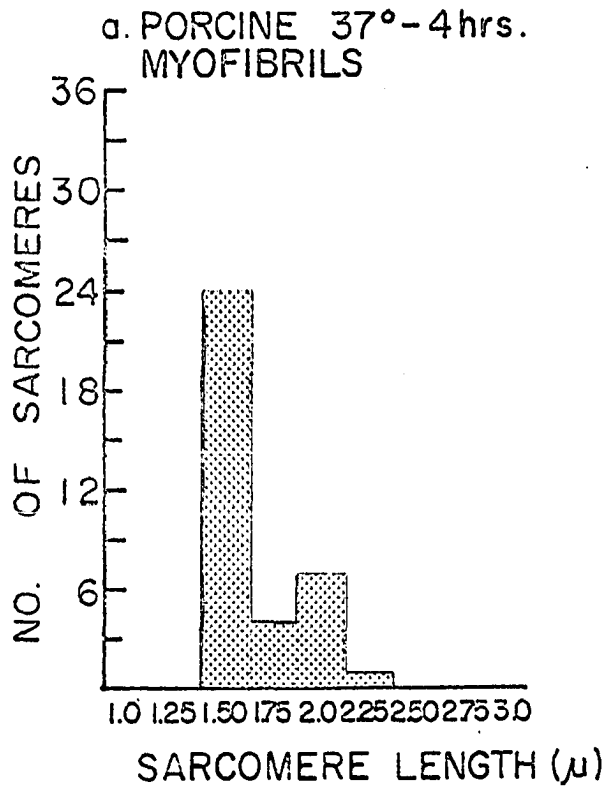cine muscle stored
at 37° for 4 hours
(24 measured).

Fig. 13c. Distribution of
sarcomere lengths
for porcine myo-
fibrils stored at
2° for 8 hours
(36 measured).

Fig. 13d. Distribution of
sarcomere lengths
for sectioned por-
cine muscle stored
at 2° for 8 hours
(25 measured).

a. PORCINE 37°-4hrs. MYOFIBRILS

b. PORCINE 37°-4hrs. SECTIONED

c. PORCINE 2°-8hrs. MYOFIBRILS

d. PORCINE 2°-8hrs. SECTIONED

corresponding structure in bovine myofibrils. If so, this suggests that the Z-line in porcine muscle was extensively degraded after 4 hours of storage at 37° whereas the Z-line of bovine muscle stored for 4 hours at 37° was not as vulnerable to this treatment. The sarcomere lengths of sectioned porcine muscle (Fig. 13a) suggest that post-mortem shortening at 37° occurs at a more rapid rate in porcine muscle than in bovine muscle (100% of porcine sarcomeres below 2.0µ after 4 hours at 37° but only 5% of bovine sarcomeres below this length after the same storage time and temperature).

Most of the myofibrils prepared from porcine muscle stored at 2° for 8 hours (Fig. 11d) exhibited the relaxed banding pattern (wide I and H-bands) even down to sarcomere lengths of 1.5µ. As is evident from their sarcomere lengths, however, (Fig. 13c), an appreciable portion of these myofibrils (33%) were supercontracted with sarcomere lengths between 1 and 1.5µ. As has already been discussed, A-band shortening is probably responsible for the presence of myofibrils having sarcomere lengths of 1.5 - 1.75µ but still exhibiting the relaxed banding pattern. Sarcomere lengths of sectioned samples from 2° - 8 hour muscle (Fig. 13d) suggest that a large amount of variability existed in the amount of shortening that had taken place. When examined more closely, it was found that myofibrils from one of the two animals studied had an average sarcomere length of 2.3µ after 8 hours at 2° whereas myofibrils

from the other animal had sarcomeres averaging 1.5μ in length

under these same conditions. A possible explanation for this

difference may be that the animal with the shortened sarcomeres

was stressed shortly before exsanguination. This would result

in a more rapid onset of rigor mortis and thereby cause a more

rapid rate of post-mortem shortening. The average sarcomere

length of sectioned muscle from this same animal at-death was

less than from the first animal (2.19μ compared to 2.62μ),

supporting the suggestion of stress before slaughter leading to

a very short delay phase.

Myofibrils isolated from porcine muscle stored at 37° for

8 hours (Fig. 11e) were identical in appearance with those

myofibrils isolated after 4 hours storage at 37°. Again the

Z-lines were present in sectioned muscle but were absent in

myofibril preparations. The major proportion of sarcomere

lengths (Fig. 14a,b) from both the myofibril and sectioned

preparations were about 1.5μ in length, indicating that under

these conditions the sarcomere lengths of sectioned muscle are

accurately preserved in the myofibril preparations.

Myofibrils prepared from porcine muscle stored at 2° for

24 hours were contracted (Fig. 11f) but did not display the

supercontracted pattern of alternating light and dark bands

observed in the 2°-24 hour bovine muscle. The sarcomere lengths

of myofibrils and sectioned muscle (Fig. 14c,d) also suggest

that 2°-24 hour porcine muscle has not shortened to the same extent as 2°-24 hour bovine muscle (average sarcomere lengths of 1.7μ for porcine, 1.2μ for bovine).

Myofibrils from porcine muscle stored at 16° (Fig. 11g) and 25° (Fig. 11h) for 24 hours were only slightly contracted and were structurally similar to their bovine counterparts. As was also observed for 16° and 25°-24 hour bovine myofibrils, sarcomere lengths of porcine myofibrils after 24 hours of post-mortem storage at either 16° or 25° (Fig. 15a,c) were slightly longer than the sarcomere lengths of sectioned muscle (Fig. 15b,d) after these conditions of post-mortem storage. About 25-30% of the myofibrils from 25°-24 hour porcine muscle did not exhibit Z-lines (Fig. 11i). This provides additional support to the suggestion that the Z-line in porcine muscle is more susceptable to degradation by post-mortem storage at higher temperatures than is the Z-line of bovine muscle.

Myofibrils from porcine muscle stored at 37° for 24 hours (Fig. 11j) exhibited the same type of banding pattern as that which has already been described for porcine myofibrils stored at 37° for 4 hours. However, myofibrils isolated after 24 hours post-mortem storage were more fragmented, as evidenced by the numerous short myofibril fragments (2 - 4 sarcomeres in length). Sectioned porcine muscle after 24 hours of post-mortem storage at 37° appeared very similar to 37°-24 hour sectioned bovine muscle. In some areas the fibers were straight and Z-lines were

Fig. 14a. Distribution of
sarcomere lengths
for porcine myo-
fibrils stored at
37° for 8 hours
(36 measured).

Fig. 14b. Distribution of
sarcomere lengths
for sectioned
porcine muscle
stored at 37°
for 8 hours (24
measured).

Fig. 14c. Distribution of
sarcomere lengths
for porcine myo-
fibrils stored
at 2° for 24
hours (36
measured).

Fig. 14d. Distribution of
sarcomere lengths
for sectioned
porcine muscle
stored at 2° for
24 hours (24
measured).

a. PORCINE 37°-8hrs.
   MYOFIBRILS

NO. OF SARCOMERES

SARCOMERE LENGTH (μ)

b. PORCINE 37°-8hrs.
   SECTIONED

NO. OF SARCOMERES

SARCOMERE LENGTH (μ)

c. PORCINE 2°-24hrs.
   MYOFIBRILS

NO. OF SARCOMERES

SARCOMERE LENGTH (μ)

d. PORCINE 2°-24 hrs.
   SECTIONED

NO. OF SARCOMERES

SARCOMERE LENGTH (μ)

Fig. 15a. Distribution of
sarcomere lengths
for porcine myo-
fibrils stored at
16° for 24 hours
(36 measured).

Fig. 15b. Distribution of
sarcomere lengths
for sectioned
porcine muscle
stored at 16° for
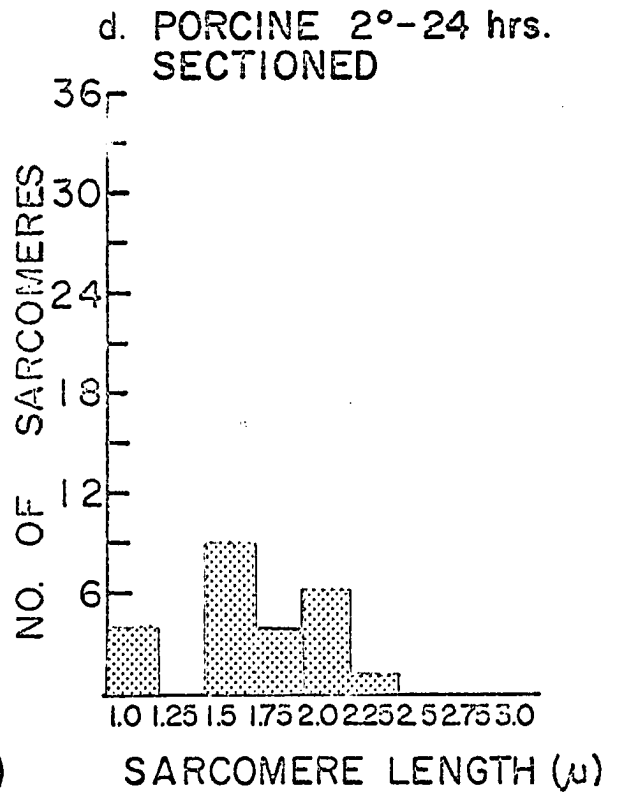24 hours
(24 measured).

Fig. 15c. Distribution of
sarcomere lengths
for porcine myo-
fibrils stored
at 25° for 24
hours (36
measured).

Fig. 15d. Distribution of
sarcomere lengths
for sectioned
porcine muscle
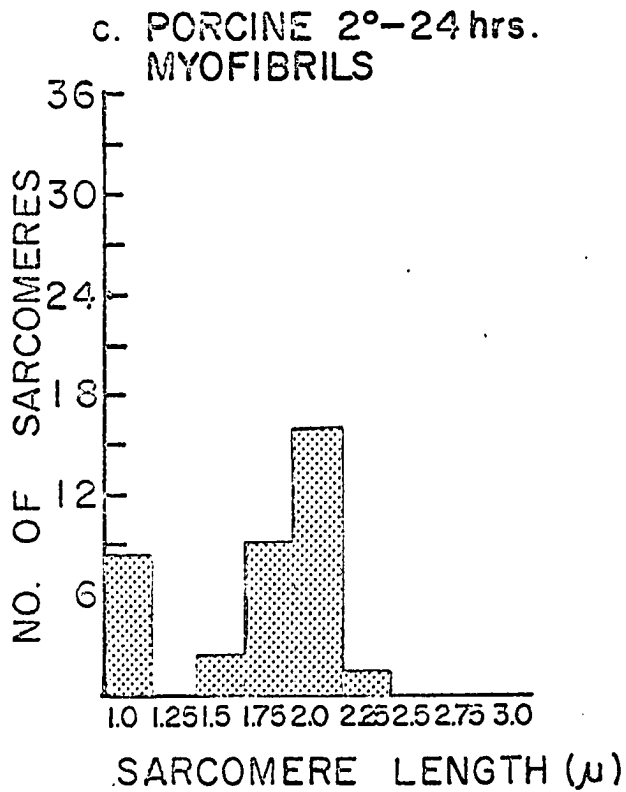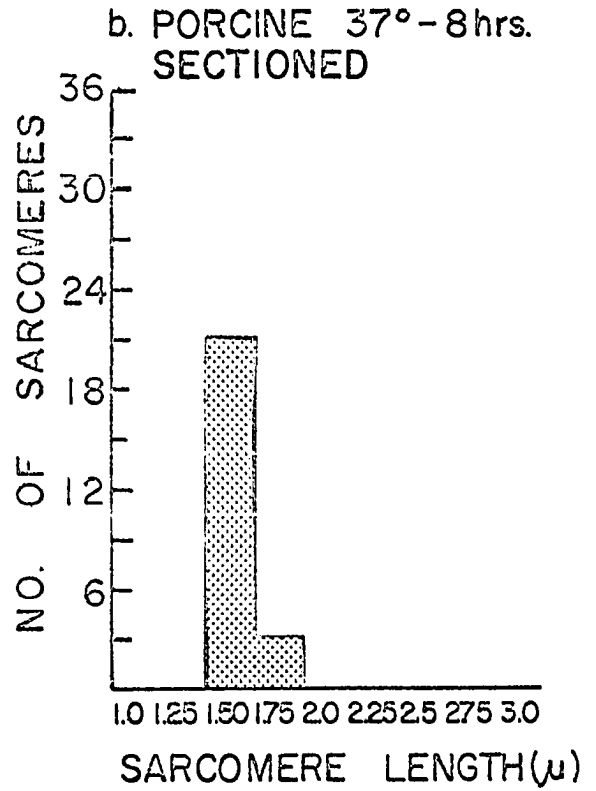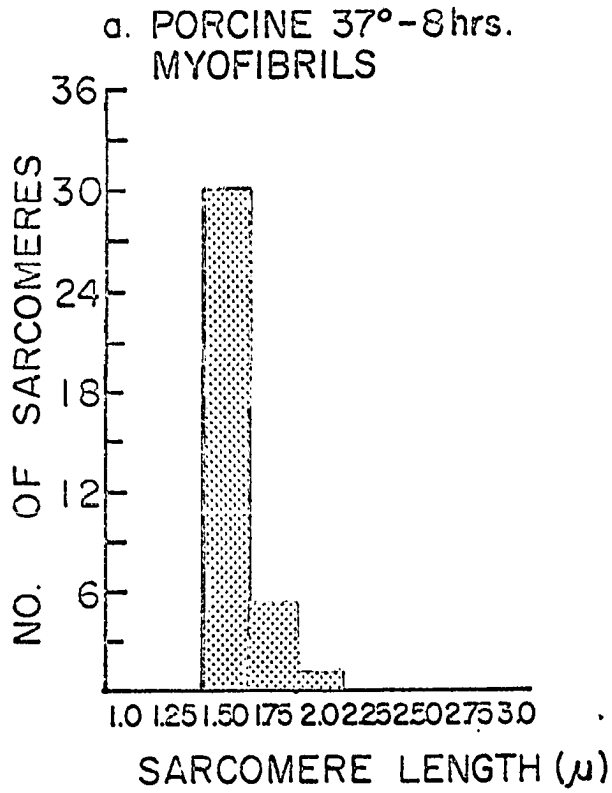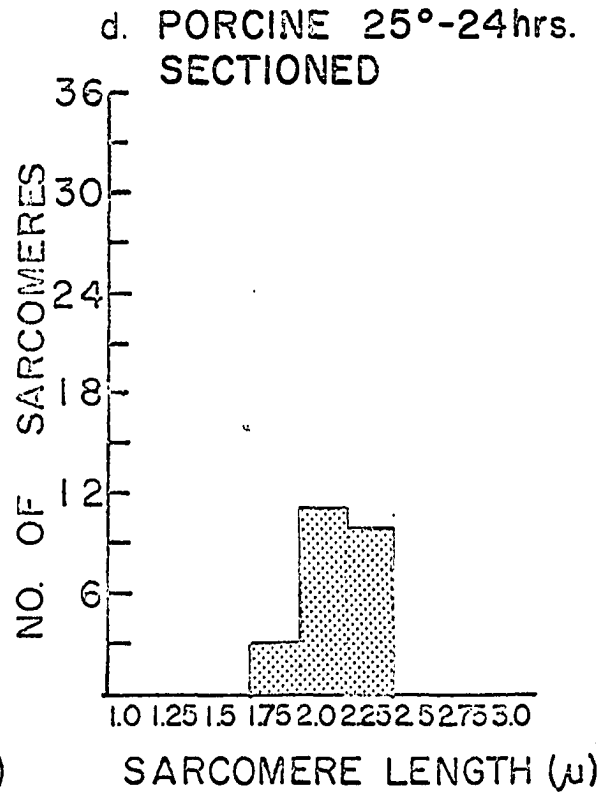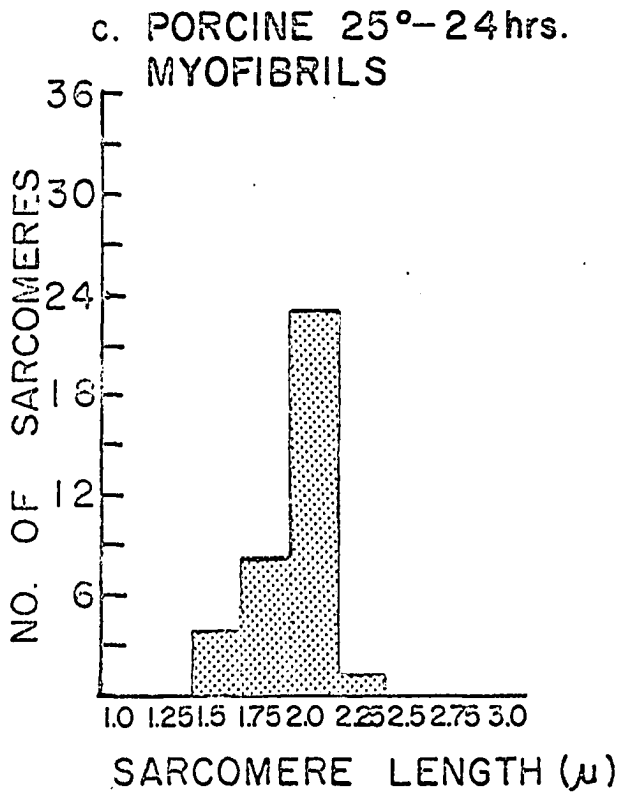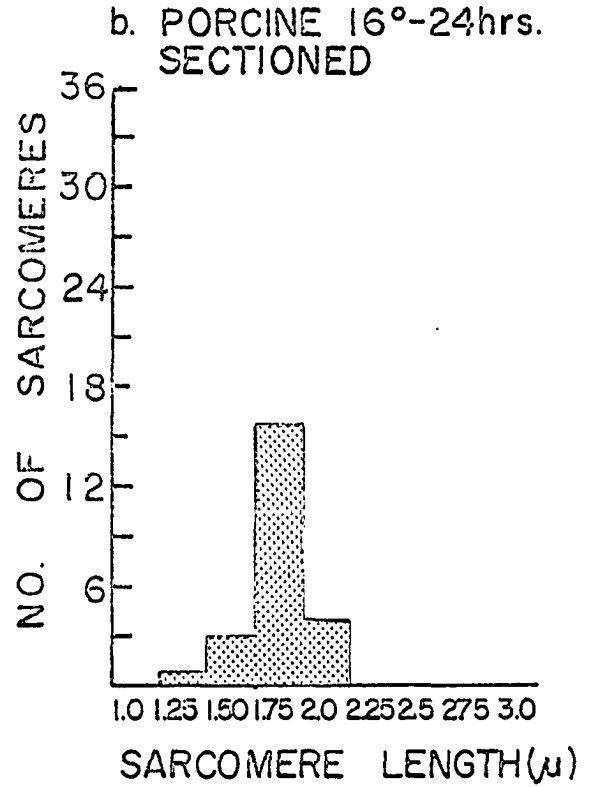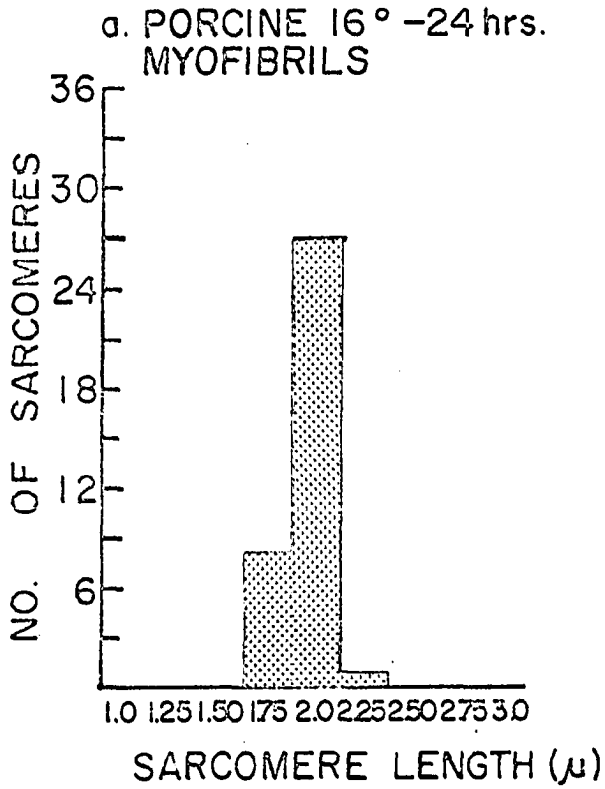stored at 25° for
24 hours (24
measured).

a. PORCINE 16° –24 hrs. MYOFIBRILS

b. PORCINE 16°-24hrs. SECTIONED

c. PORCINE 25°–24hrs. MYOFIBRILS

d. PORCINE 25°-24hrs. SECTIONED

NO. OF SARCOMERES

SARCOMERE LENGTH (μ)

observed; in other areas the fibers were wavy and fragmented

with the Z-lines no longer evident. Sarcomere lengths in the

fragmented areas were measured from the middle of the light

zone to the middle of the next light zone. This may result in

slightly high values for sarcomere lengths and may therefore

account for the apparent increase in sarcomere lengths between

8 and 24 hours at 37° (Fig. 16b).

Although it differs in degree and time course of shortening,

porcine muscle appears to exhibit the same type of post-mortem

shortening pattern (Table 2) as that observed in bovine muscle

with maximum shortening occurring at 2° and 37° and minimum

shortening occurring at 16° and 25°.

Table 2. Average sarcomere lengths (μ) of myofibrils and
         sectioned porcine muscle

	At death	2°-4 hrs.	37°-4 hrs.	2°-8 hrs.	37°-8 hrs.	2°-24 hrs.	16°-24 hrs.	25°-24 hrs.	37°-24 hrs.
myo-fibrils	1.8	1.9	1.6	1.6	1.5	1.7	2.0	1.9	1.5
sec-tioned	2.4	2.2	1.5	1.9	1.5	1.6	1.7	1.8	1.6

Rabbit muscle

The banding pattern of isolated myofibrils (Fig. 17a) and

sectioned muscle (Fig. 18a) sampled from at-death rabbit muscle

observed by phase microscopy appears to be structurally identical

to at-death bovine and porcine muscle. There was considerable A-band shortening evident in at-death rabbit myofibrils. This shortening is similar to that already discussed for at-death bovine and porcine myofibrils. Sarcomere lengths of at-death rabbit muscle (Fig. 19a,b) also correspond closely to those of at-death bovine and porcine muscle.

· Rabbit muscle stored at 2° for either 4 or 8 hours appears to be relaxed and almost identical in structure to at-death rabbit muscle, as evidenced by both sarcomere lengths (Figs. 19c, 20c) and the banding pattern shown in photomicrographs of isolated myofibrils (Fig. 17b,d) and sectioned muscle (Fig. 18b). As has been already described for 2°-4 hour and 2°-8 hour bovine or porcine myofibrils, the A-band lengths of rabbit myofibrils after 4 or 8 hours post-mortem at 2° are shorter (0.75 - 1.0μ) than the A-band lengths of sectioned rabbit muscle under these same conditions (1.25 - 1.5μ). On the other hand, sectioned samples of 2°-4 hour or 2°-8 hour rabbit muscle contrasts with both bovine and porcine muscle since muscle from the latter two species is slightly contracted after either 4 or 8 hours post-mortem at 2° (cf. Tables 1,2 and 3). After 24 hours of post-mortem storage at 2°, it appears that rabbit muscle too has undergone a small amount of shortening as evidenced by the shorter I-bands and the loss of the H-zone in both isolated myofibrils (Fig. 17f) and sectioned muscle.

Measurement of sarcomere lengths (Fig. 21c,d) confirms the impression that some shortening has occurred in the 2°-24 hour rabbit muscle since average sarcomere lengths for sectioned muscle have decreased from 2.4μ at death, 2.3μ after 4 hours, and 2.2μ after 8 hours to 1.7μ after 24 hours at 2°.

The structure of rabbit muscle stored at 37° appears very similar after either 4, 8, or 24 hours of post-mortem storage. The banding patterns observed for isolated myofibrils (Fig. 17a,e,i) from muscle stored at 37° are the same as those described for bovine and porcine muscle stored at these temperatures, although after 4 or 8 hours of post-mortem storage the Z-lines are not missing in rabbit myofibrils as they were in porcine myofibrils. However, after 24 hours post-mortem at 37°, the Z-lines are often missing from both rabbit myofibrils and sectioned muscle. This corresponds to similar observations made on bovine and porcine muscle. The appearance of sectioned rabbit muscle (Fig. 18c,d,h) is very similar to that seen in the corresponding myofibril preparations. The sarcomere lengths of rabbit myofibrils (Figs. 20a, 21c, 23a) and sectioned muscle (Figs. 20b, 21d, 23b) are the same after 4, 8 or 24 hours of post-mortem storage at 37°, and all are shortened considerably from at-death sarcomere lengths.

The appearance of rabbit muscle stored at 16° for 24 hours is the same as that of rabbit muscle stored at 25° for 24 hours. Myofibrils isolated from muscle stored for 24 hours at these two temperatures appear slightly contracted with the H-zone gone

Fig. 16a. Distribution of
          sarcomere lengths
          for porcine myo-
          fibrils stored at
          37° for 24 hours
          (35 measured).

Fig. 16b. Distribution of
          sarcomere lengths
          for sectioned
          porcine muscle
          stored at 37°
          for 24 hours
          (24 measured).

94

a. PORCINE 37°-24hrs. MYOFIBRILS

NO. OF SARCOMERES

36 30 24 18 12 6

1.0 1.25 1.5 1.75 2.0 2.25 2.5 2.75 3.0

SARCOMERE LENGTH (μ)

b. PORCINE 37°-24 hrs. SECTIONED

NO. OF SARCOMERES

36 30 24 18 12 6

1.0 1.25 1.5 1.75 2.0 2.25 2.5 2.75 3.0
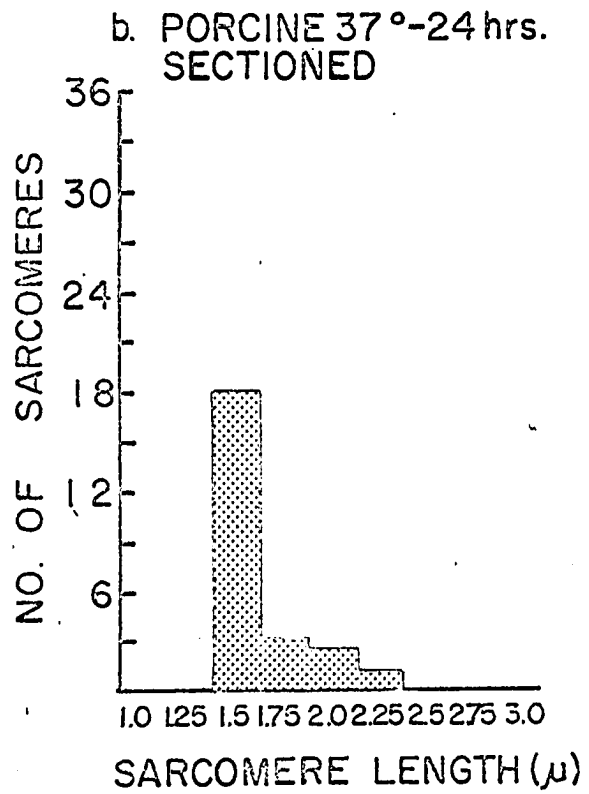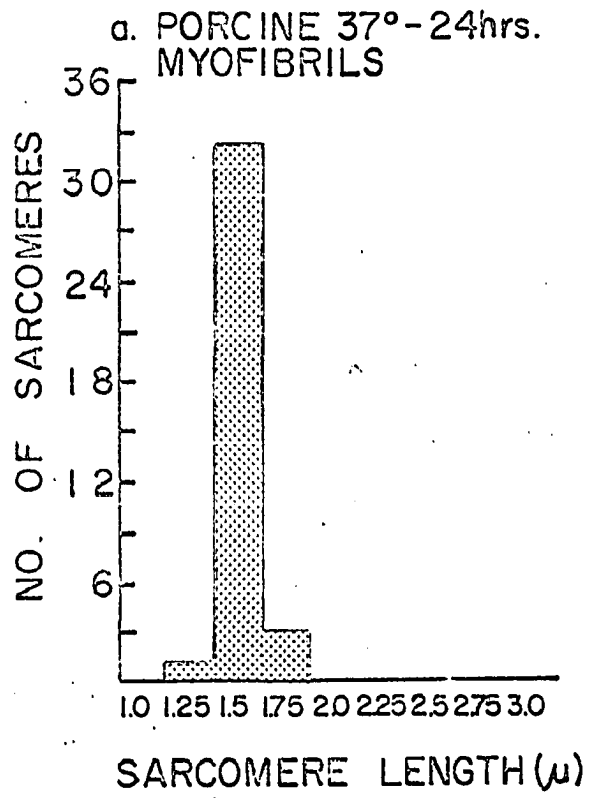
SARCOMERE LENGTH (μ)

Fig. 17a. Rabbit myofibrils
isolated at death
X 2,000

Fig. 17b. Rabbit myofibrils
isolated after
storage at 2° for
4 hrs. X 2,000

Fig. 17c. Rabbit myofibrils
isolated after
storage at 37° for
4 hrs. X 2,000

Fig. 17d. Rabbit myofibrils
isolated after
storage at 2° for
8 hrs. X 2,000

Fig. 17e. Rabbit myofibrils
isolated after
storage at 37° for
8 hrs. X 2,000

Fig. 18f. Rabbit myofibrils
isolated after
storage at 2° for
24 hrs. X 2,000

Fig. 17g. Rabbit myofibrils
isolated after
storage at 16° for
24 hrs. X 2,000

Fig. 17h. Rabbit myofibrils
isolated after
storage at 25° for
24 hrs. X 2,000

Fig. 17i. Rabbit myofibrils
isolated after
storage at 37° for
24 hrs. Note the
extensive fragmen-
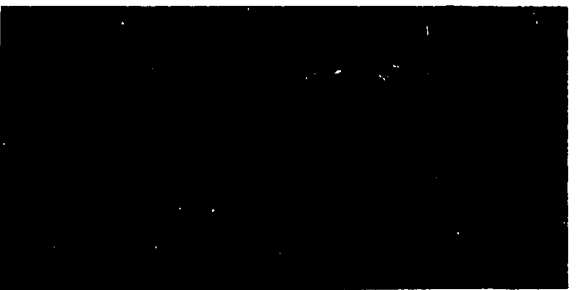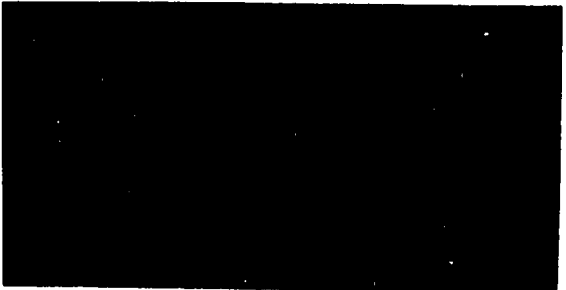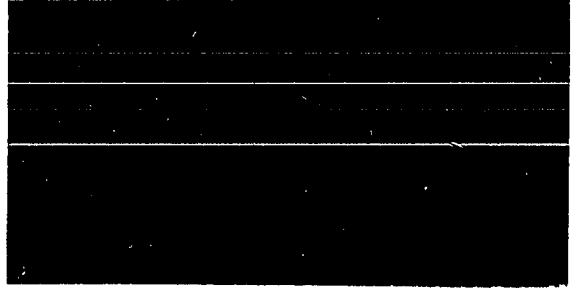tation of the myo-
fibrils. X 2,000
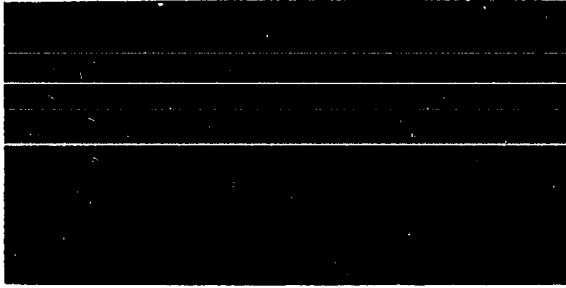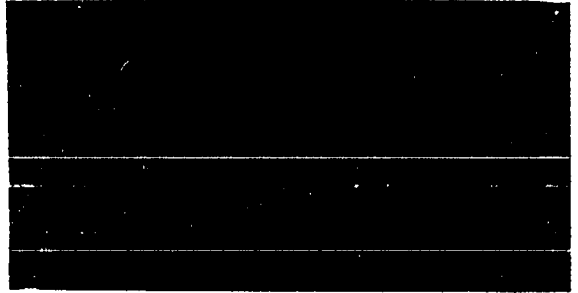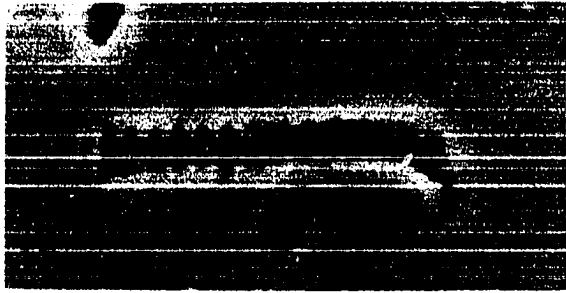
Fig. 18a. Sectioned at-death rabbit muscle exhibiting the relaxed or at-rest banding pattern. X 2,000

Fig. 18b. Sectioned rabbit muscle after storage at 2° for 24 hrs. This muscle exhibits a slightly contracted appearance. X 2,000

Fig. 18c. Sectioned rabbit muscle after storage at 37° for 4 hrs. The banding pattern is the same as observed for 4 hr.-37° myofibrils. X 2,000

Fig. 18d. Sectioned rabbit muscle after storage at 37° for 8 hrs. X 2,000

Fig. 18e. Sectioned rabbit muscle after storage at 16° for 24 hrs. This sample exhibits a severely contracted banding pattern. X 2,000

Fig. 18f. Another sample of sectioned rabbit muscle after storage at 16° for 24 hrs. Note the irregular transverse contraction bands. X 2,000

Fig. 18g. Sectioned rabbit muscle after storage at 25° for 24 hrs. X 2,000

Fig. 18h. Sectioned rabbit muscle after storage at 37° for 24 hrs. Arrows indicate fragmented appearance and loss of Z-lines. X 2,000
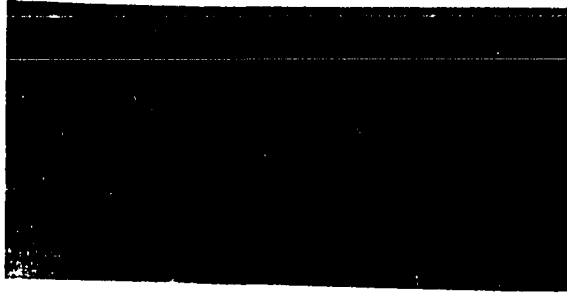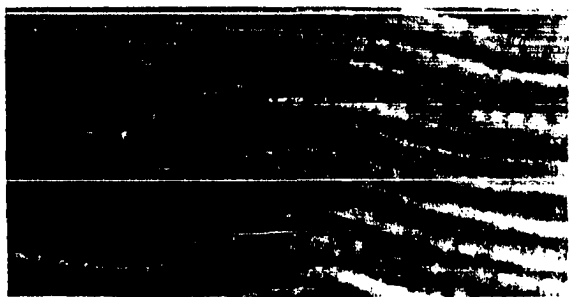
Fig. 19a. Distribution of
sarcomere lengths
for rabbit myo-
fibrils at death
(72 measured).

Fig. 19b. Distribution of
sarcomere lengths
for sectioned
rabbit muscle at-
death (37 measured).

Fig. 19c. Distribution of
sarcomere lengths
for rabbit myo-
fibrils stored at
2° for 4 hours
(36 measured).

Fig. 19d. Distribution of
sarcomere lengths
for sectioned
rabbit muscle
stored at 2° for
4 hours (22
measured).

a. RABBIT AT DEATH
MYOFIBRILS

b. RABBIT AT DEATH
SECTIONED

c. RABBIT 2°-4 hrs.
MYOFIBRILS

d. RABBIT 2°-4 hrs.
SECTIONED

Fig. 20a. Distribution of
           sarcomere lengths
           for rabbit myo-
           fibrils stored at
           37° for 4 hours
           (36 measured).

Fig. 20b. Distribution of
           sarcomere lengths
           for sectioned
           rabbit muscle
           stored at 37° for
           4 hours (24
           measured).

Fig. 20c. Distribution of
           sarcomere lengths
           for rabbit myo-
           fibrils stored at
           2° for 8 hours
           (36 measured).

Fig. 20d. Distribution of
           sarcomere lengths
           for sectioned
           rabbit muscle
           stored at 2° for
           8 hours (22
           measured).

a. RABBIT 37°-4 hrs. MYOFIBRILS

b. RABBIT 37°-4 hrs. SECTIONED

c. RABBIT 2°-8 hrs. MYOFIBRILS

d. RABBIT 2°-8 hrs. SECTIONED

Fig. 21a. Distribution of
           sarcomere lengths
           for rabbit myo-
           fibrils stored at
           37° for 8 hours
           (36 measured).

Fig. 21b. Distribution of
           sarcomere lengths
           for sectioned
           rabbit muscle
           stored at 37° for
           8 hours (24
           measured).

Fig. 21c. Distribution of
           sarcomere lengths
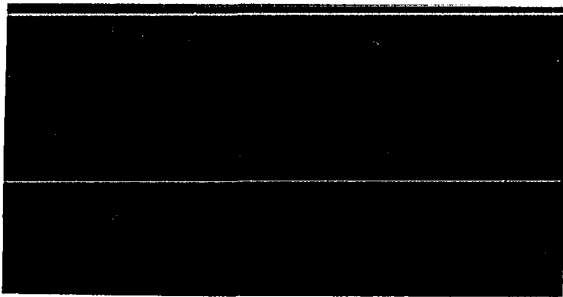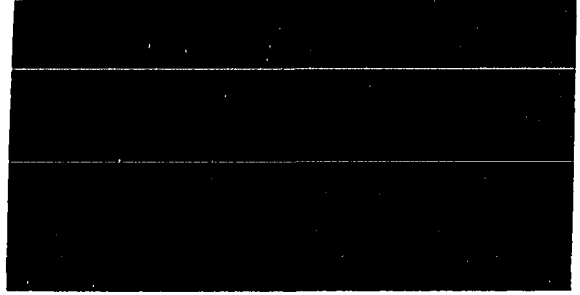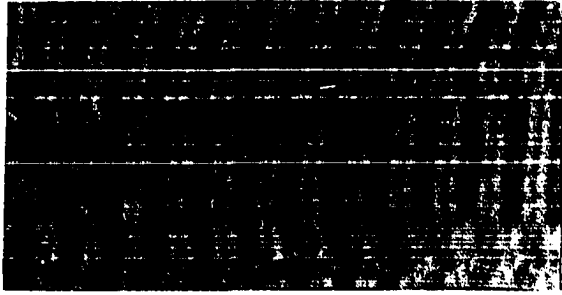   for rabbit myo-
           fibrils stored at
           2° for 24 hours
           (36 measured).

Fig. 21d. Distribution of
           sarcomere lengths
           for sectioned
           rabbit muscle
           stored at 2° for
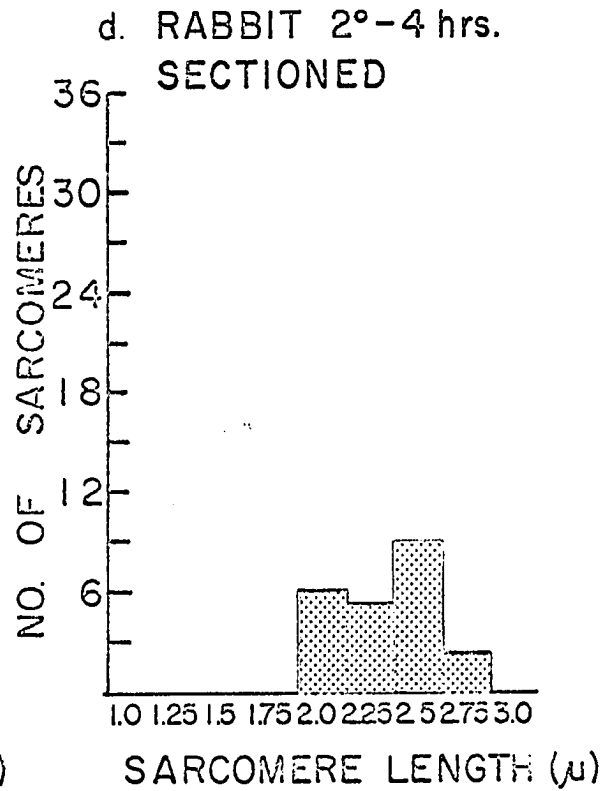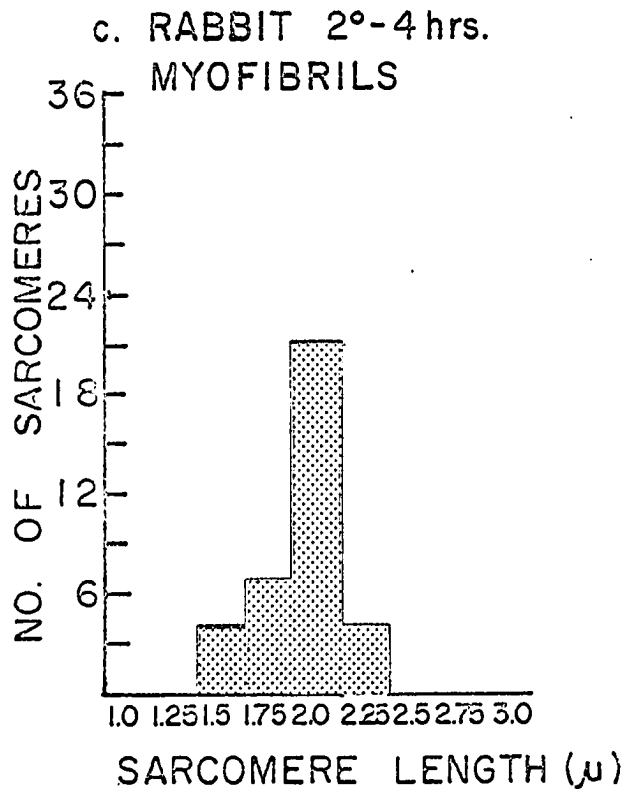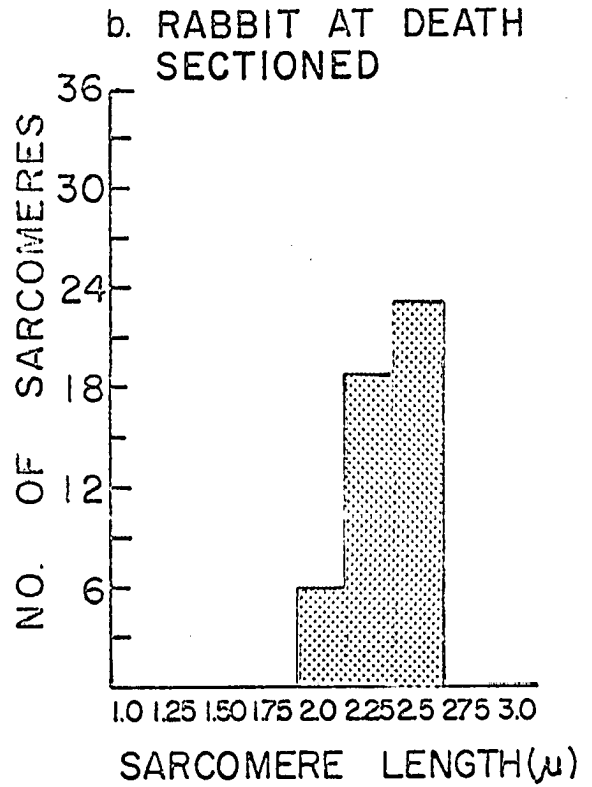   24 hours (24
           measured).

a. RABBIT 37°-8 hrs.
MYOFIBRILS

b. RABBIT 37°-8hrs.
SECTIONED

c. RABBIT 2°-24 hrs.
MYOFIBRILS

d. RABBIT 2°-24 hrs.
SECTIONED

and the I-band shortened (Fig. 17g,h). Observations on sectioned muscle (Fig. 18e,g) indicate this same banding pattern. Occasionally contraction bands are observed in 16° sectioned muscle (Fig. 18f). These irregular transverse bands were not observed in other sectioned muscle in this study. After 24 hours at either 16° or 25°, sarcomere lengths of myofibrils (Fig. 22a,c) are slightly longer than sarcomere lengths of sectioned muscle (Fig. 22b,d). This phenomenon was observed in both bovine and porcine muscle after 24 hours at either 16° or 25° and a possible explanation has already been discussed.

The average sarcomere lengths of rabbit muscle after different times and temperatures of post-mortem storage are presented in Table 3. Rabbit muscle shortens at all post-mortem storage temperatures. The sarcomere lengths of sectioned muscle suggest that in rabbit muscle, maximal shortening occurs at 37° and minimal shortening occurs at 2° although very little difference is observed between shortening at 2°, 16°, or 25°. Very little shortening is observed during the first 8 hours in rabbit muscle stored at 2° whereas all of the shortening of rabbit muscle at 37° occurs in the first 4 hours of post-mortem storage.

Table 3. Average sarcomere lengths (μ) of myofibrils and sectioned rabbit muscle

	At death	2°-4 hrs.	37°-4 hrs.	2°-8 hrs.	37°-8 hrs.	2°-24 hrs.	16°-24 hrs.	25°-24 hrs.	37°-24 hrs.
myo-fibrils	1.9	1.9	1.5	1.9	1.5	1.9	1.9	1.8	1.5
sec-tioned	2.4	2.3	1.5	2.2	1.5	1.7	1.6	1.6	1.5

Fig. 22a. Distribution of
sarcomere lengths
for rabbit myo-
fibrils stored at
16° for 24 hours
(36 measured).

Fig. 22b. Distribution of
sarcomere lengths
for sectioned
rabbit muscle
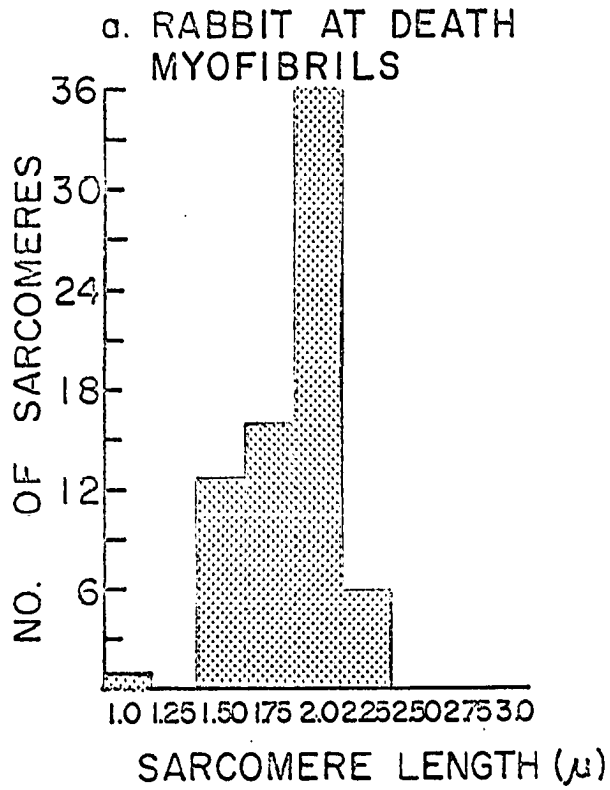stored at 16° for
24 hours (24
measured).

Fig. 22c. Distribution of
sarcomere lengths
for rabbit myo-
fibrils stored at
25° for 24 hours
(36 measured).

Fig. 22d. Distribution of
sarcomere lengths
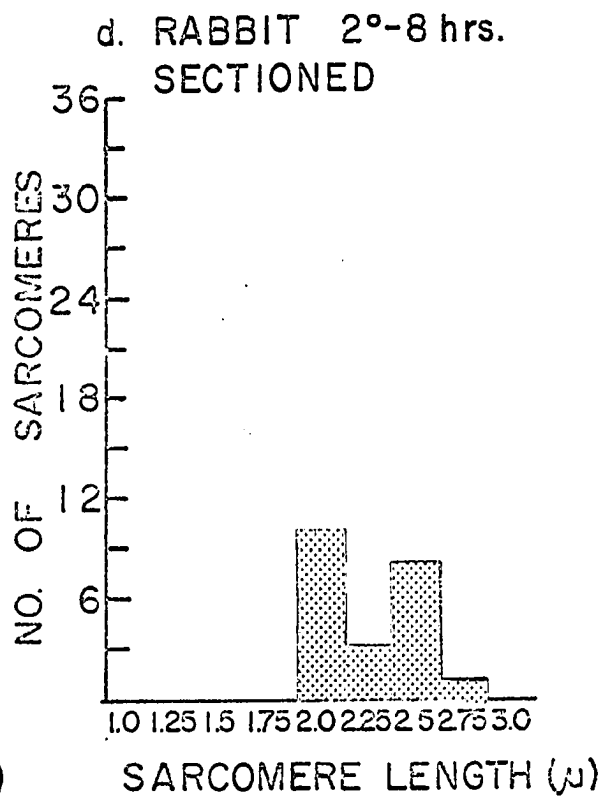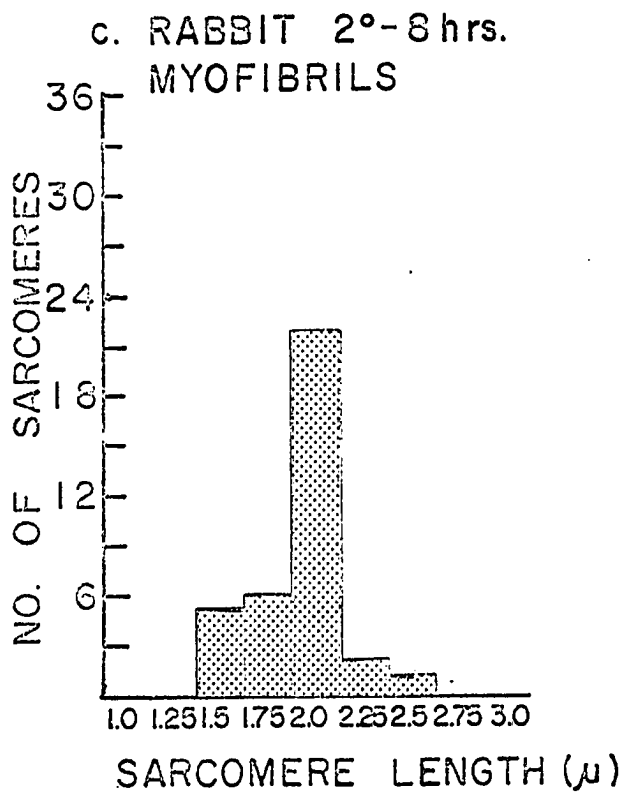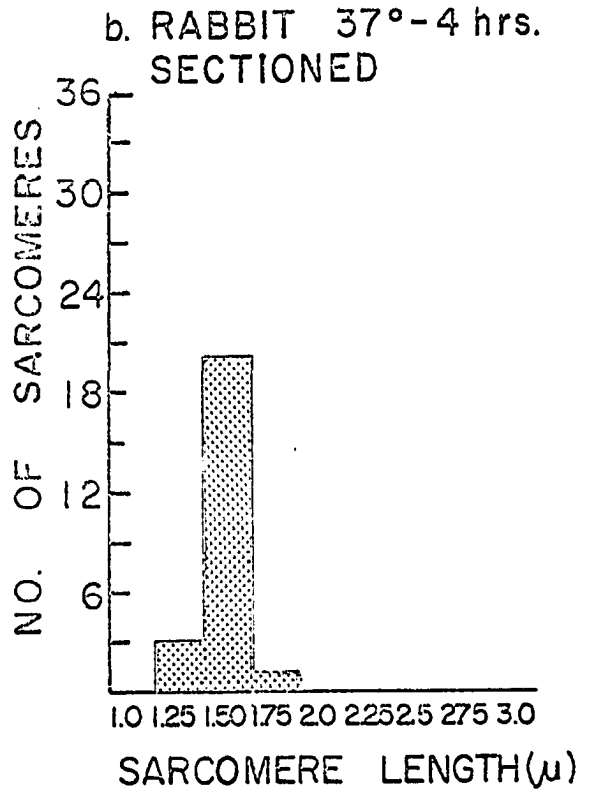for sectioned rabbit
muscle stored at
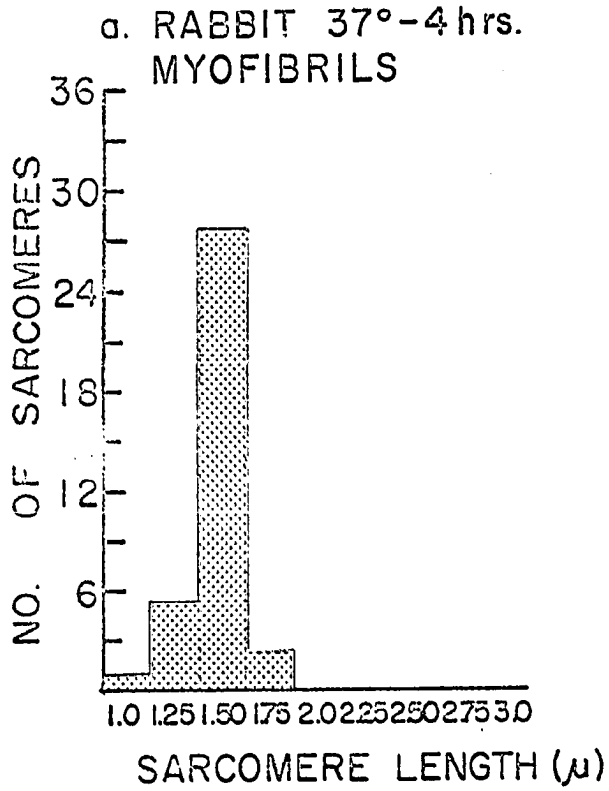25° for 24 hours
(24 measured).

a. RABBIT 16°-24 hrs. MYOFIBRILS

b. RABBIT 16°-24 hrs. SECTIONED

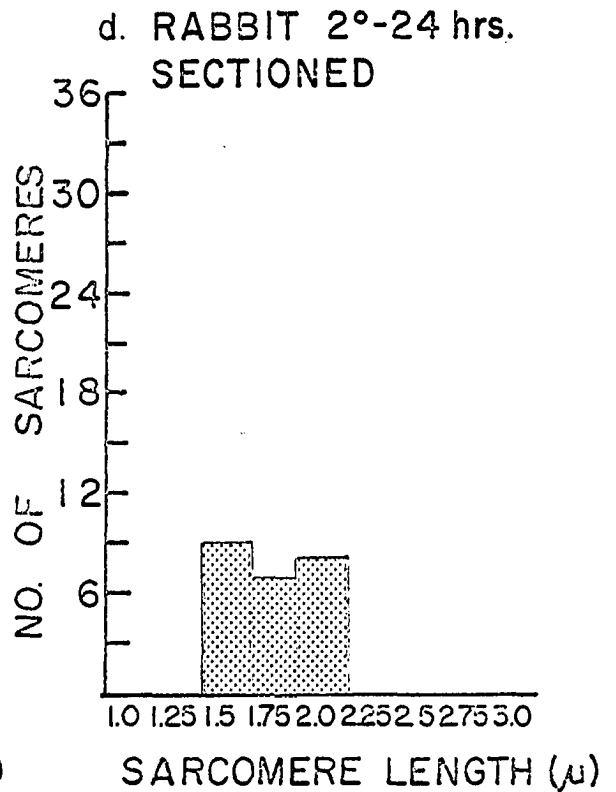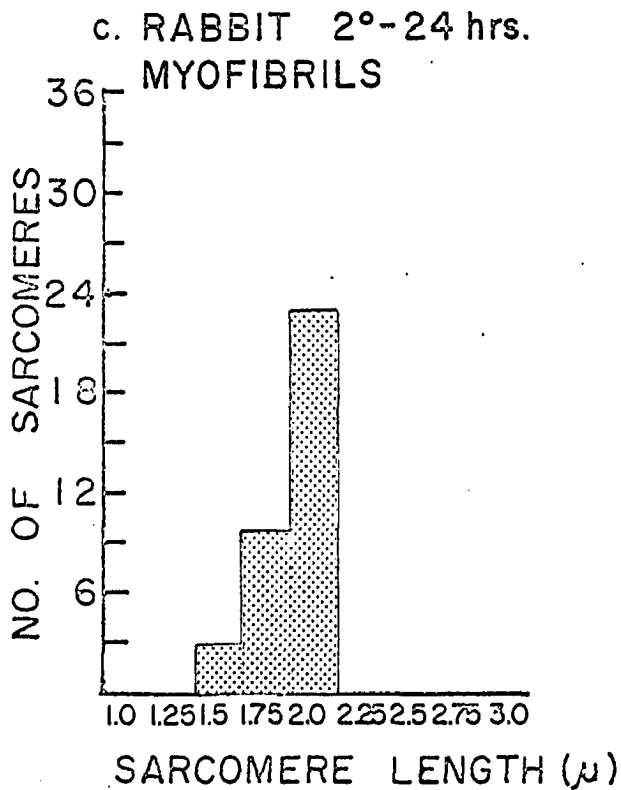c. RABBIT 25°-24 hrs. MYOFIBRILS
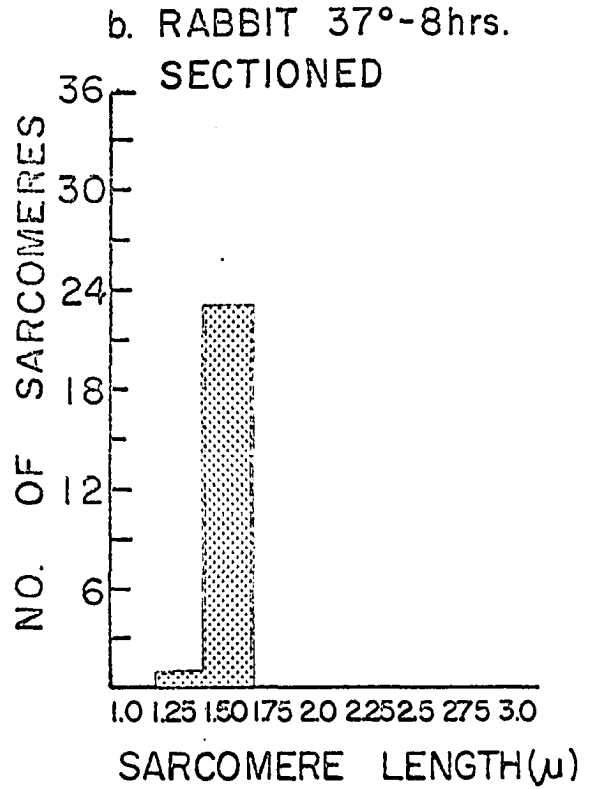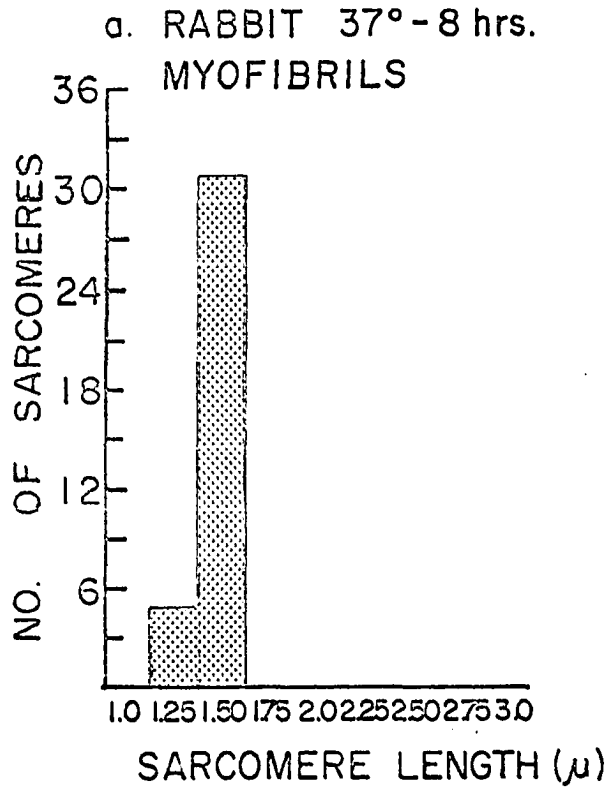
d. RABBIT 25°-24 hrs. SECTIONED
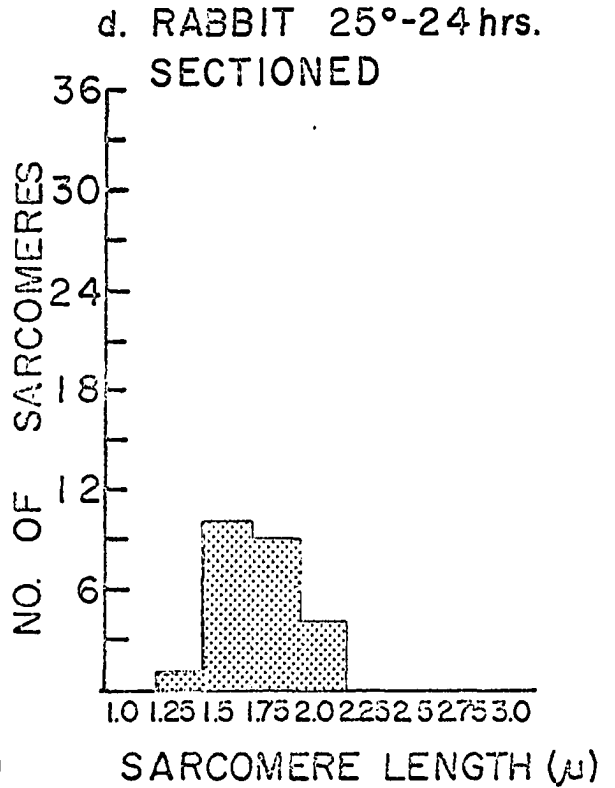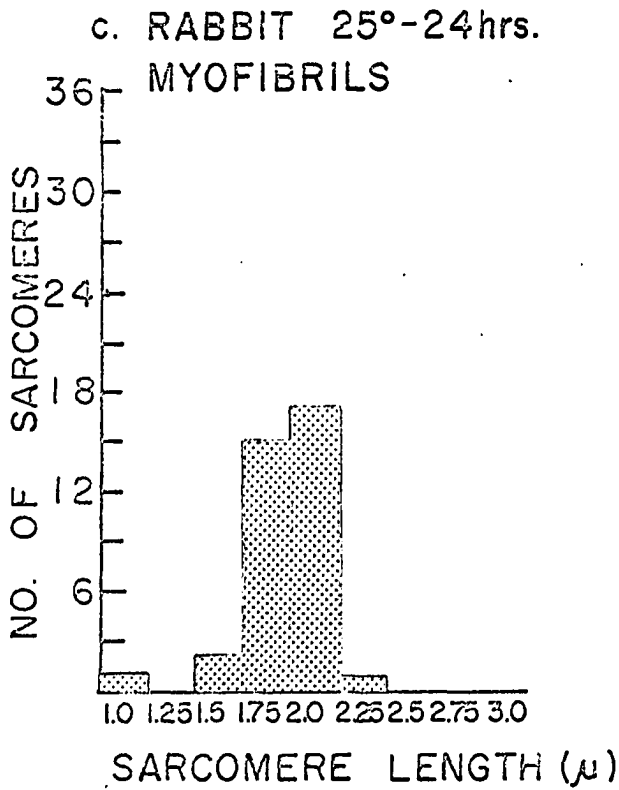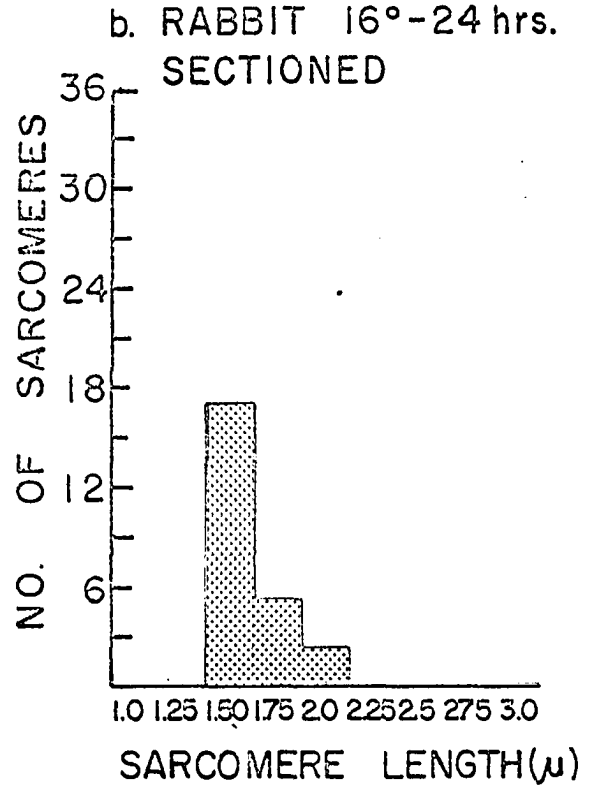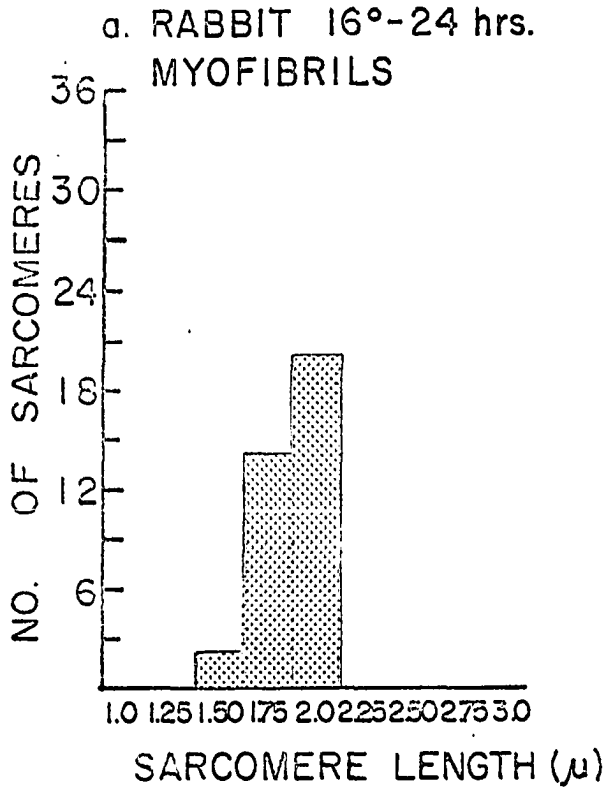
Fig. 23a. Distribution of
sarcomere lengths
for rabbit myo-
fibrils stored at
37° for 24 hours
(36 measured).

Fig. 23b. Distribution of
sarcomere lengths
for sectioned
rabbit muscle stored
at 37° for 24 hours
(24 measured).

a. RABBIT 37°–24 hrs.
MYOFIBRILS

b. RABBIT 37°– 24 hrs.
SECTIONED
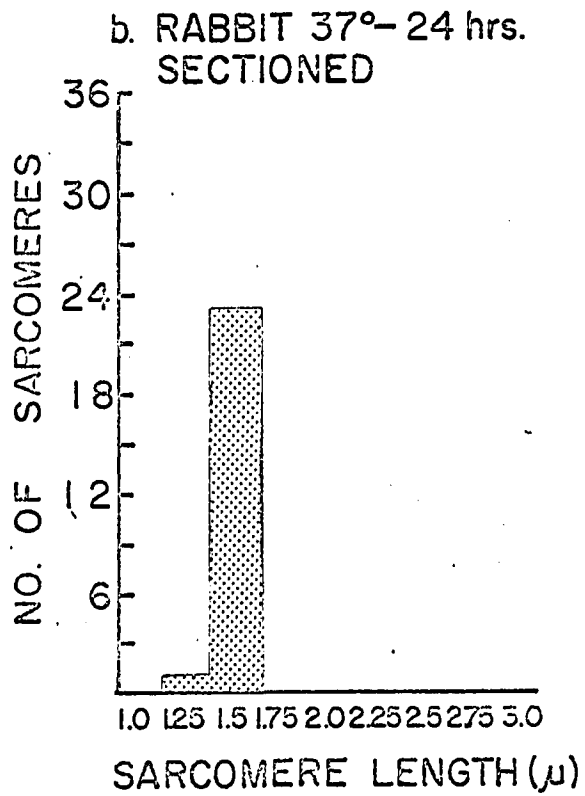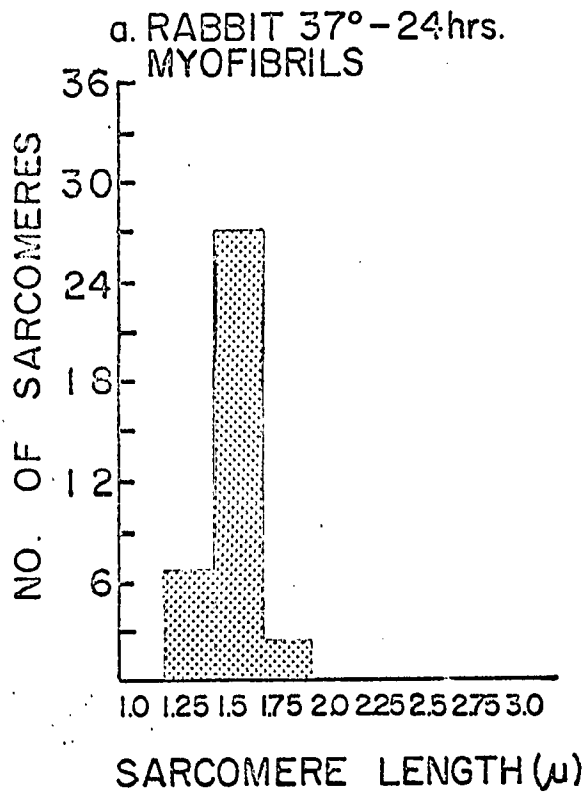
Electron Microscopy Observations

Electron microscopic observations were made on muscle which was sectioned from the same embedded samples used to make phase microscopic observations on sectioned muscle. The only difference was that samples for electron microscopy were sectioned thinner and were stained differently than the samples used for phase microscopy. Extensive measurements made on electron micrographs showed that sarcomere lengths of the electron microscopy samples were identical to those of sectioned phase microscopy samples. Furthermore, the state of contraction of the electron microscopy samples, as indicated by either sarcomere length or banding pattern, was very similar to that already described for the sectioned phase microscopy samples. Therefore, discussion of the electron microscopic results of this study will be limited to those observations made possible by the higher resolution of the electron microscope. Sarcomere lengths and banding patterns of the various samples in the electron microscope will not be mentioned except where they differ from the impressions gained by phase microscopy or where they permit a conclusion not possible with phase microscopy alone.

Bovine muscle

Bovine muscle removed immediately after death (Fig. 24) exhibited the typical banding pattern of relaxed muscle. As seen in the electron microscope, the myofibrils are constructed

of interdigitating thick and thin filaments. The thick fila-
ments lie in the center of the sarcomere and form the A-band.
That portion of the sarcomere which does not contain thick
filaments is the I-band. The light zone in the center of the
A-band, the H-zone, occurs because the thin filaments do not
overlap with the thick filaments in this area. The M-line is
seen as a dark line in the center of the H-zone and bisecting
the A-band. The Z-line is seen as a dark line to which the
thin filaments attach but do not pass through. The attachment
of the thin filaments to the Z-line results in the reported
zigzag configuration which can be observed whenever the Z-line
is sectioned in the appropriate plane (it is not readily ob-
served in Fig. 24). The sarcoplasmic reticulum is observed in
the area of the A-I junction and appears to contain a granular
material (Fig. 24). Also observed in at-death bovine muscle are
mitochondria which are structurally well preserved, with intact
cristae (Fig. 24).

After storage of bovine muscle for 4 hours at 2° (Fig.
25) the thin actin filaments have penetrated into the center
of the A-band causing loss of the H-zone, and narrowing of
the I-band. The zigzag configuration of the Z-line remains
intact after this time and temperature of post-mortem storage.
The myofibrils in bovine muscle stored at 37° for 4 hours
(Fig. 26) are also structurally well-preserved although mi-
tochondria are disrupted and the cristae are absent in many

Fig. 24. At-death bovine muscle exhibiting the
          typical relaxed banding pattern.
          X 31,500

Fig. 25. Bovine muscle sampled after 4 hrs. of
          storage at 2°. This muscle exhibits
          a slightly contracted appearance with
          the I-band narrowed and thin filaments
          passing into center of the A-band and
          masking the H-zone. X 46,000
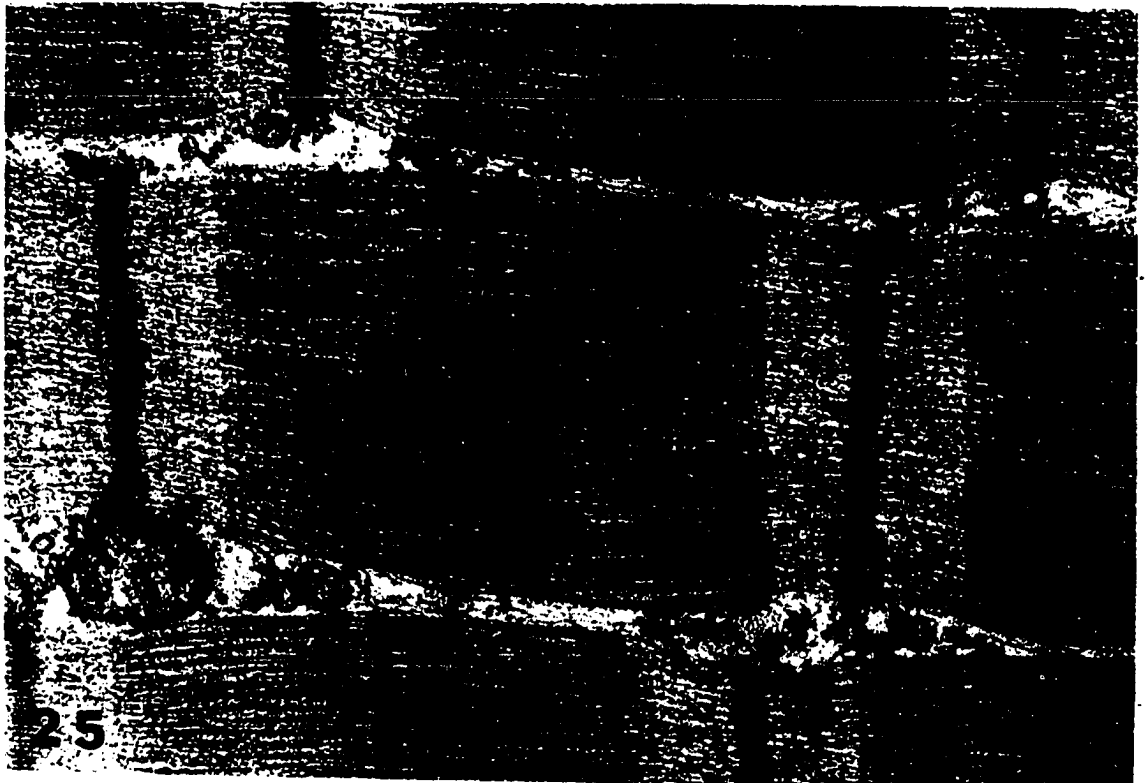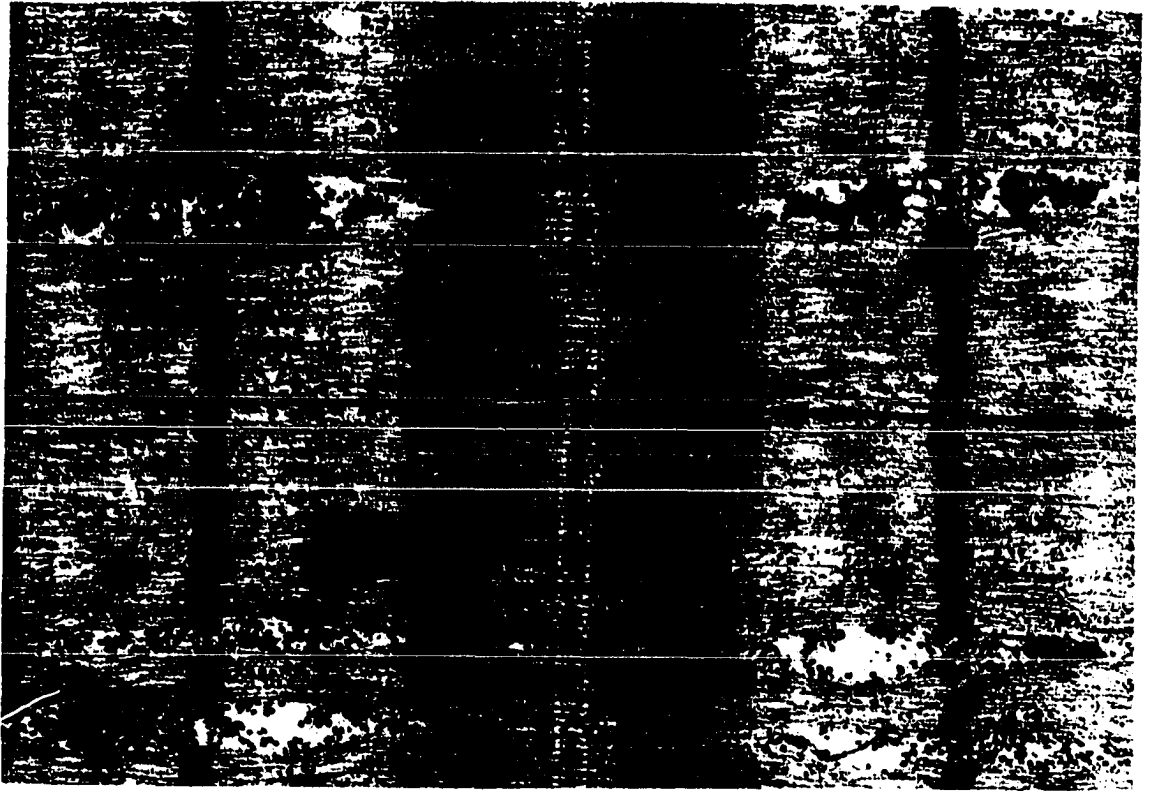
instances. About 60% of the sarcomeres in muscle stored at 2°
for 8 hours structurally resemble muscle stored at 2° for 4
hours, but the other 40% of the sarcomeres are more highly con-
tracted with the thick filaments butting up against the Z-line
(Fig. 27). After 8 hours storage at 37°, some major structural
changes were evident (Fig. 28). The Z-lines appear rather dif-
fuse and disrupted all along the length of the myofibrils. The
mitochondria and sarcoplasmic reticulum are also severely dis-
rupted. Many dense granular areas that may represent precip-
itated or crystallized Z-band material can be observed in the
neighborhood of the Z-line in the spaces between the myofibrils.
Fragmentation of the myofibrils is observed with most of the
fragmentation occurring at the level of the Z-line.

Storage of bovine muscle at 2° for 24 hours (Fig. 29)
results in the supercontracted appearance with the I-band no
longer visible and the thick filaments crumpling against or
passing through the Z-line, causing a substantial increase in
density at the level of the Z-line. The increased density
observed at the level of the Z-line gives rise to the dark
band previously described for supercontracted muscle using
phase microscopy. The light band observed by using phase optics
is the A-band, which now contains both thick and thin filaments
along its entire length, but which appears light in the phase
microscope because of the greatly increased density at the
level of the Z-line. Occasionally a less severely contracted

Fig. 26.   Bovine muscle sampled after 4 hrs. of
           storage at 37°.   The mitochondria and
           sarcoplasmic reticulum are disrupted
           but the myofibrillar structure is well-
           preserved.   X 31,500

Fig. 27.   Bovine muscle sampled after 8 hrs. of
           storage at 2°.   The myofibrils are
           contracted to the point that the thick
           filaments butt against the Z-line.
           X 45,000

116
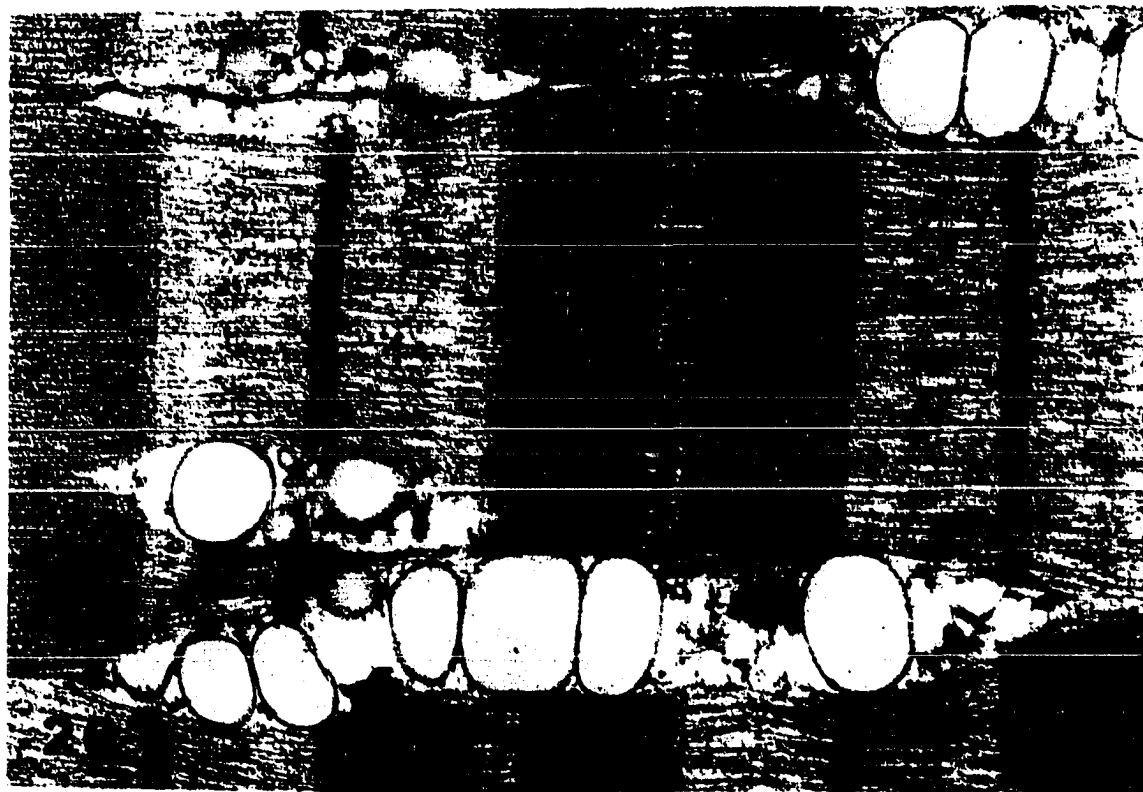
appearance was observed in the 2°-24 hour bovine muscle, but even myofibrils of this less contracted type are shortened (sarcomere lengths of 1.5-1.75μ). This less severely shortened pattern was observed in about 30% of the sarcomeres of the 2°-24 hour muscle. These sarcomeres may be congruous to the wavy areas seen by using phase microscopy on sectioned muscle after storage at 2° for 24 hours.

After 24 hours at 16° (Fig. 30), bovine muscle has shortened slightly and the thin filaments have passed into the center of the A-band and masked the H-zone. The sarcoplasmic organelles, particularly the mitochondria and sarcoplasmic reticulum, are disrupted but the zigzag structure of the Z-line remains at least partially intact. Storage at 25° for 24 hours (Fig. 31) resulted in a structural appearance similar to 16°-24 hour muscle with the notable exception that the Z-line is highly fragmented. The presence of dense granular areas at the level of the Z-line is very evident in 25°-24 hour muscle. In some cases the Z-line appears to have been completely removed by 24 hours of post-mortem storage at 25° causing fission of the myofibrils. The ultrastructure of muscle stored at 37° for 24 hours is shown in Fig. 32. The sarcomeres show much structural degradation, the Z-lines are almost completely disrupted and considerable fragmentation of the sarcomeres can be observed at the level of the Z-line.

Fig. 28. Bovine muscle sampled after 8 hrs. of
storage at 37°. The Z-line, sarcoplasmic
reticulum, and mitochondria are disrupted.
The myofibrils are shortened as evidenced
by the very short I-bands. X 31,500

Fig. 29. Bovine muscle sampled after 24 hrs. of
storage at 2°. This muscle exhibits a
typical supercontracted banding pattern
with the thick filaments crumpling at
or passing through the Z-line. X 49,000

Fig. 30. Bovine muscle sampled after 24 hrs. of
         storage at 16°. This muscle has under-
         gone some shortening since only a short
         I-band is present. X 44,500

Fig. 31. Bovine muscle sampled after 24 hrs. of
         storage at 25°. This muscle exhibits
         a contracted banding pattern with the
         Z-lines considerably disrupted.
         X 44,500
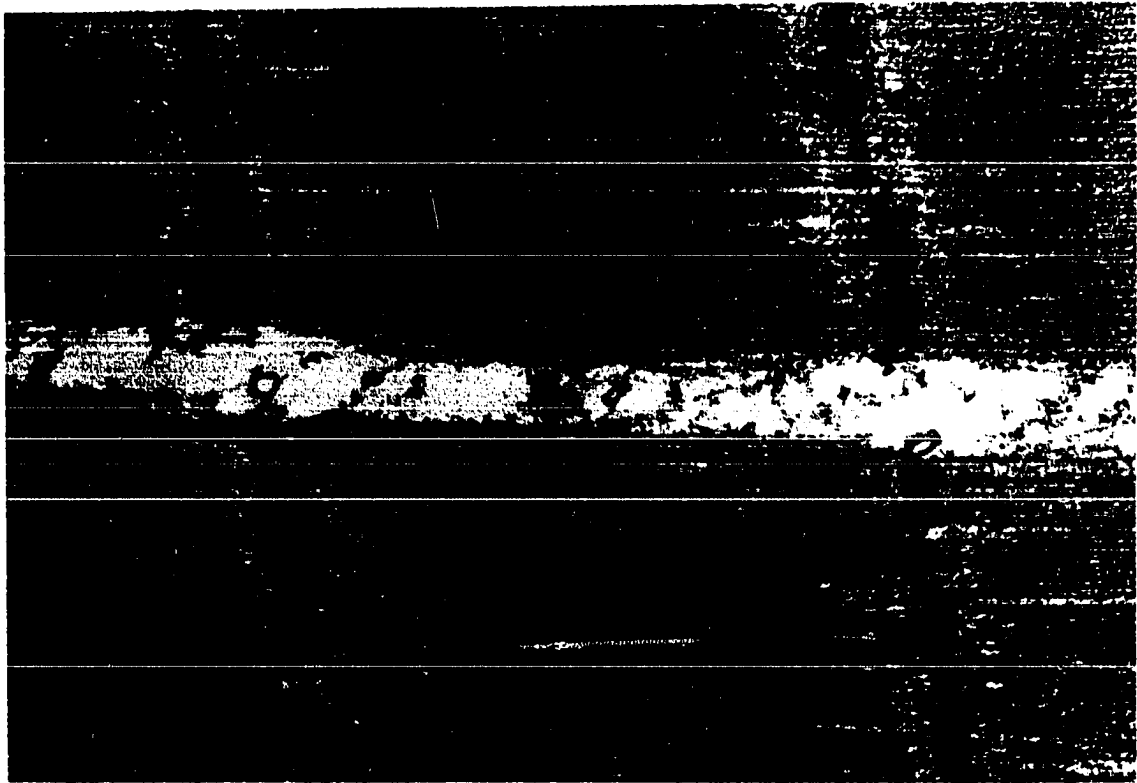
Porcine muscle

Porcine muscle sampled immediately after death (Fig. 33) exhibited the typical structure of relaxed striated muscle as it has already been described for bovine muscle. After 4 hours of post-mortem storage at 2° the H-zone of porcine muscle was no longer visible, indicating that the sarcomeres had shortened slightly (Fig. 34). Mitochondria and sarcoplasmic reticulum of 2°-4 hour porcine muscle were intact and appeared structurally identical to mitochondria and sarcoplasmic reticulum in at-death muscle. Also, in appropriately oriented sections of 2°-4 hour porcine muscle, the zigzag appearance of the Z-line was observed. On the other hand, the zigzag structure of the Z-line is disrupted and appears diffuse in porcine muscle stored at 37° for 4 hours (Fig. 35). This contrasts to the situation observed in 37°-4 hour bovine muscle where the Z-line appeared relatively intact.

Porcine muscle stored at 2° for 8 hours (Fig. 36) was shortened to the point that the thick filaments are in contact with the Z-line. The dark zone in the center of the A-band suggests that overlap of the thin filaments has occurred in the center of the A-band, indicating that the 2°-8 hour short-ening resulted from the sliding of filaments past one another in a manner analogous to that which occurs during contraction. Porcine muscle stored at 37° for 8 hours (Fig. 37) also

Fig. 32.   Bovine muscle sampled after 24 hrs. of
           storage at 37°.   The myofibrillar
           structure is somewhat disorganized with
           the Z-line disrupted.   X 51,000

Fig. 33.   At-death porcine muscle exhibiting the
           relaxed or at-rest banding pattern.
           X 49,500
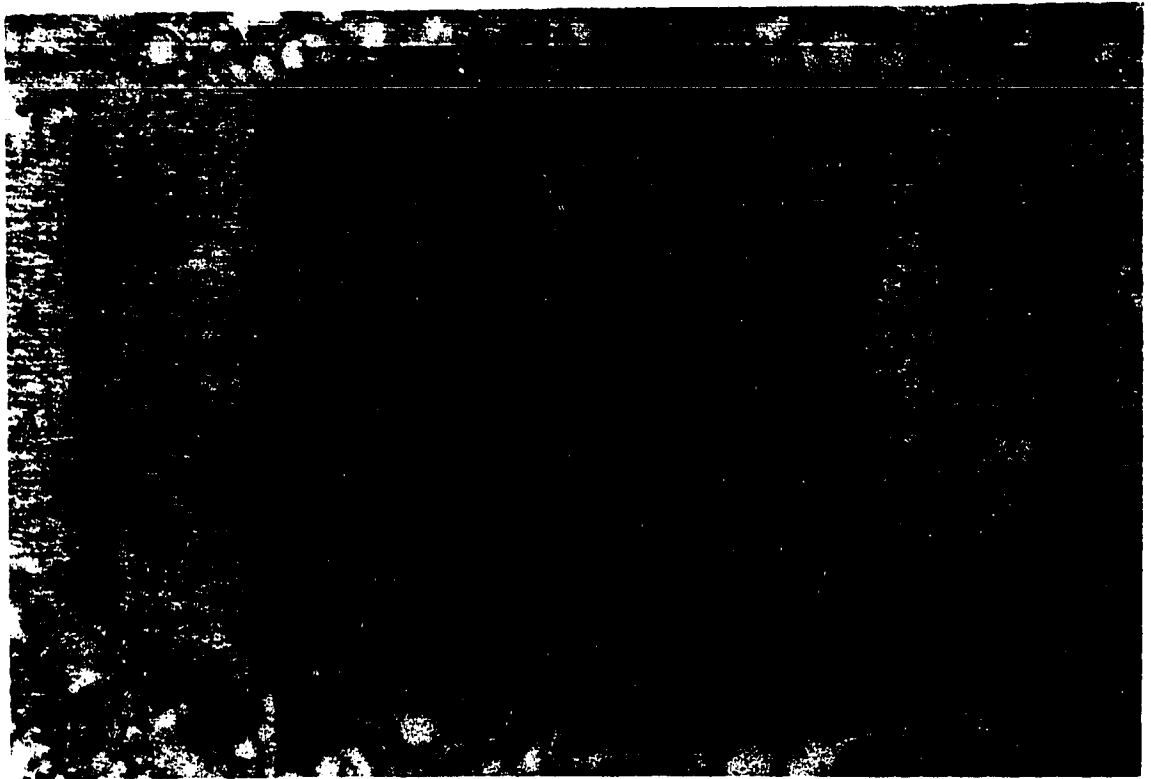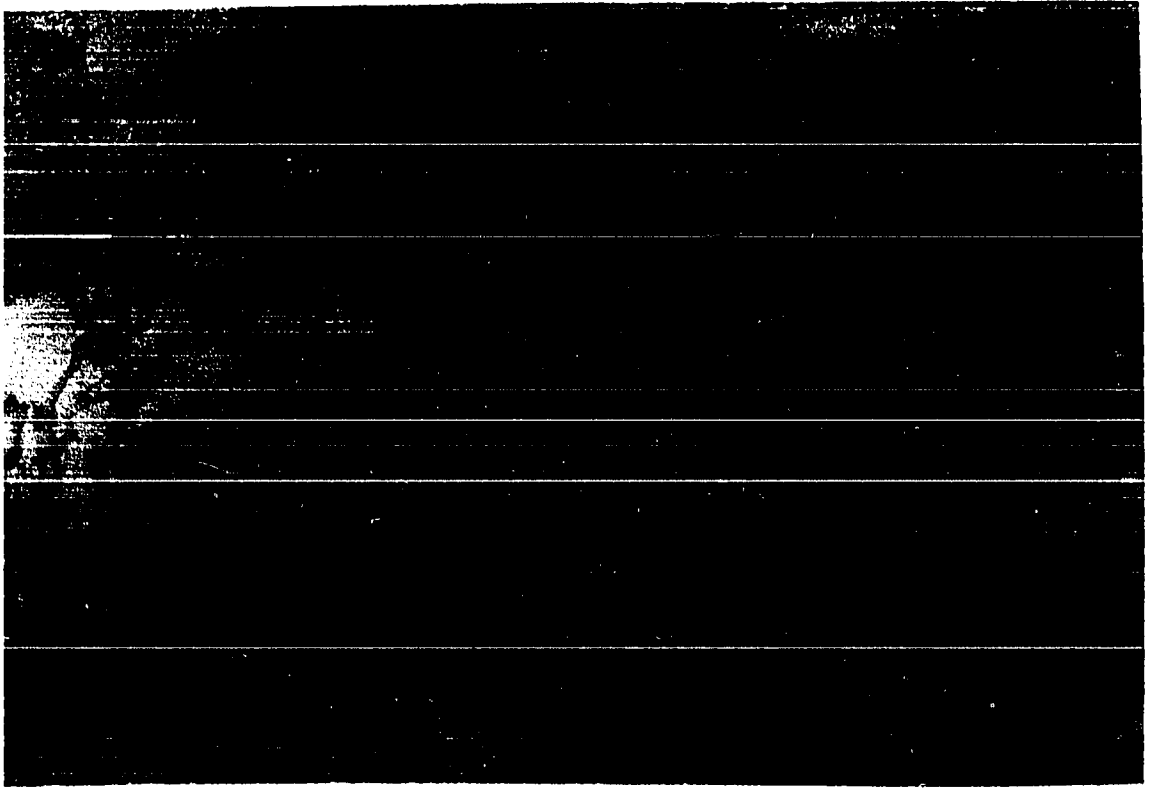
Fig. 34.   Porcine muscle sampled after 4 hrs. of
           storage at 2°. This muscle is slightly
           contracted with the thin filaments
           extending to the center of the A-band,
           thereby masking the H-zone. X 25,500

Fig. 35.   Porcine muscle sampled after 4 hrs. of
           storage at 37°. This muscle is exten-
           sively shortened and the Z-line material
           is diffuse and fragmented. X 53,500
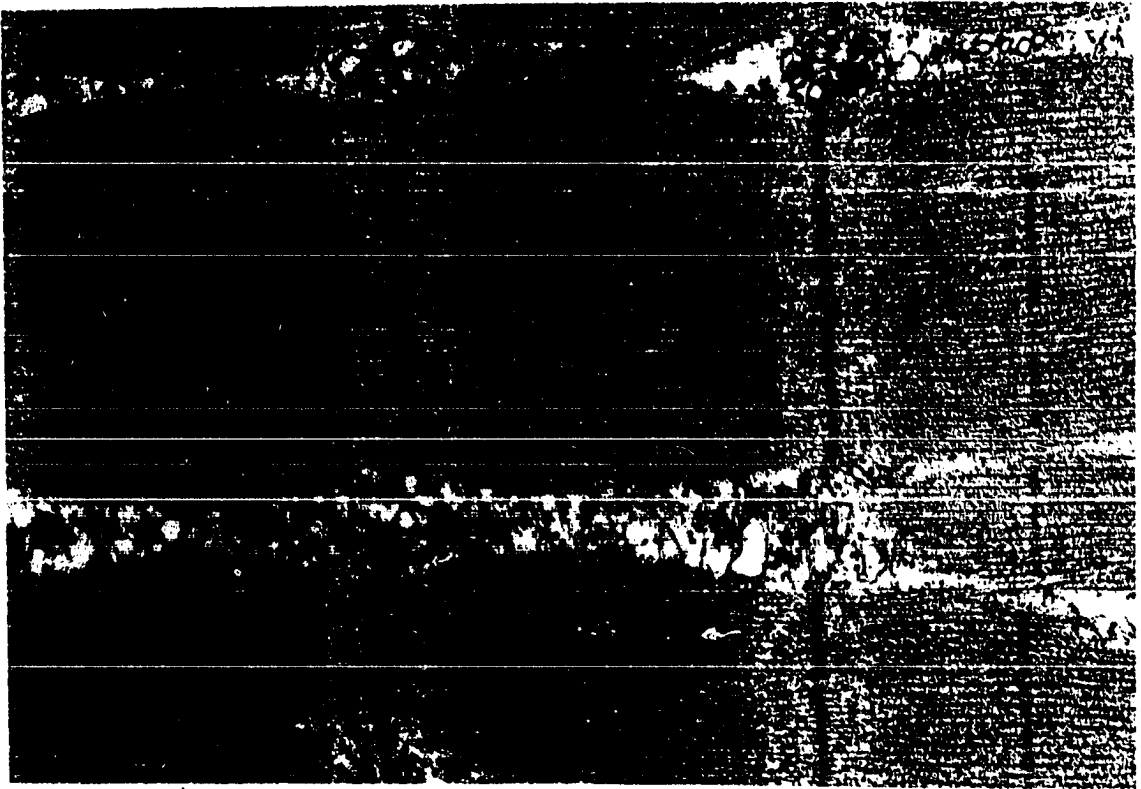
exhibited shortened sarcomeres but considerable fragmentation

of myofibrils is evident in the 37°-8 hour muscle. The darkened

area in the center of the sarcomere again suggests an overlap

of thin filaments in this region and indicates that shortening

at 37° also occurs through a sliding of filaments. The Z-line

structure and the elements of the sarcoplasmic reticulum and

mitochondria are almost completely disrupted after 8 hours at

37°. Also the dense granular material which may represent

crystallized Z-line material was evident throughout the inter-

fibrillar spaces.

Although post-mortem storage of porcine muscle at 2° for

24 hours resulted in a highly contracted appearance (Fig. 38),

this muscle was not shortened to the same extent as 2°-24 hour

bovine muscles. The 2°-24 hour porcine muscle did not show

the supercontracted banding pattern and the thick filaments

are apparently just coming into contact with the Z-line. The

overlap of thin filaments in the center of the A-band indicates

that post-mortem shortening of porcine muscle at 2° occurs as

a result of sliding of thick and thin filaments past one

another.

Storage at 16° for 24 hours (Fig. 39) results in slight

shortening of porcine sarcomeres. This shortening is not as

extensive as that observed for 2°-24 hour muscle as is indicated

by the length of the I-band remaining, and the length of overlap

Fig. 36. Porcine muscle sampled after 8 hrs. of
storage at 2°. This muscle exhibits a
highly contracted appearance with the
overlap of the thin filaments visible
in the center of the A-band. X 43,000

Fig. 37. Porcine muscle sampled after 8 hrs. of
storage at 37°. Note the large amount
of dense granular material in the
sarcoplasm surrounding the myofibrils
and the extensive disruption of the
Z-lines. X 45,000

36

Fig. 38. Porcine muscle sampled after 24 hrs. of storage at 2°. This muscle exhibits a highly contracted banding pattern with the thick filaments butting against the Z-line. X 47,000

Fig. 39. Porcine muscle sampled after 24 hrs. storage at 16°. The myofibrils are only slightly shortened as evidenced by the presence of short I-bands. Also, note the overlap of the thin filaments in the center of the A-band. X 39,000
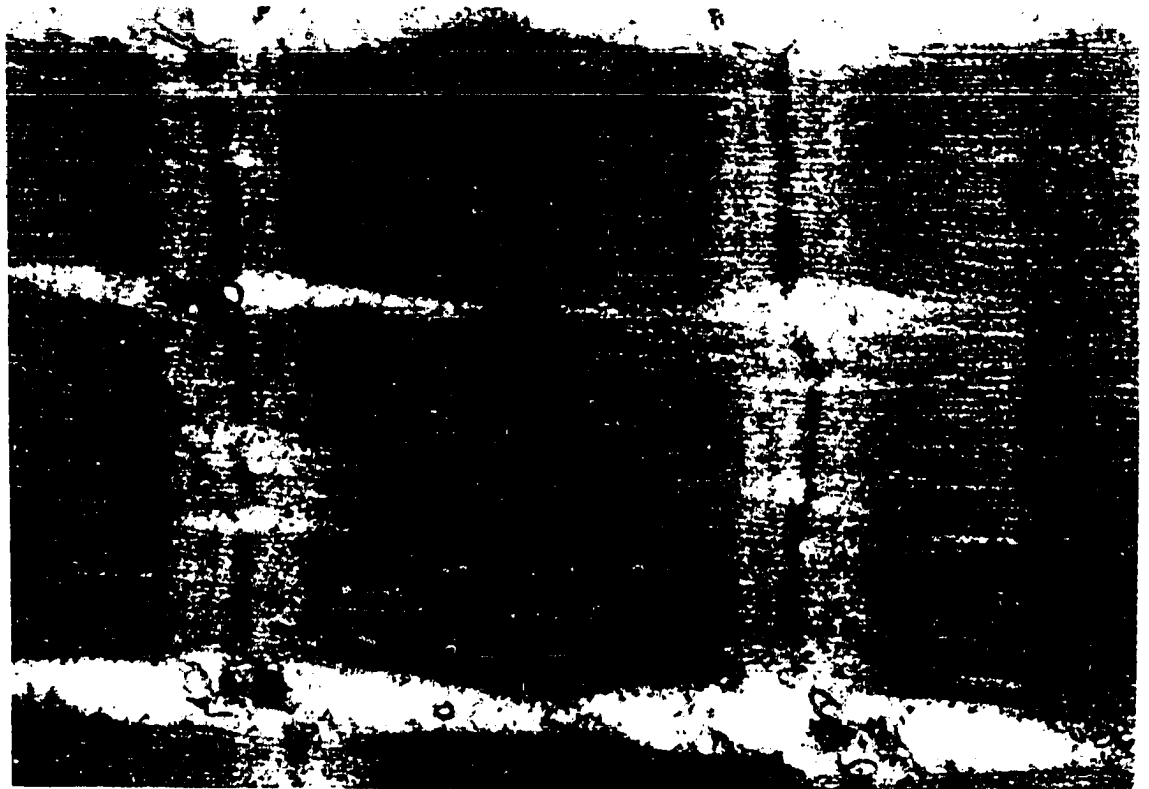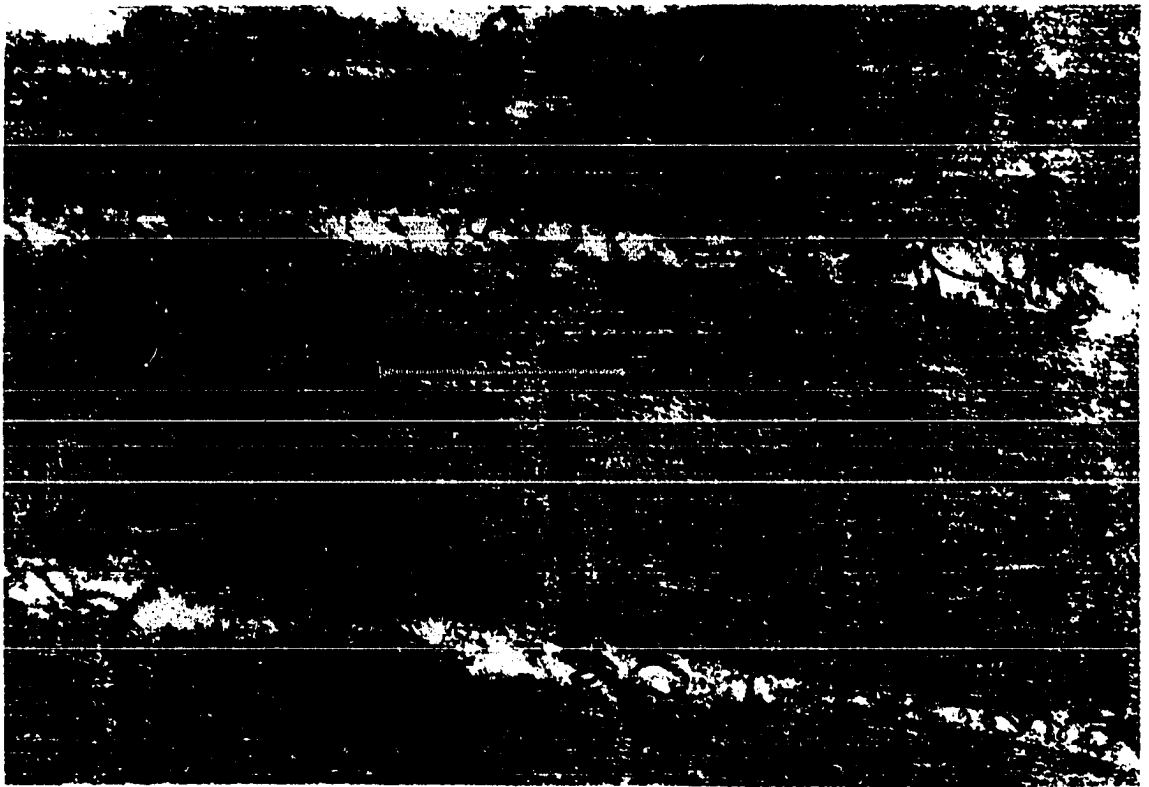
of thin filaments. It is also evident that the Z-line is
structurally intact after 24 hours storage at 16°. The effect
of 25° storage for 24 hours on porcine muscle is shown in Fig.
40. After 24 hours at 25°, the Z-line structure has been
completely disrupted and the M-line appears to be gone, sug-
gesting that these two structural entities in porcine muscle
are susceptible to degradation during storage at temperatures
above 16°. About 40% of the 25°-24 hour porcine sarcomeres
exhibited less extensive degradation than that shown in Fig.
40. These less degraded myofibrils exhibited M-lines and
remnants of a disrupted Z-line and resembled those observed in
bovine muscle stored at 25° for 24 hours (cf. Fig. 31). After
storage at 37° for 24 hours (Fig. 41) porcine myofibrils are
fragmented and the Z-line has been broken up. The overlap of
thin filaments in the center of the A-band (arrows) suggests
that shortening at 37° has occurred as a result of the sliding
of filaments. Figure 42 shows another sample of 37°-24 hour
porcine muscle depicting an area of extreme fragmentation and
disruption of the myofibrillar structure. The fragmentation
occurs principally at the level of the Z-line with some addi-
tional breaks occurring across the center of the A-band at the
level of the M-line. The dense granular material, possibly
representing "crystallized" Z-line material and previously
observed in 37°-8 hour porcine muscle, and in 37°-8 hour and
37°-24 hour bovine muscle, is again evident in the 37°-24 hour
porcine muscle.

Fig. 40. Porcine muscle sampled after 24 hrs. of
          storage at 25°. Note the absence of the
          M-line and also the highly fragmented
          Z-line. X 21,000

Fig. 41. Porcine muscle sampled after 24 hrs. of
          storage at 37°. Arrows indicate the
          overlap of thin filaments in the center
          of the A-band suggesting shortening has
          occurred by the sliding of filaments.
          Also note the areas of fragmentation and
          breaking of the sarcomeres. X 31,500
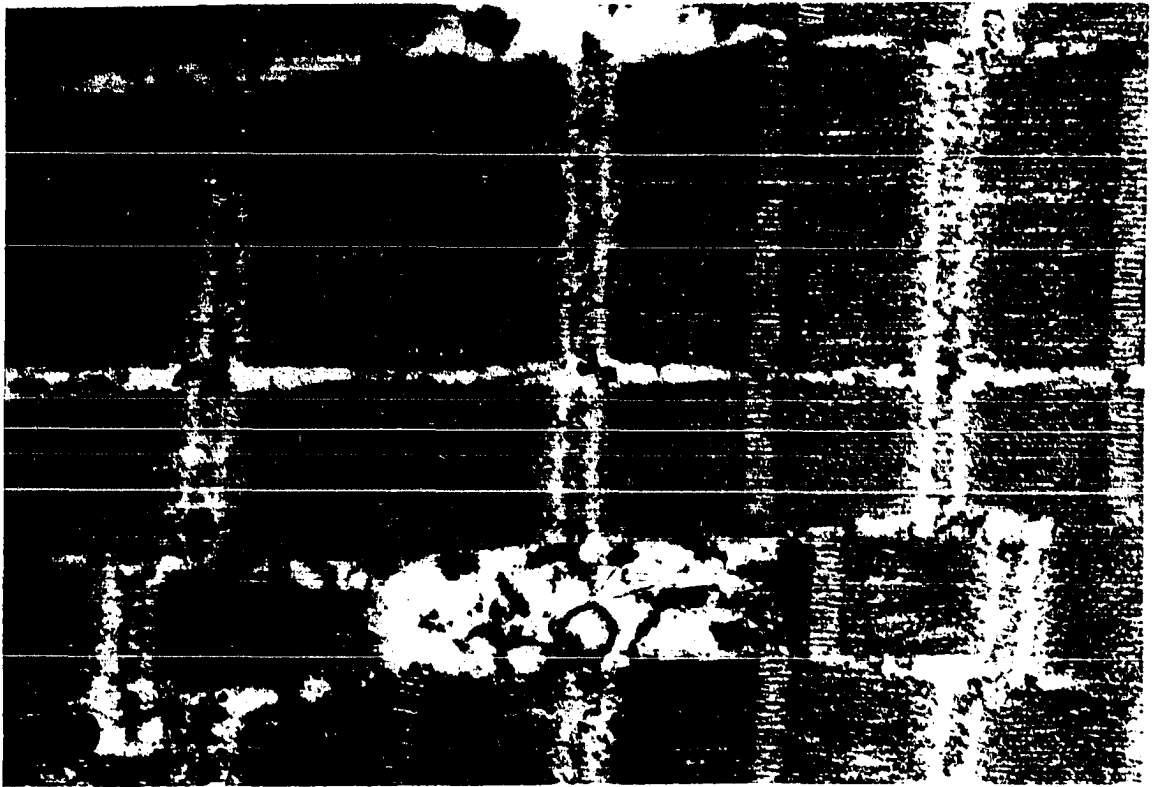
Fig. 42.   Another sample of porcine muscle
            sampled after 24 hrs. of storage
            at 37°. The sample shows a highly
            fragmented appearance with breaking
            occurring mostly at the level of
            the Z-line and in some cases also
            across the A-band. X 33,000

Fig. 43.   At-death rabbit muscle exhibiting the
            typical relaxed banding pattern. Note
            the very wide H-zone in comparison to
            at-death bovine or porcine muscle.
            X 33,000

42
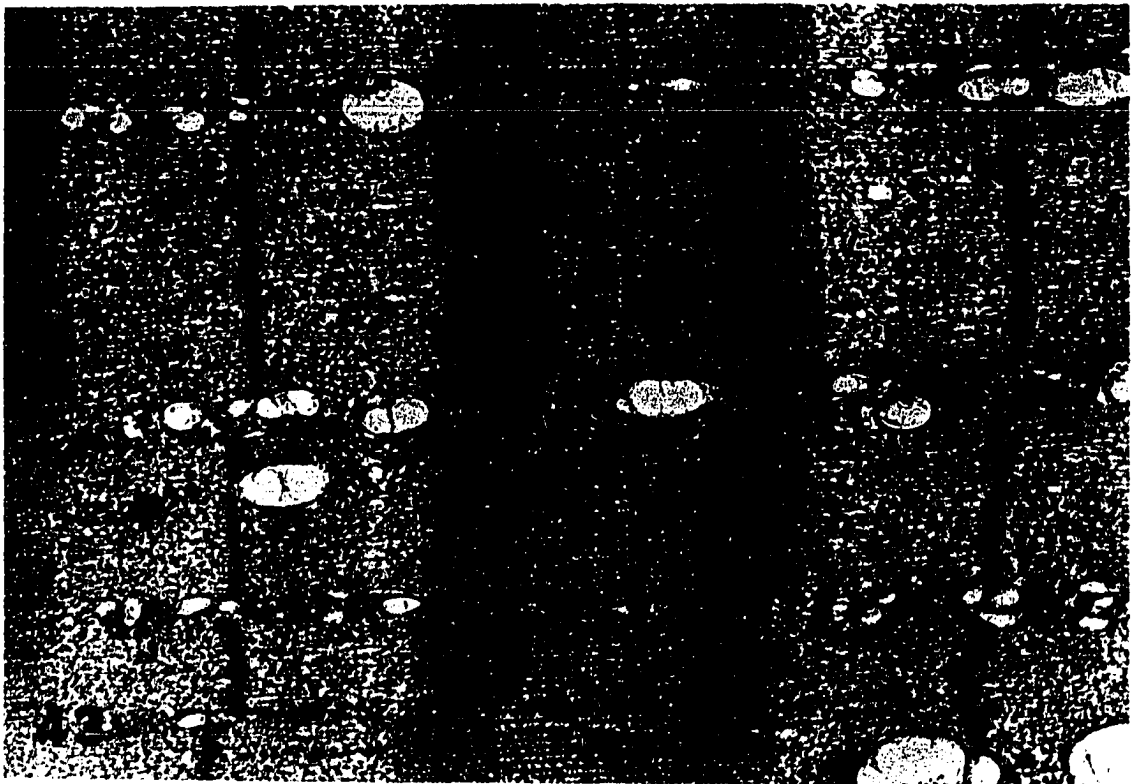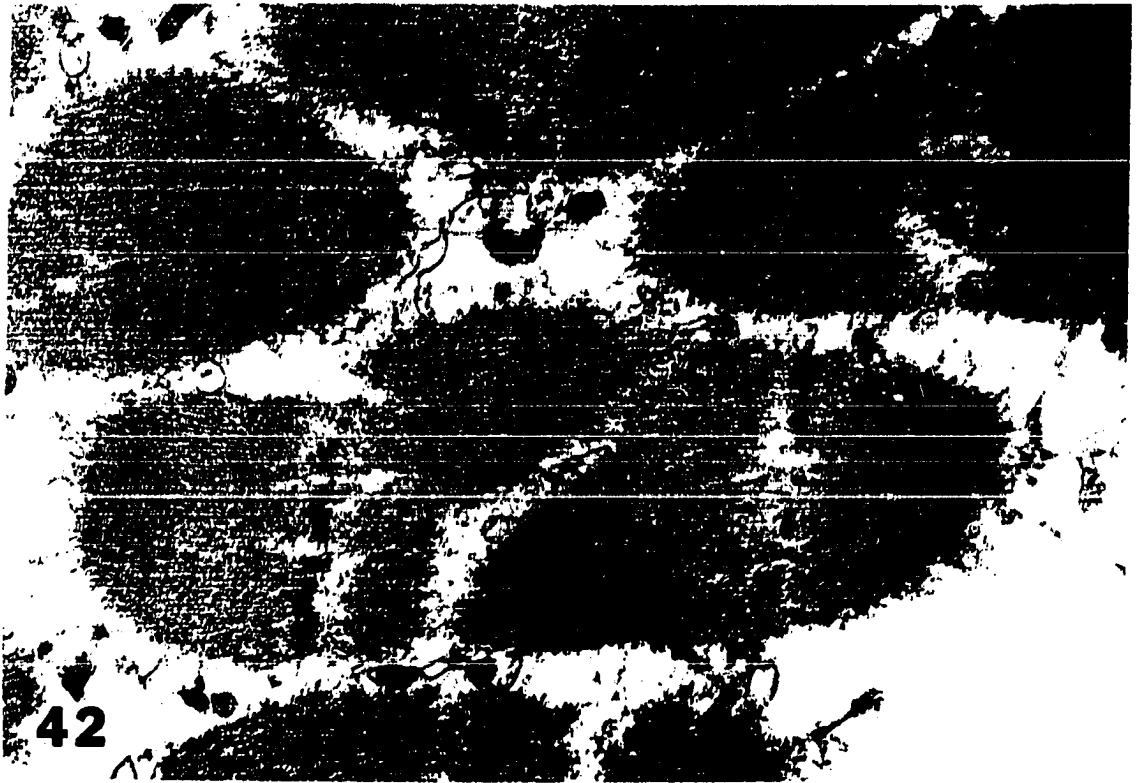
Rabbit muscle

Rabbit longissimus dorsi muscle sampled at death (Fig. 43) exhibited an ultrastructural pattern similar to that observed for either at-death bovine or at-death porcine muscle. However, it is apparent that the H-zone of at-death rabbit muscle is much wider than that observed for either of the other two species. After storage at 2° for 4 hours (Fig. 44) the H-zone in rabbit muscle was narrower than that observed immediately after death, suggesting that some shortening has occurred. The inset (Fig. 44) shows that the reported zigzag configuration of the Z-line is still intact after 4 hours at 2°. Storage of rabbit muscle at 37° for 4 hours (Fig. 45) results in substantially shortened myofibrils which are structurally degraded. As was seen also in 37°-4 hour porcine muscle, the Z-line of 37°-4 hour rabbit muscle is fragmented and broken. In addition, the sarcoplasmic organelles of 37°-4 hour rabbit muscle appear severely disrupted; the mitochondrial cristae are gone and there is considerable degradation of the sarcoplasmic reticulum membranes.

The structure of 2°-8 hour rabbit muscle is very similar to that seen in 2°-4 hour rabbit muscle with the exception that the thin filaments now pass into the center of the Z-band masking the H-zone (Fig. 46). The mitochondria and sarcoplasmic reticulum were structurally well preserved but part of the zigzag configuration has been lost. The structure of 37°-8

hour rabbit muscle is shown in Fig. 47. A marked shortening
has occurred with disappearance of the I-band and formation of
a dense region at the level of the Z-line. The Z-line is very
diffuse and broken up and the thick filaments in this area
appear to bend back on themselves or to overlap; this causes
the increased density in this region. The area of increased
density in the center of the A-band is probably caused by an
overlap of thin filaments from opposite ends of the sarcomere.
This overlap strongly suggests that shortening of rabbit muscle
at 37° also results from or is accompanied by sliding of thick
and thin filaments. In the center of this increased density
in the A-band is the M-line, bordered on either side by the
pseudo-H-zone. This banding pattern, seen in electron micro-
graphs, may explain the odd banding pattern observed by phase
microscopy of myofibril preparations from rabbit muscle stored
at 37°. During the several washes used for myofibril prepara-
tion, the disrupted Z-line may be washed out, causing formation
of a light zone where the Z-line once was. The two dark lines
observed in the center of the H-band of myofibrils may result
from the increased density due to the overlap of the thin
filaments in this area as observed in the electron microscope.
The electron micrographs indicate that the light zone seen in
the phase microscope between the two dark lines may originate
from the pseudo-H-zone and failure to resolve the M-line.

The ultrastructure of rabbit muscle stored at 2° for 24

Fig. 44. Rabbit muscle sampled after 4 hrs. of
         storage at 2°. X 31,000 Inset is a
         section of the Z-line showing that the
         zigzag configuration is still intact.
         X 91,000

Fig. 45. Rabbit muscle sampled after 4 hrs. of
         storage at 37°. The Z-line is highly
         disorganized and the myofibrils are
         substantially shortened with the thick
         filaments butting up against the
         fragmented Z-line. X 55,000
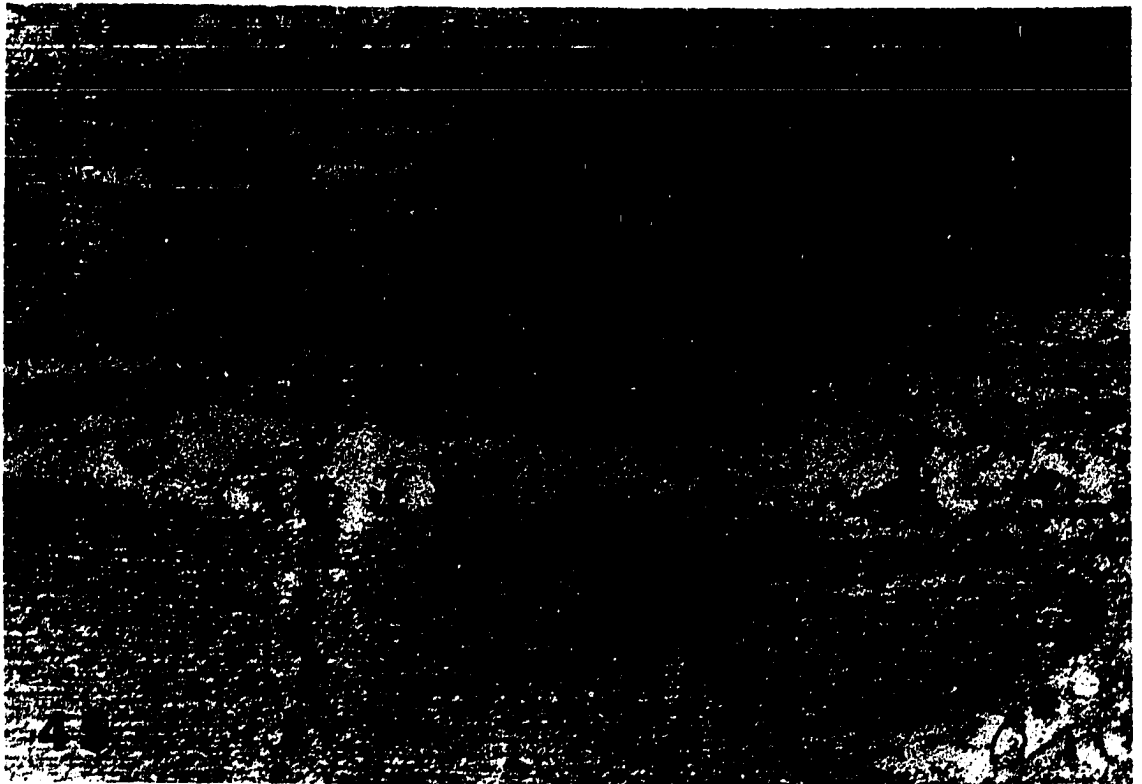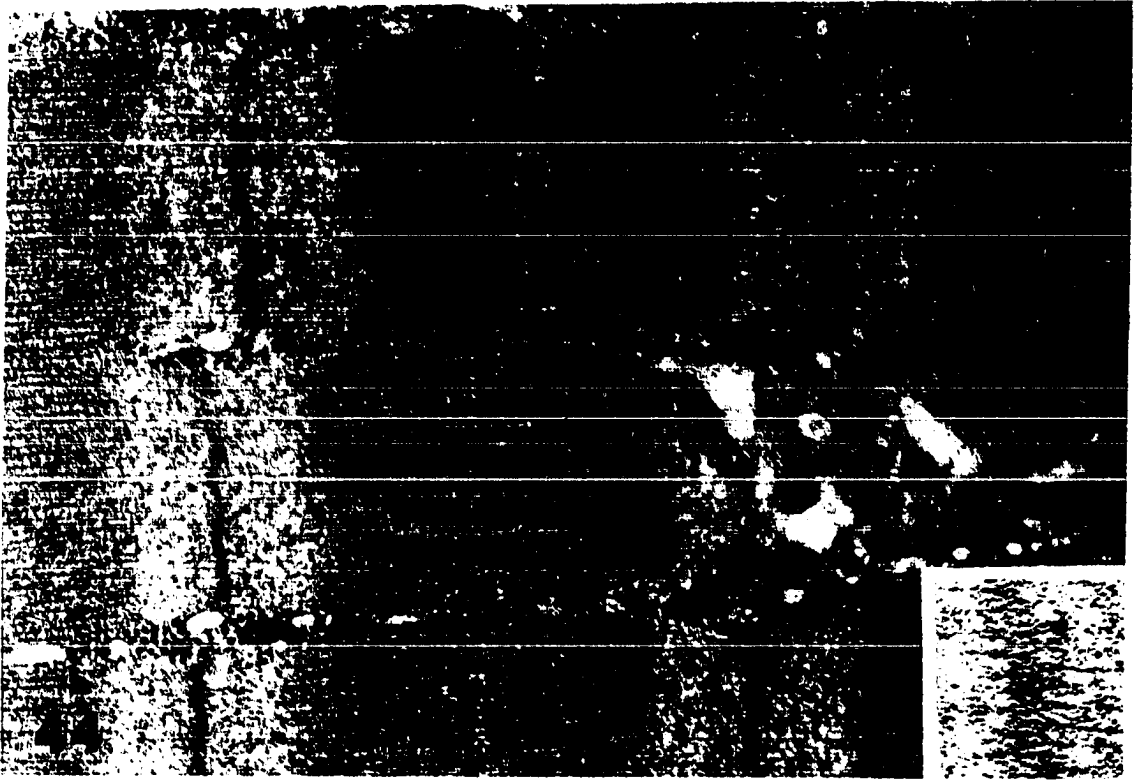
Fig. 46. Rabbit muscle sampled after 8 hrs. of
          storage at 2°. This muscle exhibits
          a relatively wide I-band but the H-
          zone is no longer evident. X 38,000

Fig. 47. Rabbit muscle sampled after 8 hrs. of
          storage at 37°. This muscle exhibits
          severely shortened myofibrils with
          thick filaments crumpling at the level
          of the Z-line. The Z-line is either
          gone or masked by the thick filaments.
          Also, note the overlap of the thin
          filaments in the center of the
          sarcomere. X 44,500
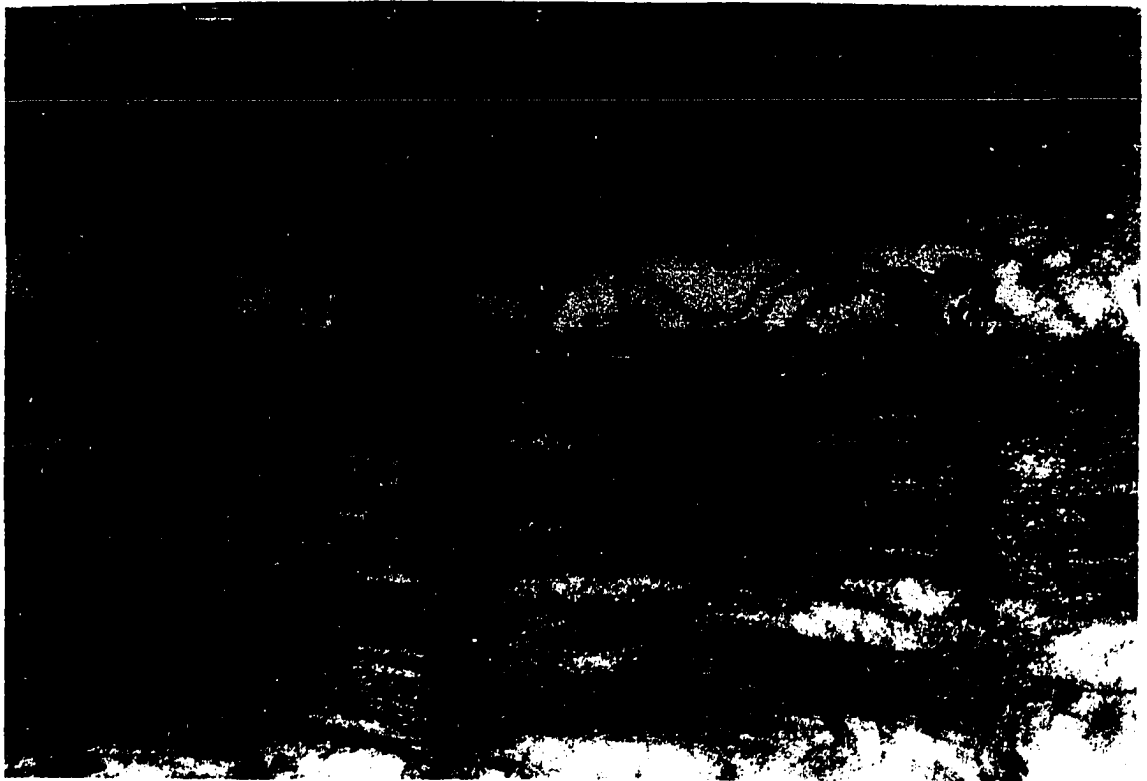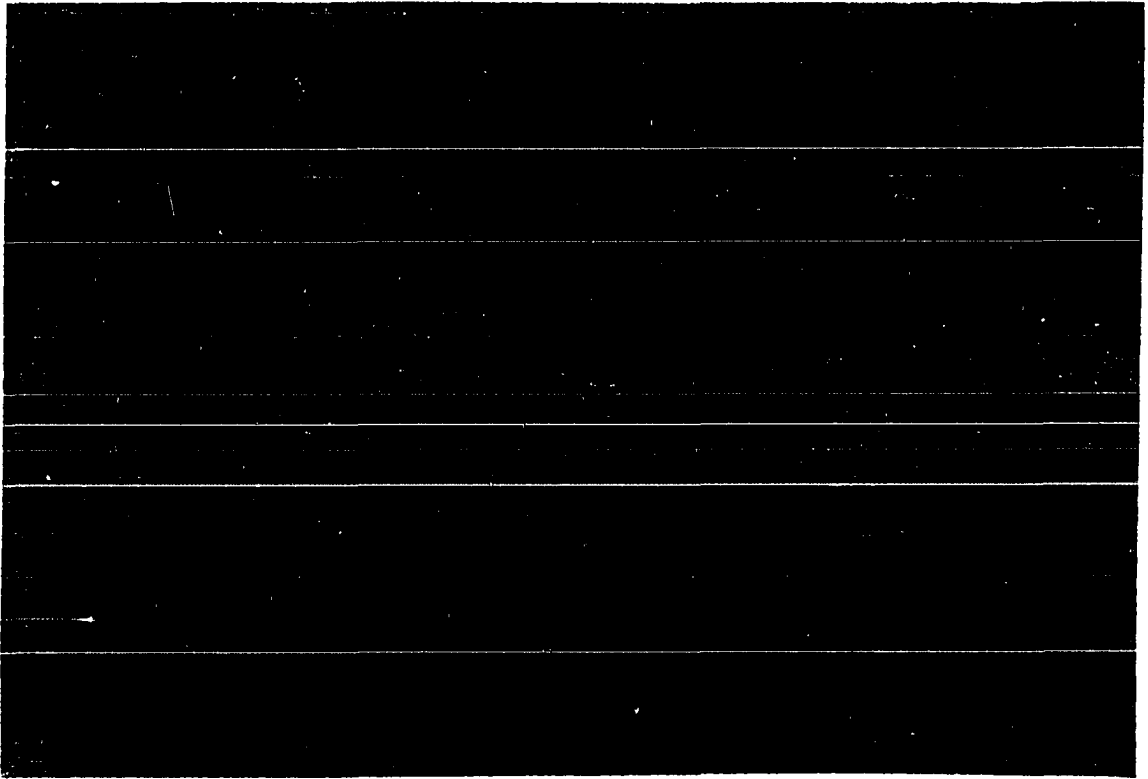
hours shows a very definite overlap of thin filaments at the
center of the A-band (Fig. 48) strongly suggesting that an
appreciable amount of shortening occurs in rabbit muscle stored
at 2°. As evidenced by the presence of thick filaments butting
up against the Z-line, more shortening occurs in muscle stored
for 24 hours at 16° (Fig. 49) than in muscle stored for 24
hours at 2°. The mitochondria and sarcoplasmic reticulum are
completely disrupted. After post-mortem storage at 25° for
24 hours (Fig. 50), the myofibrils are shortened quite severely
with the ends of the thick filaments in contact with the Z-line.
The Z-line has lost much of its structural integrity after 24
hours at 25°, and appears diffuse and disrupted in the electron
microscope (Fig. 50). Figure 51 shows an example of 25°-24 hour
rabbit muscle which has undergone severe contraction. This
type of appearance was seen several times in the 25°-24 hours
myofibrils. The sarcomeres are extremely shortened (1.1μ) and
the Z-lines are completely absent. The thick filaments of one
sarcomere overlap with the thick filaments of the next sarcomere
to give the appearance of a dark band in the region formerly
occupied by the Z-line. One very interesting structure ap-
pearing in this severely disrupted 25°-24 hour muscle is a
lattice network (arrows) which resembles the reported structure
seen in tropomyosin crystals. This observation suggests that
the 25° temperature together with the acid pH in post-mortem
muscle may have dislodged tropomyosin from its normal location

Fig. 48.   Rabbit muscle sampled after 24 hrs. of
           storage at 2°.   This muscle exhibits a
           shortened appearance with narrow I-bands
           and the presence of overlap of the thin
           filaments in the center of the sarcomere.
           X 39,000

Fig. 49.   Rabbit muscle sampled after 24 hrs. of
           storage at 16°.   The thick filaments
           extend from one Z-line to the next
           indicating substantial shortening at
           this post-mortem storage temperature.
           X 31,500
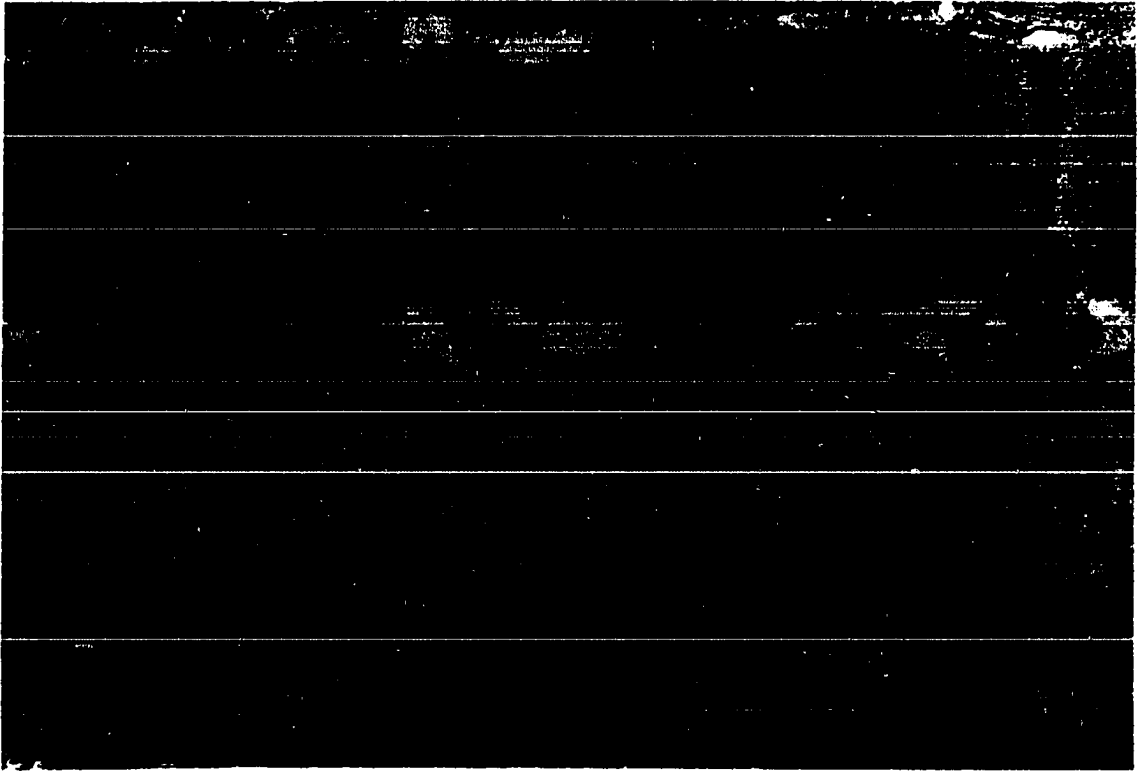
Fig. 50. Rabbit muscle sampled after 24 hrs. of
          storage at 25°. This sample is highly
          contracted with the Z-line structure
          highly fragmented. Arrow indicates
          possible precipitated Z-line material.
          X 33,000

Fig. 51. Another sample of rabbit muscle after
          24 hrs. of storage at 25°. This sample
          shows a severely contracted region with
          the thick filaments of one sarcomere
          overlapping with the thick filaments
          of the adjacent sarcomere. Arrows
          indicate crystallized tropomyosin-like
          structures. X 29,000   Inset is a
          higher magnification of the crystalline
          tropomyosin-like structure. X 62,000
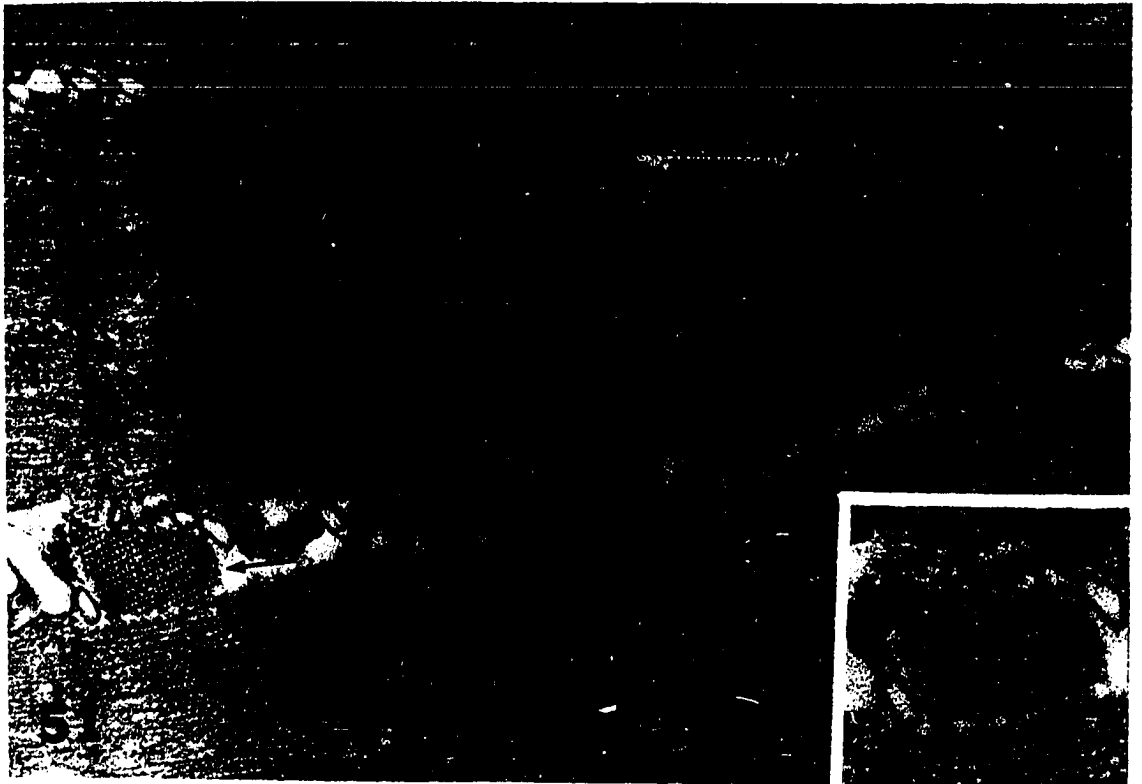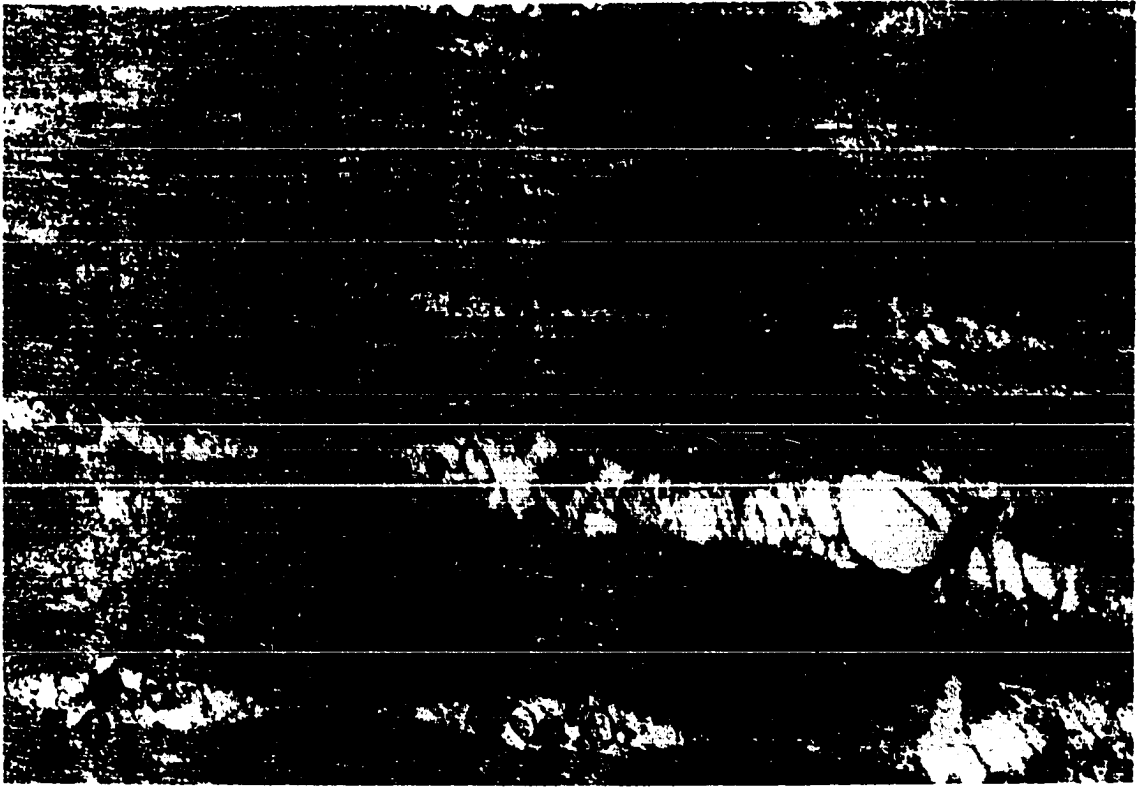
in the Z-line or thin filament and precipitated or crystallized it in the sarcoplasm surrounding the myofibril. These tropomyosin-like crystals can also be observed in Fig. 50 (arrow).

The structure of rabbit muscle stored at 37° for 24 hours is shown in Fig. 52. The Z-lines are totally disrupted and are no longer visible. Thick filaments, bending back on themselves or overlapping with filaments from the adjacent sarcomere are now evident in the area once occupied by the Z-line. The granular material observed in the sarcoplasm of 37°-24 hour rabbit muscle (arrows) may be crystallized tropomyosin but sectioned in a plane different from that shown in Fig. 51. Also, the M-lines observed in the other samples of 24 hour post-mortem rabbit muscle are absent after storage at 37° for 24 hours. The absence of the M-line and the overlap of the thin filaments in this area may cause the unusual phase banding previously described for 37°-24 hour myofibrils (cf. Fig. 17i). This same phase pattern was also seen in 37°-8 hour rabbit myo-fibrils (Fig. 17e) and it is significant that electron micro-graphs of 37°-8 hour rabbit muscle resemble those of the 37°-24 hour muscle. An explanation of the possible relationship between the absence of the M-line and the unusual phase banding pattern of 37°-8 hour and 37°-24 hour myofibrils has already been presented in the discussion of the 37°-8 hour electron micrographs.

Fig. 52. Rabbit muscle sampled after 24 hrs. of storage at 37°. Note the crumpling of the thick filaments at the level of the Z-line and the complete absence of the M-line. Arrows point out dense granular material that may represent precipitated Z-line material. X 44,000
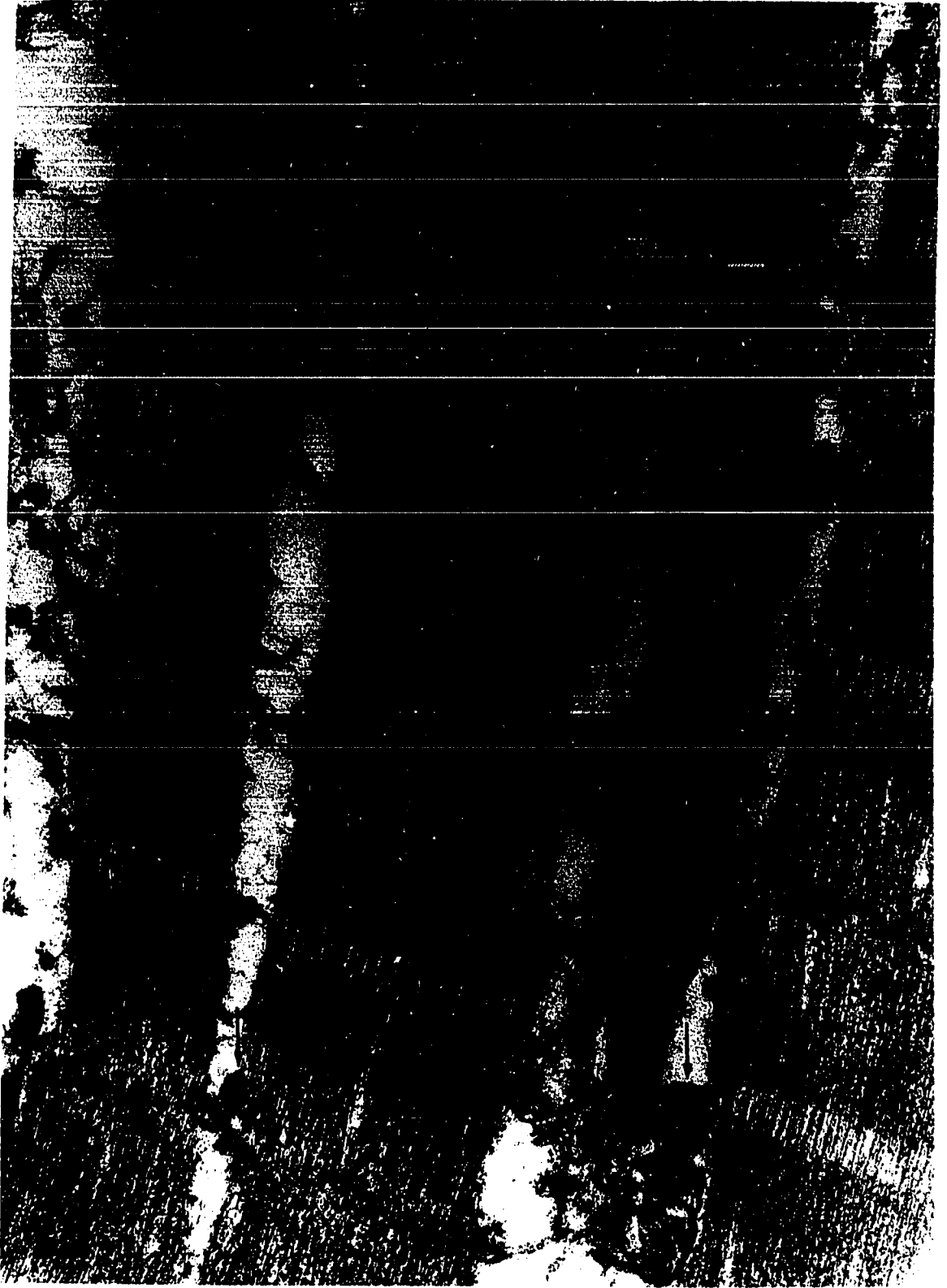
DISCUSSION

The purpose of this study was threefold: 1) to ascertain whether isolated myofibrils accurately represent the in situ structure of muscle, 2) to determine what structural changes take place in muscle during post-mortem storage at different temperatures, and 3) to compare the post-mortem structural changes of bovine, porcine and rabbit muscle. The discussion of the results of this investigation will be divided into six parts: 1) isolation of myofibrils, 2) structure of at-death muscle, 3) post-mortem storage at 2°, 4) post-mortem storage at 37°, 5) post-mortem storage at 16° and 25° and 6) comparison of myofibril structure with the structure of sectioned muscle.

Isolation of Myofibrils

The use of 0.25 M sucrose as the initial extractant has been reported to give better structural preservation of myofibrils than use of either 0.15 M KCl or 50% glycerol (Stromer and Goll 1967a). Slight modifications of the sucrose isolation procedure as it was described by Stromer and Goll (1967a) were studied to determine whether even a better structural preservation could be achieved. The effect of buffered or unbuffered wash solutions were investigated to ascertain if structural integrity of the myofibrils was affected by using unbuffered KCl wash solutions. Also, to prevent additional shortening due

to cutting and mincing during the isolation procedure, various levels of EDTA in the initial sucrose suspensions were investigated to determine which EDTA level gave the best structural preservation.

It has been known for some time that Tris buffer interferes with the biuret method of determining protein concentration. It was therefore of some value to know whether the solutions used for washing and eventual final suspension of myofibrils needed to be buffered for best preservation of the myofibril structure. It was found that porcine myofibrils isolated either in the presence or absence of Tris were identical in structure regardless of the time of post-mortem storage (at-death, 8 hours and 24 hours) at 2°.

Evidence has been reported that EDTA inhibits shortening during the onset of rigor mortis (Feinstein, 1966; Weiner and Pearson, 1966). This inhibition is due to chelation of calcium which is thought to be the ion that triggers muscle contraction and possibly also initiates rigor shortening (Nauss and Davies, 1966). Muscle obtained immediately after death of an animal is very easily stimulated by cutting or grinding. This stimulation causes severe shortening of the myofibrils and makes it impossible to determine how much post-mortem muscle will shorten past rest length. Therefore, it was necessary to find some agent which would prevent shortening of the muscle during stimulation by cutting or grinding. Because of its chelating

abilities, EDTA was chosen as such an agent. When myofibrils
were prepared from at-death, 2°-4 hour, or 2°-8 hour muscle
without any EDTA in the sucrose solution used for the initial
suspension, the myofibrils were almost entirely supercontracted.
When 1mM EDTA was used in the initial sucrose solution, 90% of
the myofibrils had a relaxed banding pattern and only 10% were
supercontracted. However, a great deal of variation in sar-
comere lengths was noted even those among myofibrils having a
relaxed banding pattern. Further analysis showed that A-band
lengths of 50% of the at-death myofibrils were considerably
shorter than normal. A-band shortening was also noted in 2°-
4 hour and 2°-8 hour muscle but the frequency of such short-
ening was lower in these muscles than in at-death muscle. It
appears that much of the variability, both in sarcomere length
and in the frequency of A-band shortening in at-death muscle
originates from the fact that membrane excitability and ATP
levels of this muscle are quite high. Thus, shearing or
grinding have a substantial excitatory effect on at-death
muscle. On the other hand, A-band and sarcomere length of
fixed and sectioned at-death muscle uniformly approximated
those of resting muscle. Sectioned muscle was tied to a glass
rod during fixation to prevent shortening. Raising the EDTA
levels of the sucrose solution used for initial suspension to 2
or 5mM did not product any further effects than those caused by

a 1mM level. When the myofibrils were prepared from 2°-24 hour muscle, no difference in structure could be detected regardless of whether EDTA was present. Also the presence and level of EDTA did not affect the structure of myofibrils isolated after 4, 8, and 24 hours of storage at 37° or after 24 hours of storage at 25°. These results strengthen the conclusion that the difficulties in accurately preserving the structure of at-death muscle were related to the membrane excitability or to ATP level of this muscle or both. Because of the results of this study 1mM EDTA was used in all subsequent preparations of myofibrils.

Structure of At-Death Muscle

Both at-death sectioned muscle and isolated myofibrils from bovine, porcine and rabbit muscle exhibited the typical relaxed banding pattern described by Huxley (1953b). Sarcomere lengths of isolated myofibrils were much shorter on the average than sarcomere lengths of sectioned muscle. This suggests that the isolation of myofibrils caused some sarcomere shortening. This shortening apparently occurs in a manner other than by the sliding of filaments because the H-zone was observed in 90% of the at-death myofibrils; the H-zone is the first structural element lost during contraction or shortening (Huxley and Hanson, 1954). As mentioned in the preceding paragraph, it was later discovered that the A-band was considerably shorter in isolated at-death myofibrils than in sectioned muscle at-death. This observation suggests that a major rearrangement of the thick

filaments has occurred as a result of the myofibril isolation procedure. Electron micrographs of bovine myofibrils and sectioned muscle (Stromer and Goll, 1967b) also show that the A-bands from isolated myofibrils exhibit much disorganization and shortening.

At-death rabbit muscle exhibited much wider H-zones when observed in the electron microscope than either at-death bovine or porcine muscle. The reason for this difference is not readily apparent and it may be an inherent difference between species.

Post-Mortem Changes at 2°

Post-mortem storage at 2° caused some shortening in all three species investigated in this study, but the shortening was far more extensive in bovine muscle than in either porcine or rabbit muscle. After 24 hours at 2° almost all bovine sarcomeres were supercontracted and had sarcomere lengths of 1.5μ or less. Porcine muscle had also shortened substantially after 24 hours at 2°; rabbit muscle was shortened the least after this time and temperature of post-mortem storage. However, contrary to the reports that post-mortem rabbit muscle does not shorten at 2°, the 2°-24 hour rabbit muscle did clearly undergo some shortening, with most of this shortening apparently occurring between 8 and 24 hours post-mortem. The 2°-24 hour bovine muscle not only shortened more extensively than rabbit and porcine muscle but also shortened sooner post-mortem.

Appreciable shortening was evident in bovine muscle already

after 4 hours at 2°. After 8 hours of post-mortem storage,

both bovine and porcine muscle had shortened extensively but

rabbit muscle had not yet undergone a large amount of shortening.

About 20 - 30% of the fibers in 2°-24 hour bovine muscle were

bent or kinked. Kinked fibers were not observed in either 2°-

24 hour porcine or rabbit muscle. This kinked structure has

previously been described in post-mortem bovine muscle by

Bendall (1960) and Newbold (1966) who both suggested that these

kinked fibers originated from some sarcomeres being depleted

of ATP without undergoing any shortening. The thick and thin

filaments in these depleted sarcomeres locked in the absence of

ATP and could not slide past one another. The shortening of

sarcomeres in adjacent fibers then forced these "locked"

sarcomeres to kink or bend without any sliding of filaments.

However, careful measurements of sarcomere lengths in the

kinked regions in this study clearly showed that these sar-

comeres had shortened considerably (1.5-1.75µ) although not to

the same extent as sarcomeres in adjacent fibers which were

straight and supercontracted. Thus, the results of this study

cast some doubt on the suggestions of Bendall and Newbold

although their hypothesis still cannot be finally rejected.

The zigzag structure of the Z-line was intact in all three

species after 8 hours of post-mortem storage at 2° and in

rabbit muscle after 24 hours of post-mortem storage. However,

the zigzag structure of the Z-line in 2°-24 hour bovine or

porcine muscle had started to deteriorate.

The results of this structural study substantiate the notion that both bovine (Locker and Hagyard, 1963) and porcine (Galloway and Goll, 1967) muscle exhibit the cold shortening effect, although cold shortening is not as extensive in porcine muscle as it is in bovine muscle. Unexpectedly, the structural results obtained in this study also clearly indicate that rabbit muscle shortens appreciably during post-mortem storage at 2°. Previous studies (Busch, 1966) have suggested that rabbit muscle does not develop any tension during post-mortem storage at 2°. However, based on the results of the present study, it now appears reasonable to suggest that muscle from all species exhibits the cold shortening effect, but that the strength and extent of this cold shortening varies among different species and possibly among different muscles within the same species. For example, all muscles studied in this investigation were unrestrained and the presence of a force so small as to be virtually undetectable by tension measurements could lead to an appreciable amount of shortening of an unrestrained fiber. The present study does not afford any clues for the origin of species differences in post-mortem shortening at 2°, nor indeed for the cause of cold shortening itself.

Post-Mortem Changes at 37°

Post-mortem storage at 37° causes a variety of structural changes in myofibrils. Already after 4 hours at 37°, the

membranous components of the cell, particularly the mitochondria and sarcoplasmic reticulum, appear structurally disrupted; after 8 hours at 37° these components are almost completely degraded. Most interesting, the zigzag structure of the Z-line is also markedly affected by post-mortem storage at 37°. The Z-lines in porcine and rabbit muscle appear to be the most labile, with the zigzag configuration in these Z-lines already gone after 4 hours at 37°, whereas the Z-lines in bovine muscle are still intact after this time of post-mortem storage at 37°. After 8 hours at 37°, the zigzag appearance is totally gone in all three species; this causes a disrupted and fragmented appearance in 37°-8 hour muscle. In many cases, the Z-line appears to be entirely missing from 37°-8 hour muscle. This occurs in all three species but is found most frequently in porcine muscle. After 24 hours of post-mortem storage at 37°, the myofibrils are even more highly fragmented than they were after 8 hours at 37°. Most of this fragmentation occurs at the level of the Z-line, probably as a result of disruption of the bonds between the Z-line and the thin filaments. The Z-lines of 37°-24 hour rabbit muscle are almost entirely missing, and a large percentage of the Z-lines are gone in 37°-24 hour porcine muscle; only a few of the Z-lines are missing from 37°-24 hour porcine muscle. The M-line also appears to be disrupted or missing after 24 hours at 37° and some fragmentation occurs at this site. This type of fragmentation is most noticeable in rabbit muscle, and the M-line in rabbit muscle also appears to be

more labile than the M-line in porcine or bovine muscle.

Considerable amounts of granular material were observed in the sarcoplasm surrounding the myofibrils of 37° muscle. In certain planes of sectioning, this dense granular material appeared structurally very similar to the Z-line and it may represent Z-line material that has been precipitated out and crystallized in the interfibrillar spaces. It is interesting that in situ conditions during post-mortem storage at 37° are ostensibly sufficiently rigorous to cause disruption and eventual removal of the Z-line, but that the Z-line material itself does not appear to be solubilized or extensively degraded under these same in situ conditions. The presence of crystallized Z-band material in the interfibrillar spaces of 37° muscle implies that it is the bond joining the Z-line to the thin filaments that is particularly labile whereas the Z-line material itself appears structurally intact after 24 hours at 37°.

Shortening at 37° occurs most rapidly in porcine and rabbit muscle. Already after 4 hours at 37°, post-mortem porcine and rabbit muscle are maximally shortened and no further shortening occurs in these species between 4 and 24 hours of post-mortem storage. Bovine muscle is only slightly shortened after 4 hours, but after 8 hours at 37°, bovine muscle is shortened to the same extent as porcine and rabbit muscle and no further shortening of 37° bovine muscle occurs between 8 and 24 hours post-mortem. Comparison with post-mortem shortening at 2° shows that both porcine and rabbit muscle shorten sooner

post-mortem at 37° than at 2°. After 4 hours post-mortem, bovine muscle was shortened to the same extent at either 2° or 37°, but after 8 hours post-mortem, bovine muscle was shortened much more extensively at 37° than at 2°. This suggests that maximal shortening occurs more rapidly at 37° than at 2°, this difference in rate of shortening being particularly marked for porcine and rabbit muscle. These ultrastructural results are in direct contradiction to the results of isometric tension studies (Busch et al., 1967; Galloway and Goll, 1967) which show that tension development at 2° occurs sooner post-mortem than tension development above 25°. This contradiction can be resolved if it is suggested that shortening at 37° initially develops very little tension; thus the initial phases of 37° shortening may easily be overlooked in isometric tension studies. Tension development during 37° shortening reaches a maximum only in the final stages of such shortening, after the cold shortening effect would already have begun. On the other hand, cold shortening, i.e. 2° shortening, is accompanied by considerable tension development even in its early stages. Thus, isometric tension experiments may detect the early phases of 2° shortening but may be sensitive to only the latter phases of 37° shortening. The ultrastructural results in this study were obtained on fiber bundles shortening under conditions of zero load; therefore these structural results cannot be directly related to isometric tension findings. Further studies are necessary before amount of isometric tension developed can be related to extent of shortening.

Post-Mortem Changes at 16° and 25°

After 24 hours of post-mortem storage at 16° and 25°, only a slight amount of shortening was observed in all three species. Although the extent of post-mortem shortening at either 16° or 25° varied slightly among the three species, for any one species, the amount of shortening at 16° was always very similar to the amount of shortening at 25°, as judged by either sarcomere lengths or banding patterns. Rabbit muscle appeared to be shortened slightly more than either bovine or porcine muscle, with the thick filaments of rabbit muscle coming into contact with the Z-line. After 24 hours of storage, bovine and porcine muscle had shortened less at either 16° or 25° than at 2° or 37°. This observation is in agreement with previous isometric tension (Busch et al., 1967; Galloway and Goll, 1967) and structural studies (Stromer et al., 1967a). On the other hand, rabbit muscle stored for 24 hours at either 16° or 25° had shortened slightly more than muscle stored for 24 hours at 2° but less than muscle stored for 24 hours at 37°. These findings are also in agreement with previous reports (Busch, 1966; Locker and Hagyard, 1963). The Z-line structure of 16°-24 hour muscle was well-preserved in all three species, whereas after 24 hours of post-mortem storage at 25°, Z-line structure was severely disrupted. This disruption was usually most severe in porcine muscle, and in some samples of 25°-24 hour porcine muscle, the

M-line was also missing. It was previously noted that the Z-line of either porcine or rabbit muscle seemed more susceptible to post-mortem degradation at 37° than the Z-line of bovine muscle; the results at 25° storage temperatures substantiate this suggestion and indicate that of the three species studied, porcine muscle may be the most sensitive to post-mortem storage at temperatures of 25° or higher.

A lattice structure, very similar to that reported for cross-sections through the Z-line (Huxley, 1963; Reedy, 1964) was occasionally observed in the interfibrillar spaces of sectioned 25°-24 hour rabbit muscle. This lattice structure is similar to that already described in 37° muscle after 8 or 24 hours of post-mortem storage, although the lattice network appears to be more clearly defined in 25° muscle than it was in 37° muscle. Part of this may be due to plane of sectioning but it is also possible that the 37° storage temperature causes some distortion in the Z-line lattice.

Comparison of Myofibril Structure with the Structure of Sectioned Muscle

Myofibrils isolated from at-death muscle exhibited a relaxed banding pattern very similar to that seen in sectioned muscle. Moreover, the sarcomere and A-band lengths were much shorter in at-death myofibrils than in at-death sectioned muscle. Measurements showed that the shorter sarcomere lengths of at-death myofibrils could be attributed, for the most part, to shorter A-band lengths in these myofibrils, although

occasionally a myofibril was observed which had been supercontracted, presumably because of the stimulation caused by the homogenization process. Shortened A-bands were also observed in myofibril preparations from $2°$-4 hour and $2°$-8 hour muscle; however, the frequency of shortened A-bands decreased with increasing time of post-mortem storage. These findings suggest that homogenization while the muscle membrane is still irritable or while ATP is still present in muscle causes considerable structural rearrangement in the A-band. Furthermore, it was noted that myofibrils from $2°$-4 hour and $2°$-8 hour muscle exhibited mostly relaxed banding patterns whereas sectioned $2°$-4 hour muscle was slightly contracted and $2°$-8 hour muscle was appreciably contracted. This result can most easily be explained by assuming that sufficient ATP remained in post-mortem muscle even after 8 hours at $2°$ to cause relaxation of the sarcomeres during homogenization in 1mM EDTA.

Myofibrils prepared from muscle stored at $37°$ for 4, 8, or 24 hours exhibit the same banding pattern and have the same sarcomere lengths as those for sectioned muscle sampled under corresponding conditions. Also, the banding pattern of $2°$-24 hour, $16°$-24 hour and $25°$-24 hour myofibrils closely resembles that of corresponding samples of sectioned muscle; however the sarcomere lengths of the $2°$-24 hour, $16°$-24 hour, and $25°$-24 hour myofibrils are slightly longer than the corresponding sarcomere lengths of sectioned muscle. This difference in sarcomere length may be due either to mechanical stress applied

to the muscle during the myofibril isolation procedure or to a slight shortening of the sectioned muscle as a result of the fixation, dehydration, and embedding processes. Page and Huxley (1963) have shown that muscle fixed in a restrained state, e.g. tied to a glass rod, does not contract, and more-over that dehydration by acetone does not cause any contraction. However, fixation in gluteraldehyde may cause 2-3% shortening of the I-filaments, but this very small amount of shortening is not sufficient to account for the observed difference in sar-comere lengths between sectioned muscle and myofibrils. It is unlikely that any other larger shrinkage effects exist since the 37°-24 hour muscle, which was taken through the same fixa-tion, dehydration, and embedding procedures as the other sam-ples of sectioned muscle, does not exhibit any apparent shrink-age and has sarcomere lengths very similar to those of isolated 37°-24 hour myofibrils. It therefore seems improbable that the shorter sarcomere lengths of the sectioned 2°-24 hour, 16°-24 hour and 25°-24 hour muscle can be explained by shrinkage of filaments alone. Instead, it appears more likely that the myofibril preparation procedure caused some alteration in sar-comere lengths of the myofibrils.

These results indicate that myofibrils do not always ac-curately reflect the in situ structure of muscle, even when precautions are taken to prevent myofibril shortening during homogenization. If EDTA is omitted from the homogenization medium, major differences result between the structure of

sectioned muscle and the structure of myofibrils isolated soon after death. Even in the presence of EDTA, at-death myofibrils do not accurately reflect the structure of sectioned muscle. Indeed, this difference in structure between myofibrils and sectioned muscle extends out to 8 hours post-mortem if the muscle is kept at 2° storage temperatures. Apparently the presence of even a small amount of ATP or a slight membrane irritability is sufficient to cause appreciable structural alterations in myofibrils during homogenization. The existence of small structural differences between myofibrils and sectioned muscle even after 24 hours of post-mortem storage at temperatures of 25° or lower indicates that myofibril structure may not accurately reflect the in situ state even when the homogenization is done in the absence of ATP. It is evident from these results that careful work on post-mortem muscle structure should be based on sectioned samples.

SUMMARY

Structural changes in bovine, porcine, and rabbit muscle during the onset of rigor mortis were studied by using phase and electron microscopy on myofibrils and sectioned muscle. Muscle samples were taken immediately after death, and the remaining portions of the excised muscles were then divided and samples stored at each of four temperatures; 2°, 16°, 25° and 37°. At 2° and 37° storage temperatures, samples were taken for electron microscopy and myofibril preparation after 4, 8 and 24 hours of post-mortem storage, whereas at 16° and 25°, samples were taken only after 24 hours of post-mortem storage.

Sectioned at-death muscle in all cases exhibited a relaxed banding pattern and had sarcomere lengths of 2.4-2.6μ. At-death rabbit muscle had much wider H-zones than either at-death bovine or at-death porcine muscle. Myofibrils isolated at-death were 90% relaxed as evidenced by their banding pattern in the phase microscope; however, sarcomere lengths of at-death myofibrils averaged about 1.8-1.9μ and some sarcomeres having a relaxed banding pattern exhibited sarcomere lengths of 1.5-1.75μ. It was found that the A-bands of many at-death myofibrils were only 0.75-1.0μ in length whereas A-band lengths in sectioned muscle averaged 1.25-1.5μ. Thus, shortened A-bands were apparently responsible for the shortened sarcomere lengths observed in those at-death myofibrils that still exhibited the relaxed banding pattern. After 4 hours of storage at 2°,

sectioned muscle from all three species exhibited a slight amount of shortening, with rabbit muscle shortened the least and bovine muscle shortened the most. Isolated myofibrils from 2°-4 hour muscle exhibited a relaxed banding pattern, but again sarcomere and A-band lengths were both shorter in myofibrils than in their sectioned counterparts. Storage at 37° for 4 hours resulted in porcine and rabbit muscle being extensively shortened whereas bovine muscle was only slightly shortened. The Z-line structure of porcine muscle, and also to a lesser degree, of rabbit muscle was disrupted and fragmented whereas bovine muscle was well preserved after 4 hours of post-mortem storage at 37°. Mitochondria and the sarcoplasmic reticulum from all three species were highly disrupted after 4 hours at 37°.

After 8 hours of storage at 2°, bovine and porcine muscle still exhibited more extensive shortening than rabbit muscle although all three species had apparently experienced some shortening between 4 and 8 hours post-mortem at 2°. The 2°-8 hour myofibrils again exhibited a relaxed banding pattern, but sarcomere and A-band lengths of the myofibrils were much shorter than those of the corresponding sectioned muscle. After 8 hours of storage at 37°, muscle from all three species was shortened to the same degree; 37°-8 hour sectioned muscle exhibited the same banding pattern and had very close to the same sarcomere lengths as those observed for isolated 37°-8 hour myofibrils. The Z-lines of 37°-8 hour muscle from all three species were

extremely fragmented, and the thick and thin filaments had lost their ordered array. Porcine myofibrils did not possess Z-lines although Z-line material was present in most of the sarcomeres in the porcine sectioned samples. However, even in the sectioned samples, the Z-line was extensively disrupted and it seems likely that the attachments between the Z-lines and the thin filaments are broken in porcine muscle after 8 hours at 37°. As a result, the Z-line is readily removed from myofibrils during washing.

Storage at 2° for 24 hours resulted in extensive super-contraction of bovine muscle, this contraction proceeding to sarcomere lengths less than 1.5μ. Rabbit muscle on the other hand was only slightly shortened to sarcomere lengths of 1.7μ; porcine muscle exhibited an intermediate amount of shortening (average sarcomere length of 1.6μ). Myofibrils isolated from each species after this time and temperature of post-mortem storage were structurally very similar to corresponding samples of sectioned muscle. After 24 hours at storage temperatures of 16° and 25°, bovine and porcine muscle was shortened less than after 24 hours at 2°, but rabbit muscle exhibited slightly more shortening at 16° or 25° than at 2°. The structure of 16°-24 hour muscle was well preserved in all species with the Z-line mostly intact. At 25°, however, the Z-line was disrupted and evidence is presented that after 24 hours at 25°, some Z-line material has been precipitated or crystallized in inter-fibrillar species in the sarcoplasm surrounding the myofibrils.

After 24 hours at 37°, myofibrils and sectioned muscle from all three species exhibited the same banding pattern and were shortened to approximately the same degree. No additional shortening of the sarcomeres occurred in any of the species between 8 and 24 hours post-mortem at 37°, but the myofibrils were more disrupted and highly fragmented after 24 hours at 37° than after 8 hours at 37°. Z-lines were frequently missing in rabbit or porcine sectioned muscle after 24 hours at 37° and were almost always absent in the 37°-24 hour porcine or rabbit myofibrils. Bovine Z-lines appeared more resistant and were usually present in both sectioned muscle and in myofibrils. Even when the Z-line was present, however, it had obviously undergone much structural deterioration and its normal zigzag structure was completely gone.

The results of this study show that regardless of storage temperature, post-mortem shortening occurs as a result of the sliding of thick and thin filaments past one another, in a manner analogous to that of contracting muscle. It is also clear from the results of this study that rabbit muscle undergoes an appreciable amount of post-mortem shortening at storage temperatures of 2°.

CONCLUSIONS

1. As measured by sarcomere lengths and changes in banding
    pattern, post-mortem shortening is similar in bovine and
    porcine muscle. Muscle from both species shortens maxi-
    mally during post-mortem storage at 2° and shortens slightly
    less during post-mortem storage at 37°. Minimum short-
    ening occurs at 16° and 25°. Rabbit muscle, on the other
    hand exhibits minimal post-mortem shortening at 2° with
    maximal shortening occurring at 37°. Rabbit muscle stored
    at 16° and 25° undergoes an intermediate amount of post-
    mortem shortening.

2. Post-mortem shortening occurs sooner post-mortem at 37°
    than at 2°. All of the post-mortem shortening of porcine
    and rabbit muscle at 37° occurs during the first 4 hours
    post-mortem and the post-mortem shortening of bovine muscle
    at 37° is completed after 8 hours. At 2°, maximal short-
    ening occurs between 8 and 24 hours post-mortem in all
    three species.

3. Electron micrographs clearly indicate that post-mortem
    shortening in all three species and at all four temperatures
    studied occurs by a sliding of thick and thin filaments
    past one another in a manner entirely analogous to the
    sliding filament theory of contraction. At any one stor-
    age temperature and for any one species, sarcomere lengths

appear quite uniform at the completion of post-mortem
shortening. This makes it unlikely that the kinked or
folded appearance sometimes observed in post-mortem muscle
arises from a locking of myofibrils at rest-length followed
by a folding of the locked myofibrils due to pressure from
adjacent, shortening fibers.

4. Post-mortem storage of muscle at 25° and 37° results in
    degradation of the Z-line. After 8 or 24 hours post-
    mortem, Z-line material can be observed in the sarcoplasm
    surrounding the myofibrils. The Z-lines in porcine muscle
    appear to be more susceptible to degradation at these
    temperatures than the Z-lines of either bovine or rabbit
    muscle. Storage temperatures of 25° or higher caused
    disorganization of thick and thin filaments and fragmen-
    tation of the myofibrils after 24 hours post-mortem.

5. Myofibrils isolated from at-death, 2°-4 hour and 2°-8 hour
    muscle exhibit unusual shortened A-bands and therefore, do
    not accurately reflect the structure of sectioned muscle
    sampled at the same times and temperatures. The structure
    of myofibrils isolated after storage at 37° for 4, 8 and
    24 hours, 2° for 24 hours, or 16° or 25° for 24 hours is
    very similar to the structure observed for sectioned muscle
    sampled at the corresponding times and temperatures.

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