THE EFFECT OF ELECTRICAL STIMULATION ON THE RATE OF POST-MORTEM GLYCOLYSIS IN SOME BOVINE MUSCLES

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CHAPTER I

INTRODUCTION

In recent years the removal of muscles from the unchilled bovine carcass has been investigated as an alternative to conventional processing methods. Potential advantages of speedy carcass processing include a more streamlined operation producing a reduced inventory and the possibility for an even flow of product through the facility. The reduction of refrigerated space required for conditioning carcasses along with the associated energy requirement presents some of the largest potential advantages of "hot" boning to the meat processor. This energy saving aspect is compounded by chilling only the edible portion of the carcass.

Muscle, if removed from its skeletal restraints before the onset of rigor mortis, will shorten considerably. This shortening produces a marked effect on the eating quality of the resulting meat. The more extensive the contraction the less tender the product. It is necessary, therefore, to induce rigor mortis prior to muscle removal. One means of hastening the onset or rigor is to subject the carcass to an elevated temperature, which accelerates post-mortem chemical changes resulting in a shorter time period before the onset of rigor. Carcasses conditioned at 16°C for as little as

three hours before muscle removal will provide meat with eating qualities comparable to meat from carcasses held for 48 hours at 1.1^{o}C (Falk, 1974; Will, 1974).

An even more rapid conditioning of meat would be achieved if the muscles of the carcass were artificially stimulated to contract. This contraction would serve to expand high energy muscle metabolities much faster than would be the case in an unstimulated carcass. Conceivably, a treatment of this type could lead to carcass fabrication immediately after slaughter. If this end were achieved, the advantages of speedy carcass processing would be realized to their maximum extent.

The objective of this study was to assess the effect of electrical stimulation of the beef carcass on the rate of post-mortem muscle glycolysis.

CHAPTER II

LITERATURE REVIEW

Structure of Muscle

Muscle is encased in connective tissue. Entire muscles are surrounded by a connective tissue sheath call the epimysium. From the deep surface of the epimysium, invaginations extend into the muscle at irregular intervals to invest bundles of muscle fibers called fasciculi. Subdivision of the larger bundles into two or three successively smaller bundles is frequently observed. The name perimysium is retained for the connective tissue surrounding the lesser fasciculi (Gould, 1973). From the perimysium, delicate connective tissue extensions pass inward to surround individual muscle fibers.

The amount of connective tissue relative to muscle fibers is much greater in some muscles than in others. This is responsible, to some extent, for differences in tenderness between muscles. The lower the connective tissue content, the more tender the cut and vice versa. In lean meat, collagenous tissue may account for three to 30 percent of the total protein (Gould, 1973).

The cellular constituent of muscle tissue is the muscle fiber, a cylindrical multinucleated structure resulting from

the fusion of several embryonic cells. The plasma membrane of the muscle fiber is covered by a polysaccharide containing coat in which several collogen fibers are embedded; the plasma membrane together with its external coat constitutes the sarcolemma of light microscopy (Constantinides, 1974).

At regualr intervals along the length of the fiber, the cell membrane invaginates to form a network of membranous channels, the "T" or transverse tubular system. The physical continuity these channels share with the exterior membrane provides a means for action potentials to be carried deep into the fiber. The "T" system is closely associated with another intracellular membranous network, the sarcoplasmic reticulum.

Unlike the "T" system, the channels of the sarcoplasmic reticulum are oriented in a longitudinal manner with the fiber and are not continuous with the "T" system or the exterior of the fiber. The sarcoplasmic reticulum binds calcium ions. When the fiber receives an action potential, these ions are released to trigger muscular contraction. The sarcoplasmic reticulum also functions in the elimination of cellular waste material and in ATP synthesis.

The contractile apparatus comprises the bulk of cellular mass and consists of aggregations of protein molecules possessing a great deal of organization. A fiber viewed with the aid of the light microscope shows alternating light and dark bands. Closer examination reveals the fiber to contain numerous fibrils, each banded and lying in register

to give the banded appearance to the fiber as a whole. The bands of the fibril are indicative of the molecular structure and mode of action of muscle.

Each fibril can be considered to be composed of a number of adjacent sarcomeres bounded on either side by a narrow, dense, protenaceous structure, the Z band. This structure consists of at least three proteins with molecular weights of 41,000; 85,000 and 95,000 daltons (Etlinger and Fischman, 1973). The 41,000 dalton protein may be actin and the 95,000 dalton molecule corresponds to the molecular weight of α-actin (Ebashi and Ebashi, 1965).

To the Z band on either side are attached many strands of protein which alone constitute the light I band on either end of the sarcomere. These strands are termed thin filaments and consist of at least three proteins. Actin is the primary protein of the thin filament (Huxley, 1957), a roughly spherical molecule with a formula weight of 41,000 - 44,000 daltons. These globular subunits are complexed into long, bead like strands. Two of these strands are twined in helical fashion and extend from the Z band toward the middle of the sarcomere (Elzinger and Collins, 1973).

Two other proteins of the thin filament are tropomyosin and troponin. Tropomyosin is a two-chain α-helical coil with a molecular weight of about 65,000 daltons (Cummins and Perry, 1972; Cohen, et al., 1973). Tropomyosins are about 400 angstroms in length and sit in the grooves of the actin helix, each tropomyosin spanning seven actins (Cummins and

Perry, 1972; Ebashi, *et al*., 1973).

Troponin was first thought to be a single protein because of its behavior in ultracentrafugation and electrophoresis (Ebashi and Kodama, 1965). However, Hartshorne and Mueller (1968) first showed that it could be separated into two components; troponin A and troponin B. There is now general agreement that troponin consists of three components of which the molecular weights are 37,000 - 40,000; 22,000 - 24,000 and 17,000 - 18,000, respectively (Schuab and Perry, 1969; Hartshorne and Pyun, 1971; Ebashi, *et al*., 1971; Greaser and Gergely, 1971).

Tropomyosin and troponin function in the regulation of contraction. One model postulates that Ca^{++} binding by troponin causes the strands of tropomyosin to change their position relative to the actin subunits. This positional change, in the case of contraction, exposes or activates binding sites on the actin subunits and allows for binding with another muscle protein, myosin (Hanson, *et al*., 1973).

Myosin is the most abundant of the myofibrillar proteins and comprises about 38 percent of the muscle protein (Giffee *et al*., 1960). Frandson (1966) reported that each myofibril contained about 2,500 myosin filaments and Bendall (1969) reported that each myofibril consisted of 180 or 360 myosin molecules.

Myosin is capable of binding calcium and potassium (Harrow and Mazur, 1966) and magnesium is normally bound to the myosin molecule. Myosin has a relatively high charge

because it contains large amounts of glutamic and aspartic acids and some dibasic amino acids.

Myosin is located in the central region of the sarcomere. Aggregates of this protein comprise a muscle structure termed the thick filament. Thick filaments alone and in combination with thin filaments make up the dense A band of skeletal muscle. The centermost area of the sarcomere where no actin filaments project is the H zone and is composed entirely of thick filaments.

Myosin from vertebrate skeletal muscle has two large polypeptide chains, each with a mass of about 200,000 daltons; and three or four smaller subunits each with a mass in the range of 20,000 daltons (Saker, 1973; Weeds and Frank, 1973). The heavy chains are folded into a rodlike α-helical conformation that extends over a length of about 1,400Å in diameter. These regions contain an ATPase which converts the phosphate bond energy of ATP into the mechanical energy of muscular contraction (Lowey and Holt, 1973). The light chains are located in the globular portion of the molecule. One class of these light chains can be removed by treating myosin with 5,5'-dithiobis-(2-nitro-benzoic acid) (DTNE) without loss of ATPase activity. More rigorous alkali treatment removes other polypeptides as well as the enzymatic capability of the molecule. (Perrie _et al_., 1973; Dreizen and Richards, 1973).

Rigor Mortis

With the destruction of the circulatory system at the time of exsanguination, muscles of a carcass with no replenishing nutrient or oxygen supply continue to metabolize stored energy sources and accumulate their end products. The ability of a muscle to contract ceases when the muscle has been depleated of energy supplying molecules. The most outstanding physical occurence in post-mortem muscle is the change from an elastic to a rigid state, rigor mortis (Marsh, 1954) after a varying period of time. The most apparent chemical change in muscle is the production of lactate which causes a decline in the pH.

Potential sources of muscular energy include: 1) muscle adenosine triphosphate (ATP); 2) muscle creatine phosphate; and 3) muscle glyogen (Bate-Smith and Bendall, 1949). Since the hydrolysis of ATP to adenosinediphosphate (ADP) is the driving force of all energy requiring muscular activities, all potential energy sources are geared to sustain ATP levels.

The rate at which ATP is hydrolyzed is obviously dependent on the muscle's energy demands. Convulsions or struggling at death utilize great quantities of ATP through the Ca^{++} activated myosin ATPase (Infante and Davies, 1962). The psoas major of well fed rabbits immobilized prior to death may take 8 hours at 17°C to begin to lose its extensibility, while the rigor onset in similar muscles from rabbits killed without immobilization begins in less than

one hour (Bendall, 1960).

Temperature plays an important role in the time course of rigor mortis. Marsh (1954) demonstrated that the rate of pH fall was progressively more rapid in bovine longissimus dorsi as the ambient temperature was raised from 7 to 43°C. Early investigators realized that increased temperature caused an increased rate of post-mortem glycolysis, which results in a shorter interval between death and the onset of rigor, and in a marked increase in shortening of the muscle during rigor at the higher temperature (Bate-Smith and Bendall, 1949; Partmann, 1963). Both of these events have important implications to be discussed later in relation to delayed chill or "hot" processing.

Bate-Smith and Bendall (1947) reported that the onset of rigor could be correlated with the disappearance of ATP and that the delay of rigor was much longer in narcotized animals than in animals allowed to struggle at death. Later Bendall (1951) stated that rapid hydrolysis of ATP began after creatine phosphate reserves had been depleted to 30 percent of the resting level.

Continuing his research on nucleotide changes in post-mortem muscle, Bendall and Davey (1957) correlated the liberations of anmonia from post-mortem tissue with the breakdown of ATP. The same authors stated that in general, rigor mortis sets in at room temperature when about 3/4 of the initial ATP content had been destroyed, whereas at 37°C the process began earlier when only about half of the ATP had disappeared.

DeFremery and Pool (1960) investigated rigor mortis in chicken muscle and confirmed earlier work on the relationship between rigor onset and ATP concentration and showed that in this species the more rapid the onset of rigor mortis, the less tender the subsequently cooked meat.

An extensive account of post-mortem chemical changes in beef muscle was published by Bodwell and others (1965) in which they reported the initial ATP concentrations of 6.4 mole/g of tissue which is in good agreement of that obtained using a similar assay by Marsh (1954) and the 5.0 μmole/g value of White, Handler and Smith (1973). Bodwell's group, however, using a bioluminescent enzyme assay obtained an initial ATP level of nearly twice the previously reported amount in beef longissimus dorsi excised within 10 minutes of slaughter. Age, breed, and handling procedures may cause this concentration to vary.

Schmidt and Briskey (1970) cast doubt on an absence of ATP being solely responsible for loss of muscle extensibility post mortem as muscle strips remained extensible in the absence of detectable ATP levels. The methods used in assaying for ATP in this study were not specific enough to permit comparison and the initial values reported were less than 2.0 mole/g. Some support for extensibility of post rigor muscles has been presented (Herliky, et al., 1972). Mouse muscles stretched in rigor and examined with the electron microscope showed a significant increase in sarcomere length and a widened "H" zone.

Immediately post-mortem creatine phosphate levels are high but decrease rapidly as ADP is rephosphorylated to ATP with the accompanying formation of creatine. Bendall (1951) found creatine phosphate content of rabbit muscle to be .80 mg/g of tissue. The same author found that ATP stores did not decrease until the creatine phosphate concentration had fallen to 30 percent of its initial level. This point was reached in about 250 minutes at 17°C, and 105 minutes at 37°C.

Bodwell and others (1965) estimated the creatine phosphate concentration of beef longissimus muscles to be 9.1 moles/g at 10 minutes post-mortem which corresponds to about three times Bendal's finding. They further reported that by 6 hours post-mortem at 3°C, post-mortem creatine phosphate had decreased to approximately 20 percent of its initial value while over 90 percent of the initial ATP remained. In a similar study on hogs, no creatine phosphate could be detected in the longissimus dorsi muscle of this species, perhaps due to the use of electrical stunning (Bodwell, et al., 1966).

The effects of various temperatures on the post-mortem metabolism of beef muscle was investigated by Cassens and Newbold (1966). They found that creatine phosphate levels fell more slowly at 15°C than at 37°C, but showed no slower change at 1°C than at 15°C.

The excision of muscles immediately after exsanguination has been shown to hasten the depletion of creatine phosphate

as well as ATP (Koch, *et al.*, 1970). The same author noted that an early excision had an adverse affect on the quality of the resulting meat. Subjecting muscles to extensibility studies on a rigorometer does not, however, deplete creatine phosphate levels at rates faster than for those not subjected to extensibility determinations (Schmidt and Briskey, 1970).

McLoughlin (1974) showed that the ante-mortem administration of the neuromuscular blocking agent curare served to elevate creatine phosphate levels post-mortem in Pietrain pigs. This confirmed earlier work (Sair, *et al.*, 1970) which reported that curarisation served to elevate creatine phosphate levels over those of untreated controls both in stress-resistant (Chester White) and in stress-susceptible (Poland Chine) pigs. These observations may be due to the relaxed state of muscles receiving the curare treatment and the subsequent reduced demand for phosphate bond energy by these muscles.

Whereas the previously discussed sources of muscular energy involve a simple storage of phosphate bond energy, muscle glycogen must be metabolized through the Embden-Meyerhof glycoltyic cycle where two molecules of ATP are produced for every glucose residue (Lehninger, 1970). The glucose residue itself is converted to two molecules of lactic acid which accumulates in post-mortem muscle and lowers its pH (Pearson, 1971).

It was erroneously believed, prior to 1950, that rigor

mortis was due to the coagulation of muscle proteins by lactic acid (Bendall, 1960) although Bate-Smith and Bendall (1947) and earlier investigators had shown the importance of ATP to the rigor process (Bendall, 1960).

Bate-Smith and Bendall (1949) confirmed that the pH decline post-mortem was a result of lactic acid accumulation in the muscle tissue by relating the two factors in a linear manner. The same authors also noted the importance of the nutritional level of the animal and the effect of exhaustion on the rigor process. Well fed, rested animals showed a long delay phase before the onset of rigor. In starved animals or those that were allowed to struggle during death, a rapid rigor onset was noted. The former instance is one in which the animals would have a high glycogen reserve and the latter would represent a glycogen depleted condition.

Marsh (1954) demonstrated the effect of temperature on the rate of fall of pH in post-mortem beef muscle. He found that increasing the temperature from $7^{o}C$ to $43^{o}C$ resulted in faster rates of pH decline. He also found no difference in ultimate pH between grades of beef.

Several parameters were followed during the development of rigor in chicken Pectoralis major muscles by deFremery and Pool (1960). The same sequence of chemical and physical events was observed in this species as for beef and rabbit. Mechanical stimulation by a feather picker was shown to accelerate the rate of pH change and ATP depletion over hand picked controls. Electrical stimulation which depletes

glycogen reserves resulted in accelerated rigor and its accompanying chemical changes.

The same authors found that when a muscle was excised and frozen immediately post-mortem, ATP and glycogen disappearance upon thawing was much more rapid than in fresh muscle. This phenomenon is termed thaw rigor. Every treatment that accelerated rigor rendered the muscles less tender.

Bodwell and others (1965) reported initially a pH value on beef longissimus dorsi of 6.99. At 48 hours post-mortem at 3 - 4°C the mean pH was 5.46. Glycogen was found to be 56.7 moles/g expressed as glucose equivalents initially, 10.0 moles/g after 48 hours and 1.4 moles/g after 480 hours. Lactic acid increased from 13.1 moles/g initially to 82.4 moles/g after 48 hours but not appreciably thereafter. The average time for the onset of rigor in beef was 12 - 15 hours after death. Again a linear relationship was observed between lactic acid accumulation and pH decline.

Cassens and Newbold (1966) reported cold shortening in beef sternomendbularis muscles excised within two hours of slaughter and held at 1°C shortened considerably while those held at 15°C shortened little, if at all. Muscles held at these temperatures did not, however, show any appreciable difference in rate of pH decrease for several hours post-mortem. The authors concluded that cold shortening was not due to glycolytic activity.

The effect of muscle excision on glycolytic rates has been investigated (Koch, et al., 1970). In porcine longissi-

mus, muscle myotomy at the time of slaughter increased the rate of glycolysis over muscles excised 45 minutes post mortem. McLoughlin (1974) reported that muscles of curarized Pietrain pigs had elevated pH values, more glycogen and less lactate than untreated pigs. However, the rate of pH change was the same in both cases. He also reported more extensive glycolysis in longissimus dorsi muscles than in semitendenosus, the former having a lower final pH and greater lactic acid production. This confirmed earlier work by McLoughlin (1970) which stated that pH dropped more rapidly in pigs slaughtered conventionally than in curarized animals and slower still in anesthetized pigs.

Environmental Effects

The environmental temperature exerts a marked effect on the time course of rigor mortis, its extent, severity, and the quality of the resulting meat. Wilson, *et al.*, (1960) found shortening in beef muscle to be much greater at 0 - 15°C than at 20 - 43°C, the higher temperature, however, provided more extensive aging which obscured toughening at the elevated temperatures. Locker and Hagyard (1963) observed minimum shortening in ox muscle when the ambient temperature was 14 - 19°C. Porcine muscle allowed to enter rigor at 2°C was less tender and exhibited more shortening than muscles held at 16°C (Forrest, *et al.*, 1969). Smith, *et al.*, (1969) examined sarcomere length in avian muscles held at selected temperatures and found maximum shortening at 20°C and minimum shortening at 12 - 18°C.

Cassens and Newbold (1967) showed that the time post-mortem before the onset of rigor mortis increased as the temperature was reduced from 37°C to 1°C. Sink, et al., (1965) observed that a short delay phase corresponded with a more severe rigor with increased shortening, whereas a longer delay before the onset of rigor causes less shortening.

From the above, it would seem that the optimum temperature for muscle to enter rigor mortis is about 16°C. This temperature causes the least amount of post-mortem shortening and increased product tenderness. Leavy, et al., (1962) proposed an explanation for this based on a change in the shape of the enzymatic (ATPase) portion of the myosin molecule at 16°C which impared the muscle's ability to shorten at this temperature.

Ramsbottom and Strandine (1949) postulated that boneless cuts removed from the bovine carcass soon after slaughter would chill faster than would the intact carcass. This was confirmed as boneless loins excised an hour after slaughter were 10 - 15°F cooler than bone-in loins at two to eight hours post-mortem. However, the muscles which were excised before chilling were less tender than those which were allowed to remain on the carcass while it was chilled.

Differences in individual muscles when processed pre-rigor were shown by Reddy (1962) who reported a decrease in tenderness of the semitendenosus but no difference in the tenderness of the longissimus dorsi and gluteus medius when compared to their conventionally processed counterpart.

Trautman (1964) reported a greater emulsifying capacity in pork processed pre-rigor. He also detected more salt soluble proteins than in conventionally processed pork. This finding was confirmed by Johnson (1969) who found a significant difference in salt soluble proteins between pre- and post-rigor muscle.

"Hot" processed hams were found by Pulliam and Kelly (1965) to have higher bacterial counts than conventionally processed hams when tested prior to smoking. After smoking bacterial counts were low in both groups. Barbe, et al., (1966) and Barbe and Henrickson (1967) found less total contamination in "hot" processed hams than in those conventionally processed. It was theorized that the more rapid processing of the "hot" hams resulted in less chance for bacterial growth to occur. Mandigo and Henrickson (1966) reported that hot processed hams, cured and smoked prior to chilling were equal or superior to conventionally processed hams when yield, tenderness, juiciness, flavor and moisture were considered. Ham muscles excised hot, cured and canned exhibited less free fluids in the can, a higher moisture content, greater shear values, more nitrosopigments, and more extensive cure diffusion than cold processed ham muscles (Reddy and Henrickson, 1969). As with pork, beef exhibits a greater emulsifying capacity when processed pre-rigor (Acton and Safle, 1969).

Organoleptic and microbial properties of hot processed bovine muscles were examined by Schmidt and Gilbert (1970).

They excised muscles at two hours post-mortem, aged them for 24 or 48 hours at 15°C in gas impermeable bags, and then froze them at -14°C. Corresponding muscles from the remaining side of the carcass were evaluated after suspension for 24 hours at 9°C. The "hot" processed muscles aged for 24 hours were similar to the controls in tenderness and "hot" muscles aged for 48 hours were more tender than muscles removed from the control side. Bacterial counts were kept within the range of 10^2 to $10^5/cm^2$ of muscle surface after 48 hours storage at 15°C in the gas impermeable bags. It was concluded that this method could produce an organoleptically acceptable product of a satisfactory microbial level.

Economic aspects of "hot" boning the bovine carcass were discussed by Brasington and Hammons (1971) who indicated that on-the-rail boning resulted in lower costs due to the use of semi-skilled workers for a portion of the operation. The operation was also reported to be more flexible, more sanitary and less fatiguing to workers as well as showing favorable yield test results.

Kastner (1972) excised muscles from bovine carcasses at two, five and eight hours post-mortem. These were compared to the muscles of the corresponding sides which were held at 2°C for 48 hours prior to fabrication. Shear force values were greater for the "hot" boned muscles removed at two and five hours post-mortem, but where not statistically different from the "cold" boned muscles when removed eight hours after death. The author considered percent loss, percent

moisture, pressed fluid ratios, color scores, percent cooking loss and flavor panel scores, as well as shear force values. He concluded that "hot" boning was feasible when the muscles were excised at five or eight hours post-mortem. The author further reported that fabrication time was reduced due to the easy excision of muscles facilitated by the pliable condition of fat and muscle.

Schmidt and Keman (1974) removed muscles from the right side of six Angus steer carcasses one hour after slaughter. The left sides remained intact at $1^{o}C$. The boneless wholesale cuts from the right sides were kept at $7^{o}C$ for four hours, then transferred to a $1^{o}C$ storage overnight, and chilled for eight days at $7^{o}C$. "Cold" boning of the left sides were accomplished after eight days chilling at $7^{o}C$. Anterior and posterior cuts of the longissimus dorsi, the psoas major, gluteus medius, semitendenosus, semimembranosus, biceps femoris, and quadriceps femoris were evaluated by taste panel and by the Warner-Bratzler shear instrument. No significant difference was found between muscles of "hot" and "cold" processed sides.

Muscles were removed from bovine steer carcasses at three, five and seven hours post-mortem by Falk, et al., (1975) who compared them to "cold" bonded muscles with respect to shear force, sarcomere length, color, taste panel preference, rate of pH decline, phychrophilic bacterial population, yield, pressed fluid ratio, cooking loss, moisture content, and fat. The longissimus dorsi excised at five

hours post-mortem and the semimembranosus excised at seven hours post-mortem were less tender than their "cold" boned counterparts although the difference was quite small. The authors concluded that "hot" processing was feasible as early as three hours post-mortem.

Will (1974) and Will and Henrickson (1976) examined the tenderness of bovine muscles excised at three, five and seven hours post-mortem with several objective and subjective measures of tenderness. They found that no major differences attributed to tenderness existed between muscles excised at those times and those allowed to remain on the suspended carcass for 48 hours.

Kastner and Russell (1975) excised bovine muscles after six, eight and ten hours at 16°C. They examined shear force, rate of pH decline, color and flavor of the resulting meat and found a conditioning period of eight hours to yield a product comparable to the conventional method.

The eating quality of "hot" boned beef was studied by Dransfield _et al._, (1976) who conducted taste panel and consumer evaluation of meat removed from the carcass within three hours of death. Nonsignificant differences were found between "hot" boned and control beef with respect to tenderness, juiciness, flavor and overall acceptability. Consumers rated the overall acceptability of hot boned beef equal to that of its conventionally fabricated counterpart for all cuts studied except the flank where hot boned beef was preferred.

Electrical Stimulation

Harsham and Daetherage were issued a United States patent for the use of electrical stimulation to increase the tenderness of meat in 1951. Electrical irritation was said to release the latent energy stored within the muscle and render it more susceptible to enzymatic digestion which produced a more tender product. The authors also demonstrated an accelerated rate of pH decline in electrically stimulated muscle.

Chicken muscle which had been electrically stimulated to exhaustion was shown by DeFrenery and Pool (1960) to have a faster rate of pH decline and an accelerated rate of ATP disappearance post-mortem. The shear values of these stimulated muscles were higher than those of unstimulated controls.

The effect of stimulation on rates of glycolysis in frog sartorius muscle was investigated by Kapatkin, *et al.*, (1964). They found that when shocks of 15 volts and two miliseconds duration were used, the rate of glycolysis measured by the rate of lactic formation increased with the number of shocks administered per minute. Hallund and Bendall (1965) also reported an increase in the rate of pH fall in the muscles of stimulated pigs. Muscles from pigs which exhibited a slow rate of post-mortem glycolysis have been shown to be more responsive to electrical stimulation than those with an intermediate rate (Forrest, *et al.*, 1966; Forrest and Brisky, 1967).

Carse (1973) investigated electrical stimulation as a means of achieving tenderness in lambs frozen soon after

slaughter. He pointed out that to prevent muscle toughening the animals needed to be suspended at 15°C for 16 hours before fabrication. A significant tenderization effect was found in lambs due to stimulation when compared to lambs frozen five hours post-mortem. Shear values were slighly lower for stimulated lambs than for animals held for 20 hours before freezing. The author also investigated electrical parameters and found that only voltage effected the rate of pH decline.

Chrystall and Hagyard (1976) used high voltage electrical stimulation to accelerate post-mortem glycolysis in lamb carcasses. When the authors compared stimulated frozen lambs to unstimulated lambs frozen at 60 minutes post-mortem, they found that shear values in the former group were about half the values obtained for the latter. In a similar experiment involving beef, Davey, et al., (1976) reported an increase in tenderness due to stimulation in the longissimus dorsi muscle. Stimulated and unstimulated sides were placed in a chill cooler at 40 minutes post-mortem and boned after 24 hours. Davey's group, therefore, used a more realistic control group than did Crystall and Hagyard (1976).

CHAPTER III

MATERIALS AND METHODS

This investigation was divided into three parts. The first study used six Hereford heifers of approximately the same weight and market grade (choice grade). These animals were delivered to the Meat Science Abattoir 24 hours prior to slaughter. After a 24 hour shrinkage period, the heifers were weighed and federally inspected (ante-mortem). Live weights of the animals ranged from 310 to 367 Kg. The animals were stunned by a captive bolt percussion stunner, bled, skinned, evicerated, split in the conventional manner and federally inspected (post-mortem).

Within an hour after death, the sides were moved into a 16°C room and each was randomly assigned to one of two treatments. The side to be stimulated was connected to a pulse generator via two leads, one a set of eight stainless steel shroud pins connected by copper wire and held in place by a teflon band. The pins were inserted into the shank musculture; principally, the gastrocnemius and distal biceps femoris. The other lead was connected to three stainless steel shroud pins which were connected by copper wire and inserted into holes drilled in the third and fifth cervical vertebrae.

The pulse generator delivered a direct current square

wave pulse with a frequency of 400 cycles per second and a duration of .5 miliseconds. The voltage was increased to 300 volts as read from a Hewlett Packard model 120 AR oscilliscope. Stimulation was initiated at one hour post-mortem and was concluded after 30 minutes.

Four muscles were sampled at one hour post-mortem, before stimulation was applied; and again at two, four, six, eight, 12 and 24 hours post-mortem. One-half inch cores were taken from the longissimus dorsi at the area of the tenth rib, from the semimembranosus at an area two inches posterior to the posterior border of the symphysis pubis, from the medial third of the supraspinatus and from the anterior third of the psoas major.

These samples were weighed and homogenized in ten volumes of doubly distilled water using a Kinematica Polytron homogenizer fitted with a three quarter inch head. The pH of the homogenate was estimated on a Corning model research pH meter fitted with a combination electrode, and calibrated before each series using pHydrion buffers of pH 5.60 and 7.00. Muscles from sides assigned to the unstimulated treatment were sampled and analysed in the same manner.

At the conclusion of the four hour post-mortem sampling both stimulated and unstimulated sides were fabricated by cutting between the fifth and sixth rib and removing the plate and flank, leaving a streamlined hind quarter on the rail.

From the forequarter the supraspinatus was dissected and

placed with the muscles of the streamlined hind quarter which were removed from the skeleton at this time. The order of removal was: tensor fascia latae, gracilis; semitendenosus; biceps femoris, semimembranosus; quadraceps complex, psoas major, gluteal muscles, and longissimus dorsi. Dissection was completed within twenty minutes of initiation. The muscles were then identified, wrapped in clear sheets of Cryovac Process Packaging, and removed to a 1.1^{o}C cooler where they remained through the duration of the sampling procedure.

The second part of this study consisted of a 2^2 factorial arrangement of treatment design conducted to study the effect of changing the method of generator contact with the side, as well as the effect of different stimulation periods. Six Hereford heifers were used, again of similar weight and grade (choice grade). The weights of these animals ranged from 285 to 391 kilograms. The animals were slaughtered as previously described.

The two stimulation durations studied were three minutes and thirty minutes. Three minutes was considered the low level of stimulation and thirty minutes the high level. Generator contact was either by probes inserted into the shank musclature or by attaching the generator leads to a side's suspensory hook. The insertion of probes in muscle was arbitrarily designated the low level of contact; attachment to the hook was designated the high level of contact.

Each side was assigned to one of four treatment combin-

ations; 1) low level of stimulation, low level of contact; 2) low level of stimulation, high level of contact; 3) high level of stimulation, low level of contact, and 4) high level stimulation, high level of contact.

Sampling, muscle removal and pH analysis were conducted as previously described. Electrical parameters were the same as in the first experiment except for the duration of the stimulus in those sides assigned to the low level of stimulation. Stimulation was initiated one hour after death.

The third experiment utilized seven animals, two steers and five heifers. Live weights ranged from 363 to 447 kilograms and the animals were approximately the same grade (choice). A speedy slaughter was performed as previously described. Sides were removed to a 16°C room and randomly assigned to one of two treatments. The sides that were stimulated were connected to the generator as described for the first experiment. One-half inch cores were taken as before from the longissimus dorsi, semimembranosus, and supraspinatus of each side at 30 minutes post-mortem (before stimulation), one, two, four, six, eight, 12 and 24 hours post mortem. Stimulation was initiated at 30 minutes post-mortem and continued for 15 minutes. Voltage, frequency, and duration of the pulse were regulated as in experiment one.

After the two hour samples were taken, the sides were fabricated into streamlined hind quarters and muscle removal was started. The muscles were removed in the manner described above and were wrapped and removed to a 1.1°C. cooler for

the duration of sampling. pH analysis was performed as previously described.

The analysis of variance and the F-test were used to analyze differences in pH. In the first study (four hour holding period), analyses of variance were calculated for each muscle. The animal by treatment interaction with five degrees of freedom was used as the error term. Means from each muscle for each sampling period were evaluated using Duncans New multiple range test.

Analyses of variance were also calculated for the differences in pH at each time period. The animal by treatment interaction with 12 degrees of freedom was used for the error term.

For the 2^2 factorial arrangement of treatments, analysis of variance were calculated for each muscle at each sampling period. Factorial effects were confounded with blocks. The intra-block error had three degrees of freedom.

Analysis of variance were compiled by muscle for the two hour holding period. The animal by treatment interaction with six degrees of freedom was used as the error term.

CHAPTER IV

RESULTS AND DISCUSSION

30 Minute Stimulation

When bovine sides were subjected to electrical stimulation, strong tetonic contractions were elicited. The sides would twist laterally away from the median plane of the carcass along which it had previously been split. These contractions lasted for 30 - 45 seconds during which time residual blood was expelled from the sides as a result of the contractions. When maximum voltage was applied at the beginning of a stimulation period, the side would respond with a sudden tetanus. This response, it was felt, could cause the side to jump from the rail. To prevent this, the voltage was adjusted to its minimum at the onset of stimulation and then elevated to its maximum resulting in a smooth, gradual contraction.

Analyses of variance by sampling period showed a nonsignificant difference ($P > .05$) between the pH of corresponding sides before stimulation. There was a nonsignificant difference in pH among the six carcasses ($P > .05$). Within sides a significant difference in pH ($P < .05$) was observed among the muscles sampled.

At two hours post-mortem, thirty minutes after stimula-

tion ceased, there was a significant difference ($P < .05$) in pH between stimulated and control sides, as well as among muscles ($P < .01$). At this time period there was also observed a significant ($P < .01$) treatment by muscle interaction. These three parameters; treatments, muscles, and treatment by muscle interaction remained significant ($P < .05$) sources of variation through the four, six, and eight hour sampling periods. At twelve hours post-mortem, the interaction was marginally significant ($P < .06$), and after 24 hours its effect was further reduced. Treatments and muscles remained important sources of variation through the 24 hour sampling period.

When these data were examined by individual muscles, the source of the muscle by treatment interection became apparent. Figures 1, 2, 3 and 4 show mean values and standard errors for the longissimus dorsi, psoas major, semimembranosus and supraspinatus muscles respectively at each sampling period. When these means were subjected to student's t tests, nonsignificant ($P > .05$) differences were found between the psoas major muscles from stimulated and unstimulated sides.

Nonsignificant differences were also found between the pH values of the supraspinatus muscle from stimulated and control sides at all sampling periods after stimulation except for 4 hour post-mortem period where a significant ($P < .05$) difference was observed. Unlike the psoas major, however, the supraspinatus pH readings, when averaged over time periods, showed a significant ($P < .01$) reduction in pH in muscles taken from stimulated sides.

pH OF L. DORSI STIMULATED
FOR 30 MINUTES, 1 HOUR POST-MORTEM

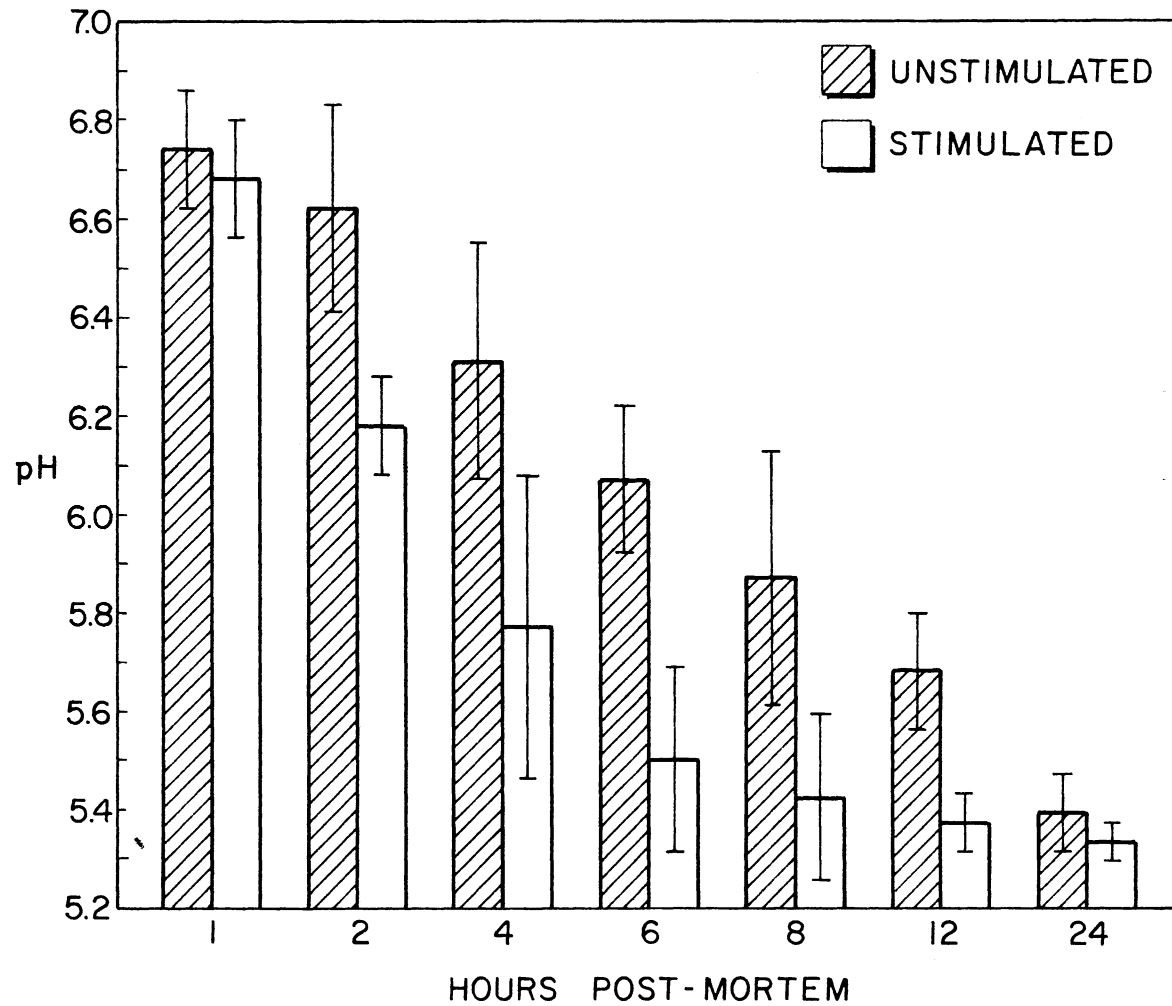

Figure 1.

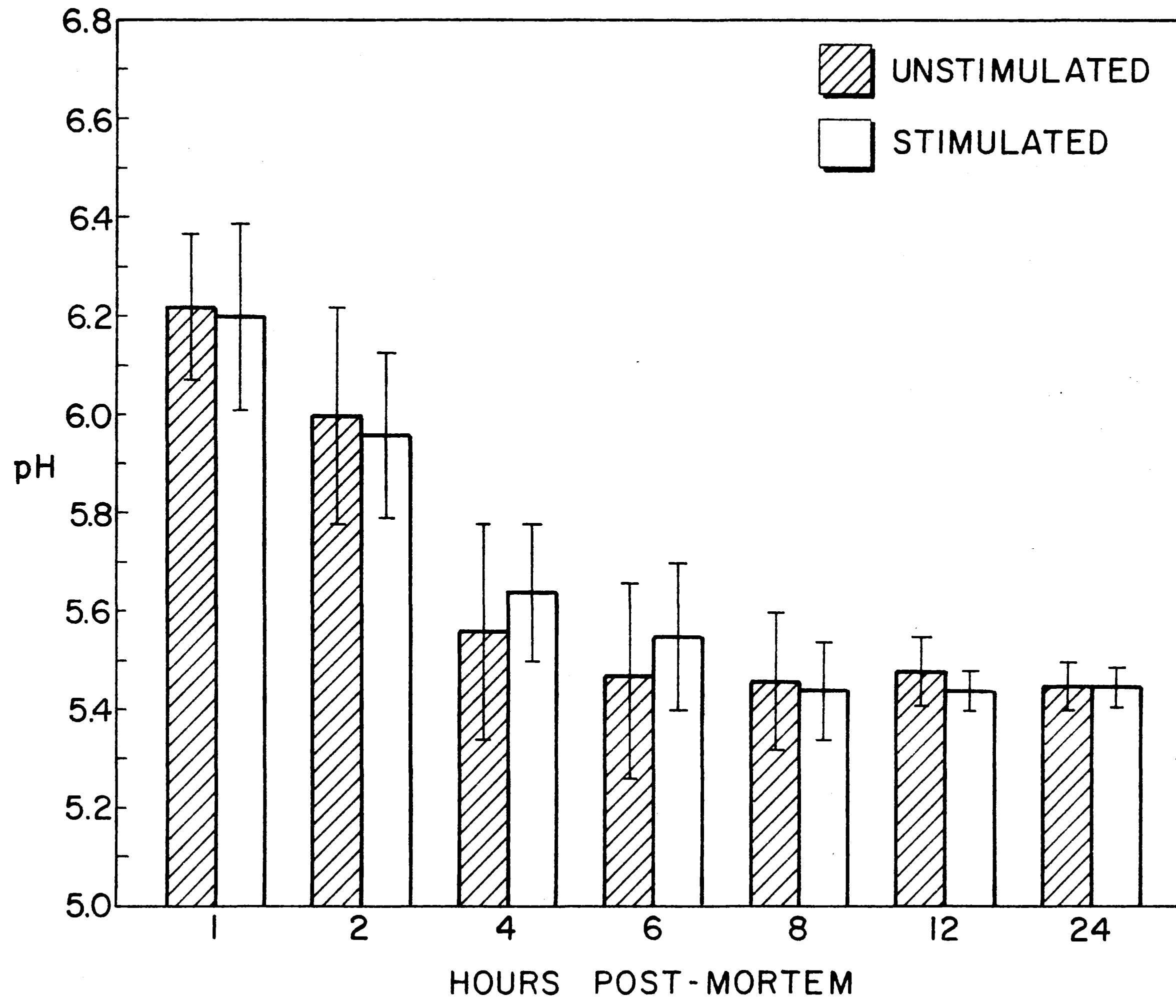

Figure 2.

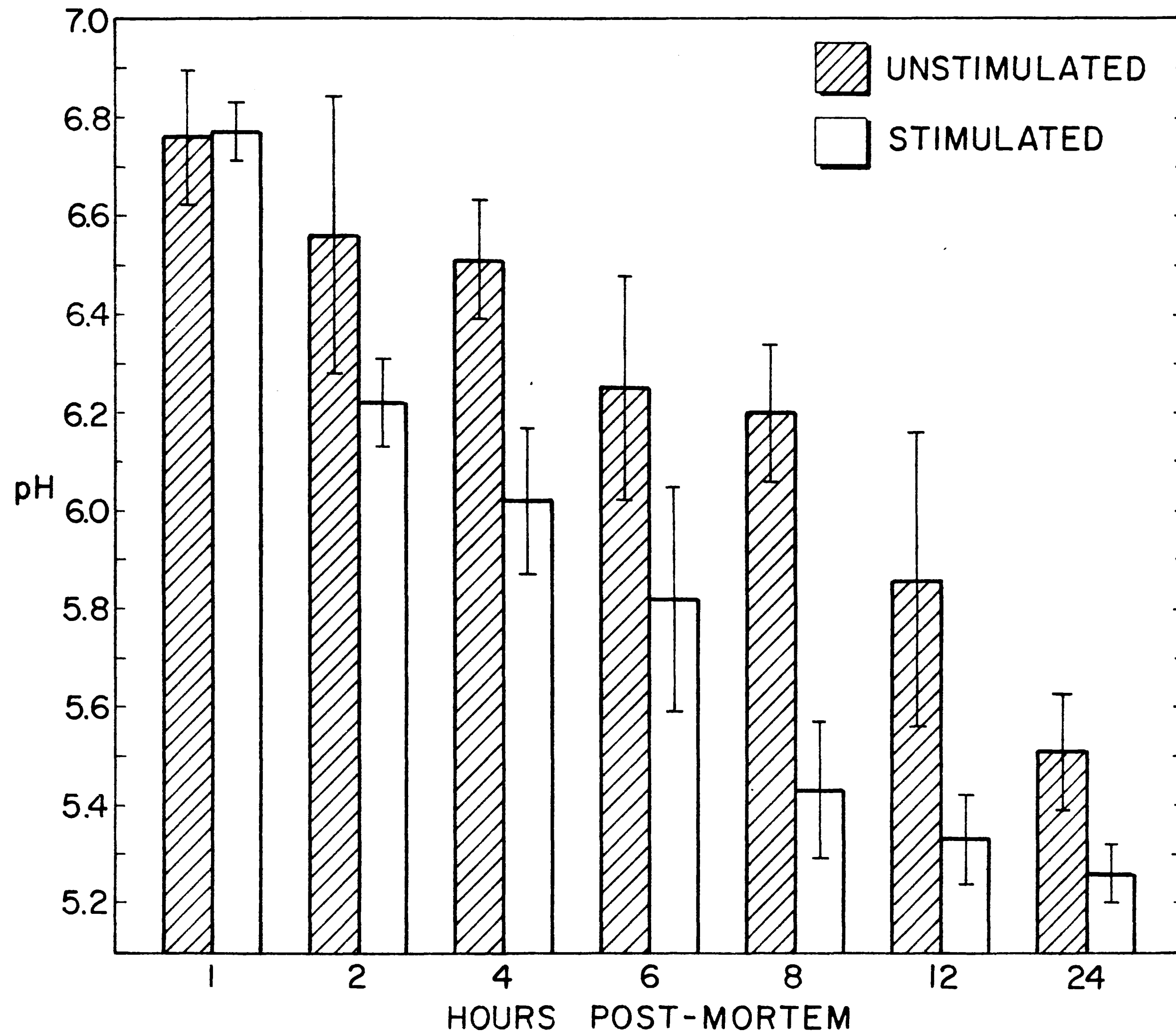

Figure 3.

pH OF SUPRASPINATUS STIMULATED
FOR 30 MINUTES, 1 HOUR POST-MORTEM

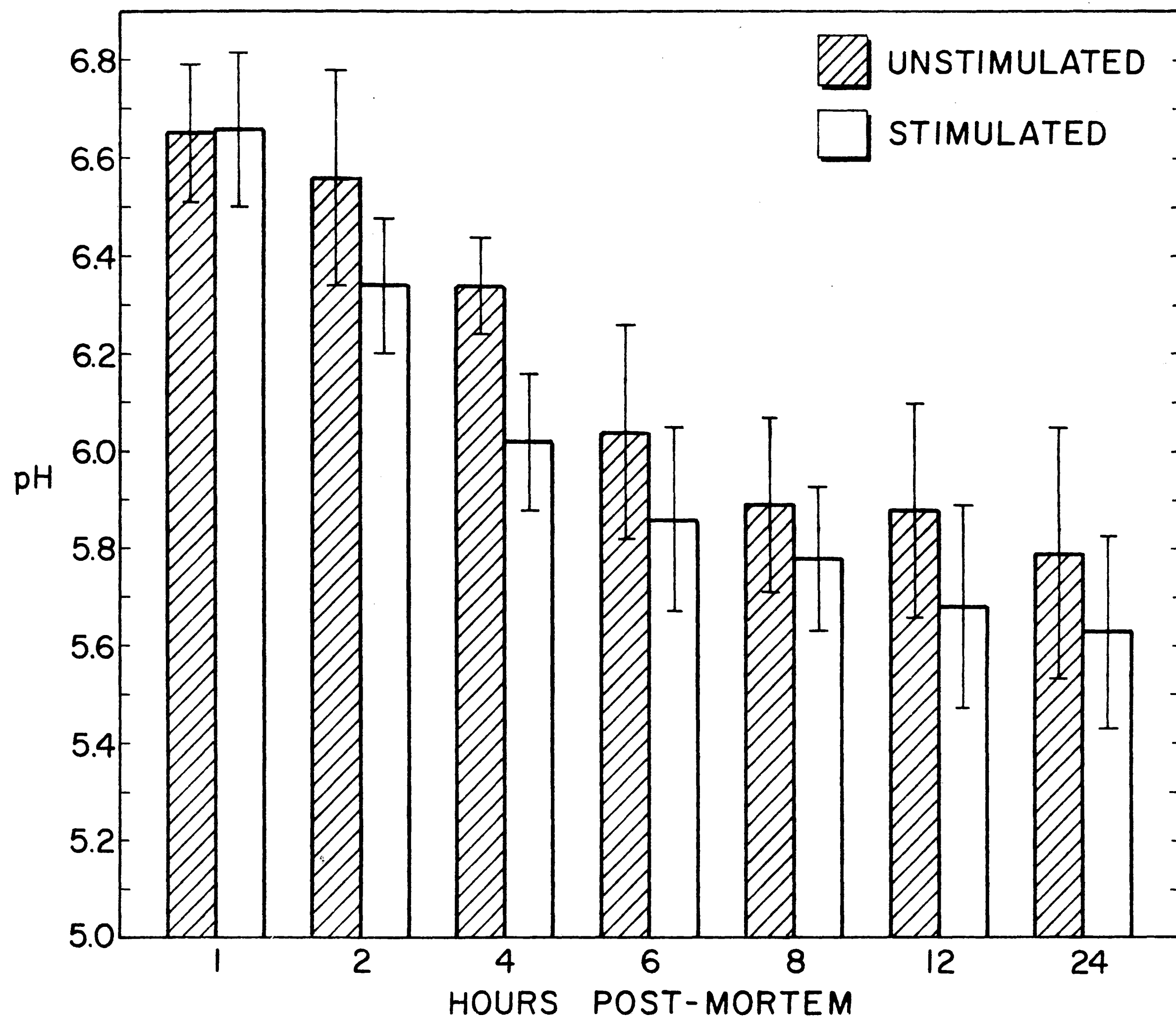

Figure 4.

The greatest response to the stimulus was elicited in the longissimus dorsi which showed a significant reduction in the pH of muscles from stimulated sides at each sampling period after stimulation with the exception of the 24 hour post-mortem sample. The semimembranosus responded similarly to the longissimus although a significant difference between the pH of muscles from stimulated and control sides persisted through the 24 hour sampling period.

The outstanding points that can be gleaned from these results are that electrical stimulation can increase the rate of post-mortem decrease of pH in bovine musculature although this effect is not uniform through all muscles and some muscles may show no effect at all.

The marked response of the longissimus dorsi and the semimembranosus might indicate that those muscles which lie along the shortest path from electrode to electrode may receive a relatively greater proportion of the stimulating current as suggested by Crystall and Hagyard (1976).

The most obvious cause for accelerated post-mortem metabolism in the muscles of stimulated sides is the contraction elicited by the stimulus. These contractions must be fueled by the hydrolysis of adenosine triphosphate (ATP) and accelerated pH decline results from lactate formation due to glycolytic activity producing ATP in response to the muscles' contractile needs. The inability of the electrical stimulus to produce an increase in pH decline of the psoas major could then be explained if one considers the method of carcass

suspension relative to this muscle.

Suspension via the achilles tendon exerts great tension on the psoas major. The contraction of this muscle in a suspended side should result in the side flexing around the hip joint, causing the side to assume a more natural posture as opposed to the extended hind leg position in the suspended situation. Stated another way; in order to contract, the psoas would have to lift the entire weight of the side from the acetabulum anterior. Such contraction may be beyond the capacity of this muscle, especially considering the extreme stretch imposed in it by normal suspension.

Even if one conceeds that the initial muscular contraction is responsible for the increased rate of pH decline during stimulation, this does not explain the continued effect observed hours after stimulation is discontinued. Bendall (1976) has suggested that the stimulus in some way damages control mechanisms of the musculature resulting in an uncontrolled release of Ca^{++} ions and a sustained ATP hydrolysis by the contractile mechanism. Later, Bendall _et al._, (1976) contends that the accelerated post stimulation pH decline may be due to elevated temperatures in the stimulated sides. Davey _et al._, (1976) reported that temperature falls faster in stimulated sides than those not subjected to this treatment although the temperature shortly after stimulation may be higher in these sides than in controls. Similar results were reported by Grusby and West (1977), and unpublished data from this laboratory also suggest that stimulated sides

exhibit a more rapid temperature decrease than unstimulated sides.

15 Minute Stimulation

The 15 minute stimulus initiated 30 minutes post-mortem was meant to evaluate a stimulation of shorter duration and to learn if beginning the current sooner post-mortem could produce a more pronounced effect in the supraspinatus than was elicited when the stimulus was applied one hour after death.

Before stimulation, 30 minutes post-mortem, significant differences ($P < .05$) were observed among animals and muscles. One hour post-mortem, 15 minutes after the cessation of stimulation, the pH of muscles from stimulated sides had declined an average of .50 units compared with a pH drop of .09 units in unstimulated controls. The difference between these values was highly significant ($P < .001$). This was the only significant difference between treatments at this sampling period.

By two hours post-mortem the difference between treatments was still highly significant ($P < .0001$). There was also a difference noted in the pH fall among the animals ($P < .05$).

At four hours after death the differences among animals was not statistically significant although a marked treatment difference persisted ($P < .001$). This treatment difference continued to be observed at six hours post-mortem. The difference among animals reappeared ($P < .05$) at this time period and a difference among muscles was seen to be margin-

ally significant ($P < .06$).

By eight hours post-mortem, in addition to the marked treatment difference ($P < .0001$), there were also significant difference noted among animals ($P < .01$), and among muscles ($P < .05$). These same parameters - treatments, animals, and muscles - were important sources of variation 12 hours after slaughter with P values less than .0005, .005 and .05, respectively.

The final sampling at 24 hours post-mortem saw significant differences among animals ($P < .05$), between treatments ($P < .05$) and among muscles ($P < .0001$).

Mean pH values and standard deviations for individual muscles are shown in figures 5, 6 and 7. The longissimus from stimulated sides showed a significant ($P < .05$) reduction in pH compared to corresponding muscles from unstimulated control sides at each sampling period after stimulation except at 24 hours post-mortem. Its behavior was similar to that of the muscles subjected to the longer stimulation time although a direct comparison is not possible due to the difference in holding times at $16^{o}C$.

Semimembranosus muscles also showed a more rapid decrease of pH in stimulated sides than in muscles taken from unstimulated controls ($P < .05$).

The supraspinatus showed a more pronounced pH decline when subjected to the 15 minute stimulus 30 minutes post-mortem than when stimulated for the longer period one hour after death. It thus appears that it is important to begin stimu-

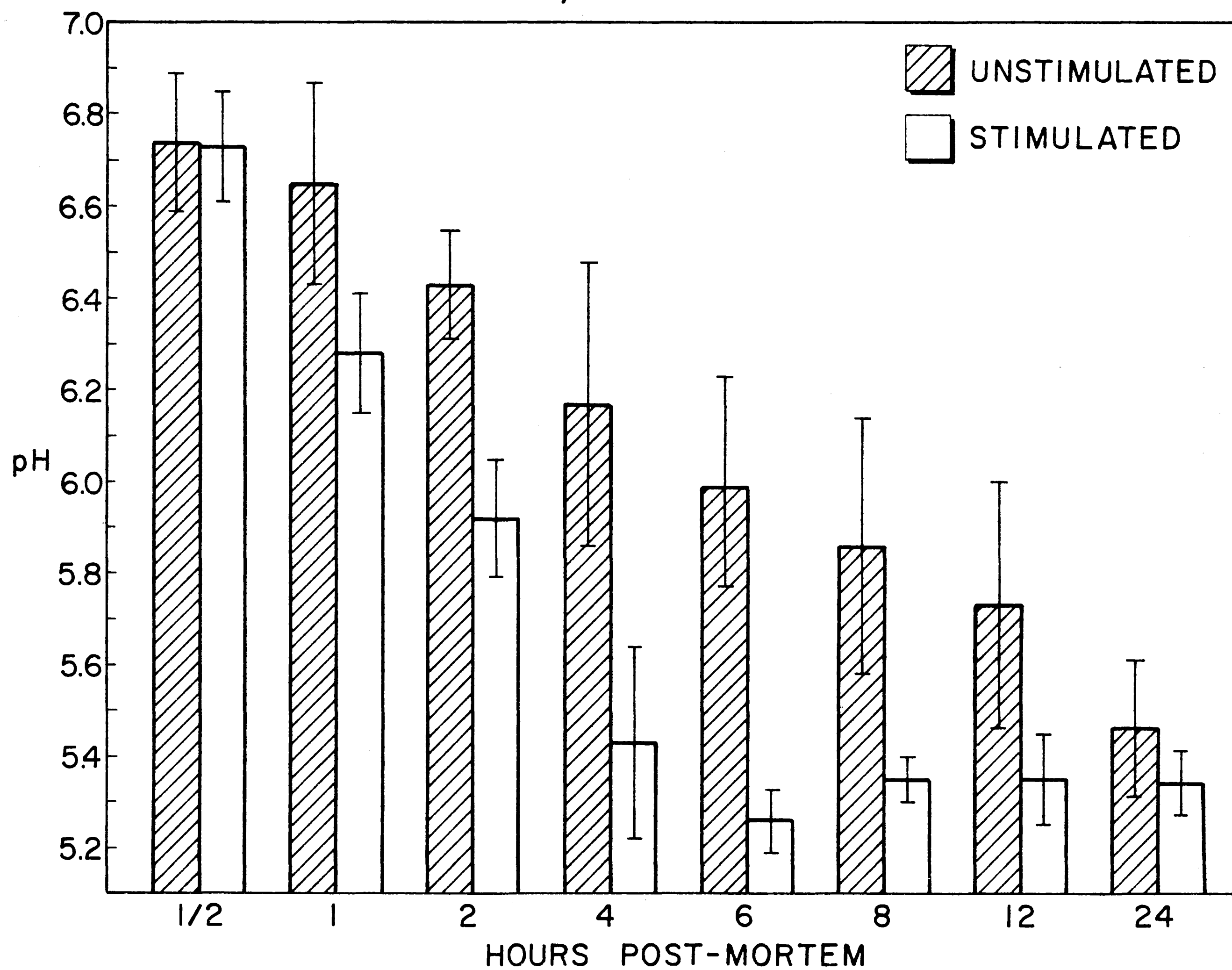

Figure 5.

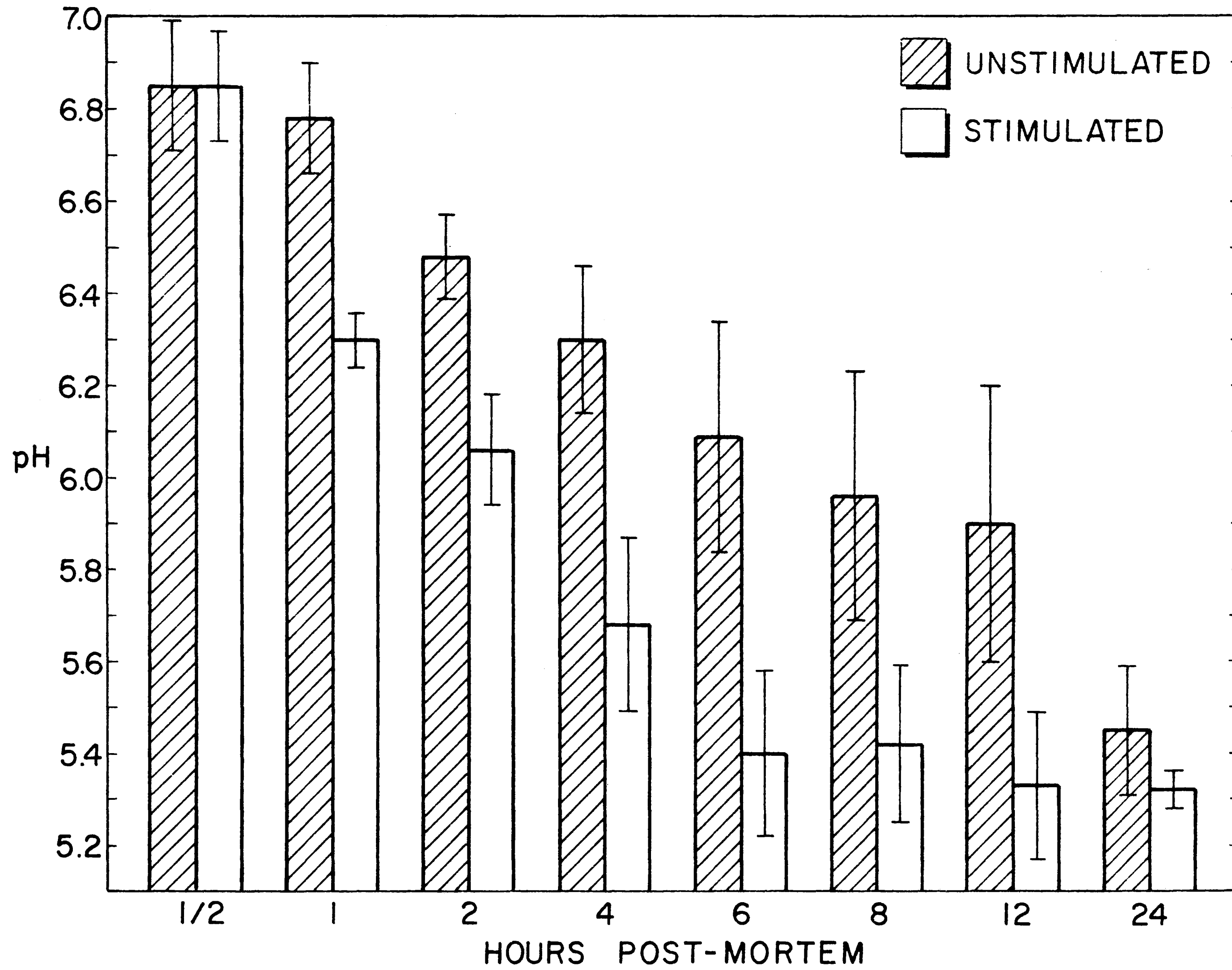

Figure 6.

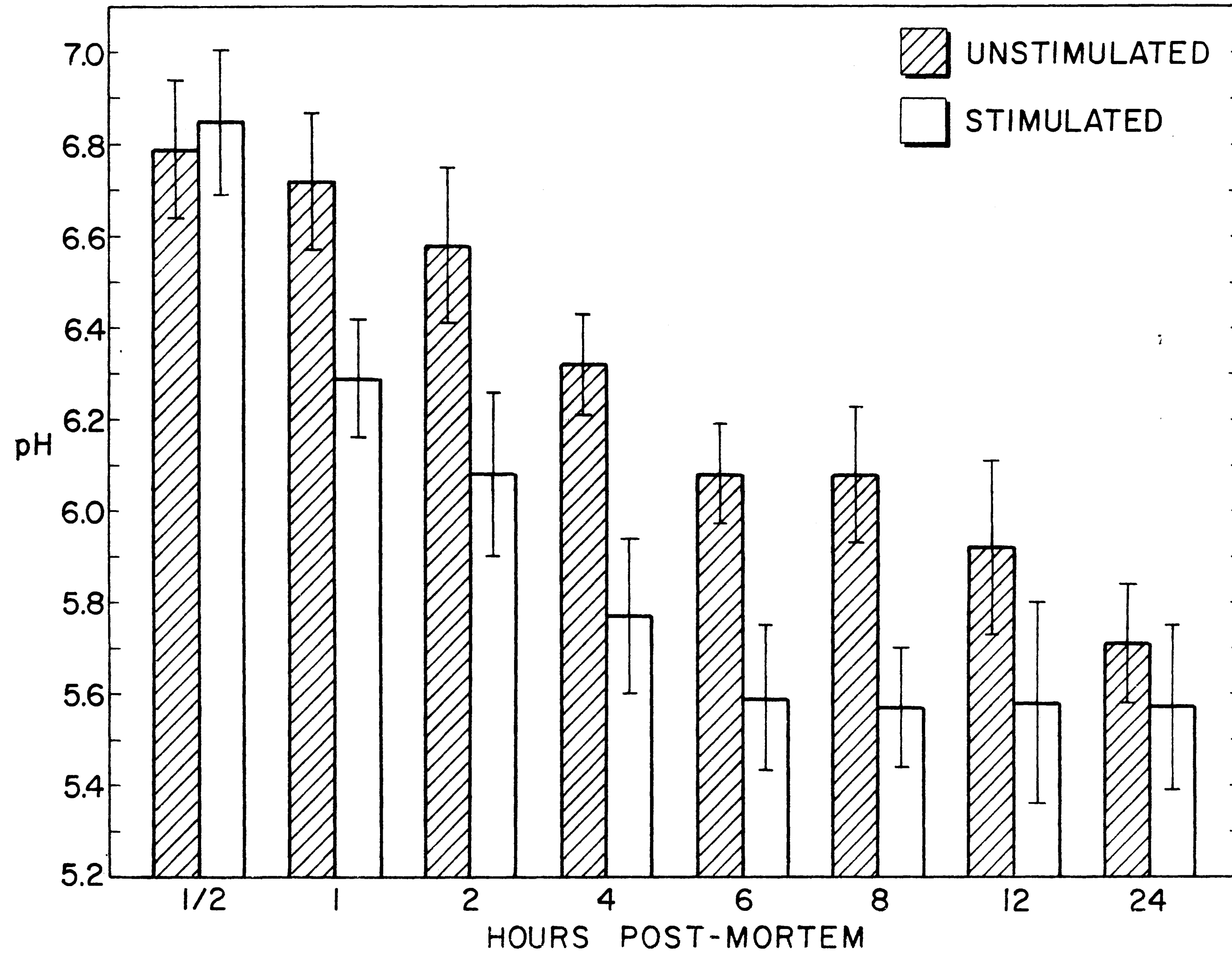

Figure 7.

lation as soon post-mortem as possible that the muscle will be in a more lifelike state and may respond to the stimulus to a greater degree.

Stimulation Duration and Contact Method

The two by two factorial arrangement of treatments was designed to compare two stimulation periods, three and thirty minutes, and two methods of carcass attachment, by pins inserted into the round and by connection to the suspensory hook. In each case, stimulation was begun at one hour post-mortem.

The longissimus displayed a treatment difference only at the 12 hour sampling period when the longer stimulation time produced a greater pH fall ($P < .05$) than the shorter stimulation period.

In the semimembranosus the treatment effects were significant ($P < .05$) at the six hour sampling period. At this time the pH drop in muscles from sides stimulated for the shorter length of time was greater than in muscles from sides receiving the longer stimulation. There was also a difference observed between the methods of contact used. The semimembranosus from sides connected to the stimulator via probes inserted into the round showed a greater pH decline at six hours post-mortem than did sides attached via the suspending hook.

Neither the psoas major nor the supraspinatus showed any significant treatment differences at any sampling period.

These results can most likely be interpreted as showing no difference between the two methods of stimulator contact or between the two stimulus durations. When a difference was detected due to length of stimulation time, it favored the shorter time in the case of the semimembranosus and the longer time in the longissimus.

Examination of the two parameters in four muscles at seven sampling periods revealed a total of 56 opportunities to declare significance. At the P = .05 level it is not unrealistic to expect the parameters to be declared falsely significant three times, especially since there is no detectable pattern or trend involved.

In light of recent literature (Bendall, 1976; Bendall _et al_., 1976; Davey _et al_., 1976) more information might have been gained regarding stimulation duration if the times selected had been considerably shorter. Thirty minutes of electrical stimualtion is almost certainly excessive and a stimulus three minutes duration accomplishes the same results. Possibly only a few seconds of the treatment would suffice, greatly streamlining the operation.

Both methods of contact to the pulse generator have advantages. Attachment to the hook is simple and prevents burning found around the area of probe insertion. When long stimulation periods are used, however, heat build up in the hook may hydrolyze the achilles tendon to gelatin creating a safety hazard. This danger would be minimal when stimulation is of a short duration.

CHAPTER V

SUMMARY

The objectives of this research were to assess the effectiveness of electrical stimulation as a method of speeding post-mortem metabolism, and to investigate the relative merits of different stimulation procedures.

In one experiment a side from each of six carcasses was stimulated for 30 minutes beginning at one hour post-mortem. The other side from each carcass was held as an unstimulated control and muscles were removed four hours after death. In another experiment seven carcasses were used. One side from each was stimulated for 15 minutes beginning 30 minutes post-mortem. The other side from each carcass was held as a control and muscles were removed at two hours post-mortem.

Six carcasses were used to investigate the effects of the application of short and long duration stimuli and two methods of attaching sides to the pulse generator.

The 30 minutes stimulation administered one hour post-mortem caused a more rapid decrease of pH in the longissimus dorsi and semimembranosus muscles as compared to that in controls. A slight increase of this rate was noted in the supraspinatus although no effect was evident in the psoas major.

Stimulation for 15 minutes begun 30 minutes after death resulted in an accelerated rate of pH decline in the muscles of stimulated sides. A more pronounced effect was noted in supraspinatus under these stimulation conditions than was observed in this muscle when stimulated for the longer period of time, an hour after slaughter. This suggested that it may be desirable to initiate stimulation as soon post-mortem as possible to elicit the greatest effect.

The application of a 30 minute stimulus at one hour post mortem resulted in no greater rate of pH fall than was observed when sides were subjected to only three minutes of stimulation. There was no difference in rate of pH fall that could be attributed to the manner in which the stimulus was introduced into the carcass.

These results suggest that a stimulus of only a few minutes may be needed to achieve the desired effect, and that the main considerations in connecting a side or carcass to a pulse generator may be safety and ease of attachment.

The pulse generator used in this research was capable of altering the frequency of the pulse and the proportion of the pulse during which the voltage was applied (duration), as well as the voltage of each pulse. This manuscript describes experiments in which the length of stimulation and mentods of carcass contact were varied. Using only these five parameters, the number of experiments necessary to examine all their effect would be quite large. The literature (Carse, 1973; Bendall, 1976; Bendall, _et al_., 1976) suggested

that only voltage is important in increasing post-mortem pH fall, but that the use of extremely high voltage is not warrented.

This research showed that the method of carcass contact is not critical and that a stimulus of only a few minutes will produce the desired results.

It would seem, then, that the mechanics of carcass stimulations do not require intensive investigation at this time. A wide variety of methods seem equally effective in speeding post-mortem metabolism. Further research is needed, however, to determine the best ways to handle electrically stimulated beef to produce a meat of high quality.

It would be useful to know the minimum time post-mortem that stimulated beef could be boned without adversely affecting its eating quality. In this respect it would be important that a short stimulation be administered immediately after exsanguination. This would allow rapid post-mortem metabolic activities to be occuring while dressing is completed.

The greatest obstacle facing the industrial application of carcass stimulation is reluctance on the part of the beef industry to abandon traditional practices. It remains to be seen if the potential advantages of time and energy saving inherent in this new method will prevail in a conservative and tradition-oriented industry.

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APPENDIX

TABLES

TABLE I

ONE HOUR ANALYSIS OF VARIANCE OF pH DECLINE IN SIDES STIMULATED FOR 30 MINUTES ONE HOUR POST-MORTEM

Source	DF	Sum of Squares	Mean Square	Observed Significance Level
Total	47	3.1155		
Main Unit Analysis	11	0.1969		
Animals	5	0.1479	0.0296	.1127
Sides	1	0.0029	0.0029	.5987
Error a	5	0.0461	0.0092	
Sub Unit Analysis	36	2.9186		
Muscles	3	2.3369	0.7790	.0150
Side x Muscle	3	0.0110	0.0036	.9964
Error b	30	0.5707	0.1902	

TABLE II

TWO HOUR ANALYSIS OF VARIANCE OF pH DECLINE IN SIDES STIMULATED FOR 30 MINUTES ONE HOUR POST-MORTEM

Source	DF	Sum of Squares	Mean Square	Observed Significance Level
Total	47	1.9668		
Main Unit Analysis	11	1.1053		
Animals	5	0.0396	0.0079	.9878
Treatments	1	0.6698	0.6698	.0335
Error a	5	0.3959	0.0792	
Sub Unit Analysis	36	0.8615		
Muscles	3	0.2047	0.0682	.0076
Treatment x Muscle	3	0.2297	0.0766	.0043
Error b	30	0.4271	0.0142	

TABLE III

FOUR HOUR ANALYSIS OF VARIANCE OF pH DECLINE IN SIDES STIMULATED FOR 30 MINUTES ONE HOUR POST-MORTEM

Source	DF	Sum of Squares	Mean Square	Observed Significance Level
Total	47	3.7449		
Main Unit Analysis	11	1.7287		
Animals	5	0.2574	0.0515	.6560
Treatments	1	1.0951	1.0951	.0124
Error a	5	0.3762	0.0752	
Sub Unit Analysis	36	2.0162		
Muscles	3	0.3020	0.1007	.0138
Treatment x Muscle	3	0.9916	0.3305	<.0001
Error b	30	0.7226	0.0241	

TABLE IV

SIX HOUR ANALYSIS OF VARIANCE OF pH DECLINE IN SIDES STIMULATED FOR 30 MINUTES ONE HOUR POST-MORTEM

Source	DF	Sum of Squares	Mean Square	Observed Significance Level
Total	47	3.5930		
Main Unit Analysis	11	1.4398		
Animals	5	0.4661	0.0932	.1117
Treatments	1	0.8295	0.8295	.0030
Error a	5	0.1442	0.0288	
Sub Unit Analysis	36	2.1532		
Muscles	3	0.4299	0.1433	.0142
Treatment x Muscle	3	0.6879	0.2293	.0014
Error b	30	1.0354	0.0345	

TABLE V

EIGHT HOUR ANALYSIS OF VARIANCE OF pH DECLINE IN SIDES STIMULATED FOR 30 MINUTES ONE HOUR POST-MORTEM

Source	DF	Sum of Squares	Mean Square	Observed Significance Level
Total	47	4.3853		
Main Unit Analysis	11	1.5312		
Animals	5	0.2270	0.0454	.0754
Treatments	1	1.2481	1.2481	.0001
Error a	5	0.0561	0.0112	
Sub Unit Analysis	36	2.8541		
Muscles	3	0.4468	0.1489	.0319
Treatment x Muscle	3	1.0760	0.3587	.0004
Error b	30	1.3313	0.0444	

TABLE VI

TWELVE HOUR ANALYSIS OF VARIANCE OF pH DECLINE IN SIDES STIMULATED FOR 30 MINUTES ONE HOUR POST-MORTEM

Source	DF	Sum of Squares	Mean Square	Observed Significance Level
Total	47	4.4679		
Main Unit Analysis	11	1.3930		
Animals	5	0.4660	0.0932	.1149
Treatments	1	0.7803	0.7803	.0013
Error a	5	0.1467	0.0293	
Sub Unit Analysis	36	3.0749		
Muscles	3	1.2144	0.4048	.0004
Treatment x Muscle	3	0.4040	0.1347	.0585
Error b	30	1.4565	0.0486	

TABLE VII

TWENTY-FOUR HOUR ANALYSIS OF VARIANCE OF pH DECLINE IN SIDES STIMULATED FOR 30 MINUTES ONE HOUR POST-MORTEM

Source	DF	Sum of Squares	Mean Square	Observed Significance Level
Total	47	4.3706		
Main Unit Analysis	11	0.4577		
Animals	5	0.2597	0.0519	.0974
Treatments	1	0.1240	0.1240	.0340
Error a	5	0.0740	0.0148	
Sub Unit Analysis	36	3.9129		
Muscles	3	2.6506	0.8835	< .0001
Treatment x Muscle	3	0.1754	1.6147	.2067
Error b	30	1.0869		

TABLE VIII

THIRTY MINUTE ANALYSIS OF VARIANCE OF pH DECLINE IN SIDES STIMULATED FOR 15 MINUTES 30 MINUTES POST-MORTEM

Source	DF	Sum of Squares	Mean Square	Observed Significance Level
Total	41	0.8581		
Main Unit Analysis	13	0.5068		
Animals	6	0.4192	0.0699	.0333
Sides	1	0.0060	0.0060	.5312
Error a	6	0.0816	0.0136	
Sub Unit Analysis	28	0.3513		
Muscles	2	0.0885	0.0442	.0272
Side x Muscle	2	0.0104	0.0052	.6150
Error b	24	0.2524	0.0105	

TABLE IX

ONE HOUR ANALYSIS OF VARIANCE OF pH DECLINE IN SIDES STIMULATED FOR 15 MINUTES 30 MINUTES POST-MORTEM

Source	DF	Sum of Squares	Mean Square	Observed Significance Level
Total	41	2.6460		
Main Unit Analysis	13	2.3109		
Animals	6	0.2891	0.0482	.8813
Treatments	1	1.9501	1.9501	<.0001
Error a	6	0.0717	0.0120	
Sub Unit Analysis	28	0.3351		
Muscles	2	0.0264	0.0132	.3306
Treatment x Muscle	2	0.0355	0.0178	.2300
Error b	24	0.2732	0.0114	

TABLE X

TWO HOUR ANALYSIS OF VARIANCE OF pH DECLINE IN SIDES STIMULATED FOR 15 MINUTES 30 MINUTES POST-MORTEM

Source	DF	Sum of Squares	Mean Square	Observed Significance Level
Total	41	3.9282		
Main Unit Analysis	13	3.3502		
Animals	6	0.7274	0.1212	.0103
Treatments	1	2.5358	2.5358	<.0001
Error a	6	0.0870	0.0145	
Sub Unit Analysis	28	0.5780		
Muscles	2	0.0520	0.0260	.4389
Treatment x Muscle	2	0.0379	0.0190	.5170
Error b	24	0.4881	0.0203	

TABLE XI

FOUR HOUR ANALYSIS OF VARIANCE OF pH DECLINE IN SIDES STIMULATED FOR 15 MINUTES 30 MINUTES POST-MORTEM

Source	DF	Sum of Squares	Mean Square	Observed Significance Level
Total	41	6.5953		
Main Unit Analysis	13	5.7547		
Animals	6	0.7519	0.1253	.2042
Treatments	1	4.6334	4.6334	.0001
Error a	6	0.3694	0.0616	
Sub Unit Analysis	28	0.8406		
Muscles	2	0.1623	0.0812	.0701
Treatment x Muscle	2	0.232	0.0116	.6586
Error b	24	0.6551	0.0273	

TABLE XII

SIX HOUR ANALYSIS OF VARIANCE OF pH DECLINE IN SIDES STIMULATED FOR 15 MINUTES 30 MINUTES POST-MORTEM

Source	DF	Sum of Squares	Mean Square	Observed Significance Level
Total	41	6.3238		
Main Unit Analysis	13	5.6229		
Animals	6	0.9990	0.1665	.0141
Treatments	1	4.4884	4.4884	$<.0001$
Error a	6	0.1355	0.0226	
Sub Unit Analysis	28	0.7009		
Muscles	2	0.1344	0.0672	.0592
Treatment x Muscle	2	0.0606	0.0303	.2572
Error b	24	0.5059	0.0211	

TABLE XIII

EIGHT HOUR ANALYSIS OF VARIANCE OF pH DECLINE IN SIDES STIMULATED FOR 15 MINUTES 30 MINUTES POST-MORTEM

Source	DF	Sum of Squares	Mean Square	Observed Significance Level
Total	41	5.1498		
Main Unit Analysis	13	4.4454		
Animals	6	1.3147	0.2191	.0099
Treatments	1	2.9760	2.9760	<.0001
Error a	6	0.1547	0.0258	
Sub Unit Analysis	28	0.7044		
Muscles	2	0.1949	0.0975	.0190
Treatment x Muscle	2	0.0112	0.0056	.7659
Error b	24	0.4983	0.0208	

TABLE XIV

TWELVE HOUR ANALYSIS OF VARIANCE OF pH DECLINE IN SIDES STIMULATED FOR 15 MINUTES 30 MINUTES POST-MORTEM

Source	DF	Sum of Squares	Mean Square	Observed Significance Level
Total	41	5.2770		
Main Unit Analysis	13	4.4833		
Animals	6	2.2218	0.3703	.0048
Treatments	1	2.0637	2.0637	.0002
Error a	6	0.1978	0.0330	
Sub Unit Analysis	28	0.7937		
Muscles	2	0.2011	0.1006	.0195
Treatment x Muscles	2	0.0745	0.0373	.1990
Error b	24	0.5181	0.0216	

TABLE XV

TWENTY-FOUR HOUR ANALYSIS OF VARIANCE OF pH DECLINE IN SIDES STIMULATED FOR 15 MINUTES 30 MINUTES POST-MORTEM

Source	DF	Sum of Squares	Mean Square	Observed Significance Level
Total	41	2.0902		
Main Unit Analysis	13	1.0602		
Animals	6	0.7012	0.1169	.0339
Treatments	1	0.2215	0.2215	.0209
Error a	6	0.1375	0.0229	
Sub Unit Analysis	28	1.0300		
Muscles	2	0.5749	0.2875	<.0001
Treatment	2	0.0164	0.0082	.6438
Error b	24	0.4387		

TABLE XVI

ANALYSIS OF VARIANCE OF pH DECLINE IN LONGISSIMUS DORSI TWO HOURS POST-MORTEM

Source	DF	Sum of Squares	Mean Square	Observed Significance Level
Total	11	1.4815		
Among Animals	5	0.9292		
A	1	0.3844	0.3844	.0851
B	1	0.1892	0.1892	.1534
AB	1	0.2809	0.2809	.2792
Reps	2	0.0747	0.0374	
Within Animals	6	0.5523		
A	1	0.0351	0.0351	.6071
B	1	0.0924	0.0924	.4215
AB	1	0.1035	0.1035	.3981
Error	3	0.3213	0.1071	

A = Duration of stimulus.

B = Method of generator contact.

TABLE XVII

ANALYSIS OF VARIANCE OF pH DECLINE IN LONGISSIMUS DORSI FOUR HOURS POST-MORTEM

Source	DF	Sum of Squares	Mean Square	Observed Significance Level
Total	11	1.8431		
Among Animals	5	1.5396		
A	1	0.3249	0.3249	.4686
B	1	0.0169	0.0169	.8584
AB	1	0.3721	0.3721	.4427
Reps	2	0.8257	0.4128	
Within Animals	6	0.3035		
A	1	0.0008	0.0008	.9273
B	1	0.0578	0.0578	.4615
AB	1	0.0004	0.0004	.9486
Error	3	0.2445	0.0815	

A = Duration of stimulus.

B = Method of generator contact.

TABLE XVIII

ANALYSIS OF VARIANCE OF pH DECLINE IN LONGISSIMUS DORSI SIX HOURS POST-MORTEM

Source	DF	Sum of Squares	Mean Square	Observed Significance Level
Total	11	0.7260		
Among Animals	5	0.4572		
A	1	0.3481	0.3481	.0511
B	1	0.0400	0.0400	.2862
AB	1	0.0306	0.0306	.3345
Reps	2	0.0385	0.0192	
Within Animals	6	0.2688		
A	1	0.1176	0.1176	.1488
B	1	0.0561	0.0561	.2743
AB	1	0.0006	0.0006	.8990
Error	3	0.0945	0.0315	

A = Duration of stimulus.

B = Method of generator contact.

TABLE XIX

ANALYSIS OF VARIANCE OF pH DECLINE IN LONGISSIMUS DORSI EIGHT HOURS POST-MORTEM

Source	DF	Sum of Squares	Mean Square	Observed Significance Level
Total	11	0.5778		
Among Animals	5	0.5066		
A	1	0.3782	0.3782	.0206
B	1	0.1122	0.1122	.0648
AB	1	0.0001	0.0001	.9214
Reps	2	0.0161	0.0161	
Within Animals	6	0.0712		
A	1	0.0136	0.0136	.3540
B	1	0.0091	0.0091	.4368
AB	1	0.0144	0.0144	.3423
Error	3	0.0341	0.0341	

A = Duration of stimulus.

B = Method of generator contact.

TABLE XX

ANALYSIS OF VARIANCE OF pH DECLINE IN LONGISSIMUS DORSI TWELVE HOURS POST-MORTEM

Source	DF	Sum of Squares	Mean Square	Observed Significance Level
Total	11	0.6942		
Among Animals	5	0.5901		
A	1	0.4290	0.4290	.1191
B	1	0.0030	0.0030	.8162
AB	1	0.0342	0.0342	.5349
Reps	2	0.1239	0.0620	
Within Animals	6	0.1041		
A	1	0.0800	0.0800	.0046
B	1	0.0128	0.0128	.0550
AB	1	0.0072	0.0072	.1054
Error	3	0.0041	0.0014	

A = Duration of stimulus.

B = Method of generator contact.

TABLE XXI

ANALYSIS OF VARIANCE OF pH DECLINE IN LONGISSIMUS DORSI 24 HOURS POST-MORTEM

Source	DF	Sum of Squares	Mean Square	Observed Significance Level
Total	11	0.5602		
Among Animals	5	0.5279		
A	1	0.4225	0.4225	.0448
B	1	0.0576	0.0576	.2341
AB	1	0.0072	0.0072	.6119
Reps	2	0.0406	0.0203	
Within Animals	6	0.0323		
A	1	0.0001	0.0001	.8876
B	1	0.0015	0.0015	.5936
AB	1	0.0180	0.0180	.1312
Error	3	0.0127	0.0042	

A = Duration of stimulus.

B = Method of generator contact.

TABLE XXII

ANALYSIS OF VARIANCE OF pH DECLINE IN PSOAS MAJOR TWO HOURS POST-MORTEM

Source	DF	Sum of Squares	Mean Square	Observed Significance Level
Total	11	0.4641		
Among Animals	5	0.3905		
A	1	0.0576	0.0576	.1787
B	1	0.2970	0.2970	.0438
AB	1	0.0081	0.0081	.5827
Reps	2	0.0278	0.0139	
Within Animals	6	0.0736		
A	1	0.0276	0.0123	.2591
B	1	0.0098	0.0276	.1294
AB	1	0.0171	0.0098	.3039
Error	3	0.0191	0.0064	

A = Duration of stimulus.

B = Method of generator contact.

TABLE XXIII

ANALYSIS OF VARIANCE OF pH DECLINE IN PSOAS MAJOR FOUR HOURS POST-MORTEM

Source	DF	Sum of Squares	Mean Square	Observed Significance Level
Total	11	0.7489		
Among Animals	5	0.4053		
A	1	0.2352	0.2353	.0190
B	1	0.1560	0.1560	.0282
AB	1	0.0049	0.0049	.4105
Reps	2	0.0092	0.0046	
Within Animals	6	0.3436		
A	1	0.2211	0.2211	.0062
B	1	0.0066	0.0066	.3170
AB	1	0.0338	0.0338	.0731
Error	3	0.0821	0.0274	

A = Duration of stimulus.

B = Method of generator contact.

TABLE XXIV

ANALYSIS OF VARIANCE OF pH DECLINE IN PSOAS MAJOR SIX HOURS POST-MORTEM

Source	DF	Sum of Squares	Mean Square	Observed Significance Level
Total	11	0.5534		
Among Animals	5	0.4243		
A	1	0.1892	0.1892	.0462
B	1	0.1892	0.1892	.0462
AB	1	0.0272	0.0272	.2310
Reps	2	0.0187	0.0094	
Within Animals	6	0.1291		
A	1	0.0264	0.0264	.2855
B	1	0.0008	0.0008	.8359
AB	1	0.0612	0.0612	.1428
Error	3	0.0470	0.0157	

A = Duration of stimulus.

B = Method of generator contact.

TABLE XXV

ANALYSIS OF VARIANCE OF pH DECLINE IN PSOAS MAJOR EIGHT HOURS POST-MORTEM

Source	DF	Sum of Squares	Mean Square	Observed Significance Level
Total	11	0.5314		
Among Animals	5	0.4778		
A	1	0.0650	0.0650	.2646
B	1	0.1225	0.1225	.1697
AB	1	0.2352	0.2352	.1000
Reps	2	0.0551	0.0276	
Within Animals	6	0.0536		
A	1	0.0036	0.0036	.7419
B	1	0.0040	0.0040	.7288
AB	1	0.0351	0.0351	.3415
Error	3	0.0109	0.0036	

A = Duration of stimulus.

B = Method of generator contact.

TABLE XXVI

ANALYSIS OF VARIANCE OF pH DECLINE IN PSOAS MAJOR TWELVE HOURS POST-MORTEM

Source	DF	Sum of Squares	Mean Square	Observed Significance Level
Total	11	0.4419		
Among Animals	5	0.2420		
A	1	0.1482	0.1482	.0878
B	1	0.0110	0.0110	.4814
AB	1	0.0529	0.0529	.2007
Reps	2	0.0299	0.0150	
Within Animals	6	0.1999		
A	1	0.0190	0.0190	.4830
B	1	0.0903	0.0903	.1801
AB	1	0.0012	0.0012	.8538
Error	3	0.0894	0.0298	

A = Duration of stimulus.

B = Method of generator contact.

TABLE XXVII

ANALYSIS OF VARIANCE OF pH DECLINE IN PSOAS MAJOR 24 HOURS POST-MORTEM

Source	DF	Sum of Squares	Mean Square	Observed Significance Level
Total	11	0.3463		
Among Animals	5	0.2402		
A	1	0.1560	0.1560	.1666
B	1	0.0100	0.0100	.6433
AB	1	0.0056	0.0056	.7253
Reps	2	0.0686	0.0343	
Within Animals	6	0.1061		
A	1	0.0001	0.0001	.9520
B	1	0.0312	0.0312	.3321
AB	1	0.0045	0.0045	.6909
Error	3	0.0703	0.0234	

A = Duration of stimulus.

B = Method of generator contact.

TABLE XXVIII

ANALYSIS OF VARIANCE OF pH DECLINE IN SEMIMEMBRANOSUS TWO HOURS POST-MORTEM

Source	DF	Sum of Squares	Mean Square	Observed Significance Level
Total	11	0.5845		
Among Animals	5	0.4461		
A	1	0.0625	0.0625	.2014
B	1	0.1225	0.1225	.1195
AB	1	0.2256	0.2256	.0705
Reps	2	0.0355	0.0178	
Within Animals	6	0.1384		
A	1	0.0171	0.0171	.4903
B	1	0.0378	0.0378	.3281
AB	1	0.0000	0.0000	1.0000
Error	3	0.0835	0.0278	

A = Duration of stimulus.

B = Method of generator contact.

TABLE XXIX

ANALYSIS OF VARIANCE OF pH DECLINE IN SEMIMEMBRANOSUS FOUR HOURS POST-MORTEM

Source	DF	Sum of Squares	Mean Square	Observed Significance Level
Total	11	0.5133		
Among Animals	5	0.2864		
A	1	0.1024	0.1024	.3310
B	1	0.0000	0.0000	1.0000
AB	1	0.0576	0.0576	.4405
Reps	2	0.1264	0.0632	
Within Animals	6	0.2269		
A	1	0.0231	0.0231	.5416
B	1	0.0496	0.0496	.3885
AB	1	0.0072	0.0072	.7270
Error	3	0.1470	0.0490	

A = Duration of stimulus.

B = Method of generator contact.

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ANALYSIS OF VARIANCE OF pH DECLINE IN SEMIMEMBRANOSUS SIX HOURS POST-MORTEM

Source	DF	Sum of Squares	Mean Square	Observed Significance Level
Total	11	0.9333		
Among Animals	5	0.2470		
A	1	0.0506	0.0506	.4649
B	1	0.0552	0.0552	.4527
AB	1	0.0121	0.0121	.7072
Reps	2	0.1291	0.0646	
Within Animals	6	0.6863		
A	1	0.2850	0.2850	.0203
B	1	0.3570	0.3570	.0149
AB	1	0.0024	0.0024	.7063
Error	3	0.0419		

A = Duration of stimulus.

B = Method of generator contact.

TABLE XXXI

ANALYSIS OF VARIANCE OF pH DECLINE IN SEMIMEMBRANOSUS EIGHT HOURS POST-MORTEM

Source	DF	Sum of Squares	Mean Square	Observed Significance Level
Total	11	0.8635		
Among Animals	5	0.5460		
A	1	0.0169	0.0169	.8119
B	1	0.0196	0.0196	.7981
AB	1	0.0484	0.0484	.6918
Reps	2	0.4611	0.2305	
Within Animals	6	0.3175		
A	1	0.0024	0.0024	.8640
B	1	0.0968	0.0968	.3215
AB	1	0.0112	0.0112	.7140
Error	3	0.2071	0.0690	

A = Duration of stimulus.

B = Method of generator contact.

TABLE XXXII

ANALYSIS OF VARIANCE OF pH DECLINE IN SEMIMEMBRANOSUS TWELVE HOURS POST-MORTEM

Source	DF	Sum of Squares	Mean Square	Observed Significance Level
Total	11	0.7295		
Among Animals	5	0.2744		
A	1	0.0961	0.0961	.1142
B	1	0.0756	0.0756	.1736
AB	1	0.0676	0.0676	.1887
Reps	2	0.0351	0.0176	
Within Animals	6	0.4551		
A	1	0.2211	0.2211	.1743
B	1	0.0220	0.0220	.6149
AB	1	0.0010	0.0010	.9126
Error	3	0.2110	0.0703	

A = Duration of stimulus.

B = Method of generator contact.

TABLE XXXIII

ANALYSIS OF VARIANCE OF pH DECLINE IN SEMIMEMBRANOSUS 24 HOURS POST-MORTEM

Source	DF	Sum of Squares	Mean Square	Observed Significance Level
Total	11	0.1641		
Among Animals	5	0.1303		
A	1	0.0006	0.0006	.9135
B	1	0.0121	0.0121	.6367
AB	1	0.0380	0.0380	.4316
Reps	2	0.0796	0.0398	
Within Animals	6	0.0338		
A	1	0.0136	0.0136	.1770
B	1	0.0004	0.0004	.7827
AB	1	0.0066	0.0066	.3081
Error	3	0.0132	0.0044	

A = Duration of stimulus.

B = Method of generator contact.

TABLE XXXIV

ANALYSIS OF VARIANCE OF pH DECLINE IN SUPRASPINATUS TWO HOURS POST-MORTEM

Source	DF	Sum of Squares	Mean Square	Observed Significance Level
Total	11	0.4902		
Among Animals	5	0.3311		
A	1	0.0000	0.0000	1.0000
B	1	0.0529	0.0529	.5999
AB	1	0.0006	0.0006	.9536
Reps	2	0.2776	0.1388	
Within Animals	6	0.1591		
A	1	0.0496	0.0496	.3229
B	1	0.0024	0.0024	.8119
AB	1	0.0003	0.0003	.9326
Error	3	0.1068	0.0356	

A = Duration of stimulus.

B = Method of generator contact.

TABLE XXXV

ANALYSIS OF VARIANCE OF pH DECLINE IN SUPRASPINATUS FOUR HOURS POST-MORTEM

Source	DF	Sum of Squares	Mean Square	Observed Significance Level
Total	11	0.4414		
Among Animals	5	0.2131		
A	1	0.0009	0.0009	.9532
B	1	0.0016	0.0016	.9377
AB	1	0.0056	0.0056	.8839
Reps	2	0.2050	0.1025	
Within Animals	6	0.2283		
A	1	0.0136	0.0136	.6301
B	1	0.0406	0.0406	.4238
AB	1	0.0312	0.0312	.4774
Error	3	0.1429	0.0476	

A = Duration of stimulus.

B = Method of generator contact.

TABLE XXXVI

ANALYSIS OF VARIANCE OF pH DECLINE IN SUPRASPINATUS SIX HOURS POST-MORTEM

Source	DF	Sum of Squares	Mean Square	Observed Significance Level
Total	11	0.7487		
Among Animals	5	0.6108		
A	1	0.1122	0.1122	.3647
B	1	0.1024	0.1024	.3820
AB	1	0.2304	0.2304	.2374
Reps	2	0.1658	0.0829	
Within Animals	6	0.1379		
A	1	0.0032	0.0032	.7650
B	1	0.0435	0.0435	.3142
AB	1	0.0015	0.0015	.8372
Error	3	0.0897	0.0299	

A = Duration of stimulus.

B = Method of generator contact.

TABLE XXXVII

ANALYSIS OF VARIANCE OF pH DECLINE IN SUPRASPINATUS EIGHT HOURS POST-MORTEM

Source	DF	Sum of Squares	Mean Square	Observed Significance Level
Total	11	1.0197		
Among Animals	5	0.8833		
A	1	0.0009	0.0009	.8664
B	1	0.1849	0.1849	.1118
AB	1	0.6480	0.6480	.0361
Reps	2	0.0495	0.0246	
Within Animals	6	0.1364		
A	1	0.0055	0.0055	.7403
B	1	0.0001	0.0001	.9640
AB	1	0.0060	0.0060	.7294
Error	3	0.1248	0.0416	

A = Duration of stimulus.

B = Method of generator contact.

TABLE XXXVIII

ANALYSIS OF VARIANCE OF pH DECLINE IN SUPRASPINATUS TWELVE HOURS POST-MORTEM

Source	DF	Sum of Squares	Mean Square	Observed Significance Level
Total	11	0.4094		
Among Animals	5	0.2409		
A	1	0.0462	0.0462	.2183
B	1	0.0841	0.0841	.1392
AB	1	0.0812	0.0812	.1432
Reps	2	0.0294	0.0147	
Within Animals	6	0.1685		
A	1	0.0379	0.0379	.4023
B	1	0.0084	0.0084	.6780
AB	1	0.0021	0.0021	.8336
Error	3	0.1201	0.0400	

A = Duration of stimulus.

B = Method of generator contact.

TABLE XXXIX

ANALYSIS OF VARIANCE OF pH DECLINE IN SUPRASPINATUS 24 HOURS POST-MORTEM

Source	DF	Sum of Squares	Mean Square	Observed Significance Level
Total	11	0.8071		
Among Animals	5	0.7693		
A	1	0.0784	0.0784	.2441
B	1	0.0992	0.0992	.2076
AB	1	0.5329	0.5329	.0510
Reps	2	0.0588	0.0294	
Within Animals	6	0.0378		
A	1	0.0171	0.0171	.1722
B	1	0.0001	0.0001	.9001
AB	1	0.0045	0.0045	.4273
Error	3	0.0161	0.0054	

A = Duration of stimulus.

B = Method of generator contact.

VITA

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