Reticular fibers of the endomysial area of bovine skeletal muscle appear to provide the framework for the sheath which surrounds individual muscle cells. Paul (1963) reported that the endomysial reticulum of biceps femoris muscle samples remained intact after heating. Crow, et al. (1964) observed that reticular fibers appeared to vary in density in uncooked samples of the longissimus dorsi muscle exhibiting extreme tenderness differences. Riley (1959) reported that mast cells are free cells present in the loose connective tissues of ox muscle. Crow, et al. (1964) observed that mast cells were closely associated with the perimysial and endomysial connective tissue of bovine skeletal muscle and appeared to be randomly distributed.

This investigation was undertaken to study histologically, the relationship of reticular fibers and mast cells to tenderness of the bovine longissimus dorsi muscle.

Materials and Methods

One hundred twenty carcasses from yearling steers were surveyed for tenderness using shear values of the longissimus dorsi muscle. Samples were excised from the area of the 12th rib two days post-mortem and aged an additional five days at 3°C. From this group 30 samples were selected. Half of the samples were taken from muscles classified as low shear value (less than 10.6 kg. on a 2.5 cm. core), and the other fifteen represented high shear value muscles (greater than 17.5 kg. on a 2.5 cm. core). These extreme shear value groups were used for relating tenderness, mast cell numbers and reticular fiber intactness.

Shear Value Determinations. Samples removed from the longissimus dorsi muscle were approximately 4 cm. thick. Each sample was standardized to a thickness of 3.2 cm. These steaks were cooked in deep fat (135°C) to an internal temperature of 70°C., and three 2.5 cm. cores were removed and sheared using the Warner-Bratzler shear machine. Samples, approximately 4 x 4 x 3 mm., were removed for reticular fiber determinations from the median core of the cooked steaks after shearing. Similar samples were removed from the median area of the adjacent uncooked portion for reticular fiber and mast cell determinations.

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Recticular Fiber Staining Procedures. Reticular fibers were stained in both cooked and uncooked samples, using a modification of the procedure described by Soule, (1962). Samples were placed immediately into 10 percent buffered (pH 7.0-7.2) neutral formalin, and fixed for a minimum of 72 hours. All samples were then dehydrated in graded ethyl alcohol baths, cleared in xylene and embedded for cross-sectioning in Paraplast (M.P. 56-58°C.). Following sectioning at 6 microns, tissue ribbons were floated out on a water bath (50-52°C.), separated and picked up on acid clean slides coated with Mayers-Albumin fixative, and allowed to dry for 12 hours. Slide-mounted sections were cleared in xylene and taken to water in graded alcohols. Staining was accomplished in one percent gold chloride for 30 minutes at 35°C., followed by dipping in distilled deionized water and transferring to a 5 percent sodium carbonate, 0.5 percent potassium hydroxide bath for 10 minutes. Slides were then transferred directly to a 5 percent potassium iodide solution for 3 minutes, followed by rinsing twice in distilled deionized water. Counter staining was carried out in a 0.25% methylene blue chloride solution for 30 seconds followed by rinsing two times in distilled deionized water. Sections were dehydrated in 95 percent ethyl alcohol, cleared in xylene and mounted in Permount.

Mast Cell Staining Procedures. Mast cells were determined in uncooked samples, utilizing a modification of the procedure described by Smith, et al., 1962. Samples were fixed for 72 hours in 95 percent ethyl alcohol: 10 percent formalin (9:1) with one percent sodium acetate added. Samples were dehydrated in 2 changes each of 95 and 100 percent ethyl alcohol for one hour, followed by clearing in xylene and embedding for longitudinal sectioning in Paraplast. The embedded tissue was sectioned serially at 6 microns, and tissue ribbons were placed directly on acid clean glass slides coated with Mayers-Albumin fixative. Slides were passed over a hot plate (55°C.) and allowed to dry for 12 hours. Tissue sections were cleared in xylene and taken to 95 percent alcohol, followed by staining in 0.25 percent Toluidine Blue 0 in 70 percent acidified ethyl alcohol (pH 1.65) for 30 minutes. Sections were decolorized in two changes of 0.5 percent acid alcohol, and counterstained in one percent Eosin Y acidified with glacial acetic acid. Following dehydration in 95 and 100 percent alcohol, sections were cleared in xylene and mounted in Permount.

Mast Cell Determinations. Twenty serial sections per sample, 6 microns thick, were placed on each slide. A net reticule (25 mm² subdivided into one mm. squares) was placed over each section to be counted. Four areas, one mm. square, were observed on each section (400X magnification), and mast cells were identified and counted. The required following calculations were made as follows:
44.

L = 2 mm, Length of square.

W = 2 mm. Width of square.

H = 6 microns = 6 x 10^{-3} mm. = Depth of section.

N = 20, Number of sections.

(L x W x H) 20 = Volume of tissue.

\[2\text{mm} \times 2\text{mm} \times (6 \times 10^{-3})\text{mm}\] \(\times 20 = 0.480\text{mm}^3\)

Duplicate samples were prepared, read and calculated. Total tissue area equaled 0.960 mm².

MC = Total mast cell numbers of duplicate samples.

\[\frac{MC}{0.960}\]

Results

Shear Values. Mean shear values for the longissimus dorsi muscle samples used in this study are shown in table 1. The t-test revealed a significant (P < .01) difference between high and low shear value groups.

<table>
<thead>
<tr>
<th>Tenderness group</th>
<th>N</th>
<th>Shear value</th>
<th>Mast Cell numbers</th>
<th>Reticular Fiber Intactness scores</th>
</tr>
</thead>
<tbody>
<tr>
<td>High S.V.(^a)</td>
<td>15</td>
<td>20.12(^c)</td>
<td>33.80</td>
<td>4.11(^d)</td>
</tr>
<tr>
<td>S.D.(^e)</td>
<td>2.41</td>
<td>43.78</td>
<td>0.65</td>
<td>0.68</td>
</tr>
<tr>
<td>Low S.V.(^a)</td>
<td>15</td>
<td>9.40(^c)</td>
<td>39.53</td>
<td>3.57(^d)</td>
</tr>
<tr>
<td>S.D.(^e)</td>
<td>0.74</td>
<td>62.24</td>
<td>0.97</td>
<td>0.50</td>
</tr>
</tbody>
</table>

\(^{a}\) S.V.; Shear value expressed as kg. of force on a 2.5 cm. core.

\(^{b}\) Refers to mast cells per cubic mm., uncooked tissue.

\(^{c}\) P < .01

\(^{d}\) P < .05

\(^{e}\) S.D.; Standard deviation
Mast Cell Numbers. The t-test revealed no significant difference between means for mast cell numbers of the high shear and low shear value groups (table 1). Correlation coefficients, relating mast cells numbers and shear values of the high and low group were not significant (table 2).

Visual observations of mast cells revealed they were in close relationship with the endomysial connective tissue. The cells appeared to vary in their affinity for Toluidine Blue 0 as dark stained mast cells were associated with very light stained cells in the same section.

Table 2. Correlation Coefficients. Comparison of Shear Values with Mast Cell Numbers and Reticular Fiber Intactness Scores.

<table>
<thead>
<tr>
<th>Independent Variable</th>
<th>High shear value group</th>
<th>Low shear value group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mast Cell numbers</td>
<td>-0.06</td>
<td>0.40</td>
</tr>
<tr>
<td>Reticular Fiber intactness</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Uncooked</td>
<td>0.28</td>
<td>0.45(^{b})</td>
</tr>
<tr>
<td>Cooked</td>
<td>-0.29</td>
<td>0.15</td>
</tr>
</tbody>
</table>

\(^{a}\) d.f. = 14
\(^{b}\) P < .05

Reticular Fiber Intactness. Means for reticular fiber intactness scores are shown in table 1. Statistical analysis revealed a significant difference (P < .05) between reticular fiber intactness score means of cooked tissues between high and low shear value groups. Reticular fiber intactness score means also differed significantly (P < .05) between cooked and uncooked tissues in the low shear value group. There was a significant relationship (r = .49) between reticular fiber intactness scores of uncooked tissue and shear values within the low shear value group (table 2). Reticular fiber intactness scores were correlated (r = .54) with mast cell numbers in the high shear value group in uncooked tissues (table 3).

Reticular fibers of cooked and uncooked tissue did not appear to vary in their affinity for gold chloride. However, it was observed on the high and low shear value cooked tissue sections that an unidentified substance with a strong affinity for methylene blue was present in and around the reticular net.
Table 3. Correlation Coefficients. Comparison of Mast Cell Numbers in Uncooked Tissue with Reticular Fiber Intactness Scores.

<table>
<thead>
<tr>
<th>Dependent variable</th>
<th>Independent Variable</th>
<th>High shear value group</th>
<th>Low shear value group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reticular Fiber Intactness</td>
<td></td>
<td>0.54&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.28</td>
</tr>
<tr>
<td>Uncooked</td>
<td>0.24</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cooked</td>
<td></td>
<td>-0.22</td>
<td></td>
</tr>
</tbody>
</table>

a d.f. = 14
b P<.05

Discussion

Reticular fiber intactness and mast cell number mean values were not significantly different between groups of uncooked tissue. When intactness and mast cell numbers were positively correlated (r = .54) in the uncooked tissue samples, cooking followed by shearing did not appear to influence reticular fiber intactness in the high shear group. However, reticular fiber intactness significantly decreased (P< .05) after cooking and shearing in the low tenderness group. Intactness was not significantly related to mast cell numbers in the low shear value uncooked tissue samples.

Perhaps bovine mast cell constituents may influence directly or indirectly the state of reticular fibers. However, measurement precision of mast cell numbers in the connective tissue area was not sufficient to consistently reflect the influence of their constituents.

LITERATURE CITED


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DR. CRAIG: I'd like to commend the three speakers on their presentations and also on the general quality of the slides. I have one announcement I'd like to make before proceeding. That is, the Chairman of the Personnel Committee, Professor Kunkle, has asked me to mention that any of you who are interested in job changes or in hiring people to check with the registration desk upstairs and the resume of the people will be there. You are asked to please not take these things away, but simply to review them on the spot. So, anybody that's looking or anybody that wants a job please go up on the second level and take care of this at your convenience. To lead the discussion of the papers which have just been presented this morning will be Dr. R. L. Henrickson of Oklahoma State University, and I will ask that when he has finished if he will handle the questions and then turn the program back to our president for the next part of the program.

DR. HENRICKSON: Thank you, Brad. You left the impression that I was going to make a speech. This is not planned, I assure you. I do want to thank the members who spoke this morning for working very closely with me and the committee, and for furnishing the papers which were turned over to Bill Sherman this morning before their presentations. They've responded excellently in this manner. I also want to extend my appreciation to the committee, which as you can see is noted in the back of your program. You heard this morning three dynamic speakers tell about the muscle cell, the fat cell, and the mast cell along with the reticular fibers. We had heard from Mr. Gillis in relation to the influence of the natural variations of breed, species, sex, etc. on the size of the muscle fiber and on the shape of the muscle fiber. You heard also of the variations that are influenced by rigor, by temperature and by tension on the muscle, regardless of whether it be artificial or whether it be natural in the carcass. Mr. Moody pointed out the structural variations of the muscle and their importance in fat deposition. Mr. Gillis pointed out this same point. The structure of the muscle seems to be of some importance in both cases. Mr. Moody pointed out that the architecture is important from the standpoint of where the arteries are located and where the fat is deposited. Then we heard from Mr. Crow in relation to the mast cells and the reticular fibers and, to some extent, the importance of the reticular fibers on tendons. I'd like at this time to open the period for questions that you might like to direct to these men. Yes?

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