Light-Microscopic Studies on Changes in the Ultrastructural Density of Skeletal Muscle Fibers Caused by Thermal Treatment

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(Text-figs. 1-5)

Thermal treatment, such as boiling and freezing, of muscle is applied not only occasionally to histological studies, but also widely to the preservation of meat. It is well known that thermal process brings about pronounced morphological changes to the structure of muscle. Many histological studies have been made on injured muscle. For instance, SAKUMA (1924), HÄGGQVIST (1932), and ABE (1932) have observed changes in boiled muscle and PLANK (1916) and CHAMBER (1932) those in frozen muscle. In addition to the structural changes, considerable changes in the ultrastructure of muscle fiber must be involved in the thermal process, since the process causes such definite alternations in the colloidal system of the muscle cell as coagulation, swelling, and disintegration of cell elements. No observation, however, has been made on these ultrastructural changes.

SEKI (1954) stated that the ultrastructural density of cell elements could be determined by examining stained specimens under the light microscope. The theory and technique of such method were elaborated by MÖLLENDORFF (1924) and SEKI (1936, 1954). The principle of the theory is explained as follows: The ultrastructural density of cell elements is estimated from the intensity of the color tone of a specimen stained with a combination of two or more acid dyes which differ in dispersity and color tone. For example, the azan method, recommended by SEKI for this purpose employs three acid dyes, orange G, azocarmine B, aniline blue. The cell elements present densely in the ultrastructure are stained with small-moleculed orange G in a yellow tone. Those present less densely are stained with moderate-moleculed azocarmine in a red tone. Those present coarsely are stained with large-moleculed aniline blue in a blue tone. In fact, the cell elements stained with azan are presented in various colors accompanied by a different degree of ultrastructural density.

The present investigation was performed, on the basis of above-metioned theory, to observe ultrastructural changes in skeletal muscle which was evoked by the thermal process, using several muscles of different animals. Comparison was also made between different methods or temperatures of boiling and freezing and the resulting changes in different muscles.

(I) MATERIALS and METHODS

The muscles used were obtained from healthy adult animals just after killing.

They consisted of the longissimus dorsi muscle of a cow and the longissimus dorsi (white muscle) and the masseter (red muscle) muscle of a rabbit as mammals, the pectoral (white muscle) and the quadriceps femoris muscle of a hen as bird, and the dorsal muscles of a golbefish, *Spheroides rubripes,* and a sciainoid fish, *Pseudosciaena undovittata,* as fish. The muscles were cut into blocks of the same shape and 1 × 1 × 0.5 cm in size.   Thermal treatment was performed in two methods, boiling and freezing.   In boiling, material was heated for ten minutes in a water bath at 30, 40, 50, 60, 70, or 100°C, the temperature being maintained exactly during the treatment. Then, it was fixed with 10% formalin. Two methods of freezing, a slow and rapid, were employed. In one method, material was frozen slowly in a refrigerator at about − 10°C for two hours.   In the other method, material was frozen rapidly in one minute with a freezing microtome.   Frozen specimens were put directly in 10% formalin to avoid a removal of drip at thawing.   Fixed specimens were imbedded in paraffin, sectioned at 7μ in thickness, and mounted on slides.   The azan method of Heidenhain was employed to examine the ultrastructure of the cell. Hematoxylin and eosin and Heidenhain's iron hematoxylin were used for the observation of the cell structure.

Sections to be compared were cut as equally thick as possible, mounted on the same slide, and stained at the same time, so that experiments might be conducted under the same conditions.

(II)   RESULTS and DISCUSSION

1.   Observation on boiled muscle.

a.   Muscle of cattle.

In unboiled control muscle, muscle fibers were presented in a red to slight yellowish-red tint with azan.   Myofibrils and cross striations of muscle fibers were poorly differentiated.

In muscle boiled at 30°C (30°–muscle for short), muscle fibers were all of the same stained color and structural aspect as those before boiled.   In other words, the ultrastructure of muscle fiber was not yet changed in boiling at this temperature.

In 40°–muscle, muscle fibers were stained red to purplish-red with an increased blue tone, as compared with 30°–muscle.   Such an increase in blue tone indicates that ultrastructural changes have occurred in fibers themselves.   In this case, it is assumed that the ultrastructure of muscle fiber has become loose because of an in-crease in affinity for the large-moleculed aniline blue.   The appearance of fibrils and cross-striations was more distinct than 30°–muscle.

In 50°–muscle, muscle fibers were stained purple to purplish-blue with a distinct increase in blue tone and fibrils, as well as cross-striations, were more distinguishable than 40°–muscle.   From a marked increase in blue tone, it is surmised that the ultra-structure of muscle fiber has become loose to a large extent.   In addition to these ultrastructural changes, microscopic structural changes, such as a partial collapse of myofibrils, took place in boiling at this temperature.

In 60°–muscle, muscle fibers were stained blue entirely. Cross-striations were noticeable in a slightly bending form, although fibrils became almost indistinct. Detailed observation disclosed that sarcolemma had been separated from the cell body due to the shrinkage of sarcoplasm and that the resulting space under the sarcolemma was filled with a granular substance which was derived from the protoplasm of muscle fibers. Intracellular connective-tissue fibers underwent degeneration to form a netlike structure. It must be noted that the ultrastructural density of muscle fiber became the lowest in boiling at this temperature, which nearly coincided with the temperature at which muscle proteins coagulated.

In 70°–muscle, muscle fibers were stained purplish-blue in a more decreased blue tone than in 60°–muscle. Decomposition of fibers was very conspicous. In short, 70°–muscle was not so coarse in ultrastructure as 60°–muscle, because that showed a more decrease in blue tone than this.

In 100°–muscle, muscle fibers were stained purple to reddish-purple in an increased red tone. Muscle fibers were apparently structureless, as a whole, owing to the disappearance of transverse and longitudinal striations of muscle fibers. The endomysial space was occupied by a large amount of disintegrated substance from myofibrils. Therefore, the ultrastructural density of 100°–muscle was higher than that of 70°–muscle, but was much lower than that of unboiled muscle. It should be noted that the muscle boiled at a temperature higher than 70°C was, in spite of spectacular damage in its structure, not so coarse in ultrastructure as that boiled at 60°C.

Generally speaking, the ultrastructural density of muscle fiber of cattle was not changed by boiling at a temperature below 30°C. It became a little lower by boiling at 40°C and much lower at 50°C, being the lowest after boiling at 60°C. After that, it showed opposite gradients of change with elevation of boiling temperature. From these results, it can be said that boiling process has an effect of making the ultrastructure of muscle fiber coarse.

As is well known, the principal constituent of muscle fiber is muscle proteins. They are composed of myogen, the globulin complex, coagulating at 50 to 60°C and myosin, the albumin complex, coagulating at 45 to 50°C. The principal factor which induces coarseness in the ultrastructure of muscle fiber may be considered coagulation of muscle proteins and other constituents. This assumption has been made from the fact stated previously that the temperature at which the ultrastructure of muscle fiber becomes the coarsest corresponds to the coagulation temperature of the above-mentioned muscle proteins.

In fact, when coagulation takes place among the cell elements of muscle fiber, including muscle proteins, and partial dissolution or swelling occurs as a result of boiling, the crevices among molecules or micelles will be enlarged. Accordingly, the ultrastructural density of the cell elements becomes low. So long as boiling temperature is high, however, coagulation is accelerated with a rise in temperature and dissolution or effusion is checked in these cell substances. So the crevices among molecules will not be enlarged so well as in the preceding case. Therefore, the ultra-

structure of the cell elements becomes denser than before.   This assumption is supported by the fact mentioned previously that muscle boiled at 100°C had a higher ultrastructural density than that at 60°C.

    b.   Muscle of rabbit.

Rabbit muscle underwent the same changes in ultrastructural density as cattle muscle when boiled.

    c.   Muscle of chicken.

Chicken muscle showed a different relationship between boiling temperature and changes in ultrastructural density from that exhibited by cattle muscle.   When stained with azan, chicken muscle revealed the following relationships between the color and boiling temperature: yellowish-red in unboiled state, purplish-red at 40°C, purple at 50°C, purplish-blue at 60°C, and purple at 70 and 100°C.   These relationships indicate that the gradient of change in ultrastructure was essentially the same in chicken muscle as in cattle muscle.   In the stainability of muscle fiber in a blue tone, chicken muscle was a little inferior to cattle muscle.
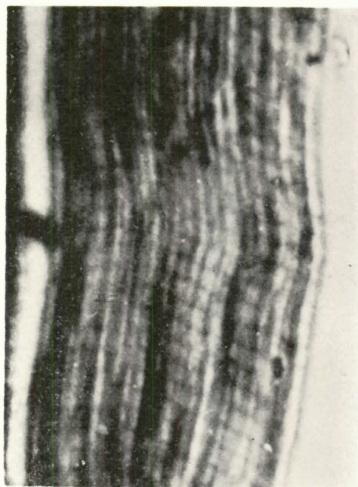

Fig. 1. Quadriceps femoris muscle of hen unboiled. Longitudinal section. Heidenhain's iron-hematoxylin staining. Myofibrils and cross-striations are poorly visible, being in a contruction stage with A disks short and I disks tall in height. × 1500.
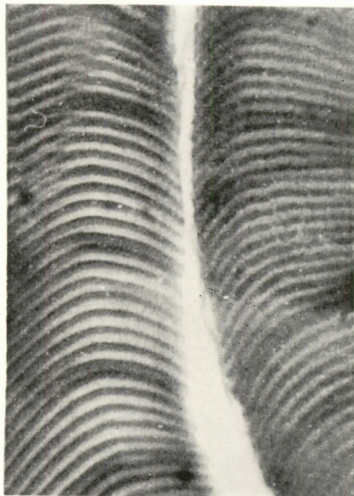

Fig. 2.   Quadriceps femoris muscle of hen boiled at 60°C for ten minutes. Longitudinal section. Azan staining. Cross-striations are distinct, bending a little. Longitudinal striations are invisible. × 1500.

As is the case with cattle muscle, cross-striations of muscle fiber were notably visualized by boiling at a definite temperature. The present report deals with detailed observation on ultrastructural changes in transverse striation caused by boiling.   In the unboiled muscle fiber, the isotropic disk (I disk) of a cross-striation was taller than the anisotropic disk (A disk), indicating a stage of contraction, and cross-striations were poorly differentiated (Fig. 1).   Stained with azan, both disks were uniformly presented in a red tone. In 40°–muscle, A disks increased in height and became

purple with a distinct blue, while I disks were red as before. The appearance of striations became somewhat distinct in general. In 50°–muscle, A disks were purplish-blue and I disks purple, making striations more distinct. Fig. 2 shows chicken muscle boiled at 60°C for ten minutes. As is clear from the figure, transverse striations are exceedingly distinct, taking a slightly bending form, with I disks increased in height, while longitudinal striations are invisible. When stained, I disks were pale purple in color and A disks dark blue. In 70°–muscle, both disks were epually purple. In 100°–muscle, these disks became to exhibit indistinct structural and staining features.

From these observations, it is noted that, of the alternating bands in transverse striations, the I disk is decreased more rapidly in ultrastructural density than the A disk when boiled at low temperature.

d. Muscle of fish.

In ultrastructural changes of muscle fiber caused by boiling, there was an essential difference between fish and the warm-blooded animals observed. When stained with azan, chicken muscle was red before boiling, purple after boiled at 40°C, purplish-blue at 50°C, purple at 60°C, and purplish-red at 70 and 100°C. From these findings, it is indicated that the ultrastructure of fish muscle became coarse after boiling at lower temperature, or 40 to 50°C, than that of muscle of the warm-blooded animal. This characteristic difference is probably derived from the well-known fact that water soluble protein which coagulates at low temperature is more abundant in fish muscle than in mammalian and chicken muscle.

e. Observation on red and white muscle.

In comparing red and white muscle, no essential difference was noticed in ultrastructural changes between them. White muscle tended to be ultrastructurally coarse by boiling at a little lower temperature than red muscle. KAWAMURA (1954) observed in frog muscle that the ultrastructural density was low in muscle rich in myofibrils and high in muscle rich in sarcoplasm. According to WEBER (1932), myogen, the globulin complex, is more abundant in white muscle than in red muscle. Therefore, the difference between both types of muscle seemed to be derived from such different chemical composition of each muscle.

It is summarized that the boiling process had an effect of making the ultrastructure of muscle fiber coarse. In more detail, the ultrastructure of muscle fiber was not influenced by boiling at a temerature below 30°C and became the coarsest by boiling at 50 to 60°C. It was no more made coarse, however, by boiling at a temperature over 70°C, although it was damaged conspicuously by boiling. Fish muscle was influenced more rapidly by boiling at lower temperature than muscle of any other animal species examined.

2. Observation on frozen muscle.

It is generally recognized that freezing of muscle brings about characteristic changes in the structure of muscle by the formation of ice crystals and that the severity of damage varies with the method or temperature of freezing. When freezing process is carried out rapidly, ice crystals of small size are formed within and outside of muscle fibers. When freezing is done slowly, ice crystals of large size are

formed only outside of muscle fibers.   In the present paper are indicated ultrastructural changes in frozen muscle.

    a.   Muscle under slow freezing.

    Morphological changes in fish muscle frozen slowly in the refrigerator are shown in Fig. 4.   As is clear from the figure, extracellular crevices are large, presenting the formation of large ice crystals along the long axis of muscle fibers.   Stained with azan, muscle fibers are presented in a yellowish red tone, being more yellowish than those of unfrozen muscle. Particularly, muscle fibers adjacent to the crevices are more yellowish than those far from them.   It is assumed, in this case, that the ultrastructure of muscle fiber has become dense, since it exhibited an increased affinity for small-moleculed orange G.   Therefore, the muscle fibers surrounding ice crystals have a denser ultratructure than those far from them.

Fig. 3. Dorsal muscle of sciainoid fish unfrozen. Longitudinal section. Azan staining. × 400.

Fig. 4. Dorsal muscle of sciainoid fish frozen slowly. Longitudinal section. Azan staining. Wide intercellular spaces have been formed along the long axes of muscle fibers by the formation of large ice crystals. Disturbed fibers are in irregular arrangement. × 400.

    In cattle and chicken muscle treated in the same manner, crevices among muscle fibers were smaller in size and the muscle fibers suffered less damage than in treated fish muscle. Tinctorial response to azan stain was of similar tendency to, but not so marked as, that of fish muscle.

    No significant difference was noticed in ultrastructural changes between red and white muscle.

    b.   Muscle under rapid freezing.

    Morphological changes in fish muscle frozen rapidly with a freezing microtome are illustrated in Fig. 5. Muscle tissue is severely broken down into netlike structure

by the formation of small ice crystals within and outside of muscle fibers.   Stained
with azan, muscle fibers are presented in a yellow tint, being obviously more yellow-
ish than those of unfrozen control muscle and slowly frozen muscle.   From these
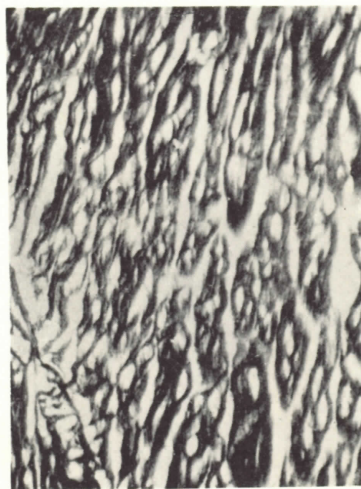

Fig. 5.   Dorsal muscle of sciainoid fish frozen rapidly.   Longitudinal section.
Azan staining.   Muscle fibers have been broken down into a netlike
structure by the formation of small ice crystals.   × 400.

results, it is concluded that rapid freezing process exercised more pronounced influ-
ence on the structure and ultrastructure of muscle fiber than slow freezing process.

The tendency observed in mammalian and chicken muscle was essentially the
same, but not so noticeable, as that in fish muscle.

There was no significant difference in ultrastructural changes between red and
white muscle.

In short, freezing process had an effect of making the ultrastructure of muscle
fiber dense, in addition to causing remarkable structural changes.   This effect was
more distinct in rapid freezing than slow freezing.   It was more intense in fish muscle
than muscle of any warm-blooded animal examined.

The ultrastructural density of muscle fiber became high after the completion of
freezing process.   This phenomenon would be interpreted by the removal of water
from the extracellular space and muscle fibers themselves as a result of formation of
ice crystals.   Deprivation of water from cell elements caused reduction in size of
crevices among molecules or micelles and, consequently, the ultrastructure of muscle
fiber became dense.

As reported by HANDA (1950) and KAWAMURA (1952), the ultrastructural densi-
ty of muscle tissue varies with animal species, age, and region of the body.   So that
deep consideration had been given in evaluating the results of experiments.

(III)   SUMMARY

Ultrastructural changes of skeletal muscle evoked by heat were examined micro-

scopically by means of the azan method.   The specimens used had been collected from several different muscles of animals of some species.   They were boiled or frozen by various methods at different temperatures.

The results obtained are summarized as follows.

1.   As a whole, boiling process had an effect of making the ultrastructure of muscle fiber coarse, while freezing process had an effect of making it dense.   The gradient of ultrastructural changes, however, varied to some extent with the kind and type of muscle.   It is assumed that the ultrastructure of muscle became coarse by boiling, mainly because the cell elements had been coagulated and partly because they had been disintegrated, and that it became dense by freezing, because the cell elements had been dehydrated.

2.   The following relationships were found between boiling temperature and ultrastructural changes of muscle fiber.

a.   The ultrastructural density of muscle fiber was not changed by boiling at a temperature below 30°C and became slightly lower at 40°C and the lowest at a temperature from 50 to 60°C.   The ultrastructure of muscle fiber boiled at a temperature over 70°C, however, was not any longer so coarse as that of muscle fiber boiled at 60°C.   The ultrastructure of muscle fiber boiled at 100°C was coarser than that of unboiled muscle fiber.

b.   White muscle fiber inclined to become coarse in ultrastructure by boiling at lower temperature than red muscle fiber.

c.   Fish muscle fiber inclined to become coarse in ultrastructure more rapidly than muscle of any warm-blooded animal examined.

3.   The following relationships were found between method of freezing and ultrastructural changes of muscle fiber.

a.   Rapid freezing made the ultrastructure of muscle fiber denser than slow freezing.

b.   Fish muscle inclined to become ultrastructurally denser through freezing process than muscle of any warm-blooded animal examined.

c.   No demonstrable difference was found in the ultrastructure after freezing process between red and white muscle.

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