CHEMICAL AND HISTOCHEMICAL OBSERVATIONS ON L. DORSI MUSCLE FROM BEEF AND PORK

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ABSTRACT

CHEMICAL AND HISTOCHEMICAL OBSERVATIONS ON *L. DORSI* MUSCLE FROM BEEF AND PORK

by Clarence Eugene Bodwell

Chemical changes in *l. dorsi* muscle of five beef carcasses were followed from less than 10 minutes after death through 20 days post-mortem. In addition, the activity of 17 specific enzymes was followed by histochemical procedures.

Initial levels of pH, glycogen and creatine phosphate were similar to previously reported values. ATP levels were more accurately estimated by an enzymic method than by acid hydrolysis procedures. Results suggest that the onset of rigor was initiated at about 12-15 hrs post-mortem. Approximately 20% and 3% of the initial glycogen remained at 96 and 480 hrs post-mortem, respectively. Glycogen was stoichiometrically degraded to lactic acid and reducing sugars under post-mortem conditions.

The histochemical activity of lactate dehydrogenase, alpha-glycerophosphate dehydrogenase, succinate dehydrogenase, isocitrate dehydrogenase, TPN diaphorase and DPN diaphorase showed a steady decrease with increasing times post-mortem. Reactions for both alcohol dehydrogenase and glutamate dehydrogenase were very weak or absent at 48 hrs post-mortem and all subsequent sampling periods.

Positive reactions for glucose-6-phosphate dehydrogenase and beta-hydroxybutyrate dehydrogenase were observed in the initial samples only. Acid phosphatase, leucine amino peptidase, and 6-phosphogluconate dehydrogenase were not detected in any of the samples.

Chemical and histochemical procedures similar to those described for beef were used to follow the changes from 0 to 24 and/or 48 hrs post-mortem in the l. dorsi muscle of eighteen pork carcasses. One side from each of thirteen pork carcasses was placed at -29°C, while the other side from each carcass was subjected to 37°C for the first 4 1/2-5 hrs post-mortem in an attempt to induce soft, watery pork.

The muscles of the pork carcasses studied exhibited marked differences in initial levels of pH, glycogen, glucose and lactic acid. Post-mortem accumulation of glucose and lactic acid was similar to that noted in beef muscle. A similar stoichiometric relation to that found in beef muscle between post-mortem decrease in glycogen and the corresponding accumulation of lactic acid and glucose existed in the carcasses from Poland China pigs. The equivalent relationship for the carcasses from Hampshire pigs was less apparent.

The 37°C treatment rarely induced soft, watery muscle as was expected from reports of previous workers. It was concluded that a low pH at a high muscle temperature per se was not a causal factor in the developement of the soft, watery condition. The loss in fibrillar water-binding capacity as the result of low pH values at high muscle temperatures reported by previous workers was confirmed. In contrast to previous studies, decreases in fibrillar water binding capacity induced by the low pH-high temperature phenomenon did not usually result in soft, watery muscle. Although the loss in fibrillar water-binding capacity is a characteristic encountered in soft, watery muscle, this characteristic by itself is not the only causal factor in the developement of this condition.

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The activity of lactate dehydrogenase, alpha-glycerophosphate dehydrogenase, succinate dehydrogenase, isocitrate dehydrogenase and DPN diaphorase was slightly weaker at both 0 and 24 hrs than similar activity in beef muscle. Alcohol dehydrogenase activity was weaker than that observed for the above enzymes, while the activity of glutamate dehydrogenase was of intermediate intensity. Glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase showed traces of activity in only a portion of the initial samples. Similarly, weak reactions for beta-hydroxybutyrate dehydrogenase were observed only at 0 hrs post-mortem. None or only weak activity was observed for malic dehydrogenase in all samples studied. Likewise, TPN diaphorase activity was unexplainably absent from all samples. Moderate levels of cytochrome oxidase activity were observed at both 0 and 24 hrs post-mortem.

UDPG-glycogen transferase was completely inactivated by the 37°C treatment, but the treatment had a less marked effect upon phosphorylase and branching enzyme. The presence of acid and alkaline phosphatase activity in muscles of 7 pork carcasses suggested that a degenerative condition existed in these muscles.

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CHEMICAL AND HISTOCHEMICAL OBSERVATIONS ON L. DORSI MUSCLE FROM BEEF AND PORK

By

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INTRODUCTION

The phenomenon of rigor mortis is extremely important in the transformation of living muscle into meat. In beef muscle, the chemical and physical changes associated with rigor mortis and the subsequent period of post-rigor aging or ripening are believed to have a strong influence on various qualitative attributes, i.e. color, tenderness, flavor, etc. (Whitaker, 1959). How these changes are related to each of the various quality factors has not been fully established. However, departures from the norm in certain characteristics, which are related to the chemical events involved in rigor, are known to result in corresponding undesirable traits (Lawrie, 1962; Bendall, 1960).

Distinct variations in the rate and extent of certain chemical changes during the post-mortem pre-rigor and rigor stages in pork muscle have been shown to have considerable influence on the quality of the resulting meat (Lawrie, 1962; Bendall, 1960). In particular, the occurrence of a low pH while muscle temperature is high has been reported to produce soft, watery pork (Bendall *et al*, 1963; Wismer-Pedersen, 1959).

Although data are available on various post-mortem chemical and physical changes in both beef and pork muscle, the causal factors remain largely unexplored. The post-mortem activity of oxidative and respiratory enzymes in muscle would appear to be potentially relevant, but this has not been studied to any extent. Given these considerations, the experimental objectives of this study were:

The phenomenon of rigor mortis is extremely important in the study

1. To determine the levels of certain chemical substances in beef muscle from shortly after death to approximately three weeks post-mortem.

2. To observe the activity of various enzymes in beef muscle during the same post-mortem period using histochemical methods.

3. To investigate similar chemical and histochemical characteristics during the first 48 hrs post-mortem in pork muscle using carcasses subjected to controlled post-mortem temperature environments, which were intended to either induce, accentuate, or retard the developement of soft, watery muscle.

REVIEW OF LITERATURE

CLASSIFICATION OF MUSCLE

Muscle is generally classified into three types--smooth, cardiac, and voluntary or striated. Cardiac muscle is restricted to the heart, while smooth muscle is found in the digestive, vascular, respiratory, and other involuntary systems. The predominating musculature of the animal body is striated muscle, and likewise, this type of muscle is the major constituent of meat. For convenience, the term muscle as used herein refers to striated or voluntary muscle.

CHEMICAL COMPOSITION OF MUSCLE

According to Bendall (1962), muscle is composed of approximately 1% ash, 3-10% fat, 21-22% non-lipid organic matter, and 70-75% water. The predominate inorganic constituents are P, K, Na, Mg, and Ca. Protein makes up about 95% of the non-lipid organic matter and constitutes about 20% of the muscle mass. Contractile proteins contribute approximately 52% of the total protein, while sarcoplasmic proteins constitute about 32%, and the stroma (connective tissue) proteins about 5-6%.

About 11% of the total nitrogen in muscle is derived from non-protein sources (Bendall, 1962). ATP (adenosinetriphosphate) and CP (creatine phosphate) account for over half of the NPN (non-protein nitrogen). Dipeptides, particularly anserine and carnosine, and free amino acids provide a small amount of NPN, but their concentration is highly variable even within muscles of a given animal (Bendall, 1962; Bate Smith, 1938; Davey, 1957, 1960a,b).

CLASSIFICATION OF MUSCLE

Muscle is generally classified into three types--smooth, cardiac,

The approximate values given herein for various constituents of muscle are subject to variation from species to species and within muscles of the same and/or different animals within a species. However, such values are in general representative of mammalian muscle (Bendall, 1962). These broad groupings obviously ignore some biologically important substances, such as vitamins and hormones. Likewise, some relatively unexplored substances such as some proteins of presumed contractile origin (Szent-Györgyi, 1960) are excluded.

STRUCTURE AND MUSCLE CONTRACTION

Several comprehensive discussions on various aspects of the structure and function of muscle have been published (Mommaerts, 1950, 1954; Szent-Györgyi, 1951, 1953, 1960; Weber and Portzehl, 1952; Dubuisson, 1954; Bailey, 1954; Hamoir, 1955; Morales and Botts, 1956; Weber, 1957; Huxley and Hanson, 1960; Bennett, 1960; Needham, 1960; Bendall, 1962; 1963; Venable, 1963). Accordingly, only a brief summary of muscle structure and function has been included.

General Structure

On a gross level, muscle is composed of a mass of tissue subdivided into increasingly smaller units by limiting interstitial and enveloping connective tissue (Walls, 1960; Bloom and Fawcett, 1963). A muscle is contained within the collagenous fascia or spimysium, which penetrates into the muscle to form the perimysium. The perimysial stroma consists of collagenous, elastic, and reticular fibers and a variety of connective tissue cells. The perimysium separates the muscle into tertiary, secondary,

and primary muscle bundles. The primary bundles are composed of varying numbers of muscle cells or fibers.

In most mammalian muscles, the nuclei are located in the sarcoplasm immediately beneath the sarcolemna. In addition to other entities, the sarcoplasm also contains the mitrochondria, the glycolytic enzymes, myoglobin, and sometimes glycogen granules and lipid droplets. The mitochondria also contain the oxidative enzymes. The sarcoplasmic fluid surrounds and fills the spaces between the highly organized contractile structures of the muscle fiber--the fibrils or myofibrils (Walls, 1960).

The Myofibrils and Filaments

When viewed in longitudinal section with a light microscope, the fibrils appear as long, parallel threads (Walls, 1960; Bloom and Fawcett, 1963). A cross-banded or striated appearance results from the presence of cylindrical segments of two main types, which alternate in repeating units along the fibril. These two segments appear as a dark band or A band, and a lighter band or I band. In the center of the I band is a narrow dark line, the Z disc. The lighter areas of the I band, on either side of the Z disc, are called the N bands. That portion of a fibril located between adjacent Z discs represents the structural unit of the fibril and is known as a sarcomere. On proceeding from one Z disc to an adjacent Z disc, three bands are apparent, an N band, the A band, and a second N band.

In electronmicrographs (H. E. Huxley, 1953,1957; Hodge et al., 1954; Huxley and Hanson, 1957b, 1960), a lighter area centrally located in the

nucleus of muscle cells or fibers.

In most mammalian muscles, the nuclei are located in the sarcoplasm immediately [illegible] the sarcolemma. In addition to other evidence, the [illegible]

A band and called the H band is apparent. The A and I bands are found to be of almost equal length in a resting fibril.

Electron microscope studies, together with data from x-ray diffraction and phase contrast or interference microscopy (Huxley and Hanson, 1954, 1957a; Hanson and Huxley, 1953, 1955; A. F. Huxley, 1957), proved that the fibril consisted of an array of distinctly organized myofilaments or filaments. These filaments were of two general types, one being about twice as thick as the other and shorter in length. These filaments, both thick and thin, overlapped along part of their lengths in ordered array within a sarcomere. This resulted in the banded or striated appearance. Within the I band, only thin filaments, bisected by the Z disc were present and within the H band only thick filaments. The portions of the A band lying on either side of the H band contained overlapping, interdigited filaments of both types.

The thick filaments are composed of myosin and the thin filaments of actin (Huxley and Hanson, 1960). A cross-section within the areas of overlap in the A band shows that each actin filament is surrounded by three filaments of myosin, while each myosin filament is surrounded by six filaments of actin. When treated with tryosin, myosin is degraded to two primary products--L or light meromyosin and H or heavy meromyosin (Mihalyi and Szent-Györgyi, 1953). The H-meromyosin contains the actin-combining and the ATPase properties of myosin. H-meromyosin has been shown to be located in the H band, while bound ATP in resting fibrils is found only in the L-meromyosin, which is located towards either end of the A band (Marshall et al., 1959). The myofilaments thus described

provide the mechanical basis for muscle contraction.

The Sarcoplasmic Reticulum

Bennett (1960) reviewed descriptions of the sarcoplasmic reticulum, which were published between 1888 and 1902. He remarked, "It is astonishing that a structure once described as accurately and as beautifully as the reticulum was-----should have so quickly become almost lost to man's knowledge". Textbooks, reviews and other papers were essentially devoid of any reference to the sarcoplasmic reticulum when it was first encountered in electromicrographs by Bennett and Porter (1953). Many studies on the reticulum have since been published (Bennett, 1955, 1956; Porter, 1956, 1961; Sjostrand, 1956; Porter and Palade, 1957). The reticulum consists of a network of membrane-limited tubules, which surround each myofibril in a continuous system. The system is composed of transverse and longitudinal tubules, which are inter-connected by thinner tubules to form "triads". In mammalian muscle, two triads are found within each sarcomere, each encircling the A band near an A band-I band junction. The triads of adjacent fibrils are inter-connected, while those of fibrils adjacent to the sarcolemna come into close contact with the sarcolemna.

Muscle Contraction

The explanation of contraction has been wrought with much controversy and opposing interpretations (Needham, 1960), but the primary questions concerning the mechanical events appear to be largely resolved (Bendall,

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1963). In a sarcomere undergoing contraction, first the H band disappears and then the N bands shorten (Huxley and Hanson, 1960). The length of neither actin nor myosin filaments changes except upon extreme shortening, when they are "obliged to do so for steric reasons occurring as a result of shortening" (Huxley and Hanson, 1960). Thus, upon contraction the thin actin filaments are pulled into the A band alongside or into the thick myosin filaments. The degree of interdigital overlapping of the two types of filaments increases as contraction progresses.

The primary contributions to an understanding of the chemical events occurring in muscle contraction are summarized below:

In the presence of ATP, a solution of actomyosin decreases in viscosity and turgidity due to dissociation of the protein into actin and myosin. Upon depletion of the ATP, actomyosin is reformed (Engelhardt and Ljubimova, 1939; Weber, 1956; Gergely, 1956). In 1941, Szent-Györgyi reported that actomyosin threads contract upon the addition of ATP (Needham, 1960). Conversely, upon suppression of ATPase activity, the addition of ATP induces extensibility of the threads. Pyrophosphate likewise induces extensibility, but not contraction, and dissociates an actomyosin solution into actin and myosin (Mommaerts, 1947). Hence, ATP was shown to have a passive "plasticizing" effect on actomyosin in the absence of active ATPase hydrolysis, while a reverse effect equivalent to contraction occurred during hydrolysis of ATP.

The plasticizing influence was found to be effected only when Mg was present in a defined concentration together with ATP in the form of a Mg-ATP complex (Bendall, 1963; Engelhardt, 1946). Thus, in the absence

of Mg and/or ATP, the actin and myosin filaments of the muscle become rigidly attached forming an actomyosin complex. Conversely, the Mg-ATP complex was shown to be necessary for contraction (Weber and Weber, 1951; Hasselbach and Weber, 1953; Bendall, 1953, 1963). Mg-ATP was thereby shown to perform two functions in the fibril---a plasticizing function essential for extensibility and to provide the active energy for contraction.

Marsh (1952) reported the presence of a relaxing factor in muscle, which completely inhibits contraction if added to washed fibrils in the presence of ATP and Mg. In the absence of the relaxing factor, contraction occurs. Ca ions suppress the effect of the relaxing factor (Bendall, 1953).

The relaxing factor is formed from granules of the triads of the sarcoplasmic reticulum (Porter and Palade, 1957; Nagai et al., 1962; Porter, 1961; Ebashi, 1961b) and acts as an ATP-driven Ca-pump (Ebashi, 1961a,b; Hasselbach and Makinose, 1962). The reticulum also serves as the conduction system for transferring impulses from the fiber membrane into the individual fibrils (Huxley and Taylor, 1955).

Bendall (1963) has succinctly summarized the phenomenon of muscle relaxation and contraction. He states, "....the Marsh relaxing factor organized in the muscle as the triads of the sarcoplasmic reticulum, exercises its control over the contractile filaments by pumping out all traces of calcium from the fibrils. The opposite process, contraction, can also be visualized as initiated by an ion-exchange process, originating at the fibre membrane as a wave of depolarization and travelling

complex was shown to be necessary for contraction (Weber and Weber, 1952; Massaglia et al. 1967; Ann. N.Y. Acad. Sci. 137, 1967). IF-ATP was thereby shown to be a [illegible] protein which [illegible] stimulating function and [illegible] the [illegible] ATP-ase.

inwards, often at the level of the Z-discs at which the triads of the reticulum are located in many muscles, as a wave of calcium ions, removed from their exchange-sites by the ionic changes accompanying the depolarization wave. The calcium ions then immediately stimulate the explosive splitting of ATP by the actin and myosin filaments, and contraction ensues. This process is reversed as the fibre-membrane is repolarized, the triads of the reticulum recapture the Ca ions, ATP floods back into the system and the conditions for relaxation are once more established."

In view of more recent data (Weber et al., 1963; Weber and Herz, 1963), this scheme requires clarification on two points. Firstly, the relaxing factor achieves its relaxing effect by reducing the concentration of Ca ions and not by removing these ions completely. Secondly, for contraction to occur Ca ions must be present. In the presence of Mg, the ATPase activity of actomyosin results from the effect of the actin on myosin, which greatly increases the relatively low rate of ATPase hydrolysis by myosin alone. Myosin ATPase is unaltered by either the relaxing factor or by the removal of Ca, while the opposite is true for actomyosin ATPase (Weber et al., 1963). Actomyosin and myofibrils contract only when complexed with Ca, and the complex is formed only when a defined concentration of Ca ions are present in the surrounding medium (Weber and Herz, 1963). The relaxing factor was thus shown to exert its effect through lowering the concentration of ionized Ca below a critical level beyond which the Ca-actomyosin complex dissociates. Since 0.1 micromole of Ca per gram of muscle is sufficient to saturate the actomyosin, many of the earlier Mg-ATP studies were unknowingly contaminated with sufficient Ca ions to facilitate contraction.

Earlier research on muscle contraction failed to prove the breakdown of ATP during contraction (Bowen, 1951, 1954; Bowen and Martin, 1958; Mommaerts, 1955; Hoeve et al., 1963). However, Cain and Davies (1962) and Cain et al., (1962) demonstrated ATP breakdown during a single contraction.

A somewhat similar but still unresolved problem is the identity of the relaxing factor in chemical terms. Tentative identifications have been made (Lorand et al., 1957; Marsh, 1960; Ells and Faulkner, 1961; Uchida and Mommaerts, 1963), but have been refuted (Briggs et al., 1959; Bendall, 1960; Parker, 1961; Lorand and Molver, 1962; Mommaerts et al., 1963; Takauji and Nagai, 1963).

The Mechanism of Contraction

Theories have been advanced for explaining the interaction of actin and myosin during contraction by Astbury (1947), Riseman and Kirkwood (1948), Pauling and Corey (1951), Hill (1953), Huxley and Hanson (1954), Huxley and Niedergerke (1954), Flory (1956a,b; 1957), and Davies (1963). The sliding filament model of Huxley has been one of the more intricately developed theories (A. F. Huxley, 1957; Huxley and Hanson, 1960; Needham, 1960). However, studies by Hoeve and Willis (1963) and Hoeve et al., (1963) indicated that the only theory fully compatible with their data on phase transitions of fibrils was that detailed by Flory (1956a,b, 1957). Flory had suggested that a phase transition from an oriented, crystalline condition to a random, amorphous state is responsible for contraction.

A more plausible and complete theory of contraction has been recently advanced by Davies (1963). This theory satisfies the major postulates of

and Cain et al., (1963). However, Cain and Nevins (1965) [illegible] during construction (Bowen, 1952, 1956; Bowen and Martin, 1952; Hammarte, 1952; Reeve et al., 1963). However, Cain and Nevins (1965) [illegible] single con-

the Huxley model as well as the requirement for a change of phase. Davies' theory, which involves both sliding and folding, can be summarized as follows:

Upon stimulation, Ca ions move from the sarcoplasmic reticulum to polypeptides on the H-meromyosin units of the myosin filaments. The Ca ions form chelate links between bound ATP on the end of the H-meromyosin polypeptides and the bound ADP of F-actin filaments. The Ca ions neutralize the electric charge on the bound ATP of the polypeptides, which then spontaneously contract to form an alpha-helix with the energy derived from hydrogen bond and hydrophobic bond formation. The contraction drags the actin filament along the myosin filament, which brings the ATP into the range of action of the H-meromyosin ATPase. The ATPase cleaves off the terminal phosphate and breaks the link. Upon rephosphorylation of the ADP, the helix is pulled out to an extended chain by the repulsion of the negative charge on the ATP and a fixed charge on the H-meromyosin. Thus, the peptide extends towards and along the actin filament. The cycle is repeated many times during a single contraction and stops when the calcium is pumped back into the sarcoplasmic reticulum.

In a subsequent paper from the same laboratory (Iyengar et al., 1964), an acto-H-meromyosin system was studied in which a polypeptide in the meromyosin was observed to undergo the postulated change in conformation under the conditions set forth in Davies' theory, i.e. an alpha-helix to a mixture of random coil and extended beta-form type of conformational change.

RIGOR MORTIS

After death muscle eventually becomes stiff and inflexible, a condition known as rigor mortis. Rigor mortis, or rigor, can be more fully defined as the collective chemical and physical events within the muscle which result in a rigid, inextensible post-mortem condition.

Observations and investigations of rigor prior to the 1930's were largely limited to those of forensic medicine. These were detailed in various editions of Taylor's "Manuel of Medical Jurisprodence" between 1844 and 1928 (Bendall, 1960). These early observations expounded the belief that the stiffening process was due to a coagulation of the water-soluble proteins of muscle. Coagulation was assumed to be caused by the accumulation of lactic acid, which resulted from the post-mortem breakdown of glycogen. Although rigor had been observed to occasionally occur at a low level of acidity (high pH or "alkaline" rigor), this was a relatively unexplained phenomenon.

In 1877, Claude Bernard observed that "alkaline" rigor developed more rapidly than acid rigor (Bendall, 1960). In 1926, Hoet and Marks described a similar phenomenon and suggested that a third change was involved, which was common to both "alkaline" and acid rigor (Bendall, 1960).

Studies on Rabbit Muscle

From 1939 to 1951, four studies on rabbit muscle established a scientific basis for the interpretation of the events occurring during rigor mortis (Bate Smith, 1939; Bate-Smith and Bendall, 1947, 1949; Bendall, 1951). Bate Smith (1939) reported a study on the changes in extensibility

After death muscle eventually becomes stiff and inflexible, a condition known as rigor mortis. Rigor mortis, or rigor, can be more fully defined as the chemical change in a fat globule within the muscle which causes [illegible]

of rabbit muscle undergoing rigor. This was the first attempt to relate chemical changes to extensibility changes during the onset and developement of rigor. He concluded that as the onset of rigor occurred, a tenfold increase in elastic modulus was observed. Connective tissue in muscle did not contribute to the stiffening during rigor. He found that in normal muscle with not less than 0.7% glycogen, the onset of rigor closely paralleled the production of lactic acid. No correlation was found between these factors in muscles which contained insufficient glycogen to bring the pH to 6.3 by production of lactic acid. The rapidity of pH decrease and the rate of onset of rigor could be influenced by antemortem exercise and/or nutritional state prior to death. He concluded almost prophetically, that the onset of rigor "is related to the loss of elasticity of the fibril, which must be associated with changes in configuration of myosin". The "myosin" of which Bate Smith (1939) spoke included actomyosin. It was further postulated that these changes were a result of the breakdown of the fiber membrane and the accompanying free exchange of ions throughout the muscle.

Later, Erdos in Szent-Györgyi's laboratory in Hungary demonstrated that ATP destruction closely paralled the developement of rigor in the rabbit (Bate-Smith and Bendall, 1947; Szent-Györgyi, 1947). Bate-Smith and Bendall (1947) showed that the onset and developement of rigor mortis was closely correlated with a decrease in ATP in both acid and "alkaline" rigor. In acid rigor, pH at death also influenced the lag period before onset of rigor, and the developement of rigor was closely paralleled by a decrease in pH. Shortening occurred only if the pH was greater than 6.2 at the onset of rigor.

In a subsequent paper Bate-Smith and Bendall (1949) concluded that ATP was necessary to prevent the muscle from passing into rigor regardless of the pH immediately post-mortem. The ATP of post-mortem muscle had a high rate of turnover and during the lag phase before onset of rigor, its breakdown was balanced by resynthesis from the glycolytic cycle. The onset of rigor began when this balance was not maintained due to insufficient glycogen. The pre-rigor lag period was long, short, or very short according to whether the muscles came from well-fed, starved, or exhausted animals, respectively. The initial pH after death was dependent upon the extent of struggling while the ultimate or final pH was determined by the level of feeding or degree of fatigue of the animal before death. The pH at onset of rigor was linearly related to the ultimate pH. Both the lag period and the time required for the onset of rigor were markedly increased by lowering the muscle temperature from 37°C to 17°C.

Bendall (1951) showed that although CP was the first chemical compound to be broken down after death, it had no effect on the onset of rigor except as a replenisher of ATP. ATP breakdown began only after 70% or more of the CP had disappeared and proceeded relatively quickly thereafter, regardless of the rate of glycolysis. The overall rate of ATP turnover depended on pH in a manner similar to that of myosin ATPase. In animals killed by stunning and decapitation, little or no CP was present immediately after death and the ATP level at the onset of rigor was approximately 50% of the level immediately after death. The decline of the ratio of resynthesis to rate of breakdown of ATP had a closer relationship to the shortening and extensibility changes than the fall in

ATP level *per se*. The latter explained the phenomenon reported by Bate-Smith and Bendall (1949) indicating that the ultimate pH and pH at the onset of rigor were linearily related. Data from calculations of various rate-ratios of the resynthesis and breakdown of ATP at different pH levels was shown to account for the variations encountered in critical ATP level with decreasing pH. The importance of the ATP rate-ratio was surmised by Bendall (1951) to be due to the requirement of small but constant levels of ATP at sites in the fibril to ensure a pre-rigor state. The requirement was lower with lower pH levels. Although the theoretical explanation was correct, Bendall (1960) concluded that the use of the ATP rate-ratio concept was unnecessary in view of subsequent knowledge on the plasticizing effects of the Mg-ATP complex. In the presence of the Marsh factor, the ATP level required for a plasticizing effect had been found to decrease with lower pH (Briggs and Portzehl, 1957). Bendall (1960) concluded that onset of rigor occurs when the ATP level is approximately 2 micromoles/g if the final pH falls below 6.3, but is about 4 micromoles/g if the pH fall is restricted.

Changes occurring during rigor mortis of other species have been found to generally adhere to the principles evolved in these studies. Research on other species includes that on the draught horse (Lawrie 1953, 1955), whale (Marsh, 1952b), pork (Wismer-Pedersen, 1959), beef (Marsh, 1954; Howard and Lawrie, 1956, 1957), chicken (deFremery and Pool, 1960, 1963) and fish (Partmann, 1963).

However, two German researchers (Hamm, 1960; Partmann, 1963) have disagreed with Bate-Smith and Bendall (1949) and Bendall (1951). They

have emphasized the role of the relaxing factor in delaying the onset of rigor as opposed to Bendall's (1951, 1960) emphasis on the decrease in ATP level. Hamm (1960) stated that the breakdown of ATP immediately after death is inhibited by the presence of the relaxing factor. During the first few hours post-mortem, the activity of calcium ions increases, and the relaxing factor is then inhibited. The myofibrillar ATPase is fully activated and the onset of rigor occurs. Partmann (1963) agreed and concluded that the rapid phase of ATP breakdown commences only after the relaxing factor is more or less inactivated by the calcium ions. He suggests that the release of Ca ions and the consequent inactivation of the relaxing factor appears to be correlated with membrane changes of the fiber.

However, Bendall (1960) concluded that less than 3% of the ATPase activity after death was due to actomyosin or myofibrillar ATPase. He presented evidence indicating that the sarcoplasmic ATPase of Kielley and Meyerhoff (1948) is the major, if not the only, active ATPase in normal post-mortem rigor. Bendall (1960) further concluded that the myofibrillar ATPase is activated only if the muscle is depolarized in some way, such as in thaw rigor, and then only to a small extent. Bendall (1963) gave further theoretical and experimental evidence for the passive role of myofibrillar ATPase during normal rigor.

The State of the Myofilaments in Rigor Mortis

Bate-Smith (1948) suggested a theory on the state of the muscle fibrils extant upon the completion of rigor and discussed a similar theory

advanced by Szent-Györgyi (1947). Bate-Smith emphasized that the two theories were complementary rather than competitive. Szent-Györgyi (1947) had suggested that actin and myosin existed in an extended form in a non-contractile interaction of the actomyosin complex. In essence, Bate-Smith (1948) was in agreement, but carried the theory further by suggesting a mechanism for the non-contractile interaction of actin and myosin. He postulated that the mechanizm consisted of interfilament cross linkages between actin and myosin.

Bendall (1960) concluded that "as rigor ensues, the actin and myosin chains combine, and the extensibility falls to that residing in the newly formed actomyosin of the A-band and the residual extensibility of the free chains of the I band", and that "rigor will result in nearly the same proportional decrease in extensibility whether the length of the muscle at the time is greater or smaller than the rest length." Using a glycerinated fiber-bundle, Bendall (1963) subsequently demonstrated that the conditions of rigor could be imposed in either the relaxed or contracted state of the muscle fiber bundle. The contraction-type filament interaction was shown to be of consequence only during thaw rigor as opposed to normal rigor.

Davies (1963) has considered the filament condition in rigor in his theory of muscle contraction. Using his model, he demonstrated how stiffness or rigidity would occur during rigor. As a result of a low ATP level, a strong attraction would develope between the positive (in the absence of ATP) binding sites on the H-meromyosin polypeptide and both the actin ADP and the fixed negative charge on the H-meromyosin. The latter would

induce contraction of the extended polypeptide. Actomyosin would be formed and a stiff, inextensible state would result.

The "Resolution" of Rigor

Marsh (1954), Bendall (1960) and Lawrie (1956) have stressed the lack of any extensibility increase after the completion of rigor mortis, and consequently the absence of any "resolution" of rigor or dissociation of actomyosin. In agreement with this, Wierbicki et al., (1956) found no evidence for actomyosin dissociation during the aging of beef. Bendall (1960) has suggested that any softening of the muscle is probably due to putrefaction. However, Zender et al., (1959) presented evidence of post-rigor softening under aseptic conditions. Lawrie (1956), Hamm (1960) and Whitaker (1959) do not preclude a possible role of ion-shifts or the action of catheptic enzymes in "post-rigor softening".

Partmann (1963) has suggested that the actomyosin may dissociate post-rigor. The interpretation by Partmann (1963) of certain experimental phenomenon as evidence for such dissociation is refuted, however, by the work of Bendall (1960) and Perry and Grey (1956).

RIGOR MORTIS IN BEEF MUSCLE

Marsh (1954) studied the relationship between extensibility and changes in ATP, CP and pH during the onset of rigor in the l. dorsi muscle of beef. In general, his results were compatible with the earlier studies on rabbit muscle (Bendall, 1951; Bate-Smith and Bendall, 1947, 1949; Bate Smith, 1939). In muscle strips held at 37°C, CP decreased to 5% or less of the TSP (total acid-soluble phosphate) before the rapid phase

of ATP disappearance and the first detectable change in extensibility occurred. Marsh (1954) also confirmed the earlier work showing that the ATP level was the primary factor controlling the onset of rigor. He demonstrated that the ATP level could be approximated from pH values of any muscle which eventually attained a pH level below 5.70.

Howard and Lawrie (1956) examined the effects of pre-slaughter treatments on the development of rigor mortis and the associated biochemical changes occurring in the muscles of Australian beef. Muscle samples, removed at 1 hr post-mortem, were analyzed for ATP, CP, TSP, glycogen and total Ca and Mg. Measurements of pH were taken at 1 and 24 hrs post-mortem. Residual glycogen was determined after the carcasses had been held for 20 weeks at -10°C. Muscle strips were also removed at 1 hr post-mortem and held at 37°C and extensibility measurements were taken to observe the onset of rigor. At the onset of rigor, samples were removed from the strips for determination of ATP levels and pH. Howard and Lawrie (1956) concluded that the general biochemical characteristics of beef muscle were similar to those of rabbit. However, neither fasting nor enforced exercise alone depleted muscle glycogen reserves sufficiently to raise the ultimate pH above normal values. This was in contrast to the effect of such treatments observed with rabbits (Bendall, 1951; Bate-Smith and Bendall, 1947, 1949; Bate Smith, 1939). On the other hand, a combination of fasting and exercise appeared to have a limited influence in raising the ultimate pH. The authors suggested that the ruminant was capable of utilizing fatty acids to a greater extent than monogastric animals, and that consequently, the glycogen reserves could be more easily

maintained. The authors concluded that of the various treatments used, the most striking was the "relaxation associated with severe hypermagnesaemia and hypercalcaemia", which respectively slowed down and accelerated the onset of rigor. Insulin-induced convulsions were found to virtually deplete muscle glycogen reserves.

In a further study (Howard and Lawrie, 1957), the ante-mortem injection of pyrophosphate appeared to accelerate the onset of rigor, which was in contrast to the plasticizing effect of *in vitro* studies. Although the authors were again unable to deplete glycogen stores by exercise or starvation, ante-mortem injection of neopyithiamin resulted in depletion of glycogen reserves. Howard and Lawrie (1957) considered this as support for the postulated role of fatty acid oxidation in beef muscle. However, they did not preclude the possibility that beef muscle contained a more efficient process for glycogen synthesis than muscles from monogastric animals.

Swift *et al.*, (1960) studied the levels of glycogen, protein, ultimate pH, and buffering capacity of eight beef muscles. Glycogen was determined at 3,10, and 28 hrs post-mortem. All muscles contained residual glycogen, even after 28 hrs post-mortem. The content was found to be largest in those muscles attaining the lowest ultimate pH values.

Fredholm (1963) reported that muscle from beef stored under refrigeration still contained 50% and 23% of their original ATP, respectively, after 3 and 5 days storage. These results were not expected in view of the work of Marsh (1954) and Howard and Lawrie (1956, 1957).

Andrews et al., (1952) determined ATPase, succinic dehydrogenase, aldolase and total glycolytic activity in beef muscle at 48 hrs, 2 weeks and 4 weeks post-mortem. The amount of glycogen at each storage period was also determined. The authors found that ATPase, succinic dehydrogenase and total glycolytic activity did not decrease. However, aldolase activity was shown to decrease to about two-thirds its 48 hrs value after 2 weeks and to one-half after 4 weeks. It was concluded that the lack of substrates in intact muscle was the limiting factor in post-mortem muscle metabolism.

RIGOR MORTIS IN PORK MUSCLE

Early studies related to rigor mortis in pork muscle are largely limited to those of Callow (1937, 1938) who conducted investigations on pork muscle from pigs that had been subjected to various ante-mortem treatments. The general effect of pH was studied and he concluded that pork muscle could be classified into two groups according to pH-structure relationships. An open structure was characterized by a moist appearance and a lower final pH, while a closed structure exhibited a dry cut surface and a higher pH.

Danish Studies

Studies on rigor mortis per se in pork muscle were not carried out until after Ludvigsen (1953) reported a condition in Danish Landrace pigs which he called "muscular degeneration" disease. He described the condition as "alterations of the musculature, appearing macroscopically as a discoloration, the altered musculature having a pale or greyish colour"

and as being "very juicy". Ludvigsen noted that the condition was especially prevalent in the l. dorsi, but that on occasion it was found in all muscles of a given animal. The pH of normal muscle was 6.8-7.0 at approximately 45 mins post-mortem, while values as low as 5.3-5.5 were found when "muscular degeneration" was present.

Wismer-Pedersen (1959) observed that the incidence of the condition described by Ludvigsen (1953) was very rare in Danish Landrace pigs and called the condition usually encountered "pale and watery pork". Wismer-Pedersen (1959) studied pH, glycogen, reducing sugars, and ATP levels at 45 mins and 20 hrs post-mortem and water-holding capacity at 20 hrs post-mortem. He concluded that the soft, watery condition was accompanied by a pH of less than 6.2 at 45 mins post-mortem. The low pH was due to a high level of lactic acid and a low level of glycogen 45 mins after slaughter, and thus was associated with very rapid post-mortem glycolysis. No differences were found in levels of ATP 45 mins post-mortem, but the soft, watery muscles showed a general decrease in water-holding capacity at 20 hrs post-mortem. The frequency of the soft, watery condition was found to be higher in summer than in winter and appeared to be influenced by the amount of stress and excitability immediately ante-mortem.

Wismer-Pedersen and Briskey (1961a) and Briskey and Wismer-Pedersen (1961a) reported four distinct types of muscle according to their post-mortem pH patterns. They reported that types 1, 2, and 3 were more or less normal while type 4 was soft and watery. The type 4 muscle exhibited a sharp, significant decrease in pH to approximately 5.1 at 1 1/2 hrs post-mortem with a subsequent elevation to 5.3-5.6. The authors further

stated that sections of l. dorsi muscle removed immediately after death and held at 37°C for 1 1/2 hrs displayed the characteristics of the type 4 muscle. Briskey and Wismer-Pedersen (1961b) concluded that the pH-time sequence and the consequent development of watery or normal tissue was dependent on a number of factors. However, only three factors were emphasized which were as follows: 1) the extent of glycogen reserves in the muscle tissue at the time of slaughter, 2) the phosphorylase activity and the state of glycogen in the tissue, and 3) the methylene-blue reduction activity of the tissue.

Wismer-Pedersen and Briskey (1961b) studied the effects of various combinations of temperature and acidity in relation to muscle structure. The authors suggested that pale, exudative pork could be produced by retaining body temperature for an extended post-mortem period. Conversely, they pointed out that rapid chilling of muscle samples, which had a low pH within 45 mins of slaughter prevented the developement of the soft, watery condition.

Bendall and Wismer-Pedersen (1962) showed that washed fibrils obtained from soft, watery pork had a lower water retention at low ionic strength and much lower extractability at high ionic strength than the fibrils from normal pork. Washed fibrils from soft, watery pork showed a greater protein content than similar fibrils from normal pork. The authors concluded that the greater fibrillar protein content in the watery fibrils was probably caused by a layer of denatured sarcoplasmic protein, which was firmly bound to the surface of the filaments. Histological evidence supported this conclusion. According to these authors, all of the changes

reported, including the characteristic gain of protein, could be artificially induced in normal muscle by allowing it to pass into rigor at 37°C. It was concluded that the immediate cause of wateriness was the combined effect of high temperature and low pH on the muscle proteins, which was in agreement with the suggestion of Wismer-Pedersen and Briskey (1961b). Bendall and Wismer-Pedersen (1962) also concluded that the depression and elevation of pH values which was reported by Briskey and Wismer-Pedersen (1961a) was a reversible phenomenon due to the effect of temperature on the pK values of charged groups on the fibrillar and sarcoplasmic proteins. They consequently refuted the suggestion of Wismer-Pedersen and Briskey (1961a) that the depression and elevation of pH was a factor in the developement of soft, watery pork.

Bendall et al., (1963) reported a further study on the rates of ATP turnover in relation to pH and onset of rigor mortis in muscle samples removed immediately after slaughter and held at 37°C. Muscle which was eventually soft and watery showed a much faster rate of ATP turnover and a shorter lag period prior to the onset of rigor. The authors were unable to explain the differences in rates, which could not be altered by various pre-mortem treatments. The authors noted that muscle which was allowed to go into rigor at a constant temperature of 37°C became watery and pale. They concluded that the probable reason for soft, watery pork was a combination of high temperature and low pH and suggested that the condition could be prevented by cooling rapidly to 30°C or below.

British Studies

Lawrie et al., (1958) reported that the ultimate pH was abnormally low in l. dorsi of exudative muscles. An ultimate pH of 5.1 or below was frequently found, with the lowest pH observed being 4.78. It was further reported that the exudative muscle had a considerably lower buffering capacity than normal muscle.

Lawrie (1960) reported a study on English pigs, mostly of the breed of Large White X Middle White. ATP, CP and pH levels and extensibility were determined on samples removed from six pigs held at 37°C under nitrogen. Glycogen and pH determinations were made on samples removed at 1 and 24 hrs post-mortem. Lawrie (1960) defined a normal l. dorsi muscle as having a pH of 6.7 at 1 hr post-mortem and an ultimate pH of about 5.5. He observed in normal muscle that the time taken for the muscles to pass from an identical pH at 1 hr post-mortem to the same ultimate pH varied 3-fold when held at 37°C. An apparently inverse relationship existed between the rate of pH decrease and the exudation of fluid. Rate of pH fall was emphasized as the major factor resulting in low water-holding capacity as opposed to the level of pH at 24 hrs post-mortem. Measurements of pH at 24 hrs in two groups of British Landrace pigs showed that they had considerably lower pH values relative to those observed for the other English pigs. Again, however, no relationship was evident between 24 hr pH and the soft exudative condition, which was in agreement with Briskey and Wismer-Pedersen (1961a,b). Lawrie observed distinct histological differences between the normal and exudative muscles in the Landrace pigs. However, these differences appeared to be primarily related to the 24 hr post-mortem pH level rather than the soft, watery condition.

American Studies

Briskey et al., (1959a,b,c; 1960) reported a decrease in glycogen in ham muscles from pigs subjected to exhaustive ante-mortem exercise. An inverse relationship was reported between initial glycogen level and 24 hr pH, water-binding capacity, and color.

Sayre et al., (1961) subjected pigs to a 0-5°C environment for 30-40 mins prior to slaughter. They reported that the treatment resulted in a decrease in initial muscle glycogen and post-mortem accumulation of lactic acid. Although color intensity of the chilled muscle was increased, the water-binding capacity was not consistently affected. The authors concluded that factors other than initial glycogen content, 24 hr pH and rate of glycolysis were important in determining water-binding capacity.

Meyer et al., (1962), using 20 Poland China and Chester White pigs, concluded that glucose tolerance was not an absolute indicator of post-mortem changes in muscle characteristics, but that there was a definite trend for those animals with a high glucose tolerance to have higher initial muscle glycogen levels and an ultimately inferior muscle quality. Sayre and Briskey (1963) reported that with the onset of rigor mortis at a pH below 5.7 and a temperature above 35°C, the muscle became extremely pale, soft and exudative and exhibited a marked loss in sarcoplasmic and fibrillar protein solubility. The latter was in agreement with the results of Bendall and Wismer-Pedersen (1962).

Sayre et al., (1963a) reported that short-term excitement and exercise immediately prior to slaughter resulted in muscle with inferior water-binding properties. Long- and short-term ante-mortem sucrose feeding

elevated initial glycogen levels and resulted in muscle that was slightly soft and pale. These authors further concluded that total phosphorylase activity was not affected by pre-slaughter treatment and did not appear to be associated with the rate of post-mortem glycolysis or with the ultimate muscle characteristics.

Sayre et al., (1963b) subjected Hampshire, Poland China and Chester White pigs to elevated environmental temperatures immediately ante-mortem. They reported that the treatment caused an increase in muscle temperature at slaughter and induced a rapid rate of pH decline and a decrease in ultimate color intensity. According to these authors, phosphofructokinase activity was not affected by ante-mortem treatments and did not appear to be related to glycolytic rate or to the condition of the muscle 24 hrs post-mortem. In contrast to the previous study (Sayre et al., 1963a), the results of ante-mortem feeding of sucrose were not uniform.

Kastenschmidt et al., (1964) studied the effects of four ante-mortem environmental temperature treatments, which were warm (42-45°C for 30-60 mins), cold (1-3°C for 30 mins), the warm followed by the cold treatment, and the cold followed by the warm treatment. The warm treatment alone was reported to induce the developement of extremely pale, soft and exudative muscle. The authors concluded that warm treatment followed by cold treatment resulted in dark, dry firm muscle. The other two treatments resulted in less marked changes.

Borchert and Briskey (1964) removed wholesale cuts immediately post-mortem and immersed them in liquid nitrogen (-195°C) for various periods of time. They concluded that immersion in liquid nitrogen with subsequent

equilibration and thawing at either -18°C or 4°C prevented the developement of pale, soft exudative muscle.

Sayre et al., (1964) concluded that when the onset of rigor mortis occurred at pH values below 5.9 with temperatures above 35°C, the l. dorsi muscle became pale and exudative. Conversely, the authors reported that if the onset of rigor mortis occurred when pH values remained above pH 6.0, the muscle was dark and firm.

Irish Studies

McLoughlin and Goldspink (1963a) reported that pale, exudative post-rigor muscle resulted if a rapid fall in pH to less than 6.0 in the l. dorsi occurred within 45 mins post-mortem. Extremely low (below 5.3) 24 hr pH values were found in many, but not all, specimens of pale, exudative muscle. The authors did not observe any histological changes characteristic of muscle degeneration in the soft, watery muscles.

McLoughlin (1963) concluded that the solubility of the sarcoplasmic and fibrillar proteins from exudative muscle was reduced at low and high ionic strengths, which is in agreement with Bendall and Wismer-Pedersen (1963). McLoughlin and Goldspink (1963b) and Goldspink and McLoughlin (1964) observed the effects of temperature and pH on the solubility of the sarcoplasmic protein. They concluded that the color of post-rigor muscle could be maintained if the temperature of the muscle was reduced to about 30°C before the pH approached 6.0. This conclusion was in agreement with the suggestion of Bendall et al., (1962).

HISTOCHEMISTRY OF STRIATED MUSCLE

Several comprehensive discussions on the developement and use of histochemical techniques have been published (Burstone, 1962; Pearse, 1960; Lillie, 1954; Danelli, 1963; Gomori, 1952; Glick, 1949). These references include detailed discussions of methods utilized for the detection of various cellular constituents and the activity of numerous enzymes in muscle. Accordingly, the present discussion is restricted to a brief description of current methods for demonstrating certain enzymic reactions which are pertinent. Furthermore, a brief account of the distribution of these enzymes in different muscle tissues is included, for example, the distribution between white and red ribers.

Methods for Detecting Dehydrogenase Activity

Nachlos et al., (1958a,b), Pearse (1957) and Hess et al., (1958) described methods for detecting the activities of the various DPN (diphosphopyridine nucleotide) and TPN (triphosphopyridine nucleotide) linked dehydrogenases. Nachlos et al., (1958a,b) utilized Nitro BT (nitro blue tetrazolium) as the final electron acceptor, while Pearse and co-workers used MTT (3- 4,5-dimethyl-thiazoyl-2 -2-5-diphenyl tetrazolium bromide).

Hess et al., (1958) described how the activity of two enzymes of the pentose shunt (glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase) and one enzyme of the beta-fatty acid oxidation cycle (beta-hydroxybutyrate dehydrogenase) could be demonstrated using MTT as the electron acceptor.

Nachlos et al., (1958a,b) detected lactate, alpha-glycerophosphate, and ethanol dehydrogenase reactions, which are indicative of glycolytic

activity utilizing Nitro BT. The same authors likewise detected the activities of three TCA (tricarboxylic acid) cycle enzymes, i.e. malate, isocitrate, and alpha-keto glutarate dehydrogenases. Since alpha-keto glutarate dehydrogenase is also a catalyst for the conversion of L-glutamic acid to alpha-keot glutarate, the reaction obtained was not regarded to be indicative of the TCA cycle activity per se.

Pearse (1960) has described the use of either Nitro BT or MTT in detecting succinic dehydrogenase activity. He has also discussed the role of this enzyme as a link between the TCA cycle and the electron transport system in addition to its involvement in the TCA cycle per se.

Methods for Detecting Respiratory Enzyme Activity

Nachlos et al., (1958a) used Nitro BT while Scarpelli et al., (1958) utilized MTT for the detection of TPN- or DPN- linked diaphorase (TPN/DPN-cytochrome c reductase) activity. Pearse (1960) has discussed the use of DPNH and TPNH as substrates, and alternatively, exogenous enzyme-substrate systems for conversion of TPN or DPN to the reduced substrate form. Moog (1943), Nachlos et al., (1958) and Burstone (1959, 1961a) demonstrated cytochrome oxidase activity by utilizing n-phenyl-p-phenylene diamine, dimethyl-p-phenylene diamine or 4-amino-1,N,N-dimethylnaphthylamine as substrate, respectively.

Methods for Detecting the Activity of Other Enzymes

A method for determining adenosine triphosphatase activity was reported by Padykula and Herman (1955a,b) while Wachstein and Meisel (1957) reported a different method. According to Padykula and Gauthier (1963)

these two methods detect myofibrillar and sarcoplasmic ATPases, respectively.

Takeuchi (1956, 1958) reported detection of the synthesis of alpha-1,4 linked glycogen from glucose-1-phosphate. The activity of the two enzymes involved, i.e. phosphorylase and branching enzyme (anylo-1,4 1,6-transglycosidase) could be individually demonstrated. Takeuchi and Glennar (1960, 1961) investigated the syntehsis of glycogen by a second pathway. This pathway involved the synthesis of an amylose-type glycogen from UDPG (uridine diphosphoglucose) by UDPG-glycogen transferase.

Procedures for the detection of LAP (leucine amino peptidase) activity were reported by Burstone and Folk (1956) and Nachlos et al., (1957, 1962) utilizing either L-leucyl-beta naphthylamide or L-leucyl-4-methoxy-2-naphthylamide as substrate. Pearse (1960) has discussed the demonstration of thiamine pyrophosphatase activity. Gomori (1952), and Burstone (1961b) have reported different methods for detecting alkaline phosphatase activity. Likewise, Gomori (1950), Grogg and Pearse (1952), and Burstone (1961b) have developed methods for demonstrating acid phosphatase reactions.

Distribution of Enzymic Activity

The concept of red and white muscles which ontain a predominance of either red or white fibers, respectively, has been recognized for many years (Needham, 1926). However, the development of histochemical methods for detecting enzymic activity has greatly facilitated elucidation of the characteristics of the two types of fibers (Dubowitz and Pearse, 1961).

Wachstein and Meisel (1955) studied the distribution of succinic dehydrogenase activity and mitochondria in various rat and rabbit muscles.

They reported considerable muscle to muscle variation in the amount of activity and in the apparent number of mitochondria present. Nachimias and Padykula (1958) found no positive correlation between fiber size in rat muscle and glycogen content, although the latter tended to be more abundant in the large white fibers. Dubowitz and Pearse (1960, 1961) reported low concentrations of oxidative enzymes and high concentrations of phosphorylase in the large white fibers of striated muscle from the human, rat, and pigeon. A reverse realtionship was observed for the small, red fibers.

Stein and Padykula (1962) observed three types of fibers in rat muscle. Type A were rich in glycogen and low in succinic dehydrogenase activity. Types B and C were high in enzyme activity and variable in their content of glycogen. According to the authors, the type A fibers were equivalent to large white fibers while types B and C represented two kinds of small red fibers.

Ogata and Mori (1963, 1964) suggested that there were three types of fibers present in mammalian muscle. Small fibers with a weak or negative phosphorylase reaction showed a high concentration of oxidative enzymes. However, large fibers showed a reverse relationship. The third type of fiber was intermediate for both phosphorylase and oxidative enzyme activity.

Pearse (1961) found a high concentration of alpha-glycerophosphate dehydrogenase in large fibers and a low concentration or absence of activity in small fibers. Thus, alpha-glycerophosphate dehydrogenase, which is not linked to a co-enzyme, paralleled phosphorylase activity.

Blancher and Van Wijhe (1962), when using a biochemical assay, found a higher level of diaphorase activity in red than in white muscle, but an equal lactate dehydrogenase activity in these muscles. Accordingly, these authors suggested that the differences between red and white fibers were probably due to inadequate diaphorase activity in the large, white fibers. However, these same workers (Van Wijhe et al., 1963; Blancher et al., 1963) later concluded that glycolytic dehydrogenases are more active in white than in red fibers. They further reported that white muscles of the guinea pig had two to three times greater alpha-glycerophosphate and lactic dehydrogenase activity than red muscles.

Hess and Pearse (1961) suggested that glycogen may be synthesized in large white fibers, via phosphorylase and branching enzyme, and in small red fibers by UDPG-glycogen transferase. However, Sasaski and Takeuchi (1963) found intense phosphorylase reaction in small fibers and a weak phosphorylase plus branching enzyme activity in fibers of large diameter.

Pearse (1961) and Blanchaer et al., (1963) have alluded to a possible relationship between the high levels of alpha-glycerophosphate dehydrogenase in the large white fibers and the postulated alpha-glycerophosphate "shuttle" (Esterbrook and Sackton, 1958). They suggested that this was possibly a preferred metabolic pathway in these fibers. Recent chemical studies (Blanchaer, 1964) on the respiration of mitochondria isolated from red and white muscles support this view.

Goldspink (1962) suggested that large fibers are merely a different phase of the small fibers. He concluded that various histochemical ob-

servations can be explained as being due to a decrease in the concentration of enzyme in the large fibers, but not the amount of total enzymic activity. However, Dubowitz and Pearse (1961) have indicated the discrepancies involved in interpreting the occurrence of large and small fibers as phases in activity of the same fiber type.

The results reported previously on the activities of various enzymes in normal muscle have been confirmed and expanded by Ogata and Mori (1963, 1964). These authors reported that red fibers of mouse muscle contained moderate to high concentrations of succinate, lactate, malate and alpha-glycerophosphate dehydrogenases, while large white fibers contained only traces. Traces of glutamate dehydrogenase activity were observed in red fibers, but no activity was observed in the small fibers. Beta-hydroxybutyrate dehydrogenase and DPN-diaphorase were moderately active in red fibers but only slightly active in large, white fibers. TPN-diaphorase activity was absent in the large fibers and present in moderate amounts in the small red fibers. TPN-isocitrate dehydrogenase and Glucose-6-phosphate dehydrogenase were not detected in either type of fiber. Similar results were generally obtained for these enzymes in cat and human muscle. Succinate, lactate, malate, alpha-glycerophosphate, and glutamate dehydrogenases were studied in dog, pig and ox muscles. Results were similar to those reported for muscles from the mouse. However, according to the authors, the white fibers of these species had higher levels of lactate, malate and glutamate dehydrogenase activity than is found in mouse muscle. Amino peptidase activity was absent in all muscles studied. Results reported for phosphorylase activity were in agreement with those of Dubowitz and Pearse (1961, 1961).

In the same studies (Ogata and Mori, 1963, 1964), all muscles observed were devoid of alkaline phosphatase activity. Acid phosphatase activity was not found using diazo dye coupling method. The latter result was in contrast to the observations of Beckett and Bourne (1958), who used a modification of Gomori's lead nitrate method (Gomori, 1952). The latter workers reported the presence in normal muscle of acid phosphatase activity in the peripheral nerves and the Golgi apparatus in both muscle fibers and connective tissue. Ogata and Mori (1963) suggested that such results were diffusion artifacts. However, Beckett and Bourne (1960) believe the two methods are each specific for a different enzyme or enzymes.

Cytochrome oxidase and both sarcoplasmic and myofibrillar ATPase have been reported to occur in normal striated muscle without any consistent pattern of distribution (Beckett and Bourne, 1960).

EXPERIMENTAL PROCEDURES

BEEF CARCASSES

This investigation was carried out to follow the chemical and histochemical changes in the l. dorsi muscle under post-mortem conditions similar to the usual commercial procedures. Previous studies (Marsh, 1954; Howard and Lawrie, 1956, 1957) on beef have primarily involved observations on muscle strips held at 37°C post-mortem.

Experimental Animals and Sampling Procedures

Five Hereford heifers weighing approximately 850 lbs were obtained from the University Farm. The animals were fasted 12 hrs prior to slaughtering at one-half hr intervals. Immediately after death, the animals were partially hoisted and an incision was made through the hide in the vicinity of the last rib. Three cores were removed from the l. dorsi muscle of each carcass using a 1-inch core borer. All initial samples were removed within 10 mins after death. Each carcass was then dressed in the conventional manner and moved into a 3-4°C cooler. Further samples were removed at 6, 12, 24, 48, 126, 288 and 480 hrs for pH, chemical and histochemical observations. Additional samples were removed for pH measurements at 72, 96, 102, 173 and 198 hrs. The holes resulting from the removal of a sample were packed with adsorbent cotton. Consecutive sampling locations were separated by 1 1/2 - 2 inches.

Immediately after removal from the carcass, samples for histochemical analysis were frozen in isopentane cooled to approximately -158°C with liquid nitrogen. Samples for glucose and glycogen analyses were

frozen in liquid nitrogen. All frozen samples were wrapped in Dow Handi-Wrap and stored at -29°C until analyzed.

Samples for lactic acid, creatine phosphate, ATP, TSP, and ortho phosphate analyses were weighed on an analytical balance and homogenized in a Waring blendor with 50 ml of cold (4°C) 7% TCA (trichloroacetic acid) solution for one min. The homogenate was filtered through Whatman No. 1 paper in the cold (4°C). The homogenizing cup was rinsed with an additional 25 ml of 7% TCA which was added to the original homogenate before filtering. The filtrate was adjusted to pH 7.1-7.3 with NaOH, made up to 100 ml with cold (4°C) distilled water and stored at -29°C until analyzed. After thawing for analyses, one-half ml chloroform was added to each sample.

Temperature Measurements

A thermocouple, attached to a Minneapolis-Honeywell temperature recorder, was inserted into the center of the l. dorsi muscle of each carcass at the approximate level of the 4th lumbar vertebra immediately after the carcass had been placed in the cooler. Temperature recordings were taken continuously throughout the first 72 hrs post-mortem.

Measurement of pH

A sample weighing approximately 2 gms was homogenized in 25 ml of 0.005 M sodium iodoacetate for one-half min in a Waring blendor. Duplicate pH measurements were made with a Beckman, Model G, pH meter.

Chemical Analyses

Creatine phosphate was determined according to the procedure of Ennor and Rosenberg (1952) and lactic acid according to the method of Barker and Summerson (1941). ATP, TSP, and inorganic phosphate analyses were made using the method of Allen (1940) as modified by Marsh (1954). ATP was also determined using a Turner fluoromenter by the bioluminescent enzymic method of Strehler and Totter (1952) and Strehler (1953).

For glucose or glycogen determinations, one-half to 1 gm of tissue was chipped from a frozen tissue block in the cold (-10°C). The tissue was introduced into a previously weighed digestion solution as outlined by Folin and Wu (1920) or an extraction solution as given by Hansen et al., (1952) for glucose and glycogen, respectively. After warming to room temperature, each solution was reweighed to obtain the sample weight. The analyses were then carried out according to the methods reported earlier herein.

Statistical Analyses

Means and standard deviations were calculated for the chemical values for each sampling period according to Snedecor (1959). An estimate of measuring error was calculated for glucose, glycogen and lactic acid determinations according to Magee (1964). In calculating this estimate, all values recorded for a given sample including all repeated analyses were utilized, even if some values appeared to be in obvious error. Accordingly, the estimates probably approach the largest expected error values.

Histochemical Procedures

Blocks of tissue which had been frozen as previously described, were trimmed and mounted on chucks with distilled water in the cold (-29°C). Sections 10-12 microns in thickness were cut on a Slee-Pearse cryostat at -16°C within one week of sampling. The sections were mounted on coverslips, and subjected to the procedures outlined by Pearse (1960) which are given below.

a) TPN diaphorase: Sections were incubated for 15-20 mins at 20-22°C in 0.1-0.3 ml of a reaction mixture which included 0.6 ml 1.1 M Na DL-isocitrate solution (pH 7.4), 0.5 ml 2.5 M Na-L-malate solution (pH 7.4), 0.3 ml 5 mg/ml solution of Nitro BT, 0.2 ml 5 mg/ml solution of TPN, 0.3 ml 0.005 M $MnCl_2$, and 1.1 ml 0.05 M veronal acetate buffer (pH 7.4). The sections were then rinsed in saline, fixed in formal-saline for 10 mins and mounted in glycerin jelly.

b) DPN diaphorase: Sections were incubated at 22°C for 15-20 mins in 0.1-0.3 ml of a reaction mixture consisting of 0.6 ml of pH 7.4 sodium lactate (0.5 M), 0.2 ml of 5 mg/ml nitro BT solution (pH 7.4), 0.2 ml of a 1.5% aqueous solution of lactic dehydrogenase, 0.3 ml of a 5 mg/ml solution of DPN, 1.0 ml of pH 7.4 phosphate buffer (0.2 M), and 0.6 ml distilled water. The sections were then rinsed briefly in 0.85% saline solution, fixed in 10% formol-saline for 10 mins, and mounted on slides in glycerin jelly.

c) Isocitrate dehydrogenase: Sections were incubated for 15-30 mins in air at 37°C in 0.1-0.3 ml of a reaction mixture consisting of 0.1 ml of sodium DL-isocitrate adjusted to pH 7.0 with Tris buffer, 0.1 ml of

0.1 M sodium cyanide solution (pH 7.2), 0.25 ml of 0.06 M phosphate buffer (pH 7.0), 0.25 ml of a 1 mg/ml Nitro BT solution, 0.1 ml of 0.05 M $MgCl_2$, 0.3 ml of distilled water and 75 mg of PVP (polyvinylpyrrolidone). The sections were then fixed in 10% formol-saline or 10% formol-calcium for 10 mins, rinsed with 10-15% ethanol, and mounted in glycerin jelly.

d) Glutamate dehydrogenase: Procedures were identical to those used for isocitrate dehydrogenase except that 1.0 M sodium-L-glutamate (monohydrate) solution buffered at pH 7.0 with phosphate buffer was utilized as substrate.

e) Glucose-6-phosphate dehydrogenase: Sections were incubated for 15 mins at 37°C in 0.1-0.3 ml of a reaction mixture, which included 0.1 ml of a 1.0 M di Na-glucose-6-phosphate solution buffered at pH 7.0 with Tris buffer, 0.1 ml of 0.1 M sodium ozide (pH 7.0), 0.25 ml of 0.2 M Tris buffer (pH 6.8-7.0), 0.25 ml of a 1 mg/ml MTT solution (pH 7.0), 0.05 ml 0.01 M Na F (pH 7.0), 0.1 ml 0.1 M TPN, 0.1 ml of 0.5 M $CoCl_2$, 0.1 ml distilled water and 75 mg PVP. If cobalt-phosphate precipitates were present, the sections were rinsed briefly (30 secs) in 1% HCl, but usually this step was not necessary. The sections were then fixed in 10% formol-saline for 10 mins and mounted in glycerin jelly containing 0.5 M cobaltous acetate.

f) Beta-hydroxybutyrate dehydrogenase: Sections were incubated for 15 mins in a reaction mixture containing 0.3 ml of a filtered 1:10 mixture of 0.5 M $CoCl_2$ and 0.06 M phosphate buffer (pH 6.8-7.0), 0.1 ml DL-beta-hydroxybutyric acid (Na salt), 0.1 ml of 0.1 M sodium ozide or anytal, 0.25 ml of MTT (1 mg/ml, pH 7.0), 0.1 ml 0.1 M DPN, 0.3 ml distilled water,

and 75 mg PVP. The sections were then fixed in 10% formol-saline for 10 mins and mounted in glycerin jelly containing 0.5 M cobaltous acetate or in plain jelly.

g) Succinate dehydrogenase: Sections were incubated for 15 mins at 37°C in a reaction mixture consisting of 2 ml of a 1:1 solution of 0.2 M phosphate buffer (pH 7.6) and 0.2 M sodium succinate, and 2 ml of 1 mg/ml Nitro BT solution (pH 7.6). The sections were then washed in saline, fixed in 10% formol-saline for 10 mins, rinsed in 15% ethanol for 5 mins and mounted in glycerin jelly.

h) 6-phosphogluconate dehydrogenase: Sections were incubated in 0.1-0.2 ml of a reaction mixture consisting of 0.1 ml of barium 6-phosphogluconate (pH 7.0), 0.1 ml of amytal or sodium ozide (0.1 M, pH 7.0), 0.25 ml of 0.2 M Tris buffer (pH 6.8-7.0), 0.25 ml of a 1 mg/ml MTT solution, 0.1 ml of 0.1 M TPN, 0.05 ml 0.5 M $CoCl_2$, 0.3 ml distilled water, and 75 mg PVP. The sections were fixed in 10% formol-saline or 10% formol-calcium for 10 mins and mounted in glycerin jelly.

i) Alpha-glycerophosphate dehydrogenase: Sections were incubated for 15 mins at 37°C in 0.1-0.3 ml of a reaction mixture consisting of 0.1 ml 0.1 M sodium-DL-alpha-glycerophosphate (pH 7.0), 0.1 ml 0.1 M sodium cyanide (pH 7.2), 0.25 ml 0.2 M Tris buffer (pH 6.8-7.0), 0.25 ml of a 1 mg/ml Nitro BT solution (pH 7.0), 0.1 ml 0.1 M DPN, 0.1 ml 0.05 M $MgCl_2$, 0.3 ml distilled water, and 75 mg PVP. The sections were then fixed in 10% formol-saline or a 10% formol-calcium solution for 10 mins and mounted in glycerin jelly.

j) Lactate and alcohol dehydrogenase: The procedures used were identical to that for alpha-glycerophosphate dehydrogenase except for the use of 0.06 M phosphate as the buffer, and 1.0 M sodium-DL-lactate or 1.0 M ethanol as substrate.

k) Acid phosphatase: Sections were post-fixed in cold (4°C) acetone for 2 hrs and incubated for 40 mins to 2 hrs in 0.1-0.3 ml of a reaction mixture containing 2 vols. of 12% sodium-beta-glycerophosphate, 1 vol. 0.1 M acetate buffer (pH 5.0-6.0), 1 vol. 2% lead acetate, and 0.3 vol. 1-5% $MgCl_2$. The sections were then rinsed in 5% sodium thiosulfate for 5 mins, and mounted in glycerin jelly.

l) Alkaline phosphatase: After drying for 1-2 hrs at room temperature, sections were incubated for 30 mins to 2 hrs in 0.1-0.3 ml of a reaction mixture consisting of 10 ml 3% sodium-beta-glycerophosphate, 10 ml 2% sodium diethyl barbiturate, 5 ml distilled water, 20 ml 2% $CaCl_2$ and 1 ml 5% magnesium sulfate. The sections were then washed in distilled water, treated with a 2% cobalt solution, washed in distilled water, treated with dilute ammonium sulfide solution, and mounted in glycerine with or without counterstaining in 1% aqueous eosin.

m) ATPase (fibrillar): Sections were incubated for 15 mins at 37°C in a freshly prepared reaction mixture adjusted to pH 9.4 with 0.1 M NaOH and containing 20 ml 0.1 M sodium barbiturate, 10 ml 0.18 M $CaCl_2$, 30 ml of distilled water and 152 mg of di-Na-ATP. The sections were then washed in three changes of 1% $CaCl_2$, transferred to 2% $CoCl_2$ for 3 mins, washed in distilled water, developed in 0.1% ammonium sulfide for 1 min, washed in distilled water, dehydrated, cleared and mounted in Permount (Fisher Chemical Company).

n) ATPase (sarcoplasmic): Sections were incubated for 15-20 mins at 37°C in a pH 7.2 reaction mixture containing 20 ml of 125 mg % ATP (di Na) solution, 20 ml 0.2 M Tris-HCl buffer (pH 7.2), 3 ml 2% Pb $(NO_3)_2$, 5 ml 0.1 M $MgSO_4$, and 4 ml distilled water. The sections were then rinsed in distilled water, developed in dilute ammonium sulfide, rinsed again in water, and mounted in glycerin jelly.

o) Thiamine pyrophosphatase: Sections were incubated at 42°C for 1 hr or at 37°C for 24 hrs in a freshly filtered reaction mixture containing 60 mg Pb $(NO_3)_2$, 45 ml 0.005 M acetate buffer (pH 5.0) and 5 ml 5% thiamine pyrophosphate. The sections were rinsed in 1% acetic acid, blotted, dried at 60°C for 5 mins, covered with a thin film of celloidin, developed in dilute ammonium sulfide, rinsed in water and mounted in glycerin jelly.

p) Leucine aminopeptidase: Sections were incubated for 2 hrs at 37°C in 0.1-0.3 ml of a reaction mixture containing 1 ml of an 8 mg/ml L-leucyl-beta-naphthylamine solution, 10 ml of sodium acetate buffer (0.1 M, pH 6.5), 8 ml of 0.85% saline solution, 1 ml of 0.02 M KCN solution, and 10 mg of Fast Blue B salt. The sections were then rinsed in 0.85% saline for 2 mins, immersed in 0.1 M cupric sulphate for 2 mins, rinsed again in saline and mounted on slides in glycerin jelly.

The activity of glucose-6-phosphate dehydrogenase was also observed in the initial samples with a Nitro BT procedure as adapted from Pearse (1960). In this procedure, sections were incubated for 30 mins at 37°C in a reaction mixture containing 0.1 ml di-Na-glucose-6-phosphate (1.0 M, pH 7.0), 0.1 ml 0.1 M amytal (pH 7.0), 0.25 ml Tris-buffer (0.2 M, pH 6.8-7.0), 0.05 ml of a 1 mg/ml Nitro BT solution (pH 7.0), 0.05 ml 0.01 M NaF

(pH 7.0), 0.1 ml 0.1 M TPN, 0.1 ml 0.05 M $MgCl_2$, and 75 mg PVP. The sections were fixed in 10% formol-saline or formol-calcium for 10 mins, and mounted in glycerin jelly.

In all cases, two or more sections were treated as outlined above. Additional sections were utilized for substrate blanks, i.e., reaction mixture containing no substrate. The commercial sources from which the various substrates, co-enzymes, etc. were obtained are listed in Appendix A, Table 1.

Over 1200 mounted sections resulted from application of the above histochemical procedures. The intensity of the reactions was evaluated by microscopic observation and rated as - (none), ± (traces), + (weak), ++ (moderate), +++ (moderately strong), and ++++ (strong).

Photomicrographs were made of representative sections using a Leitz Dialux microscope equipped with a Kodak Colorsnap 35 camera and a Vickers Instruments, Ltd. Photo-Multiplier and Automatic Integrating Timer Unit. Ansco Veraspan 35 (135 mm, A.S.A. #125) film was used.

PORK CARCASSES

Experimental Animals

Ten Hampshire gilts acquired from a private breeder were kept for two to three weeks at the Michigan State University Farm prior to slaughtering. These gilts and one Yorkshire gilt from the University herd were slaughtered in groups of 2 or 3 upon reaching a weight of 190 to 220 lbs. The animals were brought to the Meat Laboratory 2 1/2 days prior to slaughter and provided with feed and water until slaughter. The carcasses from

the ten Hampshire gilts and one Yorkshire gilt were designated as 1 through 10, and 11Y, respectively.

Six Poland China gilts were obtained from a private breeder and housed at the Meat Laboratory for 3-6 days. They were allowed free access to feed and water up to the time of slaughter. Carcasses from these gilts were designated as A through G.

Slaughtering and Sampling Procedures

The pigs were electrically stunned, shackled, hoisted, bled and processed in the usual manner. Initial samples were removed from the unevisserated carcasses at an average time of approximately 11 1/2 mins after death. The maximum time interval between death and initial sampling was 16 mins.

Carcasses 1 through 5 were dressed in the usual manner and the carcasses split down the backbone with the fatback left intact. On all other carcasses a similar procedure was followed except that on the side to be placed at -29°C the backfat over the *l. dorsi* muscle was trimmed to less than one-fourth inch thickness.

Samples were removed and treated as previously described for beef, except that chloroform was added to the TCA extracts prior to storage. In addition, samples were removed for determination of fibrillar water-binding capacity in carcasses 1 through 10 and 11Y. The sampling locations and sequence of removal of samples are listed in Appendix B, Table 1. The times post-mortem at which samples were removed for chemical analysis and pH determinations, and where applicable, histochemical determinations and fibrillar water-binding capacity are listed in Table 1.

Table 1. Approximate sampling times (mins post-mortem) in pork carcasses*

Carcass	Right side	Left side
1	10, 28, 73, 139, (193), 253, 493, 1440, 2280	33, 80, 133, 193, 253, 491, 1440, 2880
2	10, 83, 144, 236, 324, 564, 1440, 2880	80, 146, 235, 327, 564, 1440, 2880
3	47, 65, 121, 187, 251, 325, 566, 1440, 2880	12, 48, 63, 123, 190, 269, 320, 554, 1440, 2880
4	207, 308, 503, 668, 1440, 2880	14, 173, 312, 503, 668, 1440, 2880
5	11, 143, 301, 438, 633, 1040, 2880	166, 294, 438, 633, 1440, 2880
6	169, 288, 518, 1440, 2880	14, 167, 291, 516, 1440, 2880
7	16, 177, 287, 524, 739, 1440, 2880	176, 289, 526, 1440, 2880
8	182, 294, 532, 787, 1440, 2880	15, 187, 302, 532, 787, 1440, 2880
9	6, 173, 298, 525, 720, 1440, 2880	175, 305, 525, 720, 1440, 2880
10	133, 253, 493, 733, 1440, 2880	13, 120, 253, 493, 733, 1440, 2880
11	12, 266, 1440, 2880	266, 1440, 2880
A	10, 187, 329, 1440, 2880	175, 322, 1440
B	186, 333, 1440, 2880	11, 172, 322, 1440
C	10, (70), 180, 340, 1440,	(73), 175, 343, 1440
D	(70), 181, 346, 1440, 2880	10, (72), 169, 348, 1440
E	10, (89), 160, 312, 1440,	(85), 170, 303, 1440
F	(75), 172, 298, 1440, 2880	14, (74), 164, 290, 1440
G	13, (74), 175, 302, 1440, 2880	(73), 166, 293, 1440

*Underlined values are initial sampling times, and values in parenthesis are time periods at which only samples for pH were removed.

Temperature Treatments

The various temperature treatments to which individual sides were subjected are listed in Table 2.

All sides were placed at a given temperature within 13 to 27 mins after death. The treatments utilized in carcasses 1 through 5 were intended to result in a difference in ultimate muscle condition between the two sides of a given carcass. One side of each of these five carcasses was subjected to so-called "normal cooling conditions" (3-4°C), while the opposite side was subjected to one or more of three different temperature treatments as specified in Table 2. When necessary to prevent freezing, the sides held at -29°C were removed and held at 4°C for 5 to 10 min and then placed back at -29°C.

Since the procedures used for carcasses 1 through 5 did not result in marked differences in the ultimate muscle condition of the two sides of a given carcass as was expected from the observations of previous workers (Wismer-Pedersen and Briskey, 1961a; Briskey and Wismer-Pedersen, 1961a,b; Bendall and Wismer-Pedersen, 1962; Bendall et al., 1963), the two extremes in temperature (+37°C, -29°C) were subsequently employed for the remaining carcasses (6 through 10, 11Y, and A through G).

Determination of Fibrillar Water-Binding Capacity

The water-binding capacity of the fibrils was determined according to the general procedure of Bendall and Wismer-Pedersen (1962). A sample weighing approximately 2 gms was homogenized in 25 ml of a 0.04 M KH_2PO_4 buffer (pH 7.0; approximate ionic strength = 0.09) with a VirTis homogenizer, which was taken to full speed in 3 bursts of 10 seconds duration so

[illegible] temperature thermocouple by which individual alloys were [illegible] are listed in Table I.

All alloys were [illegible] after death. [illegible] tensile [illegible] two months [illegible] were [illegible]

Table 2. Temperature treatments.

Carcass No.	Treatment			
	Right side	Hours*	Left side	Hours
1	4°C	-	37°C	4 1/2-5
2	4°C	-	-29°C** +25°C	1 1 1/2
3	37°C	4 1/2-5	4°C	-
4	4°C	-	-29°C	4-4 1/2
5	4°C	-	-29°C	4-4 1/2
6 through 10, 11Y	-29°C	4-4 1/2	+37°C	4 1/2-5
A through G	-29°C	4-4 1/2	+37°C	4 1/2-5

* Number of hours a side was subjected to a given treatment prior to being placed in the 4°C cooler.

**The left side of carcass 2 was first placed in the -29°C treatment for 1 hr then moved to room temperature for 1 1/2 hrs prior to being placed in the 4°C cooler.

as to avoid excessive heating of the homogenate. Furthermore, the homogenizing flask was placed in an ice-bath during homogenization. The homogenate was centrifuged at 2000 x g for 15 mins and the supernatant discarded. The precipitated fibrils were re-mixed with 25 ml of buffer and centrifuged as before. The supernatant was discarded. The concentration of nitrogen in the washed fibrils was determined using a micro-Kjeldahl method described by the American Instrument Company (1961). Samples removed at 24 hrs post-mortem were weighed on an analytical balance before homogenization. The weight of the precipitated fibrils was also determined prior to nitrogen measurements. Grams of water retained per gm of fibrillar protein was calculated for all samples. Fibrillar protein precipitated per gm of sample was also calculated for the 24 hr samples. Calculations were made according to Bendall and Wismer-Pedersen (1962).

Temperature, pH and Chemical Determinations

Temperature was recorded and pH determinations were made according to the procedures described for beef. However, temperature measurements, were limited to the initial 6-8 hrs post-mortem.

The levels of CP, glucose, glycogen and lactic acid were determined according to the methods described for beef. The initial levels of ATP for carcasses 6 through 10 and 11Y were determined using the two methods described for beef muscle. On all other samples, ATP analyses were made by utilizing only the enzymic method.

[illegible] heating of the homogenate. Furthermore, the homogen-
[illegible] was placed in an ice-bath during homogenization. The homogen-
[illegible] centrifuged at 3000 r.p.m. [illegible] at 4°C in a refrigerated
precipitated [illegible]
be before. [illegible]
in the washed
cubed [illegible]

Histochemical Procedures

The methods described for beef were utilized to observe TPN and DPN diaphorase, as well as isocitrate, glutamate, beta-hydroxybutyrate, succinate, alpha-glycerophosphate, alcohol, and lactate dehydrogenase activity in the initial and 24 hrs post-mortem samples of carcasses 1 to 10 and 11Y. Glucose-6-phosphate dehydrogenase activity was observed using the Nitro BT method described for beef muscle.

A Nitro BT method was also used to observe the activity of 6-phosphogluconate dehydrogenase as adapted from Pearse (1960). In the same samples the activity of malate dehydrogenase and cytochrome oxidase were observed according to Pearse (1960). Methods described by Pearse (1960) were also used to observe alkaline and acid phosphatase activity in carcasses 1 through 5. These five procedures are listed below.

a) Acid phosphatase: Sections were incubated for 5 hrs at 37°C in a filtered reaction mixture containing 5 ng Na-AS-BI-phosphate, 0.10 ml dimethyl sulfoxide, 2.5 ml 0.2 M acetate buffer (pH 5.2-5.6), 35 mg Red Violet LB salt and 2 drops of 10% $MnCl_2$. Sections were washed in running water and mounted in glycerin jelly.

b) Alkaline phosphatase: Sections were air-dried for 1 to 3 hrs and incubated for 30 mins at room temperature in a reaction mixture filtered onto the sections and containing 20 mg sodium-alpha-maphthyl phosphate, 20 ml of 0.1 M Tris-buffer (pH 10.0), and 20 mg of diazote 5-chloro-o-toluidine. The sections were then washed in running water for 1-3 mins, counterstained in 0.05% anilin blue for 3 to 6 mins, washed in running water for 40 mins and mounted in glycerin jelly.

c) Cytochrome oxidase: Sections were incubated for 60 mins at room temperature in a filtered reaction mixture containing 10 mg of 1-hydroxy-2 naphthoic acid, 10 mg of N-phenyl-p-phenylene diamine, 0.5 ml ethanol, 35 ml distilled water and 15 ml Tris-buffer (0.2 M; pH 7.4). The sections were then transferred directly to 1% cobalt acetate in 10% formalin for 1 hr, washed in distilled water, and mounted in glycerin jelly.

d) 6-phosphogluconate dehydrogenase: Each section was incubated for 30 mins at 37°C in 0.1 to 0.3 ml of a reaction mixture containing 0.1 ml 0.01 M barium-6-phosphogluconate (pH 7.0), 0.1 ml sodium ozide (0.1 M, pH 7.0), 0.25 ml Tris-buffer (0.2 M; pH 6.8-7.0), 0.25 ml of a 1 mg/ml solution of Nitro BT, 0.1 ml 0.1 M TPN, 0.3 ml distilled water and 75 mg PVP. The sections were then fixed in 10% formol-calcium or formol-saline for 10 mins and mounted in glycerin jelly.

e) Malate dehydrogenase: Sections were incubated for 15-20 mins at 37°C in a reaction mixture including 0.1 ml 1.0 M L-malic acid solution (pH 7.0), 0.1 ml 0.1 M sodium cyanide solution (pH 7.2), 0.25 ml 0.06 M phosphate buffer (pH 7.2), 0.25 ml of a 1 mg/ml solution of Nitro BT (pH 7.0), 0.1 ml 0.1 M DPN solution, 0.1 ml 0.05 M $MgCl_2$, 0.1 ml distilled water and 75 mg PVP. The sections were then fixed in 10% formol-calcium and mounted in glycerin jelly.

The activity of phosphorylase, branching enzyme, and UDPG-glycogen transferase was observed on some of the initial samples of carcasses 6 through 10. Activity was detected according to the methods ofTakeuchi (1958) and Takeuchi and Glenner (1961) as described below.

a) Phosphorylase and branching enzyme: Different sections were incubated in three different reaction mixtures (A, B, C) for 1 hr at 37°C.

Reaction mixture A contained 50 mg potassium-glucose-1-phosphate, 20 mg AMP, 10 mg glycogen, 10 ml distilled water, and 10 ml 0.2 M acetate buffer (pH 5.7-5.9). Reaction mixtures B and C were identical to the above except for the addition of 5 ml ethanol or 5 ml of 0.0006 $HgCl_2$, respectively. The same three reaction mixtures with the pH adjusted to 5.0 or 6.0 were also used in some cases. Likewise, AMP and/or glycogen were omitted in similar reaction mixtures. After incubation the sections were stained in Gram's iodine and mounted in iodine-glycerin jelly or in plain glycerin jelly. Both ethanol and $HgCl_2$ inhibit branching enzyme activity. The inhibition by alcohol is strong while that of $HgCl_2$ is relatively complete. Con ersely, the inhibition of phosphorylase by either of these substances is slight. The sections stained blue to dark blue when phosphorylase activity is predominant and violet red to brownish purple when the branching enzyme predominates.

b) UDPG-glycogen transferase: Sections were incubated for 1 hr at 37°C or 1-3 hrs at 25°C in a reaction mixture containing 50 mg UDPG, 10 mg glycogen, 20 mg Versene, 10 mg glucose-6-phosphate, 14 ml distilled water, 10 ml 0.2 M Tris-buffer (pH 7.4), and 1 ml ethanol. UDPG was omitted in substrate-blank reaction mixtures. Following incubation, the sections were treated as described in the above procedures for phosphorylase and branching enzyme. A reddish purple to reddish brown color indicated UDPG-glycogen transferase activity.

With carcasses A through G, only four, and in some cases five, enzymes were studied. These included phosphorylase, branching enzyme, and UDPG-glycogen transferase, which were observed by the procedures given above. Additionally, succinic dehydrogenase and/or glutamate dehydrogenase

was/were detected using procedures previously described for beef muscle. The activity of these enzymes was observed on all initial and 2 1/2-3 hrs post-mortem samples and on a limited number of samples removed at 24 hrs after death.

Sections from all the 2 1/2-3 hr samples and on a limited number of 24 hr samples of carcasses A through G were subjected to an HCl-Orange B-Anilin Blue staining procedure as modified from Guyer (1953). Sections were fixed in 10% formalin for 10 mins, stained 6 mins in acid iron-hematoxylin, washed in distilled water, rinsed in 1% acetic acid, stained in anilin blue-orange G-HCl solution, washed in 3 changes of acetic acid, dehydrated, and cleared by an acetone-xylene sequence and mounted in Permount.

In all cases, two or more sections were treated as outlined above. Substrate blanks were also utilized as described for beef muscle. Furthermore, where feasible sections were also incubated in complete reaction mixtures containing an inhibitor. Three-tenths ml of a saturated solution of PCMB (Na-p-chloromercurobenzoate) was included for each 1.1 ml of reaction mixture as an inhibitor when observing DPN and TPN diaphorase, and isocitrate, beta-hydroxybutyrate, alpha-glycerophosphate, lactate and alcohol dehydrogenase activity. As an inhibitor for succinate dehydrogenase, 0.6 ml of a saturated solution of PCMB was included in 2 ml of reaction mixture. A concentration of 0.001 M NaN_3 was used to inhibit cytochrome oxidase activity. The commercial sources from which these substrates used in the above procedures are included in Appendix B, Table 1.

using procedures previously described for beef muscle.

of these enzymes was observed on Vl Index 1 and 2 L*3-3

Over 1350 mounted sections resulted from application of the above histochemical procedures. The intensity of the reactions was evaluated and photomicrographs were made as described for beef muscle.

Statistical Analyses

Calculations were made as previously described for beef. However, means for sampling periods were calculated only where such periods were at approximately the same time post-mortem.

Panel Evaluation

The extent of the soft, watery and pale condition of the cut loin surface of carcasses 6 through 10, 11Y, and carcasses A through G was subjectively evaluated at 48 hrs post-mortem by a four-member panel. The panel rated each loin as being either extremely, moderately, slightly, or not at all soft and watery.

RESULTS AND DISCUSSION

POST-MORTEM LEVELS AND/OR INTERRELATIONSHIPS OF CHEMICAL CONSTITUENTS, pH AND TEMPERATURE IN BEEF MUSCLE

The means and standard deviations of the values for chemical constituents and pH at various periods post-mortem are listed in Table 3. The individual values for each carcass at each time period are tabulated in Appendix A, Table 2.

Levels of pH

The average initial pH value was 6.99. The lowest initial pH value was 6.90, while the highest was 7.07. At 48 hrs post-mortem, the mean pH was 5.46 and at 480 hrs it was 5.57.

The initial pH values are in agreement with Marsh (1954), who calculated the approximate pH at death for beef muscle and found the values varied from about 6.65 to above 7.40. Similarly, the initial pH values are also in agreement with Howard and Lawrie (1956), who obtained an average beef *l. dorsi* muscle pH value of 6.74 at 1 hr post-mortem.

The values for 48 and 480 hrs post-mortem are in agreement with the final pH value of 5.50 determined under similar conditions by Howard and Lawrie (1956).

Creatine Phosphate

Creatine phosphate decreased to about 22% and 16% of the initial level (9.1 micromoles/g of muscle) at 6 and 12 hrs post-mortem, respectively. The initial CP value is in general agreement with Howard and Lawrie (1956), who reported an average value equivalent to about 7.3

Table 3. Values for chemical constituents and pH of beef muscle at various periods post-mortem[a]

Time post-mortem (hrs)	pH values[b]	Glycogen[c]	Glucose[d]	Lactic acid	ATP_1[e]	ATP_2[f]	CP	TSP	Ortho P
Initial	6.99±0.07	56.7±5.9	7.9±1.0	13.1±6.0	6.4±1.2	10.9±1.4	9.1±3.2	54.9±4.2	22.1±3.1
6	6.57±0.12	41.6±10.4	6.3±2.2	44.8±15.8	5.0±0.8	10.0±2.3	2.0±1.5	55.2±3.6	23.2±4.2
12	5.96±0.13	30.4±8.5	12.2±4.3	58.0±18.6	3.9±1.4	5.3±3.9	1.5±1.3	54.9±3.9	25.9±2.3
24	5.74±0.19	10.1±3.8	18.1±3.6	71.2±11.2	1.7±0.6	0.0 ±0.2	-	53.6±5.2	21.6±1.3
48	5.57±0.11	10.0±0.9	15.9±3.5	82.4±7.4	1.1±2.3	-	-	54.2±3.6	27.5±1.3
72	5.46±0.08	-	-	-	-	-	-	-	-
96	5.36±0.03	-	-	-	-	-	-	-	-
102	5.42±0.10	12.7±4.2	12.1±2.8	80.9±5.0	-	-	-	53.6±3.9	30.3±5.2
126	5.50±0.05	-	-	-	-	-	-	-	-
151	5.53±0.08	-	-	-	-	-	-	-	-
173	5.54±0.09	-	-	-	-	-	-	-	-
198	5.63±0.07	-	-	-	-	-	-	-	-
288	5.59±0.06	9.9±5.0	16.8±5.8	82.7±3.3	-	-	-	54.9±3.6	33.6±3.6
480	5.46±0.05	1.4±0.6	17.9±3.3	84.6±2.9	-	-	-	54.2±4.2	35.5±2.6

[a]All values are average observations on all 5 carcasses and are expressed in micromoles (microatoms for TSP and Ortho P) per gram of fresh tissue.
[b]Means ± standard deviations.
[c]Glycogen is expressed in glucose equivalents.
[d]Total reducing sugars expressed as glucose.
[e]Values obtained by acid hydrolysis.
[f]Values obtained by enzymic assay.

micromoles/g in the 1. dorsi muscle of 13 steers at 1 hr post-mortem.

ATP

The initial $ATP_{(1)}$ (acid hydrolyses) and $ATP_{(2)}$ (enzymic assay) values were 6.4 and 10.9 micromoles, respectively. The $ATP_{(2)}$ value had decreased to nil within 24 hrs post-mortem, while the $ATP_{(1)}$ value was still at a level of 1.7 micromoles/g. The $ATP_{(1)}$ value is in good agreement with that of Howard and Lawrie (1956), who reported a comparable ATP value equivalent to approximately 5.3 micromoles/g of tissue at 1 hr post-mortem, and with that of 5.6 micromoles/g at 60-80 mins post-mortem as reported by Marsh (1954).

However, the $ATP_{(2)}$ values are considerably higher than the $ATP_{(1)}$ values (Table 3). The acid-molybdate reagent of Allen (1940) used for color developement in the $ATP_{(1)}$ analysis has been shown to hydrolyze the two acid labile phosphates of ATP, and especially the terminal phosphate in varying degrees. The amount of hydrolysis is primarily dependent on temperature and time of exposure to the acid-molybdate reagent (Weil-Malherbe and Green, 1951). Since Allen's (1940) method allows the time of exposure to the acid-molybdate reagent to vary from 5 to 25 mins, values for ortho phosphate estimated by this method would be expected to include some ATP phosphate. Consequently, the initial $ATP_{(1)}$ values in the current study as well as similar values of Howard and Lawrie (1956) would be expected to be less than the true ATP value.

Conversely, when using acid hydrolysis followed by determination of free phosphate by the acid-molybdate procedure, residual ATP and ADP are

Table 4. Normal muscle of 13 steers at 1 hr post-mortem.

present at a combined level, which is less than 2 micromoles/g ($ATP_{(1)}$; Table 3). Such a residual quantity is not found when assayed with the enzymic method ($ATP_{(2)}$; Table 3). Bendall (1951) suggested that the residual ATP and/or ADP obtained by the acid hydrolysis method was due to ATP and/or ADP, which is tightly bound to the muscle protein. However, Bendall and Davey (1957) concluded that acid labile-P content was a reliable guide to true ATP and/or ADP content only in the early stages of rigor, when CP and glycolysis resynthesis maintained ATP at a fairly constant level. They further concluded that when the rate of resynthesis begins to decrease, side-reactions invalidated any simple equation for labile-P to ATP.

Furthermore, in support of the absence of detectable $ATP_{(2)}$ at 24 hrs post-mortem in the current study (Table 3), it can be calculated from data presented by Davies (1963) that less than 0.2 micromole/g of tissue of ATP and/or ADP would be bound to the muscle protein in full rigor for rabbit muscle.

Fredholm (1963) reported that 50 and 23% of the initial ATP was present in beef "gracilis-muscle" after storage at cooler temperatures for 3 and 5 days, respectively. This is not in agreement with results of the current study, where the $ATP_{(2)}$ level was negligible at 24 hrs (Table 3).

TSP and Ortho P

In the current study, the overall mean for TSP was 54.4 microatoms/g of muscle. Marsh (1954) and Lawrie (1959) reported values equivalent to

55.5 and 57.1 microatoms/g for beef muscle, respectively. Values reported for rabbit muscle have varied from 61 to over 70 microatoms/g (Bate-Smith and Bendall, 1947; Bendall and Davey (1957).

The initial level of ortho P was 22.1 microatoms/g of tissue. This level increased to 35.5 microatoms/g at 480 hrs post-mortem. Comparable values are not available for beef. However, Bate-Smith and Bendall (1947) reported an initial level equivalent to 22.9 microatoms/g and a 444 min post-mortem level (at 37°C) of 31 microatoms/g in rabbit muscle. Hence, the concentration of ortho phosphate would appear to increase 1 1/2 times after death in muscle from both beef and rabbit.

Glycogen

The initial glycogen level was 56.7 micromoles/g. This value decreased to approximately 54% at 12 hrs and to less than 20% at 24 hrs post-mortem. At 288 hrs, the glycogen was still 17.5% of the initial value, but decreased thereafter to less than 3% at 480 hrs. It seems likely that the breakdown of residual glycogen occurred as a result of the combined activity of alpha-amylase, amylo-1:6-glycosidase, and maltase, which has been suggested by Sharp (1962). Although comparable values for all the various post-mortem time periods are not available, the values found in the present study are in good agreement with the reports of previous workers at specified times post-mortem. Initial levels of glycogen calculated from the data of Howard and Lawrie (1956, 1957) are 51.9 and 51.7 micromoles/g of *l. dorsi* muscle from 13 and 5 steers, respectively. Swift *et al.*, (1960) reported glycogen levels equivalent to 40.3 micromoles/g at 2-3 hrs post-mortem.

In the current study, the 24 hr value for glycogen was 10.1 micromoles/g. This is in agreement with the results of Lawrie and Howard (1956), who reported residual glycogen levels equivalent to as much as 8.3 micromoles/g in beef muscle after being frozen at 24 hrs post-mortem and stored at -10°C for twenty weeks.

Glucose

The average glucose (total reducing sugar) level increased from 7.9 to 17.9 micromoles/g between the initial and 480 hr post-mortem sampling periods. The marked variation observed in glucose levels at intervening periods (Table 3) suggests that the amount of glucose may vary considerably from location to location, and/or it may be converted to a form other than a reducing sugar at certain times post-mortem.

The values for total reducing sugars found in the current study (Table 3) are in good agreement with those reported by Sharp and Rolfe (1958). These workers observed that free glucose and glucose-6-phosphate were present in mammalian muscle immediately post-mortem at levels equivalent to 2.8 and 2.4 micromoles of glucose per g of muscle, respectively. Only relatively small concentrations of other reducing substances were observed. Glucose-6-phosphate increased during post-mortem glycolysis to a constant level, which approached about 14 micromoles (glucose equivalents)/g of tissue. They reported an accumulation of free glucose equivalent to 5.6 and 31.6 micromoles/g in beef and pork muscle, respectively, which was held at 18-20°C for 24 hrs post-mortem. Hence, the combined value of free glucose and glucose-6-phosphate in beef muscle approached 19.6 micromoles/g. If it is assumed that a maximum of 14

micromoles of glucose-6-phosphate accumulates post-mortem per g of beef muscle, then in the current study approximately 4-5 micromoles of free sugar or other reducing substances were present at 480 hours post-mortem. Such a value is in good agreement with the observations of Sharp and Rolfe (1958).

Lactic Acid

Lactic acid, which was present at an initial concentration of 13.1 micromoles/g, had increased almost 3 1/2 times at 6 hrs post-mortem (Table 3). A further slower, but steady increase was observed up to 48 hrs to approximately 6 1/2 times the initial level. Thereafter, little or no increase was evident. Results support Marsh's (1954) suggestion that active glycolysis is complete in beef l. dorsi muscle within 36 hrs post-mortem.

Although comparable lactic acid values are not available, Howard and Lawrie (1956) suggested that a "minimum quantity" of 700 mg of glycogen per 100 g of beef l. dorsi muscle was necessary to reach an ultimate pH of 5.44. If, as these workers assumed, glycogen were completely converted to lactic acid, the value for lactic acid would be 77.8 micromoles/g of muscle at pH 5.44. Such a value is in good agreement with the results of the current study (Table 3).

Temperature

The average temperatures of the l. dorsi muscle of the five beef carcasses are given in Table 4. It would appear that the cooling process was completed for this muscle prior to 20 hrs post-mortem.

Table 4. Average post-mortem temperature of the l. dorsi muscle of five beef carcasses.

Time post-mortem (hrs)	Temperature (°C)
Initial	38.9
3	27.2
6	20.0
10	13.3
12	10.0
16	6.7
20	2.8
24	2.8
48	2.8

ATP, CP and pH

CP and ATP levels as a percent of their initial levels and their relation to pH are shown in Table 5. CP decreased to approximately 20% of its initial value within 6 hrs post-mortem. During the same period, ATP_2 had decreased by less than 10%. At 12 hrs post-mortem, ATP_2 values had decreased to about 50% of the initial value, with a concurrent average pH level of 5.96. As previously discussed, the mean temperature of the 1. dorsi muscle was 20.0, 13.3, and 10.0°C at 6, 10, and 12 hrs post-mortem, respectively.

Table 5. Levels of ATP, CP and pH during the initial 48 hrs post-mortem

Time post-mortem (hrs)	pH	ATP level as a % of the initial level		CP as a % of the initial level
		ATP_1[a]	ATP_2[b]	CP
Initial	6.99	100.0	100.0	100.0
6	6.57	78.1	91.7	22.0
12	5.96	60.9	48.6	16.5
24	5.74	26.6	0.0	-
48	5.57	17.2	0.0	-

[a]Based on acid hydrolysis values.
[b]Based on enzymic assay values.

The rapid depletion of CP prior to any significant decrease in ATP level is in agreement with the observations of Marsh (1954).

Howard and Lawrie (1956) noted that the onset of rigor in beef muscle held at 37°C occurred when 1/2 to 3/4 of the initial ATP had disappeared with a corresponding pH level of 6.02. Bendall and Davey (1957) reported that the onset of rigor in rabbit muscle occurred when 1/2 of the initial ATP was depleted in muscle held at 37°C. If held at room temperature, however, onset of rigor occurred when 3/4 of the initial level was depleted.

In the current study, it is suggested that the average time post-mortem the onset of rigor in the l. dorsi muscle was initiated was about 12-15 hrs post-mortem. Results also indicated that the developement of rigor was virtually complete prior to 24 hrs post-mortem. This observation is supported by the data on lactic acid (Table 3).

Lactic Acid and pH

The general relationship between post-mortem levels of lactic acid and pH is shown in figure 1. Increasing levels of lactic acid resulted in lower levels of pH. This is in agreement with the observations of Bate Smith (1939), and Bate-Smith and Bendall (1947) on rabbit muscle.

In the current study, 46.5 micromoles of lactic acid/g of muscle were produced for every unit decrease in pH during the initial to 24 hr period. During the same period, pH decreased from 6.99 to 5.74. This value is in good agreement with a similar increase of 44 micromoles of lactic acid/g of tissue for every unit pH decrease as calculated from the data of Howard and Lawrie (1956) in beef l. dorsi muscle. However, an equivalent value for approximately the same pH interval of 63.3 micromoles of lactic acid per g of tissue for each unit pH decrease in rabbit muscle was reported by Bate-Smith and Bendall (1949). Both the observations of Howard and Lawrie (1956) and those in the current study would suggest that the buffering capacity in beef l. dorsi muscle is considerably lower than that found in rabbit muscle within the same pH range.

Glycogen, Lactic Acid and Glucose

The relationship between post-mortem levels of glycogen, glucose (total reducing sugars) and lactic acid is shown in figure 2. The sum in micromoles of glucose, glycogen and lactic acid (expressed as glucose equivalents) is approximately constant at all times post-mortem. Deviations from a completely constant relationship are within the limits of the errors of measurements which were ± 2.2, ± 1.2, and ± 6.6 micromoles/g for glycogen, glucose, and lactic acid, respectively.

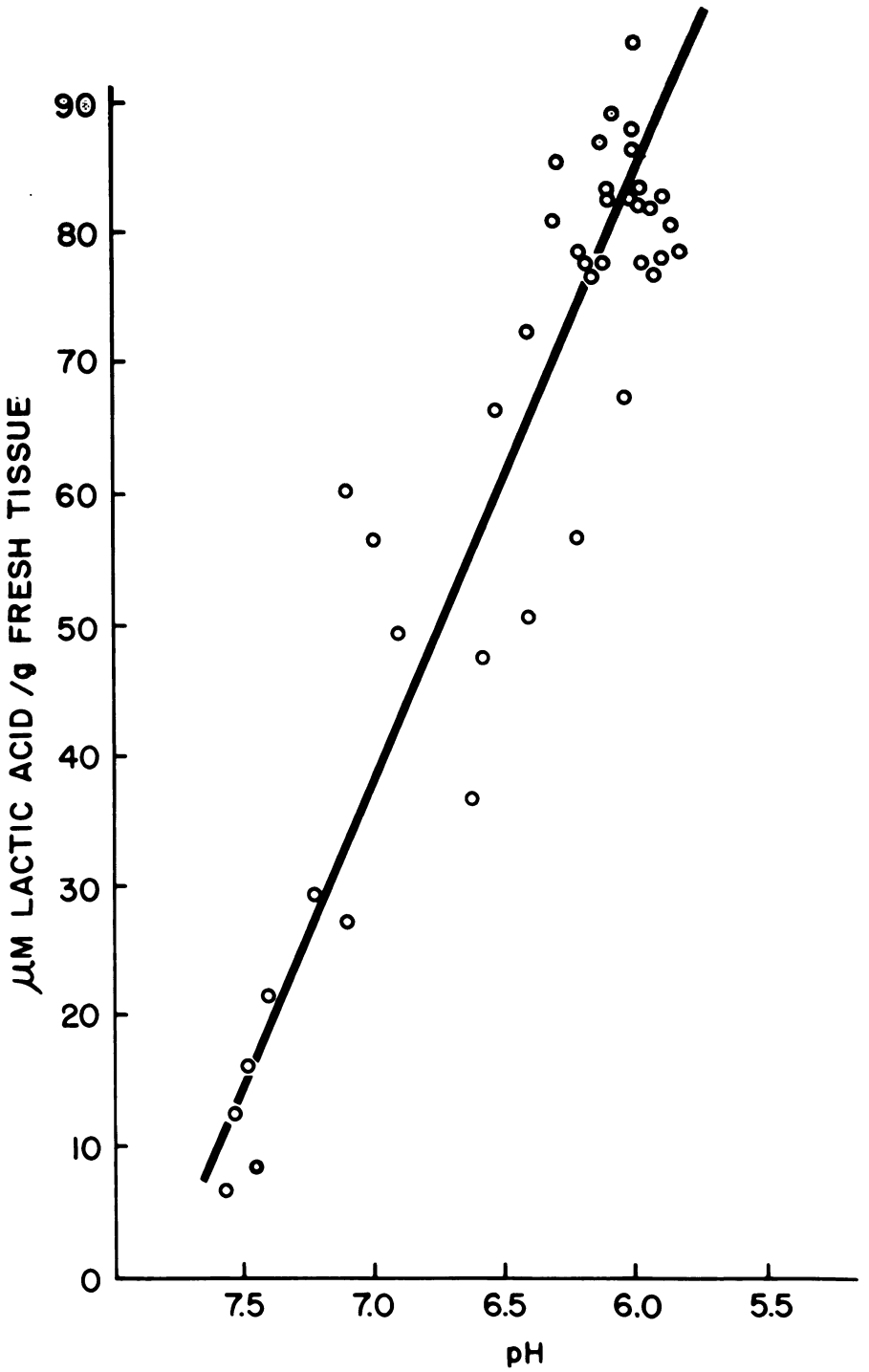


Figure 1. The relationship between pH and lactic acid in beef l. dorsi muscle.

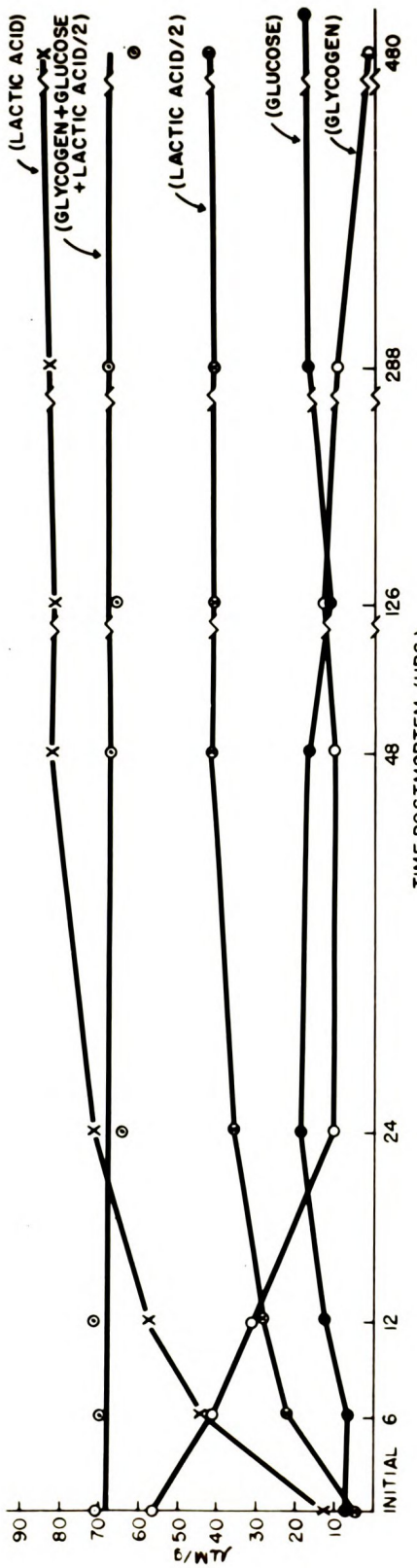


Figure 2. The relationship between post-mortem levels of glycogen, lactic acid and glucose in beef muscle (glucose, which includes total reducing sugars, and glycogen is expressed in glucose equivalents).

It is concluded that glycogen in beef muscle is degraded to glucose and lactic acid during post-mortem glycolysis in an approximately stoichiometric manner. This is in agreement with the work of Sharp and Rolfe (1958) on the accumulation of free glucose and glucose-6-phosphate. Furthermore, this conclusion supports the observation of Howard and Lawrie (1956) that in beef muscle, ultimate pH level could not be fully explained by assuming a stoichiometric degradation of glycogen to lactic acid.

CHEMICAL CONSTITUENTS, INTERRELATIONSHIPS, AND PROPERTIES OF PORK MUSCLE

Individual values for post-mortem levels of pH, glycogen, glucose, lactic acid, $ATP_{(1)}$ and $ATP_{(2)}$ for each side of carcasses 1 through 10 and 11Y are given in Appendix B, Table 2. Corresponding values for the fibrillar protein content/g of precipitate, the amount of water retained/g of fibrillar protein and the content of fibrillar protein/g of muscle at 24 hrs post-mortem are also included in Appendix B, Table 2. Likewise, individual values for post-mortem levels of pH, glycogen, glucose, lactic acid and ATP_2 for each side of carcasses A through G are tabulated in Appendix B, Table 3.

Carcasses 1 Through 5

The various temperature treatments utilized for carcasses 1 through 5 did not result in any appreciable differences between the two sides of a given carcass. The *l. dorsi* muscle surface of all five carcasses was considered normal at 48 hrs post-mortem.

Muscle Temperatures

In figure 3 are shown two typical temperature curves for two sides of a carcass. One side was subjected to -29°C immediately after death and the other to a temperature greater than 37°C. The initial temperature of the l. dorsi muscle in both sides was approximately 41°C prior to being subjected to the two different temperatures. The temperature of the side held at -29°C decreased to about 30°C after approximately 65 mins post-mortem and at 2 hrs had fallen to 19-20°C. The muscle in the side subjected to the higher temperature was still more than 35°C at 3 1/2 hrs post-mortem.

The effect of the two temperature treatments on pH value and ultimate muscle condition are given in Table 6 for the group 1 carcasses (carcasses 6 through 10) and carcass 11Y.

Table 6. Post-mortem pH and ultimate muscle condition as affected by temperature treatments of carcasses 6 through 10 and 11Y

Time post-mortem:		pH					Degree of soft, watery condition[a,b]	
		Initial	3 hrs		48 hrs			
Treatment:			-29°C	>37°C	-29°C	>37°C	-29°C	>37°C
Carcass	6	5.6	5.4	5.3	5.3	5.3	S	S to M
	7	6.7	6.5	6.2	5.6	5.3	N	S to M
	8	6.1	5.3	5.3	5.3	5.3	N	M
	9	6.4	5.7	5.4	5.3	5.5	N	N
	10	6.3	6.3[c]	5.5[c]	5.5	5.5	N	N
	11Y	6.6	6.3[d]	5.4[d]	5.7	5.5	N	N

[a]48 hrs post-mortem
[b]Rated on a scale of N = none, S = slight, M = moderate and E = extreme.
[c]Approximately 2 hrs post-mortem.
[d]Approximately 4 hrs post-mortem.

From the reports of previous workers (Wismer-Pedersen, 1959; Briskey and Wismer-Pedersen, 1961b; Bendall et al., 1963), the extremely low iniital

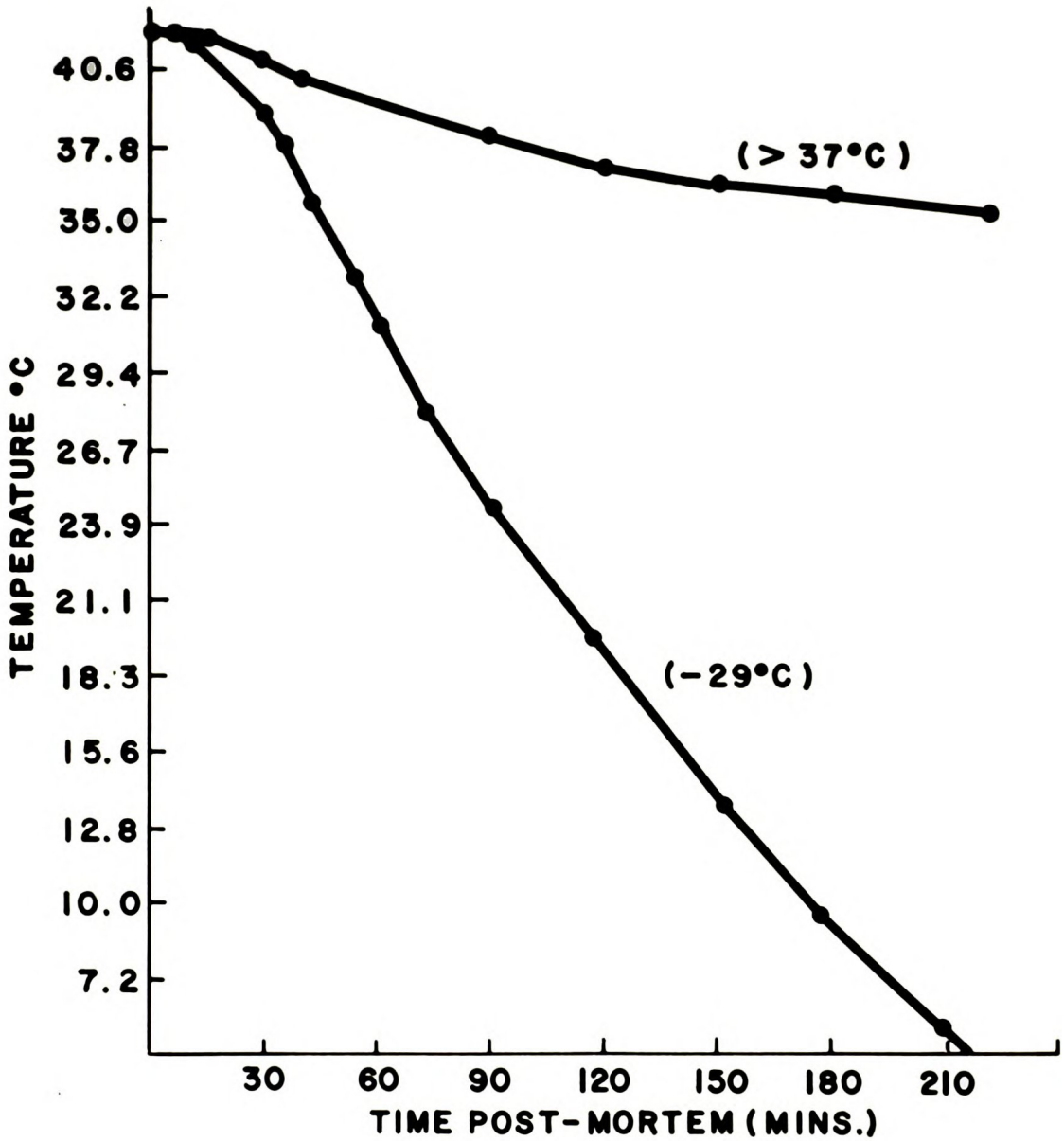


Figure 3. Post-mortem temperature of pork l. dorsi muscle of representative sides subjected to -29°C or 37°C temperatures immediately after death.

pH values for carcass 6 were expected to result in a soft, watery muscle condition observed in both sides of this carcass. Rapid cooling would not have been expected to greatly alter the muscle condition of the side placed in the -29°C treatment, since the prevailing conditions believed to have a causal relationship to the developement of the soft, watery condition were already present within 16 mins post-mortem. These conditions were the occurrence of a low pH level (less than pH 6.0) while muscle temperature was still high, i.e., above 30-35°C (Wismer-Pedersen, 1959, Briskey and Wismer-Pedersen, 1961a,b; Bendall et al., 1963).

The muscle condition of the two sides of carcass 7 were evaluated as normal, and slightly to moderately soft and watery for the -29°C and 37°C treatments, respectively. The rate of glycolysis immediately after death was not greatly different in the two sides as evidenced by the 3 hr pH levels, but differences in ultimate pH were obtained (Table 6). The high 3 hr pH value of 6.2 for the sides in the 37°C treatment was reflected in the high levels for ATP_2, and glycogen, which were much greater than the corresponding values from all other carcasses of group 1 at 3 hr post-mortem (Appendix B, Table 2). The relatively slow rate of glycolysis was expected to result in a normal muscle condition (Briskey and Wismer-Pedersen, 1961b; Bendall et al., 1963). However, the developement of the soft, watery condition in the 37°C treatment supported the observations of previous workers as to the causal role of the post-mortem pH-temperature relationship. Several workers (Briskey and Wismer-Pedersen, 1961b; Wismer-Pedersen and Briskey, 1961b; Bendall et al., 1963; Sayre et al., 1961), have suggested that rapid cooling to below 30°C before

the pH level has decreased to 5.9-6.0 or less would be expected to prevent the developement of the soft, watery condition.

Similar differences (Table 6) in ultimate muscle condition were apparent in the two sides of carcass 8. The identical pH levels in the two sides of this carcass at 3 hrs post-mortem would not necessarily preclude a faster rate of glycolysis in the 37°C side. However, the initial pH of 6.1 suggested that the pH in the side at -29°C would have declined to below 5.9-6.0 prior to any significant drop in muscle temperature.

In the two sides of carcasses 9, 10 and 11Y, marked differences were observed in 3 hr pH levels between the two treatments. The results (Table 6) on these three carcasses strongly suggested that the occurrence of a low pH at a high muscle temperature can not be equated with the routine developement of the soft and watery condition. Furthermore, these results are in disagreement with the observations of several workers (Wismer-Pedersen, 1959; Bendall and Wismer-Pedersen, 1962; Bendall et al., 1963). These investigators reported that the soft, watery condition could be induced by holding a sample of muscle removed from the carcass immediately after death at 37-41°C until it passed into rigor, and/or by merely heating a minced sample of normal muscle at 37°C for 1 1/2 hrs. A wide discrepancy would appear to exist between the observations made on small isolated samples of muscles and the results of the current study in which intact carcasses were utilized.

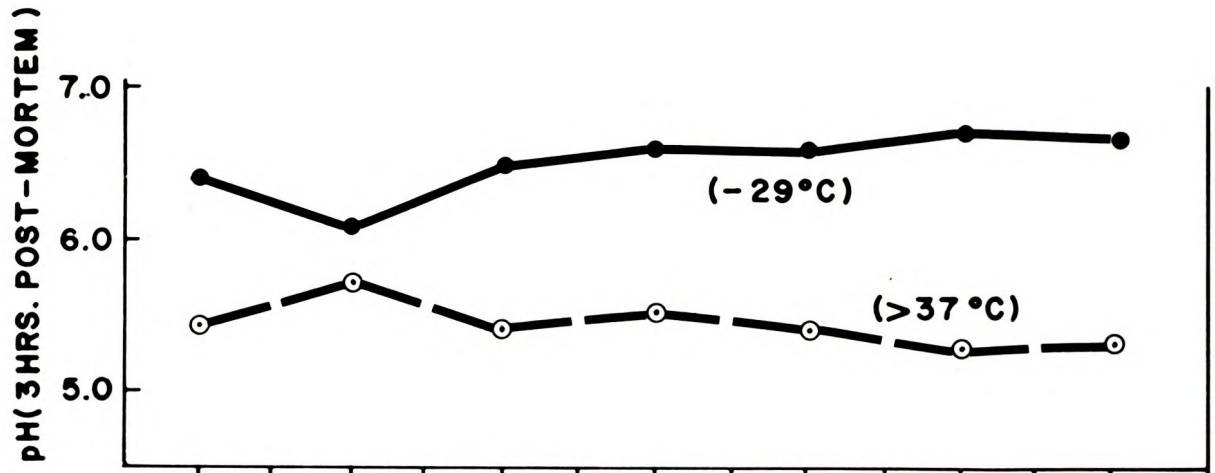
The failure to substantiate the causal effect of the low pH-high temperature relationship was further supported by the data obtained with

the group 2 carcasses (carcasses A through G). In these carcasses, the relation between 3 hr pH, temperature treatment and ultimate muscle condition at 48 hrs post-mortem is shown in Figure 4. All of these carcasses had an initial pH of 6.6 or higher. The ultimate 48 hr pH levels varied from 5.5 to 5.8 for muscles held at -29°C, while those in muscles at 37°C varied from 5.2 to 5.7 (Appendix B, Table 3). In general, the 37°C treatment greatly lowered the 3 hr pH values. However, alteration of the ultimate condition of the muscles was less apparent.

The results for carcass A followed the pattern that would be expected from the literature. The initial pH value was 6.7 (Figure 4). At 3 hrs post-mortem, the muscle at -29°C had a pH of 6.4, while the side subjected to the 37°C treatment had a pH of 5.4. The 48 hr pH values were 5.5 and 5.3 for the muscle from the low and high temperature treatments, respectively. No evidence of the soft, watery condition was found in the muscle held at -29°C while a slight to moderate degree was observed at 37°C. Similar results were obtained with carcass E (Figure 4).

In the remaining five carcasses of group 2, significant changes in ultimate muscle condition were not apparent. The data for carcass F were of particular interest. The initial pH value was 6.8. The three hr pH values were 6.7 and 5.3 for the -29°C and 37°C treatments. The eventual condition of the muscles from both treatments was assessed as being normal (Figure 4).

From the results on the two groups of carcasses, it was concluded that the occurrence of a low pH at a high temperature in pork muscle was not a causal factor in producing soft, watery muscle. However, occurrence



CARCASS:	A	B	C	D	E	F	G	TREATMENT POST-MORTEM
ULTIMATE DEGREE OF SOFT WATERY CONDITION:	NONE	NONE	NONE	NONE	SLIGHT TO NONE	NONE	NONE	-29°C
	SLIGHT TO MOD.	SLIGHT TO NONE	SLIGHT TO NONE	SLIGHT TO NONE	SLIGHT TO MOD.	NONE	SLIGHT	>37°C

Figure 4. RELATION BETWEEN pH, TEMPERATURE TREATMENT, AND ULTIMATE MUSCLE CONDITION.

of the low pH-high temperature phenomenon immediately post-mortem as a normally encountered characteristic of muscle eventually termed soft and watery is not precluded.

Properties of Fibrillar Protein

The fibrillar protein content/g of muscle and the water retained/g of fibrillarprotein from 24 hr post-mortem samples of carcasses 6 through 10 and 11Y are given in Table 7. The means and standard deviation for the grams of fibrillar protein/g of muscle were 0.0635±0.007 and 0.0970 ±0.045 grams for the -29°C and 37°C treatments, respectively. Corresponding values for grams of water retained/g of fibrillar protein were 34.77 ±11.98 and 13.25±2.91 g. The data for individual sides parallelled the observations on alterations in pH (Table 6). The -29°C treatment was ineffective in significantly altering either the fibrillar protein content or the water retention of the washed fibrils in carcass 6. The muscle in this carcass from both sides was ultimately soft and watery as previously discussed. This was expected because of the low initial pH (5.6, Table 6).

A pronounced response to the temperature treatments was observed in carcass 7, while an intermediate effect was obtained for carcass 8. The grams of water retained/g of fibrillar protein in the 37°C treatment was only 39.7 and 46.7% of that retained in the -29°C treatment for carcasses 7 and 8, respectively. A similar effect was observed in an increased content of fibrillar protein from the -29°C treatment (Table 7).

However, in carcasses 9, 10 and 11Y, the effect of the two treatments was equally as marked as that observed in carcasses 7 and 8. This

Table 7. Fibrillar protein content and water binding of washed fibrils from 24 hr post-mortem samples of carcasses 6 through 10 and 11Y.

Treatment:	Grams of fibrillar protein per gram of muscle		Grams of H_2O retained per gram of fibrillar protein		Ultimate degree of soft, watery condition[a]	
	-29°C	>37°C	-29°C	>37°C	-29°C	>37°C
Carcass 6	0.0745	0.0763	14.1	10.1	S	S to M
7	0.0579	0.1135	36.5	14.5	N	S to M
8	0.0642	0.0901	29.8	13.8	N	M
9	0.0525	0.0888	45.6	18.2	N	N
10	0.0621	0.1059	35.8	11.7	N	N
11Y	0.0698	0.1072	46.8	10.9	N	N

[a]N = none; S = slightly; M = moderately; and E = extremely.

was expecially apparent in carcass 11Y, in which the water bound/g of fibrillar protein in the muscle from the 37°C treatment was only 23.3% of that for the corresponding muscle from the -29°C treatment. In contrast to carcasses 6 and 7, both sides of carcasses 9, 10, and 11Y were assessed as being normal (Table 7).

In both carcasses 7 and 8, the 37°C treatment appeared to induce the soft, watery muscle condition. Results on these two carcasses would support the observations of Bendall and Wismer-Pedersen (1962). These workers found that the washed fibrils from muscle exhibiting the soft, watery condition had a lower water retention and that the fibrils showed a gain in protein content as compared to fibrils from normal muscle. The authors suggested that the fibrils from soft watery muscle were covered with a layer of denatured sarcoplasmic protein, which was firmly bound to the surface of the myofilaments. Bendall and Wismer-Pedersen (1962) also theorized that this was caused by the occurrence of a low pH

while muscle temperature was still high. Furthermore, they observed a similar phenomenon when muscle samples were allowed to pass into rigor at 37°C.

The effect of the 37°C treatment in increasing fibrillar protein content and in decreasing fibrillar water binding capacity in carcasses 9, 10 and 11Y is in agreement with Bendall and Wismer-Pedersen's (1962) observation that this phenomenon per se resulted from the occurrence of a low pH at a high muscle temperature. However, results from these three carcasses do not support the suggestion of Bendall and Wismer-Pedersen (1962) that the decrease in water binding capacity resulting from a low pH at a high temperature is a primary causal factor in the developement of the soft, watery condition. These results do not preclude the occurrence of decreased fibrillar water binding capacity and the concurrent gain in protein by the fibrils in soft, watery muscle.

It is suggested that factors other than the low pH-high temperature phenomenon may also have contributed to the amount of protein gained by the fibrils, and thus, the corresponding decrease in fibrillar water retention in the muscles of the pigs studied by Bendall and Wismer-Pedersen (1962). The values for grams of water retained/g of fibrillar protein by these workers were 11.22 and 6.47 grams for normal and soft, watery muscles, respectively. Such values are even lower than the values found for the muscles from the 37°C treatment in the current study (Table 7). It might be suggested that the muscles studied by Bendall and Wismer-Pedersen (1952) underwent a much sharper decline in pH immediately after death than the carcasses in the present study with a resultant accentuated

decrease in fibrillar water-binding capacity. However, observations in the current study do not support such a suggestion. For example, carcass 6 had a very high glycolytic rate immediately after slaughter which resulted in an initial pH value of 5.6. The side of this carcass which was subjected to the 37°C treatment, had a 24 hr water-binding capacity of 10.1 grams of water/g of fibrillar protein (Table 7). This value corresponds to similar values for normal muscle from the carcasses from Danish Landrace pigs studied by Bendall and Wismer-Pedersen (1962). Thus, it is suggested that muscles from Danish Landrace pigs have a generally lower fibrillar water-binding capacity than the pigs utilized in the current study. It is further suggested that another factor(s) besides the occurrence of a low pH at a high temperature and/or a sharp decline in post-mortem pH in the muscles from Danish Landrace pigs may be contributing to their greatly decreased fibrillar water binding capacity.

Creatine Phosphate

Creatine phosphate was not present in detectable amounts (>0.3 micromoles/g of tissue) in any of the initial samples of the 18 carcasses. The absence of CP may have resulted from electrical stunning, which was used prior to slaughter. Electrical stunning has been shown to accelerate the rate of post-mortem pH decline (McLoughlin, 1964a,b), which may be due in part to a rapid depletion of all CP within a few mins after death.

The absence of CP in the present study is in contrast to the results reported by Bendall *et al.*, (1963), who found average CP concentrations of 8.0 and 5.5 micromoles/g of *l. dorsi* muscle for two groups of Danish

Landrace pigs. Likewise, Lawrie (1959) reported 1 hr post-mortem levels of CP equivalent to about 7.2 micromoles/g of l. dorsi muscle in Large White X Middle White pigs. The methods of stunning were not given by Bendall et al., (1963) or Lawrie (1959). Kastenschmidt et al., (1964) reported "low creatine phosphate levels" in the l. dorsi muscle from Poland China pigs immediately after death, but did not give any values. Bendall et al., (1963) and Lawrie (1959) used an acid-molybdate procedure to determine CP, which has been shown to overestimate CP compared to other methods (Ennor and Rosenberg, 1952).

ATP

The averages and standard deviations of ATP_2 and the corresponding pH levels for various times post-mortem are given in Table 8. for the two groups of carcasses, i.e. group 1 (carcasses 6 through 10) and group 2 (carcasses A through G). The average initial pH values were 6.2 and 6.7 for groups 1 and 2, respectively. The initial ATP_2 value for both carcass groups was 5.6 micromoles/g of tissue. In the -29°C treatment more than 50 and 25% of the initial ATP_2 was present in both groups at approximately 3 and 4 1/2-5 hrs post-mortem, respectively. No ATP_2 was detected at approximately 3 hrs post-mortem in the sides held at >37°C, except in the case of carcass 7. The levels of ATP for this carcass were 8.7 and 8.9 micromoles/g of tissue for the >37°C and -29°C treatments, respectively. In support of these observations, the corresponding pH values were 6.22 and 6.51.

The average initial $ATP_{(1)}$ value (determined by acid hydrolysis) of 6 micromoles/g of tissue for the carcasses in group 1 was over 23% higher

ble 8. Post-mortem levels of ATP and pH.

rcasses:	Treatment	Average time post-mortem (mins)	pH	$ATP_{(1)}$[a] (micromoles/g)	$ATP_{(2)}$[B] (micromoles/g)
oup 1 arcasses through 10)	-	13	6.2±0.4	6.9±0.9	5.6±4.1
	-29°C	166	5.8±0.6	-	3.2±3.7
		286	5.8±0.5	-	2.6±2.4
		518	5.7±0.4	-	0.5±0.7
		745	5.4±0.3	-	0.4±0.9
	37°C	166	5.5±0.4	-	* -
		285	5.4±0.1	-	0.0
oup 2 arcasses through G)	-	11	6.7±0.1	-	**5.6±2.2
	-29°C	177	6.5±0.2	-	3.7±2.8
		323	6.4±0.1	-	1.6±2.1
		1440	5.7±0.1	-	*** -
	37°C	170	5.4±0.1	-	0.0
		317	5.4±0.1	-	0.0

P(1) determined by acid hydrolysis.
P(2) determined by enzymic assay.
Value for carcass 7 was 8.7 micromoles/g; all others = 0.0.
This value based on 6 carcasses.
1 carcass contained 0.3 micromole/g; all others = 0.0.

n the corresponding ATP_2 value (determined by enzymic assay; Table 8).
of the carcasses had $ATP_{(2)}$ levels of less than 2 micromoles/g of tissue
= 1.6 micromoles) while the average corresponding $ATP_{(1)}$ value for the
e carcasses was 6.3 micromoles/g. Hence, these $ATP_{(1)}$ values probably
lect levels of acid hydrolyzable phosphate compounds other than ATP
suggested by Bendall and Davey (1957).

However, the initial $ATP_{(1)}$ values (Table 8) are in general agreement with the values of 7.1 and 6.8 micromoles/g of 1. dorsi muscle obtained by a similar method of analysis as reported by Bendall et al., 1963) for two groups of Danish Landrace pigs. The corresponding pH values were 6.78 and 6.66 (Bendall et al., 1963).

An ATP value equivalent to less than 3 micromoles/g of Poland China dorsi muscle with a pH level of about 6.5 at "0 hrs" was calculated rom the data of Kastenschmidt et al., (1964). This is in good agreement th a similar value of 3.7 micromoles/g of Hampshire 1. dorsi muscle th a similar pH level in the current study (group 2 carcasses, Table 8), t is at variance with the results obtained for the Poland China carsses (group 1, Table 8).

In disagreement with results of the current study (Table 7), Lawrie 960) reported labile phosphate levels equivalent to about 6.9 micro-les/g of 1. dorsi muscle from Large White x Middle White pigs at 110 ns post-mortem. The corresponding pH level was 6.5. Lawrie (1960) commented on the high level of ATP found in these pigs.

cogen, Glucose and Lactic Acid

The means and standard deviations of pH, glycogen, glucose, and lac- acid at various times post-mortem for the sides of the two groups of casses are given in Table 9. The initial levels of glycogen were 28.3 75.8 micromoles/g of tissue in groups 1 and 2, respectively. Wismer-ersen (1959) reported a comparable value equivalent to 31.1 micromoles/g 1. dorsi muscle from Danish Landrace pigs at 45 mins post-mortem. The

mortem as related to temperature treatments for carcasses of groups 1 and 2.

Carcass group	Average time post-mortem (mins)	Treatment:	pH Initial	pH -29°C	pH >37°C	Glycogen (micromoles/g) Initial	Glycogen (micromoles/g) -29°C	Glycogen (micromoles/g) >37°C
1 (carcasses 6 through 10)	13		6.2±0.4			28.3±12.5		
	166			5.8±0.6	5.5±0.6		20.0±19.6	14.0±15.8
	286			5.8±0.5	5.4±0.1		15.7±16.2	2.4±1.7
	1440			5.5±0.2	5.4±0.1		6.3±8.7	0.5±0.2
	2880			5.4±0.1	5.4±0.1		2.8±3.5	0.8±0.2
2 (carcasses A through G)	11		6.7±0.1			75.8±36.6		
	174			6.5±0.2	5.4±0.1		73.8±41.1	27.6±2.48
	320			6.4±0.1	5.4±0.1		57.7±39.6	20.6±19.2
	1440			5.7±0.1	5.4±0.1		40.5±32.2	11.1±13.4

Mean pH values relative to temperature treatments for carcasses of groups 1 and 2. (continued)

Carcass group	Average time post-mortem (mins)	Treatment:	Glucose (micromoles/g) Initial	-29°C	>37°C	Lactic acid (micromoles/g) Initial	-29°C	>37°C
1 (carcasses 6 through 10)	13		12.6±3.7			54.8±21.4		
	166			15.2±7.6	17.1±5.9		67.6±28.0	83.6±18.3
	286			13.5±5.2	20.9±2.8		79.8±19.1	88.4±1.4
	1440			20.4±6.2	24.4±6.7		82.6±13.8	86.0±7.9
	2880			20.2±4.2	25.3±4.3		88.7±9.7	86.0±5.4
2 (carcasses A through G)	11		11.6±1.5			31.4±9.7		
	174			10.7±4.9	19.4±5.1		39.6±16.1	80.4±5.5
	320			16.1±3.5	27.0±9.2		46.4±5.6	80.3±6.8
	1440			15.0±3.5	35.4±15.9		75.9±6.6	80.8±4.7

rresponding pH level was 6.24. This is in good agreement with the values r group 1 carcasses (pH 6.2) in the current study, but considerably wer than comparable values in group 2 (Table 9). Also, in disagreement th the results of the current study, Lawrie (1959) reported a value uivalent to about 34.9 micromoles/g of 1. dorsi muscle from Large White Middle White pigs. The corresponding pH value was 6.7 at 1 hr post-mortem. wever, the average initial glycogen level for the group 1 carcasses ble 9) are in good agreement with an equivalent level of 33.9 micromoles of 1. dorsi muscle from Danish Landrace pigs with a corresponding pH 6.2 at 15 mins post-mortem (Briskey and Wismer-Pedersen, 1961b). These rkers classified this type of muscle as having a "sharp" post-mortem pH line.

The much lower initial levels of glycogen in the Group 1 carcasses land China pigs) than in the group 2 carcasses (Hampshire pigs) is in eement with the results of Sayre et al., (1963c). These workers found imes more glycogen in the 1. dorsi muscle of Hampshire pigs than in and China pigs.

The initial glucose levels (total reducing sugars) were 12.6 and 11.6 romoles/g of tissue in groups 1 and 2, respectively. These values are siderably lower than the equivalent values of 28.3 and 15.5 reported Wismer-Pedersen (1959) for 1. dorsi muscle from Danish Landrace carcasses, with pH levels of 6.24 and 6.76, respectively, at 3/4 hr post-em.

However, the initial glucose values (Table 9) are higher than the l of 7.4 micromoles/g of 1. dorsi muscle immediately after death called from the data of Sharp (1962).

The 24 and 48 hr glucose values varied from 24.4 to 35.4 micromoles/g muscle (Table 7). In general agreement with these values, Sharp (1962) ported glucose levels equivalent to 32.2 micromoles/g of 1. dorsi muscle ter being held at 18°C for 26 hrs and a further 144 hrs at 1°C.

The initial lactic acid levels were 54.8 and 31.4 micromoles/g of ssue for groups 1 and 2, respectively, with corresponding pH levels of 2 and 6.7. These results are in general agreement with those of Wismer-dersen (1959), who reported values equivalent to 67.8 and 37.8 micromoles of 1. dorsi muscle from Danish Landrace pigs when the respective pH vels were 6.2 and 6.7 at 45 mins post-mortem.

However, the initial lactic acid values (Table 9) were lower than values of 68.9, 68.9, and 97.8 micromoles/g of Danish Landrace 1. dorsi scle as calculated from the data of Briskey and Wismer-Pedersen (1961b). corresponding pH levels were 6.4, 6.6 and 6.2 at 15 mins post-mortem.

The 48 hr lactic acid values in the current study varied from 75.9 88.7 micromoles/g of 1. dorsi muscle. These levels are much lower than values of 124.4 to 128.9 micromoles/g of Danish Landrace 1. dorsi mus- at 24 hrs after death calculated from the data of Briskey and Wismer- ersen (1961b).

ect of Temperature on Glycogen, Glucose and Lactic Acid Levels.

The effect of the two temperature treatments on the average levels ;lucose, glycogen and lactic acid for the group 2 carcasses are shown 'igure 5. As expected, in the carcasses held at 37°C, the glycogen 1 decreased rapidly. At 3 hrs post-mortem, the accumulation of glu- was evident, while the lactic acid concentration had reached a

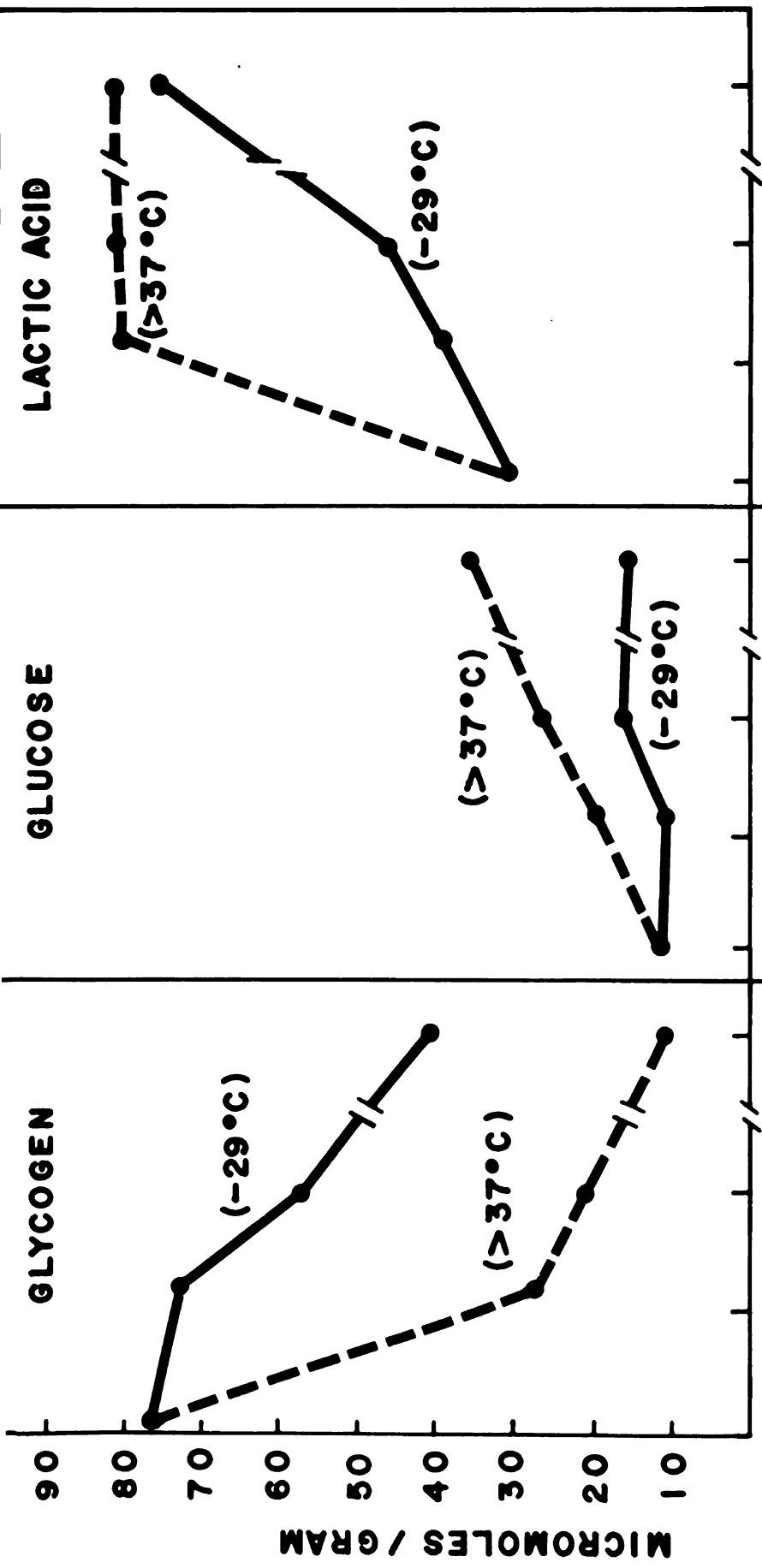


Figure 5. Effect of temperature treatment on levels of glycogen, glucose and lactic acid in carcasses A through G (Group 2).

nstant level. In the muscles from the -29°C treatment, over 90% of e initial glycogen was present at 3 hrs post-mortem and over 70% was esent at 5 hrs. For the same group, appreciable glucose accumulation d not occur until after 3 hrs. Lactic acid had attained a level of proximately 50% of that in the 37°C treatment at 5 hrs post-mortem.

gradation of Glycogen

The means and standard deviations of the sums of glycogen, glucose otal reducing sugars) and lactic acid expressed as micromoles of glu-se equivalents, and the estimates of the errors in measurement for each nstituent are given in Table 10.

ble 10. Means and standard deviations of sums of glycogen, glucose and lactic acid and estimates of measuring error for each constituent.

casses:	Group 1	Group 2
COGEN + GLUCOSE + LACTIC ACID[a]:		
Initial	68.3±7.3	103.2±33.9
-29°C treatment	67.5±2.9	98.3±35.8
37°C treatment	68.5±4.2	87.3±27.3
IMATED ERROR OF MEASUREMENT:		
Glycogen	±1.9	±4.6
Glucose	±1.4	±2.0
Lactic acid	±4.8	±4.3

ues expressed in micromoles of glucose equivalents/g of tissue.
ns are based on sums calculated for each individual sample in all es within treatments. The values for glucose used included total ucing sugars.

The values for the muscles of the carcasses in group 1 are very nilar to those obtained for beef muscle. The overall mean for beef s approximately 69 micromoles/g of muscle (Figure 2, p. 67) for these ne chemical constituents. Hence, on the basis of the data for the oup 1 carcasses (Table 10), there is nothing to suggest that glycogen s not broken down to glucose and lactic acid in a stoichiometric manner nilar to that observed in beef muscle.

However, the values from the group 2 carcasses do not completely pport the above conclusion. In these carcasses, there was a general ndency for the sums of the three components to decrease from the initial ue, to the -29°C value, and to the 37°C value, in that order (Table . This may suggest that some undetected glycolytic intermediate(s) /were accumulating, and that the level(s) was/were influenced by the perature treatments used. Alternatively, the trend for decreasing ues for the post-mortem carbohydrate pool from the group 2 carcasses merely reflect the higher errors of measurements in contrast to those group 1 (Table 10). The higher errors of measurement were probably to the high dilutions that were necessary in the glycogen determina- n for the samples from these carcasses.

TOCHEMICAL OBSERVATIONS IN BEEF MUSCLE

The relative activity of the 17 enzymes studied at 0 and 480 hrs are ılated in Table 11. The differences in intensity of enzyme activity :rved between small red, large white and intermediate fibers are in ement with established patterns of distribution (Paarse, 1960).

able 11. Relative enzyme activity in L. dorsi muscle from beef.

Enzyme	Activity[a] 0 hrs	480 hrs
LYCOLYSIS		
Lactate dehydrogenase	+ + to + + + +	+ +
α-glycerophosphate dehydrogenase	+ + to + + +	+ to + + +
Alcohol dehydrogenase	+ to + + +	+ + to +
CA CYCLE		
Isocitrate dehydrogenase	+ to + + + +	+ to + + + +
Succinate dehydrogenase	+ + to + + +	+ to + +
Glutamate dehydrogenase	+ to + +	- to ±
LECTRON TRANSPORT		
DPN diaphorase	> + + +	+ + +
TPN diaphorase	> + + +	+ to + + +
EXOSE MONOPHOSPHATE CYCLE		
Glucose-6-phosphate dehydrogenase	- to +	-
6-phosphogluconate dehydrogenase	-	-
ATTY ACID OXIDATION		
-hydroxybutyrate dehydrogenase	± to + +	-
THERS		
Acid phosphatase	-	-
Alkaline phosphatase	+ + +	+ + +
ATPase (Ca++)	+ + + +	+ + + +
ATPase (Mg++)	+ + + +	+ + + +
TPPase	+ +	+ +
Leucine amino peptidase	-	-

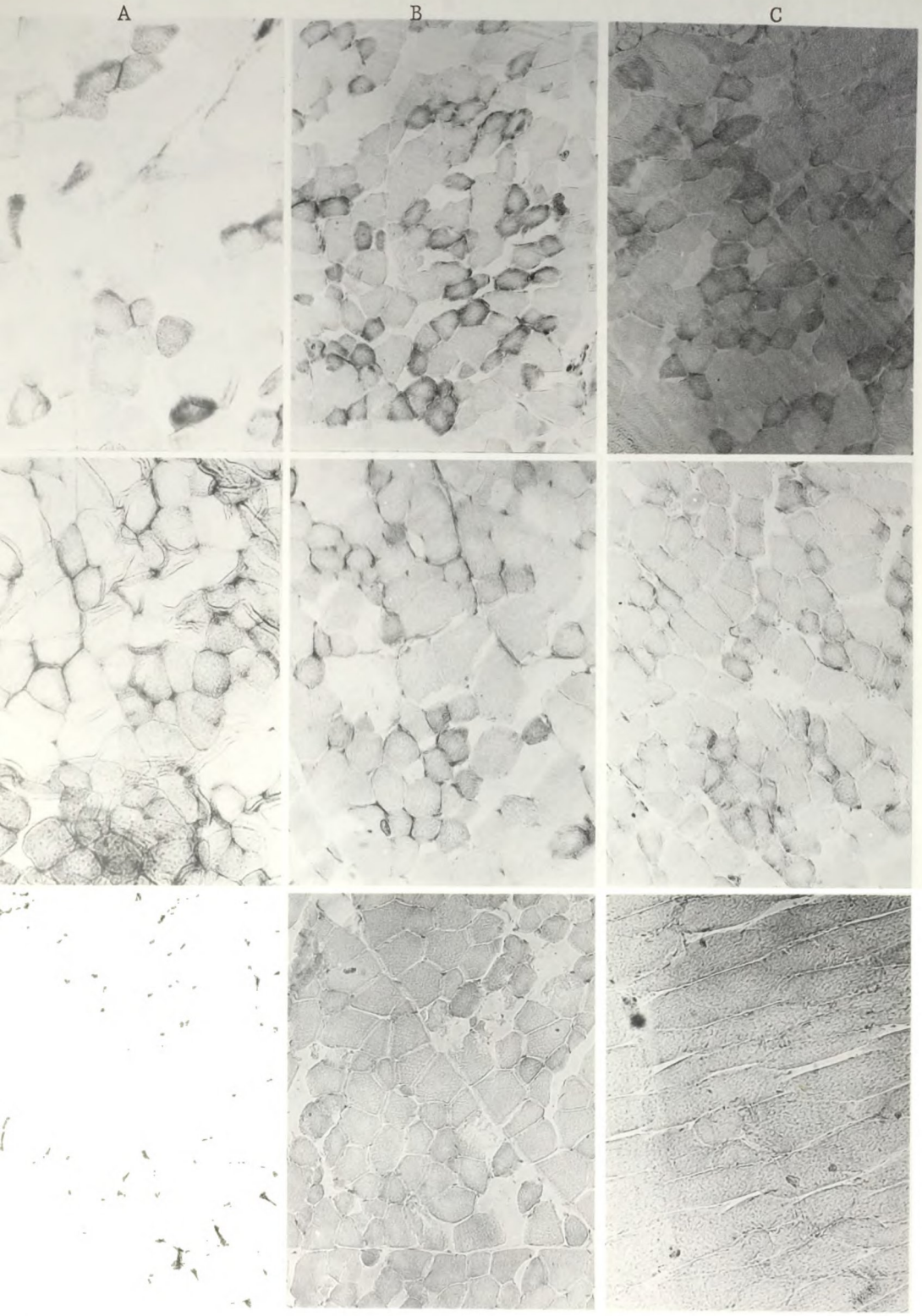
The following code was used for expressing the amount of activity:
- none, ± traces, + weak, ++ moderate, +++ moderately strong, ++++ strong.

lycolytic Enzymes

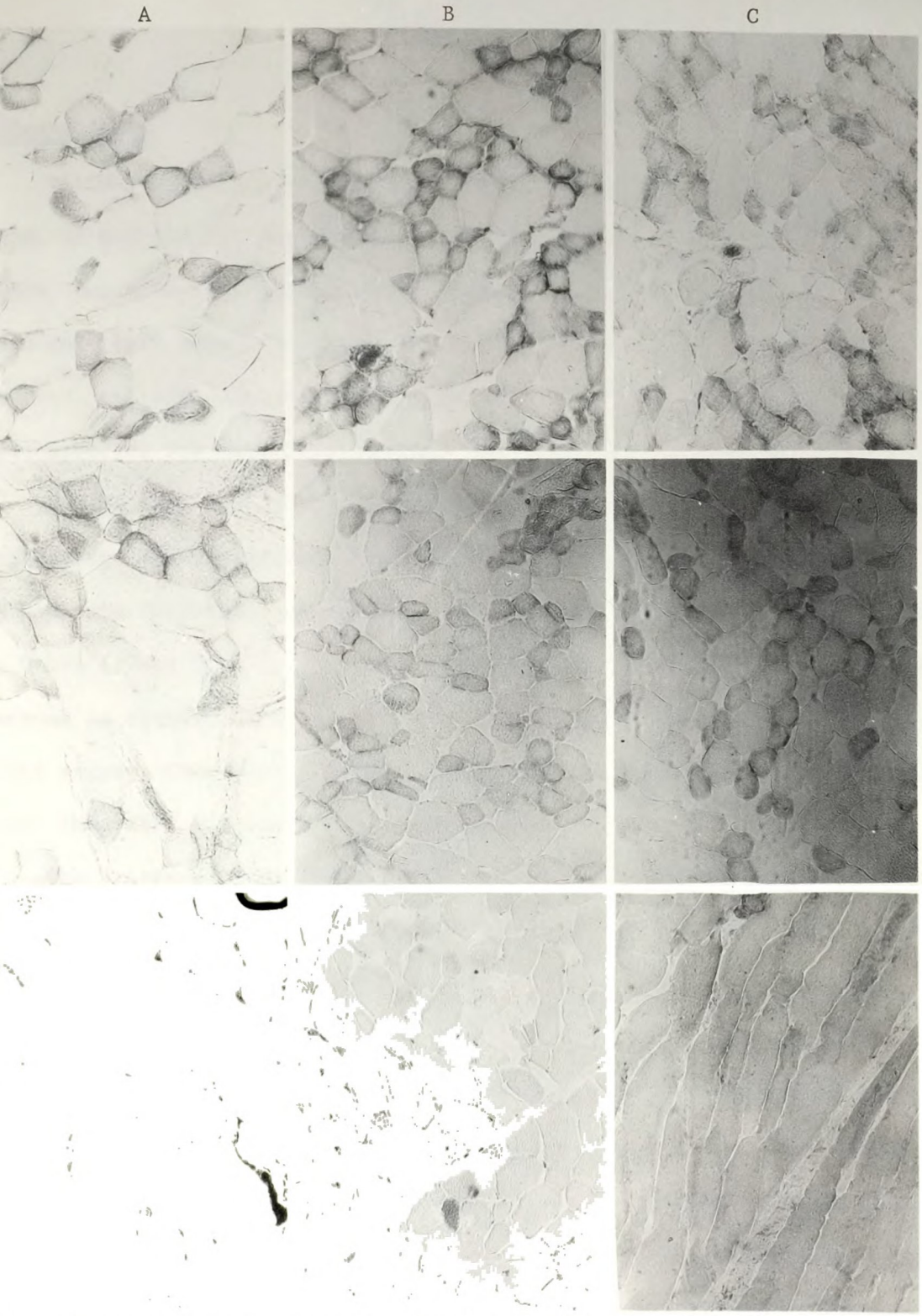
Photomicrographs of representative reactions at 0, 48 and 480 hrs ost-mortem for lactate, dehydrogenase, alpha-glycerophosphate dehydrogen-se and alcohol dehydrogenase are shown in Plate 1. Of these three gly-olytic enzymes, lactate dehydrogenase and alpha-glucogen phosphate ehydrogenase were more uniformly reactive at all times post-mortem than lcohol dehydrogenase. Alcohol dehydrogenase activity was highly variable rom carcass to carcass, especially with increasing periods of time post-rtem. Furthermore, beyond 24 hrs post-mortem, reactions for alcohol hydrogenase were usually either very weak or completely absent (Plate III B and C).

zymes of the TCA Cycle

Typical isocitrate dehydrogenase, succinate dehydrogenase, and glu-nate dehydrogenase reactions observed in 0, 48 and 480 hr post-mortem mples are shown in Plate 2. Under post-mortem conditions, isocitrate ıydrogenase appeared to be the most active enzyme of this group as well the most stable (Table 11). Succinate dehydrogenase activity was de-:ted at all times post-mortem but was highly variable in intensity. lower level of glutamate dehydrogenase activity detected at 0 hrs as pared to that observed for succinate dehydrogenase is in agreement h the observations on beef muscle of Ogata and Mori (1964). Reactions glutamate dehydrogenase were very weak or absent at 48 hrs post-mortem all subsequent sampling times (Plate 2, III B and C).



1. L. dorsi muscle from beef. Lactate (I), alpha-glycerophosphate (II), and alcohol (III) dehydrogenase activity at 0 (A), 48 (B) and 480 (C) hrs post-mortem (x 225).



2. L. dorsi muscle of beef. Isocitrate (I), succinate (II), and glutamate (III) dehydrogenase activity at 0 (A), 48 (B) and 480 (C) hrs post-mortem (x 225).

Other Enzymes

Plate 3 shows representative reactions for TPN and DPN diaphorase at 0, 48 and 480 hrs post-mortem, beta-hydroxybutyrate dehydrogenase at 0 hrs, and glucose-6-phosphate dehydrogenase at 0 hrs. The two diaphorases were both generally quite active throughout 480 hrs post-mortem. However, the DPN diaphorase reactions were uniformly stronger than the corresponding TPN reactions (Table 11).

Muscle from two of the five carcasses studied showed a weak glucose-6-phosphate dehydrogenase activity at 0 hrs (Plate 3, III A). Very weak to moderate reactions were observed for beta-hydroxybutyrate dehydrogenase at 0 hrs (Plate 3, III, B). However, no activity was detected for these enzymes in either the 24 hr post-mortem or subsequent samples. This would suggest that these enzymes are more labile under post-mortem conditions than the majority of the enzymes in the current study (Table 11).

The presence of detectable glucose-6-phosphate dehydrogenase activity was unexpected in view of the work of Ogata and Mori (1964). These investigators did not detect any activity in normal mouse, cat or human muscle. However, the activity detected for this hexase monophosphate shunt enzyme in the current study was confirmed by the Nitro BT procedure previously described. It is of interest that no activity was observed for another hexase monophosphate cycle enzyme, 6-phosphogluconate dehydrogenase (Table 11). The latter may be less stable immediately after death than glucose-6-phosphate dehydrogenase. The presence of beta-hydroxybutyrate dehydrogenase activity supports the suggestion of Howard and Lawrie (1956) that fatty acid oxidation may play an important role in the metabolism of beef muscle.

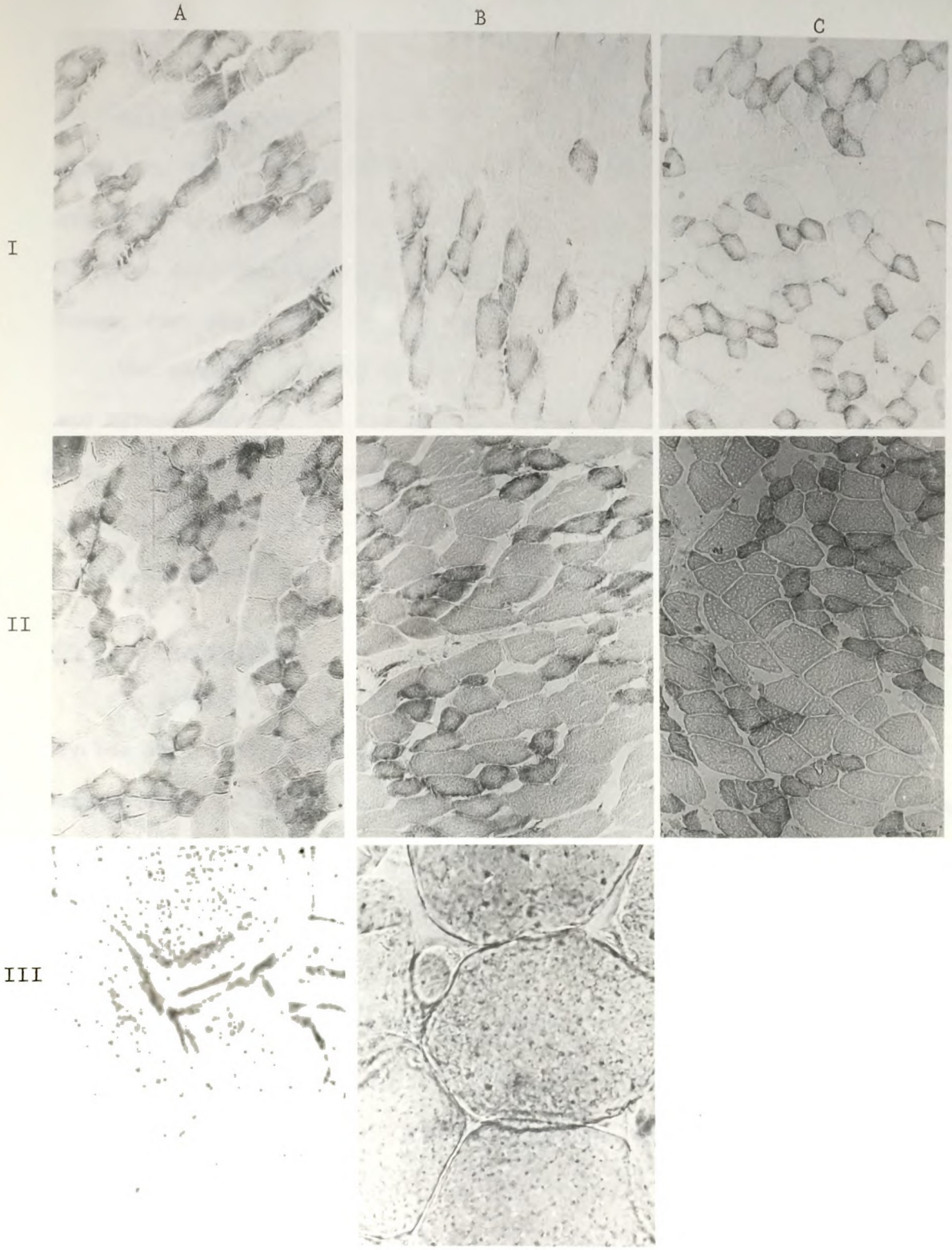


Plate 3. L. dorsi muscle from beef. TPN diaphorase (I) and DPN diaphorase (II) activity at 0 (A), 48 (B) and 480 (C) hrs post-mortem; glucose-6-phosphate (III, A) and beta-hydroxybutyrate (III, B) denydrogenase activity at 0 hrs post-mortem. Magnification: 225X.

Acid phosphatase and leucine amino peptidase activity was not detected in any of the muscles from the five carcasses (Table 11). These results are in agreement with the results of Ogata and Mori (1964) who found no acid phosphatase or leucine amino peptidase activity in normal human, cow, pig, cat or mouse muscle.

The methods used to detect alkaline phosphatase, the two ATPases and TPPase resulted in moderate to strong reactions for all samples studied. Activities were similar throughout the post-mortem period (Table 11). The Gomori (1952) method used for alkaline phosphatase resulted in a very diffuse general reaction and consequently had no significance.

General Observations

In general, the activity of the enzymes of the glycolytic and TCA cycles and of the electron transport system, which were observed in the current study exhibited a steady decrease in activity with increasing time post-mortem. Results would support the observation of Andrews et al., (1952) that the lack of substrates is the limiting factor in post-mortem metabolism in beef muscle.

HISTOCHEMICAL OBSERVATIONS IN PORK MUSCLE

The relative activity at 0 and 24 hrs post-mortem of 15 of the enzymes studied in carcasses 6 through 10 and 11Y are shown in Table 12. In general, the variation observed in activity at 24 hrs post-mortem did not appear to be related to the temperature treatment or to the pH level of a given muscle at the time of removal of a given sample.

Table 12. Relative enzyme activity in l. dorsi muscle of carcasses 1 through 10 and 11Y.

Enzyme	Activity[a] 0 hrs	24 hrs
GLYCOLYSIS		
Lactate dehydrogenase	+ to + + +	± to + +
α-glycerophosphate dehydrogenase	± to + + +	± to + + +
Alcohol dehydrogenase	- to + +	- to ±
TCA CYCLE		
Succinate dehydrogenase	+ to + + +	± to + +
Isocitrate dehydrogenase	± to + + +	- to + +
Glutamate dehydrogenase	± to + + +	- to + +
Malate dehydrogenase	- to ±	-
ELECTRON TRANSPORT		
DPN diaphorase	+ to + + +	+ to + +
TPN diaphorase	-	-
HEXOSE MONOPHOSPHATE CYCLE		
Glucose-6-phosphate dehydrogenase	- to ±	-
6-phosphogluconate dehydrogenase	± to +	-
FATTY ACID OXIDATION		
-hydroxybutyrate dehydrogenase	- to +	-
OTHERS		
Cytochrome oxidase	± to + +	± to + +
Acid phosphatase[b]	positive	positive
Alkaline phosphatase[b]	positive	positive

[a]The following code was used for expressing the amount of activity: - no activity, ± very weak, + weak, ++ moderate, +++ moderately strong, ++++ strong.

[b]Observed on carcasses 1 through 7 only.

TCA Cycle Enzymes

Succinate dehydrogenase, isocitrate dehydrogenase and glutamate dehydrogenase exhibited similar decreases in activity between 0 and 24 hrs post-mortem (Table 12). Photomicrographs of typical reactions obtained for isocitrate dehydrogenase and succinate dehydrogenase at 0 hrs and 24 hrs post-mortem from carcasses subjected to the -29°C and 37°C treatments are shown in Plate 4. However, malate dehydrogenase was observed to be either weakly active or completely inactive at 0 hrs post-mortem. In all cases no activity for this enzyme was detected at 24 hrs post-mortem.

Glycolytic Enzymes

Lactate dehydrogenase and alpha-glycerophosphate dehydrogenase activity were generally similar at both 0 and 24 hrs post-mortem (Table 12). However, individual muscles showed higher 24 hr levels of alpha-glycerophosphate dehydrogenase activity than that found for lactate dehydrogenase in any of the muscles studied. Photomicrographs representative of the reactions observed for these two enzymes are shown in Plate 5. Alcohol dehydrogenase reactions were weaker than the reactions observed for either of the other two glycolytic enzymes studied (Table 12).

Electron Transport Enzymes

Weak to moderately strong reactions were obtained when observing DPN diaphorase activity (Table 12). Typical DPN reactions are shown in Plate 4. However, TPN diaphorase activity was not detected in any of the muscles studied.

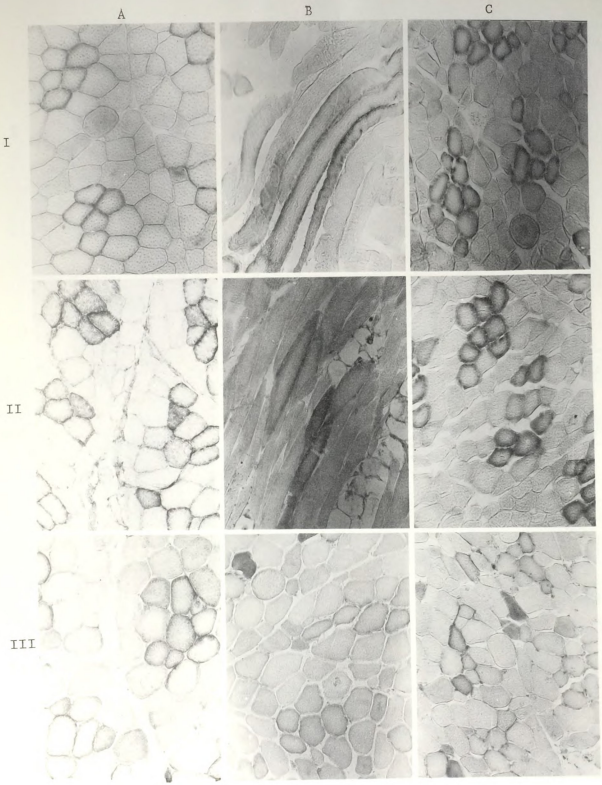


Plate 4. L. dorsi muscle from pork. Isocitrate dehydrogenase (I), succinate dehydrogenase (II) and DPN diaphorase (III) activity at 0 (A) and 24 hrs post-mortem from carcasses subjected to the -29°C (B) and 37°C (C) treatments (x 225).

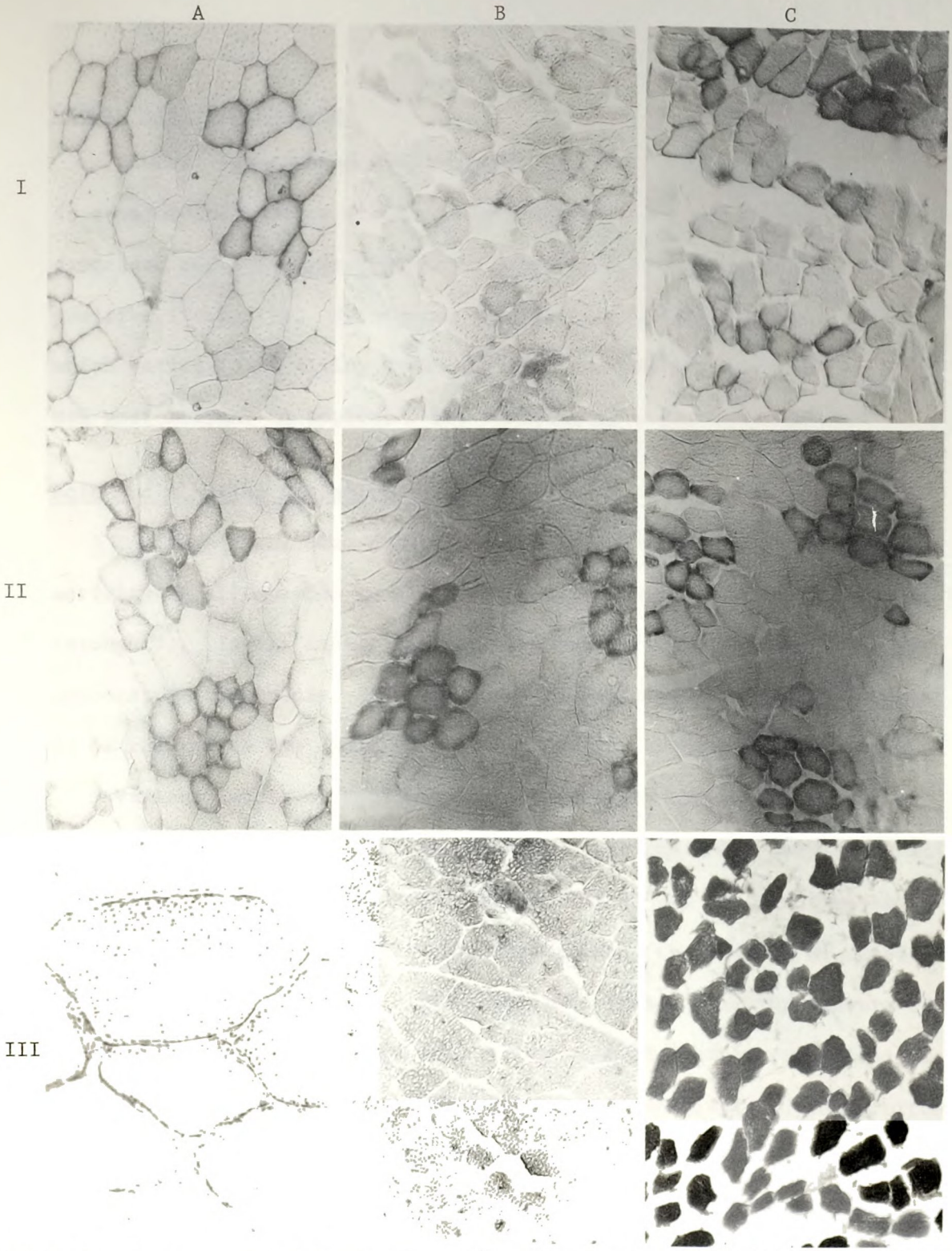


Plate 5. L. dorsi muscle of pork. Alpha-glycerophosphate (I) and lactate (II) dehydrogenase activity at 0 (A) and 24 hrs post-mortem in -29°C (B) and 37°C (C) treatments (x 225); cytochrome oxidase activity (III, A) at 0 hrs (A) post-mortem (x 860); and Aniline blue-orange C stained sections from 24 hrs post-mortem muscle in -29°C (III B) and 37°C (III C) treatments (x 225).

Other Metabolic Enzymes

Glucose-6-phosphate dehydrogenase activity was detected in traces in some of the muscles studied. Reactions for 6-phosphogluconate dehydrogenase and beta-hydroxybutyrate dehydrogenase were slightly more positive (Table 12). Cytochrome oxidase activity was detected at levels which varied in intensity from very weak to moderate at both 0 and 24 hrs post-mortem. A typical 0 hrs reaction is shown in Plate 5.

Acid and Alkaline Phosphatases

Positive areas were observed for acid and alkaline phosphatase activity in all muscles in which these enzymes were studied (carcasses 1 through 7). In all cases, the activity was not effected by any of the temperature treatments. The incidence of positive areas did not appear to be related to the appearance of the muscles at 48 hrs post-mortem.

Various levels of acid phosphatase activity are shown in Plate 6 (I A, B, and C); II A). It was concluded that the fiber showing an intense level of acid phosphatase activity (II A) was in an active state of degeneration. This conclusion was supported by the results of other workers. Fennell and West (1963) concluded that a striking feature of dystrophic muscle in mice was the strong acid phosphatase reactions of atrophic fibers and the interfibrillar connective tissues. They further suggested that acid phosphatase may function in catabolic processes. This suggestion was supported by the results of Klamer and Fennell (1962), who observed increased acid phosphatase activity in specimens of Tetrahymena during the declining phase of growth. Furthermore, Beckett and Bourne (1960) observed that groups of inflammatory cells invading degenerating fibers often showed a moderate to strong histochemical reaction for acid phosphatase.

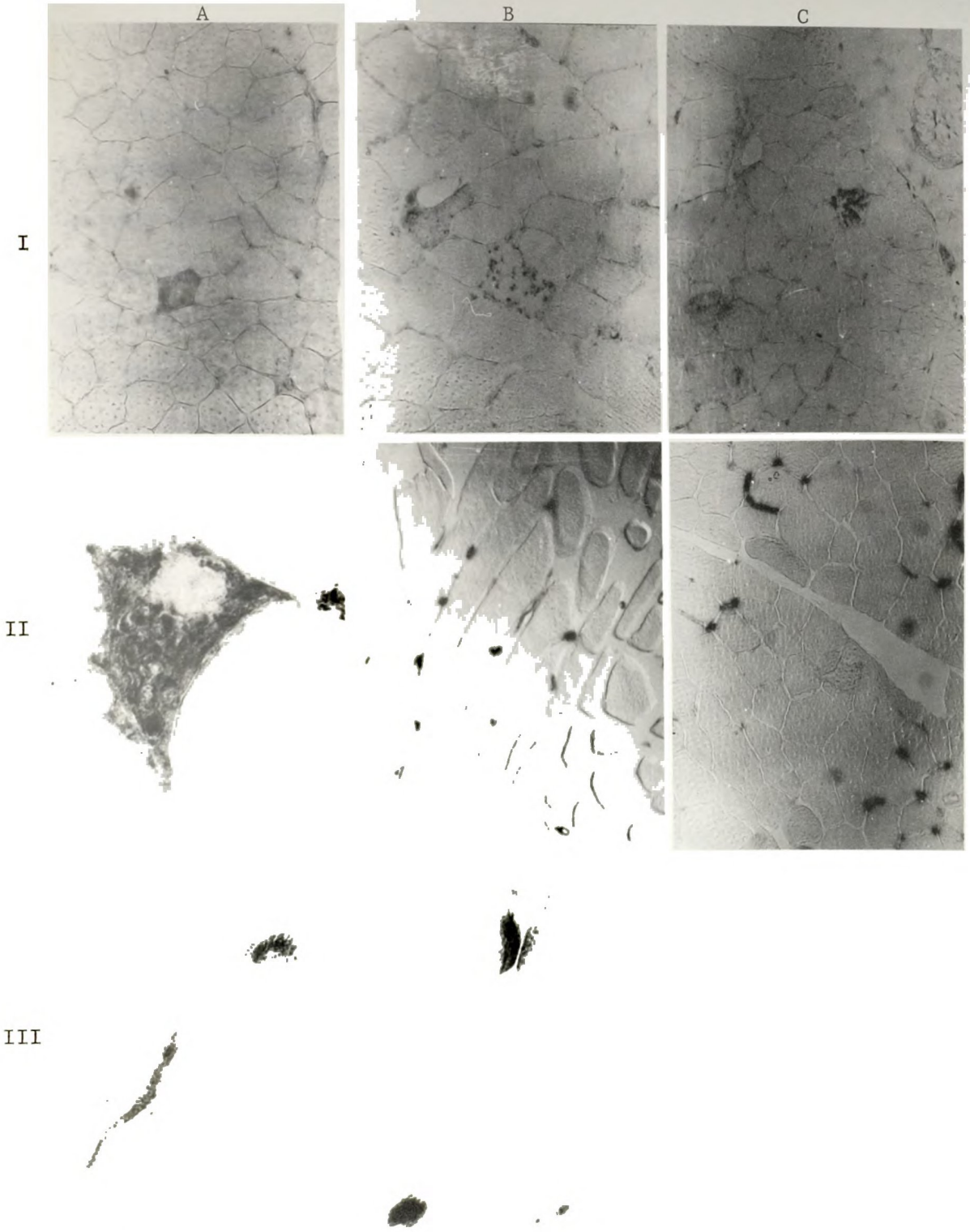


Plate 6. L. dorsi muscle of pork: varied levels of acid phosphatase activity in isolated fibers (I, A, B, and C; x 225); strong acid phosphatase activity in degenerating fiber (II, A; x 860); alkaline phosphatase activity (II, B, C; x 225); alkaline phosphatase activity in "fragmented" fiber (III A; x 860); and alkaline phosphatase activity showing developement of cross-linkages between fibers at points of high activity (III, B; x 860).

The distribution of alkaline phsophatase activity is shown in Plate 6 (photomicrographs II B, C; III A, B). The general distribution of activity is shown in the two photomicrographs at the lower magnification (II, B, C). The photomicrographs III A and B are higher magnifications of the section shown in II B (Plate 6). In photomicrograph III A, alkaline phosphatase activity is shown in a fragmented or possibly degenerating fiber. The positive area in the upper part of photomicrograph III B shows alkaline phosphatase in adjacent areas of two separate fibers. In the lower part of the same photomicrograph, a similar area is observed, but in this case the fibers appear to be joined together at the point of phosphatase activity.

It is suggested that the alkaline phosphatase activity observed is indicative of an abnormal condition in these muscles. Such a conclusion is supported by the observations of Beckett and Bourne (1960) who have noted that alkaline phosphatase activity is limited to the walls of the capillaries and the endothelial lining of the larger blood vessels in normal muscle. These workers have observed positive alkaline phosphatase activity in fibers from human muscle taken from patients with various muscular or neuromuscular disorders. However, the occurrence of the alkaline phosphatase positive fibers observed by Beckett and Bourne (1960) was not correlated with the general histological state of the muscle or with the diagnosis.

UDPG-Glycogen Transferase, Phosphorylase, and Branching Enzyme Activity

Histochemical procedures for observing phosphorylase and BE (branching enzyme or amylo-1, 4-->1,6-transglucosidase) activity were carried

out on some of the samples of carcasses 6 through 10. Some differences appeared to be present, but these differences were highly variable. Furthermore, the tissue blocks had been stored at -29°C for 2 1/2-3 wks. Consequently, the generally weak reactions as well as excessive ice crystal growth precluded any conclusions concerning the differences observed.

Phosphorylase, BE, and UDPG-glycogen transferase activity was observed in the initial sample, at approximately 3 hrs, and at 24 hrs post-mortem for carcasses A through G. Results of these observations are given in Table 13.

In the muscles from the -29°C treatment, considerable variation existed in amount of activity observed between different carcasses (Table 13). However, any relationship between activity, pH and ultimate muscle condition was not apparent. An exception to this observation was the low UDPG-glycogen transferase activity observed in carcass 6. This carcass had a pH value of 6.1 at 3 hrs post-mortem, which may suggest that the low pH level may have inactivated this enzyme.

There was no detectable UDPG-glycogen transferase activity present at 3 hrs post-mortem in any of the muscles of the sides subjected to the 37°C treatment. However, it is impossible to distinguish whether complete inactivation resulted from low pH values alone or from the combination of a low pH value and high muscle temperatures.

The variable effect of the 37°C treatment on phosphorylase and phosphorylase + BE activity (Table 13) could not be explained on the basis of muscle pH or ultimate 48 hr muscle condition.

Table 13. Levels of UDPG-glycogen transferase, phosphorylase and branching enzyme in carcasses A through G at 3 hrs post-mortem[a]

Treatment	Carcass	pH	UDPG-glycogen transferase	Phosphorylase + BE[b]	Phosphorylase[c]	Phosphorylase[d]
-29°C	A	6.4	+ to + +	+ + to + + +	+ + + to + + + +	+ + + +
	B	6.1	± to +	+ +	+ +	+ to + +
	C	6.5	+ + + +	+ + +	+ + + +	+ + + + +
	D	6.6	+ +	+ + to + + +	+ + + to + + + +	+ + +
	E	6.6	+ to + +	+ +	+ + to + + +	+ + + +
	F	6.7	+ + + + to + + + + +	+ +	+ + +	+ + +
	G	6.7	+ + + to + + + +	+ + +	+ + + to + + + +	+ + to + + + +
37°C	A	5.4	-	+	+	±
	B	5.7	-	±	-	-
	C	5.4	-	+ + +	+ + + +	+ + to + + + +
	D	5.5	-	+ + to + + +	+ + +	+ + +
	E	5.4	-	±	±	±
	F	5.3	-	+ +	+ +	+ +
	G	5.4	-	+ +	+ + +	+ + to + + + +

[a]The following code was used for expressing the amount of activity: - none, ± very weak, + weak, ++ moderate, III moderately strong, ++++ strong, and +++++ very strong.
[b]Branching enzyme.
[c]Reaction mixture contained 20% ethanol which strongly inhibits branching enzyme activity.
[d]Reaction mixture contained 10^{-4} M level of $HgCl_2$ which completely inhibits BE.

The reactions observed in the initial samples were generally similar to those found for the -29°C treatment at 3 hrs post-mortem. The reactions observed for all of the muscles at 24 hrs post-mortem generally reflected the activity at 3 hrs post-mortem. However, all reactions at 24 hrs were considerably weaker than those observed in the -29°C treatment at 3 hr post-mortem. Photomicrographs showing an extreme effect of the heat treatment on the histological condition of carcass A have been included in Plate 5 (III B, C).

General Observations

Generally, weaker activities were observed for pork muscle in the initial and the 24 hrs post-mortem samples than was observed at corresponding times for beef muscle. This observation may merely reflect a much faster pace of post-mortem chemical changes in pork muscle. The apparent absence of detectable malic dehydrogenase or TPN diaphorase activity in pork muscle may suggest that these two enzymes are quite labile under the conditions extant immediately after death in pork muscle. However, it is suggested that this observation requires further substantiation.

Although the degree of acid and alkaline phosphatase activity did not appear to be related to the ultimate muscle condition, the detection of such activity, together with the implications previously discussed, would seem to warrant further elucidation.

Studies on Beef

Chemical changes in *l. dorsi* muscle of five beef carcasses were followed from less than 10 minutes after death through 20 days post-mortem. In addition, the activity of 17 specific enzymes was followed by histochemical procedures.

Initial levels of pH, creatine phosphate and glycogen were in agreement with values reported by previous workers. Initial ATP levels averaged 10.9 micromoles/g of tissue when determined enzymically, but were considerably lower (6.4 micromoles/g) when measured by an acid hydrolyses procedure. It is believed that the latter underestimates the true ATP value found in beef muscle immediately post-mortem.

Results suggest that the onset of rigor was initiated at about 12-15 hrs post-mortem. A fairly linear breakdown of glycogen occurred during the first 24 hrs post-mortem, after which breakdown occurred at a much slower rate. When the minimum pH of 5.4 was reached at about 96 hrs after death, approximately 20% of the total initial glycogen remained. However, at 480 hrs post-mortem, less than 3% of the glycogen remained.

There was an increase in reducing sugars during the first 24 hrs post-mortem, and thereafter, little change. Lactic acid increased rapidly during the first 36-48 hrs post-mortem, with only slight further increases up to 480 hrs. It was concluded that glycogen was stoichiometrically degraded to lactic acid and reducing sugars. Of the two products, lactic acid predominated and increased some 6 1/2 times from within 10 mins after

death to 480 hrs post-mortem. During the same period of time, glucose only slightly more than doubled.

The histochemical activity of lactate dehydrogenase, alpha glycerophosphate dehydrogenase, succinate dehydrogenase, isocitrate dehydrogenase, TPN diaphorase and DPN diaphorase showed a steady decrease with increasing times post-mortem. Reactions for both alcohol dehydrogenase and glutamate dehydrogenase were very weak or absent at 48 hrs post-mortem and all subsequent sampling periods.

Positive reactions for glucose-6-phosphate dehydrogenase and beta-hydroxybutyrate dehydrogenase were observed in the initial samples only. Acid phosphatase, leucine amino peptidase, and 6-phosphogluconate dehydrogenase were not detected in any of the samples.

Pork Studies

Chemical and histochemical procedures similar to those described for beef were used to follow the changes from 0 to 24 and/or 48 hrs post-mortem in the *l. dorsi* muscle of eighteen pork carcasses. One side from each of thirteen pork carcasses was placed at -29°C, while the other side from each carcass was subjected to 37°C for the first 4 1/2-5 hrs post-mortem in an attempt to induce soft, watery pork.

The muscles of the pork carcasses studied exhibited marked differences in initial levels of pH, glycogen, glucose and lactic acid. Post-mortem accumulation of glucose and lactic acid was similar to that noted in beef muscle. A similar stoichiometric relation to that found in beef muscle between post-mortem decrease in glycogen and a corresponding accumulation

of lactic acid and glucose existed in the carcasses from Poland China pigs. An equivalent relationship for the carcasses from Hampshire pigs was less apparent.

The 37°C treatment did not consistently induce soft, watery muscle as was expected from reports of previous workers. It was concluded that a low pH at a high muscle temperature per se was not a causal factor in the developement of the soft, watery condition. The loss in fibrillar water-binding capacity as the result of low pH values at high muscle temperatures as reported by previous workers was confirmed. In contrast to previous studies, decreases in fibrillar water binding capacity induced by the low pH-high temperature phenomenon did not usually result in soft, watery muscle. Although the loss in fibrillar water-binding capacity as a result of the low pH value-high temperature relationship is a characteristic encountered in soft, watery muscle, this characteristic by itself is not a primary causal factor in the developement of this condition.

The activity of lactate dehydrogenase, alpha-glycerophosphate dehydrogenase, succinate dehydrogenase, isocitrate dehydrogenase and DPN diaphorase was slightly weaker at both 0 and 24 hrs than similar activity in beef muscle. Alcohol dehydrogenase activity was weaker than that observed for the above enzymes, while the activity of glutamate dehydrogenase was of intermediate intensity. Glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase showed traces of activity only in some of the initial samples. Similarly, weak reactions for beta-hydroxybutyrate dehydrogenase were observed only at 0 hrs post-mortem. None or only weak activity was observed for malic dehydrogenase in all samples studied.

Likewise, TPN diaphorase activity was unexplainably absent from all samples. Moderate levels of cytochrome oxidase activity were observed at both 0 and 24 hrs post-mortem.

UDPG-glycogen transferase was completely inactivated by the 37°C treatment, but the treatment had a less marked effect upon phosphorylase and branching enzyme. The presence of acid and alkaline phosphatase activity in muscles of 7 pork carcasses suggested that a degenerative condition existed in these muscles.

BIBLIOGRAPHY

Allen, R. J. L. 1940. The estimation of phosphorous. Biochem. J. 34, 858.

Andrews, M. M., Guthneck, B. T., McBride, B. H., and Schweigert, B. S. 1952. Stability of certain respiratory and glycolytic enzyme systems in animal tissues. J. Biol. Chem. 2, 715.

Astbury, W. T. 1947. On the structure of biological fibres and the problem of muscle. Proc. Roy. Soc. (London), sB 134, 304.

Bailey, K. 1942. Myosin and adenosine triphosphatase. Biochem. J. 30, 121.

Bailey, K. 1954. Structure Proteins, II. Muscle. In The Proteins, Vol. II, p. 951. Ed. H. Neurath and K. Bailey. Academic Press. N. Y.

Barker, S. B. and Summerson, W. H. 1941. The colorimetric determination of lactic acid in biological material. J. Biol. Chem. 138, 535.

Bate Smith, E. C. 1938. The buffering of muscle in rigor; protein, phosphate and carnosine. J. Physiol. 92, 336.

Bate Smith, E. C. 1939. Changes in elasticity of mammalian muscle undergoing rigor mortis. J. Physiol. 96, 176.

Bate-Smith, E. C. 1948. The physiology and chemistry of rigor mortis with special reference to the aging of beef. Adv. in Food Res. 1, 1.

Bate-Smith, E. C. and Bendall, J. R. 1947. Rigor mortis and adenosine-triphosphate. J. Physiol. 106, 177.

Bate-Smith, E. C. and Bendall, J. R. 1949. Factors determining the time course of rigor mortis. J. Physiol. 110, 47.

Beckett, E. B. and Bourne, G. H. 1958. Acid and alkaline phosphatases in normal and diseased human muscle. Acta Anat. 35, 326.

Beckett, E. B. and Bourne, G. H. 1960. Histochemistry of skeletal muscle and changes in some muscle diseases. In Structure and Function of Muscle, Vol. III, 276. Ed. G. H. Bourne. Academic Press, N.Y.

Bendall, J. R. 1951. The shortening of rabbit muscles during rigor mortis: its relation to the breakdown of adenosine triphosphate and creatine phosphate and to muscular contraction. J. Physiol. 114, 71.

Bendall, J. R. 1953. Further observations on the factor (the "Marsh" factor) effecting relaxation of ATP-shortened muscle-fibre models, and the effect of Ca and Mg ions upon it. J. Physiol. (London) 121, 232.

Bendall, J. R. 1960a. Relaxing factors in muscle. Biochem. Biophys. Research Comm. 3, 641.

Bendall, J. R. 1960b. Post mortem changes in muscle. In The Structure and Function of Muscle, Vol. III, p. 227. Ed. G. H. Bourne, Academic Press, N. Y.

Bendall, J. R. 1962. The structure and composition of muscle. In Recent Advances in Food Science. Eds. J. Hawthorn and J. M. Leitch. Vol. I, p. 58.

Bendall, J. R. 1963. Physiology and chemistry of muscle. Proc. Meat Tenderness Symposium, p. 33. Campbell Soup Co., Camden, N. J.

Bendall, J. R. and Davey, C. L. 1957. Ammonia liberation during rigor mortis and its relation to changes in the adenine and inosine nucleotides of rabbit muscle. Biochim. Biophys Acta 26, 93.

Bendall, J. R., Hallund, O. and Wismer-Pedersen, J. 1963. Post-mortem changes in the muscles of Landrace pigs. J. Food Sci. 28, 156.

Bendall, J. R. and Wismer-Pedersen, J. 1962. Some properties of the fibrillar proteins of normal and watery pork muscle. J. Food Sci. 27, 144.

Bennett, H. S. 1955. Modern concepts of structure of striated muscle. Am. J. Phys. Med. 34, 46.

Bennett, H. S. 1956. The concepts of membrane flow and membrane vesiculation as mechanisms for active transport and ion pumping. J. Biophys. Biochem. Cytol. 2 Suppli., 99.

Bennett, H. S. 1960. The structure of striated muscle as seen by the electron microscope. In The Structure and Function of Muscle, Vol. I, 137. Ed. G. H. Bourne. Academic Press, N.Y.

Bennett, H. S. and Porter, K. R. 1953. An electron microscope study of sectioned breast muscle of the domestic fowl. Am. J. Anat. 93, 61.

Blanchaer, M. and VanWijhe, M. 1962. Distribution of lactic dehydrogenase in skeletal muscle. Nature 193, 887.

Blanchaer, M. C., VanWijhe, M., and Mozersky, D. 1963. The oxidation of lactate and α-glycerophosphate by red and white skeletal muscle: I. Quantitative studies. J. Histochem. Cytochem. 11, 500.

Blanchaer, M. C. 1964. Respiration of mitochondria of red and white skeletal muscle. Am. J. Physiol. 206, 1015.

Bloom, W. and Fawcett, D. W. 1963. A textbook of Histology. W. B. Saunders, Philadelphia.

Borchert, L. L. and Briskey, E. J. 1964. Prevention of pale, soft, exudative porcine muscle through partial freezing with liquid nitrogen post-mortem. J. Food Sci. 29, 203.

Bosanquet, F. D., Daniel, P. M. and Parry, H. B. 1960. Myopathy: the pathological changes in intrinsic diseases of muscles. In The Structure and Function of Muscle, Vol. III, 336. Ed. G. H. Bourne. Academic Press, N. Y.

Bowen, W. J. 1951. Phosphorylysis of adenosine triphosphate and rate of contraction of Myosin B threads. Am. J. Physiol. 165, 10.

Bowen, W. J. 1954. Relation of maximum shortening of glycerine-treated muscle and the concentration of ATP. Am. J. Physiol. 179, 620.

Bowen, W. J. and Martin, H. L. 1958. Rate of dephosphorylation of adenosinetriphosphate and the isotonic and isometric contraction of glycerol-treated muscle. Am. J. Physiol. 195, 311.

Briggs, F. N., Kaldor, G. and Gergely, J. 1959. Participation of a dialyzable cofactor in the relaxing system of muscle. III. Substitution of pyrophosphate for the cofactor. Biochim. Biophys. Acta. 34, 224.

Briggs, F. N. and Portzehl, H. 1957. The influence of relaxing factor on the pH dependence of the contraction of muscle models. Biochim. Biophys. Acta 24, 482.

Briskey, E. J., Bray, R. W., Hoekstra, W. G., Grummer, R. H., and Phillips, P. H. 1959a. The chemical and physical characteristics of various pork ham muscle classes. J. Anim. Sci. 18, 146.

Briskey, E. J., Bray, R. W., Hoekstra, W. G., Grummer, R. H., and Phillips, P. H. 1959b. The effect of various levels of exercise in altering the chemical and physical characteristics of certain pork ham muscles. J. Anim. Sci. 18, 153.

Briskey, E. J., Bray, R. W., Hoekstra, W. G., Grummer, R. H., and Phillips, P. H. 1959c. The effect of exhaustive exercise and high sucrose regimen on certain chemical and physical pork ham muscle characteristics. J. Anim. Sci. 18, 173.

Briskey, E. J., Bray, R. W., Hoekstra, W. G., Phillips, P. H. and Grummer, R. H. 1960. Effect of high protein, high fat and high sucrose rations on the water-binding and associated properties of pork muscle. J. Anim. Sci. 19, 404.

Briskey, E. J. and Wismer-Pedersen, J. 1961a. Biochemistry of pork muscle structure. I. Rate of anaerobic glycolysis and temperature change versus the apparent structure of muscle tissue. J. Food Sci. 26, 297.

Briskey, E. J. and Wismer-Pedersen, J. 1961b. Biochemistry of pork muscle structure. II. Preliminary observations of biopsy samples versus ultimate muscle structure. J. Food Sci. 26, 306.

Burstone, M. S. 1958. The relationship between fixation and techniques for the histochemical localization of hydrolytic enzymes. J. Histochem. Cytochem. 6, 322.

Burstone, M. S. 1959. New histochemical techniques for the demonstration of tissue oxidase (cytochrome oxidase). J. Histochem. Cytochem. 7, 112.

Burstone, M. S. 1961a. Modifications of histochemical techniques for the demonstration of cytochrome oxidase. J. Histochem. Cytochem. 9, 59.

Burstone, M. S. 1961b. Histochemical demonstration of phosphatases in frozen sections with naphthol AS-phosphates. J. Histochem. Cytochem. 9, 146.

Burstone, M. S. and Folk, J. E. 1956. Histochemical demonstration of aminopeptidase. J. Histochem. Cytochem. 4, 217.

Cain, D. F. and Davies, R. E. 1962. Breakdown of adenosine triphosphate during a single contraction of working muscle. Biochem. Biophys. Res. Comm. 8, 361.

Cain, D. F., Infante, A. A. and Davies, R. E. 1962. Chemistry of muscle contraction. Nature 196, 214.

Callow, E. H. 1937. The electrical resistance and micro-structure of muscular tissue. Ann. Rept. Food Invest. Bd., p. 46. (London).

Callow, E. H. 1938. The structure of muscular tissue. Ann. Rept. Food Invest. Bd., p. 55 (London).

Danielli, J. F. 1953. Cytochemistry. Wiley and Sons, N. Y.

Davey, C. L. 1957. An ion exchange method of determining carnosine, anserine and their precursors in animal tissues. Nature 179, 209.

Davey, C. L. 1960a. The effects of carnosine and anserine on glycolytic reactions in skeletal muscle. Arch. Biochem. Biophys. 89, 296.

Davey, C. L. 1960b. The significance of carnosine and anserine in striated skeletal muscle. Arch. Biochem. Biophys. 89, 303.

Davies, R. E. 1963. A molecular theory of muscular contraction: Calcium dependent contractions with hydrogen bond formation plus ATP-dependent extensions of part of the myosin-actin cross-bridges. Nature 199, 1068.

deFremery, D. and Pool, F. 1960. Biochemistry of chicken muscle as related to rigor mortis and tenderization. Food Res. 25, 73.

deFremery, D. and Pool, M. F. 1963. The influence of post-mortem glycolysis on poultry tenderness. J. Food Sci. 28, 173.

Dubowitz, V. and Pearse, A. G. E. 1960. A comparative histochemical study of oxidative enzyme and phosphorylase activity in skeletal muscle. Histochemie 2, 105.

Dubowitz, V. and Pearse, A. G. E. 1961. Enzymic activity of normal and dystrophic human muscle: a histochemical study. J. Path. Bact. 81, 365.

Dubuisson, M. 1954. Muscular Contraction, C. C. Thomas, Springfield, Ill.

Ebashi, S. 1961a. Calcium binding activity of vesicular relaxing factor. J. Biochem. 50, 236.

Ebashi, S. 1961b. The role of "relaxing factor" in contraction-relaxation cycle of muscle. Progr. Theoret. Phys. 17, 35.

Ells, H. A. and Faulkner, P. 1961. Inhibition of myofibrillar adenosine-triphosphatase and syneresis by 1,3-diphosphoglyceric acid. Biochem. Biophys. Research Comm. 5, 255.

Engelhardt, W. A. 1946. Adenosine triphosphatase properties of myosin. Adv. in Enzymol. 6, 147.

Engelhardt, W. A. and Ljubimova, M. N. 1939. Myosin and adenosinetriphosphatase. Nature 144, 668.

Ennor, A. H. and Rosenberg, H. 1952. The determination and distribution of phosphocreatine in animal tissues. Biochem. J. 51, 606.

Esterbrook, R. W. and Sackton, B. 1958. α-glycerophosphate oxidase of flight muscle mitochondria. J. Biol. Chem. 233:1014.

Fennell, R. A. and West, W. T. 1963. Oxidative and hydrolytic enzymes of homozygous dystrophic and heterozygous muscle of the house mouse. J. Histochem. Cytochem. 11, 374.

Flory, P. J. 1956a. Role of crystallization in polymers and proteins. Science 124, 53.

Flory, P. J. 1956b. Theory of elastic mechanisms in fibrous proteins. J. Am. Chem. Soc. 78, 5222.

Flory, P. J. 1957. Crystallinity and dimensional changes in fibrous proteins. J. Cellular Comp. Physiol. 49 (Suppl. 1), 175.

Folin, A. and Wu, H. 1920. A simplified and improved method for determination of sugar. J. Biol. Chem. 41, 367.

Fredholm, H. 1963. 5'- mono-, di- and triphosphates of adenosine in beef and pork kept under refrigeration. Acta Agri. Scand. 23, 352.

Gergely, J. 1956. The interaction between actomyosin and adenosine triphosphate. Light scattering studies. J. Biol. Chem. 220, 917.

Glick, D. 1949. Techniques of Histo- and Cyto-Chemistry. Interscience, N. Y.

Goldspink, G. 1962. Biochemical and physiological changes associated with the postnatal developement of the biceps brachii. Comp. Biochem. Physiol. 7, 157.

Goldspink, G. and McLoughlin, J. V. 1964. Studies on pig muscle. 3. The effect of temperature on the solubility of the sarcoplasmic proteins in relation to colour changes in post-rigor muscle. Irish J. Agri. Res. 3, 9.

Gormori, G. 1952. Microscopic Histochemistry. Chicago Univ. Press.

Grogg, E. and Pearse, A. G. E. 1952. A critical study of the histochemical techniques for acid phosphatase, with a description of an azo-dye method. J. Path. Bact. 64, 627.

Guyer, M. F. 1953. Animal Micrology. Univ. of Chicago Press.

Hamm, R. 1960. Biochemistry of meat hydration. Adv. Food Res. 10, 355.

Hamoir, G. 1955. Fish Proteins. Advances in Protein Chem. 10, 227.

Hanson, J. and Huxley, H. E. 1953. The structural basis of the cross-striations in muscle. Nature 172, 530.

Hanson, J. and Huxley, H. E. 1955. The structural basis of contraction in striated muscle. Symposia Soc. Exptl. Biol. 9, 228.

Hansen, R. G., Rutter, W. G. and Crowe, E. M. 1952. A nephelometric method for determination of glycogen. J. Biol. Chem. 195, 127.

Hasselbach, W. and Makinose, M. 1962. ATP and active transport. Biochem. Biophys. Res. Comm. 7, 132.

Hasselbach, W. and Weber, H. H. 1953. The influence of the MB factor on the contraction of fiber models. Biochim. Biophys. Acta 11, 160.

Hess, R. and Pearse, A. G. E. 1961. Dissociation of uridine diphosphate glucose-glycogen trans-glucosylase from phosphorylase activity in individual muscle fibers. Proc. Soc. Exp. Biol. Med. 107, 569.

Hess, R., Scarpelli, D. G. and Pearse, A. G. E. 1958. The cytochemical localization of oxidative enzymes. II. Pyridine nucleotide-linked dehydrogenases. J. Biophys. Biochem. Cytol. 4, 753.

Hill, T. L. 1953. Energetics and molecular mechanisms in muscle action. Part II. Statistical thermodynamical treatment of contractile systems. Disc. Faraday Soc. 13, 132.

Hodge, A. J., Huxley, H. E. and Spiro, D. 1954. Electron microscope studies on ultrathin sections of muscle. J. Exptl. Med. 99, 201.

Hoeve, C. A. J. and Willis, V.A. 1963. Elasticity of the fibrous muscle proteins. Biochem. 2, 279.

Hoeve, C. A. J., Willis, V. A., and Martin, D. J. 1963. Evidence for a phase transition in muscle contraction. Biochem. 2, 282.

Howard, A. and Lawrie, R. A. 1956. Studies on beef quality. II. Physiological and biological effects of various pre-slaughter treatments. Tech. Paper, No. 2, p. 18; Div. Food Pres. Transp. C.S.I.R.O., Melbourne.

Howard, A. and Lawrie, R. A. 1957. Studies on beef quality V. Further observations on biochemical and physiological responses to pre-slaughter treatments. Tech. Paper, No. 4, Div. Food Pres. Transp. C.S.I.R.O., Melbourne.

Huxley, A. F. 1957. Muscle structure and theories of contraction. Progr. in Biophys. and Biophys. Chem. 7, 257.

Huxley, H. E. 1953. Electron-microscope studies on the organization of the filaments in striated muscle. Biochim. Biophys. Acta 12, 387.

Huxley, H. E. 1957. The double array of filaments in cross-striated muscle. J. Biophys. Biochem. Cytol. 3, 631.

Huxley, H. E. and Hanson, J. 1954. Changes in the cross-striation of muscle during contraction and stretch and their structural interpretation. Nature 173, 973.

Huxley, H. E. and Hanson, J. 1957a. Quantitative studies on the structure of cross-striated myofibrils. I. Investigations by interference microscopy. Biochim. Biophys. Acta 23, 229.

Huxley, H. E. and Hanson, J. 1957b. Preliminary observations on the structure of inset flight muscle. Proc. Stockholm Cont. Electron Microscopy 1956, p. 202.

Huxley, H. E. and Hanson, J. 1960. The molecular basis of contraction in cross-striated muscles. In The Structure and Function of Muscle, Vol. I, 183. Ed. G. H. Bourne. Academic Press, N.Y.

Huxley, A. F. and Niedergerke, R. 1954. Structural changes in muscle during contraction. Nature 173, 971.

Huxley, A. F. and Taylor, R. E. 1955. Function of Krause's membrane. Nature 176, 1068.

Iyengar, M. R., Glauser, S. C., and Davies, R. 1964. An ATP-induced conformational change in the acto-H-meromyosin system. Biochem. Biophys. Res. Comm. 16, 379.

Kastenschmidt, L. L., Briskey, E. J. and Hoekstra, W. G. 1964. Prevention of pale, soft, exudative porcine muscle through regulation of ante-mortem environmental temperature. J. Food Sci. 29, 210.

Kielley, W. W. and Meyerhof, O. 1948. Studies on adenosinetriphosphate of muscle. II. A new magnesium-activated adenosinetriphosphatase. J. Biol. Chem. 176, 591.

Klamer, B. and Fennell, R. A. 1963. Acid phosphatase activity during growth and synchronous division of Tetrahymena pyriformis. Exp. Cell. Res. 29, 166.

Lawrie, R. A. 1953. The onset of rigor mortis in various muscles of the draught horse muscle. J. Physiol. 121, 275.

Lawrie, R. A. 1955. Residual glycogen at high ultimate pH in horse muscle. Biochim. Biophys. Acta 17, 282.

Lawrie, R. A. 1958. Physiological stress in relation to dark-cutting beef. J. Sci. Food Agr. 9, 721.

Lawrie, R. A. 1960. Post mortem glycolysis in normal and exudative longissimus dorsi muscles of the pig in relation to so-called white muscle disease. J. Comp. Path. 70, 273.

Lawrie, R. A. 1962. The conversion of muscle to meat. In Recent Advances in Food Science, Vol. I, p. 68. Eds. J. Hawthorn and J. M. Leitch. Butterworths, London.

Lawrie, R. A., Gatherum, D. P. and Hale, H. P. 1958. Abnormally low ultimate pH in pig muscle. Nature 182, 807.

Lillie, R. D. 1954. Histopathologie Technic and Practical Histochemistry. Blakiston Co., N. Y.

Lorand, L., Molner, J. and Moos, C. 1957. Conference on the chemistry of muscular contraction, Tokyo, 1957, p. 85. Igaku Shoin Ltd., Tokyo.

Lorand, L. and Molner, J. 1962. Muscle as a tissue, p. 126. McGraw-Hill, N. Y.

Ludvigsen, J. 1953. "Muscular degeneration" in hogs (preliminary report). Proc. XVth Intern. Veterin. Cong. Part 1, Vol. 1, 602. Boktryckeri, Stockholm.

Magee, W. T. 1964. Personal communications.

Marsh, B. B. 1952a. The effects of ATP on the fibre-volume of a muscle homogenate. Biochim. Biophys. Acta 9, 247.

Marsh, B. B. 1952b. Observations on rigor mortis in whale muscle. Biochim. Biophys. Acta. 9, 127.

Marsh, B. B. 1954. Rigor mortis in beef. J. Sci. Food Agr. 5, 70.

Marsh, B. B. 1960. Identification of the muscle relaxing factor. Biochem. Biophys. Research Comm. 3, 233.

Marshall, J. M., Holtzer, H., Frick, H. and Pepe, F. 1959. The distribution of protein antigens in striated myofibrils. Exptl. Cell. Res. 7, 219 (Suppl.).

McLoughlin, J. V. 1963. Studies on pig muscle. 2. The effect of rapid post-mortem pH fall on the extraction of the sarcoplasmic and myofibrillar proteins of post-rigor muscle. Irish J. Agri. Res. 2, 117.

McLoughlin, J. V. 1964a. Observations on pale, soft exudative musculature. Proc. 17th Ann. Reciproc. Meats Conf., Madison, Wisc.

McLoughlin, J. B. 1964b. Personal communcation.

McLoughlin, J. V. and Goldspink, G. 1963a. Studies on Pig Muscle. I. Exudative pig muscle. Irish J. Agri. Res. 2, 27.

McLoughlin, J. V. and Goldspink, G. 1963b. Post-mortem changes in the colour of pig longissimus dorsi muscle. Nature 198, 584.

Meyer, J. A., Briskey, E. J., Hoekstra, W. G. and Bray, R. W. 1962. Glucose tolerance in swine as related to post-mortem muscle characteristics. J. Anim. Sci. 21, 543.

Mihalyi, E. and Szent-Györgyi, A. G. 1953. Trypsin digestion of muscle proteins. I. Ultracentrifugal analysis of the process. J. Biol. Chem. 201, 189.

Mommaerts, W. F. H. M. 1947. The reaction between actomyosin and adenosine triphosphate. J. Gen. Physiol. 31, 361.

Mommaerts, W. F. H. M. 1950. Muscular Contraction. Interscience, N. Y.

Mommaerts, W. F. H. M. 1954. The biochemistry of muscle. Ann. Rev. Biochem. 23, 381.

Mommaerts, W. F. H. M. 1955. Investigation of the presumed breakdown of adenosine-triphosphate and phosphocreatine during a single muscle twitch. Am. J. Physiol. 182, 585.

Mommaerts, W. F. H. M., Seraydarian, K. and Uchida, K. 1963. On the relaxing substance of muscle. Biochem. Biophys. Res. Comm. 13, 58.

Moog, F. 1943. Cytochrome oxidase in early chick embryo. J. Cell Comp. Physiol. 22, 223.

Morales, M. F. and Botts, J. 1956. A theory of the primary event in muscle action. In Currents in Biochemical Research, p. 609, Ed. D. E. Green, Interscience, N. Y.

Morales, M. F., Botts, J., Blum, J. J. and Hill, T. L. 1955. Elementary processes in muscle action: an examination of current concepts. Physiol. Revs. 35, 475.

Nachlas, M. M., Crawford, D. T. and Seligman, A. M. 1957. The histochemical demonstration of leucine aminopeptidase. J. Histochem. Cytochem. 5, 264.

Nachlas, M. M., Crawford, D. T., Goldstein, T. P. and Seligman, A. M. 1958. The histochemical demonstration of cytochrome oxidase with a new reagent for the Nadi reaction. J. Histochem. Cytochem. 6, 445.

Nachlas, M. M., Friedman, M. M. and Seligman, A. M. 1962. New observations on discrepancies in the histochemical localization of leucine aminopeptidase. J. Histochem. Cytochem. 10, 315.

Nachlas, M. M., Monis, B., Rosenblatt, D. and Seligman, A. M. 1960. Improvement in the histochemical localization of leucine aminopeptidase with a new substrate, L-leucyl-4-methoxy-2-naphthylamide. J. Biophys. Biochem. Cytol. 7, 261.

Nachlas, M. M., Walker, D. G., and Seligman, A. M. 1958a. The histochemical localization of triphosphopyridine nucleotide diaphorase. J. Biophys. Biochem. Cytol. 4, 467.

Nachlas, M. M., Walker, D. G. and Seligman, A. M. 1958b. A histochemical method for the demonstration of diphosphopyridine nucleotide diaphorase. J. Biophys, Biochem. Cytol. 4, 29.

Nachmias, V. T. and Padykula, H. A. 1958. A histochemical study of normal and denervated red and white muscles of the rat. J. Biophys. Biochem. Cytol. 4, 47.

Nagai, T., Uchida, K. and Yasuda, M. 1962. Some further properties of the muscle relaxing-factor system and the separation of the effective substance. Biochim. Biophys. Acta 56, 205.

Needham, D. M. 1926. Red and white muscle. Physiol. Rev. 6, 1.

Needham, D. M. 1960. Biochemistry of Muscular Action. In The Structure and Function of Muscle, Vol. II, 55. Ed. G. H. Bourne. Academic Press, N. Y.

Ogata, T. and Mori, M. 1963. A histochemical study of hydrolytic enzymes in muscle fibers of various animals. J. Histochem. Cytochem. 11, 645.

Ogata, T. and Mori, M. 1964. Histochemical study of oxidative enzymes in vertebrate muscles. J. Histochem. Cytochem. 12, 171.

Padykula, H. A. and Gauthier, G. F. 1963. Cytochemical studies of adenosine triphosphatases in skeletal muscle fibers. J. Cell Biol. 18, 87.

Padykula, H. A. and Herman, E. 1955a. Factors affecting the activity of adenosine triphosphatase and other phosphatases as measured by histochemical techniques. J. Histochem. Cytochem. 3, 161.

Padykula, H. A. and Herman, E. 1955b. The specificity of the histochemical method for adenosine triphosphatase. J. Histochem. Cytochem. 3, 170.

Parker, C. J. 1961. The role of α-glycerol phosphate in muscle relaxation. Biochem. Biophys. Research Comm. 4, 309.

Partmann, W. 1963. Post-mortem changes in chilled and frozen muscle. J. Food Sci. 28, 1.

Pauling, L. and Corey, R. 1951. Configuration of polypeptide chains. Nature 168, 550.

Perry, S. V. and Grey, T. C. 1956. A study of the effects of substrate concentration and certain relaxing factors on the magnesium-activated myofibrillar adenosine triphosphatase. Biochem. J. 64, 184.

Pearse, A. G. E. 1957. Intracellular localization of dehydrogenase systems using monotetrazolium salts and metal chelation of their formayons. J. Histochem. Cytochem. 5, 515.

Pearse, A. G. E. 1960. Histochemistry. Theoretical and Applied. Little, Brown and Co. Boston.

Pearse, A. G. E. 1961. Direct relationship of phosphorylase and mitochondrial α-glycerophosphate dehydrogenase activity in skeletal muscle. Nature 191, 504.

Porter, K. R. 1956. The sarcoplasmic reticulum in muscle cells of Amblystoma larvae. J. Biophys. Biochem. Cytol. 2 Suppli., 163.

Porter, K. R. 1961. The sarcoplasmic reticulum. Its recent history and present status. J. Biophys. Biochem. Cytol. 10, Suppli., 219.

Porter, K. R. and Palade, G. E. 1957. Studies on the endoplasmic reticulum III. Its form and distribition in striated muscle cells. J. Biophys. Biochem. Cytol. 3, 269.

Riseman, J. and Kirkwood, J. G. 1948. Remarks on the physico chemical mechanisms of muscular contraction and relaxation. J. Am. Chem. Soc. 70, 2820.

Sayre, R. N. and Briskey, E. J. 1963. Protein solubility as influenced by physiological conditions in the muscle. J. Food Sci. 28, 675.

Sayre, R. N., Briskey, E. J., Hoekstra, W. G. and Bray, R. W. 1961. Effect of preslaughter change to a cold environment on characteristics of pork muscle. J. Anim. Sci. 20, 487.

Sayre, R. N., Briskey, E. J. and Hoekstra, W. G. 1963a. Effect of excitement, fasting and sucrose feeding on porcine muscle phosphorylase and post-mortem glycolysis. J. Food Sci. 28, 472.

Sayre, R. N., Briskey, E. J. and Hoekstra, W. G. 1963b. Alteration of post-mortem changes in porcine muscle by preslaughter heat treatment and diet modification. J. Food Sci. 28, 292.

Sayre, R. N., Briskey, E. J. and Hoekstra, W. G. 1963c. Comparison of muscle characteristics in three breeds of swine. J. Anim. Sci. 22, 1012.

Sayre, R. N., Kiernat, B., and Briskey, E. J. 1964. Processing characteristics of porcine muscle related to pH and temperature during rigor mortis developement and to gross morphology 24 hr post-mortem. J. Food Sci. 29, 175.

Sasaski, M. and Takeuchi, T. 1963. Histochemical and electron microscopic observations of glycogen synthesized from glucose-1-phosphate by phosphorylase and branching enzyme in human muscle. J. Histochem. Cytochem. 11, 342.

Scarpelli, D. G., Hess, R. and Pearse, A. G. E. 1958. Cytochemical localization of oxidative enzymes. I. Diphosphopyridine nucleotide diaphorase and triphosphopyridine nucleotide diaphorase. J. Biophys. Biochem. Cytol. 4, 747.

Sharpe, J. G. 1962. Non-enzymic browning deterioration in dehydrated meat. In Recent Advances in Food Science, Vol. 2, p. 65. Eds. J. Hawthorne and J. M. Leitch, Butterworths, London.

Sharpe, J. G., and Rolfe, E. J. 1958. Deterioration of dehydrated meat during storage. In Fundamental Aspects of the Dehydration of Foodstuffs, p. 197. Soc. Chem. Ind., London.

Sjöstrand, F. S. 1956. The ultrastructure of cells as revealed by the electron microscope. Intern. Rev. Cytol. 5, 455.

Snedecor, G. W. 1959. Statistical Methods. 5th Ed. Iowa State Press.

Stein, J. M. and Padykula, H. A. 1962. Histochemical classification of individual skeletal muscle fibers of the rat. Am. J. Anat. 110, 103.

Strehler, B. L. 1953. Firefly liminescence in the study of energy transfer mechanisms. II. Adenosine triphosphate and photosynthesis. Arch. Biochem. Biophys. 43, 67.

Strehler, B. L. and Totter, J. R. 1952. Firefly luminescence in the study of energy transfer mechanisms. I. Substrate and enzyme determination. Arch. Biochem. Biophys. 40, 28.

Swift, C. E., Berman, M. D., and Lockett, C. 1960. Factors affecting the water retention of beef. II. Variation in some pH determinants among eight muscles. Food Tech. 14, 74.

Szent-Györgyi, A. 1947. The Chemistry of Muscular Contraction. Academic Press, N. Y.

Szent-Györgyi, A. 1951. Chemistry of Muscular Contraction, 2nd ed. Academic Press, N. Y.

Szent-Györgyi, A. 1953. Chemical Physiology of Contraction in Body and Heart Muscle. Academic Press, N. Y.

Szent-Györgyi, A. G. 1960. Proteins of the Myofibril. In the Structure and Function of Muscle, Vol. II, p. 1, Ed. G. H. Bourne. Academic Press, N. Y.

Takeuchi, T. 1956. Histochemical demonstration of phosphorylase. J. Histochem. Cytochem. 4, 84.

Takeuchi, T. 1958. Histochemical demonstration of branching enzyme (anylo-1,4-->1,6-transglucosidase) in animal tissues. J. Histochem. Cytochem. 6, 208.

Takeuchi, T. and Glenner, G. G. 1960. Histochemical demonstration of a pathway for polysaccharide synthesis from uridine diphosphoglucose. J. Histochem. Cytochem. 8, 227.

Takeuchi, T. and Glenner, G. G. 1961. Histochemical demonstration of uridine diphosphate glucose-glycogen transferase in animal tissues. J. Histochem. Cytochem. 9, 304.

Takauji, M. and Nagai, T. 1963. Is adenosine-3', 5'-phosphate identical with the relaxing substance? Biochem. Biophys. Res. Comm. 13, 54.

Uchida, K. and Mommaerts, W. F. H. M. 1963. Modification of the contractile responses of actomyosin by cyclic adenosine 3', 5' phosphate. Biochem. Biophys. Research Comm. 10, 1.

VanWijhe, M., Blanchaer, M. C. and Jacyk, W. R. 1963. The oxidation of lactate and α-glycerophosphate by red and white skeletal muscle: II. Histochemical studies. J. Histochem. Cytochem. 11, 505.

Venable, J. H. 1963. The histology of muscle. Proc. Meat Tenderness Symposium, p. 7. Campbell Soup Co., Camden, N. J.

Wachstein, M. and Meisel, E. 1955. The distribution of demonstrable succinic dehydrogenase and of mitochondria in tongue and skeletal muscle. J. Biophys. Biochem. Cytol. 1, 483.

Wachstein, M. and Meisel, E. 1957. Histochemistry of hepatic phosphatases at a physiologic pH. With special reference to the demonstration of bile canaliculi. Am. J. Clin. Path. 27, 13.

Walls, E. W. 1960. The microanatomy of muscle. In The Structure and Function of Muscle, Vol. I, 21. Ed. G. H. Bourne. Academic Press, N. Y.

Weber, H. H. 1957. The biochemistry of muscle. Ann. Rev. Biochem. 26, 667.

Weber, A. 1956. The ultracentrifugal separation of L-myosin and actin in an actomyosin sol under the influence of ATP. Biochim. Biophys. Acta 19, 345.

Weber, A. and Herz, R. 1963. The binding of calcium to actomyosin systems in relation to their biological activity. J. Biol. Chem. 238, 581.

Weber, A., Herz, R. and Reiss, I. 1963. On the mechanism of the relaxing effect of fragmented sarcoplasmic reticulum. J. Gen. Physiol. 46, 679.

Weber, H. H. and Portzehl, H. 1952. Muscle contraction and fibrous muscle proteins. Adv. in Protein Chem. 7, 161.

Weber, A. and Weber, H. H. 1951. Zur Thermodynamik der Kontraktion des Fasermodells. (English Summary). Biochim. Biophys. Acta 7, 339.

Weil-Malherbe, H. and Green, R. H. 1951. The catalytic effect of molybdate on the hydrolysis of organic phosphate bonds. Biochem. J. 49, 286.

Whitaker, J. R. 1959. Chemical changes with aging of meat with emphasis on the proteins. Adv. in Food Res. 9, 1.

Wismer-Pedersen, J. 1959. Quality of pork in relation to rate of pH change post mortem. Food Res. 24, 711.

Wismer-Pedersen, J., and Briskey, E. J. 1961a. Rate of anaerobic glycolysis versus structure in pork muscle. Nature 189, 318.

Wismer-Pedersen, J., and Briskey, E. J. 1961b. Relationship of postmortem acidity and temperature. Food Tech. 15, 232.

Zender, R., Lataste-Dorolle, C., Collet, R. A., Rowinski, P. and Mouton, R. F. 1958. Aseptic autolysis of muscle: biochemical and microscopic modifications in rabbit and lamb muscle during aseptic and anaerobic storage. Food Res. 23, 305 (1958).

APPENDIX

Appendix A. Table 1. Sources of chemicals for histochemical procedures.

Chemical	Source*
AMP (adenosine-5'-phosphate; Na-salt, crystalline; from muscle	Sigma
ATP (adenosine-5'-triphosphate; di Na, 3 H_2O, crystalline)	Sigma
Diazote 5-chloro-o-toluidine	Dajac
DPN (beta-diphosphopyridine nucleotide)	Sigma
Fast Blue B salt (O-dianisidine, tetrazotized)	Sigma
Fast red violet LB salt	Sigma
Glucose-6-phosphate (di Na salt)	Sigma
Glucose-1-phosphate (di-K salt)	Sigma
Glucose-1-phosphate (di-Na salt)	Sigma
L-Glutamate (Na)	Sigma
DL-alpha-Glycerophosphate	Sigma
beta-Glycerophosphate (di-Na)	Sigma
Glycogen (from rabbit)	Mann
DL-beta-Hydroxybutyrate (Na salt)	Sigma
DL-isocitrate (tri-Na)	Sigma
Lactic dehydrogenase	Sigma
DL-Lactate (Na salt, 60% syrup)	Sigma
L-Malic acid (Na)	Sigma
MTT (MTT tetrazolium)	Sigma
1-Hydroxy-2-Naphthoic acid	Kodak
Nitro BT (Nitro blue tetrazolium)	Sigma
N-Phenyl-p-phenylene diamine	Kodak
6-Phosphogluconate (Ba)	Sigma
Sodium-AS-BI-phosphate	Sigma
Succinate (Na)	Sigma
Thiamine pyrophosphate (Cl)	Mann
TPN (triphosphopyridine nucleotide, Na salt)	Sigma
UDPG (uridine-5'-diphosphoglucose, Na salt)	Sigma

*Sigma: Sigma Chemical Co., 3500 DeKalb St., St. Louis 18, Mo.
Dajac: Borden Co. Chemical Division, 5000 Langdon St., Philadelphia 24, Pa.
Kodak: Eastman Kodak Co., Rochester 3, N. Y.
Mann: Mann Research Laboratories, 136 Liberty St., New York 6, N.Y.

Appendix A. Table 2. Post-mortem levels of chemical constituents, and pH for individual beef carcasses at various periods post-mortem

	Time post-mortem (hrs)	Carcass 1	2	3	4	5
pH:						
	Initial	6.90	6.95	7.07	6.98	7.03
	6	6.41	6.50	6.73	6.60	6.60
	12	5.80	5.92	6.07	5.90	6.13
	24	5.60	5.80	6.02	5.53	5.73
	48	5.47	5.50	5.67	5.50	5.72
	72	5.43	5.60	5.40	5.40	5.48
	96	5.35	5.38	5.40	5.37	5.32
	102	5.42	5.58	5.40	5.35	5.33
	126	5.45	5.52	5.45	5.52	5.55
	151	5.47	5.48	5.49	5.67	5.52
	173	5.46	5.50	5.48	5.68	5.58
	198	5.55	5.59	5.60	5.72	5.69
	288	5.63	5.65	5.50	5.58	5.58
	480	5.47	5.40	5.50	5.50	5.42
Glycogen:						
(mg/g)	Initial	10.20	9.30	9.81	7.44	9.29
	6	6.58	4.34	9.47	7.32	6.06
	12	3.66	4.79	7.22	3.44	5.53
	24	1.45	2.18	2.39	0.65	1.54
	48	1.68	1.70	1.84	1.40	1.50
	102	2.32	3.52	3.03	2.85	1.56
	288	1.56	1.06	3.06	1.62	0.69
	480	0.40	0.28	0.28	0.12	0.23
Glucose:						
(mg/g)	Initial	1.52	1.49	1.18	1.63	1.32
	6	1.30	0.95	1.76	0.87	0.80
	12	2.12	3.18	1.45	2.74	1.47
	24	3.66	3.98	2.26	3.25	3.14
	48	3.52	3.51	2.81	2.35	2.18
	102	3.02	2.31	1.85	1.83	1.90
	288	3.89	4.35	2.60	2.26	2.00
	480	3.79	3.20	3.66	3.21	2.26
Lactic acid:						
(mg/g)	Initial	1.93	0.76	0.60	1.46	1.13
	6	4.50	5.13	2.66	5.48	2.39
	12	7.34	6.57	4.30	4.57	3.33
	24	7.05	7.75	6.02	6.09	5.14
	48	6.96	8.57	7.02	7.46	7.11
	102	6.88	8.04	7.06	7.30	7.14
	288	7.80	6.97	7.46	7.47	7.52
	480	7.35	7.49	7.97	7.96	7.44

Appendix A. Table 2. Post-mortem levels of chemical constituents, and pH for individual beef carcasses at various periods post-mortem (continued)

	Time post-mortem (hrs)	Carcass 1	2	3	4	5
ATP_1 (mg P/g)	Initial	.328	.425	.517	.388	.309
	6	.330	.283	.383	.228	.270
	12	.138	.207	.368	.267	.222
	24	.080	.051	.125	.104	.156
	48	.050	.040	.189	.055	.007
ATP_2 (micromoles /g)	Initial	10.1	12.6	10.7	9.2	11.8
	6	9.9	8.5	11.0	7.3	13.2
	12	0.0	2.5	6.7	7.9	9.4
	24	0.0	0.0	1.4	0.5	1.3
	48	0.0	0.0	0.0	0.0	0.0
TSP (mg P/g)	Initial	1.703	1.879	1.547	1.770	1.600
	6	1.837	1.776	1.556	1.688	1.676
	12	1.743	1.886	1.642	1.673	1.569
	24	1.584	1.914	1.620	1.689	1.483
	48	1.573	1.868	1.640	1.686	1.629
	102	1.637	1.866	1.561	1.671	1.585
	288	1.736	1.880	1.590	1.662	1.631
	480	1.479	1.878	1.701	1.680	1.655
Ortho P (mg P/g)	Initial	0.6274	0.6570	0.6090	0.6693	0.8497
	6	0.6487	0.6044	0.6640	0.7494	0.9329
	12	0.8440	0.7234	0.7490	0.8156	0.8789
	24	0.6782	0.7047	0.6930	0.6796	0.5923
	48	0.8044	0.8505	0.8190	0.9134	0.8833
	102	0.7576	0.7713	1.0530	1.1052	1.0124
	288	1.0336	0.8772	1.0500	1.0617	1.1902
	480	1.0095	1.0215	1.1580	1.1407	1.1867

Appendix B. Table 1. Sampling locations* on pork carcasses

Carcass	Right side	Left side
1	1L, 9T, 6L, 13T, 3L, 4/5L, 2T, 11T**	3L, 11T, 6L, 13T, 9T 7T, 1L, 4/5L
2	7T, 9T, 6L, 13T, 1L, 11T, 8T	5L, 13T, 4/5L, 3/4L, 11T, 1L, 7T
3	6L, 9T, 11T, 4/5L, 7T, 3L, 13T, 1L, 5L	1L, 3L, 11T, 4/5L, 6L, 13T, 7T, 9T, 7T
4	4/5L, 2/3L, 1L, 6L, 7T, 9T	2/3L, 4/5L, 1L, 6L, 7T, 9T
5	4/5L, 11T, 1L, 9T, 2/3L, 14T	2/3L, 11T, 1L, 9T, 4/5L, 14T
6	2/3L, 11T, 1L, 9T, 4/5L, 14T	Same as in right side
7	"	"
8	4/5L, 7T, 1L, 6L, 2/3L, 9T	"
9	"	"
10	6L, 4/5L, 2/3L, 1L, 13/14T, 9T	"
11	"	"
A through G	2/3L, 11T, 1L, 9T, 4/5L, 14T	"

* L = lumbar vertebra; T = thoracic vertebra. Numbers refer to vertebra numbers, i.e., 1 = 1st, 2 = 2nd, etc.
**Listed in order of sampling.

Appendix B. Table 2. Post-mortem levels of pH, glycogen, glucose, lactic acid, ATP, fibrillar protein content, and water-binding capacity for each side of pork carcasses 1 through 10 and 11Y.

Carcass	Treatment	Time post-mortem (mins)	pH	Glycogen (mg/g)	Glucose (mg/g)	Lactic acid (mg/g)	ATP_2[a] (micro-moles/g)	g fibrillar protein per g precipitate	g H_2O per g fibrillar protein	Precipitated fibril protein as % of sample wt.
1	-	10	-	6.59	1.79	4.58	4.2	.0551	17.15	-
	+4°C	28	-	4.31	2.20	5.99	1.9	.0545	17.35	-
		73	-	2.44	2.06	7.78	0.0	.0623	15.05	-
		139	-	2.33	2.72	6.14	0.0	.0383	25.11	-
		193	-	-	-	-	-	-	-	-
		253	-	0.35	3.19	7.49	-	.0455	20.98	-
		493	-	0.90	2.96	6.47	-	-	-	-
		1440	-	0.10	4.78	6.68	-	.0485	19.62	-
		2880	-	0.11	4.07	7.58	-	-	-	-
	+37°C	33	-	3.97	1.79	5.34	-	.0521	18.19	-
		80	-	1.43	2.50	7.31	3.9	.0527	17.98	-
		133	-	2.23	2.00	5.33	0.0	.0365	26.40	-
		193	-	0.83	3.20	7.19	0.0	-	-	-
		253	-	0.07	4.04	7.89	-	.0497	19.12	-
		491	-	0.60	4.43	7.83	-	-	-	-
		1440	-	0.00	4.78	7.54	-	.0617	15.21	-
		2880	-	0.05	5.66	7.68	-	-	-	-
2	-	10	-	7.38	2.78	6.25	3.4	.0323	29.96	-
	+4°C	83	-	1.26	2.64	7.96	0.0	.0575	16.39	-
		144	-	0.70	4.17	8.08	0.0	.0599	15.70	-
		236	-	1.93	5.97	7.43	-	.0431	22.20	-
		324	-	0.15	6.32	6.38	-	.0713	13.03	-
		564	-	0.17	5.10	8.26	-	-	-	-
		1440	-	0.07	5.61	7.08	-	.0743	12.46	11.34
		2880	-	0.03	5.75	7.73	-	-	-	-

[a] ATP_2 determined by enzymic assay. Initial values for $ATP_{(1)}$ (acid hydrolyses) for carcasses 6 through 10 and 11Y were 1.2, 10.3, 1.8, 8.9, 5.6 and 10.3 micromoles/g of tissue, respectively.

Appendix B. Table 2. Post-mortem levels of pH, glycogen, glucose, lactic acid, ATP, fibrillar protein content, and water-binding capacity for each side of pork carcasses 1 through 10 and 11Y. (continued)

Carcass	Treatment	Time post-mortem (mins)	pH	Glycogen (mg/g)	Glucose (mg/g)	Lactic acid (mg/g)	ATP_2[a] (micro-moles/g)	g fibrillar protein per g precipitate	g H_2O per g fibrillar protein	Precipitated fibril protein as % of sample wt.
2	+25°C	80	-	3.67	5.04	7.64	0.0	.0653	14.31	-
		146	-	0.24	5.38	8.84	0.0	.0563	16.76	-
		235	-	0.66	6.35	9.30	-	.0461	20.69	-
		327	-	0.53	7.88	8.62	-	.0773	11.94	-
		564	-	0.15	6.90	7.89	-	-	-	-
		1440	-	0.06	8.00	7.95	-	.0743	12.46	12.30
		2880	-	0.00	6.92	8.10	-	-	-	-
3	-	12	-	10.48	1.88	4.54	-	.0365	26.40	-
	+4°C	48	-	6.87	1.70	6.63	2.7	-	-	-
		63	-	1.98	3.74	7.22	0.0	.0264	36.88	-
		123	-	5.41	2.44	7.42	0.0	.0287	33.84	-
		190	-	3.78	3.76	7.31	-	.0276	35.23	-
		269	-	1.74	3.96	5.80	-	.0335	28.85	-
		320	-	0.11	5.49	7.70	-	.0605	15.53	-
		554	-	0.39	4.95	8.62	-	-	-	-
		1440	-	1.09	4.97	7.87	-	.0563	16.76	11.55
		2880	-	0.00	5.54	7.31	-	-	-	-
	+37°C	47	-	6.87	1.94	6.49	1.5	-	-	-
		65	-	1.87	3.56	7.69	0.0	.0389	24.71	-
		121	-	0.58	4.22	7.95	0.0	.0419	22.87	-
		187	-	2.90	3.68	7.50	-	.0293	33.13	-
		251	-	0.07	4.90	8.15	-	.0563	16.76	-
		325	-	0.58	5.40	8.37	-	.0653	14.31	-
		566	-	0.17	5.76	7.66	-	-	-	-
		1440	-	0.03	7.85	10.57	-	.0551	17.15	11.89
		2880	-	0.00	6.79	6.83	-	-	-	-

[a] ATP_2 determined by enzymic assay. Initial values for $ATP_{(1)}$ (acid hydrolyses) for carcasses 6 through 10 and 11Y were 1.2, 10.3, 1.8, 8.9, 5.6 and 10.3 micromoles/g of tissue, respectively.

Appendix B. Table 2. Post-mortem levels of pH, glycogen, glucose, lactic acid, ATP, fibrillar protein content, and water-binding capacity for each side of pork carcasses 1 through 10 and 11Y. (continued)

Carcass	Treatment	Time post-mortem (mins)	pH	Glycogen (mg/g)	Glucose (mg/g)	Lactic acid (mg/g)	ATP_2[a] (micro-moles/g)	g fibrillar protein per g precipitate	g H_2O per g fibrillar protein	Precipitated fibril protein as % of sample wt.
4	-	14	6.08	6.27	2.90	6.62	2.6	.0749	12.35	-
	+4°C	207	5.38	1.75	3.84	7.50	0.0	.0671	13.90	-
		308	5.29	3.54	4.26	8.58	0.0	.0814	11.27	-
		503	5.40	2.85	3.72	8.32	-	.0856	10.68	-
		668	5.34	2.33	3.64	8.33	-	-	-	-
		1440	5.39	0.80	4.70	8.75	-	.0988	9.12	12.93
		2880	5.28	0.70	5.56	8.84	-	-	-	-
	-29°C	173	5.44	2.63	2.85	8.07	0.0	.0790	11.66	-
		312	5.36	1.39	4.18	7.82	0.0	.0952	9.50	-
		503	5.42	1.57	4.69	7.67	-	.0778	11.85	-
		668	5.32	1.40	3.86	7.75	-	-	-	-
		1440	5.31	0.00	5.32	10.52	-	.0934	9.71	12.76
		2880	5.31	0.56	5.62	8.10	0	-	-	-
5	-	11	6.01	2.61	2.36	5.55	4.7	.0755	12.25	-
	+4°C	143	5.45	1.27	3.26	9.45	0.0	.0808	11.38	-
		301	5.53	1.69	3.03	8.18	0.0	.0767	12.03	-
		438	5.46	1.07	3.33	7.48	-	.0778	11.85	-
		633	5.38	0.10	3.92	7.60	-	-	-	-
		1440	5.41	0.00	4.71	8.71	-	.0964	9.37	13.59
		2880	5.35	0.09	3.73	8.12	-	-	-	-

[a] ATP_2 determined by enzymic assay. Initial values for $ATP_{(1)}$ (acid hydrolyses) for carcasses 6 through 10 and 11Y were 1.2, 10.3, 1.8, 8.9, 5.6 and 10.3 micromoles/g of tissue, respectively.

Appendix B. Table 2. Post-mortem levels of pH, glycogen, glucose, lactic acid, ATP, fibrillar protein content, and water-binding capacity for each side of pork carcasses 1 through 10 and 11Y. (continued)

Carcass	Treatment	Time post-mortem (mins)	pH	Glycogen (mg/g)	Glucose (mg/g)	Lactic acid (mg/g)	ATP_2[a] (micro-moles/g)	g fibrillar protein per g precipitate	g H_2O per g fibrillar protein	Precipitated fibril protein as % of sample wt.
5	-29°C	166	5.49	0.42	3.47	8.55	0.0	.0725	12.79	-
		294	5.59	1.72	2.58	6.25	0.0	.0755	12.25	-
		438	5.47	0.90	3.55	6.89	-	.0886	10.29	-
		633	5.63	1.33	3.52	6.87	-	-	-	-
		1440	5.35	0.17	3.57	6.04	-	.0749	12.35	9.36
		2880	5.37	0.20	3.62	8.16	-	-	-	-
6	-	14	5.63	2.14	2.71	6.32	1.2	.0281	34.58	-
	+4°C	169	5.35	0.43	4.08	8.10	0.0	.0719	12.91	-
		286	5.23	0.24	3.47	8.98	0.0	.0737	12.57	-
		517	5.20	0.20	4.28	8.18	-	.0713	13.03	-
		746	5.26	0.19	4.35	7.10	-	-	-	-
		1440	5.34	0.14	4.93	6.54	-	.0677	13.77	7.77
		2880	5.32	0.02	4.85	7.53	-	-	-	-
	+37°C	167	5.30	0.22	3.19	7.61	0.0	.0826	11.11	-
		291	5.24	0.25	3.91	7.85	0.0	.0838	10.93	-
		516	5.27	0.09	3.82	6.27	-	.0773	11.94	-
		746	5.30	0.16	4.30	6.02	-	-	-	-
		1440	5.25	0.07	4.76	6.70	-	.0850	10.77	7.97
		2880	5.29	0.09	3.94	7.46	-	-	-	-
7	-	16	6.67	8.24	1.44	2.54	10.3	.0305	31.79	-
	+4°C	177	6.51	9.00	0.93	2.64	8.9	.0299	32.45	-
		287	6.18	7.76	1.14	4.43	3.7	.0299	32.45	-
		524	5.91	4.39	1.40	8.33	0.0	.0281	34.59	-

[a] ATP_2 determined by enzymic assay. Initial values for $ATP_{(1)}$ (acid hydrolyses) for carcasses 6 through 10 and 11Y were 1.2, 10.3, 1.8, 8.9, 5.6 and 10.3 micromoles/g of tissue, respectively.

Appendix B. Table 2. Post-mortem levels of pH, glycogen, glucose, lactic acid, ATP, fibrillar protein content, and water-binding capacity for each side of pork carcasses 1 through 10 and 11Y. (continued)

Carcass	Treatment	Time post-mortem (mins)	pH	Glycogen (mg/g)	Glucose (mg/g)	Lactic acid (mg/g)	ATP_2[a] (micro-moles/g)	g fibrillar protein per g precipitate	g H_2O per g fibrillar protein	Precipitated fibril protein as % of sample wt.
7	+4°C	739	5.87	3.30	1.54	5.62	0.0	-	-	-
		1440	5.59	3.88	2.40	5.82	-	.0270	36.04	6.04
		2880	5.58	1.58	2.96	6.66	-	-	-	-
	+37°C	176	6.22	7.26	1.68	4.66	8.7	.0305	31.79	-
		289	5.37	0.95	3.23	7.90	0.0	.0611	15.37	-
		526	5.32	0.50	4.72	7.67	0.0	.0509	18.65	-
		739	5.41	0.14	4.68	7.37	-	-	-	-
		1440	5.30	0.12	4.52	7.34	-	.0653	14.30	11.84
		2880	5.34	0.16	4.72	8.10	-	-	-	-
8	-	15	6.07	4.03	3.13	7.37	1.8	.0275	35.36	-
	+4°C	187	5.29	0.43	4.14	8.84	0.0	.0784	11.76	-
		302	5.35	1.50	3.20	8.08	0.0	.0413	23.20	-
		532	5.28	0.28	3.62	8.28	-	.0479	19.10	-
		787	5.28	0.24	4.50	8.12	-	-	-	-
		1440	5.25	0.23	4.68	8.66	-	.0393	24.50	6.70
		2880	5.29	0.15	3.28	8.54	-	-	-	-
	+37°C	182	5.28	0.42	4.64	8.58	0.0	.0868	10.50	-
		294	5.40	0.23	4.59	7.83	0.0	.0802	11.50	-
		532	5.30	0.14	5.02	8.16	-	.0695	13.40	-
		787	5.43	0.16	5.28	8.50	-	-	-	-
		1440	5.38	0.09	6.12	8.32	-	.0713	13.00	9.40
		2880	5.34	0.15	5.83	8.43	-	-	-	-

[a] ATP_2 determined by enzymic assay. Initial values for $ATP_{(1)}$ (acid hydrolyses) for carcasses 6 through 10 and 11Y were 1.2, 10.3, 1.8, 8.9, 5.6 and 10.3 micromoles/g of tissue, respectively.

Appendix B. Table 2. Post-mortem levels of pH, glycogen, glucose, lactic acid, ATP, fibrillar protein content, and water-binding capacity for each side of pork carcasses 1 through 10 and 11Y. (continued)

Carcass	Treatment	Time post-mortem (mins)	pH	Glycogen (mg/g)	Glucose (mg/g)	Lactic acid (mg/g)	ATP_2[a] (micro-moles/g)	g fibrillar protein per g precipitate	g H_2O per g fibrillar protein	Precipitated fibril protein as % of sample wt.
9	-	6	6.42	5.57	2.07	3.85	8.9	.0449	21.30	-
	+4°C	175	5.65	4.35	2.50	6.05	3.6	.0377	25.50	-
		305	5.72	2.98	2.07	6.90	4.3	.0365	26.40	-
		525	5.90	3.65	2.59	4.81	1.3	.0293	33.10	-
		720	5.47	1.79	2.86	6.06	2.1	-	-	-
		1440	5.65	0.82	2.86	7.61	-	.0216	45.30	5.46
		2880	5.32	0.47	3.41	8.50	-	-	-	-
	+37°C	173	5.36	2.76	3.01	8.20	0.0	.0760	12.20	-
		298	5.43	0.37	3.70	8.10	0.0	.0641	14.60	-
		525	5.42	0.44	4.47	7.48	-	.0605	15.50	-
		720	5.45	0.08	5.46	7.92	-	-	-	-
		1440	5.49	0.13	3.94	8.01	-	.0521	18.20	9.26
		2880	5.45	0.13	4.20	7.39	-	-	-	-
10	-	13	6.30	5.54	2.00	4.59	5.6	.0311	31.20	-
	+4°C	120	6.31	3.83	2.08	4.78	3.5	.0407	23.60	-
		253	6.27	1.68	2.24	7.48	5.1	.0383	25.10	-
		493	5.97	3.02	2.65	5.05	1.1	.0258	37.80	-
		733	5.83	3.26	2.92	5.66	0.0	-	-	-
		1440	5.46	0.58	3.50	8.56	0.0	.0276	35.20	9.40
		2880	5.50	0.27	3.10	8.71	-	-	-	-

[a] ATP_2 determined by enzymic assay. Initial values for $ATP_{(1)}$ (acid hydrolyses) for carcasses 6 through 10 and 11Y were 1.2, 10.3, 1.8, 8.9, 5.6 and 10.3 micromoles/g of tissue, respectively.

Appendix B. Table 2. Post-mortem levels of pH, glycogen, glucose, lactic acid, ATP, fibrillar protein content, and water-binding capacity for each side of pork carcasses 1 through 10 and 11Y. (continued)

Carcass	Treatment	Time post-mortem (mins)	pH	Glycogen (mg/g)	Glucose (mg/g)	Lactic acid (mg/g)	ATP_2[a] (micro-moles/g)	g fibrillar protein per g precipitate	g H_2O per g fibrillar protein	Precipitated fibril protein as % of sample wt.
10	+37°C	133	5.53	1.94	2.85	8.57	0.0	.0455	21.00	-
		253	5.55	0.32	3.54	8.09	0.0	.0767	12.00	-
		493	5.50	0.23	4.78	7.43	-	.0838	10.90	-
		733	5.30	0.25	4.68	7.88	-	-	-	-
		1440	5.51	0.07	2.83	8.34	-	.0796	11.60	11.04
		2880	5.54	0.15	4.10	7.34	-	-	-	-
11Y	-	12	6.55	6.34	1.96	2.85	10.3	.0287	33.80	-
	+4°C	-	-	-	-	-	-	-	-	-
		-	-	-	-	-	-	--	-	-
		266	6.34	2.95	1.96	5.93	0.0	.0222	44.10	-
		-	-	-	-	-	-	-	-	-
		1440	5.73	0.69	3.36	6.81	-	.0228	42.90	7.27
		2880	5.73	-	-	-	-	-	-	-
	+37°C	-	-	-	-	-	-	-	-	-
		-	-	-	-	-	-	-	-	-
		266	5.40	0.23	2.99	8.06	0.0	.0581	16.20	-
		-	-	-	-	-	-	-	-	-
		1440	5.47	0.20	3.30	7.66	-	.0856	10.70	15.07
		2880	5.47	-	-	-	-	-	-	-

[a] ATP_2 determined by enzymic assay. Initial values for $ATP_{(1)}$ (acid hydrolyses) for carcasses 6 through 10 and 11Y were 1.2, 10.3, 1.8, 8.9, 5.6 and 10.3 micromoles/g of tissue, respectively.

Appendix B. Table 3. Post-mortem levels of glucose, glycogen, lactic acid, ATP and pH for each side of carcasses A through G[1]

Carcass	Treatment	Time post-mortem (mins)	pH	Glycogen[2] (mg/g)	Glucose[3] (mg/g)	Lactic acid (mg/g)	ATP[4] (micro-moles/g)
A	-	10	6.69	14.08	2.22	4.21	-
	-29°C	187	6.43	14.26	0.98	4.77	0.0
		329	6.35	9.31	2.70	3.90	0.0
		1440	5.72	8.36	3.22	7.33	-
		2880	5.52	-	-	-	-
	>37°C	165	5.42	4.79	3.56	7.93	0.0
		312	5.34	4.36	5.72	8.08	0.0
		1440	5.33	00.75	9.30	7.73	-
B	-	11	6.61	2.86	2.09	3.84	3.6
	-29°C	186	6.10	1.17	2.54	5.84	0.0
		333	6.29	0.90	3.36	5.14	0.0
		1440	5.80	0.00	2.41	6.22	-
		2880	5.67	-	-	-	-
	>37°C	172	5.70	0.10	1.52	7.48	0.0
		322	5.64	0.28	1.80	6.60	0.0
		1440	5.67	0.12	1.77	7.64	-
C	-	10	6.86	20.13	1.65	1.71	3.8
	-29°C	70	6.79	-	-	-	-
		180	6.45	21.89	0.78	1.66	4.0
		330	6.42	17.82	2.96	4.22	1.6
		1440	5.68	13.80	3.93	6.81	0.0
		2880	5.52	-	-	-	-
	>37°C	63	6.40	-	-	-	0.0
		170	5.38	9.34	4.24	7.04	0.0
		343	5.31	8.72	6.30	6.79	-
		1440	5.34	5.89	8.58	6.67	-

[1]All chemical values expressed on a per gram of fresh tissue basis.
[2]Expressed in glucose-equivalents.
[3]Total reducing sugars expressed in glucose equivalents.
[4]Determined enzymatically.

Appendix B. Table 3. Post-mortem levels of glucose, glycogen, lactic acid, ATP and pH for each side of carcasses A through G[1] (continued)

Carcass	Treatment	Time post-mortem (mins)	pH	Glycogen[2] (mg/g)	Glucose[3] (mg/g)	Lactic acid (mg/g)	ATP[4] (micro-moles/g)
D	-	10	6.79	9.39	2.29	2.53	7.2
	-29°C	70	6.84	-	-	-	-
		181	6.56	9.32	1.42	3.43	4.0
		346	6.35	5.35	2.68	4.26	1.0
		1440	5.67	2.15	2.25	6.34	0.0
		2880	5.49	-	-	-	-
	>37°C	72	6.52	-	-	-	-
		169	5.49	1.16	3.80	7.52	0.0
		348	5.36	0.22	3.90	7.42	-
		1440	5.40	0.00	4.08	7.38	-
E		10	6.69	10.33	2.26	2.45	3.5
	-29°C	89	6.54	-	-	-	-
		160	6.57	8.66	2.06	3.72	4.8
		312	6.45	4.57	3.73	3.96	2.8
		1440	5.61	2.37	2.26	6.44	0.0
		2880	5.47	-	-	-	-
	>37°C	85	6.43	-	-	-	-
		170	5.37	0.49	3.64	6.48	0.0
		289	5.37	0.39	4.30	6.51	-
		1440	5.33	0.14	4.70	6.77	-
F	-	14	6.78	17.59	1.83	2.63	8.3
	-29°C	75	6.62	-	-	-	-
		172	6.72	16.77	2.76	2.07	7.2
		298	6.59	17.13	1.76	3.49	5.7
		1440	5.66	10.15	2.44	6.81	0.3
		2880	5.70	-	-	-	-
	>37°C	74	6.40	-	-	-	-
		164	5.32	8.81	3.55	6.76	0.0
		290	5.25	6.43	5.44	7.80	-
		1440	5.23	2.40	7.74	7.13	-

[1]All chemical values expressed on a per gram of fresh tissue basis.
[2]Expressed in glucose-equivalents.
[3]Total reducing sugars expressed in glucose equivalents.
[4]Determined enzymatically.

Appendix B. Table 3. Post-mortem levels of glucose, glycogen, lactic acid, ATP and pH for each side of carcasses A through G[1] (continued)

Carcass	Treatment	Time post-mortem (mins)	pH	Glycogen[2] (mg/g)	Glucose[3] (mg/g)	Lactic acid (mg/g)	ATP[4] (micro-moles/g)
G	-	13	6.78	21.22	2.40	2.42	7.4
	-29°C	74	6.84	-	-	-	-
		175	6.66	20.93	3.00	3.40	5.9
		302	6.45	17.75	3.14	4.23	0.0
		1440	5.62	14.21	2.48	7.88	-
		2880	5.60	-	-	-	-
	>37°C	60	6.35	-	-	-	-
		166	5.38	10.18	4.14	7.34	0.0
		293	5.28	5.60	6.56	7.40	-
		1440	5.30	4.62	8.51	7.60	-

[1]All chemical values expressed on a per gram of fresh tissue basis.
[2]Expressed in glucose-equivalents.
[3]Total reducing sugars expressed in glucose equivalents.
[4]Determined enzymatically.