Changes in the Stainability of Muscle with Azan in the Course of Putrefaction

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(Text-figs. 1-2)

It is very important, from the viewpoint of food sanitation, to investigate various changes in the course of putrefaction of meat. Numerous studies have been made on the postmortem changes of muscle chemically, bacteriologically, and morphologically. In the field of morphological observations, ITIKAWA (1949) studied horse meat with regard to macroscopical changes in color, luster, humidity, odor, hardness, and taste and microscopical changes in cross striations, myofibrils, nuclei, and cytoplasm of muscle cells during a period from immediately after death to the onset of putrefaction. Little has been reported, however, on changes of the stainability of muscle in the course of putrefaction. KIZUKA *et al.* (1955) studied histochemically postmortem changes in meat by means of alizarin S and Schiff's reaction and reported that the stainability of muscle fibers was profoundly affected by the change of the pH value of muscle after death.

The present investigation was performed, upon the theory on "the ultrastructural density of tissue" elaborated by MÖLLENDORFF (1924) and SEKI (1936, 1954), to observe changes of the color of muscle stained by the azan method which employs three acid dyes with different dispersity and color. On the other hand, the degree of putrefaction of the same specimen used for histological examination was estimated chemically by measuring the amount of volatile basic nitrogen (VBN) by Conway's method and compared with the change of stainability. As a few reports have been made by SEKI and his collaborators in this field, this paper deals with some new findings since then.

(I) MATERIALS AND METHODS

The materials used were the dorsal muscle of a globefish, *Spheroides rubripes,* and the femoral muscle of a dog. The chemical composition was a little different between these muscles. The materials were cut into small blocks about $2 \times 1 \times 0.5$ cm in size and placed in a Petri dish, which was floated in a larger dish filled with water to prevent the muscle blocks from drying. Room temperature was about 28 °C in this experiment, so that putrefaction was accelerated remarkably in muscle. In the case of fish muscle, a specimen was harvested just after death (for control) and 7, 30, 50, and 80 hours after death, respectively. It was cut into two equal parts, of which one was fixed

in 10% formalin solution for histological examination and the other used for chemical study. In the case of dog muscle, specimens were collected immediately after death (for control) and 10, 35, 55, 85, and 100 hours after death and dealt with in the same manner as described for fish muscle.

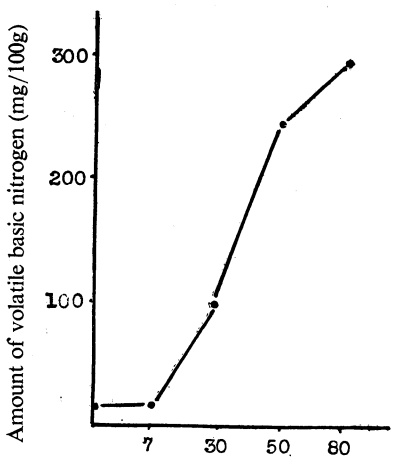
The fixed blocks were embedded in paraffin and sectioned 10μ thick. Sections from each block were mounted on a single slide. Heidenhain's azan staining method was employed to observe changes in the stainability of muscle, and Heidenhain's iron-hematoxylin and regular hematoxylin and eosin were used for the observation of the cell structure in general.

Sections to be compared were cut in as equal thickness as possible, mounted on the same slide, and stained at the same time, so that experiments might be conducted under the same condition.

The other half of same block was used to determine the amount of VBN by Conway's method for chemical estimation of the degree of putrefaction of the muscle.

(II) RESULTS

1. Observation on fish muscle



Text-fig. 1. Changes in the amount of volatile basic nitrogen in fish muscle after death.

In the control specimen harvested just after death, cross striations and myofibrils of muscle fibers were more or less clearly detectable histologically. Stained with azan, muscle fibers appeared in a red tint and interstitial connective-tissue fibers in a blue tint. Text-fig. 1 shows changes in the amount of VBN in fish muscle. The VBN level was 14.2mg/100g (hereinafter, simply mg) in the control muscle.

In the specimen harvested 7 hours after death (7-hour specimen for short), muscle increased in hardness and seemed to be in the stage of rigor mortis. Histologically, cross striations and myofibrils were more distinct than those of the control. No other morphological changes were seen in this stage. The color of muscle stained with azan was more yellowish in tint than that of the control. VBN was not increased in amount, remaining 14.5 mg. It should be noted that, although some change occurred in the staining color of muscle, there was no change in its VBN amount.

In the 30-hour specimen, muscle became soft again owing to the lysis of rigor mortis and was juicy with a slightly putrid odor. No histological changes were noticed, except a partial colliquation and disappearance of connective tissue fibers. Nevertheless, muscle fibers in this stage were stained purplish-red with a distinct increase of a purple tone. The level of VBN was elevated extremely, reaching 92.9 mg. It is of interest that a

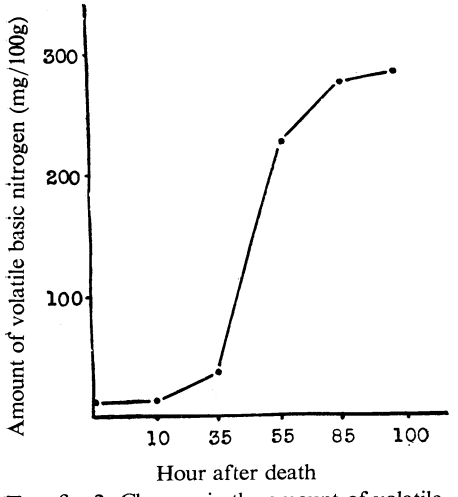
remarkable change in staining color occurred in accordance with the elevation of VBN level.

In the 50-hour specimen, muscle emanated a putrid odor and was softened with remarkable colliquation. Histologically, cross striations and myofibrils were hardly visible and degenerative changes, such as pyknosis and disappearance of nuclei, were shown, so that the muscle tissue became homogeneous. Muscle fibers became colliquative in highly putrefied portions of the specimen, which were stained reddish-purple diffusely or purple entirely with azan. The amount of VBN was increased to 235.7 mg.

In the 80-hour specimen, muscle was collapsed and colliquated to lose its original form. Histologically, the sarcolemma melted and myofibrils liquefied entirely. The muscle in this period was stained purplish-blue to blue. The amount of VBN was 298.4 mg.

In short, the color of fish muscle stained with azan was red immediately after death and changed to orange at the stage of rigor mortis, purplish-red 30 hours after, purple 50 hours after, and at last purplish-blue or blue 80 hours after death.

2. Observation on dog muscle



Text-fig. 2. Changes in the amount of volatile basic nitrogen in dog muscle after death.

In the control muscle, cross striations and myofibrils were observed distinctly. When stained with azan, it was red with a little more yellowish tint than fish muscle at the same period. Text-fig. 2 shows changes in the VBN amount of dog muscle. The VBN level of the control specimen was 10.0 mg.

In the 10-hour specimen, muscle became rigid owing to the effect of rigor mortis. Cross striations and myofibrils were seen more clearly in it than in the control specimen. The color of muscle stained with azan was red with a more yellowish tint than in the control. The VBN level was maintained at 11.4 mg.

In the 35-hour specimen, muscle became soft owing to the lysis of rigor mortis. Histologically, connective-tissue fibers gradually decreased in amount and muscle fibers were more or less distinct in appearance, although cross striations and myofibrils were invisible. Stained with azan, the muscle fibers were purplish-red with a decreased purple tone, compared with those of fish muscle at the same period of harvesting. The amount of VBN was 34.4 mg, about three times as large as that of the control muscle.

In the 55-hour specimen, muscle produced a bad odor and became soft and juicy. Interstitial connective-tissue fibers showed a partial fusion or disappeared. Moreover, fusion of muscle fibers occurred and muscle tissue appeared homogeneous. The amount of VBN increased rapidly to 231.1 mg. The muscle in this period was stained reddish-

purple with azan.

In the 85-hour specimen, muscle putrefied entirely. Histologically, the fusion of sarcolemma and liquefaction of myofibrils were so marked that the muscle tissue appeared uniform as a whole. When stained with azan, the muscle tissue was reddish-purple or purple. The VBN level reached 273.1 mg.

In the 100-hour specimen, muscle became muddy. The muscle tissue liquefied entirely and stained purple or bluish-purple diffusely. The amount of VBN was 280.0 mg.

In short, the color of dog muscle stained with azan was red just after death, yellowish-red 10 hours after, purplish-red 35 hours after, reddish-purple 55 hours after, purple 85 hours after, and purple or bluish-purple 100 hours after death.

Changes in the color of muscle stained with azan in the progress of putrefaction showed a similar tendency in dog muscle as in fish muscle. When observed in detail, however, the changes were not so rapid and distinct in dog muscle as in fish muscle.

(III) DISCUSSION

Changes in the stainability of muscle with azan in the progress of putrefaction were observed histologiaclly and compared with those in the VBN level determined chemically. Both fish and dog muscles used in the present investigation were stained red with azan when harvested just after death, yellowish-red in the stage of rigor mortis, and purplish-red about 30 hours after death. Afterwards, the color of muscle became purple to blue with the progress of putrefaction.

The azan method employs three acid dyes, azocarmine B, orange G, and anilin blue. Of these dyes, according to ŌKURA *et al.* (1950), orange G producing an orange tint is of the highest dispersity, anilin blue producing a blue tint is of the lowest, and azocarmine B producing a red tint is of moderate dispersity. In this investigation, it was due to azocarmine B that the control muscle was stained red, to orange G that the specimen in the stage of rigor mortis was stained yellowish-red, and to anilin blue that the specimen in the course of putrefaction was stained gradually from purple to blue.

According to SEKI's theory on "the ultrastructural density of tissue", such changes in stainability with azan are explained as follows: The ultrastructural density of muscle tissue becomes gradually coarse with the progress of putrefaction. The coarser the muscle ultrastructurally, the larger-moleculed dye it may be stainable with. OIYAMA (1950) observed in various tissues that the ultrastructural density of tissue element became coarse with the progress of retrogressive degeneration. Similar findings were reported in detail by SEKI (1951) who dealt with pancreatic cells showing postmortem changes very quickly.

The findings in the present investigation essentially agree with those of the abovementioned authors. They contain, however, some noticeable points. First, the muscle in the stage of rigor mortis had a more yellowish tint than the control specimen harvested just after death. It is assumed that, in that stage, the ultrastructural density of muscle tissue became dense because of narrowing of clefts among molecules or micelles of the tissue by coagulation of protein particles. Consequently, muscle fibers are stain-

able only with a small-moleculed dye such as orange G. Secondly, the muscle was stained purple after the lysis of rigor mortis. This is probably because the ultrastructural density became coarse at this time. Thirdly, there was a remarkable change in stained color at the early stage of putrefaction when the amount of VBN was about 30 mg. It is considered that the ultrastructural density of muscle tissue suddenly coarse at this period. It is noted that changes in color by azan staining, or more precisely, changes in the ultrastructural density of muscle tissue were related to the degree of putrefaction as measured chemically.

As mentioned above, there were some differences in the change of stained color between fish and dog muscle. HANDA (1950) and KAWAMURA (1952) stated that the ultrastructural density of muscle tissue varied with species, age, and region of the body of an animal. It is well known that water-soluble protein is more abundant in fish than in mammalian meat. A rapid, distinct change of the color of fish muscle is probably due to such differences in chemical composition of muscle.

(IV) SUMMARY

Changes in color of muscle stained with azan in the course of putrefaction were examined histologically, from the viewpoint of ultrastructural density, and compared with the amount of volatile basic nitrogen (VBN) of muscle measured chemically by Conway's method.

The results obtained are summarized as follows:

1. The color of muscle as stained with azan was red just after death when no rigor mortis appeared, and gradually became purplish-red, purple, purplish-blue, and finally blue in accordance with the degree of putrefaction of muscle. Such changes in color were not due to those of pH value but to a gradual change of the ultrastructural density of muscle tissue with the progress of decomposition of meat, which allows a large-moleculed dye to invade into the tissue.

2. During the period of rigor mortis, any changes could not be demonstrated in the histological picture and the amount of VBN of muscle. However, the color of muscle stained with azan at this period had a more yellowish tint than that of the control muscle harvested just after death. It is considered that the ultrastructural density of muscle tissue became dense in this stage.

3. In the period when the VBN level reached about 30mg/100 g and which was regarded as an early stage of putrefaction, muscle was stained purplish-red with a purplish tone. It is assumed that the ultrastructural density of muscle tissue became coarse in this stage.

4. The muscle with a VBN level of about 100mg/100 g and showing remarkable morphological changes was stained purple. The ultrastructural density of muscle tissue in this stage seems to have become coarser.

5. The putrid muscle with a VBN level of more than about 250mg/100g was stained bluish-purple or blue.

6. There was a slight difference between fish and mammalian muscle in the change

of staining color by azan with the progress of putrefaction. In general, fish muscle showed a more rapid and distinct change in color than mammalian muscle when stained.

From these results, it may be said that the azan staining method is available, to some extent, for histological estimation of the freshness of meat.

Acknowledgment. The author wishes to thank Prof. Y. TSUJI of the Hiroshima University, Laboratory of Veterinary Science, to which he belongs, for helpful encouragement given to this work.

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