POST MORTEM PROTEOLYTIC CHANGES AFFECTING MYOFIBRILLAR PROTEINS

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The changes in tenderness of beef and poultry muscle as a function of time post mortem are well documented. Initially, in the pre-rigor state, the muscles are very tender and, if beef is cooked rapidly within an hour after slaughter, the meat is as tender as if it were aged for 144 hours. However, it is quite tough if cooked at 24 hours post mortem (Paul et al., 1952). Poultry is apparently similar to beef in terms of post mortem tenderness changes; however, the time involved is much shorter. (Beef may require 10-20 days to reach maximum tenderness while poultry requires 12 to 24 hours (de Fremery and Pool, 1960).

While the chemical events associated with the increase in toughness of meat (rigor) are, in general, well-understood (Bendall, 1960, 1963), the events which lead to an increase in tenderness on aging are not well-understood. One might postulate that the increase in tenderness on aging is due to (1) resolution of rigor mortis, (2) changes in the connective tissue content of the muscle, (3) changes in the hydration of the proteins as influenced by changes in pH and ionic atmosphere and, (4) proteolytic enzyme action on the myofibrillar proteins.

There is no experimental evidence to support the first two postulations. Marsh (1954) reported that beef muscle in full rigor held under nitrogen at 70°C showed no increase in extensibility after 7 days or after 24 hours at 37°C. Bate-Smith and Bendall (1956) have reported similar results for rabbit muscle held at 30°C. Therefore, it appears to be erroneous to speak of the "resolution of rigor mortis" as this implies the reversal of the process by which rigor occurred. There appears to be no evidence that during the tenderness increase there is a conversion of actomyosin back to actin and myosin. Wierbicki et al. (1954) have shown that there is no change in the connective tissue content of meat on aging. This observation should be contrasted to the changes found in the connective tissues of meat after treatment with proteolytic enzymes such as papain, ficin or bromelain (Wang et al., 1957; Miyada and Tappel, 1956; El-Gharbawi and Whitaker, 1963). Ficin can hydrolyze elastin quite readily even at low temperatures (El-Gharbawi and Whitaker, 1963; Yatco-Manzo and Whitaker, 1962), while collagen is not hydrolyzed by these enzymes unless it is first denatured (Hinricks and Whitaker, 1962; El-Gharbawi and Whitaker, 1963). Birkner and Auerbach (1960) have reported changes in the collagen but not elastin content of meat on aging but they concluded that the changes probably do not account for increased tenderness on aging.

This brings us to the third postulation. The work of Deatherage and Hamm (1960), Hamm and Deatherage (1960 a, b), Wierbicki et al.
(1957a, b), Hamm (1960), Bendall and Wismer-Pedersen (1962) as well as others leave little doubt that there are changes in the hydration of meat proteins during aging. These changes in hydration are brought about by changes in the pH and ionic atmosphere of the muscle on aging. Some of this increase in hydration could also be the result of proteolytic enzyme action as we have found that we could increase the water binding capacity of meat by 30 to 50% by treatment with the proteolytic enzyme ficin (see Hamm, 1960, p. 393). Dr. Wismer-Pedersen will cover the subject of meat protein hydration in the next talk so we shall not dwell further on this postulation.

The fourth possibility for explaining the increase in tenderness of meat on aging is through the action of proteolytic enzymes on the myofibrillar proteins. It is now generally accepted that most living cells contain small subcellular particles called lysosomes. The number of lysosomes per cell is dependent upon the function of the cell. These lysosomes contain a large number of hydrolytic enzymes including the cathepsins. In order for the enzymes to be released to act upon the cellular constituents the lysosomal membrane must be broken. The strength of this membrane appears to be a function of the health of the animal and the ease with which lipid peroxidation can take place. One would expect that after death and during the aging process many of these membranes would be broken.

There are two approaches that have been used to attempt to understand the role of the cathepsins in meat tenderization. The first involves the demonstration of hydrolytic changes in the proteins during the aging process and the second the isolation of the cathepsins and a study of their properties. In both approaches one must be careful that what he is dealing with is the result of action of the proteolytic enzymes of the muscle and not of the proteolytic enzymes of microbial origin.

Zender et al. (1958) have demonstrated that there are electrophoretic changes in the properties of the proteins of rabbit and lamb muscle on aging under aseptic conditions. Fischer (1963) reported that there was a correlation between the increase in a water-soluble protein fraction of chicken muscle as separated on a DEAE-cellulose column and tenderness as measured by a taste panel. Weinberg and Rose (1960) found that the extractable nitrogen increased during aging of chicken muscle. High dosage irradiation of unheated meat generally leads to a mushy texture and the liberation of tyrosine which is attributed to the action of proteolytic enzymes (Nickerson et al. 1950; Doty and Wachter, 1955; Kirn et al., 1956; Drake et al., 1957). Histologically, a disappearance of the cross-striations and the appearance of transverse breaks is found on aging (Birkner and Auerbach, 1960; Paul et al., 1944). Whether these histological changes are the result of catheptic action is not clear. It is well-known that after rigor poultry muscle increases in tenderness much more rapidly than does beef muscle. There should be some correlation between the amount of proteolytic enzyme activity in a tissue and the rate at which tenderness develops if the enzymes are involved. Bandock-Yuri and Rose (1961), working with chickens killed and dressed under commercial conditions, found less catheptic activity in chicken than in beef muscle. However, we have found that chicken leg and breast muscle contain considerably more catheptic activity than does beef round (Doyle and Whitaker, 1964). On the other
hand, Wierbicki and Deatherage (1956) and Locker (1960) found very little, if any, evidence for proteolysis occurring during aging. Small injuries to muscle tissue result in rapid clearance of the tissue by the increased number of phagocytic cells (Field, 1960). Whether these phagocytic cells come from the vascular system or from the muscle system is not clear. However, it is clear there is a great increase in proteolytic activity.

The demonstration of hydrolytic changes in the complex protein system of muscle during aging is extremely difficult. The usual classical procedures for measuring an increase in amino or carboxyl groups as the result of peptide bond splitting are not sensitive enough. Large fragmentation of the molecules must occur before the detectable changes in amino or carboxyl group content are above experimental error. One, therefore, must rely upon the more modern techniques of electrophoresis and ion-exchange chromatography. Even these alone are not sufficient. If a change in the electrophoretic and/or chromatographic properties of a protein fraction is detected during aging, it must then be demonstrated that the change is due to a splitting of peptide bonds by proteolytic enzymes rather than due to denaturation, complexation or deamidation. In other words, one must demonstrate a decrease in molecular weight and the appearance of new end terminal groups. Sephadex gel filtration should prove to be a valuable method for detecting changes in molecular weight (Whitaker, 1963).

While Balls in 1938 reported the presence of proteolytic enzymes in beef muscle, most of the work on the cathepsins has been carried out on enzymes from the spleen and kidney. The proteolytic enzyme content of muscle is extremely small (less than 0.01%) and is mixed up with all the proteins normally found in muscle. The purification of the cathepsins from muscle is extremely difficult. Table I summarizes some of the properties of Cathepsins A, B, C and D from beef spleen. In addition, tissues contain several peptidases including enzymes similar to pancreatic leucine aminopeptidase and carboxypeptidase A (Smith, 1948). In muscle, it is fairly well-established that there are two groups of proteolytic enzymes with distinct pH optima, one at pH near 5 and the other at 8 to 9 which are capable of hydrolyzing the muscle proteins (in rats, Koszalka and Miller, 1960; in beef, Landmann, 1963; and in chicken, Doyle and Whitaker, 1964). We have been particularly perplexed by the fact that there is little or no action on casein or hemoglobin of the proteolytic enzymes acting at the alkaline pH. The presence of cathepsins B and C in beef muscle has been demonstrated by Landmann (1963), cathepsin A in beef muscle by Bodwell and Pearson (1964) and cathepsins A and B in chicken muscle by Doyle and Whitaker (1964). The purification procedure used for chicken cathepsin in our laboratory is shown in Table II.

This type of work has established two important points; one, proteolytic enzymes are present in muscle and second, the pH optima of some of these is within the range of meat. However, the role, if any, which these play in meat tenderization is still not answered. Bendall (1960) has indicated that "it appears to be unnecessary to search for any other cause for the accompanying resolution than the proliferation of putrefactive organisms within the muscle tissue."
REFERENCES


Wierbicki, E., V. R. Cahill, and F. E. Deatherage. 1957a. Effects of added sodium chloride, potassium chloride, calcium chloride, magnesium chloride and citric acid on meat shrinkage at 70° C. and of added sodium chloride on drip losses during freezing and thawing. Food Technol. 11, 74.


**Table I**  
Endopeptidases of Animal Tissues

<table>
<thead>
<tr>
<th>Name</th>
<th>Specificity</th>
<th>pH optimum</th>
<th>Ref.</th>
</tr>
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<tbody>
<tr>
<td>Cathepsin A</td>
<td>Homospecific with pepsin</td>
<td>5.4 (Cbz-GT)²</td>
<td>Fruton and Bergmann (1939)</td>
</tr>
<tr>
<td>Cathepsin B (-SH)</td>
<td>Homospecific with trypsin</td>
<td>5 (BAA)²</td>
<td>Greenbaum and Fruton (1957)</td>
</tr>
<tr>
<td>Cathepsin C (-SH)</td>
<td>Homospecific with α-chymotrypsin</td>
<td>3.5 (hemoglobin)</td>
<td>Gutmann and Fruton (1948);</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5-6 (GPA)²</td>
<td>Fruton &amp; Mycek (1956);</td>
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<td></td>
<td></td>
<td></td>
<td>Tallen et al. (1952);</td>
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<td></td>
<td></td>
<td></td>
<td>De la Haba et al. (1959)</td>
</tr>
<tr>
<td>Cathepsin D</td>
<td>Same specificity as pepsin on oxidized B chain of insulin, does not split Cbz-GT²</td>
<td>3.1 (hemoglobin)</td>
<td>Press et al. (1960)</td>
</tr>
<tr>
<td>Muscle proteinase</td>
<td>Does not hydrolyze the specific substrates of pepsin, trypsin, or α-chymotrypsin</td>
<td>4 (hemoglobin)</td>
<td>Snoke &amp; Neurath (1950)</td>
</tr>
</tbody>
</table>

¹ Activators

² The following abbreviations are used: BAA, α-benzoyl-L-argininamide; Cbz-GT, carbobenzoxy-L-glutamyl-L-tyrosine; GPA, glycyl-L-phenylalaninamide.
Table II
Purification Procedure for Chicken Skeletal Muscle Cathepsins
(Doyle and Whitaker, 1964)

<table>
<thead>
<tr>
<th>Procedure</th>
<th>Activity recovered (%)</th>
<th>Specific activity</th>
<th>Purification (fold)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Supernatant liquid from 80,000 x g centrifugation of homogenate</td>
<td>100</td>
<td>0.01</td>
<td>1</td>
</tr>
<tr>
<td>2. pH adjusted to 4.2, 5% saturated ammonium sulfate added, supernatant liquid saved</td>
<td>100</td>
<td>0.08 - 0.1</td>
<td>8-10</td>
</tr>
<tr>
<td>3. Fraction between 45 and 70% saturated ammonium sulfate</td>
<td>60</td>
<td>0.1 - 0.25</td>
<td>10-25</td>
</tr>
<tr>
<td>4. First chromatography on CM-cellulose (450 ml/hr., pH 5.2)</td>
<td>60</td>
<td>0.5 - 4.0</td>
<td>50-400</td>
</tr>
<tr>
<td>5. Second chromatography on CM-cellulose (100 ml/hr., pH 5.2)</td>
<td>26</td>
<td>9.4</td>
<td>940</td>
</tr>
</tbody>
</table>

*Activity measured on urea denatured hemoglobin at pH 4.6 and 35.0°C.*

DR. SAYRE: Thank you, Dr. Whitaker, for a very interesting presentation. I think we had better get on with the next speaker now. We again are quite fortunate to have with us Dr. Wismer-Pedersen. He received his Ph.D. degree from McGill University and has been working for several years at the Danish Meat Research Institute on the biochemistry of post mortem changes in muscle. This past year he has been a visiting professor at Michigan State University and soon will return to the Danish Meat Research Institute. It gives me great pleasure to present Dr. Wismer-Pedersen.

DR. WISMER-PEDERSEN: Thank you.