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Histological Differences in the Muscles of Full, Half and Rough Fed Steers

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This work was conducted as a part of the national project, "Cooperative Meat Investigations", formerly designated as "A Study of the Factors Which Influence the Quality and Palatability of Meat". Among the factors to be dealt with in this phase of the work is the histologic relationship between the texture and quality of meat from cattle of different degrees of finish. The size of the muscle fibers, and the amount and distribution of connective tissue, lipoids, glycogen and fats in the carcasses of fat, half fat and thin cattle have been studied in detail.

LITERATURE

In 1909, 1910 and 1911 Bell[1 2 3] studied the occurrence of fat in epithelia and in muscle fibers of the psoas and erector spinae muscles. He found fat droplets in the muscle fibers of 7 to 28 centimeter calf fetuses, but none was observed in the muscle fibers of older fetuses. In view of these observations he thinks that the fat in the muscle fibers was not merely stored but was probably required in the metabolism of the fiber in the early stages of its development. No fat was found in 3 to 7 centimeter fetuses or in the muscles of slaughtered beeves, except in one very thin steer. The presence of fat in the muscle of this steer was considered a condition of fatty degeneration or infiltration. He concluded that the fat droplets found inside epithelia, cartilage, and muscle fibers were independent of the nutritive condition of the animal.

In 1915 Bell[4] described a method for differentiating between neutral fats and lipoids. Small pieces of tissue were fixed in a mixture of 100 cc. of 10% aqueous solution of potassium bichromate and 5 cc. of glacial acetic acid. The fixed tissue was embedded in paraffin, and stained with a saturated solution of Sudan III. This method was sufficient to demonstrate any fat droplets that could be found in frozen sectional material.

Smith and Mair[5 6] are conservative about the value of the bichromate-hematoxylin method for the differentiation of fats. They found that cerebroside, a constituent of the medullary sheath, was stained by this method.

In 1904 Traina[7] studied the presence of intra-epithelial fat in marasmic cadavers and in rabbits killed by starvation. In the rabbit fat was found in the pancreas, lachrymal gland, suprarenal, thy-

roid, testis, liver and kidney. In rabbits which died of starvation, the intra-epithelial fat was not reduced in quantity, though epithelial cells were reduced to half the normal size and the connective tissue fat was almost completely used up. The condition in man did not affect the intra-glandular fat, though the connective tissue fat was very greatly reduced. He concluded that there was no connection between the nutritive condition of the individual and the intra-peritoneal fat.

In 1912 Greene[8] found that fat was stored in large quantities in superficial muscles situated along the sides of the salmon. Fat droplets were found within the sarcoplasm and just beneath the sarcolemma. He concluded that this muscle has the power of lipogenesis strongly developed.

In 1916 Bullard[9] found that the cardiac fibers of mammals, both fetal and adult, normally contain droplets of neutral fat. The fat droplets were arranged in longitudinal and transverse rows in the sarcoplasm between the myofibrillae. Large droplets were found in the Q band and smaller droplets in the J band. Muscle fibers which contained very little fat were found side by side with fibers heavily loaded with it. In states of inanition the normal visible fat gradually decreased in quantity. When fatty foods were given there was a pronounced increase in fat in the muscle. The phospholipins (lecithin and related compounds) of cardiac muscle were found in true interstitial granules (mitochondria) and were neither markedly decreased in inanition nor increased when fats were given in the foods. He is of the opinion that neutral fat droplets in cardiac muscle do not arise from true interstitial granules.

In 1907 Kemp and Hall[10] studied the relation of fat to the muscle fiber, both histologically and chemically, in the fattening of beeves. Osmic acid, Sudan III, and Scharlach R were the stains used. The muscles were sectioned fresh and after fixation in formalin. No fat was found in the muscle fibers of even the fattest animals. On the other hand some of the very lean meat yielded more fat by extraction than could be accounted for by the fat which showed under the microscope.

Wallbaum[11] in 1899 found fat inside the muscle fibers of normal children in about three-fifths of the cases examined, and in about the same per cent of rachitic children.

Rosenfeld[12] is of the opinion that in the epithelium of the kidney the fat is derived mostly from destruction of intracellular lipoids or from structural changes of the cytoplasm.

In 1906 and 1908 Smith[13] [14] studied the process of staining fat with basic aniline dyes. Carbonic acid was used to hydrolize neutral fats into fatty acids and glycerine. The fatty acids thus formed stained with basic dyes. Nile blue was found to stain fatty acids blue and neutral fats red.

MATERIALS AND METHODS

Samples of muscle for study were obtained from representatives of three groups of yearling cattle fed in the University of Missouri experimental feed lots. The cattle were all "good" to "choice" grade Hereford steer calves at the outset. One group was full fed from November to June, 196 days, on a ration of shelled corn, cottonseed meal (43% protein), legume hay, and corn silage; these being fat enough to grade "choice" slaughter cattle when the samples used were taken. A second group was fed a similar ration except the grain was limited to one-half the quantity fed the cattle in the first group. These cattle naturally consumed more roughage than the cattle in the first group. The time in the feed lot was the same. They were scarcely fat enough to sell as slaughter cattle when marketed and graded "low good". The third group was carried for a similar time on a ration of legume hay and corn silage without grain. They were in good feeder cattle condition when samples were taken and graded as "medium" slaughter cattle.

TABLE 1.—CATTLE FROM WHICH CARCASSES WERE STUDIED. ALL ABOUT FOURTEEN MONTHS OLD WHEN SLAUGHTERED

		Weight	Slaughter Grade
Full fed 196 days	— Steer No. 20	750	"low choice"
	— Steer No. 19	795	"choice"
	— Heifer No. 4	725	"choice"
Fed half a grain ration plus roughage 196 days	— Steer No. 517	635	"low good"
	— Steer No. 136x	550	"low good"
	— Heifer No. 538	630	"low good"
Fed roughage only 196 days	— Steer No. 136	450	"low medium"
	— Steer No. 589	490	"medium"

As soon as possible after the animals were killed samples of muscle for study were taken from the mid-portion of gracilis, the short head of the triceps and from the longissimus dorsi at the level of the twelfth rib. Small cubes were removed from these muscles in the right half of each animal and were fixed in the following fluids: Bouin's, Zenker's, Ciaccio's, Carnoy's, absolute alcohol, Ziegwallner's, 10% formalin, 5% formol diluted with saline and mercuric chloride plus 40% formol. Slender slips of muscle from the same areas were macerated in 20% nitric acid for periods of two to four days.

Nine days after refrigeration, samples of the same muscles from the left half of the carcasses were fixed in a like manner. All of the muscular tissue with the exception of that fixed in 10% formalin and 5% formol diluted with saline, was embedded in paraffin (melting point 55 and 60 degrees Centigrade) and sectioned at a thickness of 10 micra.

The paraffin embedded sections were stained with Mallory's triple connective tissue stain, hematoxylin-eosin, Ciaccio's, Van Giesen's, Best's carmine and a modification of the Langhan's iodine method.

For identification of true fats frozen sections were cut 25 micra in thickness with a freezing microtome, stained in methylene blue, Herxheimer's Scharlach R, and Nile blue. Some sections were counter-stained in Delafield's hematoxylin. By these methods true fats are intensely stained; cholesterin, cholesterin esters, and fatty acid mixtures are less intensely stained. Other lipoids as a rule are not stained.

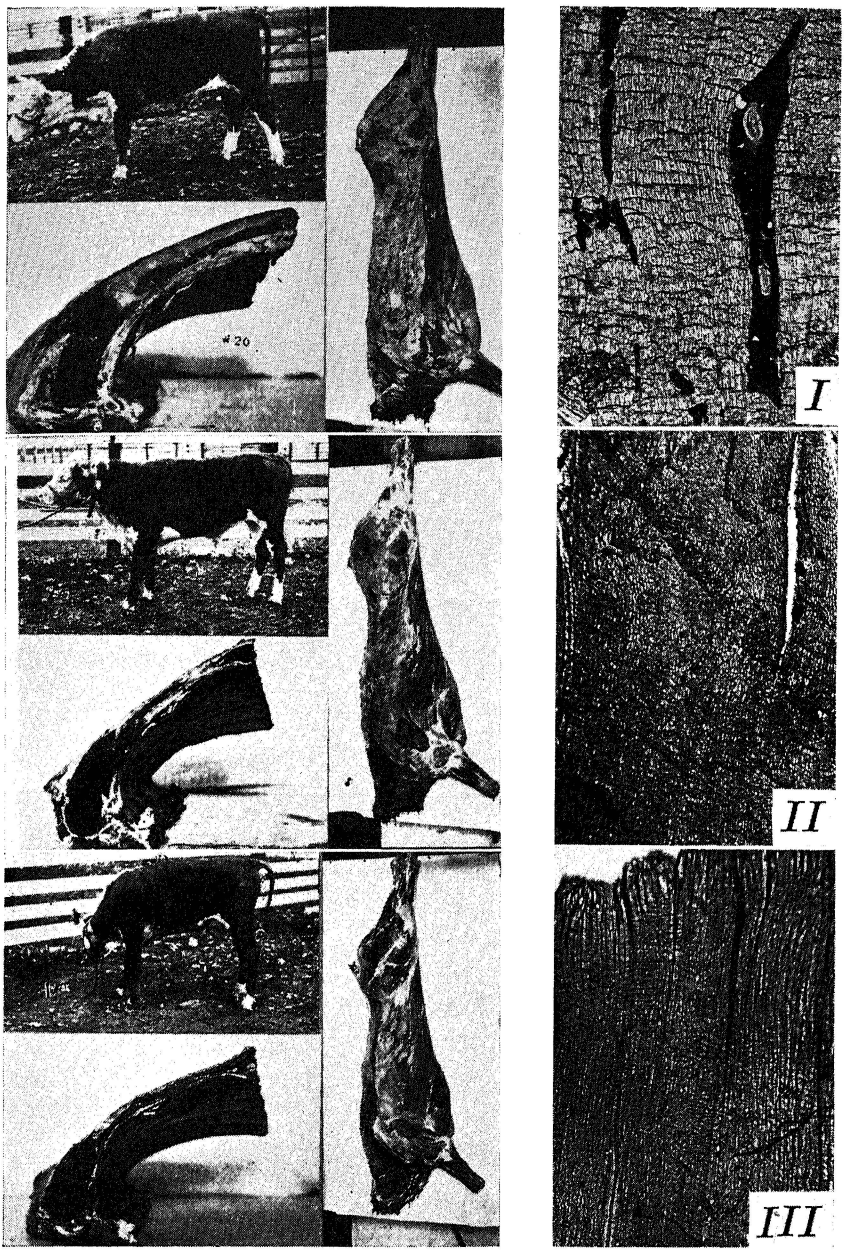
Tissues fixed in Ciaccio's fluid were stained in a saturated solution of Sudan III, counter-stained in Delafield's hematoxylin and mounted in Apathy's medium. This method gives a positive stain for masked fatty compounds (in this case, lipoids).

For the identification of glycogen two methods were used, the iodine technique and the Best's carmine method. In the modified Langhan's iodine method, sections were stained in Lugol's solution, differentiated in oil of origanum and mounted in balsam. Best's carmine method differs from the former in that the paraffin sections are placed over-night in a one per cent solution of celloidin in absolute alcohol, stained with Best's carmine solution, differentiated, and mounted in balsam. This method, although not so specific, yields a permanent stain, while the iodine technique does not. The saliva test was used on control sections of liver and muscle as a means of checking the efficiency of the stains.

For demonstrating both elastic and collagen fibers Van Giesen's and Mallory's connective tissue stains were used. In the former method the sections were stained in a picro-fuchsin mixture. This method renders white fibrous connective tissue red, and elastic tissue and muscle yellow. In the latter method the sections were stained in a solution of aniline blue and orange G. This technique renders the collagen and retiform connective tissue fibers blue and the elastic fibers red.

The tissues fixed in Bouin's fluid were stained heavily with hematoxylin and counter-stained with eosin. Over-staining with hematoxylin made individual muscle fibers stand out distinctly because the connective tissues stained a deeper blue than did the muscle fibers. The muscle for the most part underwent only slight shrinkage and was in good shape for measuring the fibers. The number of muscle fibers in an area 0.207 mm.^{2} were counted. The principle used in making the counts was essentially that used in making a differential blood count. The counts were made from twenty-five cross section areas of the gracilis and longissimus dorsi muscles of animals numbered 19, 4, 589, 136 and 538 and the average number of fibers in a square calculated.

The macerated muscle fibers were either washed in distilled water and mounted in glycerine or stained over-night in alum cochineal, washed in water, dehydrated and mounted in balsam. The diameters of 200 such muscle fibers were measured and the average size calculated.



There are here shown photographs of each of three steers at the time of slaughter (14 months of age), of the 11th rib, of the chilled carcass, and on the right, photomicrographs of longitudinal sections (x40) of the longissimus dorsi muscle at the level of the twelfth rib stained with Herxheimer's Scharlach R.

I. Steer No. 20, fed a full ration and roughage 196 days, live weight 750 pounds. Note the great thickness of fat on the rib cut, and the large amount of fat between the muscle fibers in the photomicrograph. (fat shows intensely black).

II. Steer No. 517, fed a half grain ration and roughage 196 days, live weight 635 pounds. Note the intermediate amount of fat showing in the rib cut, and the relatively small amount of fat between the muscle fibers (3 black spots along the right margin of the white streak in the upper right portion of the photomicrograph).

III. Steer No. 136 fed on roughage without grain 196 days, live weight 450 pounds. Note the small amount of fat in the rib cut. There was no fat found between the muscle fibers (longissimus dorsi) in the sections studied microscopically.

OBSERVATIONS

The results with glycogen stains were not very definite, although small amounts were seen as fine granules and amorphous masses scattered irregularly throughout the protoplasm of the muscle fibers taken from the freshly killed animals. Sections from the refrigerated meat showed very little or no glycogen in the muscle fibers. This was probably due to this product having been transformed into sugar, a substance not detected by histo-chemical methods.

The individual muscle fibers as seen in transverse sections had such irregular shapes that accurate measurements were very difficult. Since it seemed desirable to obtain for comparison some estimate of their relative sizes, the number of fibers in 25 given areas (as mentioned under "Materials and Methods") was counted and the average number per square computed. By this method sections of muscle from the half-fed animals were found to contain a greater number of muscle fibers per unit area than was found in the muscle of the full-fed animals. In other words, the muscle fibers of the full-fed animals were larger in diameter than those of the half-fed animals. The average numbers of fibers counted in the given area of transverse sections of the longissimus dorsi muscles (at the level of the twelfth rib) of the full and half-fed animals were 9.7 and 12, respectively. The average numbers of fibers counted from sections of the gracilis muscle of the full-fed cattle were 10.6 and for the half-fed ones, 13.2.

A more exact method of size determination was the measurement of the diameters of the isolated muscle fibers. Two hundred macerated fibers from each muscle were carefully measured and the average size determined. Such measurements of the muscle fibers before refrigeration showed a definite relation in size to the adiposity of the animals. Those of the three full-fed cattle averaged 61 micra and were 33% larger than those of the rough-fed steers which averaged 41.24 micra in diameter. The muscle fibers of half-fed animals were intermediate in size between the above extremes, measuring 51.74 micra (Table 2). The above measurements were taken from the gracilis muscle.

TABLE 2.—AVERAGE DIAMETERS IN MICRA OF THE ISOLATED MUSCLE FIBERS OF STEERS

Muscle	Full-fed	Half-fed	Rough-fed
Longissimus dorsi	64.23	60.48	48.40
Gracilis	61.00	51.74	41.24
AVERAGE	62.61	56.11	44.82

Macerated fibers from the longissimus muscles at the level of the twelfth rib of steers full-fed (No. 20), half-fed (No. 517) and rough fed (No. 136), showed a similar gradation in size.

In view of the results obtained by both methods of muscle fiber measurement it is very obvious that the size of the fiber is in part somewhat related to the nutrition of the animal. What then in the nutrition of the animal is responsible for this size variation of fibers? Is their size somewhat proportional to the amount of stored fats, lipoids and glycogen, or are there some other factors to be considered? In this work true fats were in no case found within the muscle fibers, so we may safely eliminate this storage product as one of the possible factors. Further research will be necessary to clarify this problem.

Frozen sections stained with Nile blue, Scharlach R, and methylene blue showed fat globules (true fats) in the connective tissue between the muscle fibers. The amount of fat present varied considerably in the different groups of cattle and seemed to be somewhat proportional to the amount of nutrients consumed. There were numerous areas composed of large fat globules distributed between the muscle fibers of the full-fed animals (Fig. I), such an abundance in fact that the stained fatty masses were observed grossly in all sections studied. Sections of muscle from the half-fed animals contained relatively much less fat (Fig. II). In addition, the fat globules in the latter animals were smaller in size, and distributed singly here and there between the muscle fibers. In the case of the rough-fed steers the fat was so scarce that often a single globule would not be seen in several sections (Fig. III). It is noteworthy that in all of the animals studied, true fats were in no case seen within the muscle fibers.

Sections from the refrigerated meat presented microscopic pictures of fat very similar to those just described. From this observation it seems reasonable to conclude that cold storage of short duration (nine days) does not alter the true fats in character, distribution or amount, at least in so far as determined by these histochemical methods.

Histologic study showed the connective tissue to consist mainly of collagen and retiform fibers. It was distributed between the muscle fibers uniting them into fasciculi and also serving to support the blood vessels. The fibers of this tissue were so small and irregular in their course that it was difficult to draw any definite conclusions as to the relative amount. However, it seems that there is no appreciable difference in the amount, distribution and character of the connective tissue present in the muscles of the animals studied. The histologic picture in the refrigerated meat was much the same.

CONCLUSIONS

1. The muscle fibers of the full-fed animals were greatest in diameter, those of the rough-fed steers were smallest, and those from the half-fed were intermediate in size.

2. Glycogen was found in small amounts in the fresh muscle fibers of all animals studied.

3. In the refrigerated meat glycogen was not demonstrated.

4. True fats were found in abundance in the connective tissue of the muscles of the full-fed cattle while only traces were found in the rough-fed steer.

5. True fats were not demonstrated within the muscle fibers.

6. The fats were apparently unchanged by cold storage of short duration.

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