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QUALITATIVE PROPERTIES OF BEEF FROM BULLS AND STEERS

By

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ABSTRACT

QUALITATIVE PROPERTIES OF BEEF FROM BULLS AND STEERS

By

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Three experiments were conducted to evaluate the effects of dietary regimen, Ralgro implants and early postmortem storage temperature on various qualitative properties of beef from bulls and steers. Ralgro implants had no effect (P>.05) on performance, carcass traits, palatability traits or total and soluble collagen of beef-type bulls. Low energy diets decreased (P<.05) live weight, average daily gain, feed efficiency, carcass fatness and quality grades and panel tenderness and overall acceptability of Holstein bulls. Diet had no effects (P>.05) on juiciness, flavor, shear force values or total and soluble collagen. While high temperature aging had no effect (P>.05) on acidic and neutral proteinase activities, it enhanced (P<.05) alkaline proteinase activity. Acidic and neutral proteinase activities were lowest (P<.05) from 16 to 48 h postmortem and increased slightly by 168 h, but alkaline proteinase activity was not affected (P>.05) by time postmortem. Aging temperature did not affect meat tenderness (P>.05).

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INTRODUCTION

The bulk of existing data suggest that intensive feeding improves the tenderness of meat (Zinn et al., 1970a; Bowling et al., 1977; Aberle et al.,1981; Tatum, 1981). This effect is indirect, probably by increasing carcass weight and fatness, thereby, reducing the deleterious effects of rapid postmortem chilling practices (Smith et al., 1976). It is also suggested that intensive feeding increases collagen solubility (Aberle et al., 1981; Wu et al., 1981b) which is thought to be more related to differences in tenderness than is collagen content (Carmichael and Lawrie, 1967; Cross et al., 1973b; Berry et al., 1974).

Diet-health controversies involving meat and animal fats have received tremendous publicity, and consumers are concerned about the relations between life-threatening diseases and the consumption of fat meat (Dikeman, 1982). Thus, because of this and other economic considerations, there is a growing consumer demand for

meat that has less fat, yet, is palatable, healthful and inexpensive. This demand will intensify rather than diminish according to Kauffman (1982). Because of these changes in consumer desires and the escalating costs of production, it is impractical to adhere to the practice of intensive feeding to produce expensive fat insulation. (Tatum, 1981). Shortening of the finishing period, production of leaner carcasses and the use of technology appear inevitable to produce desirable quality meat.

Bull carcasses constitute a potential solution to this dilemma. It is generally accepted that bulls are superior to steers in average daily gain, feed efficiency and in decreased amounts of fat (Turton, 1969; Field, 1971; Seideman et al., 1982). Although steers are generally superior to bulls in tenderness (Field, 1971; Seideman et al., 1982), the differences are small when compared at young ages (13 to 14 mo) (Field et al., 1966; Rhodes, 1969). Differences in flavor and juiciness ratings are generally small or nonexistent (Field, 1971; Seideman et al., 1982). Dairy bulls offer a great potential for beef production because they may be considered as a by-product and, therefore, lack sensitivity to supply and demand of beef (Cartwright, 1983).

Hormonal growth stimulants are believed to shorten the number of days on feed (Hathaway et al.,

1973), improve the daily gain of bulls (Lamm et al., 1980; Johnson et al., 1984) and improve the quality grade of bulls (Wierbicki et al., 1956; Raltson, 1978; Johnson et al., 1984). On the other hand, they do not affect meat tenderness (Forrest, 1975; Gregory et al., 1983; Johnson et al., 1984). Growth stimulants can be used to improve daily gains, shorten the number of days on feed and to produce a suitable amount of fat cover on bull carcasses to offset rapid chilling effects without being counterproductive to the leanness of meat.

This study has been undertaken to investigate: 1) The effect of hormonal implants on the quality and quantity of meat from beef-type bulls; 2) The acceptability of meat from Holstein bulls as affected by dietary energy density; 3) The effects of dietary energy density and hormonal implants on collagen content and solubility in young bulls.

REVIEW OF LITERATURE

Factors Influencing Meat Quality

Tenderness is probably the most important quality attribute to the consumer of meat (Mackintosh et al., 1936; Cover et al., 1944; Hiner et al., 1955; Parrish et al., 1962; Deatherage, 1963; Jeremiah and Martin, 1982). Since flavor and juiciness are less variable and can be compensated for by the use of spices, tenderness remains the main criterion for judging quality. For these reasons research workers have devoted considerable effort to the study of factors involved in tenderness of meat. The first attempts were possibly those of a group of workers of the Hygiene Institute of Wurzburg, Germany, i.e. Schindler (1895), Schepilewsky(1899) and Lehmann(1907), (cited by Mackintosh et al., 1936) who obtained evidence that tenderness is closely related to the connective tissue content.

Since then, research workers have been concerned with the problem of meat quality. The attempts made have been twofold: 1) to establish ways and means of measuring meat quality, especially tenderness, and 2) to identify the factors that contribute to differences in meat tenderness. Excellent reviews have been written during the past

several decades by Blumer(1963), Pearson(1966), Szczesniak and Torgeson(1965), and Asghar and Pearson(1980). These authors reviewed the work on muscle structure and chemistry as it related to tenderness. Asghar and Pearson(1980) successfully categorized the factors contributing to meat tenderness into structural factors, chemical factors, biophysical factors and factors related to ante- and post-mortem treatments. The structural factors (i.e., size of the muscle bundles, muscle fiber diameter, sarcomere length and wavy fibers) and the biophysical factors (i.e., muscle pH, total water content, water holding capacity and expressible juice) will not be included in this review, since none of these measurements were made in this study.

Chemical factors

Fat. Considerable emphasis has been placed on the relationship of fat content to quality characteristics of meat. The results are contradictory. Helser et al. (1930) reported that fat animals were more tender and juicier than unfinished ones. Panelists, however, favored the flavor and aroma of roasts from animals without much finish. They suggested that the additional fat on the roast seemed to add a very pronounced fatty odor and

flavor, which was not pleasing to the majority of the panelists. Barbella et al. (1939) reported opposite results regarding the desirability of the flavor. Mackintosh et al. (1936) observed that changes in tenderness seem to be related to carcass grade and quantity of marbling. Husaini et al. (1950a) reported a low but significant correlation between marbling and tenderness. Cover et al. (1956) measured fatness by physical separation, marbling scores and quantity of ether extract, and reported that tenderness and juiciness were more closely associated with ether extract than with other fat measurements. Palmer et al. (1958) reported a highly significant correlation between marbling and tenderness. Other workers substantiated the importance of marbling as a predictor of tenderness (Batcher and Dawson, 1960; Zessin et al., 1961; Murphy and Carlin, 1961; Weir et al., 1962; Henry et al., 1963). Field et al. (1966) observed that higher marbling scores in bulls were closely associated with higher sensory tenderness ratings than those in steers and heifers when age is held constant. Smith et al. (1970) reported that increasing percentage of intramuscular fat was correlated with higher ratings for juiciness, tenderness and overall satisfaction in ovine primal cuts. However, intramuscular fat content did not appear to be consistently related to flavor desirability. Berry et al. (1974) reported that increased percentage of fat was

associated with increased tenderness. More recently the work of Westing and Matsushima (1976) and Jennings et al. (1976,1978) emphasized the positive relationship between the degree of marbling and tenderness.

Hostetler et al. (1936) have shown that fatness is not an important indicator of the quality attributes of beef. This finding is further supported by the work of Hankins and Ellis (1939), who obtained strong evidence that variations in tenderness are caused mainly by factors other than fatness. This same conclusion has been reported by Ramsbottom et al. (1945). Cover et al. (1944) concluded that fatness as an indicator of tenderness in lamb may be regarded as of doubtful practical value. Husaini et al. (1950b) found no correlation between tenderness at 3 or 15 days postmortem and carcass grade or intramuscular fat content. This is in contradiction to their earlier work (Husaini et al.,1950a). Wierbicki et al. (1955,1956) failed to obtain a relationship between marbling and tenderness. They decided that marbling is a sex and breed associated characteristic. This decision was later confirmed by Sleeth et al. (1958). The extensive study of Cover et al. (1958) involving 203 steers also supported these findings. This group of workers reported that coefficients of correlation for tenderness with carcass grade, separable fat and ether extract were low and inconsistent

as to sign. Judge et al. (1960) also failed to find a significant correlation between marbling and tenderness and they concluded that marbling was related to juiciness in pork. In lamb muscle, Batcher et al. (1962) reported that neither the amount of separable fat nor intramuscular fat content affected tenderness,juiciness or flavor scores of the cooked meat. The association between marbling and tenderness was found to vary with animal age. Tuma et al.(1962) reported that marbling did not enhance tenderness of steaks from 18-month-old cattle, but in the 42- and 90- month-old cattle, tenderness was more highly associated with higher degrees of marbling. On the other hand, they found that taste panel flavor and juiciness did not appear to be related to marbling score. Others have confirmed that marbling is more related to juiciness than to tenderness in pork (Onate and Carlin, 1963) and in beef (Romans et al., 1965).

The work of Suess et al. (1966b) clearly indicated the very low relationship of marbling to meat quality. They showed that marbling, grade and bone maturity, whether considered simultaneously or singly, were associated with less than 7% and 11% of the variation in tenderness and juiciness, respectively. These observations are supported by Crouse et al. (1978) who reported that the percentage of intramuscular fat in longissimus muscle and adjusted fat thickness independently accounted for only 2 to 3% and

6 to 8% of the variation in tenderness and juiciness, respectively. They suggested that an increase of 30 units in marbling is required to increase panel tenderness one unit. These ideas were substantiated recently by Tatum et al. (1982) who suggested that large differences in marbling would be required to effect a detectable change in palatability. Jost et al. (1983) reported that marbling alone accounted for only .4% of the variation in tenderness and did not remain in the predictive equation for estimating flavor or juiciness. Others, however, failed to find a close association between intramuscular fat and tenderness or juiciness (Cross et al.,1973b; Reagan et al., 1975).

Connective Tissue Proper. Connective tissue proper is an inherent component of meat. It constitutes the fibrous network that holds the muscle fibers, other cells and structures (vascular and nerve elements) found in muscle in place. Connective tissue proper is made up of a sparse population of cells, ground substance and the fibers (mainly collagen and to a much lesser extent, elastin). The ground substance, which is composed of glycosaminoglycans and proteoglycans, has received almost no attention as to its relationship to meat quality. Collagen is the most abundant constituent of connective

tissue proper in muscle and has been found to be associated with toughness of meat.

The early work was focused on the total amount of collagen present in muscle. The first attempts to separate collagen quantitatively from muscle were those of the German group of workers (Schindler, 1895; Schepilewsky, 1899; Lehman, 1907) who were cited by Mackintosh et al. (1936). They devised a mechanical and a chemical method for the separation of connective tissue from the rest of the flesh and concluded that tenderness was closely related to the connective tissue content of the flesh. This finding seemed to fascinate many researchers. Mackintosh et al. (1936) obtained a low but significant correlation between collagen nitrogen and panel tenderness as well as between shear values and collagen nitrogen. They concluded that the three variables were measuring some factor related to tenderness and probably the same property. Hiner et al. (1955) concluded that both collagen and elastin fibers are factors influencing tenderness. Nottingham (1956) confirmed these findings and reported a high correlation between connective tissue content and shear force measured parallel to the fibers. Other workers used hydroxyproline as a measure for the amount of connective tissue. Parrish et al. (1962) suggested that hydroxyproline was a better measure of tenderness in low-

quality steaks than in high-quality steaks. Prost et al. (1975a) reported large difference in hydroxyproline due to the carcass quality grade but failed to obtain differences due to the age or sex of the animals.

Many dissenting views are reported regarding the lack of significant correlations between collagen content and tenderness of meat. Mitchell et al. (1928) observed nonsignificant differences in connective tissue content due to sex or age of the animals. They also found no relationship between the ordinary market grade of beef carcasses and the connective tissue content of the lean of the ribeye or of the round muscles. They concluded that insofar as the tenderness of meat is related to its content of connective tissue, their results lend no support to the belief that the appearance, texture and firmness of its lean gives reliable information concerning its tenderness. Those conclusions are substantiated by Ramsbottom et al. (1945), Hershberger et al. (1951), Wilson et al. (1954), Griswold (1958a,b), Paul (1962a,b), Carpenter et al. (1963), and Ritchey and Hostetler (1964). Cover and Smith (1956) reported that collagen content was associated with tenderness only when the same muscle was cooked by different methods but not when different muscles were compared by the same method of cooking. Shrimpton and Miller (1960) could not explain differences in tenderness of broilers by their connective tissue content. Other

workers, using hydroxyproline as a measure of collagen content, found that it was not a critical measure of tenderness (Wierbicki et al. 1955; Hunsley et al. 1967; 1971). Field (1968) and Field et al. (1970b) reported nonsignificant correlations between tenderness measures and either collagen content, labile collagen or epimysial thickness. Purchas (1972) showed that a 1% increase in nonconnective tissue tenderness produced the same result as a 10% increase in connective tissue tenderness.

Another approach used by researchers is measuring the alkali-insoluble proteins as an index of connective tissue. Husaini et al. (1950a,b) obtained a high correlation between tenderness and alkali-insoluble proteins. They concluded that connective tissue as represented by alkali-insoluble protein is in part responsible for tenderness differences. Loyd and Hiner (1959) measured the hydroxyproline content of the alkali-insoluble protein fraction and showed a significant correlation of this measure with two measurements of tenderness. A dissenting view was reported by McClain et al. (1965b) who did not find significant differences in alkali-insoluble collagen between tenderness groups

Wilson et al. (1954) reported that samples from steers and cows contained less collagen and elastin than veal, although veal was more tender than the older groups.

They discussed the probability that veal connective tissue might have different chemical or physical properties. This was confirmed by the findings that total collagen is not related to age of the animal (Kauffman et al.1964; Ritchey et al.1964; Hunsley et al. 1967,1971; Reagan et al., 1976). These observations together with the lack of significant relationship between collagen content and tenderness drew the attention to the properties of collagen. Therefore, collagen solubility and cross-linking were extensively studied. Kauffman et al. (1964) concluded that tenderness measures were not significantly related to collagen solubility or the amount of total connective tissue. McClain et al. (1965a) reported that although age difference in soluble collagen were evident, acid- and salt- soluble collagen in foreshank muscles of cattle did not differ significantly between tender and less tender groups. Smith et al. (1970) confirmed these conclusions and reported no correlation between tenderness and collagen solubility. However, Cross et al.(1972) reported a significant correlation between tenderness and the percentage of soluble collagen. Cross et al. (1973a,b) concluded that the percentage of soluble collagen was more closely related to panel scores for the amount of detectable connective tissue than was total collagen. Berry et al. (1974), however, associated both decreased collagen content and increased percentage of soluble

collagen with increases in beef tenderness. Reagan et al. (1975) concluded that collagen content was closely related to subjective measures, while collagen solubility was more closely related to objective measures of tenderness. Other workers have successfully correlated tenderness with collagen solubility (Herring et al., 1967; Field and Pearson 1969; Field et al.,1970c; Williams and Harrisson, 1978). Most recently Wu et al. (1981b) showed that high energy diets increased collagen solubility but they failed to find differences in quality measures due to feeding regimen. Hall and Hunt (1982), however, reported a small and inconsistent effect of dietary regimen on total and soluble collagen, and found a nonsignificant correlation between measures of collagen solubility and palatability traits.

Earlier, the excellent work of Goll et al. (1962;1963;1964a,c,d) on the age-associated changes in the properties of bovine collagen, clearly indicated the existence of more frequent or stronger cross-linkages within and among the molecules of more mature collagen. Such cross-links may affect the rate of solubilization of collagen and thereby exert an effect upon meat tenderness independent of the total amount of connective tissue present. Other workers confirmed those findings (Hill,1966; Carmichael and Lawrie,1967; Kruggel et al.,1970;

Shimokomaki et al.,1972; McClain,1976). Other workers have reported on the effects of aging and heating on the properties of collagen. Winegarden et al.(1952) interpreted their results to mean that cooking steaks and roasts to rare and medium doneness (55 to 65 C) brings about little, if any, change in the connective tissues of the muscles studied. They also reported that aging connective tissues for 35 days as compared to 10 days increased the tenderness of the connective tissue only slightly. deFremery and Streeter al. (1969) concluded that postmortem tenderization of chicken meat is not related to changes in connective tissue. Although McClain et al. (1970) found a 50% and a 34% decrease in yield of the bovine and porcine intramuscular connective tissue, respectively, after 72 h of aging, they did not find marked differences in the heat absorbed during thermal shrinkage or the thermal shrinkage temperature of the connective tissue isolated. They concluded that the connective tissue isolated had not been greatly altered. Kruggel and Field (1971) reported a decrease in the amount of soluble collagen from muscles aged 21 d. They suggested that this may be due to molecular changes in intramuscular connective tissue, and that stretching may have exposed the collagen molecules to physical or enzymatic changes resulting in the cleavage of cross-links. Those results were generally confirmed by Pfeiffer et al. (1972). Other

workers, however, reported that postmortem aging had no effect on connective tissue solubility and that the tenderization brought about by aging is not accompanied by significant changes in collagen solubility (Pierson and Fox,1976; Jerimiah and Martin,1982). Thermal behavior of connective tissue was also considered by many workers. Winegarden et al. (1952) reported that connective tissue softened, lost weight, shortened, decreased in width, and increased in thickness during heating, and that the degree of changes increased as the time and temperature of heating were increased, and as the collagen content of the tissues increased. Later Goll et al. (1962) found the thermal shrinkage temperature increased with animal age, i.e., 55 C for veal to 70 C for aged cows. Ritchey and Cover (1962) and Ritchey et al. (1963) reported a considerable decrease in collagen nitrogen as cooking temperature was increased. Field et al. (1970a) observed a decrease in the melting point of epimysial and intramuscular collagen after 24 h of aging. Recently, Judge et al. (1981) found a decrease in the thermal shrinkage temperature of bovine collagen due to carcass electrical stimulation. They proposed hat this reduction in thermal stability may result from a decrease in the number or strength of collagen cross-links. Judge and Aberle (1982) observed an increase in the thermal

shrinkage temperature of bovine intramuscular collagen due to the age of the animal, and a decline due to postmortem aging.

Intracellular Components. As the problem of tenderness has been attacked on many fronts, the role of the intracellular components has been studied. Those include intracellular proteins, nonprotein nitrogenous compounds and ions. Husaini et al. (1950a,b) failed to find any correlation between tenderness and soluble protein fractions. Wierbicki et al. (1954) using Sorensen's buffer for extraction observed a relation between tenderness and the percentage of total nitrogen extracted. They suggested that increases in tenderness with postmortem aging may be related to the dissociation of actomyosin or some similiar protein changes which increase protein extractability. Later work by Wierbicki et al. (1956) did not support this suggestion. They presented evidence which indicated that tenderness may be related to the degree of hydration of meat proteins. Carpenter et al. (1961) concluded that extractable nitrogen was not a reliable indicator of tenderness. Hill (1962) compared the fiber composition of tough and tender muscles from beef, lamb and pigs. He suggested that, on a stroma-nitrogen-free basis, the greater myofibrillar nitrogen content and

the smaller sarcoplasmic nitrogen content are to some extent reflected in greater toughness of the beef semitendinosus compared to the longissimus muscle. Extending his argument he suggested that the greater sarcoplasmic and lower myofibrillar nitrogen contents of lamb and pig muscles, when compared with beef muscles, are reflected to some degree in the greater tenderness of the muscles of these two species.

Hegarty et al.(1963) indicated that myofibrillar protein solubility was highly correlated with tenderness as determined by shear and panel measurements. Aberle and Merkel (1966) indicated that the amount of extractable myofibrillar nitrogen was negatively related to Warner-Bratzler shear values as aging proceeded. Similiar results were reported by Khan and VanDenBerg (1964), Dikeman et al. (1970), and Ma and Addis (1973). Goll et al. (1964b), however, stated that protein solubility did not appear to be related to tenderness, thereby, confirming the reports of Husaini et al. (1950a,b). Other workers reported that the myofibrillar proteins are in some way related to the tenderness of meat (Purchas, 1972; Cross et al., 1973a,b; Marsh, 1977). Muscle hemoglobin and myoglobin are also studied as possible determinants of tenderness. Although Husaini et al. (1950b) detected no relation of muscle hemoglobin to tenderness after 3 d postmortem, they

observed a significant correlation at 15 d as tenderness increased between 3 and 15 d Wierbicki et al. (1955) reported that color (cyanometmyoglobin) showed no relationship to tenderness, except that it may reflect some age or sex differences. Tuma et al. (1963) confirmed this finding by measuring color by Photovolt Reflectometer. Other reports claimed that a significant but an inconsistent relationship exists between muscle color (myoglobin) and tenderness (Martin et al.,1971; Berry et al.,1974). Some of the enzymes involved in the cell metabolism also have been studied. DeFremery and Pool (1963) reported that intravenous injections of sodium iodoacetate, which inhibits phosphoglyceraldehyde dehydrogenase, resulted in poultry meat which was more tender without aging. Although Lee et al. (1976) could not find any relevance of creatine phosphokinase (CPK) and lactate dehydrogenase (LDH) concentrations to the tenderness of broilers; Martin et al. (1977) reported that plasma cocentrations of CPK at slaughter were significantly less for the tough group. Anglemier et al. (1961) reported a significant correlation of .78 between tenderness and alkaline phosphatase activity.

Nonprotein nitrogenous compounds present in muscle tissue also has received a great deal of attention as to their role in explaining differences in tenderness. Husaini et al. (1950a) failed to identify any correlation

between tenderness scores and the nonprotein nitrogen fraction. Khan and VanDenBerg (1964) observed a decrease in the nonprotein nitrogen fraction during the onset of rigor mortis. They postulated that during the post-rigor tenderization period amino acids and peptides increased in meat as a result of proteolysis. Aberle and Merkel (1966) reported that soluble nonprotein nitrogen increased significantly during postmortem aging. Earlier Ma et al. (1961) observed that the tender longissimus and psoas muscles contained more leucine-isoleucine than the less tender semitendinosus.

Ante- and Postmortem Factors

Breed and Sex Effects. The relation between breeding and tenderness has long been an issue of debate. Differences in palatability factors due to breeding were reported by (Purchas,1972; Luckett et al.,1975b; Winer et al.,1981). In all three studies the Brahman breed was involved. It is noticeable in the literature that differences in tenderness exists between breeds whenever Brahman or crosses involving Brahman were studied and compared with other breeds. Wheeling et al. (1975) reported significantly lower shear values for Hereford x Angus steers when compared to Galloway x Hereford-Angus, Brown

Swiss x Hereford, or Charolais x Hereford steers. However, sensory panel ratings for tenderness, flavor and juiciness were not significantly different between those crosses. Other reports, however, suggested no significant differences in palatability attributes due to breeding (Husaini et al.,1950b; Jeremiah et al.,1976; Jeremiah and Martin,1977; Berry et al,1977; Lewis et al.,1977; May et al.,1977; Johnson et al.,1984). It is interesting to note that Suess et al. (1966a) reported significant sire differences in tenderness for the semimembranosus but not for the longissimus muscle; conversely significant sire differences existed for longissimus juiciness but not for the semimembranosus. Jeremiah and Martin (1982) reported that bovine breeds may differ in total intramuscular collagen content of certain muscles, however such differences may not be of practical significance.

The consumer demand for lean, yet tender meat, and excess fat from steers and heifers compared to bull carcasses have attracted attention to the effects of sex on meat quality and quantity. Many workers assumed that sex condition has little effect on tenderness and that the differences are perhaps not commensurate with increased cost of producing meat from castrates, (Wierbicki et al.,1955,1956; Palmer,1963; Suess et al.,1966a; Warwick et al,1970; Prost et al.,1975b; Arthaud et al.,1977; Landon et al.,1978). Others have observed that steers produce

more marbling, higher grades and more tender meat than bulls. Hunsley et al. (1967,1971) reported that age and sex are of equal importance in affecting tenderness. Field et al. (1966) could not detect any significant differences between bulls and steers or heifers in the age range of 300 to 399 d. Beyond that range, however, differences started to widen in favor of steers and heifers. Riley et al. (1983) indicated that bulls and steers which had at least 7.6mm fat thickness did not differ in 28 out of 30 possible comparisons of palatability characteristics. Differences in the connective tissue content between sexes were not evident to Mitchell et al. (1928) and Prost et al. (1975a). Most studies show that intact males are superior to castrates and females in cutability, feed efficiency and the amount of lean to fat, and that differences in tenderness are very small to hinder the production of meat from intact males (Turton,1969; Brannang,1969; Rhodes,1969; Field,1971; Seideman et al.,1982).

Nutritional Effects. With few exceptions, there seems to be a general agreement between researchers that high planes of nutrition produce carcass with greater fat cover, higher marbling scores, larger ribeye areas and higher quality grades. Animals normally perform well

under high plane of nutrition, gain faster and dress out higher than animals on low planes of nutrition. Chemically, carcasses from animals fed on high planes of nutrition, have higher ether extract and lower moisture in their longissimus muscles than animals on lower planes of nutrition (Wanderstock and Miller,1948; Jacobson and Fenton,1956; Oltjen et al.,1971; Utley et al.,1975; Wheeling et al.,1975; Dinius et al.,1976; Bowling et al.,1977; Dinius and Cross,1978; Harrison et al.,1978; Summers et al.,1978; Young and Kauffman,1978; Aberle et al.,1981; Bidner et al.,1981; Ridenour et al.,1982). Hershberger et al. (1951) reported no relationship between the rate of gain and quality grades or yield of edible meat in cattle. Bidner et al. (1981) reported that although grain feeding increased subcutaneous fat and marbling, it did not significantly influence quality or yield grades. In contrast, Ridenour et al. (1982) reported that a high concentrate diet throughout the growing and finishing phase did not significantly affect slaughter and carcass weight, fat thickness, skeletal and lean maturity or yield grade. No significant differences in animal performance or carcass characteristics due to protein sources (Greathouse et al.,1974) or protein level (Kelly et al.,1963) have been reported. Increased length of time on high energey diets has been found to increase the percentages of crude protein, ether extract, crude ash,

and to decrease moisture (Zinn et al.,1963). Zinn et al.(1970a) found that the length of time on feed increased average daily gain up to 180 d, and marbling and carcass grade up to 240 d.

The effect of feeding regimen on the quality characteristics of meat is more controversial. A number of studies have indicated that high energy diets produced meat of superior quality traits. These traits include higher panel scores for tenderness, juiciness, flavor and overall desirability, lower shear force values, brighter lean color and whiter fat color. (Wanderstock and Miller,1948; Graham et al.,1959; Shrimpton and Miller,1960; Dube et al.,1971; Bowling et al.,1977; Summers et al.,1978; Aberle et al.,1981). It is interesting to note that several studies showed significant differences in tenderness due to nutritional regimen only when tenderness was measured by a taste panel rather than by shear force measurements (Meyer et al.,1960; Harrison et al.,1978). At least two studies showed a highly significant age x nutritional regimen (Graham et al.,1954) or age x d on feed (Zinn et al.,1970a) interactions that affected quality traits. This is conceivable because grass-finished cattle require more time (Oltjen et al.,1971; Bidner et al.,1981) to reach a certain end point, e.g., similiar weight.

On the other hand, a number of studies indicated nutritional regimen had no effect on meat tenderness, measured either subjectively or objectively, and that it had no effect on the other quality characteristics, i.e., juiciness, flavor intensity, color of fat, or cooking losses (Hershberger et al.,1951; Jacobson and Fenton,1956; Wheeling et al.,1975; Dinius et al.,1976; Dinius and Cross,1978; Young and Kauffman,1978; Smith et al.,1979; Burson et al.,1980; Bidner et al.,1981; Wu et al.,1981b; Hall and Hunt,1982; Crouse et al.,1984). Kelly et al. (1963) studied the effects of different levels of protein in the diet and reported that there was no marked or consistent effect of protein level on quality traits. Others conclude that feeding to similiar carcass composition (Young and Kauffman,1978) or adjusting to either constant weight or longissimus muscle fat content (Smith et al.,1977) masks the effects of nutrition on the quality attributes of meat.

An earlier investigation by Smith et al. (1976) suggested that deposition of increased quantities of subcutaneous or intramuscular fat increased tenderness via changes in postmortem chilling rate. Increased fat decreases the rate of temperature decline (insulation effect), enhances the activity of autolytic enzymes, lessens the extent of myofibrillar shortening and thereby increases the ultimate tenderness. Following this logic

Tatum (1981) questioned whether tenderness was nutritionally controlled. He reviewed the reports of several workers and concluded that although there is some indication that nutrition directly influences various intrinsic properties of postmortem muscle, the bulk of existing data suggest that intensive feeding improves tenderness by increasing carcass weight and fatness, thereby reducing the susceptibility to rapid postmortem chilling and cold-induced toughening.

Data on the effects of high energy feeding on collagen content and solubility are not consistent. Burson et al. (1981) reported that steaks from cattle fed low energy density diets for 91 d, or fed medium or high energy diets for 56 d had the least amount of detectable connective tissue compared to those from cattle fed submaintenance diets. Other studies suggested that feeding high energy diets resulted in higher collagen solubility in the longissimus muscle (Aberle et al.,1981; Wu et al.,1981b). Hall and Hunt (1982), however, concluded that dietary regimen had a small and inconsistent effect on total collagen and measures of collagen solubility.

Effects of Hormonal Growth Stimulants. Hormonal growth stimulants have been studied extensively. These include diethylstilbesterol, Compudose Synovex, melengestrol

acetate, Rapigain, Zeranol and Ralgro. The effect of these growth stimulants on daily gain and carcass composition has been well documented. Implantation with growth promotants has been reported to shorten days on feed by an average of 28 d (Hathaway et al.,1973), and 42 d (Greathouse et al.,1983). It is generally agreed that implantation improved the daily gain for steers and heifers (Ray et al.,1969; Hathaway et al.,1973; Nichols and Lesperance,1973; Nicholson et al.,1973; Ward et al.,1978; Lamm et al.,1980). For bulls, however, the results regarding daily gain and feed efficiency are inconsistent. Lamm et al.(1980) reported that during the growing-finishing period, daily gain was not affected by implant treatment. Greathouse et al.(1983) observed that average daily gain and feed efficiency of implanted bulls improved 6.5 to 10.4% and 7.9 to 8.1%, respectively. Hormone implantation generally increased fat deposition in bulls raising their quality grade (Wierbicki et al.,1956; Forrest,1975; Ralston,1978; Johnson et al.,1984) and decreased fat deposition in steers lowering their quality grades (Wierbicki et al.,1956; Hathaway et al.,1973; Forrest,1975). Gregory et al.(1983), however, did not observe differences in composition between unimplanted bulls and bulls implanted twice with 36mg Zeranol, i.e., at birth and 70 d later, or once with 72mg at birth. According to Ward et al. (1978) the time of implantation is

critical. These workers reported that steers receiving Ralgro implants twice during the nursing period were heavier than unimplanted steers, and that both steers and heifers responded significantly to implants during the growing phase. They also found that both heifers and steersdid not respond significantly to implants during the finishing phase. This finding was confirmed by the work of Lamm et al.(1980).

There is a general agreement that growth stimulants have no effect on palatability attributes. Wierbicki et al.(1956) stated that diethylstilbesterol treated animals tended to have slightly more hydroxyproline(collagen) in their meat and were slightly less tender than untreated controls. Greathouse et al. (1983), however, reported that tenderness was higher for Ralgro implanted bulls than for nonimplanted bulls. They also observed that the detectable connective tissue scores were higher in steaks from implanted bulls. Other studies showed no differences in quality factors between implanted and unimplanted animals (Forrest, 1975; Gregory et al., 1983; Johnson et al., 1984).

MATERIALS AND METHODS

1. Animals:

Sixty-four wholesale ribs from two different groups of bulls were used in this study. Sixteen ribs were obtained from seven Angus and nine Polled Hereford bulls which were on an experiment designed to evaluate the effect of Ralgro implantation on the carcass characteristics of intact bulls. Those bulls were born between February and May 1981. Eight bulls were randomly implanted with 36 mg Ralgro per animal at the start of the experiment and after 70 d on feed. The other eight bulls served as controls. All bulls received a diet consisting of corn silage, high moisture corn and a protein mineral supplement (table 1.1). The ten older bulls were on feed for 173 d and the six younger bulls were on feed for 204 d. The bulls were slaughtered at an average live body weight of 458.2 kg. Carcass measurements were taken after a 48 h chilling period. Rib roasts (9th to 12th rib) were removed from the right side of each animal, sealed in polythene bags and frozen in a -30 C blast freezer until used.

Table 1.1. COMPOSTION OF THE DIET FED TO THE CONTROL AND RALGRO IMPLANTED BEEF-TYPE BULLS[a]

Ingredient	Dry Matter %	Crude Protein %	% of Diet, DM Basis
Corn silage	33.4	8.0	49.9
High moisture corn	68.6	9.8	35.4
Protein-mineral supplement	89.8	48.3	14.7

Fomulation of Protein-mineral Supplement

Ingredient	Wt., kg
Soybean meal	848.18
Limestone	41.82
Trace mineral salt	15.45
Rumensin 60 premix	1.70
Vitamin A premix	1.02
Vitamin D premix	.91

a - bulls were fed in groups of 8.

Another 48 ribs were obtained from carcasses in an experiment designed to study the effect of dietary energy level on the performance of Holstein bulls. Holstein bull calves were purchased at 120 d of age. They were started on a fermented alfalfa halage diet for 15 d. Five bulls were killed and their ribs (9th to 12th rib) were retained to provide initial composition and to serve as an initial group. The rest of the bulls were assigned to one of four experimental diets. The facilities available did not allow handling more than 24 bulls at one time. The experiment was then repeated six times utilizing a total number of 144 bulls. Each time the 24 animals were divided between the four experimental diets, namely, 10%, 40%, 70% and 85% corn silage (dry matter basis). High moisture corn and a protein supplement were added to provide the rest of the dry matter. All bulls received 16% crude protein for the first 120 d, which was reduced to 13% thereafter until the end of the feeding period. Table 1.2 shows the energy density of these diets. All bulls were initially implanted with 36 mg Ralgro per animal. This implantation was performed to stimulate the average daily gain and to improve feed efficiency. Each time the 24 bulls were slaughtered, ribs from two animals from each of the four diets were saved for this study making a total number of

Table 1.2 ENERGY DENSITY OF THE FEEDLOT DIETS FED TO HOLSTEIN BULLS

Item	Corn Silage, % (DM)[a]			
	10	40	70	85
	Growing phase[b]			
Crude protein, %	16	16	16	16
NE m, Mcal/kg	.42	.39	.36	.34
NE g, Mcal/kg	.28	.28	.23	.22
	Finishing phase[c]			
Crude protein, %	13	13	13	13
NE m, Mcal/kg	.44	.40	.37	.34
NE g, Mcal/kg	.29	.26	.24	.22

[a] Bulls in the 85% silage group received no grain.

[b] Growing phase = from the start of experiment to 295.4 kg of live weight.

[c] Finishing phase = from 295.4 kg live weight to the end of experiment (290 d).

12 bulls from each of the four diets.

The bulls were killed in a commercial slaughter plant after 290 d on feed. All carcasses were electrically stimulated using 450 volts, 20 bursts of 2 sec duration each. Carcass measurements were obtained after a 48 h chilling period. Ribs (9th to 12th rib section) were removed from the right side of the carcasses, sealed in polythylene bags and frozen at -30 C until used.

2. Tenderness evaluation:

Four steaks (3.75 cm thick) were removed from each rib roast starting at the 12th rib, after the removal of the exposed end (approximately 1.2 cm on each end). The steaks were thawed overnight in a refrigerator at 4C. Three steaks were cooked to an internal temperature of 60C on a Farberware openhearth electric broiler. The steaks were then cooled to 4C overnight in a refrigerator. Two steaks were used for the subjective quality evaluations by a 12-member semi-trained taste panel. Three cores (2.5 cm diameter) were removed from each of the two steaks. Each core was divided into two pieces after removing the cooked surfaces. Four pieces of meat, representing each of four animals, were presented to each panelist in each session. Scoring was made for tenderness, juiciness, flavor and overall satisfaction, on a hedonic scale of 1 to 9 (9 =

extremely tender, juicy, flavorful or desirable and 1 = extremely tough, dry, bland or undesirable). The third steak was used for objective tenderness evaluation by a Warner-Bratzler Shear apparatus. Seven to ten cores (1.25 cm diameter) were removed from each steak and sheared once.

3. Proximate analysis:

The soft tissues (lean and fat) from the fourth steak were separated from the bone and ground using a Kitchenaid grinder equipped with a 4 mm plate. Grinding was performed three times to ensure sample homogeniety. The ground samples were kept in plastic bags in a 30 C freezer. Moisture, ether extractable fat and Kjeldahl nitrogen were determined according to the methods reported by AOAC (1980). Protein was determined by multiplying Kjeldahl nitrogen by 6.25. These determinations were expressed as percentages on wet sample weight basis.

4. Total and Soluble Collagen

The ground samples described above were thereafter powdered with dry ice in a -30 C freezer (IKA Universalmumle - M20 mill). This powdering was undertaken to ensure greater sample homogeneity. The extraction of the heat labile salt-soluble collagen was then performed by methods outlined by Hill (1966). For hydrolysis, an

equal volume of 12 N HCL was added to the supernatant. The residue was suspended in 10 ml distilled water and an equal volume of 12 N HCL was added. Thus, the final concentration of HCL was 6 N for the hydrolysis of both fraction. Samples were hydrolyzed in an autoclave (American Sterilizer Co.) for 6 h at 120C and 1.05 kg/cm^2 pressure. Samples were removed, allowed to cool and approximately 3g of activated charcoal (14 to 60 mesh) were added and the tubes shaken thoroughly. Contents of each tube were filtered through Whatman No. 2 paper into a flat bottom evaporation flask rinsing tubes and filler papers several times. Samples were then evaporated to dryness under vacuum with a rotary-evaporator at 70C. Five mL distilled water and 3 drops of .02% methyl red were added to each flask followed by titration to a milky color with 1.0 N NaOH. Samples were then filtered into Volumetric flasks through Whatman No. 2 paper rinsing flasks and paper with distilled water. Samples were diluted to contain between 1 and 5 mg hydroxyproline/mL. Hydroxyproline determination was performed by the methods outlined by Bergman and Loxley (1963). The chemicals used were obtained from Sigma Chemical Co., St. Louis, Mo. Hydroxyproline was converted to collagen by the factor 7.25 (Goll et al., (1963). Hydroxyproline content, total collagen and soluble collagen were expressed as mg/g on

wet tissue basis and on protein basis (Kjeldahl N x 6.25).

5. Statistical analysis

For the the first group of bulls, which received the Ralgro treatment, data were analyzed on a completely random design to compare the implanted and the unimplanted bulls. For the second group of bulls, which received the four experimental diets, the data were reduced on a randomized complete block design with time as the blocking factor because the experiment was repeated six times (Gill, 1978).

RESULTS AND DISCUSSION

I. Effects of Ralgro Implants on Performance, Carcass and Meat Quality characteristics of Bulls

Performance data of the control and Ralgro implanted bulls after 174 days are presented in Table 1.3. No differences were noted between the two groups of bulls in final live weight or average daily gain (ADG) ($P>.05$). These results agree with the observation of Lamm et al. (1980). Hathaway et al. (1973) observed that DES or Zeranol implants shortened days on feed by an average of 28 d. Their results were confirmed by other investigators (Nicholson et al., 1973; Greathouse et al., 1983) who observed that implants improved daily gain. The results presented here (Table 1.3) do not support these observations. Since the bulls are fed in groups, data on daily DM intake and feed efficiency represent pen averages. Therefore, statistical analysis on these data was not possible and no definitive conclusion can be stated. Carcass characteristics for the control and Ralgro implanted bulls are presented in Table 1.4. Differences between the two groups of bulls were not signifigant ($P>.05$) for any of the carcass measurements shown. These data agree with observations reported earlier (Lamm et al., 1980; Gregory et al., 1983). Other

Table 1.3 MEAN PERFORMANCE OF THE CONTROL AND RALGRO IMPLANTED BULLS DURING THE GROWING PERIOD[a]

Item	Control	Implants	SE[b]
Initial age,d	239.5	245.2	9.5
Initial live weight, kg	225.3	218.5	21.2
Final live weight, kg	457.1	455.7	25.9
Average daily gain, kg	1.33	1.36	.07
Daily DM intake, kg[c]	7.12	6.83	
Feed DM/gain, kg[c]	5.35	5.02	

[a] Measurements taken at 174 d after the start of of the feeding experiment.

[b] SE = standard error of a treatment mean.

[c] Animals are fed in two groups of 8. Means reflect pen averages, statistical analysis was not possible.

investigators have reported that hormonal treatment increased fat deposition in bulls and raised their quality grade (Wierbicki et al., 1956; Forrest, 1975; Johnson et al., 1984). However, there are differences between these studies as to the hormone or growth promotant used, the concentration and/or the frequency of implanting. Hathaway et al. (1973) reported that while DES decreased the fat percentage and increased the moisture percentage in steer carcasses, Zeranol seemed to have no effect on these traits. The results obtained by one hormone are not consistent with those of other hormones that are used. The season of the year during which the trial is performed appears to be important. Ray et al. (1969) compared MGA, DES, Synovex-H and Rapigain-1 in winter and summer trials. They concluded that none of these agents was effective in improving feedlot performance of steers and heifers in summer trials, while significant effects were observed in winter trials.

Meat quality characteristics for the control and Ralgro treated bulls are shown in table 1.5. Differences in panel tenderness, juiciness, flavor and overall desirability ratings were not significant ($P>.05$), neither were differences in shear force. These results are in general agreement with the bulk of evidence suggesting that hormonal treatment has no effect on meat quality factors (Forrest, 1975; Gregory et al., 1983; Johnson et

Table 1.4. MEAN EFFECTS OF RALGRO IMPLANTS ON THE CARCASS CHRACTERISTICS OF BEEF-TYPE BULLS[a]

Item	Control	Ralgro implants	SEof mean
Live weight, kg	457.9	458.5	16.9
Hot carcass weight, kg	279.5	281.8	12.8
Longissimus area, cm	78.4	81.4	4.0
Fat thickness cm	.76	.78	.06
Marbling score[b]	34	35	.9
Quality grade[c]	13	13	.5
Yield grade	2.1	2.1	.15

a No differences were found between means for all traits (P>.05).

b Marbling scores (devoid=0 to 10 - practically devoid=10 to 20 - traces=20 to 30 - slight= 30 to 40 .... etc).

c Carcass quality grades (Std 10 to 12, Gd 13 to 15, Choice 16 to 18, Prime 19 to 21).

al., 1984). The results presented here, however, disagree with those of Wierbicki et al. (1956) who observed that DES treated bulls and steers tended to be slightly less tender than their untreated counterparts. This observation was not consistent with their finding that DES tended to upgrade bull carcasses. Greathouse et al. (1983) reported that panel flavor intensity scores were higher for steaks from Ralgro implanted bulls than for those from nonimplanted bulls. They also observed that tenderness scores were higher for implanted bulls compared to unimplanted controls (slaughter weight 454 kg).

The discrepancy between the observations of this group and the results presented in Table 1.5 might be due to the frequency of implantation. Greathouse et al. (1983) implanted their bulls with 36 mg of Ralgro at average ages of 3, 123, 198, 324 and 425 d. Bulls in this experiment were implanted twice, i.e., at 242 d and at 312 d of age. Some chemical measurements on the 9th rib section of the control and Ralgro treated bulls are presented in Table 1.6. Again no effect of Ralgro treatment is evident for any of these measurements ($P>.05$). These results agree with others (Hathaway et al. 1973; Greathouse et al., 1983). Gregory et al. (1983) showed an increase in the fat percentage of the longissimus muscle when the bulls were implanted twice with 36 mg of Ralgro; i.e., at birth and at 70 d, as

Table 1.5. MEANS AND STANDARD ERRORS OF QUALITY CHARACTERISTICS FOR THE CONTROL AND RALGRO IMPLANTED BULLS[a]

Trait	Control	Ralgro Implants	SE
Panel tenderness[b]	6.51	6.65	.28
Panel juiciness[b]	6.29	6.44	.21
Panel flavor[b]	6.43	6.47	.1
Panel overall satisfaction[b]	6.48	6.48	.13
Warner-Bratzler shear force, kg	4.52	4.44	.43

[a] No differences were found for any of the measurements ($P>.05$).

[b] Hedonic scale (1 to 9) 1= extremely tough, dry, bland or undesirable; 9= extremely tender, juicy, flavorful or desirable.

compared with untreated bulls, but no differences in moisture or protein percentages were shown. Few studies have been conducted to investigate the effects of hormonal treatment on collagen content and solubility. The results presented here (Table 1.6) show no effect of Ralgro treatment on collagen content or salt-solubility as measured by hydroxyproline (P>.05). The results reported by Wierbicki et al.(1956) showed a tendency for increased hydroxyproline content with DES implantation. This is, however, only a trend with weak significance. Other investigators reported no significant differences in amount of panel detectable connective tissue between controls and implanted bulls (Gregory et al., 1983; Johnson et al., 1984).

Simple correlation coefficients between meat quality characteristics and some of the carcass traits and chemical measurements are presented in Table 1.7. The correlation coefficients are very low and insignificant (P>.05). Moreover many of them are inconsistent as to their sign; i.e., some traits correlate negatively with both panel tenderness and shear force. However, no generalization is made due to the small number of animals in this experiment; i.e., 16 bulls.

Table 1.6. EFFECT OF RALGRO IMPLANTS ON SOME CHEMICAL COMPOSITION DATA OF THE 9th RIB SECTIONS OF BULL CARCASSES[a]

Trait	Control	Ralgro Implants	SE[b]
Moisture, %	54.7	55.5	.93
Ether extract, %	27.7	26.9	1.13
Protein, %	16.8	16.8	.31
Soluble collagen[c]	8.15	8.25	5.5
Total collagen[c]	18.88	18.05	7.6
Soluble collagen[d]	48.7	48.9	3.2
Total collagen[d]	112.7	106.9	4.3

[a] No differences between the means for any of the traits found (P>.05).

[b] SE = Standard error of treatment mean.

[c] Expressed as mg/g wet tissue.

[d] Expressed as mg/g protein.

It seems that hormonal implantation (36 mg/bull) at 242 and at 312 d of age was not effective in producing differences in performance, carcass traits or meat quality attributes.

II. Effects of Dietary Energy Density on Carcass and Meat Quality Characteristics of Holstein Bulls

Effects on Animal Performance. The effects of dietary energy density on the performance of Holstein bulls are shown in Table 1.8. Bulls that received the 85% corn silage diet had a lower average live weight ($P<.05$) compared to those that received 70%, 40%, or 10% corn silage. ADG was higher ($P<.05$) for the 10% silage group compared to the 85% group. Feed consumed per kg of weight gain was lower ($P<.05$) for the 10% group compared to the other three groups which did not differ from each other in feed efficiency. These results agree with the bulk of evidence indicating that animals on an all forage diets were lighter in live weight (Crouse et al., 1984), had lower ADG (Herrshberger et al., 1951; Dinius and Cross, 1978; Summers et al., 1978; Bidner et al., 1981) and required more days on feed to reach the end point compared to concentrate fed animals (Oltjen et al., 1971; Young and

Table 1.7. SIMPLE CORRELATIONS BETWEEN MEAT QUALITY ATTRIBUTES AND SOME CARCASS TRAITS AND CHEMICAL COMPOSITION DATA

Trait	Tender-ness	Juici-ness	Flavor	Overall Satis-faction	Shear-force
Fat thickness	-.25	-.4	.06	-.34	.11
Marbling score	-.17	-.09	-.01	-.16	-.19
Quality grade	-.07	-.01	-.09	-.11	-.24
Moisture, %	.19	.20	.32	.20	.09
Ether extract,%	-.16	-.21	-.26	-.18	-.11
Protein, %	-.13	.16	-.14	-.08	.18
Collagen :					
soluble[a]	.1	.1	.28	.01	-.22
total[a]	-.08	.06	.08	-.10	.03
soluble[b]	.12	.07	.30	.02	-.26
total[b]	-.04	-.002	.11	-.09	-.02

a expressed as mg/g on a wet tissue basis.

b expressed as mg/g of protein.

Table 1.8. MEANS AND STANDARD ERRORS OF LIVE WEIGHT, AVERAGE DAILY GAIN (ADG) AND FEED EFFICIENCY FOR HOLSTEIN BULLS FED DIETS DIFFERING IN PERCENTAGE OF CORN SILAGE

Item	Corn Silage, %				
	10	40	70	85	SE[a]
Live weight, kg[b]	499.70[c]	503.79[c]	482.58[c]	443.94[d]	8.62
ADG, kg	1.27[c]	1.25[c,d]	1.16[c,d,e]	1.11[e]	.03
Feed/gain, kg	2.34[c]	2.56[d]	2.59[d]	2.62[d]	.04

[a] SE = standard error of treatment mean.

[b] Values indicate the final weights.

[c,d,e] Means on the same row having different superscripts differ ($P<.05$).

Kauffman, 1978; Bidner et al., 1981).

Effects on Carcass Traits. Carcass traits for the four dietary groups are presented in Table 1.9. Hot carcass weight followed the same trend as live weight with the 85% corn silage group having lower carcass weight ($P<.05$) than the other three groups which did not differ from each other in carcass weight. No differences in ribeye area ($P>.05$) were found between the four dietary groups. This observation does not agree with other reports because low energy diets have been shown to result in smaller ribeye area measurements compared to high energy diets (Harrison et al., 1978; Aberle et al., 1981). These investigators, however, used steers in their studies rather than bulls.

Bulls in both the 10% and 40% corn silage groups have greater fat thickness at the 12th rib, higher marbling scores and quality grades, and higher yield grades ($P<.05$) than bulls in the 85% corn silage group. These data agree with the findings of many investigators (Oltjen et al.,1971; Utley et al., 1975; Dinius et al., 1976; Harrison et al., 1978; Summers et al., 1978; Aberle et al., 1981; Ridenour et al., 1982). Young and Kauffman (1978) reported that grain feeding increased fat thickness compared to corn silage or haylage-corn silage diets, but did not affect intramuscular fat estimated as marbling or ether extractable fat. These investigators had fed their

Table 1.9. EFFECT OF DIETARY ENERGY DENSITY ON CARCASS TRAITS OF HOLSTEIN BULLS

	Corn Silage, %				
Trait	10	40	70	85	SE[a]
Hot carcass weight, kg	296.40[d]	282.92[d]	280.34[d]	252.12[e]	7.03
Ribeye area, cm	77.9	77.6	77.9	73.9	2.1
Fat thickness, cm	.25[d]	.25[d]	.24[d,e]	.18[e]	.02
Marbling score[b]	28.3[d]	28.7[d]	25.7[d,e]	21.3[e]	1.03
Quality grade[c]	12.5[d]	12.6[d]	12.2[d]	11.1[e]	.3
Yield grade	1.83[d]	1.85[d]	1.62[d,e]	1.52[e]	.09

[a] SE = standard error of the treatment mean.

[b] Marbling score (Devoid 0 to 10, practically devoid 10 to 29, traces 20 to 30, slight 30 to 40 ... etc).

[c] Quality grade (Standard 10, 11 and 12; Good 13, 14, and 15; Choice 16, 17 and 18; Prime 19,20 & 21).

[d,e] Means on the same row having different superscipts differ ($P<.05$).

steers to approximate similiar composition (mean ultrasonic fat thickness) which may explain the discrepancy in their results compared to others. Bidner et al. (1981) reported that while grain feeding increased subcutaneous fat thickness and marbling, it did not significantly influence quality or yield grades. These results are surprising, especially with regard to quality grades. They discussed the possibility that this discrepancy might be due to the coding system used for marbling and quality grade rather than biological differences in the two traits.

Effects on Meat Quality Attributes. Subjective and objective measurements of meat quality of bulls from the four dietary treatments are presented in Table 1.10. Panel tenderness scores for the bulls in the 70% and 85% corn silage groups were lower ($P>.05$) than those for the 10% silage group. Tenderness scores for the bulls in the 40% group were intermediate. Panel juiciness and flavor are not different ($P<.05$). Panel scores for overall satisfaction were not different for the bulls in the 10%, 40%, and 70% corn silage groups. However, panel scores for this trait for bulls in the 85% corn silage group were lower ($P<.05$) than those in the 10% silage group. Shear force measurements did not support taste panel scores, since no differences ($P>.05$) were evident in shear force

Table 1.10. EFFECT OF NUTRITIONAL REGIMEN ON QUALITY CHARACTERISTICS OF BEEF FROM HOLSTEIN BULLS

Trait	Corn Silage, %				
	10	40	70	85[b]	SE[a]
Panel tenderness	6.75[b,c]	6.07[c,d]	5.77[d]	5.78[d]	.23
Panel juiciness	6.18[b]	5.99	6.09	5.83	.18
Panel flavor	6.41[b]	6.40	6.37	6.12	.15
Panel overall satsifaction	6.58[b,c]	6.27[c,d]	6.05[c,d]	5.85[d]	.17
Shear force, kg	5.61	6.11	6.29	6.14	.31

[a] SE = standard error of the treatment mean

[b] Hedonic scale 1 to 9 (1=extremely tough, dry, bland or undesirable. 9=extremely tender, juicy, flavorful or desirable).

[c,d] Means on the same row with different superscipts differ ($P<.05$).

among groups. The panel results agree with earlier reports which suggest that feeding high energy diets results in more tender steaks (Wanderstock and Miller,1948; Bowling et al.,1977; Harrison et al.,1978; Summers et al.,1978; Aberle et al.,1981) While most of these investigators reported differences in flavor, juiciness and shear force due to nutritional regimen, the data presented here do not confirm their observations. However, Harrison et al.(1978) also reported that shear force measurements did not support the differences in panel tenderness scores due to nutritional regimen in longissimus muscle samples from steers. The lack of significant differences in flavor and juiciness scores may be unique to bulls, which were the object of this study, since the other investigators cited used steers in their studies. Other researchers also have reported that dietary regimen had no effect on meat palatability (Hershberger et al.,1951; Burson et al.,1980). Wu et al.(1981b) reported that nutritional regimen had little effects on palatability of meat from steers. The steers fed high energy diets were about 4 mo older than the grass-fed steers. Since tenderness is influenced by animal age, the effect of age might have masked the dietary effects in the study reported by these researchers. In other studies, it was also observed that nutritional regimen had no effect on meat palatability when animals are fed to similiar live weights (Dinius et

al.,1976; Bidner et al.1981) or to similiar fat thickness over the longissimus muscle (Young and Kauffman,1978; Crouse et al.,1984. None of these studies used bulls to show the effects of dietary energy density on eating quality of meat. It is known that the meat from steers and heifers is less variable in tenderness compared to that from bulls. This may explain why nutritional effects on meat palatability were not observed in this study. Moreover, feeding to achieve a constant fat thickness or weight may negate the effects of factors involved in tenderness differences such as rapid chilling and cold-induced toughening or the effects of rate of heat dissipation on proteolytic enzyme activity.

Effects on Chemical Composition. Chemical composition data of the 9th rib sections are shown in Table 1.11. It is evident that the bulls that received diets containing 10 or 40% corn silage had lower moisture ($P<.05$) and higher ether extractable fat ($P<.05$) than those that received the 70 or 85% corn silage diets. Bulls in the 85% corn silage group had a higher protein content ($P<.05$) than those in the 10 and 40% groups, while bulls in the 70% group were intermediate. Similiar results have been reported by other investigators (Jacobson and Fenton,1956; Wheeling et al.,1975; Summers et al.,1978). Young and Kauffman (1978) observed that nutritional

Table 1.11. MEAN EFFECT OF NUTRITIONAL REGIMEN ON THE 9th RIB SECTION CHEMICAL COMPOSITION FROM HOLSTEIN BULL CARCASSES

Trait	Corn Silage,%				
	Initial Group[a]	10	40	70	85	SE[b]
Moisture, %	74.5	59.1[f]	59.6[f]	63.3[e]	63.5[e]	.90
Fat, %	4.1	21.3[e]	20.9[e]	16.4[f]	15.8[f]	1.2
Protein, %	20.5	18.3[f]	18.6[f]	19.3[e,f]	20.4[e]	.5
Soluble collagen[c]	8.3	10.3	8.7	10.0	8.2	1.2
Total collagen[c]	24.9	22.9	20.2	22.9	21.3	2.2
Soluble collagen[d]	40.4	57.6	47.0	51.3	41.9	5.0
Total collagen	121.8	128.2	108.7	116.9	109.4	11.6

[a] Initial group includes five bull calves slaughtered at the start of the experiment

[b] SE = standard error of the treatment mean.

[c] Expressed as mg/g wet muscle tissue.

[d] Expressed as mg/g on muscle protein basis.

[e,f] Means on the same row with the same superscript are not different (P>.05); those having different superscript differ (P<.05).

regimen had no effect on marbling or ether extractable fat despite its effects on fat thickness. This is probably due to the fact that these investigators fed the animals to a similar carcass composition based on ultrasonic readings of fat thickness. The young bulls (i.e., initial group of five calves) killed at the start of the experiment had considerably higher moisture percentage and lower ether extractable fat in their 9th rib sections than the bulls at the end of the experiment. Although the means for the initial group are not statistically comparable to the means of the experimental groups, it is generally accepted that muscles of young animals have a higher moisture and lower fat content than those of older animals (Wilson et al.,1954).

Table 1.11 shows that diet had no effect (P>.05) on the amount of total collagen and salt-soluble collagen. These results agree with others suggesting that dietary regimen has no effect on collagen content (Aberle et al.,1981; Wu et al.,1981b; Hall and Hunt,1982; Crouse et al.,1984). Burson et al.(1980) observed that high-energy diets resulted in a decreased amount of connective tissue detectable by a panel. They did not use a chemical method to determine the collagen content to confirm their panel results. On the other hand, Crouse et al. (1984) found no difference in the amount of panel detectable connective tissue between dietary treatments.

It has been reported that high energy diets increased collagen solubility especially the salt soluble fraction (Aberle et al.,1981; Wu et al.,1981b). The results presented here (Table 1.11) did not confirm these findings, but they are in conformity with the results reported by Hall and Hunt (1982) who observed that dietary regimen had small and inconsistent effects on total collagen and measures of collagen solubility in A-maturity bovine carcasses. The young calves (initial group) contained approximatly 14% more collagen (on a wet tissue basis) compared to the mean of the other groups (although statistical comparison was not possible). Similiar results have been reported previously (Wilson et al.,1954).

Simple correlations between meat palatability attributes and some carcass traits and chemical composition data are presented in Table 1.12. Total and salt-soluble collagen were significantly related to panel tenderness scores ($P<.05$). Total collagen also was related to panel overall acceptability ($P<.05$). All other correlations are not significant ($P>.05$). Shear force had a low correlation with fat thickness ($P=.1$) and flavor scores were poorly associated ($P=.1$) with percentage protein of the 9th rib section. Panel scores were not significantly correlated with fat thickness, marbling, quality grade, ether extractable fat or moisture content.

Measures of collagen content and collagen solubility were not significantly correlated with W-B shear force measures, despite their significant correlation with panel tenderness scores. Evidence for and against the relationship of palatability attributes to measures of fatness (fat thickness, marbling and ether extract percentage) or to quality grades abounds in the literature. These results support the evidence against high correlations between meat palatability traits and measures of fatness or quality grades. Likewise the correlations between palatability attributes and measures of collagen content and solubility are quite low and of little practical significance in this study. Others have reported that shear force measurements were not closely related to total connective tissue content (Field, 1968; Cross et al.; 1973b). In fact, Cross et al. (1973b) suggested that shear force values are more closely related to muscle fiber properties than to connective tissue components of cooked meat. Reagan et al. (1975) suggested that total collagen was more closely related to subjective measures of tenderness. The results presented in Table 1.12 are in agreement with these findings. Reagan et al. (1975) suggested that total collagen was more closely related to subjective measures of tenderness than solubility of collagen, while collagen solubility was more closely related to objective measures of tenderness.

Table 1.12. SIMPLE CORRELATIONS BETWEEN MEAT QUALITY AND CARCASS TRAITS AND CHEMICAL COMPOSITION OF HOLSTEIN BULLS FED VARIOUS LEVELS OF ENERGY

Trait	Panel				
	Tender-ness	Juici-ness	Flavor	Overall Accept	Shear Force
Fat thickness	-.06	.09	.003	.05	.22
Marbling	.08	.14	.14	.14	.09
Quality grade	.02	.13	.14	.10	.13
Mositure, %	-.11	-.12	-.05	-.13	-.11
Ether extract,%	-.06	.09	.01	.08	.15
Protein, %	.08	.02	.24*	.05	-.20
Solublecollagen	.32*	-.01	-.08	.23	-.03
Total collagen	.33**	.06	.08	.30**	.01

* P<.1.

** P<.05.

From the results presented herein for Holstein bulls, it is evident that up to 40% corn silage in the diet is not detrimental to the meat quality traits. Except for lower ether extract content and panel tenderness score, even 70% corn silage in the diet did not adversly affect any of the other variables studied. It appears from these data that acceptable qualitative properties can be expected with up to 50% corn silage in the diet. Considering this fact and the fact that dairy beef production is a by-product and lacks sensitivity to supply and demand of beef (Cartwright, 1982); beef production from Holstein bulls could have a great impact on lower beef prices and greater consumer acceptability.

SUMMARY

Effects of Ralgro implants on performance, carcass and meat quality characteristics of beef-type bulls were evaluated in trial 1. Sixteen bull calves (7 Angus and 9 Polled Hereford) were divided into two groups. One group was implanted twice with 36 mg of Ralgro per animal (at 242 and 312 d of age) the other group served as controls. Animals were fed a diet consisting of corn silage, high moisture corn and a protein mineral supplement. Bulls were slaughtered at an average weight of 458 kg and an average age of 14 mo. After carcass composition data were obtained, palatability and chemical composition (moisture, ether extract and protein percentages, and total and soluble collagen) also were determined on steaks from the longissimus muscle (9th to 12th rib section). Ralgro implants had no effect (P>.05) on live animal performance or carcass traits. Meat tenderness whether determined by panel or Warner-Bratzler shear force was not affected (P>.05) by Ralgro treatment, neither was panel juiciness, flavor or overall satisfaction scores. Ralgro implants had no effect (P>.05) on chemically determined moisture, ether extractable fat or protein percentages. It also did not affect the amounts of total or salt-soluble collagen

as determined by hydroxyproline analysis. Simple correlations between meat palatability measures and carcass traits and chemical measurements were nonsignificant (P>.05) and inconsistent as to their signs.

In a second trial 48 Holstein bulls were divided equally between four dietary treatments (10%, 40%, 70% and 85% corn silage having energy densities of .71, .65, .59 and .57 Mcal/Kg in the growing phase and .73, .67, .60 and .57 Mcal/Kg in the finishing phase, respectively). Bulls were slaugthered at 14 mo of age. Carcass composition data were obtained and then palatability and chemical composition as described above were performed on steaks (9th to 12th rib section). High energy diets improved (P<.05) live weight, average daily gain, feed effeciency, carcass fatness and quality grades. High energy diets also improved (P<.05) panel tenderness and overall satisfaction scores, but did not affect (P>.05) panel juiciness and flavor scores or Warner-Bratzler shear force. High energy diets increased (P<.05) ether extractable fat and decreased (P<.05) moisture and protein percentages, but had no effect (P>.05) on the amounts of total or salt-soluble collagen.

It is concluded from trial 1 that Ralgro implants (36 mg/bull at 242 and 312 d of age) had no effects on performance, carcass traits or meat palatability

characteristics of beef-type bulls. Corn silage in diets for Holstein bulls up to 40% had no detrimental effects on performance, carcass traits or meat quality traits(trial 2).

CHAPTER 2

EFFECTS OF EARLY POSTMORTEM STORAGE TEMPERATURE ON PROTEINASE ACTIVITY AND TENDERNESS OF BEEF LONGISSIMUS MUSCLE

INTRODUCTION

High temperature storage during the first few hours postmortem has been shown to enhance tenderness of meat as compared to the conventional low temperature chilling practices employed by the meat industry (Smith et al., 1971; Parrish et al., 1973; Fields et al., 1976; Marsh et al., 1981). In search of the causes for the observed improvement in tenderness, several factors have been investigated including cold-induced toughening (Locker, 1960a; Locker and Hagyard, 1963; Marsh and Leet, 1966), and more extensive proteolysis under high temperature aging conditions (Moeller et al., 1976; Wu et al., 1981a; Yates et al., 1983; Dutson, 1983). The effects of carcass chilling on cold-induced toughening was not studied in this research project and will not be discussed further.

A number of techniques have been used to observe proteolytic changes in muscle proteins and relate them to differences in meat tenderness. Chemical assessments of proteolysis (Locker, 1960b; Davey and Gilbert, 1966; Parrish et al., 1969) failed to prove that proteolysis is a major factor determining meat tenderness. In the last

two decades, however, more sensitive methods have been employed, hence ultrastructural studies revealed that very specific and limited degradation does occur in muscle during postmortem storage (Takahashi et al., 1967; Henderson et al., 1970). Thus, the use of electron microscopy and SDS-Polyacrylamide gel electrophoresis has led to the detection of a number of proteolytic changes occurring in myofibrils including the disruption of the Z-disks and Z-disk-I-band structure (Henderson et al., 1970; Stromer et al., 1976), as well as the appearance of a protein bands at the 30K-dalton position on gels (Mac Bride and Parrish, 1977; Olson and Parrish, 1977; Penny and Ferguson-Pryce, 1979; Parrish et al., 1981). This protein band has been found to arise from troponin-T degradation (Dabrowska et al., 1973; Olson et al., 1977). Since many proteolytic enzymes, active in the acidic, neutral and alkaline pH range, were found to be associated with skeletal muscles (Goll et al., 1983; Pearson et al., 1983); these ultrastructural changes have been attributed to activity of these enzymes. Since these proteolytic changes are few and limited, and concentration of protein hydrolases in muscle tends to be rather low compared to other tissues (Pennington, 1977); highly sensitive procedures need to be used to detect enzyme activity and the changes on myofibrils.

The objectives of this study were to 1) use a sensitive method (release of radioactivity from a labeled substrate) to detect the small changes in enzymatic activity. 2) Compare the proteinase activity under high and low temperature aging conditions over time postmortem. 3) Relate differences in proteinase activity to differences in tenderness of beef steaks.

REVIEW OF LITERATURE

It has long been known that high temperature aging produces more tender meat as opposed to aging at low temperature, i.e., 0 to 5 C. Smith et al.(1971), Parrish et al. (1973) and Fields et al. (1976) indicated that conditioning of beef carcasses at 14 to 19 C for 12 to 24 h increased the rate of postmortem tenderization when compared with conventional chilling at 0 to 2 C. Smith et al. (1979) observed that conditioning of beef carcasses at 13 C for 8 h produced more tender longissimus steaks as opposed to chilling at 3 C. Shear force measurement, however, did not support the taste panel data. This tenderness enhancement was observed for grass-fed steers but not for steers fed concentrate or forage in drylot. Marsh et al. (1981) indicated that tenderness of loin steaks from beef was strongly influenced by muscle temperature during the first 3h after slaughter. Maintenance of temperature at about 37 C within the longissimus muscle during this 3h period, whether by heavy fat cover or by ambient-temperature manipulation resulted in appreciable tenderness enhancement.

Proteolysis of muscle proteins was proposed long ago as an important factor contributing to postmortem changes in muscle (Hoagland et al., 1917) and has since been

suggested by many researchers to be the primary mechanism of tenderization. Although tenderness has been shown to increase gradually as the storage time postmortem increased (Deatherage and Harsham,1947; Ramsbottom and Strandine,1949; Goll et al.,1964b; Whitaker,1964; Dutson and Lawrie, 1974; Dransfield et al., 1981; Etherington,1981), very little increase was observed in chemical constituents of aged meat due to proteolysis (Locker,1960b; Davey and Gilbert,1966; Parrish et al.,1969), which led these workers to doubt the importance of proteolysis in postmortem storage tenderization. While Zender et al. (1958) observed almost complete autolytic breakdown of texture in lamb and rabbit muscle, Sharp (1963) suggested that autolysis in postmortem muscle was restricted to the sarcoplasmic protein fraction. He did not detect any changes in the solubility of collagen or the fine structure of the myofibrils during 6 mo of storage.

Earlier studies, some of which are cited above, focused mainly on the chemical constituents of aged meat thought to result from proteolytic breakdown. These include free N-terminal groups, free amino acids and nonprotein nitrogen. Ultrastructural studies (Takahashi et al.,1967; Henderson et al.,1970) and the similarities between tryptic digestion and postmortem storage (Goll et

al.,1971) brought about a change in the experimental strategies. Later studies focused on the detection of breakdowns in the polypeptide chains of muscle structural proteins to elucidate the effects of postmortem proteolysis. This new strategy has led to many suggestions about the relation between proteolysis and increased tenderness during postmortem storage and the nature of the proteases involved.

A number of proteolytic enzymes found in the muscle tissue are identified and characterized. Although many criteria have been used to classify these enzymes (Barrett,1980; Bird et al.,1980; Goll et al.,1983), classification according to their optimum pH will suit the purpose of this study. Accordingly, these proteolytic enzymes will be classified into three groups: 1) Acidic proteinases; 2) Neutral proteinases; and 3) Alkaline proteinases.

Acidic Proteinases

Most of these proteinases are located in lysosomes and they include the group called cathepsins. Of the many lysosomal proteinases known, only seven have been shown to exist inside skeletal muscle cells. Using direct cytochemical studies, Bird et al. (1978) showed that cathepsins B and D were localized in skeletal muscle cells. Moreover, the activities of Cathepsins B,D,H and L

have been detected in skeletal muscle cell culture homogenates (Bird et al.,1981). Cathepsins A and C and lysosomal carboxypeptidase B are thought to be found in muscle cells because they are found in virtually all lysosomes. Thus, the evidence for their presence in muscle lysosomes is only suggestive at present. Schwartz and Bird (1977) clearly demonstrated that cathepsins B and D degrade myosin at pH 5.2 and 4.0, respectively, and degrade actin at pH 5.0 with cathepsin D being the most active. They also estimated the amounts of myosin and these two catheptic enzymes in muscle and concluded that there is enough of these two enzymes to degrade all myosin in skeletal muscle cells in 6 to 9 d. Cathepsins A and C exhibit activity only with synthetic peptides and derivatives of dipeptides, respectively, so they are not likely to play a major role in meat aging (Pearson et al.,1983).

Studies with purified cathepsin L have demonstrated that it has a greater ability to degrade proteins than cathepsin B. Recent estimates indicate that cathepsin L has 10 times, and cathepsin H 5 times, greater activity with myosin as substrate compared to cathepsin B (Bird and Carter,1980). The activity of cathepsin C was reported to be low in skeletal muscle relative to other tissues. In muscle it has been demonstrated to be less than 5% of the

activity in spleen (Bouma and Gruber,1964).

Other lysosomal enzymes also may possibly be involved in meat tenderization. Among these, Cathepsins G and N, although they have not been detected in skeletal muscle cells, they both can degrade collagen (Etherington, 1981; Goll et al., 1983). Lysosomal Beta-glucuronidase and Beta-galactosidase have been shown to be associated with collagen degradation and the high temperature aging enhancement of tenderness (Moeller et al, 1976; Dutson and Yates, 1978; Wu et al., 1981a).

Neutral Proteinases

Proteolytic activity in the neutral pH range has been documented in muscle tissue (Bird and Carter, 1980). Only a few of the enzymes active in this pH range have been purified and studied. One of these is the neutral serine proteinase described in smooth muscle by Beynon and Kay (1978). This enzyme is not found to be located inside skeletal muscle cells, but is found in other unidentified cells in muscle tissue (Goll et al., 1983). Another group of enzymes active at neutral pH is the mammalian collagenases described by Harris and Cartwright (1977). The most studied neutral proteinase is the Calcium - dependent proteinase generally known as Calcium-activated factor (CAF). Of the enzymes found to be active in the

neutral and alkaline pH range, CAF is the only one shown to be located inside striated muscle cells and more specifically it is found in the Z-disk region of the myofibril (Ishiura et al., 1980; Dayton and Schollmeyer, 1981). These researchers suggested that this enzyme may be involved in myofibril degradation. It has been shown that CAF disrupts the Z-disk structure of myofibrils (Busch et al., 1972), and this action has been demonstrated to result in meat tenderization (Penny et al., 1974). Penny (1974) suggested that the observed destruction of Z-disks by CAF is due to its digestion of the alpha-actinin, rather than the actin component. Although several investigators agreed that CAF has two polypeptide chains of 80K and 30K daltons (Dayton et al., 1975; 1976a; Waxman and Krebs, 1978; Hathaway et al., 1982; Mellgren et al., 1982), other investigators suggest that it has only one 80K dalton polypeptide (Ishiura et al., 1978; Azanza et al., 1979). The original form of CAF purified by Dayton et al. (1976a) was found to be optimally active in the pH range 6.5 to 8.0 and at 1 to 2 mM Ca concentration. It had little activity below .1 mM Ca^{2+} and was not activated by other divalent cations. It also required a reduced sulfhydryl group for optimal activity (Dayton et al., 1976b; Waxman, 1978).

Many investigators have demonstrated that CAF has a very limited degradative specificity (Penny, 1974;

Dayton et al., 1975; 1976b; Waxman, 1978; Ishiura et al., 1979). Although Penny (1974) reported that alpha-actinin was a substrate for CAF and suggested that the destruction of the Z-disks was due to the digestion of alpha-actinin; Dayton et al. (1975); 1976 b) demonstrated that CAF had no activity against actin, alpha-actinin, troponin-C or myosin. They found that CAF is active against C-protein, tropomyosin, troponin-I and troponin-T. Dayton et al. (1976 b) suggested that CAF may have a physiological role in disassembly of intact myofibrils during metabolic turnover of myofibrillar proteins. Ishiura et al. (1979) have shown that CAF degrades myosin heavy chain and alpha-actinin only when the ratio of the enzyme to these proteins was raised (more than 1:800). It is generally agreed that CAF removes the Z-disk, but the mechanism has not been agreed upon. The digestion of troponin-T or desmin by CAF does not explain the removal of the Z-disks because both proteins are not in the Z-disk. These findings stimulated the reinvestigation of the proteins found in the Z-disk. The possibility exists that the Z-disk might contain protein(s) not yet recognized, whose digestion might explain the removal of the Z-disk by CAF. Recently, Nagainis and Wolfe (1982) reported an unusual form of actin resides in the Z-disk, which is different from thin-filament actin in isoelectric pH, solubility in

KI and antigenicity in mice. They suggested that the hydrolysis of this Z-disk actin by CAF may explain the dissociation of the Z-disk.

The problem with the originally purified CAF is that it requires millimolar concentrations of Ca^{2+} for optimal activity. The concentrations of intracellular Ca^{2+} , however, rarely rise above 10uM (Marban et al., 1980; Lee et al., 1980). Recently, the efforts of many investigators have culminated in the isolation of a form of CAF active at micromolar concentrations of Ca^{2+}, (Mellgren, 1980; Kubota et al., 1981; Szpacenko et al., 1981; Dayton et al., 1981). Cottin et al. (1981 a,b) were able to purify this low Ca^{2+} requiring form of CAF together with its inhibitor from rabbit skeletal muscle. This form of CAF, termed low-Ca^{2+} CAF, is thought to be similar to the original CAF but modified in an as yet unknown way such that its negative charge at pH 7.5 and its Ca^{2+} requirement for maximal activity are reduced. Others, however, thought that this form is produced from the original CAF by limited autolysis (Suzuki et al., 1981a; 1981b; Hathaway et al., 1982). Whether this low-Ca^{2+} CAF is a distinct enzyme or a form produced by autolysis of CAF, its presence in muscle explains how CAF could be active at physiological conditions. The in vivo regulation possibilities of this enzyme in living animals is discussed by Goll et al. (1983).

Alkaline Proteinases

Several studies of proteolytic activities in muscle in the pH range 7.5 to 12 have been described in the literature. In the first of these studies Koszalka and Miller (1960a) observed that rat skeletal muscle homogenates prepared in 2% KCL and incubated at 37C exhibited maximal autolysis at pH 8.5 to 9.0 as measured by the release of trichloroacetic acid-soluble substances which absorb light at 280nm. Later Koszalka and Miller (1960b) purified and characterized an enzyme active optimally at pH 8.5 to 9.0. Since then several of these proteolytic activities have been reported which include: the alkaline myofibrillar proteinase with an optimum pH range of 8.0 to 12.0 (Holmes et al., 1971); muscle alkaline proteinase active at pH 9.0 to 10.5 (Noguchi and Kandatsu, 1971; 1976); the rat myofibrillar proteinase described by Mayer et al., (1974); the alkaline serine proteinase with an optimum pH of 7.5 to 8.5 (Katunuma et al., 1975); the myosin light-chain proteinase described by Bhan et al. (1978); and the myosin-cleaving proteinase reported by Murakami and Uchida (1978; 1979). Most of these enzymes possess the same properties, thus, it is likely that they are the same enzyme or originate from one enzyme. Recent evidence (Dahlmann et al., 1982) has

indicated that the alkaline serine proteinase (Katunuma et al., 1975) and the muscle alkaline proteinase (Noguchi and Kandatsu, 1976) are identical and cross-react immunologically with mast cell chymase 1.

Several investigators using different methods have indicated that most of these alkaline proteolytic activities are not located inside muscle cells but rather in mast cells associated with the connective tissue matrix. These studies have indicated that the alkaline serine proteinase (Woodbury et al., 1978a; 1978b; Dahlmann et al., 1982), the muscle alkaline proteinase (Dahlmann et al., 1982) and the myosin-cleaving proteinase (Kuo et al., 1981) are located in mast cells. Other studies using compound 48/80, which is known to degranulate mast cells, have shown that most, if not all, alkaline proteinases originate from mast cells and not from the muscle cells themselves (Park et al., 1973; Drabikowski et al., 1977; McKee et al., 1979; Libby and Goldberg, 1980). The fact that these enzymes are not located inside muscle cells, together with their alkaline pH optima, leave many doubts as to their involvement in postmortem tenderization. It is well known that postmortem muscle never has a pH in this range. Instead the pH falls from near 7.4 shortly after death and reaches an ultimate value of about 5.2 to 5.5 approximately 1 to 2d postmortem.

Endogenous Inhibitors of Proteinases

All of the proteolytic activities seem to be under rigorous control in living cells. Present evidence suggests that specific endogenous inhibitors are synthesized in cells and used as one of the many ways to control their proteolytic enzymes. Inhibitors for many of the known proteolytic enzymes have been isolated. Two peaks of cathepsin B inhibitory activity have been isolated by Schwartz and Bird (1977). A specific inhibitor to the neutral serine proteinase was detected by Beynon and Kay (1978) during the course of purifying this enzyme. This inhibitor was later purified to homogeneity and characterized by Carney et al. (1980). Nolan et al. (1978) characterized a specific inhibitor to collagenases of mammalian origin. Shortly after the purification of CAF, it was discovered that muscle cells also contain an inhibitor specific for CAF (Okitani et al., 1976; Waman and Krebs, 1978; Otsuka and Goll, 1980). This inhibitor can completely inhibit both the high-Ca^{2+} and the low-Ca^{2+} CAF (Szpacenko et al., 1981). Specific inhibitors for several of the alkaline proteases have also been described (Noguchi et al., 1974; Katunuma et al., 1976). These inhibitors cause some problems when assaying enzymic activity in crude muscle extracts.

Proteolysis in Postmortem Muscle

Rather than continuing to use the different chemical measurements of proteolysis, new techniques have been employed (electron microscopy and electrophoresis), in an effort to determine the nature of postmortem changes and whether these changes could be attributed to proteolysis (Goll et al., 1970; 1971). The application of these techniques has produced new insights into the events of postmortem tenderization. The most important general finding is that the myofibrils remain largely intact even after long periods of postmortem storage. After storage at 2 C or 16 C for 13 d Goll and Robson (1967) were able to prepare myofibrils from bovine skeletal muscle that retained normal contractile properties as measured by their ability to superprecipitate when ATP was added or by their Mg-modified ATPase activity. Stromer and Goll (1967) observed that this same muscle has clear and microscopically normal banding pattern after 13 d of storage, when observed by electron microscopy. These findings were further supported by the findings of several others using SDS-polyacrylamide gel electrophoresis (Arakowa et al., 1976; Samejima and Wolfe, 1976; Yamamoto et al., 1977; Penny, 1980; Etherington, 1981). They reported that no degradation of myosin heavy chains occurred in muscle stored 0 to 4 C. Others reported a small amount of myosin heavy chain degradation in muscle

stored at 40 C (Arakawa et al., 1976; Samejima and Wolfe, 1976; Yamamoto et al., 1979) or at -20 C (Yamamota et al., 1977). All of these findings supported the earlier results that limited proteolysis occurred during postmortem storage (Locker, 1960b; Davey and Gilbert, 1966; Parrish et al., 1969).

The second important general observation during postmortem aging is the increase in width and loss of the Z-disks and, at higher storage temperatures degradation of the M-Line (Henderson et al., 1970; Penny, 1980; Etherington, 1981). The third observation is that the actin-myosin interaction seems to change during postmortem aging. Although the nature of this change is not known, its occurrence is indicated by several observations. An increase in ATPase activity of myofibrils or actomyosin preparations during postmortem storage has been reported (Goll and Robson, 1967; Arakawa et al., 1970; Ikeuchi et al., 1978). Actomyosin prepared from postmortem muscle has a faster rate of superprecipitation in response to ATP than actomyosin from fresh muscle (Arakawa et al., 1976; Goll et al., 1970). Actomyosin prepared from muscle stored for 7 d dissociates at a lower ATP concentration than that from fresh muscle (Fujimaki et al., 1965). Additionally, shortened sarcomeres gradually lengthen as postmortem storage proceeds in the absence of ATP (Gothard

et al., 1966; Stromer et al., 1967). This observation supports the conclusion of Fujimaki et al. (1965) that the interaction between actin and myosin becomes weaker with aging time.

The fourth and probably the most consistent observation regarding the postmortem changes in myofibrillar proteins is the gradual appearance of 34 K-, 30 K- and 27 K-dalton protein bands in SDS- polyacrylamide gels (Dabrowska et al., 1973; Samejima and Wolfe, 1976; Yamamoto et al., 1977; Olson and Parrish, 1977; Olson et al., 1977; Macbride and Parrish, 1977; Penny and Ferguson-Pryce; 1979; Parrish et al., 1981; Ouali et al., 1983). It is uncertain which of the myofibrillar proteins gives rise to these 30 K-dalton bands. Several investigators, however, showed that the degradation of troponin-T produces polypeptides having molecular weights near 30K dalton (Dabrowska et al., 1973; Olson et al., 1977; Yamamoto et al., 1977). Furthermore, the decrease in the intensity of troponin-T band with the concomitant increase in the 30 K dalton band (Samejima and Wolfe, 1976; Olson et al., 1977) is highly suggestive as to the origin of the 30 K-dalton band.

Proteolytic Enzymes Involved in Tenderization

The observations described above led to some conclusions about which enzymes are most likely to be

involved in postmortem tenderization. The bulk of the evidence implicates CAF as being responsible for most of the changes which lead to postmortem tenderization (Penny, 1976; Olson et al., 1977; Penny and Ferguson-Pryce, 1979; Ouali and Valin 1981; Ouali et al., 1983) at storage temperatures of 0 to 4 C. During frozen storage or aging at temperatures above 16 C catheptic enzymes may contribute to tenderization by acting in concert with CAF. The most probable enzymes are cathepsins L, H and B (Goll et al., 1983). Limited degradation of collagen by proteinases outside the muscle cell (Goll et al., 1970; Etherington, 1981) or by a muscle lysosomal enzyme system (Kopp and Valin, 1981) also could contribute to postmortem tenderization.

Degradative Changes Causing Tenderization

Although it has been shown that some degradation occurs in the sarcoplasmic protein fraction of muscle (Goll et al., 1970; Sharp, 1963), this degradation would not contribute directly to increases in tenderness (Goll et al., 1983). Zender et al. (1958) observed almost complete breakdown of texture in rabbit and lamb muscle during aseptic storage at 25 and 37 C. In later studies, however, the bulk of the evidence suggests that very limited proteolysis, if any, occurs. Recent studies

employing electrophoresis techniques have shown that the principal degradative change of postmortem myofibrils is the loss of troponin-T and the concomitant appearance of the 30 K-dalton fragment (Dabrowska et al., 1973; Olson et al., 1977). Other investigators suggested that there is a very high relationship between tenderness and the degradation of troponin-T (Olson and Parrish, 1977; Macbride and Parrish, 1977; Penny and Dransfield, 1979; Parrish et al., 1981). Another observation is the loss of Z-disk integrity (Henderson et al., 1970) as well as the fractures seen between the Z-disk and the I-band (Penny, 1980; Etherington, 1981). These degradative alterations also could contribute to tenderness. Most of the available evidence suggested that CAF is the enzyme responsible for the disruption of the Z-disk structure, however, the specific protein(s) of the Z-disk which are degraded to cause this disruption are not known. Penny (1974) suggested that destruction of the Z-disk by CAF is due to its digestion of alpha-actinin. This has been disproved because Dayton et al.(1975) found that CAF had no effect on alpha-actinin. The intensity of the alpha-actinin band after SDS-PAGE of myofibrils prepared from conditioned meat was found to be the same as that of myofibrils from fresh meat (Penny and Ferguson-Pryce, 1979; Penny, 1980). There is evidence, however, that the

bonding of alpha-actinin to the Z-disk structure is weakened during aging. Alpha-actinin was found to be more easily extracted from aged myofibrils than from at-death myofibrils (Cheng and Parrish, 1978). Thus, there appears to be some factor involved in the bonding of alpha-actinin to the Z-disk structure which is modified during aging but it is not the alpha-actinin-actin association. Nagainis and Wolfe (1982) suggested that the Z-disk contains a different form of actin which is highly susceptible to CAF digestion, and that the degradation of this form of actin explains the removal of the Z-disk by CAF. Cytoskeletal proteins also have been shown to be degraded during conditioning. King et al. (1981) observed degradation of connectin occurred during heating of muscle homogenates (pH 5.5). They questioned the contribution of connectin to meat toughness as this protein is extensively degraded in cooked meat. Young et al. (1981) reported the partial loss of connectin and the disappearance of desmin due to proteolysis during storage. They suggested that the disintegration of the cytoskeletal network can account for the postmortem changes in the physical properties of muscle and for the increased tenderness of stored meat.

Effect of Temperature and pH on Tenderness.

Earlier studies have demonstrated that high

temperature conditioning produces more tender meat as opposed to the conventional chilling at 0 to 2 C (Smith et al., 1971;1979; Parrish et al., 1973; Fields et al., 1976). Recent work has been concentrated on the effect of aging temperature and pH on the release, distribution and activity of proteolytic enzymes. Marsh et al. (1981) have reported that maintenance of temperature at about 37C in the longissimus muscle during the first 3 h postmortem, results in appreciable tenderness enhancement and that early postmortem muscle pH also affects tenderness significantly. Marsh (1983) reviewed the research on the relationship of muscle pH to tenderness and concluded that high muscle pH, especially during the first several hours postmortem, enhances tenderness possibly by extensive proteolysis by neutral proteinases. Moeller et al. (1976) reported that high temperature aging enhances the disruption of the lysosomal membrane as evidenced by a significant increase in the percentage of free enzyme activity 12 h postmortem for both cathepsin C and Beta-glucuronidase. They concluded that some of the differences in tenderness produced by high temperature aging are possibly associated with the increased free lysosomal enzymes during the first 12 h postmortem. Wu et al. (1981a) observed a greater release of Beta-galactosidase and Beta-glucuronidase, regardless of the time postmortem, with high temperature aging. Yates et al.

(1983) observed that incubation of bovine muscle at 37 C promoted extensive proteolytic changes in myofibrillar proteins, and that degradation of myosin and troponin-T were the most noticeable changes. They also noted that while troponin-T and alpha-actinin were altered at pH 7.0, more troponin-T and myosin degradation occurred at pH 5.4 and 37 C than at pH 7.0 and 4C. These studies were reviewed by Dutson (1983) who indicated that increased postmortem temperature produces more tender muscle and increases the disruption of troponin-T, myosin, Z-disks, connectin and gap filaments. He also stated that high pH increases the activity of CAF and low pH increases the activity of lysosomal cathepsins and that both high and low pH increase the degradation of troponin-T, Z-disk, gap filaments and connectin.

It seems that high temperature during the early postmortem hours is essential for the activity of CAF which is enhanced by the near neutral pH at that time. This high temperature is also essential for the rupture of lysosomal membranes and release of their enzymes. Later as the pH falls, the environment becomes more conducive for the lysosomal enzymes for further proteolysis of the myofibrillar proteins which was begun initially by CAF.

MATERIALS AND METHODS

Five steers from the Beef Cattle Research Center at Michigan State University, ranging in live weight from 450 to 560 kg were slaughtered for use in this study. The hot carcass weights ranged from 292 to 368 kg. Only one steer was slaughtered on any given day and dressed by conventional practices. The right side of each carcass was transferred to a cold room (2 to 3 C) at 1 h postmortem while the left side was kept at room temperature (20 to 22 C) for 16 h and then transferred to the cold room. These temperatures represented the conventional carcass chilling procedures used in the meat industry and high temperature aging conditions, respectively. Samples (1.27 cm diameter core) were removed from the lumbar and caudal half of the thoracic portion of the right and left side longissimus muscle at 1, 4, 8, 16, 24, 48 and 168 hours postmortem.

Before removing the muscle samples at each time, the temperature of the longissimus (lumber and caudal half of the thoracic region) muscle was measured by inserting a Koch model 1365 thermometer into the muscle. Muscle pH of the right and left longissimus was monitored at each of the sampling times. One g of fresh muscle was weighed in a Corex tube and 5 ml of 5 mM sodium idoacetate were

added. This mixture was then homogenized using a Brinkman polytron and the pH of the slurry was measured with a Beckman model 3560 digital pH-meter.

For the measurement of enzymatic activity, muscle samples were extracted by the methods outlined by Rothig et al. (1978). Three hundred to 400 mg of fresh muscle were weighed into preweighed Corex tubes. The tubes were then placed on ice and 7 ml of cold (1:1 v/v) glycerol: .05M Tris-HCL buffer (pH 7.4), containing 1M KCL and .2% (w/v) Triton x100 (Appendix I), were added. The glycerol was used to keep the activity of enzymes from deteriorating during storage. The samples were then homogenized with a Brinkman polytron at a setting of 7, with 4 bursts of 20 sec each allowing the samples to cool on ice for 30 sec after each burst. The homogenized muscle samples were centrifuged for 10 min at 3000 x g. and 4 C in a Sorval superspeed RC2-B refrigerated centrifuge. Supernatants were decanted into large tubes through a single layer of cheesecloth The precipitate was rehomogenized and centrifuged as described above and the supernatants were pooled. The muscle extracts were then diluted with buffer such that the protein concentration in each was less than 10 mg/ml. Extracts were stored at -30 C until assayed for enzymatic activity.

Bovine hemoglobin (Sigma Chemical Co. St Louis, MO)

was labeled according to the methods described by Roth et al (1971). Five hundred mg batches of bovine hemoglobin were weighed and each batch was dissolved in 15 ml of mega pure water and its pH adjusted to 6.1 with .1N NaOH. Fifty μCi of $K^{14}CNO$ (52 mCi/mmol, Amersham Corporation, Arlington Heights, IL) dissolved in 10 ml of mega pure water were added to the dissolved hemoglobin, i.e., 2 ml per batch. The mixture was incubated in a waterbath at 50 C for 2 h and stored thereafter at 4 C overnight. Two ml of mega pure water containing 20 micromol of cysteine hydrochloride (pH 6.1) were added to each batch and the mixture incubated in a waterbath of 37 C for 2 hours. These mixtures were then transferred to dialysis tubing (6000 to 8000 MW cutoff) and dialyzed in a cold room (4 C) for 48 h against 9 changes of deionized water. The batches were then pooled and stored at -30 C until used. Radioactivity of labeled hemoglobin was determined by counting (in a Beckman LS-3133P Liquid Scintillator) .1 ml of labeled hemoglobin in 10 ml ACS scintillation liquid (Amersham Corporation, Arlington Heights, IL). Radioactivity was found to be 9000 to 10,000 cpm and after precipitation with perchloric acid (PCA) the radioactivity was found to be 400 to 700 cpm. This is the radioactivity which was not associated with labeled hemoglobin or was released by acid treatment.

Enzymatic activity was measured as the radioactivity released from CN-labeled hemoglobin according to the methods originally outlined by Roth et al. (1971) and later used by Rothig et al. (1978). The standard incubation system was determined after preliminary experiments. Accordingly, .4 ml of muscle extract, .125 ml of labeled hemoglobin and .4 ml of a citrate-phosphate-borate buffer containing .5 M KCL (Appendix II) used to monitor the pH; were incubated in a shaking waterbath at 37 C for 2 h. Then an equal volume (.925 mL) of cold PCA was added to stop the reaction. To monitor the radioactivity released from hemoglobin by factors other than proteolysis, control mixtures devoid of muscle extracts were incubated along with the assay mixtures and the muscle extracts were added after PCA precipitation. All the tubes were then centrifuged at 11,000 x g for 30 min in the Sorval Centrifuge mentioned earlier. PCA was then precipitated by adding .5 ml of concentrated potassium citrate solution to the supernatants and recentrifugation at 11000 x g for another 30 min. Five tenths ml aliquots of the supernatants were counted in 10 ml of ACS scintillant. All the assay and control mixtures were run in duplicates and the averages were determined. The counts in the control mixtures were substracted from those of the assay mixtures to give the radioactivity released from hemoglobin by enzymatic

hydrolysis. Enzymatic activity in the muscle extracts was measured at acidic (4.9), neutral (7.6) and alkaline (9.2) pH. Choice of these pH values was based on preliminary experiments which showed three peaks of enzymatic activity at pH (4.9, 7.6 and 9.2) when the muscle extracts had been assayed in the pH range 3.0 to 10.0 (details described in the results section).

Protein concentration was determined in the muscle extracts according to the method of Lowry et al. (1951). The enzymatic activity was then expressed as blank corrected cpm/mg protein of muscle extract.

At 168h one steak (3.8 cm thick) was removed from the 12th rib region of each side. Steaks were cooked on a Farberware open hearth electric broiler to an internal temperature of 65 C. The cooked steaks were then cooled overnight at 4 C. Shear force was determined on cores (1.3 cm diameter) by a Warner-Bratzler shear force instrument. Each core was sheared twice across the muscle fibers. Tenderness of each beef side was then determined as the average of 16 shear force readings.

The data were analyzed as a randomized complete block design with repeated measurement (Gill, 1978). The data for shear force (tenderness) were analyzed as a randomized complete block design (Gill, 1978) because tenderness was determined once at the end of the storage

period (168 hours) for each side.

RESULTS AND DISCUSSION

In preliminary experiments, three ratios of crude muscle extract to CN-labeled hemoglobin (8:5, 16:5 and 32:5) were assayed to determine the incubation medium. These data are shown in Figure 2.1. The selection of the 2 h incubation period was based on the findings of Mulvaney (1984) who showed that for a 2 h incubation, linear activity was observed for all ratios of substrate to muscle extract tested. Figure 2.1 shows that only the 16:5 ratio provided relatively linear activity. This ratio (16:5) was used for all subsequent assays, i.e. .4 ml of muscle extract to .125 ml of CN-labeled hemoglobin.

In other preliminary experiments, the pH dependence of proteolytic activity of bovine longissimus muscle extracts was assayed in the pH range from 3.0 to 10.0, in increments of .5 pH units. Figure 2.2 shows three peaks of activity one each in the acidic, neutral and alkaline pH regions, i.e., pH 5.0, 7.5, and 9.0, respectively. Each peak activity was then assayed to further identify each pH optima by using five pH values, i.e., two pH values on each side of the peak observed in Figure 2.2, in increments of .1 pH units. Figure 2.3 presents the optimum pH for each of the three activities which were 4.9, 7.6 and 9.2 for the acidic, neutral and alkaline proteolytic activities, respectively. These three pH

FIGURE 2.1. EFFECTS OF INCUBATION TIME AND THE RATIO OF MUSCLE EXTRACT TO ^{14}CN-LABELED HEMOGLOBIN UPON PROTEINASE ACTIVITY AT pH 5.0. MUSCLE EXTRACT: LABELED HEMOGLOBIN RATIOS, * = 8:5; * = 16:5; & = 32:5.

FIGURE 2-1

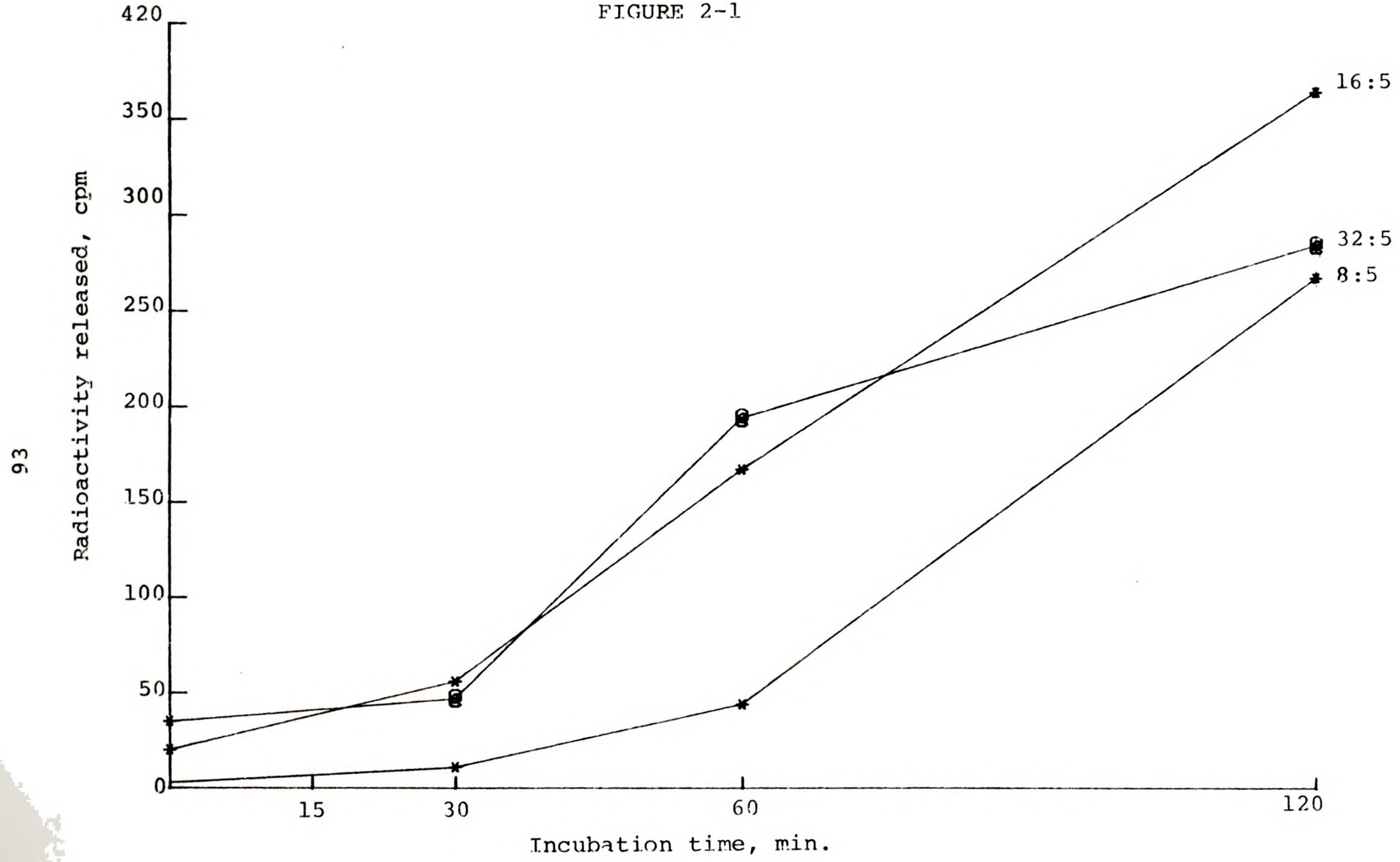

93
Radioactivity released, cpm
420
350
300
250
200
150
100
50
0
15
30
60
120
Incubation time, min.
16:5
32:5
8:5

FIGURE 2.2. pH DEPENDENCE OF PROTEOLYTIC ACTIVITY IN CRUDE LONGISSIMUS MUSCLE EXTRACTS FROM BEEF.

FIGURE 2.2

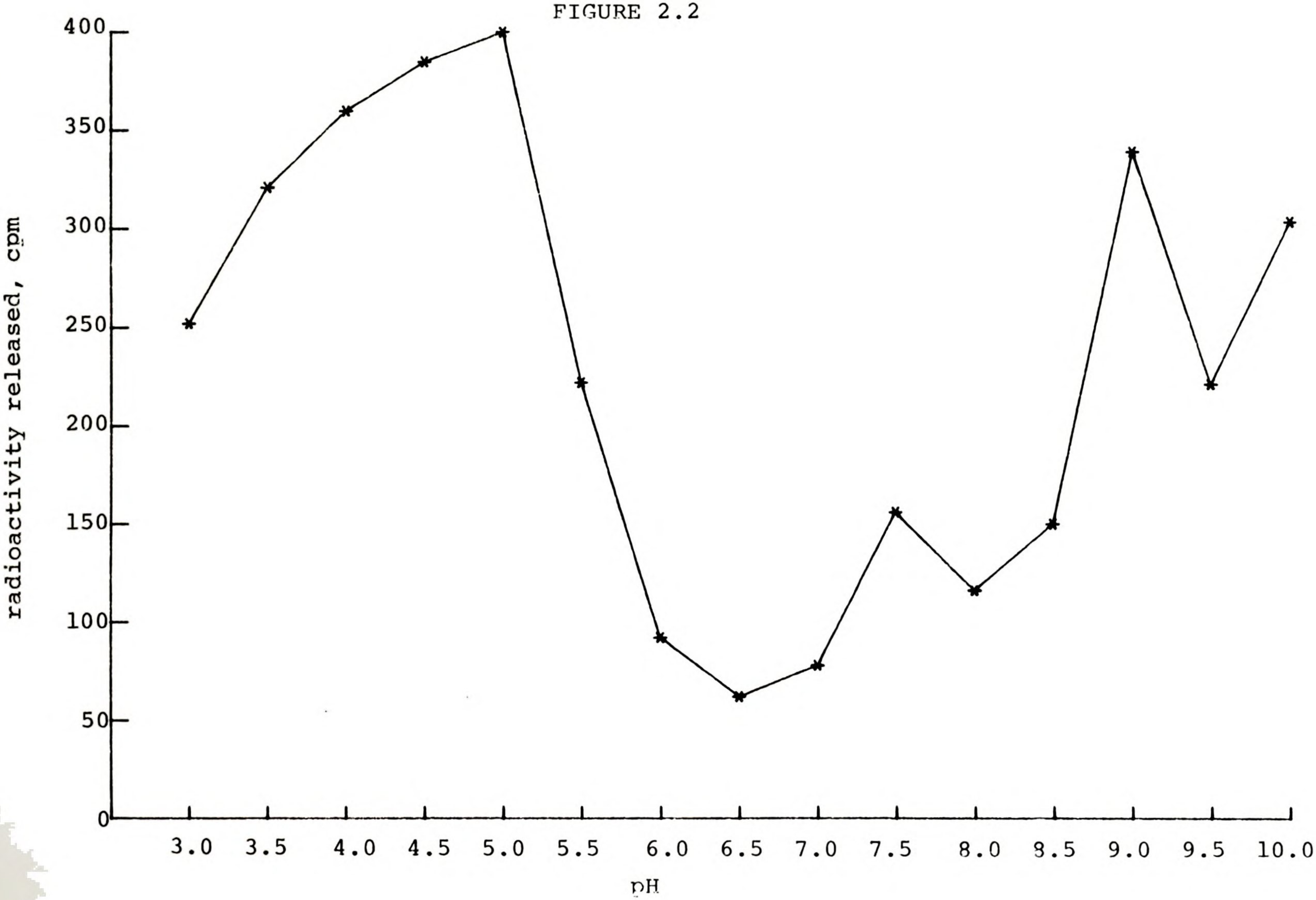
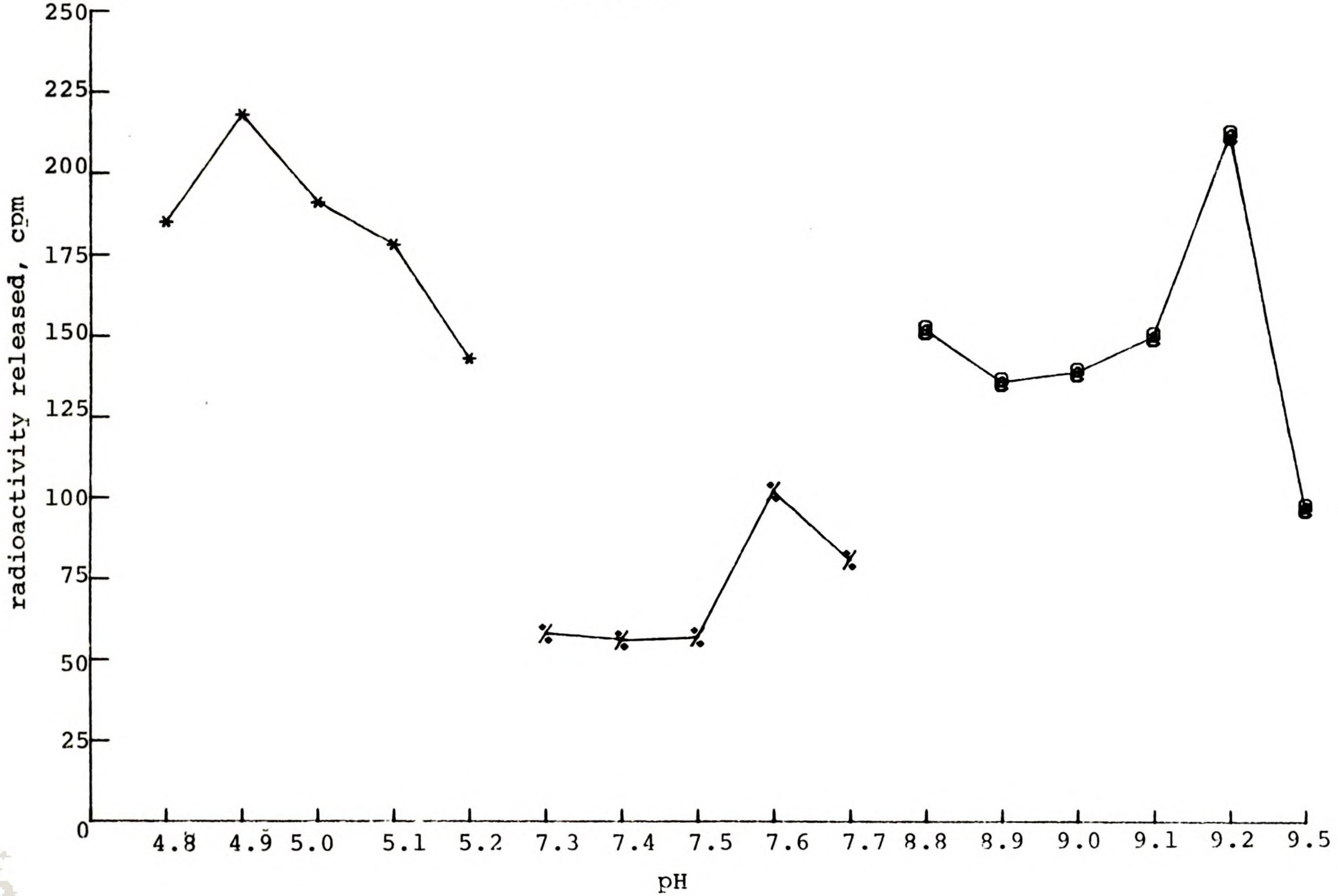

radioactivity released, cpm
400
350
300
250
200
150
100
50
0
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.5
7.0
7.5
8.0
8.5
9.0
9.5
10.0
pH

FIGURE 2.3. pH DEPENDENCE OF PROTEOLYTIC ACTIVITY IN CRUDE MUSCLE EXTRACTS. DETERMINATION OF THE EXACT PEAKS OF PROTEOLYTIC ACTIVITY IN THE A) ACIDIC pH RANGE, B) NEUTRAL pH RANGE AND C) ALKALINE pH RANGE.

FIGURE 2.3


radioactivity released, cpm
250
225
200
175
150
125
100
75
50
25
0
4.8
4.9
5.0
5.1
5.2
7.3
7.4
7.5
7.6
7.7
8.8
8.9
9.0
9.1
9.2
9.5
pH

values were used to assess the acidic, neutral and alkaline proteolytic activities of all muscle samples in this study. The above pH optima are at variance with observations in rats (Rothig et al., 1978) and pigs (Mulvaney, 1984). In these two studies they found that peak proteolytic activity occurred at pH 3.8, 7.0 and 8.5. This discrepancy may be due to the fact that these researchers did not adjust the final pH in their assay tubes after adding the muscle extract. In this study it was observed that the Tris-HCL buffer used to prepare the muscle extracts changed the pH of the final incubation medium. Thus adjustment of the pH in the incubation medium was found to be necessary.

Temperature decline in the longissimus muscle of the sides aged at the high temperature (HT) and low temperature (LT) is shown in Figure 2.4. Both the LT and HT sides maintained muscle temperatures above room temperature for at least 8 h postmortem. From 16 hour and until 24 h postmortem the HT sides maintained longissimus muscle temperatures 3 to 5 C above the LT sides. However by 48 h postmortem the difference in temperature between the two treatments had narrowed to 1.5 C which was maintained throughout the remainder of the experimental period to 168 hours. Postmortem decline in longissimus muscle pH for the HT and LT treatments is shown in Figure

FIGURE 2.4. LONGISSIMUS MUSCLE TEMPERATURE MEASURED AT VARIOUS TIMES POSTMORTEM FOR THE HIGH TEMPERATURE (*=HT) AND LOW TEMPERATURE (+=LT) AGED BEEF SIDES.

FIGURE 2.4

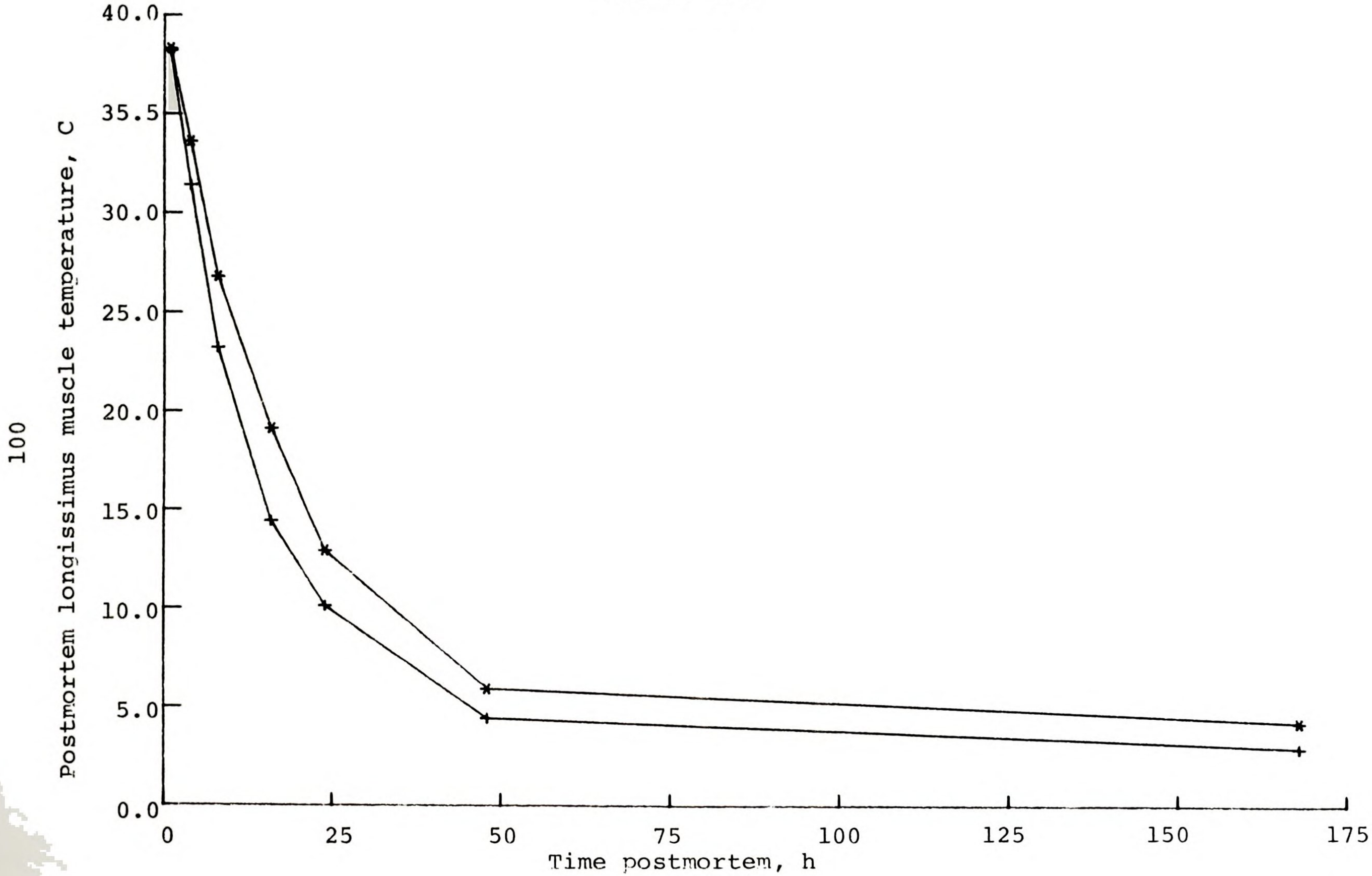
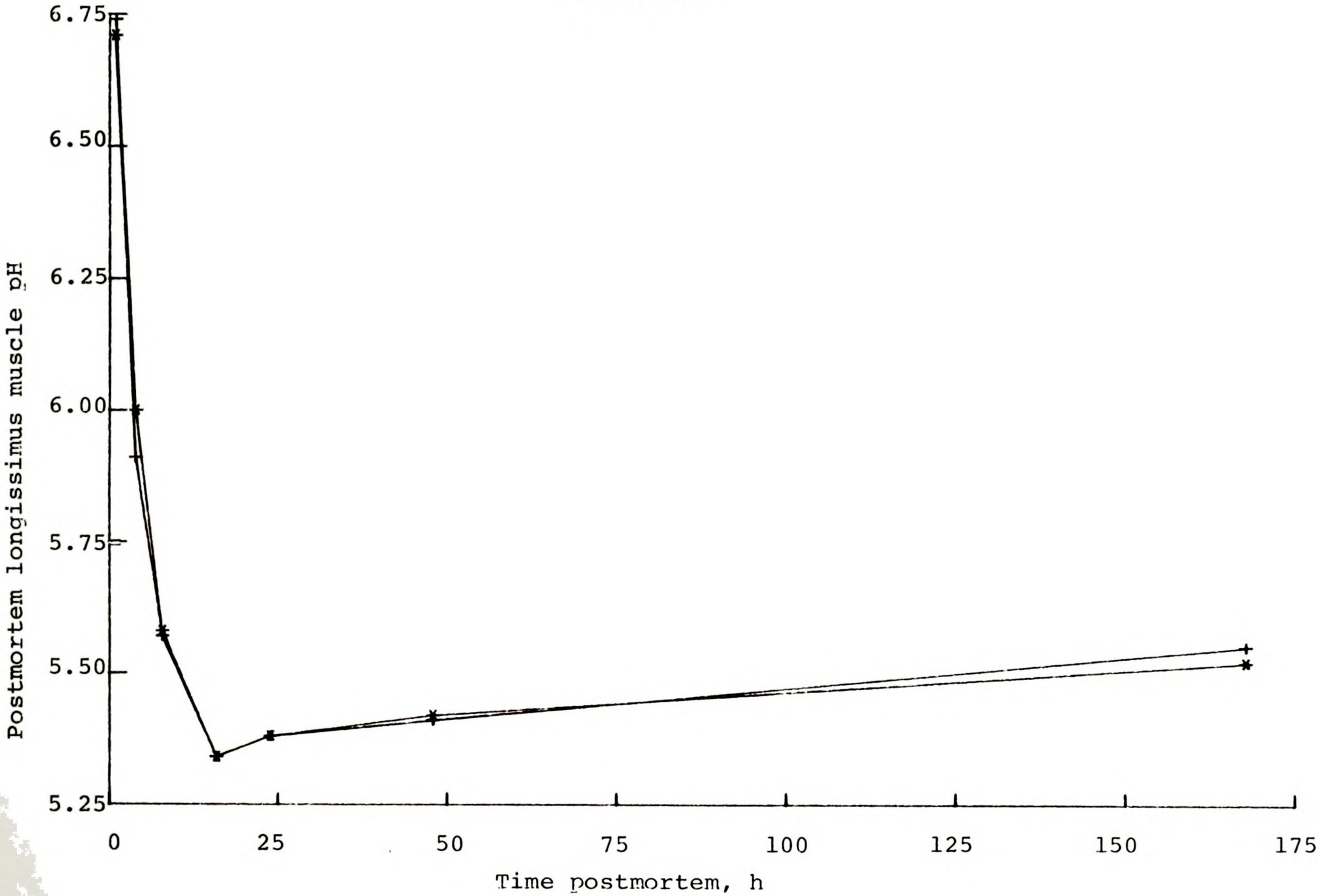
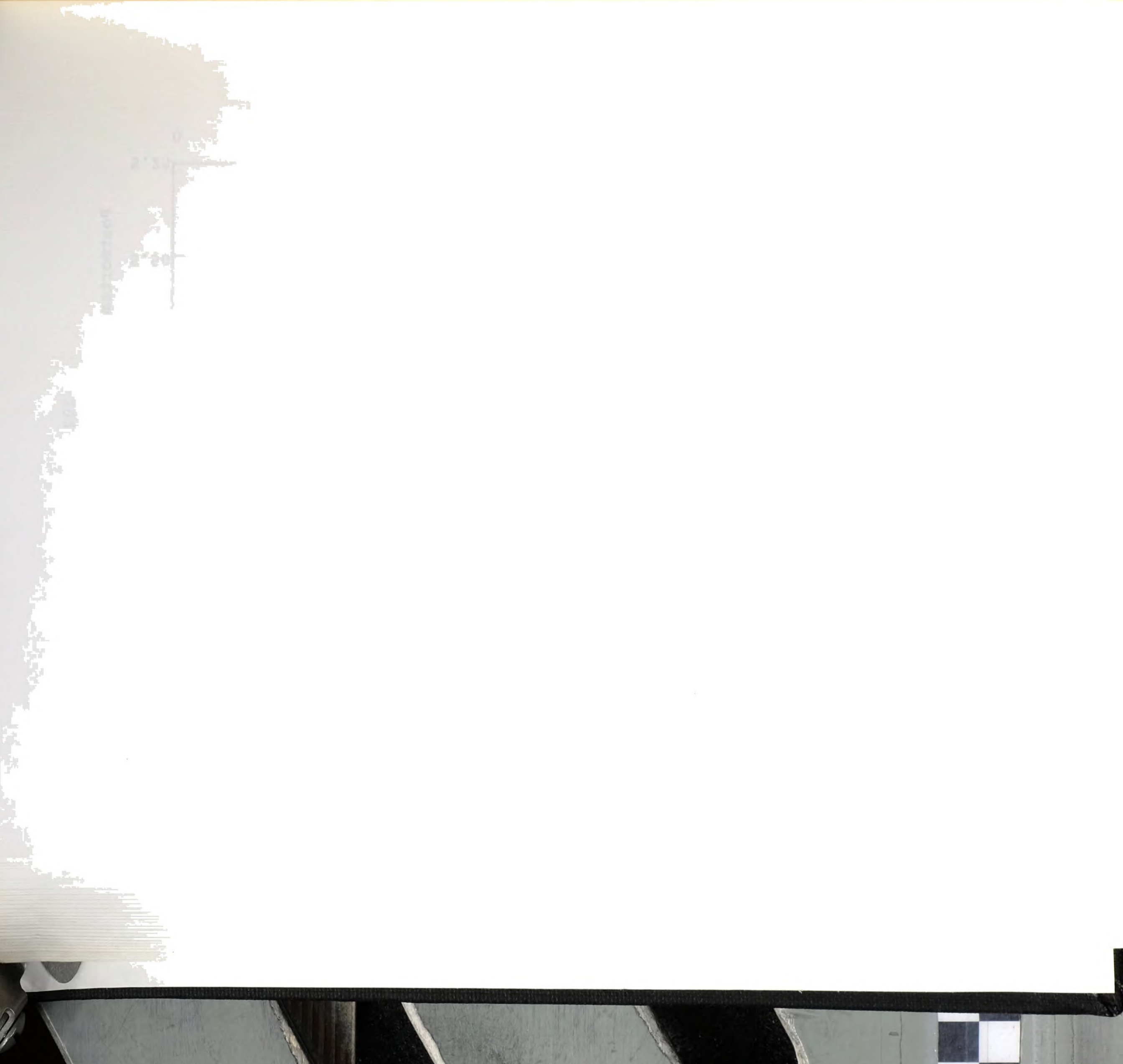

40.0
35.5
30.0
25.0
20.0
15.0
10.0
5.0
0.0
Postmortem longissimus muscle temperature, C
100
0
25
50
75
100
125
150
175
Time postmortem, h

FIGURE 2.5. POSTMORTEM pH DECLINE OF THE LONGISSIMUS MUSCLE OF THE BEEF SIDES AGED AT HT AND LT.

FIGURE 2.5


6.75
6.50
6.25
6.00
5.75
5.50
5.25
Postmortem longissimus muscle pH
0
25
50
75
100
125
150
175
Time postmortem, h



2.5. The effect of treatment on longissimus muscle pH was not significant (P>.05). It is evident that muscle pH of both treatments decreased sharply to its lowest value (5.34) at 16 h postmortem and then rose slowly thereafter.

Tenderness Measurements. Tenderness measured by Warner-Bratzler shear, was determined on 1.27 cm diameter cores from cooked steaks from the longissimus muscle of eight beef sides, i.e., four from each treatment. The average shear force for each longissimus muscle as well as the treatment means and standard error are presented in Table 2.1. No difference (P>.05) in shear force due to the aging temperature was observed. With the exception of steer No. 2, longissimus steaks from the HT side had lower shear force values than those from the LT side. The small differences between treatments may be due to the fact that all the steers used in this study graded choice. Additionally, muscle pH and temperature were remarkably similar between the beef sides held at HT and LT conditions for the first 16 hours postmortem. The limited number of beef sides used in this study precludes drawing any conclusions.

Acidic Proteinase Activity. Figure 2.6 shows the acidic proteinase activity for the HT and LT aged sides, as assessed under optimal conditions in vitro (pH 4.9,

TABLE 2.1 MEAN SHEAR FORCE VALUES FOR THE LONGISSIMUS MUSCLE STEAKS FROM BEEF SIDES AGED AT HIGH AND LOW TEMPERATURES[a]

	Early Postmortem Treaetment	
	High Temperature Side	Low Temperature Side
Steer Number		
2	4.3	3.4
3	4.6	6.1
4	5.4	5.6
5	4.1	4.7
Treatment Mean	4.60	4.95
SE .47		

a Each value is the average of 16 shear force determinations
b SE = standard error of treatment mean.

FIGURE 2.6. ACIDIC PROTEINASE ACTIVITY IN CRUDE LONGISSIMUS MUSCLE EXTRACTS AS AFFECTED BY AGING TEMPERATURE (HT VS LT) AND TIME POSTMORTEM. THE ASSAY WAS CONDUCTED AT pH 4.9 AND 37C. HT = *, AND LT = +.

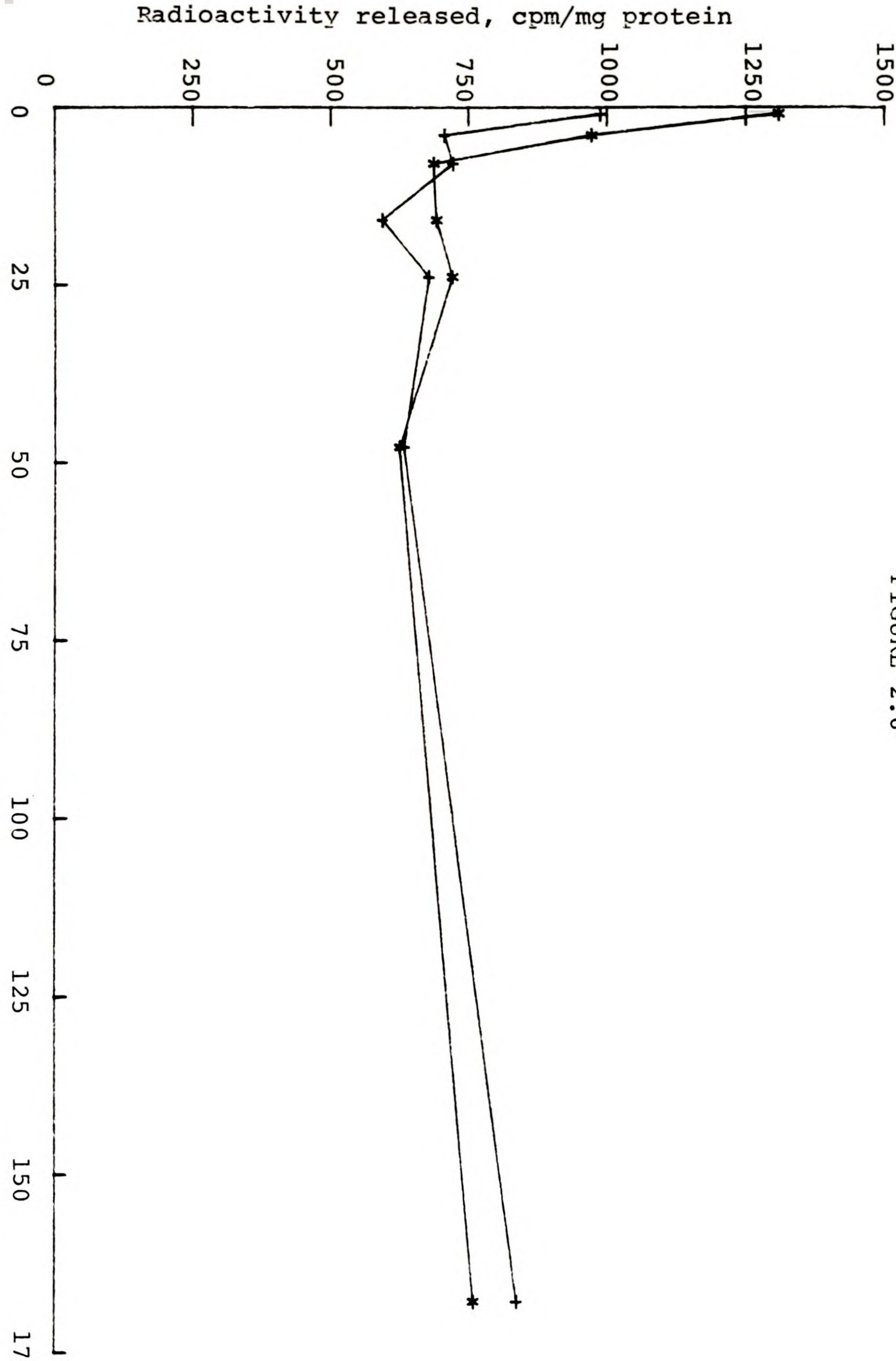

Radioactivity released, cpm/mg protein
0
250
500
750
1000
1250
1500
0
25
50
75
100
125
150
175
Postmortem time, h

FIGURE 2.6

37 C). Analysis of variance showed that the interaction between the early postmortem temperature treatment and time postmortem was nonsignificant (P>.05). This indicates that the time trend of the acidic proteinase activity for the two aging temperatures was not different from parallel. The effect of aging temperature on the acidic proteinase activity also was nonsignifigant (P>.05). These findings do not agree with observations reported by others. Wu et al. (1981a) observed that HT incubated muscle samples had a greater release of lysosomal enzymes (Beta-glactosidase and Beta-glucuronidase), regardless of the time postmortem as compared to samples held at 2 C. Yates et al. (1983) reported that incubation of bovine muscles at 37 C promoted more drastic proteolytic changes in myofibrillar proteins than in muscles incubated at 4 C. In a review of the literature Dutson (1983) concluded that elevated postmortem temperature increased the activity of enzymes which cause disruption of myofibrillar proteins. In the present study the effect of storage time was significant (P<.1). Specific comparisons of the combined (HT and LT) time period means (Table 2.2) showed that the acidic proteinase activity at 16 h and 48 h was lower than that at 1 h postmortem (P<.1). A nonsignificant increase in the proteinase activity was observed at 168 h postmortem

compared to 48 h. The activity at 168 h approached that at 4 h postmortem.

Neutral Proteinase Activity. The neutral (pH 7.6)

TABLE 2.2. COMBINED MEANS OF THE HT AND LT ACIDIC, NEUTRAL AND ALKALINE PROTEINASE ACTIVITIES AT EACH POSTMORTEM TIME PERIOD

	Postmortem Time Period, h						
Proteinase	1	4	8	16	24	48	168
Acidic	1148[a]	839[a,b]	705[a,b]	643[b]	700[a,b]	629[b]	798[a,b]
Neutral	493[c]	300[c,d]	308[c,d]	279[d]	281[d]	253[d]	329[c,d]
Alkaline	324	228	216	220	214	222	255

[a,b]Means in a row with the same superscript do not differ ($P<.1$).

[c,d]Means in a row with the same superscript do not differ ($P<.05$).

proteinase activity is presented in Figure 2.7. Analysis of variance showed that, like the acidic proteinase activity, the interaction between the aging temperature and time period postmortem was not significant (P>.05). There also was no difference in neutral proteinase activity between the HT and LT aged sides. The effect of time period postmortem was significant (P<.05, Table 2.2). Comparison of time period means shows that the neutral proteinase activity at 16, 24 and 48 h postmortem was lower than that a 1 h. Like the acidic proteinases a slight but nonsignificant increase in neutral proteinases activity was observed at 168 hours compared to that at 16, 24 and 48 h.

Alkaline Proteinase Activity. The activity of alkaline (PH 9.2) proteinases for the two aging temperatures is presented in Figure 2.8. Again temperature x period interaction was not significant (P>.05). Analysis of variance showed that the alkaline proteinase activity remained unchanged over postmortem time (P>.05) for both the HT and LT aging treatments (table 2.2). However, HT aging had enhanced activity (P<.05),i.e., longissimus muscles from the HT aged sides tended to have higher (18%) alkaline proteinase activity than the LT aged sides.

FIGURE 2.7. NEUTRAL PROTEINASE ACTIVITY IN CRUDE LONGISSIMUS MUSCLE EXTRACTS AS AFFECTED BY AGING TEMPERATURE (HT VS LT) AND TIME POSTMORTEM. THE ASSAY WAS CONDUCTED AT PH 7.6 AND 37C. HT = *, ANFD LT = +.

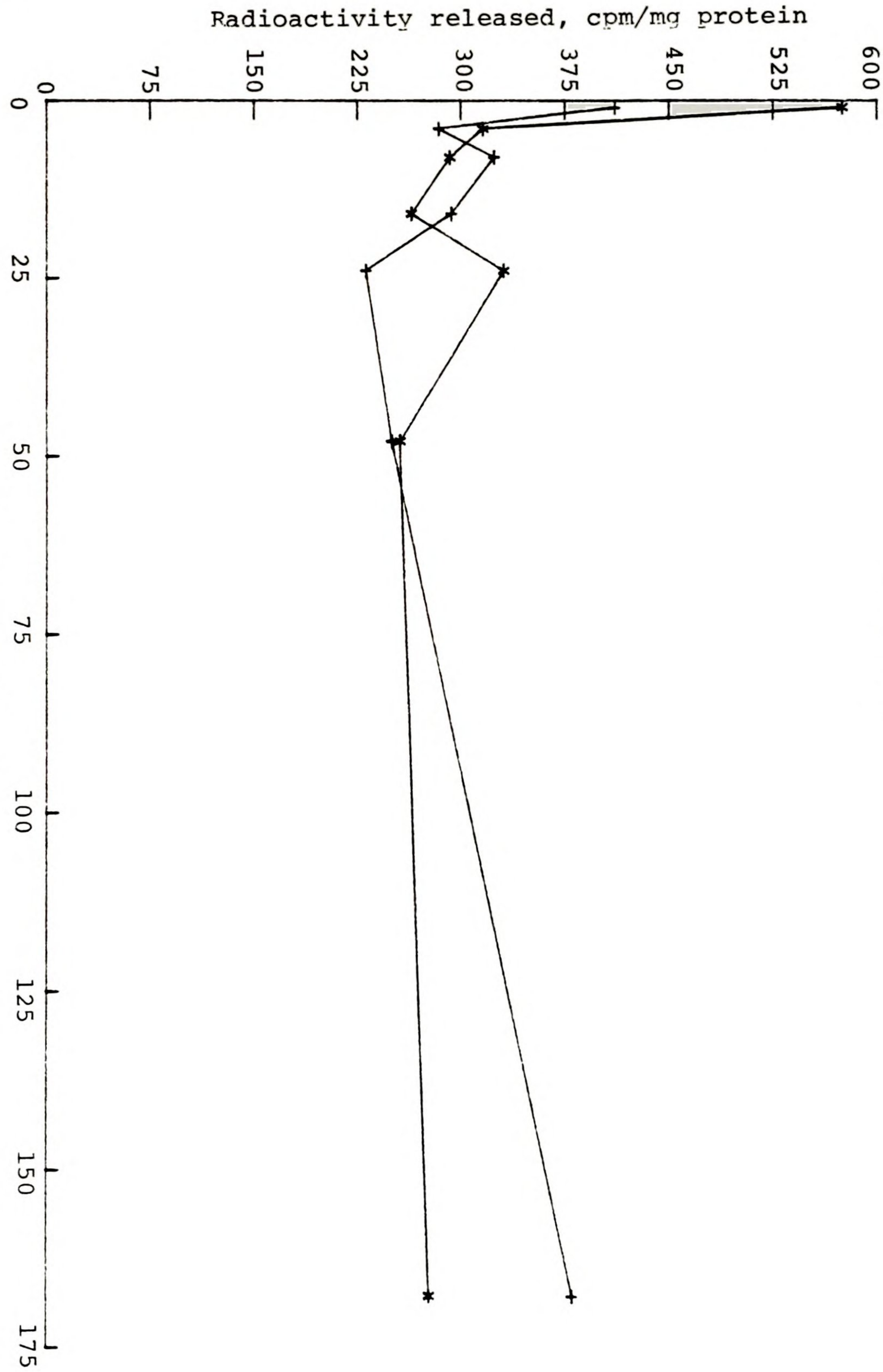
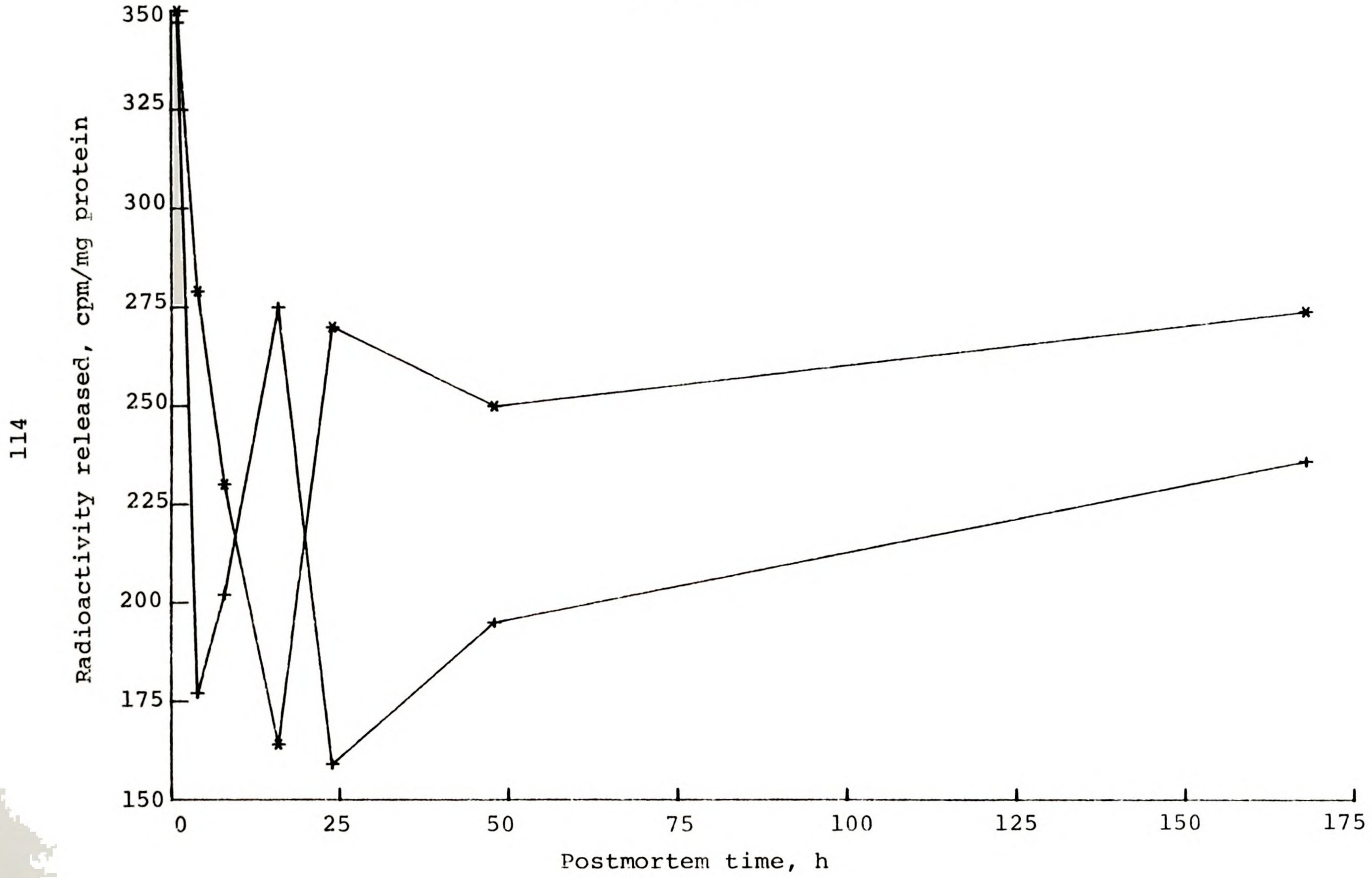

Radioactivity released, cpm/mg protein
0
75
150
225
300
375
450
525
600
0
25
50
75
100
125
150
175
Postmortem time, h

FIGURE 2.7

FIGURE 2.8. ALKALINE PROTEINASE ACTIVITY IN CRUDE LONGISSIMUS MUSCLE EXTRACTS AS INFLUENCED BY AGING TEMPERATURE (HT VS LT) AND TIME POSTMORTEM. THE ASSAY WAS CONDUCTED AT PH 9.2 AND 37C. HT= *, AND LT = +.

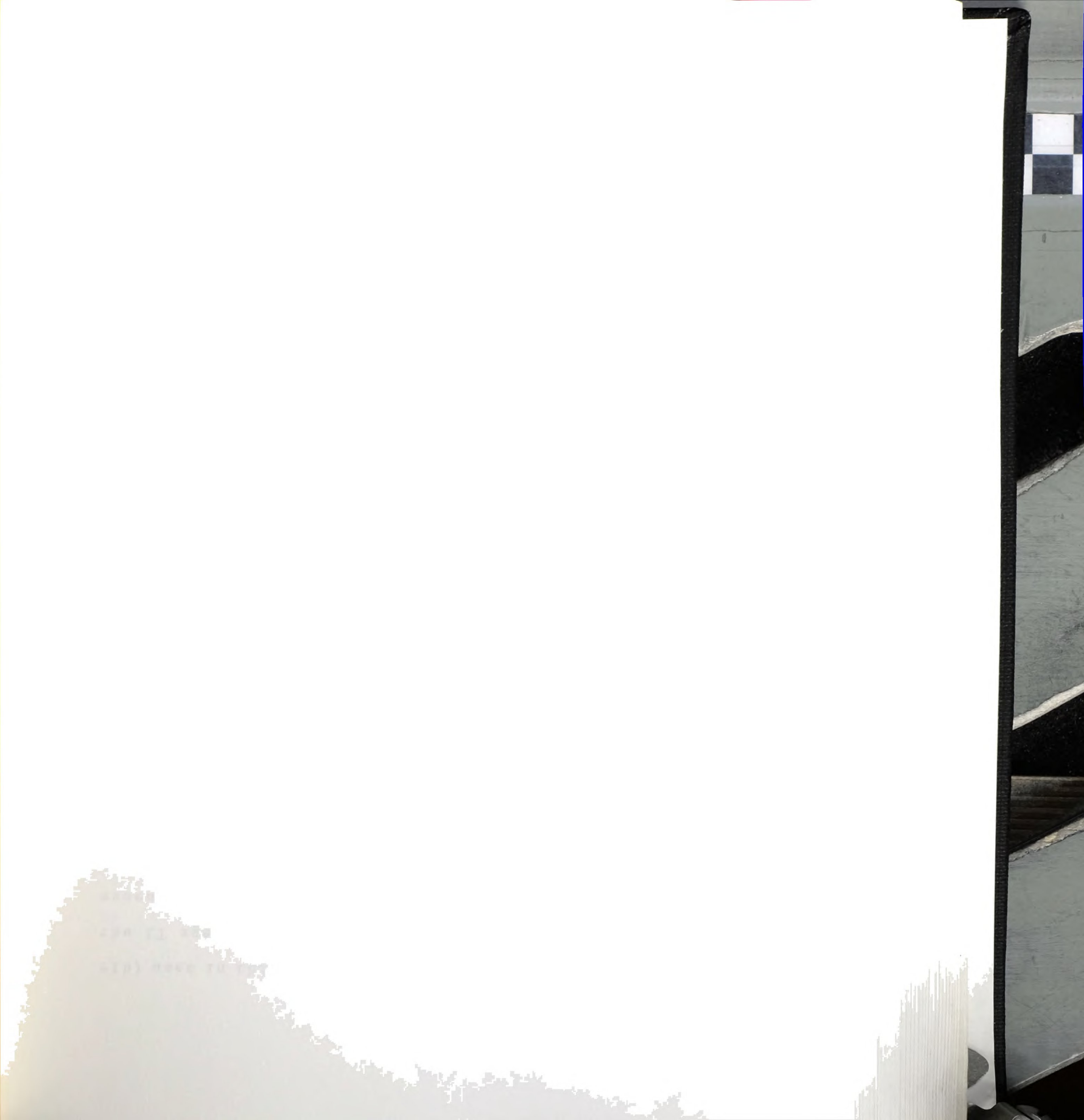
FIGURE 2.8


114
Radioactivity released, cpm/mg protein
350
325
300
275
250
225
200
175
150
0
25
50
75
100
125
150
175
Postmortem time, h

Surprisingly neither the rate nor the extent of the pH fall was different for the two aging temperature treatments. Marsh(1954) reported that, in the range of 7 to 43 C, the rate at which the pH of ox longissimus muscle fell postmortem diminished with decrease in temperature of the muscle. Cassens and Newbold (1967) reported similiar results for ox sternomandibularis muscle. The latter authors also showed that the extent to which muscle pH falls was influenced by temperature; the principal effect being the marked lowering of the ultimate pH in the temperature range of 5 to 25 C as compared to lower or higher temperature ranges. Lawrie (1962) also reported similiar results for beef muscle. However, these observations were made on muscles excised from the carcasses. This causes the muscles to attain the experimental temperature very quickly. The results of the pH fall postmortem reported here (Figure 2.5) were based on pH measurements on samples taken from the longissimus muscles while they were still on carcasses. Considering the fact that the muscle temperature remained high up to 16 h postmortem, i.e., 19.1 and 14.4 C for the HT and LT aged sides, respectively, it is not surprising that aging temperature had no effect on the rate or the extent of pH fall postmortem. The relatively fat carcasses (approximantly 1.5 cm average fat thickness at the 12th

rib) used in this experiment slowed the chilling rate of the LT aged sides such that the rate of pH change was essentially equal to that of the HT aged sides.

While no differences in tenderness (Warner-Bratzler shear) were observed between the HT and LT aged sides, others have reported different results for carcasses exposed to these aging conditions. Smith et al. (1971), Parrish et al. (1973) and Fields et al. (1976) reported that aging of beef carcasses at 14 to 19 C for 12 to 24 h produced more tender steaks than aging at 0 to 2 C. Similiar results were reported recently (Marsh et al.,1981; Dutson, 1983). These observations , however, were not applicable to carcasses from cattle that received high planes of nutrition. Smith et al. (1976) reported that increased quantities of fat: 1) decreased the rate of temperature decline either by insulation or via an increase in carcass mass, 2) enhanced the activity of autolytic enzymes or increased the duration of active proteolysis, 3) lessens the extent of myofibrillar shortening and thereby increased the ultimate tenderness of cooked meat from fatter carcasses compared to those with less fat. Smith et al. (1979) observed that aging at 13 C improved the taste panel tenderness and juiciness of beef longissimus steaks as opposed to conventional chilling at 3 C. These results were true only for thin carcasses from grass-fed steers. These differences



produced by different aging temperatures were not evident in carcasses from steers that had received high concentrate diets for 49 d and 98 d or those fed a high forage diet for 98 d compared to the grass-fed group. Moreover, even for the grass-fed steers, shear force comparisons did not support the taste panel data. Similiar observations were reported by Parrish et al. (1969) for beef carcasses held at 15 C for 48 h vs carcasses chilled at 2 C when samples were evaluated at 4 or 7 d postmortem.

The lack of differences in shear force values for the HT and LT aged steer sides (Table 2.1) is in agreement with the above results, i.e., (Smith et al., 1976; Smith et al.,1979; Parrish et al.,1969). The failure to detect differences in tenderness may be due to the fact that the steer carcasses in this experiment were fat and had high marbling scores as all five steers graded U.S. Choice. The temperature decline curves (Figure 2.4) indicate that the LT aged sides maintained longissimus muscle temperatures above 14 C for at least 16 h postmortem. This may explain why no differences in tenderness existed between the HT and LT aged sides. Shear force measurements may also be less sensitive in detecting differences in tenderness compared to taste panels as was evident in the observations reported by Smith et al. (1979).

The lack of significant differences between the HT

and LT aged sides for acidic and neutral proteinase activities does not agree with many reported observations. The optimal temperature for many enzymes is near 37 C (Bird and Carter,1980; Beynon and Kay, 1978; Katunama et al.,1975; Koszalka and Miller,1960). Although the activity of CAF is higher at 37 C than at 25 C for the first 15 min of incubation, the activity is more stable at 25 C for up to 12 h of incubation (Dayton et al.,1976b). Higher temperatures were also observed to cause greater release of lysosomal enzymes than at 2 C (Moeller et al., 1976; Wu et al.,1981a). Accordingly, higher enzymatic activities should prevail at higher temperatures than at lower temperatures. Many other workers observed that degradation of myofibrillar proteins was more extensive at higher than at lower temperatures (Arakawa et al.,1976; Robbins et al.,1979; Yates et al.,1983; Dutson,1983). The data in Figures 2.6 and 2.7, for the acidic and neutral proteinase activities indicate that activity of both proteinase systems declined as muscle temperature decreased for both aging temperature treatments. The failure to observe differences between the HT and LT aging treatments may be due to the fact, already mentioned, that the temperature of the muscles in both treatments remained above 14 C for at least 16 h postmortem. Moreover, the differences in muscle temperature between the two treatments were small although statistically significant, i.e., 2.1,3.6, and 4.7

C at 4, 8, and 16 h. postmortem. These small muscle temperature differences might contribute to the difficulty of detecting differences in the acidic and neutral proteinase activities. This becomes even more obvious if the effect of time period postmortem on the acidic and neutral proteinase activities is considered. In comparing the time period means for both proteinsae activities, the activity at 4 and 8 h postmortem was not different from that at 1 hour ($P>.05$). Enzyme activity was lower ($P<.05$) at 16 h postmortem when muscle temperature had decreased to 19.1 and 14.4 C These data suggest that temperature differences at least 15 C are required to observe differences in enzymatic activity of the acidic and neutral proteinases. Most of researchers mentioned above used intact muscles or muscle strips removed from the carcass shortly after death (Wu et al., 1981a; Yates et al., 1983) or even isolated myofibrils (Arakawa et al., 1976; Robbins et al., 1979) in their studies. These systems reach the desired temperature quickly. The comparisons were often made between conventional chilling temperatures of 2 C and high temperatures of 22, 25, 35 or 37 C, i.e., large temperature differences. These factors together with the diversity of assay methods for assessing enzymatic activity may, partially, explain the discrepancy in the results reported herein and those of others.

Although most of the above mentioned studies have assessed proteinase activities under optimal conditions of temperature and pH, these conditions are not necessarily those which prevail in postmortem muscle. Thus, most of these studies including this study rely on in vitro systems for assessing protease activites. Different substrates for purified protease have been used by these researchers. Which include synthetic substances (Moeller et al., 1976; Wu et al., 1981) purified myofibrils (Dayton et al., 1976b; Robbins et al., 1979) and casein (Dayton et al., 1976b). Other researchers have used purified myofibrils and after autolysis employed electrophoresis to show the changes in myofibrillar proteins attributable to proteolysis (Arkawa et al., 1976; Yates et al., 1983). This leaves doubt as to whether these studies are comparable and whether the study reported herein, which used CN-labeled hemoglobin as a substrate is comparable to them. Moreover, in all the studies which employed purified enzymes (Dayton et al., 1976b; Moeller et al., 1976; Robbins et al., 1979; Wu et al., 1981) endogenous protease inhibitors are excluded from the assay mixtures. This adds to the difficulty of comparing other studies with the present study since crude muscle homogenates were used herein which have all the inhibitors and muscle proteins present. All these factors might explain the discrepency of the results reported here

and earlier studies on proteinase activity.

The alkaline proteinase activity produced different results than the acidic and neutral proteinases (Figure 2.8). While time postmortem (up to 168 h) had no effect ($P>.05$) on alkaline proteinase activity, HT aging enhanced ($P<.05$) the activity of this proteinase system. The authors who have reported enzymes active in the alkaline pH range conducted their assays at 37 C (Koszalka and Miller, 1960b; Mayer et al., 1974; Katunuma et al., 1975; Murakami and Uchida, 1978). They did not compare enzyme activities at different temperatures. In one study (Noguchi and Kandatsu, 1976) it was determined that a rat alkaline protease had its maximum activity at 57 C. It is somewhat surprising that small differences in temperature, i.e., HT vs LT aged sides caused detectable differences in the alkaline enzyme system in favor of the HT aging, while large differences in temperature, i.e.,1 h postmortem vs 16, 24, 48 and 168 hours did not significantly affect enzyme activity. The practical significance of the alkaline proteases, due to their high pH optimum (9.2), probably is not important in postmortem tenderization of meat.

It is concluded from this study that while storage time postmortem was more important than aging temperature for the acidic and neutral proteinase activities, aging

temperature was an important factor affecting the alkaline proteinase activity. Neither the aging temperature nor postmortem proteolytic activity were important factors in determining tenderness (Warner-Bratzler shear) of longissimus muscle steaks from these high quality beef carcasses.

SUMMARY

The effects of early postmortem storage temperature on proteinase activity and tenderness of beef longissimus muscle were evaluated on five steer carcasses. The right side of five U.S. Choice grade steers were placed in a 2 C cooler 1 h postmortem (low temperature, LT), while the left side were held at 22 C for 16 h and then transferred to the cooler (high temperature, HT) and held at 2 C thereafter. The activity of the acidic, neutral and alkaline proteinases was measured (at optimal conditions, i.e., 37C and pH 4.9, 7.9 and 9.2, respectively) on crude longissimus muscle extracts using CN-labeled hemoglobin at 1, 4, 8, 16, 24, 48 and 168 h postmortem. Samples of the longissimus muscle were taken at each of these periods and extracted in .05M Tris-HCL buffer, pH 7.3, containing 1M KCL and .2% Triton-X100. The ratio of the crude muscle extracts to labeled hemoglobin to a citrate-phosphate-borate buffer (pH monitor) was 16:5:16. Proteinase activity was determined as radioactivity released from labeled hemoglobin after acid precipitation and expressed as blank corrected cpm/mg protein.

Steaks (5 cm thick) from the 12th rib region were removed at 168 h for Warner-Bratzler shear force determination. The absence of period x temperature interaction indicated that the time trend in proteinase

activity for the two temperature treatments was not different from parallel (P>.05). Activity of the acidic and neutral proteinases was not affected (P>.05) by temperature treatment, however, HT enhanced (P<.05) alkaline proteinase activity. Activity at 16 h as compared to 1 h postmortem was lower (P<.05) for the neutral proteinase and tended to be lower (P=.01) for the acidic proteinase. Time postmortem had no effect (P>.05) on alkaline proteinase activity. Aging temperature had no effect (P>.05) on tenderness (shear force).

It is concluded that while postmortem storage time is more important than aging temperature in affecting the acidic and neutral proteinase activities, aging temperature is an important determinant of alkaline proteinase activity. Neither aging temperature nor proteinase activity were important factors in determining tenderness of good quality steaks in this study.

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APPENDICES

APPENDIX I

Preparation of homogenization buffer (For muscle homogenates used to determine the proteolytic activity). 1:1 (v/v) glycerol: Tris-HCL buffer (pH 7.3) containing 1M KCL and 0.2% (w/v) Triton x 100

1) Calculation

pK for Trisma base = 8.2

since pH = pK + log (conjugate base/ conjugate acid)

so = pH - pK = log (base/acid)

= -.9

i.e. the ratio of conjugate base to conjugate acid is .112:.888

2) Stock solutions:

.5M Trisma base : .5x 121.1 = 60.55 g/1

.5M Tris-HCL : .5x 158 = 79 g/1

3) Preparation of one liter of buffer:

a) Trisma base	.112x 100	=	11.2	ml
b) Tris-HCL	.888x 100	=	88.8	ml
c) KCL		=	74.55	g
d) Triton-x 100		=	2.0	g

Mix all ingrediants in a large beaker. Add about 100 ml of mega pure water and stir. transfer solution to 500ml

of mega pure water and stir. transfer solution to 500ml volumetric flask and add water to volume. Transfer to a 1 liter volumetric flask and bring to volume with glycerol. Rinsing of glassware is very important.

APPENDIX II

Preparation of assay buffer (used to monitor the pH in the enzymatic activity assays).

Citrate-phosphate-borate buffer containing .5M KCL. To desired pH.

Stock solutions (one liter each)

1) 1N NaOH - 40 g/1.
2) H PO - 35ml of 85% H PO .
3) Citric acid - 70 g/1.
4) .1N HCL containing .5M KCL - 8.333 ml of 12 N HCL + 37.275 g KCL/1 (for pH adjustment)

Preparation of buffer (one liter) from stock solutions listed above.

a) 100 ml H PO
b) 100 ml Citric acid
c) 343 ml 1N NaOH

Weigh out:

d) 3.54 g Boric acid
e) 37.275 g KCL

Mix in a large beaker and stir to dissolve boric acid and KCL. Transfer to a 1000ml volumetric flask. Bring to volume.

To have the buffer at a desired pH, mix 20ml (or

To have the buffer at a desired pH, mix 20ml (or multiples thereof) of this buffer with a certain volume of the .1N HCL stock solution listed above. These volumes are determined from a table reported by Teorell and Stenhagen (1938) as shown below.

For each 20ml batch of the buffer:

pH values used in the preliminary and final experiments.

Preliminary Experiment

Desired pH	ml of .1N HCL
3.0	56.50
3.5	53.20
4.0	50.50
4.5	47.80
5.0	45.18
5.5	42.36
6.0	39.42
6.5	36.06
7.0	32.65
7.5	29.87
8.0	28.02
8.5	26.60
9.0	23.75
9.5	20.52
10.0	17.92

Final Experiment

4.9	49.68
7.6	29.43
9.2	22.38