# ULTRASTRUCTURAL CHANGES IN POSTMORTEM PORCINE MUSCLE

Thesis for the Degree of Ph. D.

MICHIGAN STATE UNIVERSITY
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1971

This is to certify that the

thesis entitled

ULTRASTRUCTURAL CHANGES IN POSTMORTEM

PORCINE MUSCLE

presented by

Thayne R. Dutson

has been accepted towards fulfillment
of the requirements for

Ph.D. degree in Food Science

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Date April 5, 1971

O-7639

ABSTRACT

ULTRASTRUCTURAL CHANGES IN POSTMORTEM

PORCINE MUSCLE

by Thayne R. Dutson

The primary objective of this study was to provide information on the ultrastructural changes that take place in postmortem porcine skeletal muscle tissue. Muscle samples were taken from 10 normal and 10 PSE pigs (pigs with pale, soft and exudative musculature) at 15 minutes and 24 hours postmortem for electron microscopic examination. In addition, samples were removed at 45 minutes postmortem for measurement of ion concentration by the electron microprobe in relaxed and contracted muscle. Aseptic samples were also taken from one pig, after which they were ground, inoculated and incubated with Pseudomonas fragi and examined with the electron microscope. Bacteria grown on non-muscle media were prepared and examined with the electron microscope, and compared with those in muscle.

The ultrastructural pattern for normal muscle at 15 minutes postmortem was characterized. Red, intermediate and white fibers were identified on the basis of similarities to these three fiber types in the rat. The width of the Z lines were approximately 1200, 775 and 625 Å for red, intermediate and white fibers, respectively. In addition, the Z lines were denser in red fibers and least dense in white fibers. Red fibers had a greater number of mitochondria, containing more abundant and more closely packed cristae, whereas, white fibers had the smallest number of mitochondria with less frequent and more open structured cristae. The intermediate fibers had more mitochondria than white fibers, but less than red fibers and their structural appearance was in between that for the other two fiber types.

In all fiber types, some mitochondria were located just beneath the sarcolemma, and others were paired at the Z line. However, only the red and intermediate fibers contained mitochondria in interfibrillar rows. The sarcoplasmic reticulum for red and intermediate fibers was tortuous and tubular in the region of the H-zone, whereas, it formed open tubules or flattened sac-like structures in white fibers. Connections of T tubules with the sarcolemma were also evident for all fiber types.

At 24 hours postmortem, the ultrastructure of normal porcine muscle was markedly altered as compared to that at 15 minutes postmortem. Loss of some Z line material was observed, and the mitochondria were considerably disrupted. The sarcoplasmic reticulum was extremely disrupted with no triads or transverse tubules being evident. However, the differences between fiber types were still easily discernible. There was less postmortem disruption in red fibers than in intermediate or white fibers.

Muscle from PSE pigs at 15 minutes postmortem was different from normal muscle and more variable in appearance. All three fiber types from PSE muscle at 15 minutes postmortem showed more disruption than normal muscle, but appeared similar to normal muscle at 24 hours postmortem. Although some fibers from each type were similar to normal muscle, the amount of disruption was generally less in red type fibers.

At 24 hours postmortem, PSE muscle showed more disruption than normal muscle, but the differences were not as great as at 15 minutes postmortem. PSE myofibrils exhibited a more granular appearance.

Cryostat sections gave better tissue preservation than freeze drying for analyzing pig muscle with the electron microprobe. Very little difference was found in the concentration of the various elements in different

areas of muscle tissue. There was, however, a slightly higher concentration of certain elements (particularly potassium) in perimysial connective tissue. Due to the high cost of microprobe analysis and only small differences in elemental concentrations, only initial samples were analyzed.

Pig muscle tissue inoculated and incubated with Pseudomonas fragi showed marked disruption of the myofibrils as compared to uninoculated incubated controls. After incubation, the inoculated samples showed almost complete absence of material in the H zone, marked disruption of the A band (probably myosin) and some loss of dense material from the Z line. These changes suggest that proteolysis had occurred.

Bacteria that were observed in spoiled muscle tissue exhibited protrusions or blebs on the outer surface of the cell walls. The blebs appeared to form globules that migrated into the muscle mass. Bacteria grown in non-muscle media did not produce these blebs, which indicates they were induced by growth on muscle tissue. It is postulated that the globules may contain a protease, which is released upon coming in contact with the muscle mass.

# ULTRASTRUCTURAL CHANGES IN POSTMORTEM PORCINE MUSCLE

By

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A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Department of Food Science and Human Nutrition

1971

ACKNOWLEDGEMENT

The author wishes to express his appreciation to his major professor, Dr. A. M. Pearson, for his guidance and support throughout the research program and for his assistance in the preparation of the thesis.

Appreciation is expressed to Dr. G. C. Spink for his instruction in electron microscopy and for serving on the guidance committee. Appreciation is also expressed to Dr. R. A. Merkel for serving on the guidance committee and for providing pig muscle for these studies. The author also wishes to thank the other members of his guidance committee Dr. R. A. Fennell and Dr. E. J. Benne.

Special thanks are expressed to fellow graduate students for their assistance and encouragement during the research program and to Darlene Vander Kolk for assisting in preparation of the photographs.

The author is especially grateful to his wife, Joyce, and son, Bradley, for their continuous understanding and encouragement.

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# INTRODUCTION

The chemical and physical changes that take place in pork muscle from the time of death until it reaches the consumer are extremely important in determination of meat quality. Pale, soft and exudative (PSE) muscle, the state of contraction and amount of microbial contamination all have a marked influence on the final product.

The PSE condition is characterized by rapid postmortem glycolysis, which results in abnormal accumulation of lactic acid and a low pH while the internal muscle temperature is still high. The effect of high temperature and low pH on muscle proteins appears to be the primary factor in producing PSE muscle. There also seems to be a difference in some of the ultrastructural components, such as the sarcoplasmic reticulum, between PSE and normal muscle (Greaser et al., 1969).

The state of muscle contraction at completion of rigor mortis has a marked effect on tenderness. The amount of magnesium ions, and particularly the amount of calcium ions present in the myofibril, influence the extent of contraction. Calcium is sequestered in the sarcoplasmic reticulum in relaxed muscle, and thus the sarcoplasmic reticulum plays a major role in determining the state of contraction.

Bacterial growth markedly affects consumer acceptability and furthermore is significant from the public health standpoint. More recently, it has been shown that bacterial growth may alter some of the major muscle proteins (Borton, 1970; Hasegawa et al., 1970; Tarrant et al., 1971).

Information is not available in the literature on the normal ultrastructure of pig muscle, particularly for the sarcoplasmic reticulum, mitochondria and other ultrastructural components at different postmortem times. Ultrastructural differences between red and white muscle fibers for the rat have been described by Gauthier (1970), but have not been well described in pig muscle.

The primary objective of this study was to provide information on the relationship of ultrastructural changes in pig muscle to meat quality. The major objectives were to be accomplished by:

(1) Establishing a normal ultrastructural pattern for pig muscle as compared to other species, including differences found between red and white muscle fibers at different postmortem times.

(2) Determining the ultrastructural differences and similarities between normal and PSE muscle.

(3) Measuring the amount and position of various ions in the muscle tissue during contraction and relaxation.

(4) Determining the ultrastructural changes that take place in muscle tissue during bacterial spoilage.

# LITERATURE REVIEW

## Structure of Skeletal Muscle

### Gross Structure

Although skeletal muscles are highly differentiated for performance of numerous types of movement, a basic structural pattern is common to all muscles (Lawrie, 1966). In reviewing the gross structure of skeletal muscle, Lawrie (1966) stated that muscle as a whole is surrounded by a sheath of connective tissue known as the epimysium. From the epimysium, septa of connective tissue penetrate into the muscle separating a number of fibers into bundles. The connective tissue around each bundle of fibers is called the perimysium. Lawrie (1966) also stated that a fine connective tissue framework passes inward from the perimysium, and finally forms a sheath around each individual muscle fiber. This sheath is called the endomysium.

Underneath the connective tissue of the endomysium are the individual muscle fibers, which are multinucleated cells having a diameter of 20 to 80 μ and a length of up to 47 cm in some muscles (Bendall, 1969). Within the sarcolemma (plasma membrane of the muscle cell) are the contractile elements of the muscle cell, the myofibrils, and the sarcoplasm, which includes mitochondria, fat droplets, the sarcoplasmic reticulum, transverse tubules, glycogen granules and components normally found in other cells (Bloom and Fawcett, 1962).

Myofibrils

The myofibrils are primarily made up of the proteins, myosin and actin, which are directly involved in the contraction process and are arranged together into thick and thin filaments (Bendall, 1969). The thick filaments, consisting of longitudinally oriented myosin molecules, give rise to the high density and anisotropic A bands. The thin filaments give rise to the less dense isotropic I bands and are primarily made up of aggregates of actin molecules arranged as double stranded chains (Bendall, 1969).

The thick and thin filaments are arranged into small longitudinally repeating compartments called sarcomeres, which account for the cross striated appearance of skeletal muscle (Bendall, 1969). Each sarcomere is made up of a Z line (which bifurcates the I band), one half of an I band (containing only thin filaments), an A band (containing an overlap of thick and thin filaments), an H zone in the center of the A band where only thick filaments are present, and another half of an I band (Bloom and Fawcett, 1962; Bendall, 1969). In addition to myosin and actin, other proteins found in the myofibril are troponin, tropomyosin and β-actinin, which are primarily located along the thin filaments, and α-actinin, which is located along the Z line (Maruyama and Ebashi, 1970). The thick and thin filaments slide past each other during contraction and relaxation, causing a decrease in the length of the sarcomere during contraction or an increase during relaxation (Bendall, 1969).

Sarcoplasmic Reticulum and Transverse Tubular System

The internal membrane system of muscle fibers consists of two rather distinct basic parts: (1) the sarcoplasmic reticulum (SR); and (2) the transverse tubular system (T system), which are predominantly composed of longitudinal and transverse elements, respectively (Sandow, 1970; Peachey, 1970; Hoyle, 1969). According to Sandow (1970) and Hoyle (1969), the exact nature of the SR and T system varies considerably between different species. Differences in the SR and T systems also exist between the various fiber types found in skeletal muscle (Gauthier, 1970; Sandow, 1970).

The T system is responsible for propogation of the action potential inwards to the innermost fibrils of the muscle cell, and thus conveys stimulation from the myoneural junction to the center of the muscle fiber (Bendall, 1969; Peachey, 1970; Sandow, 1970). The T system is derived from the sarcolemma during embryonic development (Ezerman and Ishikawa, 1967), and apparently retains its connection being virtually an inward extension of the sarcolemma (Bendall, 1969; Peachey, 1970).

The first demonstration in vertebrate skeletal muscle of the continuity between the sarcolemma and the T system was made by Franzini-Armstrong and Porter (1964) in fish muscle. They showed in photomicrographs that the T system has direct connections with the plasma membrane and is open to the external fluids. Direct observation of sarcolemmal invagination to form the T system has not been made in frog or mammalian skeletal muscle, but Huxley (1964) and Page (1964) found evidence that the T system

opens directly to the outside of the fiber by soaking muscle in ferritin before fixing. Ferritin is an electron-dense, iron-containing protein that can not penetrate the sarcolemma. Upon finding ferritin particles within the lumen of the T system, these authors concluded that the T system is directly continuous with the external portion of the muscle cell.

The sarcoplasmic reticulum (SR) is derived from the rough-surfaced endoplasmic reticulum during embryonic development (Ezerman and Ishikawa, 1967), and is thus an internal membrane system. According to Peachey (1970), the SR varies considerably as a result of physiological function and size of the muscle cell. He stated that the larger the diameter of a muscle cell and the faster its cycle of contraction and relaxation, the more extensively developed is the SR. This reflects the function of the SR, which is thought to involve the release of $Ca^{++}$ into the myofibrils during activation of contraction and its removal during relaxation (Hodgkin and Horowicz, 1960; Peachey and Porter, 1959; Peachey, 1966, 1970).

The membranous SR is made up of connections between cisternae and tubules that are organized in a repeating fashion along the sarcomeres, with one repeating unit per sarcomere in amphibian muscle and two repeating units per sarcomere in mammalian and some fish muscles (Peachey, 1970; Revel, 1964). The repeating SR units encircle each myofibril, are arranged end to end and extend the entire length of the myofibril (Peachey, 1965; Revel, 1964). At each end of the repeating SR units a dilated cisternae called the terminal cisternae is located (Porter and Palade, 1957; Peachey,

1965). These terminal cisternae are in very close apposition to the transverse tubules of the T system, with one terminal cisternae on either side of each transverse tubule.

The combination of a terminal cisternae, a transverse tubule and a terminal cisternae is called a triad, and is the probable site at which the excitation impulse is transferred from the T system to the SR (Sandow, 1970; Peachey, 1970; Bendall, 1969). In frog muscle, the triads are located at the area of the Z line, with the transverse tubule being located almost directly over the Z line and the terminal cisternae covering most of the I band on either side (Peachey, 1965). Arising from the terminal cisternae are longitudinal tubules of the SR that traverse laterally along the myofibrils in the region of the A bands (Peachey, 1965). Peachey (1965) also showed that the SR is in the from of a perforated sheet at the center of the A band, which he called the fenestrated collar. He postulated that the function of the fenestrated collar is to increase the surface area of the SR in this region, thus increasing its $Ca^{++}$ sequestering ability (Peachey, 1965, 1968, 1970).

In mammalian muscle, there are two triads per sarcomere, which are located at the junction of the A and I bands (Peachey, 1970; Gauthier, 1969, 1970; Revel, 1964; Pellegrino and Franzini, 1963). The terminal cisternae on one side of each triad (the side adjacent to the A band) leads into longitudinal tubules, which traverse laterally along the myofibrils in the region of the A band, connecting in the H band area with the longitudinal tubules and leading into the terminal cisternae on the

other end of the A band (Gauthier, 1969, 1970; Revel, 1964). The nature of the SR in the H band area seems to vary with fiber type and consists of an elaborate network of narrow tubules to flattened sacs, which in some cases are fenestrated (Gauthier, 1969).

The terminal cisternae on the other side of each triad (the side closest to the Z line) leads into longitudinal tubules that connect in the area of the Z line with longitudinal tubules arising from the terminal cisternae on the other side of the Z line (Gauthier, 1969, 1970; Revel, 1964). Literature reports do not include a description of the exact nature of the SR in the area of the Z line, but in most cases it seems to be somewhat similar to the SR in the H band area (Gauthier, 1969, 1970; Revel, 1964).

Other Components

The nuclei of muscle cells are located just under the sarcolemma, show a rather uniform chromatin content and have a prominent nucleoli (Bloom and Fawcett, 1962; Gori et al., 1967). Many mitochondria are present in most muscle cells, but the type, location and number of mitochondria vary with fiber type (Bloom and Fawcett, 1962; Gori et al., 1967; Gauthier, 1970). Glycogen granules, ribosomes, Golgi net and other organelles make up the rest of the sarcoplasm (Bloom and Fawcett, 1962; Gori et al., 1967). The amount of sarcoplasm present in muscle cells is apparently different for various fiber types (Bloom and Fawcett, 1962).

## Red, White and Intermediate Muscle Fibers

Differences that exist in the color of various skeletal muscles have been recognized for many years (Dubowitz, 1970). Some of the reasons for these differences, however, remained a mystery until histochemical and ultrastructural observations were made on the grossly and apparently red and white muscles (Padykula, 1952; Ogata and Mori, 1964; Pellegrino and Franzini, 1963; Padykula and Gauthier, 1963; Gauthier, 1970).

The development of histochemical techniques for determination of specific enzymes in tissues enabled researchers to differentiate between red and white muscle fibers on the basis of the activity of their metabolic enzymes (Dubowitz and Pearse, 1960; Stein and Padykula, 1962; Ogata and Mori, 1964). Dubowitz and Pearse (1960), on the basis of the reciprocal activity of phosphorylase and dehydrogenases in muscle fibers, suggested the division of muscle fibers into two types. They classified these fibers as type I and type II, with type I fibers being rich in dehydrogenases and weak in reaction for phosphorylase, while type II fibers were rich in phosphorylase and weak in reaction for dehydrogenases.

Stein and Padykula (1962) defined three fiber types (A, B and C) in the rat based on the activity of succinic dehydrogenase. The A type fibers were those that showed a weak reaction, the C type fibers were those that showed a strong reaction and the B type fibers were intermediate between the other two for succinic dehydrogenase activity. The A type fibers described by Stein and Padykula (1962) correspond to the type II fibers of Dubowitz and Pearse (1960), while the B and C type fibers together correspond to their type I fibers.

By correlating the relative activities of many different enzyme systems in muscle fibers, Romanul (1964) was able to recognize eight different fiber types. These eight fiber types could be divided into three groups, which corresponded to red, white and intermediate or A, B and C type fibers as described by Stein and Padykula (1962). Other workers (Gauthier, 1969, 1970; Nyström, 1968; Dubowitz, 1970) have stated that there are three major fiber types that can be recognized in mammalian skeletal muscle. The most descriptive and simple terminology (Gauthier, 1969) to be applied to these three fiber types is red (those fibers showing strong reactions for succinic dehydrogenase and other oxidative enzymes), white (those fibers low in oxidative enzyme activity but high in phosphorylase, glycogen and other glycolytic parameters) and intermediate (those fibers being intermediate in staining for succinic dehydrogenase and other oxidative enzymes). Gauthier (1969, 1970) states that although there are three distinct fiber types in mammalian skeletal muscle, both red and intermediate fibers contribute to the redness of intact muscle.

Analysis of mammalian skeletal muscle using the electron microscope has demonstrated that different fiber types can be distinguished on the basis of ultrastructural differences (Padykula and Gauthier, 1963; Pellegrino and Franzini, 1963; Gauthier and Padykula, 1966; Gauthier, 1969, 1970). Gauthier (1970) stated that three distinct types of skeletal muscle fibers (red, white and intermediate) can consistently be identified on the basis of mitochondrial content, width of the Z line and the structure of the sarcoplasmic reticulum. The red fibers have abundant closely

packed mitochondria beneath the sarcolemma and in rows between some myofibrils. The mitochondria in red fibers are dense with closely packed cristae. The intermediate fibers have a mitochondrial distribution somewhat the same as the red fibers, except that they are slightly fewer in number, less dense and have fewer cristae (Gauthier, 1969, 1970). In the white fibers of the rat, there are few if any mitochondria located beneath the sarcolemma or in interfibrillar rows, with the predominant type of mitochondria being those paired at the Z line (Gauthier, 1969, 1970).

The width of the Z line is one of the most distinguishing characteristics of the different fiber types (Gauthier, 1969). Red fibers have the widest Z line averaging 634 Å, with the white fibers having the thinnest Z line, averaging 339 Å. The intermediate fibers have a Z line width intermediate between the red and white fibers with an average of 433 Å, with the most striking difference being between the red and intermediate fibers.

According to Gauthier (1969, 1970), the form of the SR is also different between fiber types. This difference occurs at the region of the H zone, where the red fibers show a network of narrow longitudinal tubules and the white fibers exhibit a more compact arrangement of parallel tubules, which are sometimes expanded to form flattened sacs. These sacs appear fenestrated in some areas. Gauthier (1969, 1970) also stated that it is possible to recognize the three different fiber types at the ultrastructural level by the consistant differences existing in the Z line, mitochondria and the SR. Gauthier (1970) also stated that the cytological characteristics of a particular fiber type have been incorrectly attributed to some form of

experimental or pathological alteration because the differences existing between fiber types have not been fully considered or understood.

Many studies have been concerned with different fiber types in pig skeletal muscle (Beecher et al., 1965, 1968; Moody and Cassens, 1968; Cooper et al., 1969; Schmidt et al., 1970). These studies, which are primarily concerned with histochemical and biochemical differences between fiber types, have shown that the various fiber types in pig skeletal muscle are similar to those found in other mammalian muscles. Muir (1970) reported a difference in the mitochondrial content of red and white muscle fibers in electron microscope sections of pig muscle, but made no mention of other differences that are known to occur between red and white muscle fibers. No other reports on pig muscle were found in the literature describing the differences between red, white and intermediate fibers on an ultrastructural level.

## Postmortem Changes In Muscle Ultrastructure

Lawrie (1966) has stated that stoppage of blood circulation at the time of death initiates a complex series of changes in muscular tissue, which include loss of the oxygen supply, failure of nervous and hormonal regulation and destruction of osmotic equilibrium. These changes cause a chain reaction that results in many physical and chemical alterations in muscle tissue (Lawrie, 1966; Cassens, 1966; Goll et al., 1970).

### Pale, Soft and Exudative Muscle

When oxygen is depleted by the tissues after blood flow ceases at death, adenosine triphosphate (ATP) can no longer be produced by oxidative

phosphorylation. Replenishment of ATP is then taken over by postmortem glycolysis, which occurs much more rapidly in some cases than others (Briskey, 1964; Cassens, 1966; Lawrie, 1966). Rapid postmortem glycolysis results in an accumulation of lactic acid and a reduced pH (Briskey, 1964). In pig muscles where rapid glycolysis occurs at a high temperature, meat quality is diminished (Briskey, 1964; Cassens, 1966). Pig muscles showing low quality due to rapid glycolysis following death are pale in color, soft in texture, exude water and have been described as pale, soft and exudative (PSE) by Briskey (1964).

Using the electron microscope, Cassens et al. (1963) observed normal muscle and muscle from pigs induced to become PSE by subjecting them to high antemortem temperatures (42-45°C). They found that normal muscle showed gradual distruption of certain sarcoplasmic components during the first 24 hours postmortem with no marked alteration of the myofibrils. Postmortem disruption of the sarcoplasmic components occurred in the PSE muscle at a more rapid rate than for normal muscle. The myofilaments of PSE muscle also had a more granular appearance than normal samples. Greaser et al. (1969) also using the electron microscope studied the myofibrillar fractions from PSE and normal muscle at 0 and 24 hours postmortem. They found no differences between PSE and normal myofibrils in the 0 hour samples, but a marked difference was found at 24 hours. The PSE fractions had wider Z lines, and a more granular appearance of the myofilaments.

Greaser and coworkers (1969a, b, c, d) observed the postmortem changes occurring in the SR of pig muscle. They found that the calcium accumulating

ability of the SR fragments decreased with time postmortem, and that SR fragments from PSE muscle had less calcium accumulating ability than similar fragments from normal muscle. These authors also stated that the difference in calcium accumulating ability between SR fragments from normal and PSE muscle were inherent, and probably did not reflect differences in the rate of postmortem change.

Koch et al. (1970a, b) have shown that the time of sampling after death also influences the rate of postmortem change. They reported that muscles excised at death had less glycogen and more lactic acid than companion muscles on the opposite side of the carcass that were not excised until 15 minutes postmortem. Thus, more glycolysis had taken place in zero hour samples due to excision than in similar samples excised at 15 minutes after death. These authors also found that myotomy at or near the time of death tended to decrease ultimate muscle quality.

Rigor Mortis

Another phenomenon that occurs in muscle tissue as a result of ATP breakdown is rigor mortis (Goll et al., 1970; Newbold, 1966; Stromer and Goll, 1967; Bendall, 1951). According to Goll et al. (1970), rigidity in rigor mortis arises from attempted shortening of muscles on opposing sides of the same bone. Isometric tension development in postmortem muscle is caused by the postmortem release of calcium from the SR (Goll et al., 1970). Goll et al. (1970) also stated that loss of extensibility, which keeps the muscle in its shortened state, is brought about by the disappearance of ATP and the irreversible formation of actomyosin bonds.

Lawrie (1966) attempted to relate meat tenderness to the number of actomyosin bonds formed during rigor mortis. He stated that muscles with a large actin and myosin filament overlap at the time of rigor completion were less tender than similar muscles with a smaller amount of overlap.

A difference in the time course of rigor mortis has been found for red and white muscles, with completion being established earlier in white than for red muscles (Beecher et al., 1965; Schmidt et al., 1970). Beecher et al. (1965) stated that sarcomere length of post-rigor muscle is altered by the extent of contraction during rigor mortis, which was given as a possible cause for the difference in sarcomere length between red and white muscles. Schmidt et al. (1970) have shown that muscles from stress-susceptible pigs have a shorter time course of rigor mortis than corresponding muscles from stress resistant pigs. However, they did not measure sarcomere length in this study.

Postmortem Aging

Goll et al. (1970), Stromer and Goll (1967a, b) and Stromer et al. (1967) have found at least two types of changes that take place in the myofibrils of skeletal muscle during postmortem storage. One change found was disruption and degradation of the Z line, while the other was a weakening of the actin-myosin interaction. Results reported by Goll et al. (1970) indicate that Z line degradation is caused by calcium being released when the SR loses its ability to accumulate calcium. The authors then stated that the cause of postmortem weakening of the actin-myosin interaction is not yet clear, but might be due to postmortem changes in actin and myosin themselves.

Davey and Dickson (1970) have also demonstrated that changes take place at the Z lines in postmortem skeletal muscle. They found a weakening of the myofibrillar structures at the junction of the I band and the Z line. They also concluded that the Z line undergoes progressive changes and loses ground substance as aging proceeds.

## Muscle Contraction

The normal sequence of events leading to contraction of muscle begins with depolarization of the sarcolemma and generation of an action potential, which is initiated by a nerve impulse at the myoneural junction (Adrian, 1970; Bendall, 1969). According to Bendall (1969), the action potential is transmitted to the internal parts of the muscle cell by the T system. Bendall (1969) also stated that from this point onward the exact chain of events is not known, except that the final result is the release of calcium ions, probably from their storehouse in the SR. Adrian (1970) stated that very little is known about the nature of the potential change across the transverse tubular wall during internal transmission of the action potential, and that even less is known about the way in which the change in potential causes release of calcium from the SR.

By using murexide, which changes color upon binding calcium, Jöbiss and O'Connor (1966) obtained direct evidence that calcium is released into the sarcoplasm when the action potential arrives at the triad region of the myofibrils. Combination of calcium with murexide was determined photometrically.

Using the murexide technique on isolated SR fragments, Nakamaru and Schwartz (1970) have shown that the calcium accumulating ability of the SR decreases as pH increases from 5.9 to 7.8. These authors also found that the SR fragments rapidly released accumulated calcium as the pH was raised from 6.6 to 7.5. They then suggested that intracellular changes in hydrogen ion concentrations might also result in calcium release in vivo. However, Bendall (1969) stated that the physiological resting pH of muscle is 7.4, a pH value, which according to Nakamaru and Schwartz (1970), would result in low calcium binding by the SR fragments.

Bendall (1969) concluded that even though many quantitative aspects of the calcium release into the myofibrils are uncertain, it is clear that mere inhibition of the calcium pump could not be the mechanism for calcium release, since calcium diffusion from the SR is not sufficiently rapid. When the stimulus for contraction reaches the SR, calcium ions are released to give a free concentration of about $10^{-5}$M, although a concentration of $5 \times 10^{-6}$M is probably sufficient for a maximal response. There is then an explosive activation of actomyosin adenosine triphosphatase (ATPase), which hydrolyzes ATP. Contraction takes place as the actin and myosin filaments slide by each other (Bendall, 1969).

Maruyama and Ebashi (1970) stated that troponin in the absence of calcium has a depressing effect on the interaction between actin and myosin. They found that troponin has a high affinity for binding calcium, which then no longer inhibits the interaction of actin and myosin. Consequently they suggested that the triggering action of calcium is accomplished by binding

to troponin, thus, allowing actin and myosin to interact. The splitting of ATP, when actin and myosin intereact, provides the energy for the thick and thin filaments to slide past each other, thereby, causing shortening (Bendall, 1969; Huxley, 1969; Gergely, 1970). The exact mechanism by which this is accomplished is not fully understood, but seems to involve a conformational change in the myosin molecule--either at the site of its interaction with actin or near one of its hinge regions (Bendall, 1969; Huxley, 1969; Gergely, 1970).

In order for the myofibrils to return to their resting condition, the free calcium ions must be reduced to a concentration of about $10^{-7}$M (Bendall, 1969). According to Bendall (1969), the removal of calcium from the myofibrils is accomplished by some part of the SR, probably the terminal cisternae.

In studying calcium uptake by isolated fragments of the SR, Hasselbach (1964) found that calcium uptake was dependent upon the splitting of ATP and the calcium concentration outside the vesicles. On the basis of the above information, the fact that certain concentrations of other components are needed for calcium uptake and an assumption that a calcium carrier molecule exists within the membrane, Hasselbach (1964) proposed the following sequential mechanism for calcium uptake: (1) A phosphoryl group is transferred from ATP to the carrier molecule at the outer surface of the membrane in the presence of calcium. (2) When the carrier is phosphorylated, its affinity for calcium is greatly increased and a calcium-carrier-phosphate complex is formed. (3) This complex diffuses to the inner surface of the membrane where it is dephosphorylated, causing a reduction in the affinity of the carrier for calcium. (4) Calcium is then liberated inside of the

vesicles in spite of the high calcium concentration in their interior.

Weber et al. (1966) have also proposed a mechanism for calcium uptake by SR vesicles. They suggested that active transport by the SR membrane occurs in somewhat the same manner as outlined by Hasselbach (1964). They proposed that two calcium molecules are bound to the membrane carrier for each ATP molecule hydrolyzed and that part of the calcium is bound to the inside of the vesicle membrane. They stated that a small amount of calcium may also be bound on the exterior of the vesicle membrane. Although Weber et al. (1966) did not rule out the possibility that ATP may be hydrolyzed on the internal vesicle membrane, they suggested that this is not likely since ATP and ADP must then diffuse rapidly across the membrane. They also included an explanation of the calcium flux across the membrane and attributed the outflow of calcium to diffusion.

Bendall (1969) stated that the mechanism for transport of ions through any biological membrane is a matter of speculation, and as yet, none of the postulated carriers have been isolated. He also stated that even though the mechanism of calcium transport proposed by Weber et al. (1966) clarifies some of the facts, it does not explain other observations. Bendall (1969) then proposed a possible mechanism for calcium accumulation in the SR, which involves ATP induced shape changes in the membrane protein molecule at the site of calcium binding. He stated that these proteins could turn themselves "inside out, or outside in" and thus carry the attached calcium with them and release it on the inside of the vesicles.

Muscle tissue can be caused to contract by stimuli other than those of a nervous type, which include electrical impulses, postmortem changes

and myotomy (Bendall, 1969; Goll et al., 1970; Koch et al., 1970a, b). Apparently the contraction mechanism is the same for these stimuli as for nervous impulses and is initiated by the release of calcium into the myofibrils (Bendall, 1969; Goll et al., 1970).

## Electron Microprobe X-ray Analysis

The electron microprobe x-ray analyzer and its use in studying elemental distribution in biological tissues has been described by Mellors (1964). He stated that the electron microprobe is a relatively new instrument, which gives precise nondestructive elemental chemical analysis in localized tissue areas.

The principles upon which this instrument operates have been described by Mellors (1964). A beam of electrons with high acceleration potential arising from an electron gun is focused on the flat surface of a conductive sample. Some energy from the incident beam is taken up by the atoms of the sample, which raises their energy state. On return to normal energy levels, the atoms emit x-rays whose wavelengths are characteristic of each particular atom and whose intensities are proportional to the concentration of that atom. The x-ray spectra arise from the removal and replacement of inner shell electrons in the atom and are affected very little by chemical combinations of the atoms.

According to Engel et al. (1968) the emitted x-rays can be sorted out by means of a number of curved crystal spectrographs, and the source element identified on the basis of its x-ray characteristics. These authors also stated that the electron microprobe is able to present electron scanning

displays of sample current and back scattered electrons, permitting the recording of micrographs which demonstrate the distribution of each element present, as well as the topography of the sample.

The electron microprobe x-ray analyzer was originally used for analysis of metals. As new applications appeared in the literature, whole new fields of research opened up in chemistry, solid state physics, biology and other areas (Birks, 1963).

Studies have been conducted using the electron microprobe to determine the distribution of various elements in animal tissues, such as copper in Wilson's disease of the cornea (Tousimis and Adler, 1963), calcium and phosphorus in human cortical bone (Mellors, 1964) and sodium deposits in the lamina propria of mouse vas deferens (Lane and Martin, 1969).

In studying the chick chorioallantoic membrane, which actively transports and sequesters calcium, Coleman et al. (1970) used the electron microprobe to show that certain ectodermal cells are involved in the sequestering process.

Engel et al. (1970) used the electron microprobe to detect specific elements deposited in muscle tissue using a histochemical reaction invisible under light microscopy. The histochemical reaction was for ethylenedinitrilotetraacetic acid (EDTA)-activated ATPase, which has a strong reaction in type I fibers and a weak reaction in type II fibers. The elements deposited by the reaction are calcium and phosphorus. Microprobe analysis showed high amounts of both calcium and phosphorus in type I fibers and very little in type II fibers. The authors stated that a slight amount of

potassium and very little sodium was present in muscle fibers incubated in the histochemical media. They also found very little detectable calcium and phosphorus in muscle tissue not incubated in the histochemical media. No other reports of electron microprobe analysis in muscle tissue were found in the literature.

## Meat Spoilage

### Effects of Spoilage Organisms on Meat

Several workers (Kirsh et al., 1952; Ayres, 1960; Adams et al., 1964; Jay, 1967; Stringer et al., 1969) have concluded that the predominant bacteria contributing to fresh meat spoilage are the Pseudomonas and Achromobacter species. Ayres (1960) found the pseudomonads were the only species that increased in numbers on refrigerated (0-10°C) beef. He also found the pseudomonads to be responsible for slime production. Adams et al. (1964) found fresh fish were contaminated with various genera, but the pseudomonads were the primary cause of spoilage.

According to Jay (1970), the earliest signs of spoilage in ground meat are off odors and tackiness, which indicate the presence of bacterial slime. Jay (1970) stated that sliminess is due to both bacterial growth and "loosening of meat structural proteins".

Jay (1966) indicated beef allowed to spoil at refrigerator temperatures does not undergo significant proteolysis. Jay and Kontou (1967) concluded that amino acids and nucleotides rather than the primary beef proteins support the growth of beef spoilage bacteria. However, Jay (1970) indicated that primary muscle proteins may be attacked by spoilage bacteria

after the carbohydrates, free amino acids and nucleotides have been depleted. This is in agreement with a report by Lerke et al. (1967), in which they reported no significant proteolysis of fish muscle until spoilage had become evident.

Margitic and Jay (1970) found a change in the antigenicity of salt-soluble beef muscle proteins due to spoilage, but stated their findings indicate incomplete breakdown. Rampton et al. (1970) reported no measureable effect of five species of microorganisms and a mixed culture from fresh hamburger on the myofibrillar proteins of pig muscle. However, they showed there was a decrease in the amount of certain non-protein ultraviolet absorbing compounds due to bacterial growth.

In studying the effects of bacteria on porcine muscle, Borton et al. (1970a) reported no changes in protein solubility of samples inoculated with Pediococcus cerevisiae or Micrococcus luteus. They did, however, find changes in the protein solubility of samples inoculated with Leuconostoc mesenteroides and Pseudomonas fragi. Leuconostoc mesenteroides caused a decrease in the amount of salt soluble proteins at 20 days storage. Pseudomonas fragi caused an increase in both the water-soluble and salt soluble proteins, a decrease in the insoluble proteins and a large increase in nonprotein nitrogen.

Borton et al. (1970b) further reported that salt-soluble extracts of porcine muscle inoculated with Pseudomonas fragi showed a loss in the number of protein bands on starch-urea gel and disc-urea gel electrophoresis. They then concluded Pseudomonas fragi caused some proteolysis of the myofibrillar proteins. However, they found that salt-soluble extracts of porcine muscle inoculated with three other species were not altered.

Hasegawa et al. (1970a) found that Clostridium perfringens caused marked proteolysis of both the sarcoplasmic and urea-soluble proteins of porcine muscle. Other species studied caused little or no change in these proteins. Hasegawa et al. (1970b) found extensive proteolysis of porcine sarcoplasmic proteins by Pseudomonas fragi and some breakdown by Leuconostoc mesenteroides. They also found considerable breakdown of urea-soluble proteins in pig muscle by Pseudomonas fragi and Pediococcus cerevisiae.

Ultrastructure of Bacteria

Weibe and Chapman (1968a, b) have studied the ultrastructure of various pseudomonads with the aid of the electron microscope. They found pseudomonads have an irregular undulant cell wall, a nearly planar cell membrane, show loosely packed ribonucleoprotein particles throughout the periphery of the cytoplasm and contain axially disposed DNA.

Some of the strains of pseudomonads studied by Weibe and Chapman (1968a, b) contained protrusions or bleblike evaginations of the cell wall. The blebs were shown to be induced in some strains by specific physiological and nutritional conditions. Although Weibe and Chapman (1968a, b) stated that these blebs were probably not artifactual, that possibility was not completely eliminated. The functional significance of the blebs was not determined.

Knox et al. (1966) showed that Esherichia coli grown on lysine-limited media secreted a lipopolysaccharide in the form of protrusions or blebs. The blebs were formed by evaginations of the cell wall and later pinched off to form globules in the media surrounding the cells.

Hitchins and Sadoff (1970) in electron microscope sections have also observed blebs or protrusions of the cell wall for Azotobacter vinelandii. They noted that the blebs ultimately broke free and formed globules of various sizes. The globules were implicated in forming the capsule of encysting cells. Hitchins and Sadoff (1970) also stated that good preservation of morphological events was obtained on using glutaraldehyde and osmium tetroxide as a fixing agent.

# EXPERIMENTAL METHODS

## Experimental Animals

A total of 37 pigs were used in this study. Sixteen Yorkshire and 20 Hampshire pigs were obtained from Michigan producers that were known to have a high incidence of the PSE condition in their herds.

Five normal and five PSE pigs were selected from each breed (Yorkshire and Hampshire) on the basis of 45 minute pH and transmission value (a measure of the water soluble protein extractability) at 24 hours postmortem. The five pigs from each breed with the highest pH and lowest transmission values were classified as normal, while the five animals from each breed with the lowest pH and highest transmission values were classified as PSE. These 20 pigs (10 normal and 10 PSE) were used for electron microprobe analysis of muscle ion distribution and for electron microscopic analysis of normal and PSE muscle ultrastructure.

One additional pig obtained from the Michigan State University Swine Farm was used to ascertain the effects of meat spoilage organisms upon the ultrastructure of muscle.

## Slaughtering Procedures

All pigs of the Hampshire and Yorkshire breeds were hoisted by the hind leg and killed without stunning by sticking anterior to the sternum, thus severing the carotid artery and the jugular vein. After death samples were taken and the carcasses were eviscerated, skinned, washed and placed in a 4°C cooler.

The pig used to study meat spoilage was stunned electrically, shackled and hoisted by one hind leg. The area of the neck just anterior to the sternum was cleaned with a solution of phisohex bacteriocidal soap. The pig was stuck and bled using a knife that had been sterilized in a steam-heated sterilizer. It was then scalded, dehaired, eviscerated and cleaned in the normal manner. The unsplit carcass was rinsed with alcohol, flamed to further sterilize and to remove the excess alcohol and was then placed in a 4°C cooler for 24 hours.

## Measurement of pH

At 45 minutes postmortem, a muscle sample was excised from the left longissimus muscle of all Yorkshire and Hampshire pigs in the region of the third or fourth lumbar vertebrae. Sample pH was measured with a Corning model 12 expanded scale pH meter by placing the electrodes directly on the surface of the muscle. Six readings were made and used to calculate an average pH value for each animal.

## Transmission Value

The procedure used to obtain transmission values was the same as that outlined by Weatherspoon (1969). Samples were taken at 24 hours postmortem from the left longissimus muscle of all Yorkshire and Hampshire pigs in the region of the 10th to 11th thoracic vertebrae. These samples were used to determine transmission values, which were one of the two indicators of muscle quality.

Ten grams of finely ground muscle was placed in a 50 ml graduated centrifuge tube and diluted with cold distilled water to a final volume of 40 ml. The mixture was stirred and then stored at 40°C for 20 hours, at which time it was remixed and centrifuged at 3000 rpm for 20 minutes at 4°C. The supernatant was filtered through Whatman No. 1 filter paper. A 1 ml aliquot of the filtrate was added to each of two test tubes, one of which contained 5 ml of 0.15M phosphate-citrate buffer at pH 4.6 and 20°C, while the other (used as a blank) contained 5 ml of distilled water at a timperature of 20°C. Both solutions were held at 20°C for 30 minutes. Percent transmission was read against a blank on a Baush and Lomb Spectronic 20 colorimeter at 600 nm.

## Preparation of Fresh Pig Muscle for Electron Microscopic Examination

### Sampling

Samples of the right *longissimus* muscle from all Yorkshire and Hampshire pigs were removed at 15 minutes postmortem in the area of the fourth and fifth lumbar vertebrae. Samples were also taken at 24 hours postmortem from the left *longissimus* muscle of the same pigs in the region of the 10th to 11th thoracic vertebrae. These samples were cut into strips 3 mm in cross section and 1 cm long. The muscle strips were then placed in glutaraldehyde fixative.

Samples were not taken at the time of death because drastic postmortem changes have been shown to be caused by sampling immediately following death (Koch *et al.*, 1970).

Fixation and Embedding

A modification of the procedure described by Sjöstrand (1967) was used for glutaraldehyde fixation. Muscle strips were fixed in a 1.25% glutaraldehyde solution made up in sodium phosphate buffer as shown in Appendix 1. The pH of the fixing solution was 7.4, and the osmolarity was adjusted to 415 miliosmolar by adding sodium chloride.

After fixation for two hours, the samples were washed for 30 minutes in two changes of sodium phosphate buffer containing sodium chloride. The pH of the washing buffer was 7.4 and the osmolarity was 440 miliosmolar. The washing buffer was made up as shown in Appendix 2.

The samples were transferred from the washing buffer to a 1% osmium tetroxide solution in veronal acetate buffer. The pH of the osmium tetroxide solution was 7.4. The osmolarity was adjusted to the tonicity of blood (300 miliosmolar) by adding appropriate amounts of sodium, potassium and calcium chloride. The osmium tetroxide fixative was prepared according to procedures described by Sjöstrand (1967), which are outlined in Appendix 3. Samples were fixed for one hour in this fixative.

After fixation, samples were dehydrated by placing them in 25, 50, 75 and 95% ethanol for 10 minutes each. They were then placed in two changes of 100% ethanol for 15 minutes. The tissue was transferred from 100% alcohol to propylene oxide (two changes of 30 minutes each), and then into a 1:1 mixture of propylene oxide and epon for 12 hours.

After the tissues were removed from the propylene oxide-epon mixture, they were trimmed to approximately 3 mm in length and 1 sq mm in cross

section. They were then transferred to 00 size gelatin capsules containing pure epon. The method for preparing the epon is given in Appendix 4. The capsules containing the samples were placed in a desiccator under a slight vacuum at room temperature for 12 hours. They were then placed in an oven at 60°C and allowed to harden for 36 hours. The hardened epon blocks containing the muscle tissue were stored under vacuum in a desiccator at room temperature until further use.

Sectioning and Staining

Epon embedded tissue blocks were trimmed by hand with a razor blade. The tissues were then sectioned on glass knives to a thickness of 600 to 800 Å using an LKB ultramicrotome. Sections were picked up from the knife boat on uncoated 300-mesh copper grids.

The tissue sections were stained by floating the grids on a saturated solution of uranyl acetate for 30 minutes and rinsing thoroughly with distilled water (Sjöstrand, 1967). Sections were then stained for five minutes in a solution of lead citrate (Reynolds, 1963), which was made up as described in Appendix 5. After lead citrate staining, the sections were washed with 0.02N sodium hydroxide followed by distilled water. After drying, the sections were ready for electron microscopic examination.

Observation and Photography of Specimens

Grids containing stained sections were placed in a Philips EM-300 electron microscope and observed at an accelerating voltage of 60 KV. At

least five grids were observed for each sample and representative photographs were taken on Kodak Estar thick Base-70 mm film. The film was developed for three minutes in Kodak D-19 developer, washed in running water (30 sec), fixed for four minutes in Kodak Fixer, washed in running water for 30 minutes, washed in distilled water for two minutes and dried. All the solutions utilized had a temperature of 20°C.

Ilford Ilfoprint rapid stabilization paper was exposed from the negatives using a Durst S-45-EM enlarger. The prints were developed in an Ilford model 1501 rapid stabilization processor and selected prints were fixed in Kodak fixer, washed and dried on a ferrotype dryer.

## Electron Microprobe Analysis

### Sampling Procedure

The entire caput mediale tricipitis muscle was removed at 15-45 minutes postmortem from both sides of selected Yorkshire and Hampshire carcasses. Care was taken to avoid mechanically induced contraction.

The left muscle was contracted by stimulating it with a Grass Electrical Stimulator. This was accomplished by means of a clamp connected with a lead wire to the positive and negative terminals, respectively. One clamp was placed on the origin of the muscle while the other clamp was placed on the insertion. The stimulator was turned on causing a tetanic contraction of the muscle. The frequency, duration and voltage of stimulation used was 85 per second, 75 miliseconds and 10 volts, respectively. The contracted muscles were immediately frozen by immersing them with clamps attached, into liquid nitrogen. The stimulator was left on during freezing.

The right muscle was frozen in liquid nitrogen without stimulation, and thus, was in a relaxed state.

Sample Preparation

Two methods of sample preparation were used for electron microprobe analysis. One method involved freeze-drying of the sample and the other involved frozen sections.

Freeze Drying and Fixation. The frozen muscles were placed on a table in a -25°C freezer and small pieces were removed with a boning knife. The pieces were trimmed to a size of about 1 cm long, 3 sq mm in cross section and placed in a large glass tube (55 mm in diameter and 38 cm long) that had been equilibrated to -40°C in a diethyl oxalate-dry ice mixture. This tube had a standard taper 60/50 ground glass top and was one fourth filled with histowax. A vacuum pump was then connected to the top of the tube and freeze drying of the tissue was accomplished over a 48 hour period.

After freeze drying was complete, the tube was removed from the diethyl oxalate-dry ice mixture and paraformaldehyde or osmium tetroxide vapors were introduced into the vacuum through a stopcock. The tissue was fixed for four hours while still under vacuum. After fixing, the histowax was melted by placing the bottom of the tube in a heating mantle and wrapping the upper part of the tube with heating tape. The melted histowax was allowed to infiltrate the tissue for three hours under vacuum. After infiltration the muscle samples were removed from the freeze drying apparatus and embedded in fresh melted histowax.

The histowax blocks containing the tissue were trimmed and 10 μ thick sections were cut on a Leitz rotary microtome. These sections were placed on polished carbon discs with Haupt's adhesive. The sections on the discs were placed in two changes of xylene for 3 minutes each to remove the wax. They were coated with a thin layer of carbon and observed in the electron microprobe.

Frozen Sections. The frozen muscles were placed on a table in a -25°C freezer and pieces about 5 $mm^3$ were removed. These pieces were then frozen to a S.L.E.E. cryostat sample holder with a few drops of distilled water. The sample holder containing the tissue was then placed on the microtome of the S.L.E.E. cryostat.

Sections were cut at 10 μ, placed on glass coverslips and left in the cryostat at -18°C in the presence of a silica gel drying agent until they were dry (about 12 hours). The sections were coated with a thin layer of carbon and observed in the electron microprobe.

## Analysis of Samples

Carbon coated sections were placed under vacuum in an ARL-electron microprobe x-ray analyzer-scanning electron microscope. Secondary electron images, elemental x-ray emission images and line scans of elemental x-ray emission were recorded at an accelerating voltage of 15 KV and 0.01 μamps. Recordings of the oscilliscope images of secondary electron and x-ray emission patterns were preserved on polaroid film. Line scans of elemental x-ray emission were recorded on an x-y recorder.

## Electron Microscopic Examination of Meat Spoilage

### Sampling

At 24 hours postmortem, the carcass used to study meat spoilage was laid on a table covered with Kraft paper, the shoulder and ham sections were removed and the middle portion of the carcass was placed so the dorsal midline was accessible. A cut was made with a sterile knife through the backfat along the dorsal midline, with subsequent cuts being made perpendicular to the midline. The backfat was removed and sections of the *longissimus* muscle were excised and placed in a sterilized container. The above operations were performed in a clean room free from excessive air currents. The operator wore sterile disposable gloves during the entire procedure.

### Inoculation and Incubation of Muscle

Sections of *longissimus* muscle were ground through a sterilized prechilled grinder, placed in presterilized sample jars and covered with a loose fitting sterile lids. Inoculated samples were treated identical to control samples except that 10 ml of inoculation media, prepared by diluting a 48 hour pure culture of *Pseudomonas fragi* (American Type Culture Collection) 100 fold, was added to 1000 gm of muscle during grinding.

Both the uninoculated control and inoculated samples were incubated at 10°C for 0, 8 and 20 days. At the end of each storage period, samples were removed from the jars and prepared for electron microscope analysis and bacterial counts.

Bacterial Counts

Bacterial numbers were obtained using the methods described by the American Public Health Association (1966). Eleven grams of sample were blended with 99 ml of sterile water in a presterilized blendor. After making appropriate dilutions, 1.0 or 0.1 ml of the slurry was pipetted into sterile disposable petri dishes. APT (all-purpose plus tween) agar was used as the plating media. The plates were incubated at 25°C for 48 hours. The colonies were then counted and recorded as the log bacteria per gram of tissue.

Growth of Bacteria on Non-Muscle Media

In order to ascertain if alterations in bacterial ultrastructure occur due to growth on muscle tissue, bacteria were grown on APT (media containing no intact muscle tissue) and non-protein media (a media in which protein material is absent). The non-protein media contained amonium chloride as a nitrogen source, glucose as a carbon source and was made up as outlined in Appendix 6. The bacteria grown in APT broth were incubated at 10°C for 6 days. Those grown in non-protein media were incubated at 10°C for 12 days. After incubation the bacteria were prepared for electron microscopic examination.

Fixation and Embedding

Samples of control and inoculated muscle tissue were removed from the surface of the ground muscle mass at the end of each incubation period. These samples were then cut into small pieces (no larger than 3 $mm^3$), fixed and embedded as previously described for electron microscopic examination.

A 4 ml sample of the bacteria was removed from both the APT and non-protein media. Each sample was added to 10 ml of a 1.25% glutaraldehyde fixative (Appendix 1) in a graduated centrifuge tube. After two hours of fixation, the bacteria were centrifuged at 2,000xg for 10 minutes. The supernatant was discarded. The bacteria were resuspended in 14 ml of washing buffer (Appendix 2). The remaining fixation, dehydration and embedding procedure was identical to that described earlier for muscle tissue, except that the bacteria were centrifuged and resuspended each time the solution was changed. The bacteria were embedded in flat embedding molds (LKB Instruments, Inc.) instead of gelatin capsules.

Sectioning, Staining, Observation and Photography

Sectioning, staining, examination and photography of all samples were the same as previously described for electron microscopic examination.

## RESULTS AND DISCUSSION

### Muscle Quality

The 45 minute pH and 24 hour transmission values obtained for the PSE and normal pigs of the Yorkshire breed are shown in Table 1. The same information for the Hampshire pigs is shown in Table 2. The 45 minute pH and 24 hour transmission values for all pigs of both breeds are shown in Appendix 7.

Table 1. Values for pH at 45 minutes and transmission values at 24 hours postmortem for PSE and normal Yorkshire pigs.

| PSE Group | | | Normal Group | | |
|---|---|---|---|---|---|
| Animal No.[a] | % Transmission[b] | Muscle pH[c] | Animal No.[a] | % Transmission[b] | Muscle pH[c] |
| Y-5 | 67.0 | 5.50 | Y-1 | 8.0 | 6.80 |
| Y-9 | 79.5 | 5.50 | Y-2 | 10.5 | 6.80 |
| Y-11 | 74.0 | 5.56 | Y-6 | 11.0 | 6.70 |
| Y-12 | 46.0 | 6.35 | Y-7 | 18.0 | 6.80 |
| Y-16 | 42.0 | 6.23 | Y-14 | 15.0 | 6.68 |
| Mean | 61.7 | 5.82 | Mean | 12.5 | 6.76 |

[a]Y = Yorkshire
[b]Transmission values were measured as described by Weatherspoon (1969).
[c]pH was measured by direct readings on the muscle surface.

The mean pH values at 45 minutes postmortem were 5.82 and 6.76 for the PSE and normal Yorkshire pigs, respectively, while the corresponding values for the Hampshire pigs were 6.27 and 6.68. The mean transmission

values at 24 hours postmortem were 61.7 and 12.5 for the PSE and normal Yorkshire pigs, respectively. The corresponding values for the Hampshire pigs were 52.0 and 8.8.

Table 2. Values for pH at 45 minutes and transmission values at 24 hours postmortem for PSE and normal Hampshire pigs.

| PSE Group | | | Normal Group | | |
|---|---|---|---|---|---|
| Animal No.[a] | % Transmission[b] | Muscle pH[c] | Animal No.[a] | % Transmission[b] | Muscle pH[c] |
| H-3 | 37.0 | 5.78 | H-4 | 7.0 | 6.70 |
| H-8 | 70.0 | 6.02 | H-5 | 7.0 | 6.55 |
| H-9 | 60.0 | 6.57 | H-6 | 6.0 | 6.65 |
| H-10 | 54.0 | 6.58 | H-15 | 12.0 | 6.66 |
| H-16 | 39.0 | 6.40 | H-17 | 12.0 | 6.86 |
| Mean | 52.0 | 6.27 | Mean | 8.8 | 6.68 |

[a]H = Hampshire
[b]Transmission values were measured as described by Weatherspoon (1969).
[c]pH was measured by direct readings on the muscle surface.

Since pH and transmission values were used as a basis for dividing the pigs into their respective groups, the differences in pH and transmission values for normal and PSE pigs were obvious. Selection of PSE and normal pigs on this basis is justified by the results of other authors (McLoughlin and Goldspink, 1963; Aberle and Merkel, 1968; Koch et al., 1969a, b) who have shown low muscle pH at 45 minutes postmortem and high transmission values at 24 hours postmortem to be associated with the PSE condition. Examination of the data shows that the PSE and normal pigs were

generally quite well delineated from each other. This was more evident for transmission values than for pH readings, which suggests that transmission values may be a better measure of muscle quality than 45 minute pH.

## Ultrastructure of Normal Pig Muscle Fibers

### Myofibrils

Based on the width of the Z lines, three main groups of myofibrils were observed; those having very wide (approximately 1,200 Å) Z lines as shown in Figure 1, those having Z lines of intermediate width (approximately 775 Å) as shown in Figure 2, and those having relatively narrow (approximately 625 Å) Z lines as shown in Figure 3. These three groups correspond to myofibrils from red (wide Z lines), intermediate (Z lines of intermediate width) and white (narrow Z lines) fibers of the rat as described by Gauthier (1970). She indicated that consistent differences in Z line width between different fiber types established it as a valid criterion for distinguishing between red, intermediate and white muscle fibers at an ultrastructural level.

On this basis, the differences in Z line width between pig muscle fibers was used to classify them as red, intermediate or white fibers. An electron micrograph of adjacent red and white fibers is shown in Figure 4. The gross differences that can be seen in these fibers enables one to easily identify the different fiber types.

The width of the Z lines reported by Gauthier (1970) for rat muscle were roughly half that of those found in the present study for pig muscle.

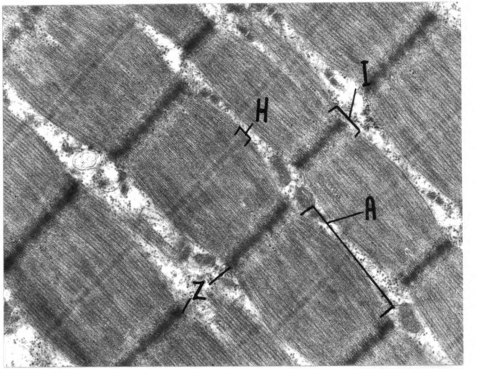


Figure 1. Electron micrograph of pig Y-7 showing characteristic myofibrils from normal red muscle fibers at 15 minutes postmortem. A = A band, I = I band, H = H zone and Z = Z line. X 22,600.

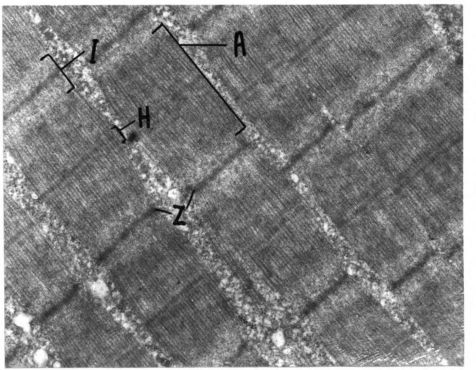


Figure 2. Electron micrograph of pig H-4 showing characteristic myofibrils from normal intermediate muscle fibers at 15 minutes postmortem. A = A band, I = I band, H = H zone and Z = Z line. X 22,400.

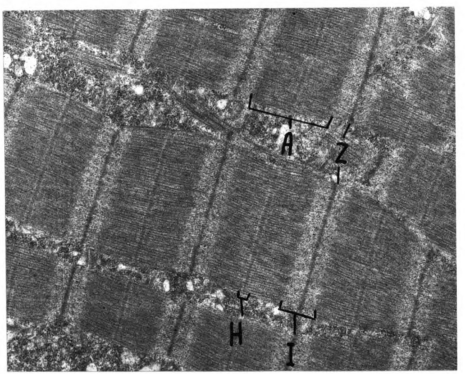


Figure 3. Electron micrograph of pig Y-2 showing characteristic myofibrils from normal white muscle fibers at 15 minutes postmortem. A = A band, I = I band, H = H zone and Z = Z line. X 16,300.

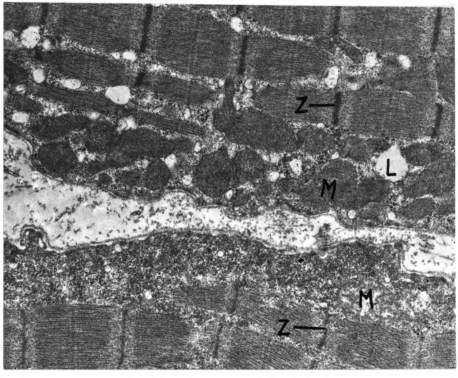


Figure 4. Section of pig Y-2 showing a normal red and a white muscle fiber from the longissimus muscle at 15 minutes postmortem. The characteristic ultrastructure of each fiber type is evident. L = lipid droplets, Z = Z line, M = mitochondria. X 15,100.

A search of the literature has failed to reveal any previous reports showing differences in the width of Z lines between various fiber types in pig muscle.

Although differences were noted in the size of the I band between myofibrils from various muscle fibers, variability in the size of the I band was not consistently the same for any one fiber type. The differences in I band size may reflect the amount of contraction present in the fibers at the time of fixation.

The myofibrils observed in this study exhibit the characteristic banding pattern that occurs in skeletal muscle from other vertebrates, except for a very few fibers that showed contracture bands (Figure 5) similar to those shown by Cassens et al. (1963b). These authors reported that contracture bands could be produced by cutting a muscle, which is the probable cause of such bands in the present study. The number of contracture bands produced in this study was probably minimized by taking samples at 15 minutes postmortem rather than immediately after death.

Mitochondria

Electron micrographs of representative mitochondria from red, intermediate and white muscle fibers at 15 minutes postmortem are presented in Figures 6, 7 and 8, respectively.

Figure 6 shows mitochondria from characteristic red fibers. Examination reveals the mitochondria are large, show very closely packed cristae and are dense in appearance. The red fibers contain large numbers of mitochondria, which are found either in groups just beneath the sarcolemma, arranged in interfibrillar rows or in pairs at the Z line.

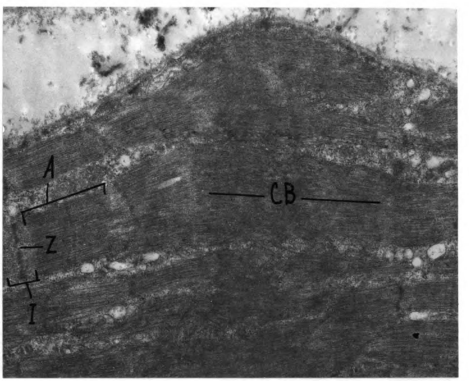


Figure 5. Electron micrograph of pig H-15 showing a contracture band (CB) in a normal muscle fiber at 15 minutes postmortem. Z = Z line, A = A band, and I = I band. X 16,300.

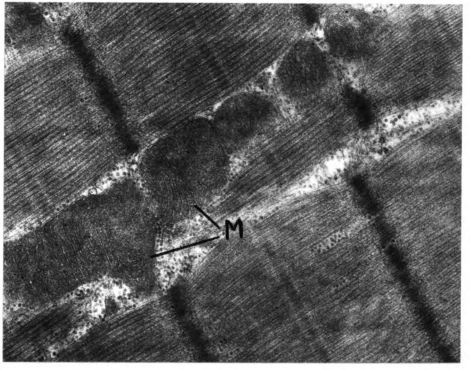


Figure 6. Electron micrograph of pig Y-17 showing characteristic mitochondria (M) from normal red fibers at 15 minutes postmortem. X 36,300.

Figure 7 shows mitochondria from intermediate fibers which have approximately the same distribution as for the red fiber (Figure 6), but they are less dense in appearance and have less closely packed cristae. It was also observed that intermediate fibers had fewer mitochondria than red fibers.

As can be seen in Figure 8, mitochondria from white fibers appear somewhat similar to those found in intermediate fibers (Figure 7), except they have less closely packed cristae and are smaller in size. Mitochondria occurred much less frequently in white fibers than in the other fiber types. Most of the mitochondria in white fibers appeared to be located between the myofibrils at the Z line. However, a limited number were also observed near the sarcolemma. Mitochondria were absent in some sections of white muscle fibers.

Mitochondria differences between red, intermediate and white fibers of the pig were essentially the same as those observed by Gauthier (1970) in muscle fibers from the rat. However, there appeared to be less mitochondria in the white muscle fibers of the pig as compared to that of the rat as described by Gauthier (1970). Muir (1970) has also reported a very low mitochondrial content for white fibers from pig muscle as compared to red fibers. However, he made no mention of ultrastructural differences in appearance of the mitochondria from the different fiber types.

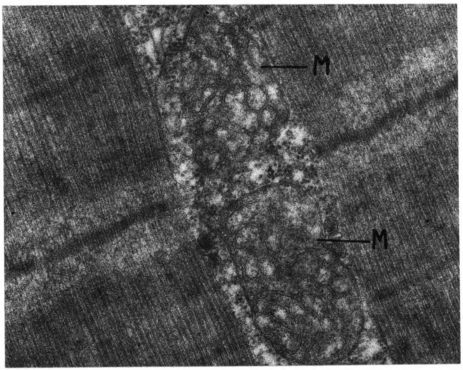


Figure 7. Electron micrograph of pig H-4 showing characteristic mitochondria (M) from a normal intermediate fiber at 15 minutes postmortem. X 40,700.

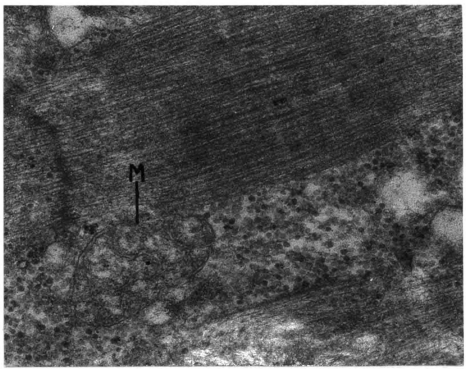


Figure 8. Electron micrograph of pig H-4 showing a characteristic mitochondria (M) from a normal white fiber at 15 minutes postmortem. X 57,200.

Sarcoplasmic Reticulum and T System

Electron micrographs showing the characteristic ultrastructure of the SR and T system in normal pig muscle fibers are shown in Figures 9, 10, 11 and 12.

As can be seen in Figure 9, the triads in pig muscle are located at the junction of the A-I band, which is in agreement with reports by Peachey (1970) for other mammalian species. Figure 9 also shows an invagination of the sarcolemma to form a transverse tubule. A terminal cisternae of the SR can be seen on either side of the invagination.

Connection of a transverse tubule with the plasma membrane is shown in Figure 10. The basement membrane shown in Figure 10 is continuous along the outside of the cell and does not invaginate with the plasma membrane in forming the transverse tubule. Material from the SR can be seen in close apposition to the transverse tubule as it passes between two adjacent mitochondria.

These results indicate that the transverse tubules of the T system are continuous with the exterior surface of the muscle fiber in the pig. This is in agreement with results reported by other authors (Franzini-Armstrong and Porter, 1964; Ezerman and Ishikawa, 1967; Peachey, 1970), who have demonstrated the continuity of transverse tubules with the exterior of muscle cells from other species.

Figures 11 and 12 show the fine structure of the SR between transverse tubules in white and intermediate muscle fibers, respectively. The SR in the white fibers tended to have larger longitudinal tubules than that from red or intermediate fibers. In the region of the H zone, longitudinal

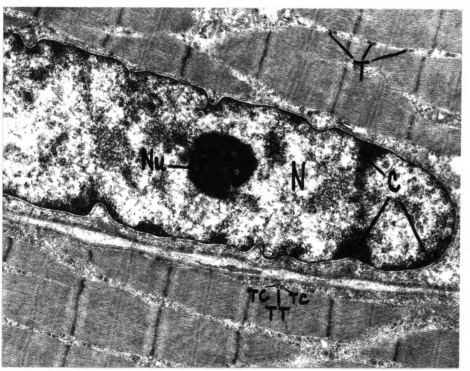


Figure 9. Electron micrograph showing portions of two intermediate fibers from pig H-6. N = nucleus, Nu = nucleolus, C = chromatin, T = triads, TT = transverse tubules, TC = terminal cisternae. X 11,560.

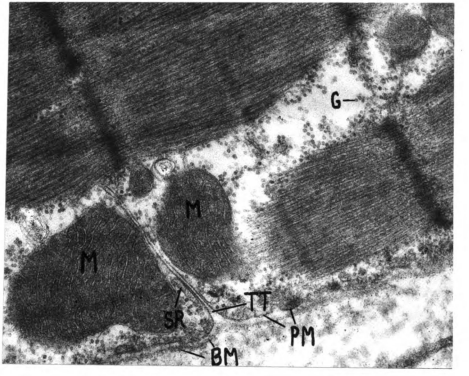


Figure 10. Section through a normal muscle fiber from pig Y-17 showing a sarcolemmal invagination. TT = transverse tubule, SR = sarcoplasmic reticulum substance, PM = plasma membrane, BM = basement membrane, M = mitochondria and G = glycogen granules. X 37,900.

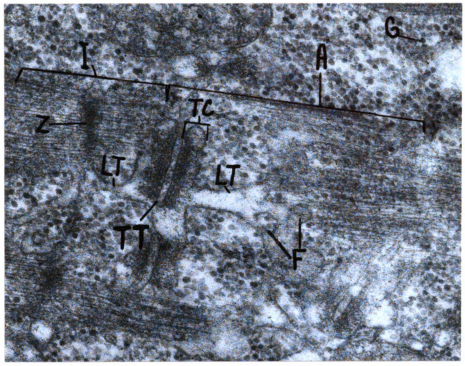


Figure 11. A section along the edge of a myofibril from a normal white muscle fiber from pig H-4. LT = longitudinal tubule, TT = transverse tubule, TC = terminal cisternae, F = fenestrations, G = glycogen granules, I = I band, A = A band and Z = Z line. X 57,200.

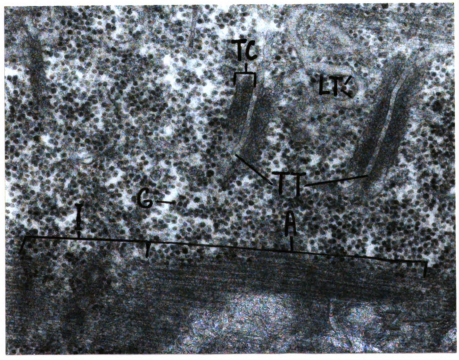


Figure 12. A section along the edge of a myofibril from a normal intermediate muscle fiber of pig H-5. LT = longitudinal tubule, TC = terminal cisternae, TT = transverse tubules, G = glycogen granules, A = A band, I = I band and Z = Z line. X 49,500.

tubules of white fibers formed open sac-like structures, which sometimes appeared to be fenestrated. On the other hand, the longitudinal tubules of red and intermediate fibers retained more of a tortuous tubular appearance in this region. These results agree with those of Gauthier (1970), who found the SR from red fibers of rat muscle was in the form of elaborate narrow tubules in the H zone, and the SR from white fibers appeared as flattened sacs.

Figures 11 and 12 also show that the cisternae are in close apposition to the transverse tubules. Dense granular material was also found in the terminal cisternae. This is in agreement with Muir (1970), who observed dense granules in the terminal cisternae of pig muscle fibers. Other authors (Porter and Palade, 1957; Peachey, 1970) have reported the presence of dense granules in the terminal cisternae of muscle fibers from other species.

Reports describing the ultrastructure of the SR in various fiber types from pig muscle were not found in a search of the literature.

Other Components

Numerous glycogen granules were present in all normal muscle at 15 minutes postmortem. The white and intermediate fibers tended to have a larger amount of glycogen than the red fibers. This can be seen by comparing the red fiber in Figure 10 with the white and intermediate fibers of Figures 11 and 12, respectively. This is in contrast to the results of Beecher et al. (1965), who reported the glycogen content of various red and white muscles of the pig to be essentially the same. However, present

results are in agreement with the report of Koch et al. (1970a, b), who found more glycogen to be present in pig longissimus (white) muscle than in pig rectus femoris (red) muscle. Beatty and Bocek (1970) have also shown more glycogen to be present in white than in red muscle from the monkey.

The nuclei of pig muscle cells are large, ellipsoidal and contain a prominent nucleolus as shown in Figure 9. The chromatin material tends to be more dense around the periphery of the nuclear membrane. These same general features have been described for muscle cell nuclei by Slatterback (1966) and Gori et al. (1967).

## Ultrastructure of Postmortem Pig Muscle

### Myofibrils

Electron micrographs showing representative myofibrils from red, intermediate and white fibers of normal pig muscle at 24 hours postmortem are presented in Figures 13, 14 and 15, respectively. Myofibrils from all three fiber types show the same banding pattern as was observed in samples at 15 minutes postmortem.

The width of the Z lines at 24 hours postmortem was essentially the same for each fiber type as at 15 minutes postmortem. However, some Z line material was absent from the myofibrils of the intermediate and white fibers at 24 hours postmortem. The areas showing disrupted Z lines are presented in Figures 14 and 15. The Z lines of myofibrils from red fibers (Figure 13) had less postmortem disruption than those from intermediate and white type fibers.

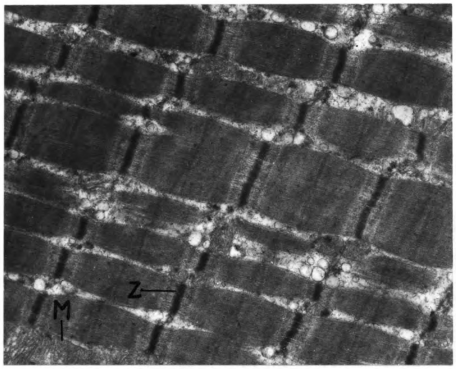


Figure 13. Electron micrograph of representative red fiber myofibrils from pig H-6 at 24 hours postmortem. M = mitochondria and Z = Z line. X 16,600.

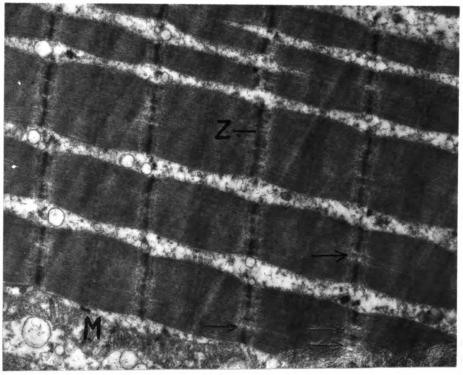


Figure 14. Electron micrograph of representative intermediate fiber myofibrils at 24 hours postmortem from pig H-6. M = mitochondria, Z = Z line and arrows point to Z line disruption. X 16,600.

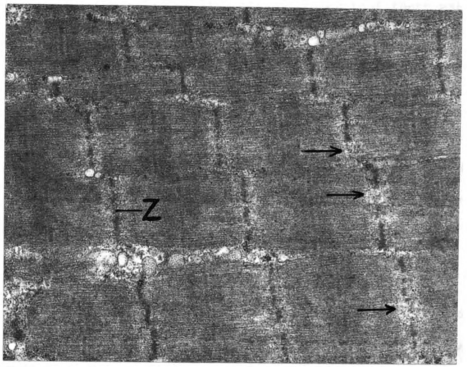


Figure 15. Electron micrograph of representative white fiber myofibrils at 24 hours postmortem from pig Y-2. Z = Z line and arrows point to Z line disruption. X 22,400.

These results agree with those reported by Greaser et al. (1969a), who showed a loss of Z line material in myofibrillar fractions of normal pig muscle at 24 hours postmortem. Davey and Dickson (1970) and Goll et al. (1970) have also reported a loss of Z line material during aging of muscle. However, Cassens et al. (1963a) stated that there was no evidence of any alteration in the myofibrils from normal pig muscle during 24 hours postmortem holding. None of these reports mentioned any differences between the Z lines from various fiber types.

Greaser et al. (1969a) gave no explanation as to the cause of postmortem Z line disruption. However, Davey and Dickson (1970) and Goll et al. (1970) stated that release of calcium by the SR might be responsible for Z line disintegration.

Mitochondria

An electron micrograph showing both a red and an intermediate fiber is presented in Figure 16. Characteristic mitochondria for each fiber type at 24 hours postmortem are also clearly shown. An electron micrograph of a white fiber and its characteristic mitochondria is presented in Figure 17.

The mitochondria from 24 hour postmortem red fibers (Figure 16) have a much more open structure than similar mitochondria from red fibers at 15 minutes postmortem (Figure 6). The cristae appear to be intact and are still closely packed at 24 hours postmortem, but a large amount of the ground substance has disappeared.

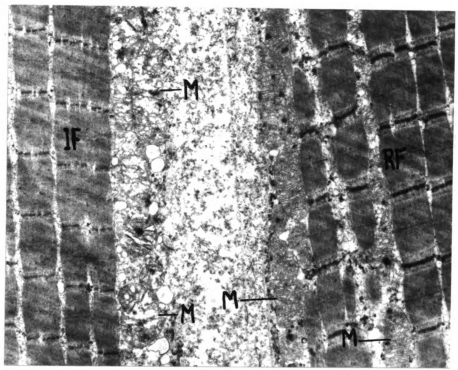


Figure 16. Electron micrograph showing both a red fiber (RF) and an intermediate fiber (IF) at 24 hours postmortem from pig H-6. M = mitochondria. X 8,800.

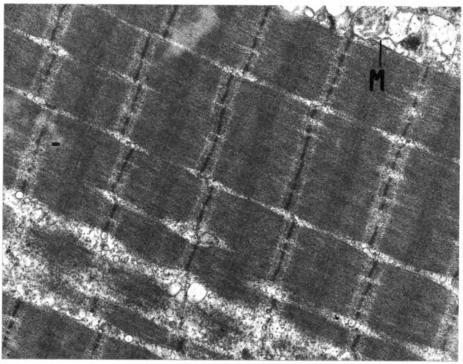


Figure 17. An electron micrograph of a white muscle fiber at 24 hours postmortem from pig Y-2. M = mitochondria. X 13,200.

Mitochondria from intermediate fibers (Figure 16) appear much more disrupted at 24 hours postmortem than similar mitochondria from red fibers (Figure 16). The cristae show considerable disruption and most of the ground substance has disappeared. The two fibers in Figure 16 illustrate the differences between the mitochondria of the two fiber types.

Figure 17 shows a mitochondrion from a white fiber at 24 hours postmortem. A few cristae are evident, but most of the internal structure is absent. It was difficult to distinguish mitochondria in white muscle fibers at 24 hours postmortem. This could be due to either their relatively small numbers or to an almost complete postmortem disruption.

Cassens et al. (1963a) have reported disruption of mitochondria for normal pig muscle at 24 hours postmortem. However, they did not mention differences in mitochondria from various fiber types. Greaser et al. (1969a) reported much more disruption of mitochondria in a fraction isolated at 24 hours postmortem than was found in a similar fraction isolated immediately after death. However, they found some mitochondria to be fairly well preserved at 24 hours following death. No mention of possible differences in mitochondria from various fiber types was made by these authors.

Results of the present study suggest that postmortem disruption of mitochondria, as found in isolated mitochondrial fractions by Greaser et al. (1969a), also occurs in intact muscle. However, mitochondria from red fibers appeared to be less susceptible to postmortem disruption than those from intermediate or white fibers, which could account for the presence of

intact mitochondria as observed by Greaser et al. (1969a). Mitochondria from white fibers seem to be the most susceptible to postmortem disruption.

Sarcoplasmic Reticulum and T System

Electron micrographs showing the SR and T system of red, intermediate and white fibers at 24 hours postmortem are presented in Figures 18, 19 and 20, respectively. The SR from red fibers retained some of its tubular appearance as can be seen in Figure 18. In the region of the A band there appears to be remnants of longitudinal tubules. In the region of the A-I band junction, terminal cisternae appear to be present.

The SR in the other two fiber types, (white and intermediate shown in Figures 19 and 20, respectively) has lost its original appearance and all that remains are vesicular structures. No triads or transverse tubules were evident at 24 hours postmortem in any of the fiber types.

Cassens et al. (1963a) have reported that disruption of sarcoplasmic components occurs in normal pig muscle at 24 hours postmortem. However, no specific reference was made to the SR. Greaser et al. (1969a) reported no marked change in the isolated heavy SR fraction of muscle at 24 hours postmortem. They did, however, notice a change in the isolated light SR fraction. In another study Greaser et al. (1969b) showed that a marked postmortem decrease occurred in the calcium accumulating ability of both the light and heavy SR fractions of muscle.

In the present study, marked structural alterations occurred in the SR of intact muscle at 24 hours postmortem. This could be a factor in lowering the calcium accumulating ability of the SR, which was reported earlier by Greaser et al. (1969b).

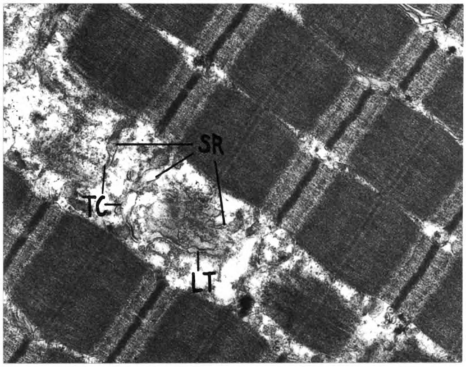


Figure 18. Electron micrograph showing the SR of a red fiber at 24 hours postmortem from pig Y-7. SR = sarcoplasmic reticulum, LT = possible longitudinal tubules and TC = possible terminal cisternae. X 19,900.


Figure 19. Electron micrograph showing the SR of an intermediate fiber at 24 hours postmortem from pig H-6. V = vesicular type structures that probably originate from the SR. X 13,300.

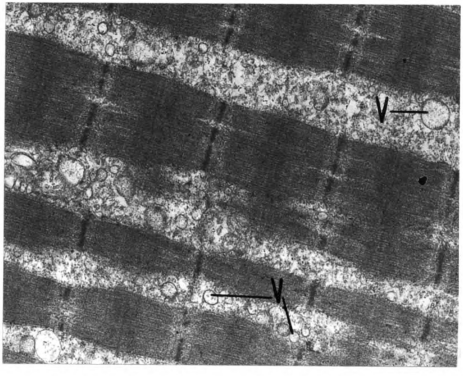


Figure 20. Electron micrograph showing the SR of a white fiber at 24 hours postmortem from pig Y-2. V = vesicular type structures that probably originate from the SR. X 19,900.

Other Components

There were no observable changes in the nuclei of any of the fiber types at 24 hours postmortem. Few, if any, glycogen granules were still present in any of the fibers at 24 hours postmortem. This was true for red, intermediate and white fibers. Results agree with other authors (Briskey, 1964; Koch et al., 1969a, b), who have shown that postmortem glycolysis reduces glycogen to a very low level prior to 24 hours following death.

Comparison of the Ultrastructure of PSE and Normal Pig Muscle

Myofibrils

Fifteen Minute Postmortem Samples. Figures 21, 22 and 23 show myofibrils from PSE muscle at 15 minutes postmortem for red, intermediate and white fibers, respectively. There was no difference in the appearance of myofibrils for the three fiber types from normal (Figures 1, 2 and 3) and PSE muscle at 15 minutes postmortem. However, more contracture bands were present in PSE muscle (Figure 24). As shown in Figure 24, fibrillar structure was continuous throughout the contracture bands.

Results of the present study agree with those of Greaser et al. (1969a), who found no difference between myofibrils from PSE and normal animals immediately after death. However, their report was based upon isolated myofibrillar fractions rather than intact muscle.

Muir (1970) reported numerous localized areas resembling contracture bands to be present shortly after death in PSE muscle from Pietrain pigs.

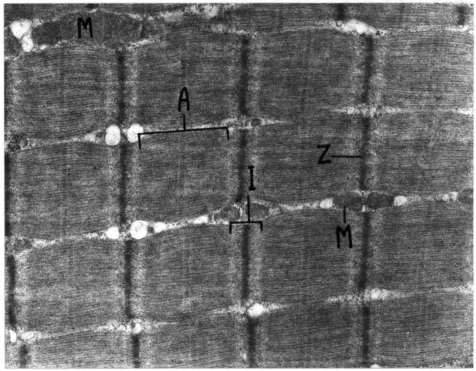


Figure 21. Electron micrograph showing myofibrils of a red fiber from PSE muscle (pig Y-12) at 15 minutes postmortem. Z = Z line, A = A band, I = I band and M = mitochondria. X 18,900.

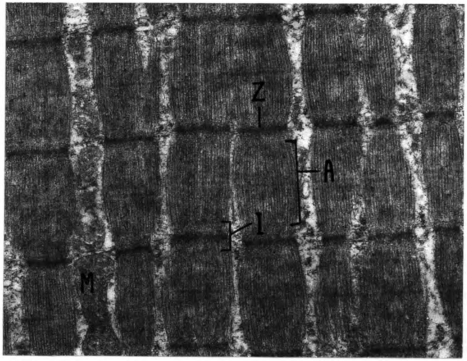


Figure 22. Electron micrograph showing myofibrils of an intermediate fiber from PSE muscle (pig Y-12) at 15 minutes postmortem. Z = Z line, A = A band, I = I band and M = mitochondria. X 18,900.

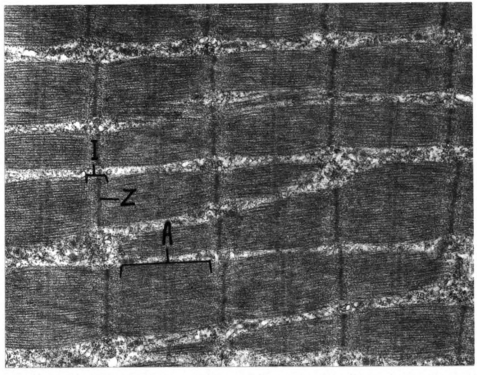


Figure 23. Electron micrograph showing myofibrils of a white fiber from PSE muscle (pig Y-12) at 15 minutes postmortem. Z = Z line, A = A band and I = I band. X 18,900.

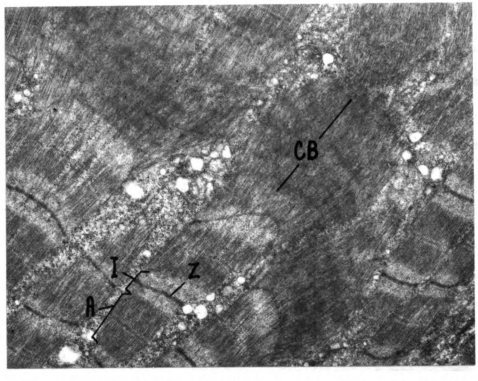


Figure 24. Electron micrograph showing a contracture band (CB) from PSE muscle (pig H-8) at 15 minutes postmortem. Z = Z line, A = A band and I = I band. X 12,600.

He attributed these bands to coagulation of myofibrillar and sarcoplasmic proteins due to the rapid postmortem fall in pH. In contrast, Cassens et al. (1963b) previously reported that contraction bands in muscle tissue are caused by violent contraction, which produces mechanical disturbance of the fibrillar system rather than by a precipitation of the proteins. These conclusions were based partially on the fact that continuity in fibrillar structure was evident throughout the contraction bands. Results of the present study agree with those of Cassens et al. (1963b), and also indicate that PSE muscle is more susceptible to contracture banding than normal muscle.

Twenty Four Hour Postmortem Samples. Electron micrographs showing the ultrastructure of myofibrils from red, intermediate and white fibers at 24 hours postmortem are presented in Figures 25, 26 and 27, respectively. The gross appearance of these myofibrils is much the same as that described earlier for normal myofibrils at 24 hours postmortem (Figures 13, 14 and 15). There does, however, seem to be more disruption in the myofibrils of PSE muscle, particularly for the red type fibers. A slight increase in granularity of PSE myofibrils was also noticed. Some separation of the filaments in the A band of a red fiber can be seen in Figure 25. Figure 28 shows a higher magnification of the myofibrils from an intermediate fiber. Separation of the A band filaments can be seen as well as some disruption of the Z line.

Contracture bands were found in a few fibers of PSE muscle at 24 hours postmortem as can be seen in Figure 29. This is in contrast to normal muscle at 24 hours postmortem, where no contracture bands were observed.

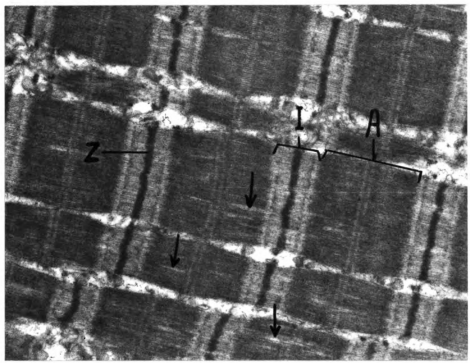


Figure 25. Electron micrograph showing myofibrils of a red fiber from PSE muscle (pig Y-9) at 24 hours postmortem. Arrows mark areas of A band filament separation. Z = Z line, A = A band and I = I band. X 18,900.

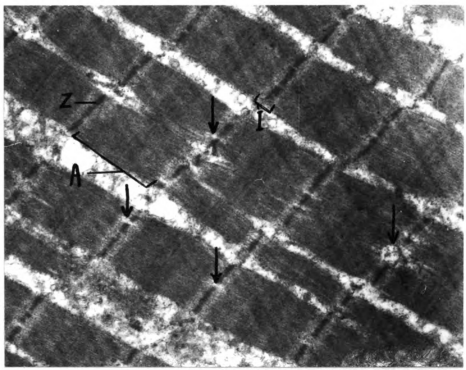


Figure 26. Electron micrograph showing myofibrils of an intermediate fiber from PSE muscle (pig H-9) at 24 hours postmortem. Arrows mark areas of Z line disruption. Z = Z line, A = A band and I = I band. X 18,400.

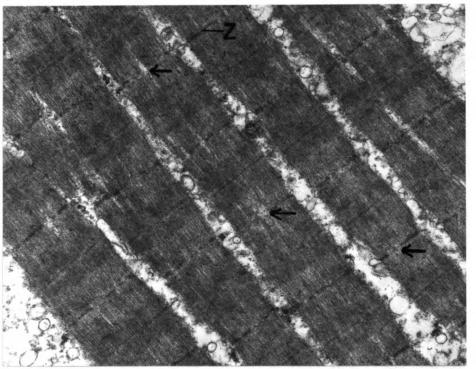


Figure 27. Electron micrograph showing myofibrils of a white fiber from PSE muscle (pig H-10) at 24 hours postmortem. Arrows mark areas of Z line disruption. Z = Z line. X 19,300.


Figure 28. Electron micrograph showing a high magnification of myofibrils from a PSE muscle intermediate fiber (pig Y-12) at 24 hours postmortem. Arrows mark areas of filament separation. Z = Z line and A = A band. X 34,100.

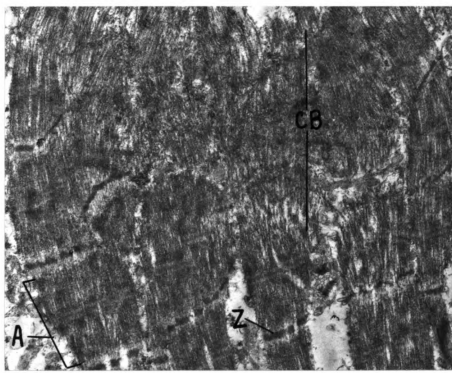


Figure 29. Electron micrograph showing a contracture band (CB) from PSE muscle (pig H-3) at 24 hours postmortem. Z = Z line and A = A band. X 19,300.

Cassens et al. (1963a) reported some disruption of myofibrils from antemortem heat-induced PSE muscle at 24 hours postmortem. This is in agreement with the results of the present study, where disruption of PSE myofibrils at 24 hours postmortem was observed.

Greaser et al. (1969a) reported a difference between the isolated myofibrils from PSE and normal muscle at 24 hours postmortem. They stated that myofibrils from PSE muscle showed wider Z lines, and the myofibrils had a more granular appearance than similar normal muscle. No difference in Z line width between PSE and normal myofibrils was found in the present study on comparing all three fiber types. This suggests that differences in the width of the Z line observed by Greaser et al. (1969a) could have been the result of comparing different fiber types. However, observations in the present investigation confirm the slight difference in granularity between PSE and normal myofibrils as reported earlier by Greaser et al. (1969a).

The presence of contracture bands in PSE myofibrils at 24 hours postmortem agrees with a study by Cassens et al. (1963b), in which they reported that formation of contracture bands is reversible if postmortem glycolysis is normal. However, the band formation is irreversible, if postmortem glycolysis is rapid and rigor occurs rapidly.

## Mitochondria

Fifteen Minute Postmortem Samples. An electron micrograph showing mitochondria from a red and an intermediate PSE muscle fiber at 15 minutes postmortem is shown in Figure 30. Mitochondria from the red fiber show

closely packed cristae and a fairly dense appearance, but have a more open structure than mitochondria from normal red fibers (Figure 6). Mitochondria from the intermediate PSE fiber at 15 minutes postmortem show less closely packed cristae and are less dense in appearance than mitochondria from normal intermediate fibers (Figure 7).

An electron micrograph showing mitochondria of a white fiber from PSE muscle at 15 minutes postmortem is presented in Figure 31. These mitochondria have an extremely open structure and show considerable disruption. As compared to mitochondria from normal white fibers, their structure corresponds more nearly to that of normal fibers at 24 hours postmortem.

The appearance of mitochondria from the three PSE fiber types at 15 minutes postmortem was more varied than that for normal fibers at the same time period. Mitochondria of some PSE red fibers had a structure nearly identical to that of normal red fibers at 15 minutes postmortem (Figure 10), while mitochondria from some similarly treated red fibers were nearly the same as those from normal 24 hour red fibers (Figure 16). Similarly, great variation was found in mitochondria of 15 minutes postmortem white and intermediate fibers from PSE muscle.

In general, mitochondria of all three fiber types from PSE muscle at 15 minutes postmortem showed much more disruption than those of corresponding fiber type from normal muscle. Also mitochondria from PSE red fibers were less susceptible to disruption than mitochondria from PSE white and intermediate fibers.

Greaser et al. (1969a) have shown that mitochondria isolated from PSE muscle shortly after death have a more disrupted appearance than mitochondria

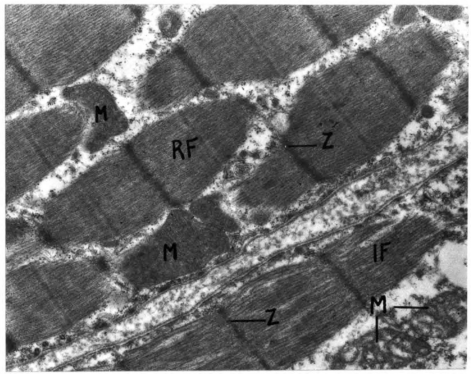


Figure 30. Electron micrograph showing mitochondria of red and intermediate fibers from PSE muscle (pig Y-11) at 15 minutes postmortem. IF = intermediate fiber, RF = red fiber, Z = Z line and M = mitochondria. X 18,900.

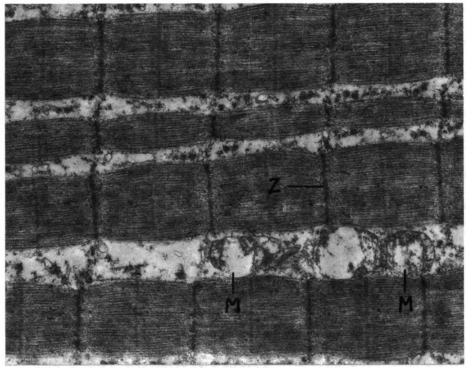


Figure 31. Electron micrograph showing mitochondria of a white fiber from PSE muscle (pig Y-12) at 15 minutes postmortem. M = mitochondria and Z = Z line. X 19,400.

isolated from normal muscle at the same time interval. Results obtained in the present study also show that more disruption occurred in the PSE mitochondria. Variation in the appearance of mitochondria from PSE muscle probably reflects a difference in the rate of postmortem change, since other features (SR and glycogen) of fibers with disrupted mitochondria are more nearly like normal muscle at 24 hours postmortem. However, the possibility that an inherent difference existed could not be ruled out.

Twenty Four Hour Postmortem Samples. Figures 32, 33 and 34 show mitochondria from red, intermediate and white fibers, respectively. All fibers shown are from PSE muscle at 24 hours postmortem. Mitochondria from all three fiber types showed considerable disruption. In most cases, mitochondria from PSE muscle at 24 hours postmortem had a less distinct structure than mitochondria from normal muscle at the same postmortem time (Figures 16 and 17). Red fiber mitochondria showed less disruption than mitochondria from white and intermediate fibers.

Results of this study agree with those of Greaser et al. (1969a), who showed extreme disruption of mitochondria in isolated mitochondrial fractions from PSE muscle at 24 hours postmortem. However, the amount of disruption on using intact muscle in the present study was not as great as that found by Greaser et al. (1969a).

## Sarcoplasmic Reticulum and T System

Fifteen Minute Postmortem Samples. The SR from a red fiber of PSE muscle (Figure 35) shows considerable disruption and appears to have open

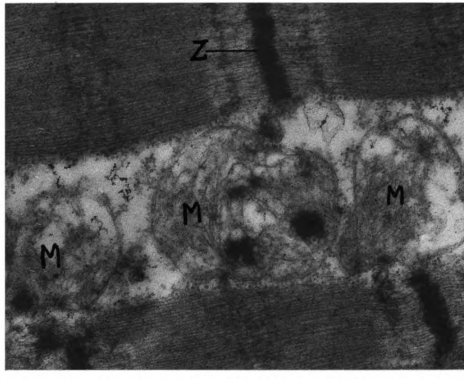


Figure 32. Electron micrograph showing mitochondria of a red fiber from PSE muscle (pig Y-11) at 24 hours postmortem. Z = Z line and M = mitochondria. X 46,200.

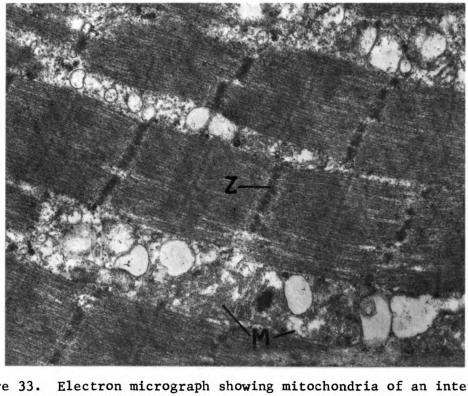


Figure 33. Electron micrograph showing mitochondria of an intermediate fiber from PSE muscle (pig H-9) at 24 hours postmortem. Z = Z line and M = mitochondria. X 23,800.

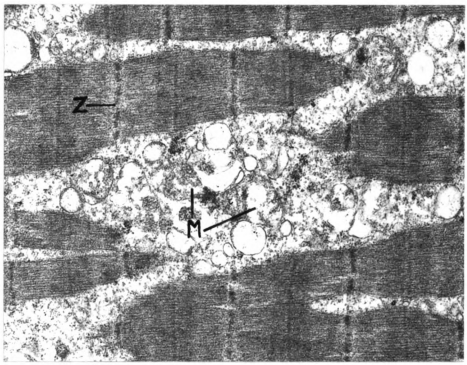


Figure 34. Electron micrograph showing mitochondria of a white fiber from PSE muscle (pig H-10) at 24 hours postmortem. Z = Z line and M = mitochondria. X 21,400.

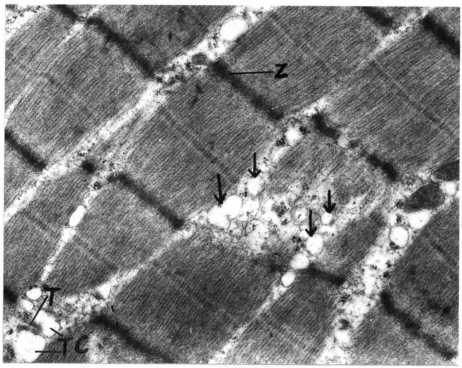


Figure 35. Electron micrograph showing the SR of a red fiber from PSE muscle (pig Y-11) at 15 minutes postmortem. Arrows point to vesicles or open tubules. Z = Z line, T = triad and TC = terminal cisternae. X 22,400.

tubules or vesicular type structures between the myofibrils. There is still some evidence of triads at 15 minutes postmortem, and the terminal cisternae are generally distended. The SR in some red fibers from PSE muscle at 15 minutes postmortem does not show much disruption, and appears to be almost the same as in normal red fibers at 15 minutes postmortem.

The intermediate fiber in Figure 36 shows marked disruption of the SR, with the tubules between the triads being almost completely absent. The triads are clearly evident. However, the SR of intermediate fibers from 15 minute postmortem PSE muscle was not always so disrupted. In some cases, the SR from intermediate fibers appeared almost the same as for normal 15 minute samples.

The white fiber shown in Figure 37 also shows extreme disruption of the SR, with very little structure being evident between the triads. Nevertheless, some triads are still evident. The large amount of variability in the SR ultrastructure among various white fibers from PSE muscle at 15 minutes postmortem was similar to that for intermediate and red fibers. Disruption of the SR in some fibers of each type could be due to rapid postmortem changes or to an inherent difference in the individual fibers.

Cassens et al. (1963a) have reported rapid postmortem disruption in the sarcoplasmic components of antemortem heat-induced PSE muscle, but no specific mention was made as to the effect on the SR. Results of the present study agree with those of Cassens et al. (1963a) in that rapid postmortem disruption of the sarcoplasmic components was clearly evident. The present study also showed that specific disruption of the SR occurred in

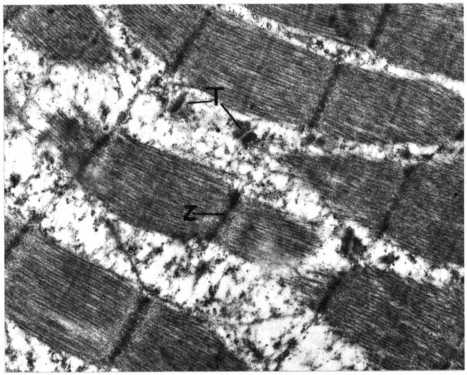


Figure 36. Electron micrograph showing the SR of an intermediate fiber from PSE muscle (pig Y-11) at 15 minutes postmortem. Z = Z line and T = triad. X 22,400.

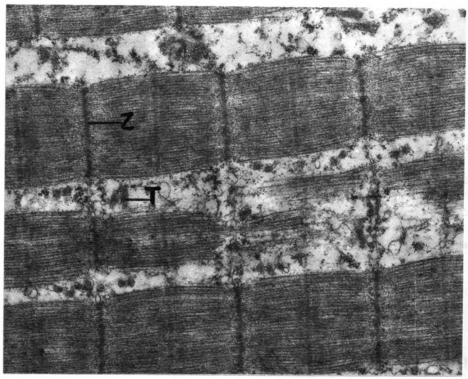


Figure 37. Electron micrograph showing the SR of a white fiber from PSE muscle (pig Y-12) at 15 minutes postmortem. Z = Z line and T = triads. X 22,700.

15 minute postmortem PSE muscle. Similarly, Greaser et al. (1969b) have shown isolated SR fractions of PSE muscle rapidly lose their calcium accumulating ability after death. The decrease could be due to disruption of the membranes, which was demonstrated to occur in the present study.

Twenty Four Hour Postmortem Samples. Electron micrographs showing the SR of red, intermediate and white fibers from PSE muscle at 24 hours postmortem are presented in Figures 38, 39 and 40, respectively. As can be seen, the SR and T system are almost completely absent in all fiber types. The appearance of the SR for each fiber type is almost the same as for normal muscle at 24 hours postmortem (Figures 18, 19 and 20). The SR of PSE muscle does, however, seem to be somewhat more disrupted, particularly in the red fibers. The SR for all PSE fibers was similar in appearance at 24 hours postmortem.

Greaser et al. (1969a) found no difference in the appearance of the heavy SR fraction isolated from PSE muscle at 24 hours postmortem as compared to the normal muscle fraction at the same postmortem time. Their results plus those of the present study indicate that postmortem disruption is greater in PSE muscle and occurs at an earlier postmortem time. Nevertheless, normal muscle ultimately showed approximately the same amount of disruption by 24 hours postmortem.

Other Components

No differences were observed in the nuclei from PSE and normal muscle at either 15 minutes or 24 hours postmortem. The amount of glycogen present in PSE muscle showed extreme variation at 15 minutes postmortem. Those

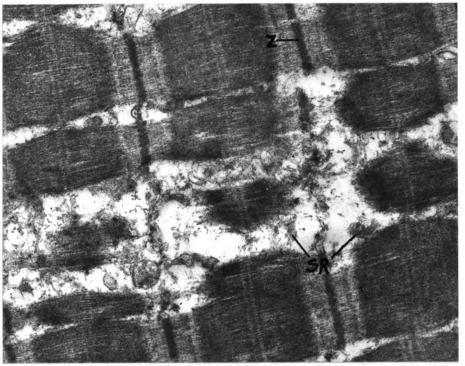


Figure 38. Electron micrograph showing the SR of a red fiber from PSE muscle (pig Y-9) at 24 hours postmortem. Z = Z line and SR = possible remnants of sarcoplasmic reticulum. X 20,900.

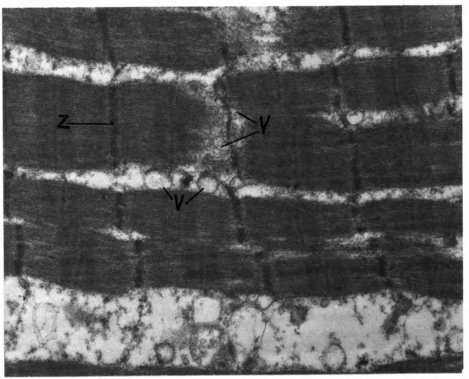


Figure 39. Electron micrograph showing the SR of an intermediate fiber from PSE muscle (pig H-9) at 24 hours postmortem. Z = Z line and V = vesicular remnants of the SR. X 20,900.

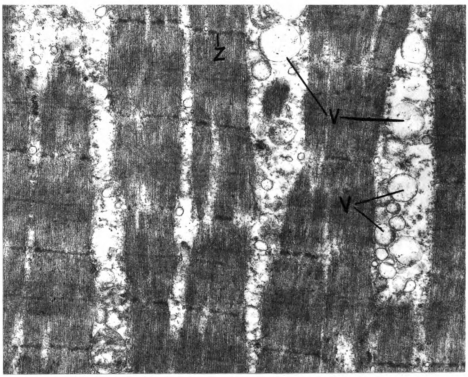


Figure 40. Electron micrograph showing the SR of a white fiber from PSE muscle (pig H-10) at 24 hours postmortem. Z = Z line and V = vesicular remnants of the SR. X 17,000.

fibers showing the most disruption of other components (mitochondria and SR) contained less glycogen. This indicates a faster rate of postmortem glycolysis. By 24 hours postmortem, however, there was little or no observable glycogen present in either PSE or normal muscle fibers.

## Electron Microprobe Analysis of Pig Muscle

Since the electron microprobe can be used to measure relative concentrations of elements in localized tissue areas, it was decided to use this instrument to measure the sodium, potassium, calcium and magnesium distribution in contracted and relaxed muscle tissue.

A secondary electron image of longitudinally sectioned pig muscle tissue is presented in Figure 41. This sample was prepared by freeze drying. Figure 42 shows a sample sectioned on the cryostat. The sample prepared by freeze drying (Figure 41) shows considerable tissue disruption, making it difficult to distinguish individual cells. The sample sectioned on the cryostat (Figure 42) shows fairly intact cells with myofibrils or groups of myofibrils being evident. The normal striations present in skeletal muscle upon observation by transmission methods cannot be seen, but the general topography is clearly evident. Since the amount of disruption was extensive in the freeze dried sample, sections prepared on the cryostat were used for all subsequent electron microprobe studies.

The lack of preservation in freeze dried tissues could be due to incomplete freeze drying, because freeze drying has previously been reported to give good tissue preservation (Pearse, 1968).

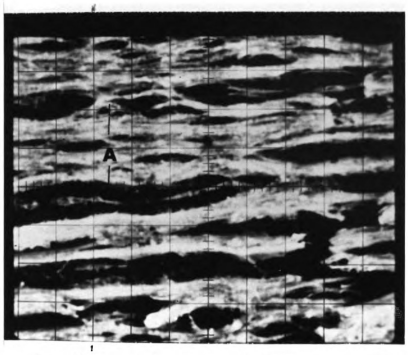


Figure 41. A secondary electron image of relaxed muscle from pig H-8 prepared by freeze drying. A marks the probable boundaries of a muscle fiber. X 550.

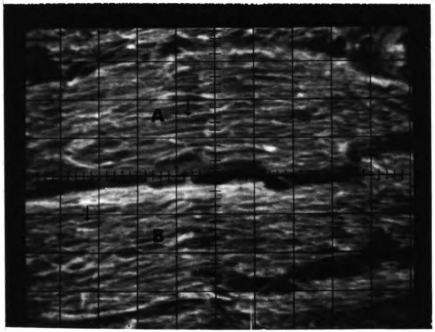


Figure 42. A secondary electron image of relaxed muscle from pig H-8 prepared by cryostat sectioning. A and B point out separate fibers and arrows mark individual myofibrils. X 850.

Preliminary samples were analyzed to ascertain the amount of elemental variation in pig muscle tissue. A secondary electron image of low magnification is presented in Figure 43 and shows a group of muscle cells between two areas containing perimysial connective tissue. A line scan of x-ray emission for sodium, potassium, calcium and magnesium was taken from a fixed area marked in Figure 43. The results are presented in Figures 44, 45, 46 and 47 for sodium, potassium, calcium and magnesium, respectively. The scan for individual elelents does not give absolute concentrations but is relative within the same scan.

The line scan for sodium (Figure 44) shows that areas of low concentrations occurred at scan positions of 0.5, 2.3 and 7.4. All of these values correspond to areas in which organized tissue appears to be absent. Two areas of higher sodium concentration occurred at scan positions of 1.8 and 6.8. These two areas appear to be localized in connective tissue. Another area of relatively high sodium concentration was present at a scan position of 5.8 and appears to correspond to muscle tissue. Upon examination of the area, there was no evidence for any specific structures that might contain high amounts of sodium.

The line scan for potassium (Figure 45) shows a relatively constant potassium concentration, except that a high amount occurred at a scan position of 6.8. This value appears to be associated with connective tissue. However, the other area of connective tissue at a scan position of 1.8 had a low concentration of potassium. Thus, connective tissue *per se* does not always contain a high concentration of potassium.

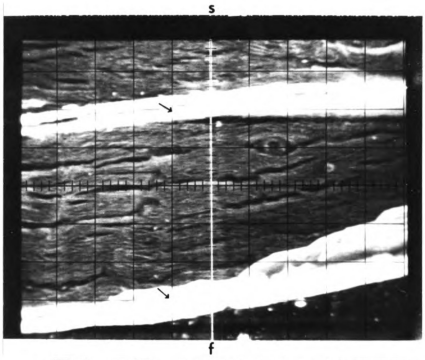


Figure 43. A secondary electron image of relaxed muscle from pig H-8 prepared by cryostat sectioning. The line from s to f marks the area of a line scan and the letters a and b mark connective tissue. X 200 (Each major division = 40μ).

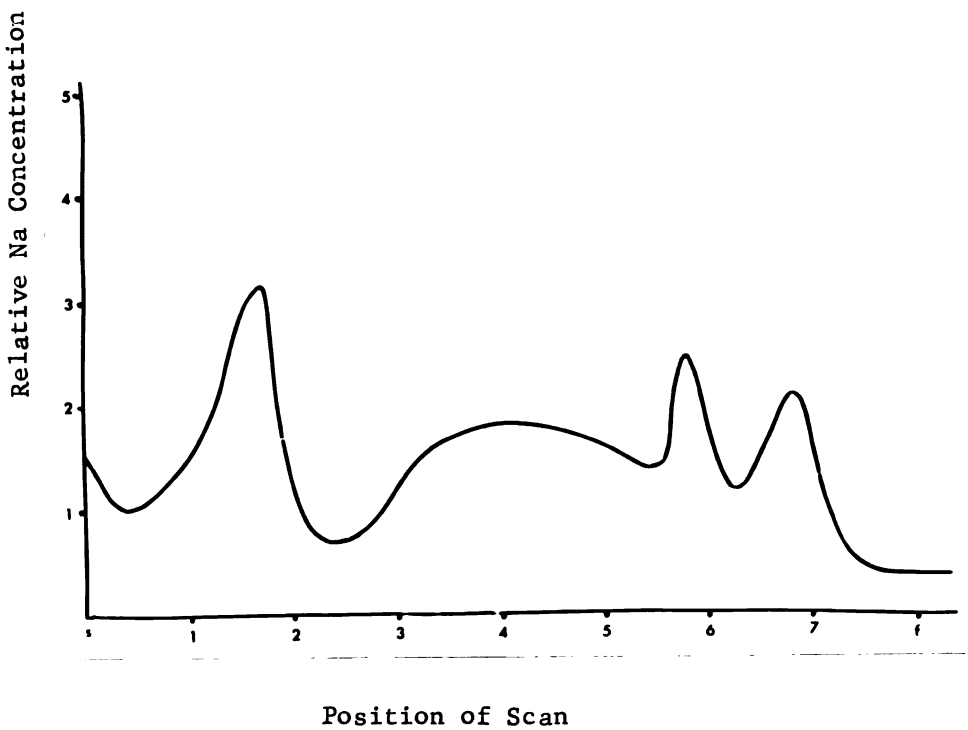


Figure 44. A recording of the sodium intensity along the line from s to f as shown in Figure 43. The numbers on the abscissa correspond to each major division along that line.

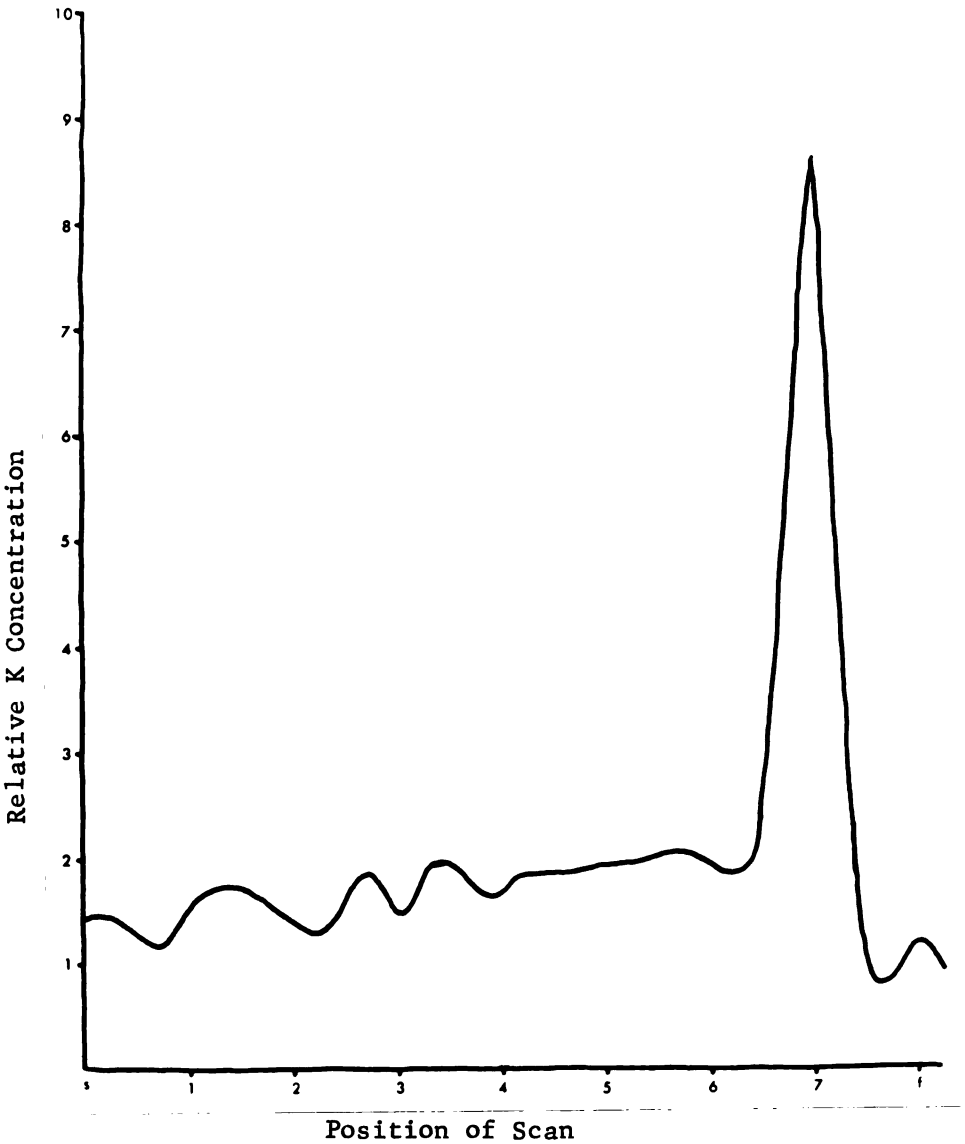


Figure 45. A recording of the potassium intensity along the line from s to f as shown in Figure 43. The numbers on the abscissa correspond to each major division along that line.

The line scan for calcium as shown in Figure 46, shows low concentrations at scan positions of 0.5, 2.3 and 7.4. All of these areas appear to be essentially devoid of organized tissue. Three areas containing a relatively high concentration of calcium can be seen at scan positions of 3.5, 5.1 and 6.8. The areas at scan positions of 3.5 and 5.1 are found in muscle tissue. Evidence of specific structures that might be responsible for the high calcium concentration in these areas were not apparent. The area at a scan position of 6.8 is localized in connective tissue.

The scan for magnesium (Figure 47) shows much the same pattern as for calcium, with low values at scan positions of 2.3 and 7.4, and relatively high concentrations at scan positions of 3.5, 5.1 and 6.8.

A photograph of the potassium x-ray emission for the tissue shown in Figure 43 was taken to determine the distribution of potassium. The photograph is presented in Figure 48 and shows a relatively high concentration of potassium in the connective tissue area marked by b in Figure 43. The other connective tissue area (area a in Figure 43) showed a relatively small amount of potassium, particularly where the line scan was made.

The differences in elemental concentration found between the various tissue areas were generally small. This could be due to an even distribution of elements throughout the tissue or perhaps be the result of movement of elements during tissue preparation. There did, however, seem to be a higher concentration of elements (particularly potassium) in the area of connective tissue marked by b in Figure 43.

In studying muscle tissue with the electron microprobe, Engel (1968) found very little sodium and calcium to be present. He further indicated

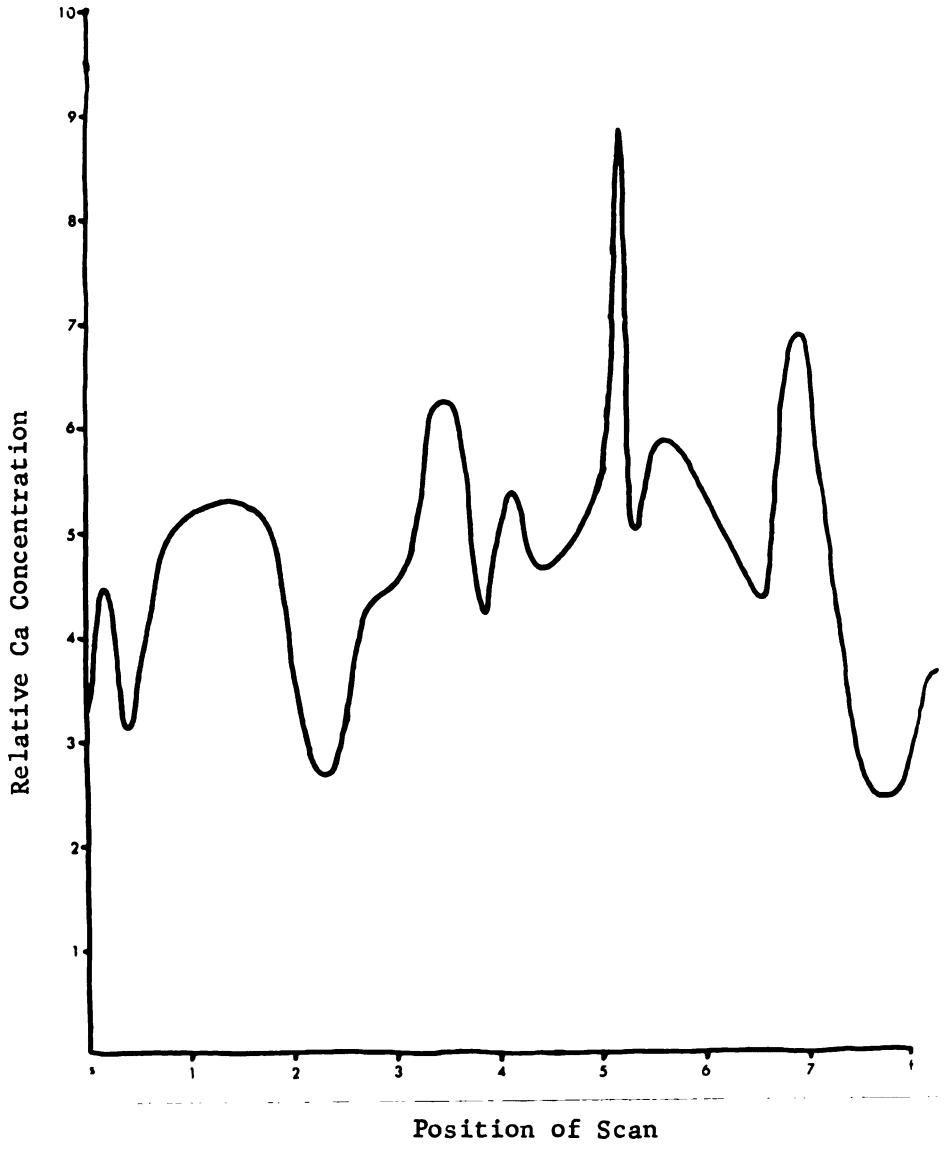


Figure 46. A recording of the calcium intensity along the line from s to f as shown in Figure 43. The numbers on the abscissa correspond to each major division along that line.

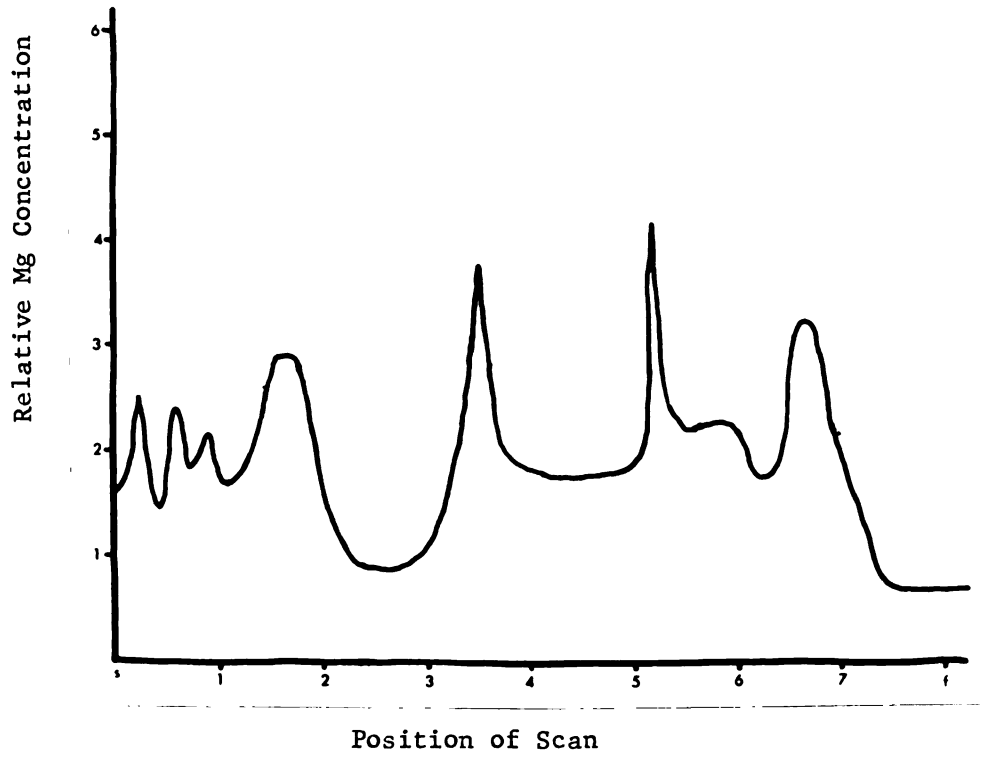


Figure 47. A recording of the magnesium intensity along the line from s to f as shown in Figure 43. The numbers on the abscissa correspond to each major division along that line.

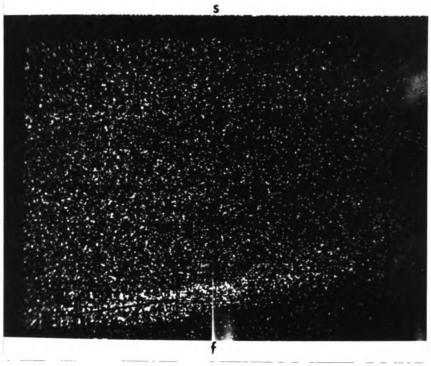


Figure 48. A photograph of the potassium x-ray emission over the area shown in Figure 43. Arrows mark areas of connective tissue. The white spots show areas of potassium localization.

that there was no difference in the concentration of these elements in different tissue areas. He did, however, find more potassium in muscle tissue than sodium and calcium. He did not find any differences in potassium concentration for various muscle fibers. The results of the present study agree in that very little difference was noted in the elemental concentration for different tissue areas.

Since electron microprobe analysis was expensive and results showed only small differences in the concentration of various elements, microprobe analysis was discontinued. The differences in the concentrations of the various elements were small between different tissue areas and did not appear to be related to specific structures. However, this does not show that elemental concentrations are the same in all areas of muscle tissue. A more extensive study using more samples and new advancements in microprobe design might provide more meaningful insight into this problem.

## Changes in the Ultrastructure of Meat During Spoilage

### Bacterial Growth

The log bacterial numbers per gram of muscle were 5.05, 9.48 and 10.34 for the inooulated samples after incubation for 0, 8 and 20 days, respectively. Bacterial growth was not detected in control samples incubated for 0, 8 and 20 days. Inoculated samples appeared to be spoiled after incubation for 8 days. However, a greater amount of spoilage was evident after 20 days incubation. The major portion of bacterial growth appeared to be on the surface of the ground meat samples. This is not surprising, since Pseudomonas fragi is an aerobe.

Bacterial counts were not taken for the APT and non-protein media. However, large numbers of bacteria were present in both media on observation under a phase contrast microscope.

Changes in Muscle Ultrastructure Due to Spoilage

There were no discernible differences in the ultrastructural appearance of control and inoculated samples at 0 days incubation. The ultrastructural appearance of both control and inoculated muscle at 0 days was the same as that previously described for normal pig muscle at 24 hours postmortem, except for the fact that some fibers and myofibrils were ruptured-presumedly due to grinding.

Although more bacterial growth and more spoilage was evident in samples inoculated and then incubated for 20 days, the ultrastructure did not appear to be any different than that after inoculation and incubation for 8 days. This probably reflects the fact that most spoilage occurs on the outer surface of the muscle mass, since Pseudomonas fragi is an aerobic organism. Thus, it appears that the maximum amount of tissue disruption had occurred on the surface of the sample by the end of 8 days incubation.

Marked alteration of the myofibrillar ultrastructure occurred in muscle inoculated and incubated with Pseudomonas fragi as can be seen by comparing Figures 49 and 50. The myofibrils (Figure 50) showed an extremely disrupted appearance, particularly in the A band region. Furthermore, the H zone is almost devoid of the original material. Few if any thick (myosin) filaments are still evident. Most of the dense material from the Z line was also lost. The actin filaments of the I band are fairly distinct,

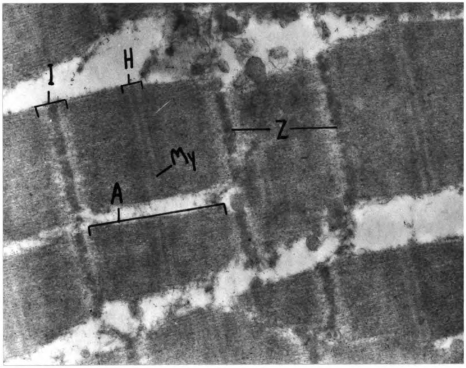


Figure 49. Electron micrograph of myofibrils from uninoculated pig muscle incubated at 10°C for 20 days. Z = Z line, A = A band, I = I band, H = H zone and My = myosin filaments. X 28,600.

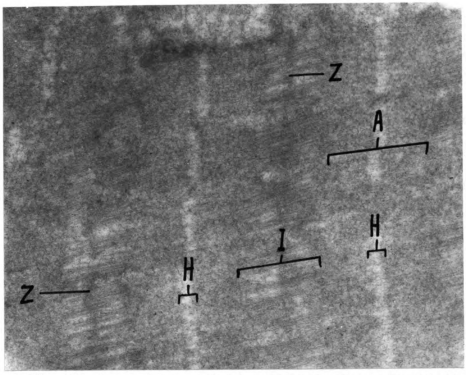


Figure 50. Electron micrograph of myofibrils from pig muscle inoculated with Pseudomonas fragi and incubated for 20 days at 10°C. Z = Z line, A = A band, I = I band and H = H zone. X 28,600.

however, and appear to extend into the A band, where they become less apparent and show a more granular appearance (Figure 51). The granular appearance in this region may be due to remnants of myosin filaments that remain attached to the actin filaments.

A cross section of myofibrils from uninoculated control muscle is presented in Figure 52. Both myosin and actin filaments are clearly evident. Figure 53 shows a similar cross sectional area for muscle that has been inoculated and incubated with Pseudomonas fragi. Myosin filaments are no longer evident, although a few actin filaments can still be seen.

Tarrant et al. (1971a) showed no significant change in the myofibrillar fractions of muscle inoculated with Pseudomonas fragi at 8 days of incubation, but found a significant decrease in this fraction after 20 days. However, they did find some change in the electrophoretic banding pattern of myofibrillar proteins after 8 days of incubation. They then concluded that Pseudomonas fragi grew only on the surface of the ground meat sample, thus, proteolysis was not detected until spoilage had proceeded further and affected the entire mass of meat. However, results of the present study suggest that proteolysis commences at an earlier stage as shown by the marked alterations in the myofibrils after 8 days incubation. Early myofibrillar disruption would likely be easier to detect by electron microscopy, since the samples were taken from the outer surface of the muscle mass where bacterial growth was greatest.

Results of the present study show that breakdown of myofibrils occurred as a result of spoilage by Pseudomonas fragi, and support the

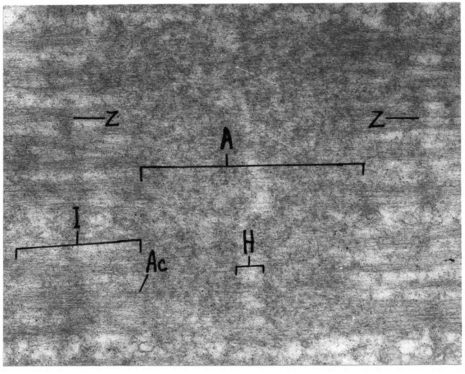


Figure 51. Electron micrograph showing one sarcomere of a myofibril from pig muscle after inoculation with Pseudomonas fragi and incubation for 20 days at 10°C. Z = Z line, A = A band, I = I band, H = H zone and Ac = actin filaments. X 58,900.

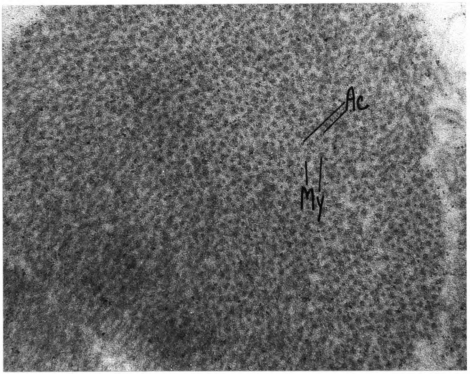


Figure 52. Electron micrograph showing a cross section of a myofibril from uninoculated pig muscle after incubation at 10°C for 20 days. Ac = Actin filaments and My = myosin filaments. X 113,400.

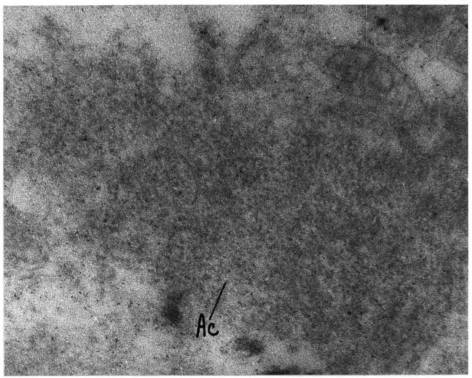


Figure 53. Electron micrograph showing a cross section of a myofibril from pig muscle inoculated with Pseudomonas fragi and incubated at 10°C for 20 days. Ac = Actin filaments. X 113,400.

results of other authors (Borton et al., 1970a, b), who have shown proteolysis of myofibrillar proteins occurs in inoculated muscle. In addition, results of the present study show specific disruption of components in the A band region, probably myosin, and removal of material from the Z line as a consequence of bacterial growth. Tarrant et al. (1971b) have isolated a proteolytic enzyme from muscle inoculated and incubated with Pseudomonas fragi. This enzyme may be the cause of the myofibrillar disruption found in the present study.

Bacterial Ultrastructure

Bleblike evaginations or protrusions were found to be present on the surface of Pseudomonas fragi organisms that were observed in spoiled muscle. These blebs can be seen in Figure 54. They contain dense granular material, and appear to be present on the entire surface of the bacteria.

A higher magnification of a bacterial cell in the process of division occurring in spoiled muscle tissue is presented in Figure 55. Globules containing dense granular material identical or similar to that in the blebs can be seen in close proximity to the cell. These globules may be formed from the surface blebs. The globules, however, are not apparent within the muscle mass. It is postulated that the absence of the intact globules within the muscle mass may be due to rupture of these globules on contact with muscle tissue, thus, releasing their contents. However, the possibility that muscle tissue masks the globular structure can not be ruled out on the basis of present evidence.

A very high magnification of a surface bleb is presented in Figure 56. As can be seen in this figure, the blebs contain small granules and

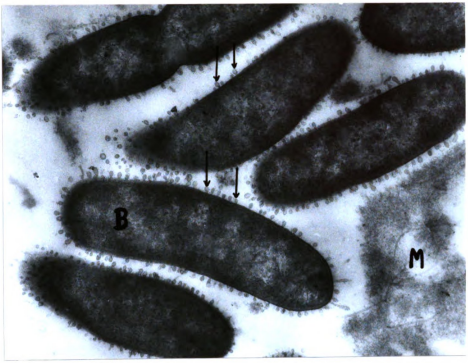


Figure 54. Electron micrograph showing Pseudomonas fragi organisms that were present in muscle tissue that had been inoculated and incubated at 10°C for 8 days. B = bacterial cell, M = remnants of muscle tissue and arrows point to bleblike evaginations from the cell wall. X 43,700.

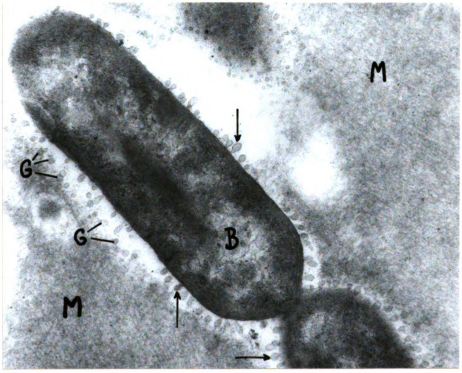


Figure 55. Electron micrograph of a dividing Pseudomonas fragi organism that was present in muscle tissue that had been inoculated and incubated at 10°C for 8 days. B = bacterial cell, M = remnants of muscle tissue, G = globules formed from surface blebs and arrows point to surface blebs. X 58,000.

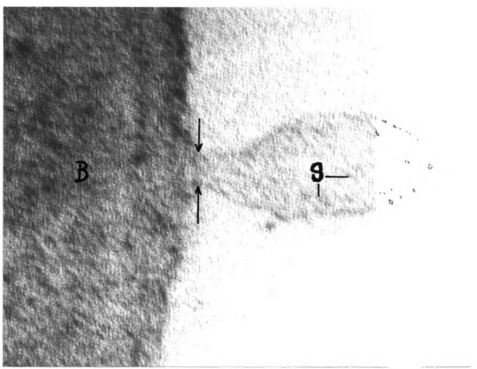


Figure 56. Electron micrograph showing a very high magnification of a bleblike evagination from the surface of a Pseudomonas fragi organism that was present in muscle tissue that had been inoculated and incubated at 10°C for 8 days. B = bacterial cell, g = dense granular material and arrows point to the apparent continuation of the bleb membrane with the outer surface of the cell wall. X 918,000.

are surrounded by a membrane, which appears to be continuous with the outer surface of the bacterial cell wall.

Knox et al. (1966) have shown a lipopolysaccharide to be secreted in globules that were formed from blebs on the surface of Escherichia coli organisms. Hitchins and Sadoff (1970) have also shown that material involved in formation of a cyst capsule was released by Azotobacter vinelandii in protrusions or blebs that later separated from the cell to form globules. The blebs observed in the present study have the same appearance as those described by Knox et al. (1966) and Hitchins and Sadoff (1970) which indicates that they may contain a specific substance (possibly a protease).

Certain nutritional and physiological conditions have been shown to induce the formation of blebs on the cell wall of some pseudomonads (Weibe and Chapman, 1968a, b). Thus, it is possible that the formation of blebs in the present study may be the result of growing the bacteria in muscle tissue.

To determine if bleb formation was caused by growing the bacteria on muscle tissue, bacteria were grown in APT and non-protein media. The bacteria grown in these media were prepared for electron microscopic examination in an identical manner to those grown in muscle tissue. Electron micrographs of bacteria grown in APT and non-protein media are presented in Figures 57 and 58, respectively. The Pseudomonas fragi organisms grown on non-muscle media did not contain any blebs or surface evaginations (Figures 57 and 58). Thus, results of the present study suggest that the

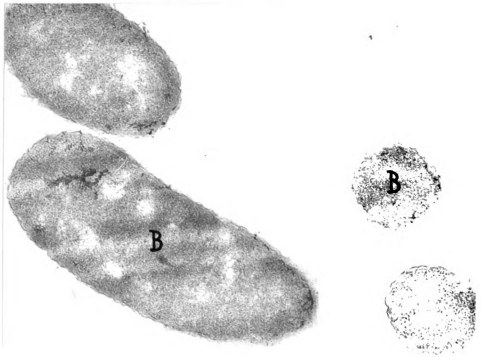


Figure 57. Electron micrograph showing Pseudomonas fragi organisms that were grown in APT media at 10°C for 6 days. B = bacterial cell. X 45,600.

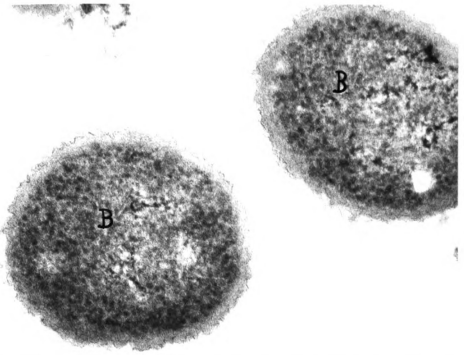


Figure 58. Electron micrograph showing Pseudomonas fragi organisms that were grown in non-protein media at 10°C for 12 days. B = bacterial cell. X 125,800

bleblike evaginations and globules are formed on the surface of Pseudomonas fragi organisms as a result of their being grown on muscle tissue.

Results of the present study show that marked alteration in the myofibrillar ultrastructure occurs due to the growth of Pseudomonas fragi on muscle tissue. It is postulated that the material contained in the blebs may be a protease, and thus, could be responsible for myofibrillar disruption.

## SUMMARY

Ultrastructural changes in porcine muscle as influenced by postmortem aging, by the PSE (pale, soft and exudative) muscle condition and by bacterial spoilage were investigated. In addition, the position and relative concentrations of sodium, potassium, calcium and magnesium ions were determined in contracted and relaxed muscle.

At 15 minutes postmortem, muscle fibers were classified as red, white and intermediate on the basis of their ultrastructural similarity to these same classes in rat muscle. Red fibers had dense appearing Z lines about 1,200 Å in width, whereas, the Z lines from white fibers were much less dense and were approximately 625 Å wide. Intermediate fibers were about 775 Å in width and contained Z lines of intermediate density. More mitochondria were present in red fibers than in white or intermediate fibers, with white fibers having the smallest number. Mitochondria were located at the Z line, in interfibrillar rows and just beneath the sarcolemma for red and intermediate fibers. White fibers contained mitochondria at the Z line and beneath the sarcolemma, but none were observed in interfibrillar rows. Cristae of red fiber mitochondria were abundant and closely packed, whereas, cristae of mitochondria from white fibers were fewer in number and more open in structure. The structure of mitochondria from intermediate fibers was intermediate between that of red and white fibers. The SR formed tortuous tubules in the region of the A band for red and intermediate fibers, but appeared as flattened sacs or open tubules in white fibers.

At 24 hours postmortem, the three fiber types were still evident with the relationship of Z line width and mitochondrial distribution remaining the same as at 15 minutes postmortem, There was, however, a marked disruption of the SR and of mitochondria in all fiber types. Some loss of Z line material in white and intermediate fibers was also apparent at 24 hours. Red fibers appeared to be less susceptible to postmortem disruption than intermediate or white fibers.

There was considerable variation in the extent of disruption for all fiber types from PSE muscle at 15 minutes postmortem. In general, all fiber types from 15 minute PSE muscles had a structure more nearly like that of normal muscle at 24 hours postmortem. There were, however, a few fibers that showed very little disruption and appeared similar to normal muscle at 15 minutes postmortem. Red fibers in PSE muscle showed less disruption at 15 minutes postmortem than white or intermediate fibers. Muscle from PSE animals also had a larger number of contraction bands at 15 minutes postmortem than normal muscle.

A slightly more granular appearance of the myofibrils was observed in PSE muscle at 24 hours postmortem than in normal muscle at the same time. PSE muscle was more disrupted than normal muscle at 24 hours postmortem, but the difference was not as great as at 15 minutes.

Samples sectioned on the cryostat were found to have better tissue preservation upon electron microprobe analysis than samples prepared by freeze drying. Electron microprobe analysis showed very little difference in the concentration of various elements between different tissue areas. Because of the minor differences in ion concentrations between

various tissue areas and the high cost of microprobe operation, only preliminary samples were analyzed.

The myofibrils from pig muscle that had been incoulated and incubated with Pseudomonas fragi showed an extremely disrupted appearance as compared to uninoculated controls. There was an absence of most of the dense Z line material, and the H zone was almost completely devoid of the original material. Myosin filaments could no longer be observed in the A band, which had taken on a very granular appearance. However, actin filaments were still evident.

Small surface evaginations or blebs were observed on the cell walls of most Pseudomonas fragi organisms. Globules were also observed in the immediate area surrounding the bacteria. The fact that the globules and blebs appeared to contain the same granular type material indicates that the globules were formed by the blebs. Pseudomonas fragi organisms that were grown on non-muscle media did not have any bleblike structures. It was postulated that the bacteria exert their action by secreting a substance-probably a protease-into the blebs. The blebs appear to form globules, which then disintegrate upon contact with the muscle tissue.

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# APPENDIX

Appendix 1. Schedule for preparation of 1.25% glutaraldehyde fixative.

| Compound | Amount | Final molarity |
|---|---|---|
| $Na\ H_2\ PO_4 \cdot H_2O$ | 1.8 gm | 0.007M |
| $Na_2\ H\ PO_4 \cdot 7\ H_2O$ | 23.25 gm | 0.041M |
| NaCl | 5.0 gm | 0.043M |
| 50% Commercial glutaraldehyde | 50 ml | |
| $H_2O$ | 1975 ml | |

Appendix 2. Schedule for preparation of glutaraldehyde washing buffer.

| Compound | Amount | Final molarity |
|---|---|---|
| $Na\ H_2\ PO_4 \cdot H_2O$ | 1.8 gm | 0.013M |
| $Na_2\ H\ PO_4 \cdot 7\ H_2O$ | 23.25 gm | 0.081M |
| NaCl | 5.0 gm | 0.086M |
| $H_2O$ | 925 ml | |

Appendix 3. Schedule for preparation of 1% osmium tetroxide fixative.

Stock solution A

| | |
|---|---|
| Sodium Acetate | 9.714 gm |
| Veronal-sodium | 14.714 gm |
| Distilled $H_2O$ to make | 500 ml |

Stock solution C

| | |
|---|---|
| Sodium Chloride | 40.25 gm |
| Potassium Chloride | 2.1 gm |
| Calcium Chloride | 0.9 gm |
| Distilled $H_2O$ to make | 500 ml |

Add 10 ml of Solution A, 3.4 ml of solution C and 26 ml of distilled water. Adjust pH with 0.1N HCl (approximately 11 ml).

Appendix 4. Procedure for preparation of epon embedding material.

Mixture A

94 g DDSA[1]
80 g Epon 812
Mix thoroughly

Mixture B

78 g NMA[2]
100 g Epon 812
Mix thoroughly

Add together 7 grams of mixture A, 3 grams of mixture B and 0.14 grams of DMP-30[3]. Mix for 5 minutes.

[1]Dodecenyl Succinic Anhydride

[2]Nadic Methyl Anhydride

[3]Dimethyl Amino Methyl Phenol

Appendix 5. Schedule for preparation of lead citrate stain.

| | |
|---|---|
| Lead Nitrate Pb $(NO_3)_2$ | 1.33 gm |
| Sodium Citrate $Na_3$ $(C_6H_5O_7)\cdot 2\ H_2O$ | 1.76 gm |
| $H_2O$ | 30 ml |

Shake above mixture for 30 minutes.
Add 8.0 ml of 1N Na OH and dilute to 50 ml with $H_2O$.

Appendix 6. Schedule for preparation of non-protein media for Pseudomonas fragi.

Stock solution A

| | |
|---|---|
| 1% | $NH_4Cl$ |
| 0.5% | glucose |
| 0.2% | $Na_2HPO_4$ |

Stock solution B

| | |
|---|---|
| 4% | $Mg\ SO_4 \cdot 7\ H_2O$ |
| 0.2% | NaCl |
| 0.2% | $Fe\ SO_4 \cdot 7\ H_2O$ |
| 0.72% | $Mn\ Cl_2 \cdot 4\ H_2O$ |

Media is prepared by adding 10 ml of stock solution B to 500 ml of stock solution A. The pH of this media is 7.4.

Appendix 7. Values for pH at 45 minutes and transmission values at 24 hours postmortem for all pigs of the Yorkshire and Hampshire breeds.

| Animal No. | % Transmission | Muscle pH | PSE or Normal[1] |
|---|---|---|---|
| | Yorkshire Pigs | | |
| Y-1 | 8.0 | 6.80 | N |
| Y-2 | 10.5 | 6.80 | N |
| Y-3 | 34.0 | 6.50 | |
| Y-4 | 22.0 | 6.50 | |
| Y-5 | 67.0 | 5.50 | PSE |
| Y-6 | 11.0 | 6.70 | N |
| Y-7 | 18.0 | 6.80 | N |
| Y-8 | 13.0 | 6.40 | |
| Y-9 | 79.5 | 5.50 | PSE |
| Y-10 | 10.0 | 6.06 | |
| Y-11 | 74.0 | 5.56 | PSE |
| Y-12 | 46.0 | 6.35 | PSE |
| Y-13 | 20.0 | 6.38 | |
| Y-14 | 15.0 | 6.68 | N |
| Y-15 | 14.0 | 6.58 | |
| Y-16 | 42.0 | 6.23 | PSE |
| | Hampshire Pigs | | |
| H-1 | 37.2 | 6.50 | |
| H-2 | 18.6 | 6.60 | |
| H-3 | 37.0 | 5.78 | PSE |
| H-4 | 7.0 | 6.70 | N |
| H-5 | 7.0 | 6.55 | N |
| H-6 | 6.0 | 6.65 | N |
| H-7 | 32.0 | 6.40 | |
| H-8 | 70.0 | 6.02 | PSE |
| H-9 | 60.0 | 6.57 | PSE |
| H-10 | 54.0 | 6.58 | PSE |
| H-11 | 18.0 | 6.80 | |
| H-12 | 35.0 | 6.55 | |
| H-13 | 22.0 | 6.40 | |
| H-14 | 29.0 | 6.58 | |
| H-15 | 12.0 | 6.66 | N |
| H-16 | 39.0 | 6.40 | PSE |
| H-17 | 12.0 | 6.86 | N |
| H-18 | 17.0 | 6.64 | |
| H-19 | 13.0 | 6.40 | |
| H-20 | 12.0 | 6.51 | |

[1]PSE = Pale, soft and exudative, N = normal, a blank space indicates the pig was not classified as to muscle condition and was not utilized for final analysis.