THE EFFECT OF ELECTRICAL STIMULATION ON DELAY CHILLED BOVINE CARCASSES

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THE EFFECT OF ELECTRICAL STIMULATION ON DELAY

CHILLED BOVINE CARCASSES

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CHAPTER I

INTRODUCTION

Many changes in the fabrication of the bovine carcass to retail cuts have taken place in the past three decades. Traditionally, sides of beef are chilled twenty-four hours at the slaughter facility before being transported to retail outlets. The sides or quarters of beef are then fabricated into retail cuts.

A modification of this processing method, termed "boxed beef", is being accepted by an ever-increasing segment of the industry. This process entails the breakdown of the chilled side of beef into wholesale cuts at the slaughter facility. The wholesale cuts are vacuum packaged, placed in boxes, and further processed on arrival at the retail outlets. Even though the "boxed beef" approach is more efficient than the traditional method in certain respects, limitations of this process do exist.

The next logical step in processing procedures by the meat industry relates to the fabrication of the beef carcass prior to chilling. This method involves the separation or seaming of muscle or muscle systems from bone and excess fat prior to chilling. The economic importance of hot muscle boning has been enhanced in recent years by ever-increasing utility, labor, transportation, and operating costs of previously discussed processing methods.

Economic advantages of hot muscle boning of the beef carcass are

varied (Will, 1974; Noble, 1977; Pierce, 1977; Kastner, 1977; McCollum, 1977). From a consumer's standpoint, muscle boning followed by chilling produces a boneless, closely trimmed product that lends itself well to marketability. However, before on-the-rail muscle removal of the warm carcass will be termed commercially feasible, a suitable eating quality must be provided in the end product.

Working toward this goal, the use of electrical stimulation is being studied as a means of accelerating rigor mortis in the muscles of the beef carcass. The lowering of pH and the depletion of ATP (adenosine triphosphate) are two chemical events which are closely interrelated in the transformation of muscle to meat. The rate and extent of post-mortem decline in pH and ATP at the onset of rigor mortis greatly influence the use of muscle for food. Therefore, it is felt that the key to success in hot muscle removal is accelerating the onset of rigor mortis.

The objectives of these experiments were: (1) to develop a procedure whereby the amount of ATP present in striated bovine muscle could be determined; (2) to monitor the effectiveness of electrical stimulation in causing the decline in ATP in selected muscles; (3) to measure the sarcomere length in stimulated and control muscles; and (4) to document the ultrastructural post-mortem changes associated with electrically stimulated muscles.

CHAPTER II

THE EFFECT OF ELECTRICAL STIMULATION ON ATP DEPLETION AND SARCOMERE LENGTH IN DELAY CHILLED BOVINE MUSCLE[1,2,3]

Summary

This study was undertaken to assess the effectiveness of electrical stimulation as a means of speeding post-mortem metabolism as measured by ATP (adenosine triphosphate) depletion in delay chilled bovine carcasses.

Six animals of similar weight and age were used in this study. Electrical stimulation was initiated 30 minutes post-mortem. The stimulated side received a square-wave pulse of a magnitude (peak) of 300 volts at 400 cycles/second (frequency), with a duration of 0.5 milliseconds and a current of 1.9 amps. ATP measurements were taken at 8 time periods (0.5, 1.0, 2.0, 4.0, 6.0, 8.0, 12.0, and 24.0 hours) post-mortem. Results indicated muscles from the stimulated sides of beef

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[1]Journal article of the Agriculture Experiment Station, Oklahoma State University, Stillwater.

[2]P. A. Will, R. L. Henrickson, R. D. Morrison and G. V. Odell.

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exhibited significantly faster reductions of ATP than unstimulated controls. Electrical stimulation is an effective means of speeding postmortem glycolysis and rigor mortis.

Shear force data published by Pierce (1977) indicated increased tenderness from electrically stimulated sides of beef. However, the measurement of sarcomere lengths from the same research animals revealed no significant differences between electrically stimulated and unstimulated sides. This indicated that some mechanism, in addition to the prevention of "cold shortening", brought about the tenderization of the meat.

Introduction

Electrical stimulation of the meat carcass is not a new idea developed in recent years. Benjamin Franklin in 1749, in writings published by Lopez and Hurbert (1975), discovered that electrical stimulation of turkey carcasses made the resultant meat "uncommonly tender." Franklin's discovery was once again applied by Harsham and Deatherage (1951) in a patent which found electrically stimulated meat promoted tenderization (U.S. Patent 2,544,681). The use of an electrical current for tenderization was once again confirmed by Carse (1973) in working with lamb carcasses. Since 1973 several research projects have been initiated studying different aspects of electrical stimulation as it affects the muscle systems of the carcass (Chrystall and Hagyard, 1975; Davey et al. 1976; Bendall et al. 1976; Gilbert et al. 1976; Smith et al. 1977; Shaw and Walker, 1977). The application of an electrical current to freshly slaughtered beef carcasses has been shown to increase the rate of glycolysis and reduce the time for onset of rigor mortis

(Carse, 1973; Locker et al. 1975; Davey et al. 1976; McCollum and Henrickson, 1977; Shaw and Walker, 1977).

Working toward the goal of removal of muscle and muscle systems prior to chilling, the application of an electrical stimulus may have a great influence on the use of muscle for food. The rate and extent of post-mortem decline in pH and ATP at the onset of rigor mortis is reflected in major variations in tenderness of the resulting meat. The objective of this study was to assess the effectiveness of electrical stimulation as a means of speeding post-mortem metabolism as measured by ATP (adenosine triphosphate) depletion in delay chilled bovine muscle. In addition, sarcomere lengths for electrically stimulated and unstimulated muscles were examined in relation to tenderness of the resultant meat.

Materials and Methods

Six Hereford steers of similar weight (363 to 407 kg) and grade were slaughtered and the carcasses split in the conventional manner. Both sides were placed in a temperature control chamber cooled to 16^{o}C with circulating air. At thirty minutes post-mortem, the stimulated side received an AC square-wave pulse of a magnitude (peak) of 300 volts. The frequency of the stimulus was 400 cycles/second with a duration of 0.5 millisecond and a current of 1.9 amps. This stimulus was applied to the side of beef for a period of 15 minutes. The control side received no electrical stimulation. Cylindrical samples measuring ATP from the longissimus dorsi (LD), semimembranosus (SM), and supraspinatus (SS) muscles were taken with a 1.9 centimeters hand coring device at eight time periods (0.5, 1.0, 2.0, 4.0, 6.0, 8.0, 12.0, and 24.0 hours post-

mortem). The longissimus dorsi samples were taken from the 10th rib; semimembranosus samples were removed from an area 2 inches posterior to the broder of the symphysis pubis. The supraspinatus samples came from the medial third of that muscle.

Both the control and stimulated sides of beef were fabricated into streamline hindquarters 2 hours post-mortem. Individual muscles were then removed from the hindquarters and placed in Cry-o-vac plastic wrap. These muscles were placed in a room at $1^{o}C$ for an additional 46 hours.

To eliminate ATP losses, samples were immediately stored in liquid nitrogen (LN_2) until extracted. ATP concentration was determined by homogenizing the 1.5 g frozen samples with a Kinematica Polytron. A double (2x) extraction of muscle samples using 0.4 M perchloric acid ($HClO_4$) and centrifugation for 10 minutes at 15,000 X G was followed by neutralization using 5 N KOH. The neutralized extract (pH range of 6.8 to 7.2) was stored in an ice bath for 1 hour before being centrifuged at 15,000 X G for 10 minutes. The neutralized supernatant was frozen using a dry ice, acetone freezing bath. Samples were held at $-20^{o}C$ for 12 hours. Duplicate samples for all time periods from a specific animal and muscle were analyzed as an assay unit. The Firefly Lantern Extract (Sigma Chemical Company) and a Model 3000 Integrating Photometer (SAI Tech. Co., La Jolla, California) were used to quantitate ATP concentrations (Marshall, 1977).

Results and Discussion

Table I shows data relating to sarcomere length. Data from all muscles studied (LD, PM, SM, and ST) indicated no significant differences between the control and stimulated samples. Published work by Pierce (1977) indicated increased tenderness in muscles from electrically

TABLE I

EFFECT OF ELECTRICAL STIMULATION ON SARCOMERE LENGTH

Treatment	Muscle	n	DF	Average	Mean Square
Control	LD	60		1.70	
Stimulation	LD	60	5	1.65	0.07
Control	PM	60		3.22	
Stimulation	PM	60	5	3.44	1.75
Control	SM	60		1.76	
Stimulation	SM	60	5	1.65	0.08
Control	ST	60		1.90	
Stimulation	ST	60	5	2.03	0.15

Longissimus Dorsi (LD)

Psoas Major (PM)

Semimembranosus (SM)

Semitendinosus (ST)

a = process mean square

n = number of observations

stimulated sides of beef (Warner-Bratzler shear and taste panel evaluation). However, in the present study no statistical differences between control and stimulated sarcomere lengths were shown to exist. This indicates that a simple prevention of cold shortening as expressed by Carse (1973); Locker (1976); Chrystall and Hagyard (1975) (1976); Davey et al. (1976); Chrystall (1976); Shaw and Walker (1977) as the explanation for tenderization found in the electrically stimulated muscles should be seriously questioned (Smith et al. 1977; Bowling et al. 1978).

The post-mortem depletion of ATP in stimulated and unstimulated muscles is presented in Figures 1, 2, and 3. The ATP content at the 0.5 hour showed no significant differences between stimulated and unstimulated semimembranosus (4.38 mg ATP/g tissue stimulated to 4.74 mg ATP/g tissue unstimulated), longissimus dorsi (3.65 to 3.68), and supraspinatus (3.78 to 3.83) muscles. The effect of electrical stimulation was to increase the subsequent decline of ATP in the semimembranosus (Figure 1), longissimus dorsi (Figure 2), and supraspinatus (Figure 3) muscles. The ATP concentration was significantly lower ($P < 0.001$) in the stimulated SM, LD, and SS muscles at 1.0, 2.0, 4.0, 6.0, and 8.0 hours post-mortem. At the 12.0 hour time period, significance ($P < 0.05$) was indicated in the SM and LD muscles with the converse being true in the SS. Nonsignificant differences in the SM, LD, and SS muscles were indicated at the 24 hour sampling period (Figures 1, 2 and 3). The accelerated depletion of ATP in electrically stimulated muscles follows closely published work on rabbit and lamb, Bendall (1976); beef, Bendall et al. (1976); and lamb, Bowling et al. (1978).

Using a criteria set by Bendall et al. (1976) of pH $\leq$ 6 and ATP levels $\leq$ 50 percent of its initial level as a safe limit below which

Figure 1. Percentage of ATP Depletion in Stimulated and Unstimulated Semimembranosus Muscle. Each point is an Average of 12 Observations

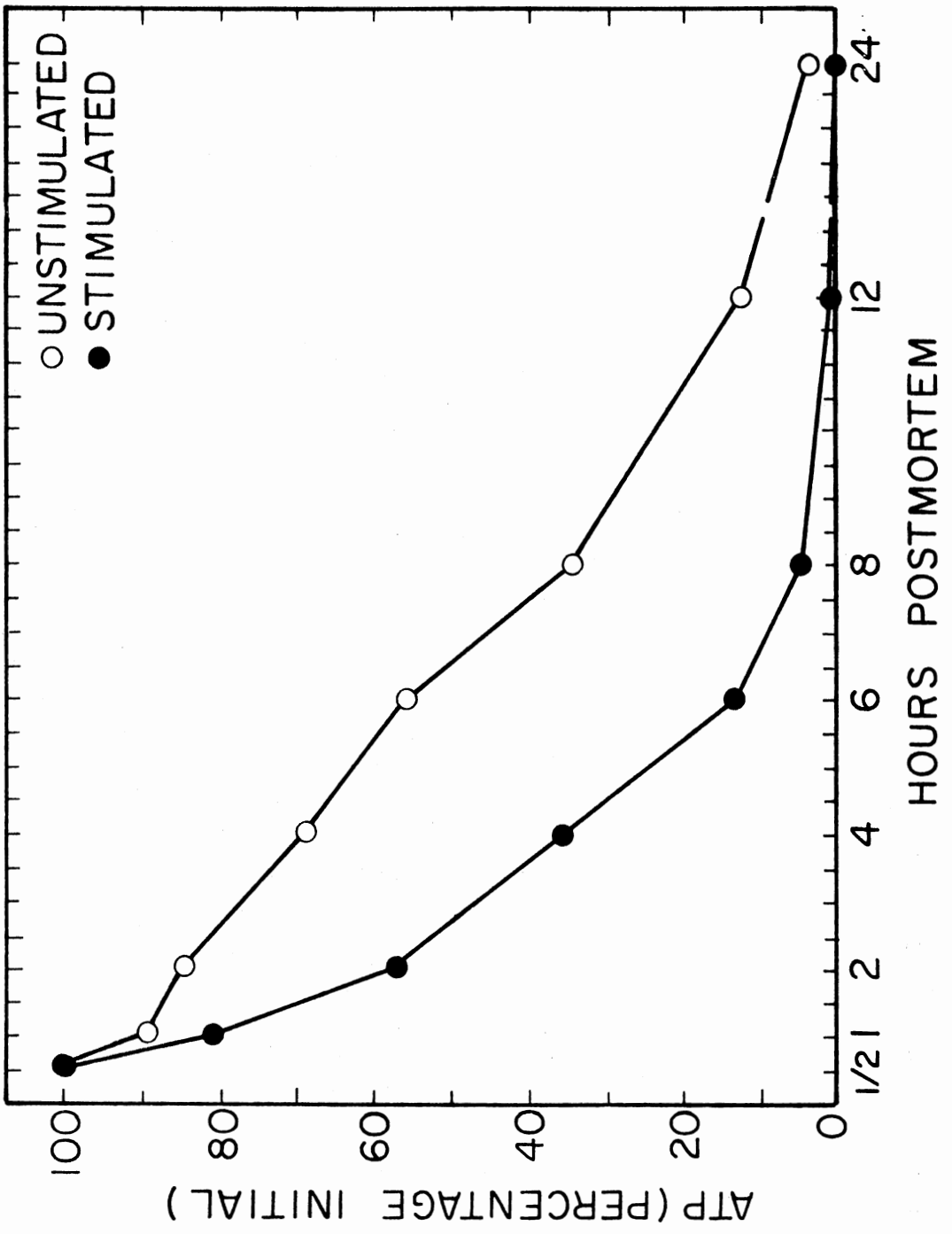

UNSTIMULATED
STIMULATED
ATP (PERCENTAGE INITIAL)
100
80
60
40
20
0
1/2
1
2
4
6
8
12
24
HOURS POSTMORTEM

Figure 2. Percentage of ATP Depletion in Stimulated and Unstimulated Longissimus Dorsi Muscle. Each Point is an Average of 12 Observations

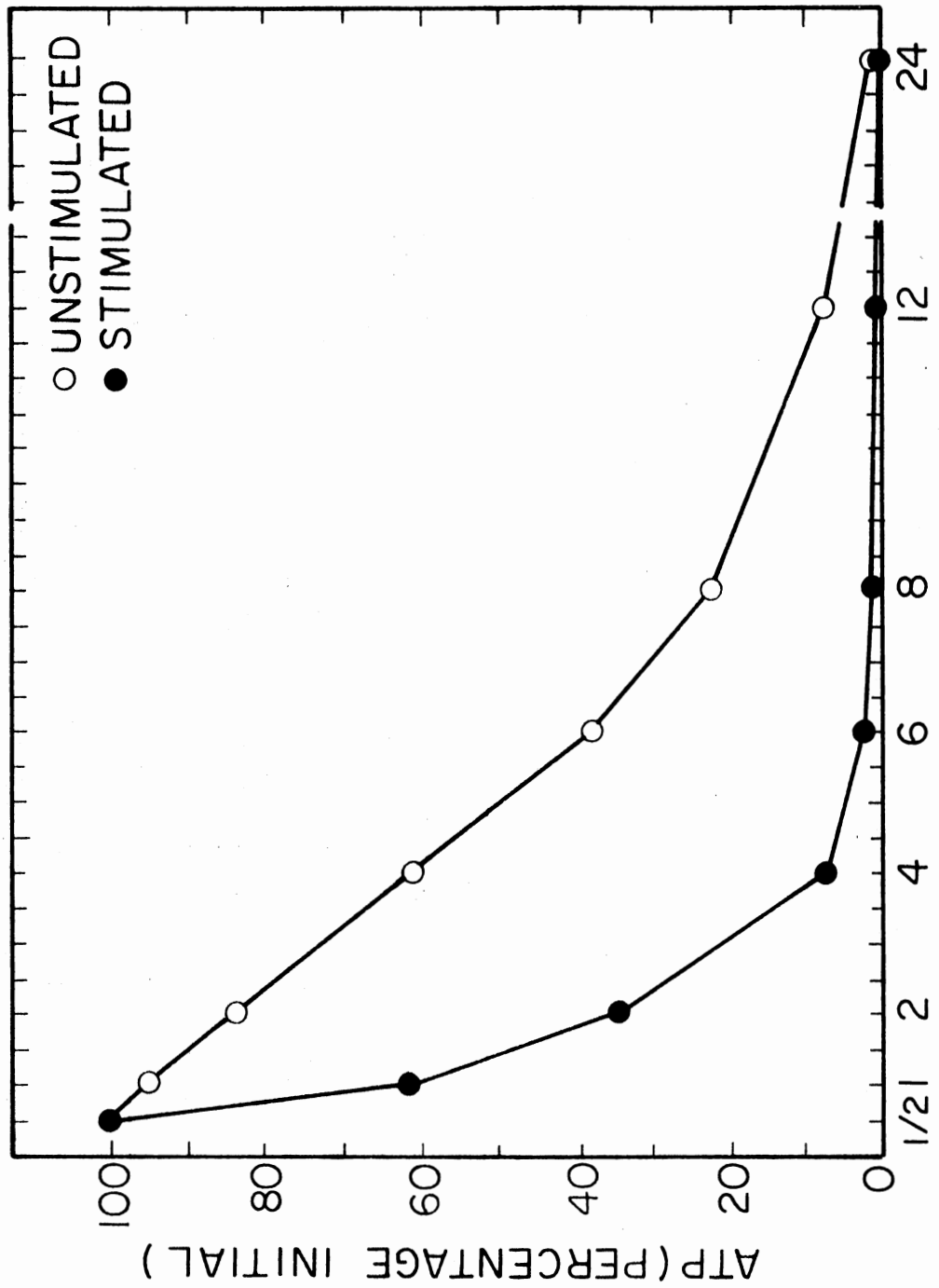
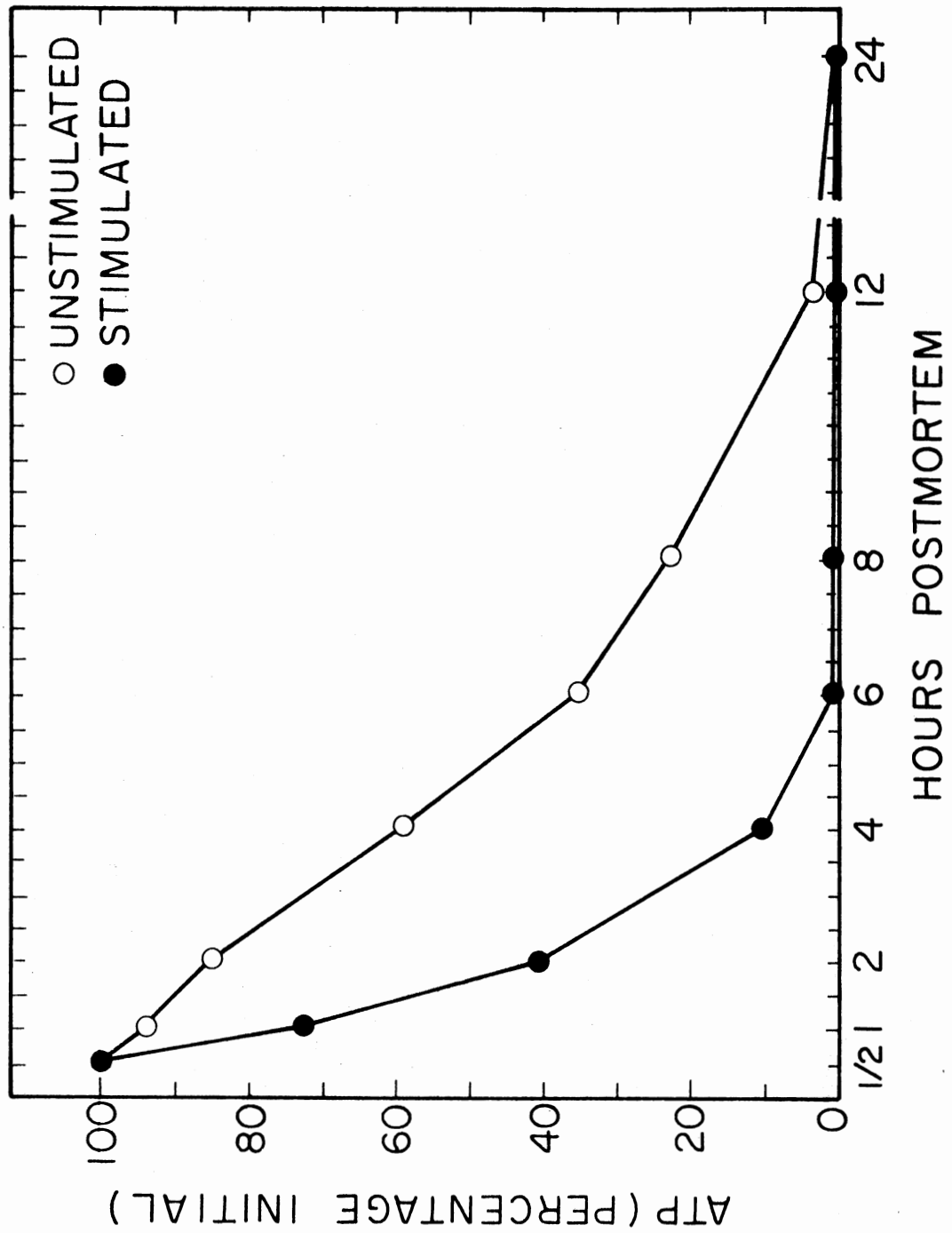

UNSTIMULATED
STIMULATED
ATP (PERCENTAGE INITIAL)
100
80
60
40
20
0
1/21
2
4
6
8
12
24
HOURS POSTMORTEM

Figure 3. Percentage of ATP Depletion in Stimulated and Unstimulated Supraspinatus Muscle. Each Point is an Average of 12 Observations


UNSTIMULATED
STIMULATED
ATP (PERCENTAGE INITIAL)
100
80
60
40
20
0
1/2
1
2
4
6
8
12
24
HOURS POSTMORTEM

rapid cooling can be initiated, a potential savings in time can be realized in stimulating beef carcasses (Figures 1, 2 and 3). The stimulated SM muscle had an immediate drop in ATP to below 50 percent by 2.75 hours post-mortem, while the control reached this value by 6.75 hours. This represents 59 percent savings of time in reaching a comparable ATP level in the unstimulated control (Figure 1). A similar savings of 70 percent in the longissimus dorsi (Figure 2) and 60 percent in the supraspinatus (Figure 3) muscles were realized compared to the control.

These findings relating to time savings lend credence to the concept of fabrication of beef carcasses prior to chilling and are associated with the onset of rigor mortis.

Conclusions

This research clearly demonstrates that the application of an electrical stimulus to freshly slaughtered beef carcasses increases the post-mortem metabolism of muscles of the carcass and initiates the onset of rigor mortis at an earlier time sequence. The importance of cold shortening has been well documented in the production of less tender meat by conventional processing methods; however, the extent of its influence in electrically stimulated carcasses is in question.

The use of electrical stimulation by the future red meat industry lends itself to the concept of fabrication of the carcass to boneless cuts prior to chilling. This accelerated processing prior to chilling would encourage early packaging of the meat in a vacuum wrap. Early packaging would have the advantages of saving space and energy, avoiding weight loss or contamination, allowing additional aging periods, and giving an attractive appearance to the resultant meat product.

CHAPTER III

ULTRASTRUCTURAL POST-MORTEM CHANGES IN ELECTRICALLY STIMULATED BOVINE MUSCLES[1,2,3]

Summary

Alterations in the morphology of beef longissimus dorsi, psoas major, semitendinosus, and supraspinatus muscles induced by electrical stimulation were studied at the light and electron microscopic levels. Samples of control and electrically stimulated muscles were removed from the carcass at 0.5-, 1-, 6-, and 24-hours post-mortem, processed, and embedded in plastic. Changes induced by electrical stimulation were compared with those in normal autolysis in control muscle.

Light microscopic examination revealed contraction bands and intracellular edema in the stimulated muscle samples while the control showed no sign of morphological change. At the electron microscopic level, electrical stimulation caused specific structural changes in the muscles.

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[1]Journal article of the Agricultural Experiment Station, Oklahoma State University, Stillwater.

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[3]Department of Animal Sciences and Industry, Department of Physiological Sciences, and Department of Energy, Washington, D.C.

Swollen sarcoplasmic reticulum, mitochondria, and T-tubes were observed in the stimulated muscle samples. In addition, contraction banding, intracellular edema, disintegration of the myofibril, and other morphological deviations were also observed.

These data indicated that mechanisms other than the prevention of "cold shortening" bring about tenderization of the resultant meat and led to the conclusion that electrical stimulation accelerated normal muscle autolysis.

Introduction

The application of an electrical current to freshly slaughtered beef carcasses has been shown to increase the rate of glycolysis and reduce the time for onset of rigor mortis (Carse, 1973; Locker et al. 1975; Davey et al. 1976; McCollum and Henrickson, 1977; Shaw and Walker, 1977; Will et al. 1978a). These biochemical and physical events have been attributed to the prevention of the development of detrimental effects of "cold shortening" and enhancement of the tenderness of the resulting meat (Carse, 1973; Locker, 1976; Chrystall and Hagyard, 1975; 1976; Davey et al. 1976; Chrystall, 1976). These conclusions were likewise reached by Gilbert et al. (1976) working with pre-rigor hot boned bovine carcasses.

Reduced "cold shortening" is one of the major factors given by these researchers as contributing to the tenderization due to electrical stimulation. Work by Pierce (1977) showed that steaks from electrically stimulated sides of beef had significantly lower objective shear force values and preferred ranking by panelists in comparison to control steaks. Sarcomere lengths were not significantly different

between control and electrically stimulated sides, indicating no difference in cold shortening (Will, 1978). Based on these experiments, it was determined that additional research investigating physical makeup of the striated muscle fiber as it related to the effect of electrical stimulation was warranted. Therefore, the ultrastructural changes resulting from electrical stimulation were compared to those due to normal autolysis in four bovine muscles.

Materials and Methods

Four steers of similar weight and grade were slaughtered and the carcasses split in the conventional manner. Both sides were placed in a temperature control chamber cooled to 16°C with circulating air. At thirty minutes post-mortem, the stimulated side received a square-wave pulse of a magnitude (peak) of 300 volts, 400 cycles/second (frequency) with a duration of 0.5 millisecond and a current of 1.9 amps for a period of 5 minutes, while the control side received no electrical stimulation. Cylindrical samples from the longissimus dorsi (LD), psoas major (PM), semitendinosus (ST), and supraspitatus (SS) muscles were taken with a 1.9 cm hand coring device at a 0.5-, 1-, 6-, and 24- hours post-mortem. Both the control and stimulated sides of beef were fabricated into streamline hindquarters 2 hours post-mortem. The four muscles studied were placed in Cry-o-vac transparent wrap and held at 1.1°C until the 24 hour sample was taken.

The muscle samples were fixed immediately in cold (4°C), 2% glutaraldehyde in 0.27M cacodylate buffer, pH 7.4 for 2 hours. After washing in the same buffer, the samples were then fixed for 1 hour in 2% O_sO_4 in the cacodylate buffer. Dehydration was followed by embedment

in Epon resin. Thick sections, 1-2 μm, were stained with Mallory's Azure II blue (Richardson et al., 1960) and observed with a light microscope. Thin sections (silver) were stained with uranyl acetate and lead citrate (Reynolds, 1963) and then observed with a Philips EM 200 electron microscope.

Results and Discussion

Longissimus dorsi

Light Microscopy. The inspection of light micrographs at 0.5-hours post-mortem revealed no morphologic differences in striated muscle between control and experimental samples. At 1 hour, no changes in the morphology of the control were evident (Figure 4A), but distinct contraction bands were observed in the electrically stimulated longissimus dorsi muscle (Figure 4B) and by 6 hours, intracellular edema was also present (Figure 4D). In the 6-hour control samples, these changes were not observed (Figure 4C). At 24 hours, intracellular edema was noted in the control; however, no contraction banding was present (Figure 4E). In the 24-hour experimental samples (Figure 4F), the amount of intracellular edema and contraction bands was similar to that present at the 6-hour treatment.

Electron Microscopy. There were no detectable differences between control and experimental treatments at the 0.5-hour period. The nuclei appeared normal, but a slight dilation of the sarcoplasmic reticulum was present in both control and experimental samples.

At 1 hour, no nuclear changes were observed in the control muscle, but the sarcoplasmic reticulum was dilated (Figure 5A). In the experi-

Figure 4. Light Micrographs of Longissimus Dorsi. A: 1-Hour control. No morphologic changes (300 X). B: 1-hour stimulated. Note contraction bands, arrow (300 X). C: 6-hour control. No morphologic changes (200 X). D: 6-hour stimulated. Note slight intracellular edema and contraction bands, arrow (200 X). E: 24-hour control. Note intracellular edema (200 X). F: 24-hour stimulated. Note intracellular edema and contraction bands, arrow (200 X).

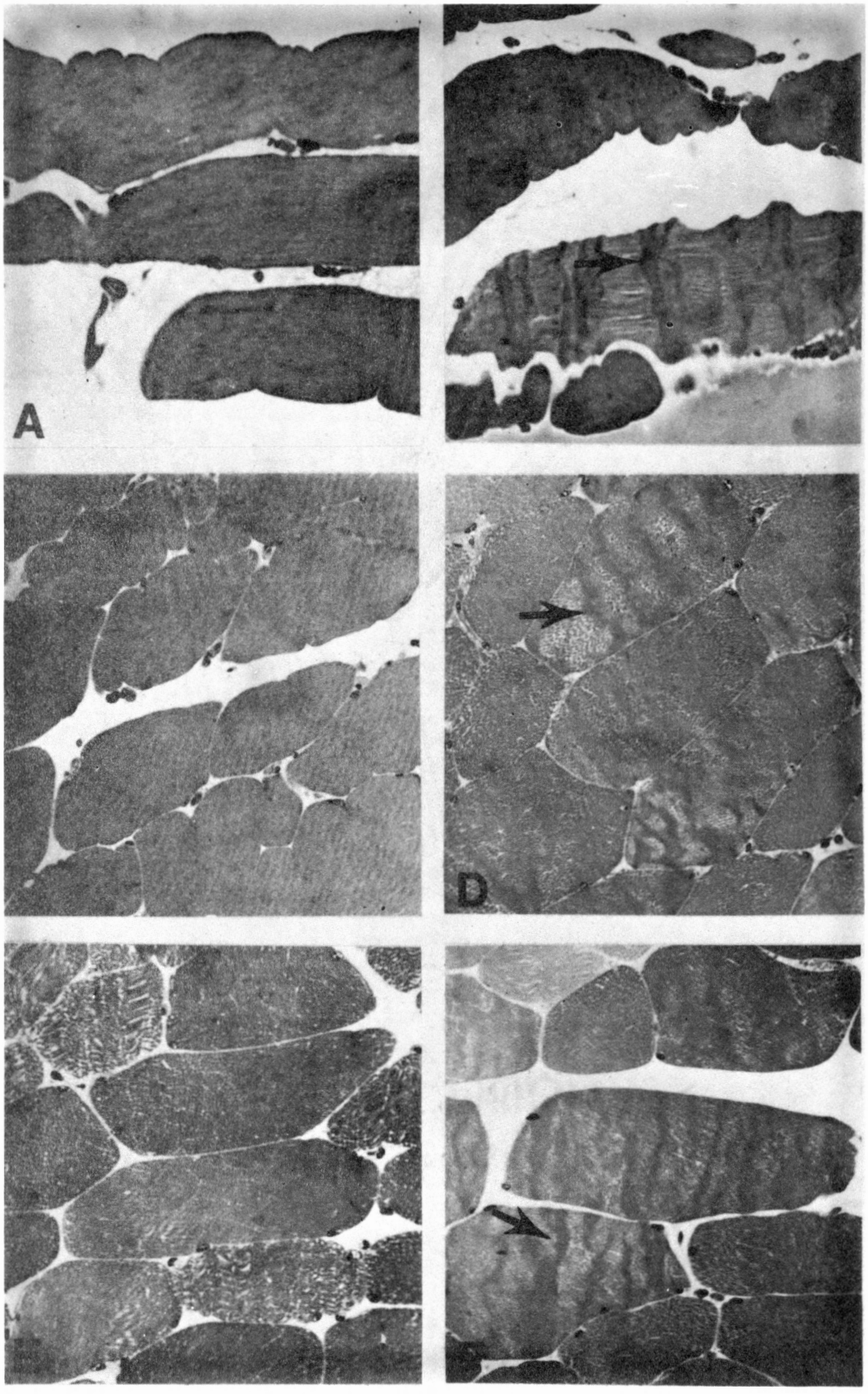
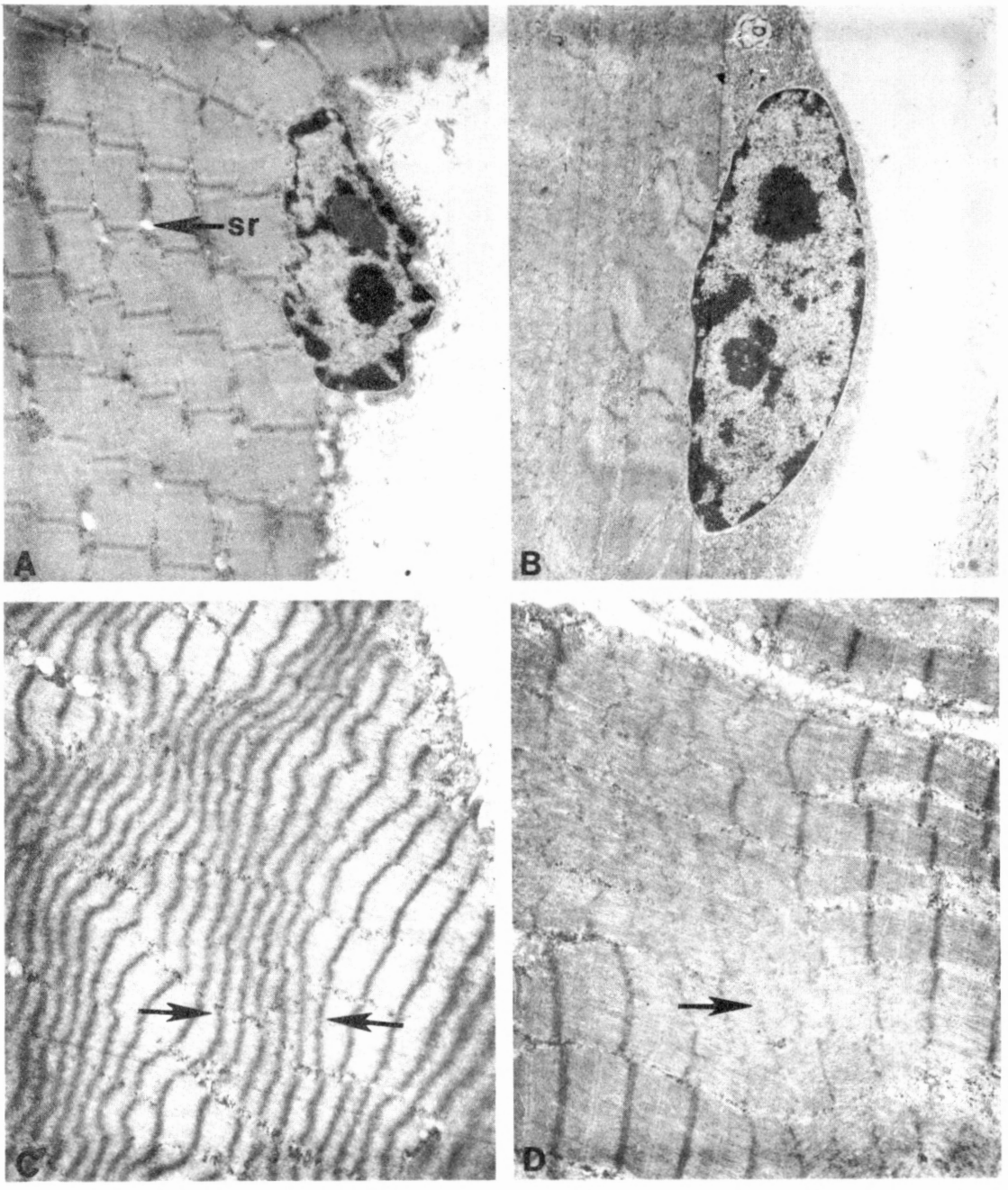

A
D

Figure 5. Electron Micrographs of Longissimus Dorsi. A: 1-hour control. Nucleus appears normal but sarcoplasmic reticulum is slightly dilatated [sr] (9,500 X). B: 1-hour stimulated. No change in nuclear morphology (8,500 X). C: 1-hour stimulated. Arrows indicate edges of contraction band. Note stretched areas on either side (8,000 X). D: 1-hour stimulated. Note area of sarcomere disruption, arrow (11,000 X).

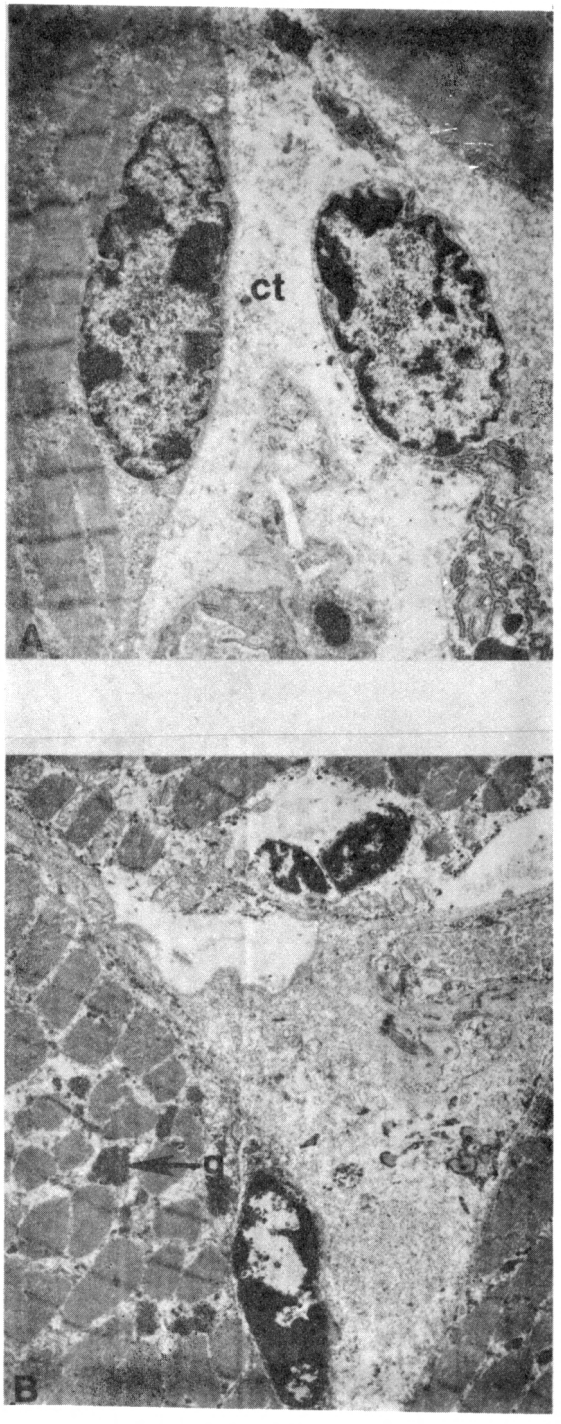

sr
A
B
C
D

mental muscle, there was no difference in the nuclear morphology when compared to the control (Figure 5B). Contraction bands in combination with stretched areas were observed in the myofibrils from the electrically stimulated muscle (Figure 5C). In addition, areas of disruption of the sarcomere integrity were observed which appeared to be due to Z-line disintegration (Figure 5D).

In the 6-hour control, a slight intracellular edema and a flocculent material in the connective tissue were noted. However, the integrity of the nucleus and sarcomere was maintained (Figure 6A). In the stimulated muscle the chromatin material in the nucleus was markedly clumped along the nuclear membrane and around the nucleolus (Figure 6B). An accumulation or clumping of structures resembling glycogen were observed in slightly swollen areas of the cytoplasm (Figure 6B). Likewise, intracellular edema was also shown to be present at this time period (Figure 6B).

At 24 hours the control exhibited intracellular edema and swollen and ruptured mitochondria (Figure 7A). The experimental treatment revealed a host of abnormalities not present in the control. These included a granular appearance of the connective tissue, swelling of the sarcoplasmic reticulum, a general swelling of the entire muscle cell, and a pyknotic nucleus (Figure 7B). In addition, the mitochondrial outer membrane and cristae were swollen and ruptured (Figure 7C). The capillary system servicing the muscle cell was also found to be damaged (Figure 7C). In addition, a separation of the sarcolemma and basal lamina from the cytoplasm was noted (Figure 7D). There was also a loss of sarcomere integrity, breakdown of myofibril and myofilaments (Figure 7E), and an accumulation of glycogen as seen in the 6-hour experimental

Figure 6. Electron Micrographs of Longissimus Dorsi. A: 6-hour control. Nuclear morphology and sarcomere arrangement are normal. Note flocculant material in the corrective tissue [ct] (8,000 X). B: 6-hour stimulated. Note clumping of chromatin in nuclei and accumulation of glycogen [g] (6,000 X)


ct
A
c
B

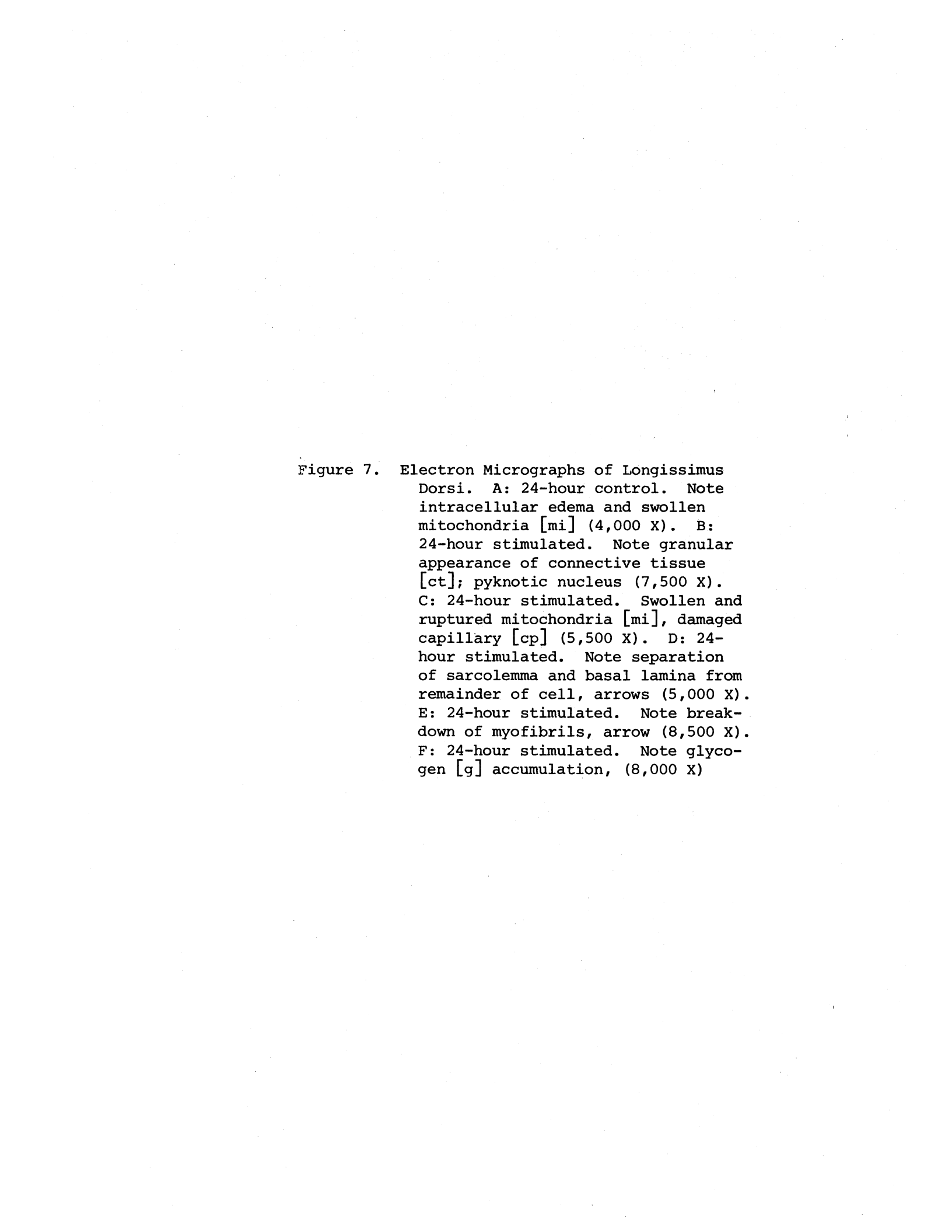
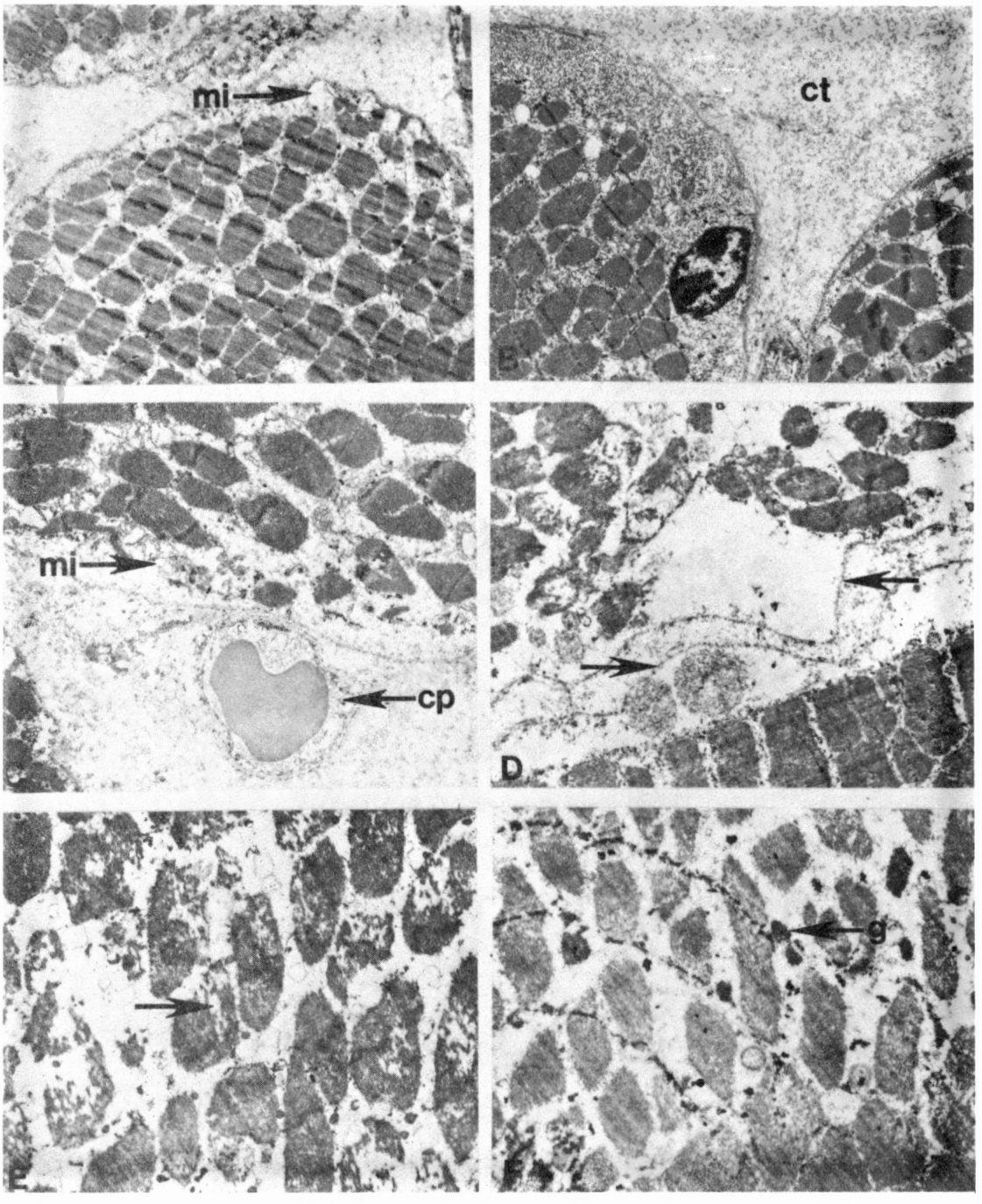


Figure 7. Electron Micrographs of Longissimus Dorsi. A: 24-hour control. Note intracellular edema and swollen mitochondria [mi] (4,000 X). B: 24-hour stimulated. Note granular appearance of connective tissue [ct]; pyknotic nucleus (7,500 X). C: 24-hour stimulated. Swollen and ruptured mitochondria [mi], damaged capillary [cp] (5,500 X). D: 24-hour stimulated. Note separation of sarcolemma and basal lamina from remainder of cell, arrows (5,000 X). E: 24-hour stimulated. Note breakdown of myofibrils, arrow (8,500 X). F: 24-hour stimulated. Note glycogen [g] accumulation, (8,000 X)


mi
ct
B
mi
cp
D
g

treatment (Figure 7F).

Supraspinatus

Light Microscopy. Observations of light micrographs indicated that the control samples of this muscle taken at all time intervals (0.5, 1, 6, and 24 hours) were morphologically intact. No morphologic changes were observed in the 0.5- or the 1-hour experimental muscle. Slight intracellular edema was the only morphological change observed in the experimental treatment, and it was present at both 6 and 24 hours.

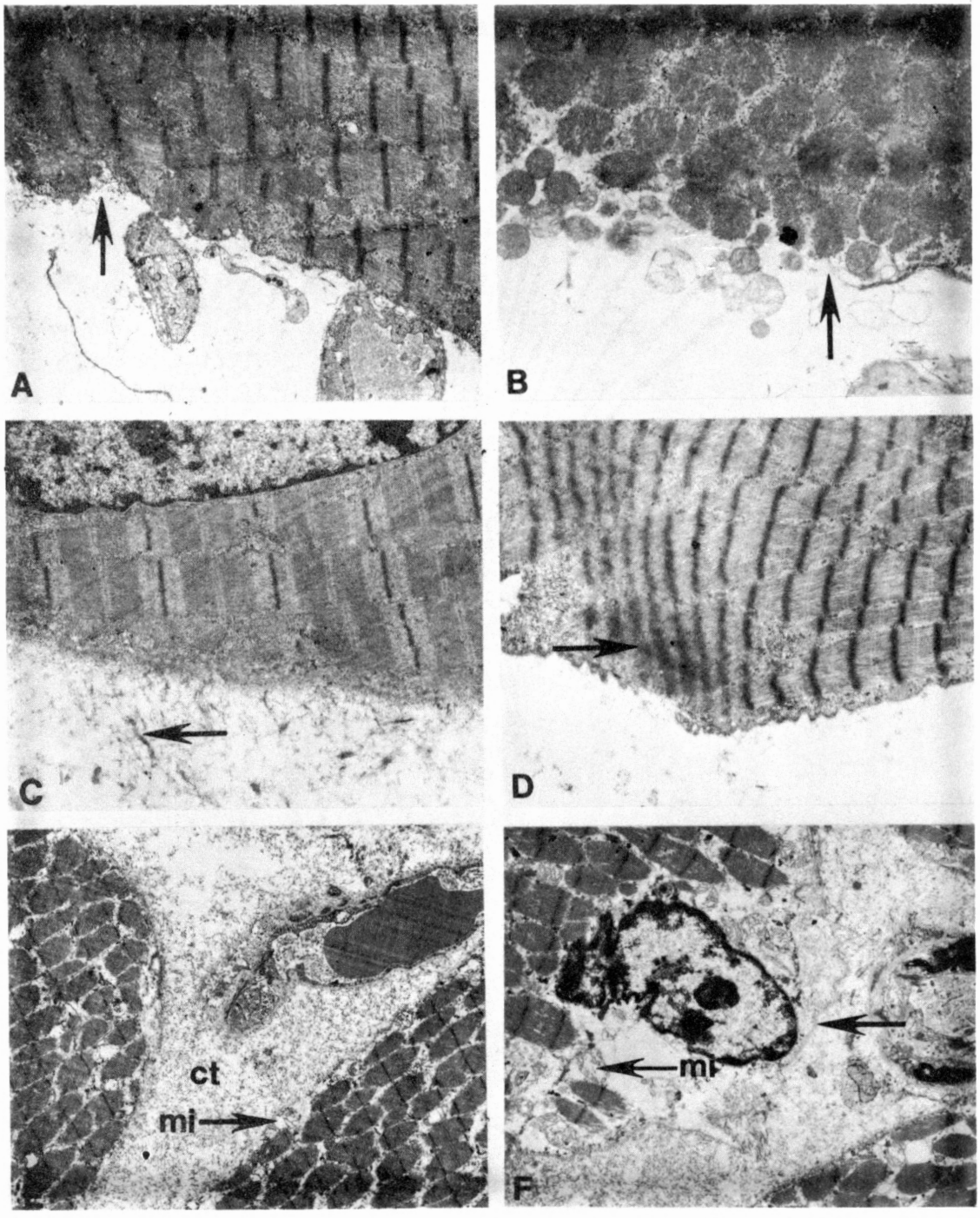
Electron Microscopy. Electron micrographs of the control and experimental treatments showed localized rupture of the sarcolemma at the 0.5 time period (Figures 8A and 8B). All other intracellular organelles appeared morphologically normal.

At 1 hour it appeared that there was a disruption of collagen and an indistinct sarcolemma present in the control treatment (Figure 8C). Contraction bands and localized rupture of the sarcolemma were also evident in the electrically stimulated sample at 1 hour (Figure 8D).

At 6 hours swollen mitochondria with ruptured cristae were evident in both treatments. Similar morphological changes present at 1 hour were present at 6 hours in the electrically stimulated samples.

The control at 24 hours showed swelling of the mitochondria and a granular material in the connective tissue (Figure 8E). By 24 hours, the sarcolemma of experimental muscle was broken, but the basal lamina was intact (Figure 8F). Intracellular edema was present as well as swollen mitochondria containing ruptured cristae (Figure 8F). The nucleus appeared slightly pyknotic and glycogen was clumped in the

Figure 8. Electron Micrographs of Supraspinatus. A: 0.5-hour control (7,500 X). B: 0.5-hour stimulated (8,000 X). Both A and B show localized rupture of the sarcolemma, arrows. C: 1-hour control. Note disrupted collagen, arrow, and indistinct sarcolemma (10,000 X). D: 1-hour stimulated. Note area of contraction bands arrow (7,500 X). E: 24-hour control. Note granular connective tissue [ct]; swollen and ruptured mitochondria [mi] (4,000 X). F: 24-hour stimulated. Note presence of basal lamina and absence of sarcolemma, arrow; note intracellular edema, swollen mitochondria [mi] and slightly pyknotic nucleus (6,000 X)


A
B
C
D
ct
mi
mi
F

cytoplasm of the muscle cell (Figure 8F).

Psoas Major

Light Microscopy. At all time periods, the control muscle samples appeared normal. Experimental muscle had only slight intracellular edema at 0.5, 1, 6, and 24 hours.

Electron Microscopy. At 0.5 hour the control muscle showed only slight intracellular edema (Figure 9A). In the experimental muscle, the nucleus, myofilament arrangement, and mitochondria appeared normal, but the sarcolemma was pulled away and there was a lot of intracellular edema (Figure 9B). Also, localized rupture of the sarcolemma was observed in some cells.

At 1 hour the mitochondria were swollen (Figure 10A) and the nucleus in the control muscle appeared slightly pyknotic. The experimental muscle contained abnormal sarcomere structure along with alteration of the Z-line symmetry (Figure 10B).

By 6 hours the control showed swollen and ruptured mitochondria and lifting way of basal lamina with rupture of sarcolemma integrity (Figure 11A). The experimental muscle showed the above conditions plus breakage of sarcolemma and basal lamina; also, the basal lamina appeared to be lifted away from the main part of the cytoplasm (Figure 11B).

Twenty-four hour control muscle samples revealed conditions similar to those present at 6 hours. Experimental samples showed extensive lifting of the basal lamina and highly pyknotic nuclei (Figures 12A and 12B).

Figure 9. Electron Micrographs of Psoas Major. A: 0.5-hour control. Normal morphology with only slight edema (9,000 X). B: 0.5-hour stimulated. Note separation of sarcolemma, arrow, and intracellular edema (7,000 X)

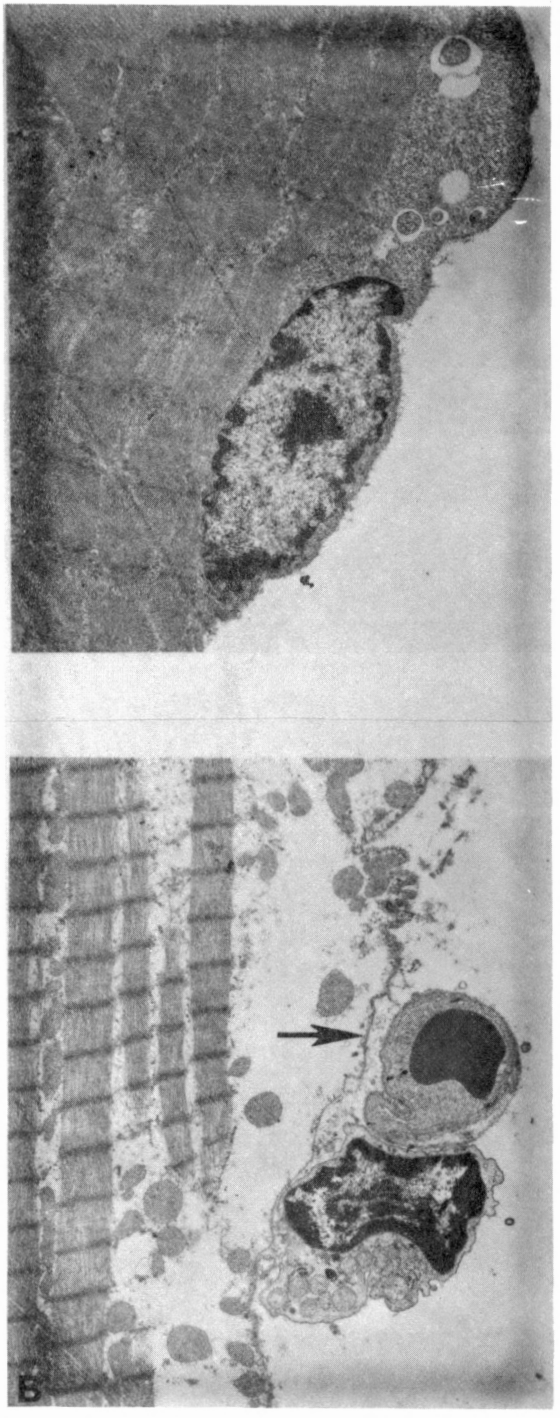

Б

Figure 10. Electron Micrographs of Psoas Major. A: 1-hour control. Swollen mitochondria [mi] (13,000 X). B: 1-hour stimulated. Z lines [Z] are disoriented and abnormal sarcomere structure (13,500 X)

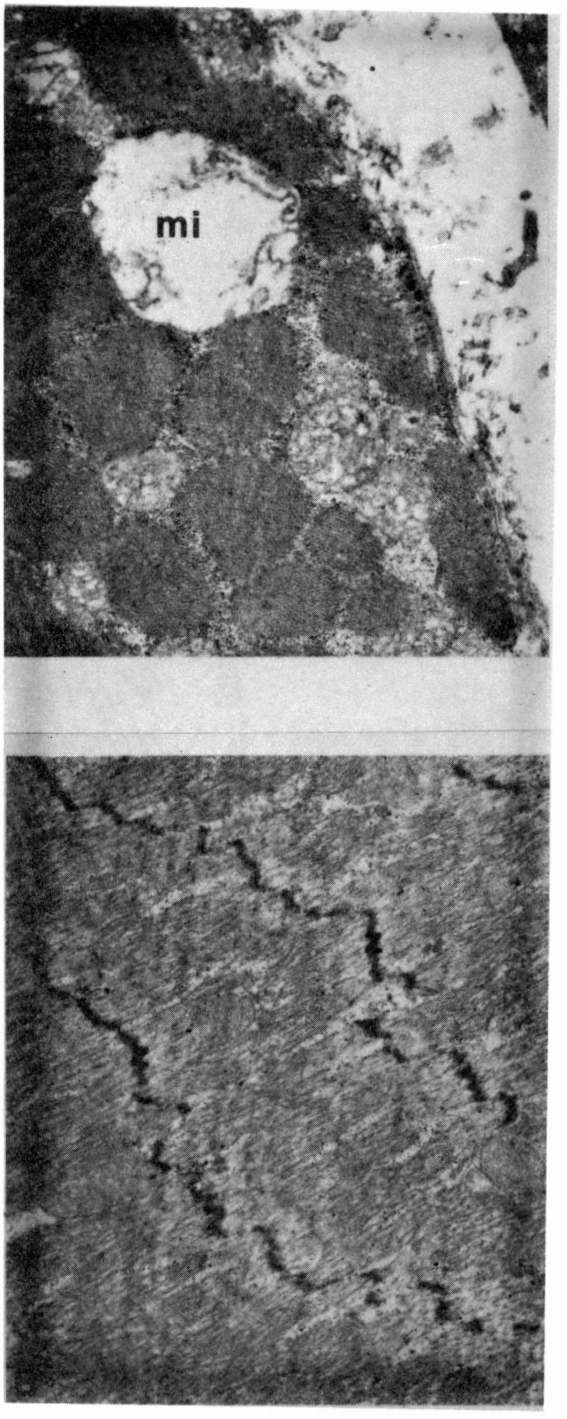

mi

Figure 11. Electron Micrographs of Psoas Major. A: 6-hour control. Swollen and ruptured mitochondria [mi]; arrow indicates ruptured sarcolemma; basal lamina [bl] (6,000 X). B: 6-hour stimulated. Note basal lamina [bl] which has separated from the cell (6,000 X)

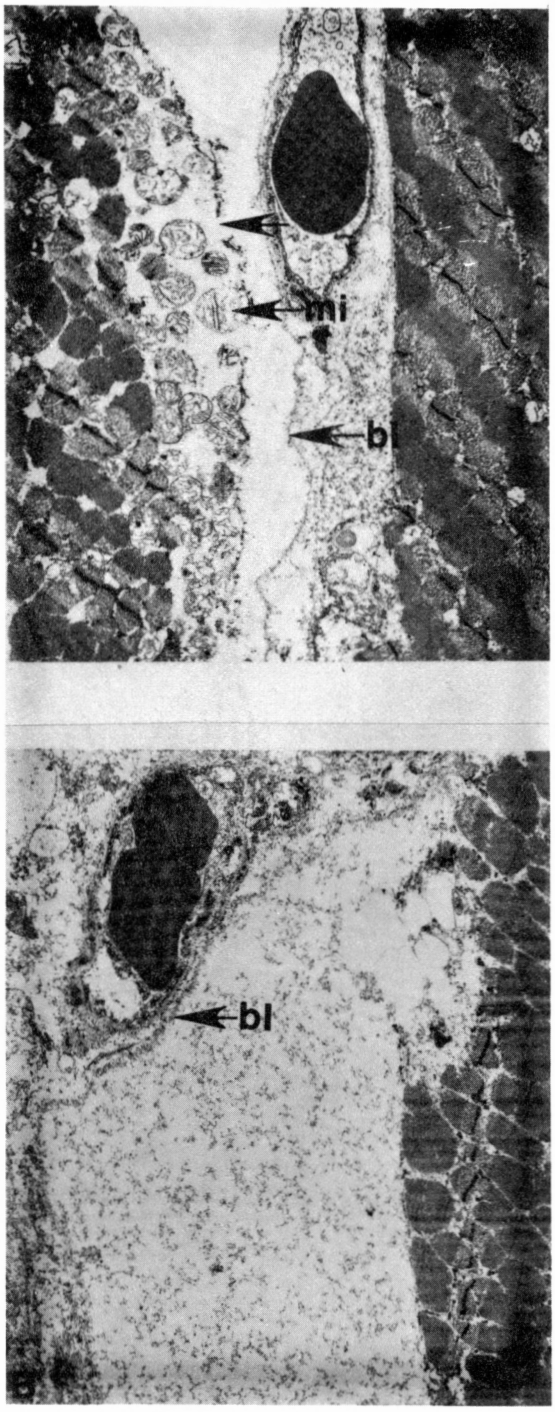
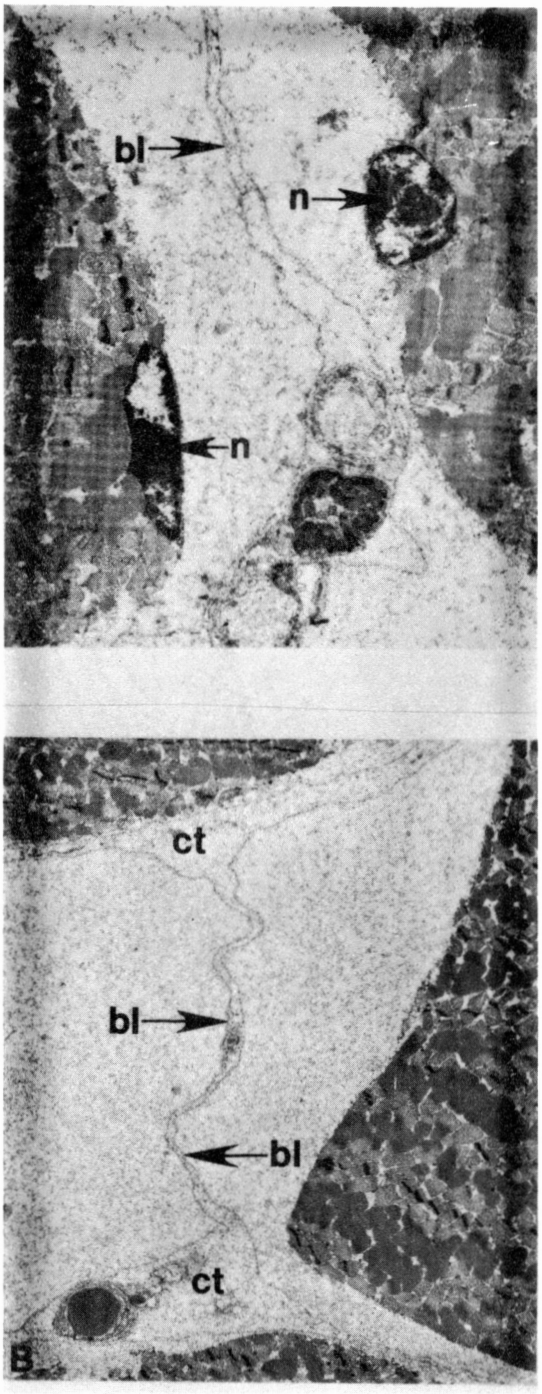

mi
bl
bl

Figure 12. Electron Micrographs of Psoas Major. 24-hour stimulated. A: Note separation of basal lamina [bl] and pyknotic nuclei [n] (5,500 X). B: Note extensive separation of basal lamina from muscle cell, arrows; connective tissue [ct] (3,500 X)


bl
n
n
ct
bl
bl
ct
B

Semitendinosus

Light Microscopy. Control samples at the 0.5-, 1-, 6-, and 24-hour time periods had similar morphological appearances. The experimental treatment showed development of slight intracellular edema at the 6- and 24-hour periods. No additional morphological changes were evident at the light level in the electrically stimulated samples.

Electron Microscopy. At 0.5 hours, no changes were observed in either treatment at the electron microscope level. At 1 and 6 hours, slight swelling of the sarcoplasmic reticulum and mitochondria were observed in both treatments. At 24 hours swollen mitochondria, sarcolemma breakdown, and debris in the connective tissue were noted in both treatments.

The two primary structural alterations induced by the electrical stimulation of beef muscle were contraction bands and accelerated autolysis. Contraction bands were observed only in the longissimus dorsi and supraspinatus muscles at 1, 6, and 24 hours and at 1 hour, respectively.

Contraction bands have been observed under a host of pathological conditions such as ischemia, cardiomyopathy, catecholamine toxicity, (Adomian et al. 1978), hemorrhagic shock, (Leet et al. 1975; Adomian et al. 1976), catecholamine infusion, open heart surgery (Adomian et al. 1976), malignant hyperthermia (Isaacs et al. 1973; Reske-Nielsen, 1973; Fenoglio and Irey, 1977; Britt and Kalow, 1970) cold shortening (Marsh et al. 1974) and electrical stimulation (Dutson et al. 1977). Work by Adomian et al. (1978) indicates that contraction bands may be considered a morphological index of pathology in myocardial tissue obtained 40

minutes post-mortem. These contraction bands were interspersed between the normal cross striations within the myofibril. Fenoglio and Irey (1977) found these bands in cardiac muscle associated with myofibrillar degeneration or myofiberlysis characterized by dissolution of myofilaments disruption of sarcomeres and sarcolemmal breaks. Dissolution of myofilaments and disruption of sarcomeres can be observed following prolonged autolysis. However, even after 12 hours of autolysis, sarcolemmal break were not observed (Buja and Roberts, 1974). Therefore, contraction bands and sarcolemmal breakage were not directly due to autolysis or ischemia.

The autolysis of control muscle as observed in this investigation agrees with previously published data (Dutson et al. 1974; Cassens et al. 1963; Greaser et al. 1969a; Henderson et al. 1970; Abbott et al. 1977; and Gann, 1974).

The experimental longissimus dorsi showed signs of accelerated autolysis as early as 1 hour post-mortem. Disruption of sarcomere integrity (I-band) at the 1-hour post-mortem period related to 192 hours post-mortem muscle degeneration reported by Abbott et al. (1977). A similar autolysis in I-band has been reported by Cassens et al. (1963); Greaser et al. (1969a); and Parrish (1977).

At 6 hours post-mortem, the stimulated treatment showed signs of acute cell injury as the pyknotic nuclei are highly evident (Price et al. 1964; Trump and Ericsson, 1965). The swelling and intracellular edema present in the stimulated treatment may possibly be explained by reported losses of water-holding capacity of the muscle proteins (Bendall and Wismer-Pedersen, 1962) and the escape of sarcoplasmic protein from the fiber into the intrafiber space (Bendall, 1973).

At 24 hours intracellular edema and swollen and ruptured mitochondria were present in the experimental as well as control. The lifting away of the sarcolemma and basal lamina from the cytoplasm in the experimental treatment muscle agreed with published data of porcine muscle 48 hours post-mortem (Abbott et al. 1977). The ultrastructure of electrically stimulated muscle 24 hours post-mortem showed accelerated morphological degeneration in comparison to the normal autolysis of control muscle tissue. The breakdown of mitochondria, mitochondrial membrane, sarcoplasmic reticulum, the T-tubule system, myofibril, myofilaments, and pyknotic nucleus indicated a physical alteration of the muscle cell. These changes may be partially explained by work of Dayton et al. 1976 and Dutson, 1977 both researchers indicating that at lower pH readings lysosomal catheptic activity is probably responsible for changes in subcellular organelles. In addition, calcium activated factor (CAF) activity which Abbott et al. 1977 indicated as being partially responsible for the normal autolysis of muscle has its greatest activity at a neutral pH and is not thought to be active at an acid pH (Dayton et al. 1976, Dutson, 1977). Therefore, it would appear that electrical stimulation through accelerating pH decline increases the free activity of the lysosomal enzymes.

The morphological changes occurring in the nuclei, sarcoplasmic reticulum, and myofibrils of the control semitendinosus agreed with results published by Abbott et al. 1977.

The experimental treatment at 24 hours post-mortem showed signs of speeded muscle autolysis. The breakdown of the sarcolemma 24 hours in the experimental treatment corresponded to a similar response reported in normal autolysis of muscle 48 hours post-mortem (Abbott et

al. 1977).

Ultrastructural changes in the control muscle appeared to degenerate at a slightly slower rate than that of the experimental treatment. However, the change in the stimulated supraspinatus muscle through 24 hours appeared not to be as marked as observed in the other muscles studied.

Samples taken from the psoas major control revealed a steady progression of autolysis which was at a faster rate than the longissimus dorsi, supraspinatus, and semitendinosus muscles. This is in agreement with pH data by McCollum (1977). His results showed that psoas major muscle from stimulated and non-stimulated treatments had a significantly faster drop in pH as compared to other muscles studied. A difference in the time of onset of autolysis was observed between the control and experimental treatments. The electrically stimulated muscle showed signs of accelerated autolysis from the standpoint of extensive lifting of the sarcolemma, highly pyknotic nuclei, swollen and ruptured mitochondria, and abnormal sarcomere structure.

The mitochondria of striated muscle are commonly used in determining viable from injured fibers. Deviations from normal mitochondrial makeup in studying the normal autolysis of muscle are of non-mitochondrial induced nature (Price, 1973). The autolysis of muscle indicates swelling of the mitochondria during normal rigor onset and death (Abbott et al. 1977; Dutson et al. 1974). Marked mitochondrial swelling in electrically stimulated longissimus dorsi, supraspinatus, semitendinosus muscles were noted. This agrees with published data (Devine, 1974).

Myofibril disintegration was noted in the longissimus dorsi and

psoas major muscles. This autolysis of myofibril organization occurs in normal disintegration of striated muscle; however, it occurs at a much faster time sequence in electrically stimulated muscle (Abbott et al. 1977; Henderson et al. 1970; Cassens et al. 1963; Greaser et al. 1969a; and Dutson et al. 1974). It is our belief that one of the major effects of electrical stimulation is an acceleration of the normal autolysis of muscle.

The relationship of electrical stimulation of carcasses and resulting tenderness of the meat product has been explained as a simple prevention of "cold shortening" (Carse, 1973; Locker, 1976; Chrystall and Hagyard, 1975; Davey et al. 1976; Chrystall, 1976; Gilbert et al. 1976).

Locker and Daines (1974), studying frozen beef muscle, used a phase contrast microscope to conclude that "electrical stimulation caused no apparent damage." Chrystall and Hagyard (1975) indicated no gross or ultrastructural changes occurred in lamb muscles receiving 3,600 volts of electricity; however, considerable tenderization was derived from the stimulus. These findings suggest that tenderness from electrical stimulation is due to a prevention of "cold shortening" and not an induction of some physical change in the muscles of the carcass.

Research published by Pierce (1977) indicated increased tenderness from the electrically stimulated carcasses. However, the measurement of sarcomere lengths revealed no significant differences (Will, 1978). This indicated that some mechanism, in addition to the prevention of "cold shortening", brought about the tenderization of meat. This tenderness was derived in part from physical or structural changes induced by electrical stimulation in the muscle. This was partially explained by the accelerated autolysis seen in stimulated striated muscles and

was an active rather than passive effect as proposed by "cold shortening" alone.

CHAPTER IV

SUMMARY OF RESULTS

Recent research dealing with the removal of muscle systems before initial chilling of the bovine carcass has suggested that this process offers economic advantages to the meat industry. For optimum efficiency, a carcass would be fabricated immediately after slaughter. However, there are metabolic activities which must proceed in the muscles of the carcass before fabrication can be initiated. This study was undertaken to assess the effectiveness of electrical stimulation as a means of speeding post-mortem metabolism as measured by ATP (adenosine triphosphate) depletion, and sarcomere length in delay chilled bovine carcasses. In addition, ultrastructural changes resulting from electrical stimulation were compared to those due to normal autolysis in four bovine muscles. In the first portion of the study, six animals of similar weight and age were used. Electrical stimulation was initiated 30 minutes post-mortem for 15 minutes. The stimulated side received a square-wave pulse of a magnitude (peak) of 300 volts at 400 cycles/stimulus (frequency) for a duration of 0.5 milliseconds and a current of 1.9 amps for a period of 15 minutes. The control side received no electrical stimulation. ATP measurements were taken at 8 time periods (0.5, 1.0, 2.0, 4.0, 6.0, 8.0, 12.0 and 24.0 hours) post-mortem. Muscle samples to determine sarcomere lengths were taken 48 hours post-mortem. The second study utilized four animals of similar weight and

age. Stimulation procedures were similar to the first study except that the stimulation period was for 5 minutes instead of 15 minutes.

In the first study, muscles from the stimulated sides of beef exhibited significantly faster reductions of ATP than did the unstimulated controls. It was concluded that electrical stimulation is an effective means of speeding post-mortem glycolysis and onset of rigor mortis. Published work by Pierce (1977) indicated increased tenderness in muscles from electrically stimulated sides of beef (Warner-Bratzler shear and taste evaluation). However, no statistical difference between control and stimulated sarcomere lengths were shown to exist. Data from the ultrastructural study indicated that mechanisms other than the prevention of "cold shortening" brought about tenderization of the resultant meat and led to the conclusion that electrical stimulation accelerated normal muscle autolysis. The combination of ATP, sarcomere length, and ultrastructural data led to the conclusion that electrical stimulation will permit the holding period between slaughter and muscle excision to be reduced.

Further basic research determining the current density of electricity passing through each muscle of the carcass is needed. Likewise, the effect of electrical stimulation on the mechanism of calcium uptake and binding in the sarcoplasmic reticulum and mitochondria may help explain the accelerated ATP decline.

Further applied research dealing with electrical stimulation and delay chill processing should investigate the optimum conditions for electrical stimulation of the beef carcass. This would include both the most efficient and best from a practical standpoint (i.e. that most feasible for use in industry). In addition, research dealing with

electrical stimulation and delay chill processing should be conducted, looking at the aging in vacuum packaged meat cuts, the emulsifying ability in ground meat for sausage manufacture, and the color stability in retail cuts.

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APPENDIXES

TABLE II

ANALYSIS OF VARIANCE OF SARCOMERE LENGTHS IN STIMULATED AND UNSTIMULATED DELAY CHILLED PSOAS MAJOR MUSCLE

Source	DF	Sum of Squares	Mean Squares
Total Corrected	119	31.20	
Animal	5	11.87	2.37
Stimulation	1	1.45	1.45
Animal x Stimulation (experimental error)	5	8.74	1.75
Sarcomere (Animal Stim.)	108	9.14	0.08

TABLE III

ANALYSIS OF VARIANCE OF SARCOMERE LENGTHS IN STIMULATED AND UNSTIMULATED DELAY CHILLED SEMITENDINOSUS MUSCLE

Source	DF	Sum of Squares	Mean Squares
Total Corrected	119	5.73	
Animal	5	1.62	0.32
Stimulation	1	0.57	0.57
Animal x Stimulation (experimental error)	5	0.73	0.15
Sarcomere (Animal Stim.)	108	2.81	0.03

TABLE IV

ANALYSIS OF VARIANCE OF SARCOMERE LENGTHS IN STIMULATED AND UNSTIMULATED DELAY CHILLED SEMIMEMBRANOSUS MUSCLE

Source	DF	Sum of Squares	Mean Squares
Total Corrected	119	3.78	0.03
Animal	5	0.71	0.14
Stimulation	1	0.36	0.36
Animal x Stimulation (experimental error)	5	0.42	0.08
Sarcomere (Animal Stim.)	108	2.29	0.02

TABLE V

ANALYSIS OF VARIANCE OF SARCOMERE LENGTHS IN STIMULATED AND UNSTIMULATED DELAY CHILLED LONGISSIMUS DORSI MUSCLE

Source	DF	Sum of Squares	Mean Squares
Total Corrected	119	5.55	0.05
Animal	5	3.40	0.68
Stimulation	1	0.06	0.06
Animal x Stimulation (experimental error)	5	0.36	0.07
Sarcomere (Animal Stim.)	108	1.73	0.02

TABLE VI

ANALYSIS OF VARIANCE OF ATP DATA IN STIMULATED AND UNSTIMULATED DELAY CHILLED SEMIMEMBRANOSUS MUSCLE

Source	DF	Sum of Squares	Mean Squares
Total Corrected	191	631.58	3.31
Main Unit Analysis			
Animal	5	29.56	5.91
Stimulation	1	50.81	50.81
Animal x Stimulation (Error A)	5	6.60	1.32
Subunit Analysis			
Time	7	463.68	66.24
Stimulation x Time	7	19.05	2.72
Animal x Time	35	41.48	1.19
Animal x Stimulation x Time	35	20.17	0.58
AT + AST (Pooled Error B)	70	61.65	0.88

TABLE VII

ANALYSIS OF VARIANCE OF ATP DATA IN STIMULATED AND UNSTIMULATED DELAY CHILLED LONGISSIMUS DORSI MUSCLE

Source	DF	Sum of Squares	Mean Squares
Total Corrected	109	396.06	2.07
Main Unit Analysis			
Animal	5	6.17	1.23
Stimulation	1	41.39	41.39
Animal x Stimulation (Error A)	5	0.99	0.20
Subunit Analysis			
Time	7	303.94	43.42
Stimulation x Time	7	25.09	3.58
Animal x Time	35	9.93	0.28
Animal x Stimulation x Time	35	8.35	0.24
AT + AST (Pooled Error B)	70	18.28	0.26

TABLE VIII

ANALYSIS OF VARIANCE OF ATP DATA IN STIMULATED AND UNSTIMULATED DELAY CHILLED SUPRASPINATUS MUSCLE

Source	DF	Sum of Squares	Mean Squares
Total Corrected	191	446.06	2.34
Main Unit Analysis			
Animal	5	9.27	1.85
Stimulation	1	34.51	34.51
Animal x Stimulation (Error A)	5	0.67	0.13
Subunit Analysis			
Time	7	358.81	51.26
Stimulation x Time	7	24.42	3.49
Animal x Time	35	11.17	0.32
Animal x Stimulation x Time	35	6.95	0.20
AT + AST (Pooled Error B)	70	18.13	0.26

VITA

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