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THE COLLAGEN ARCHITECTURE AND IMMUNOHISTOCHEMICAL PROPERTY IN BOVINE SKELETAL MUSCLES

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Background:

The tenderness of muscle fibers rapidly takes place during postmortem time, while that of connective tissue gradually occurs (Nishimura et al., 1998). The mechanism of the meat weakening is still uncertain. It is considered that the mechanical stability of connective tissue depends on the existence of several types of collagens and binding proteins, such as laminin and fibronectin.

Objectives:

The present study focuses on connective tissue structure, especially the collagen architecture and the immunohistochemical localization of several types of collagens and binding proteins. The collagen architecture was observed with the scanning electron microscopy (SEM) of the maceration method and the localization of these proteins was immunohistochemically demonstrated.

Materials and methods:

The biceps femoris muscles of Japanese Brown steers were utilized in the present study. For SEM observation, the muscles were fixed with 3% glutaraldehyde buffer solution for 2 days. Then they were macerated in 8% NaOH solution for 4-5 days at 25℃ and rinsed in distilled water for 4 days (Ohtani, 1987; Tabata et al., 1994). After treatment with 1% tannic acid followed by 1% osmium tetraoxide, the materials were dehydrated in a graded series of ethanol, and then treated with t-butyl alcohol. The freeze-dried specimens were coated with Pt-Pd and examined under a JEOL JCM-5400 scanning electron microscope. For immunohistochemical observation, we used polyclonal antibodies raised against type I collagen, type III collagen, type IV collagen, laminin and fibronectin. The muscles were frozen in dry-iced acetone and cut into 8-μm-thick sections. The sections were incubated with several antibodies and then treated with a secondary antibody conjugated with FITC. The colored specimens were examined under a Nikon RCM-8000 fluorescence microscope.

Results and discussion:

Each of muscle fibers that were digested was enclosed with the endomysium and many muscle fibers were encircled with the perimysium in SEM specimens (Fig. 1a). The collagen fibrils, 50-100 nm in diameter, in the endomysium oriented circular around muscle fibers (Fig. 1b), while those in perimysium showed longitudinal directions (Fig. 1c). The former fibrils scattered separately, on the other hand, hundreds of the latter fibrils accumulated and made thick collagen bundles. It was considered that the tenderness of connective tissue in meats might not mainly depend on the endomysium but on the perimysium. Immunohistochemical specimens showed that the type I (Fig. 2a) and type III collagens (Fig. 2b) were demonstrated both in endomysium and perimysium. The type IV collagen (Fig. 2c) and laminin (Fig. 2d) were only in the endomysium corresponding to the external lamina. The fibronectin (Fig. 2e) was found diffusely in the endomysium and perimysium. The immunological results strongly suggested that the muscle fibers were connected with the type IV collagen, a main component of external lamina. The laminin were supposed to act as a binding protein between the muscle fibers and the type IV collagens. The type I and III were the main collagenous proteins in the connective tissue of meats and might be adhered by the fibronectin. From the above results we considered that the meat tenderness depended on the perimysium having many thick collagen bundles that were the type I and III collagens. Furthermore we sepculated that the weakning of connective tissue after postmortem time could be resulted from the degradation of these types of collagen or of the binding proteins.

Conclusion:

The endomysium was encircled by thin collagen fibrils and the perimysium was composed of thick collagen bundles in the SEM

specimens. The immunofistochemical results suggested that the connective tissue in meats was composed of several types of collagens and binding proteins. The external lamina of muscle fibers was composed of the type IV collagen and the laminin. The Type I and III collagens were observed both in the endomysium and the perimysium. The fibronectin was diffusely localized in the endomysium and the perimysium.

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Literature cited:

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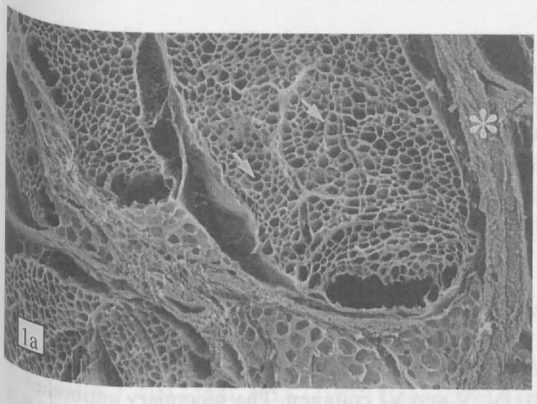
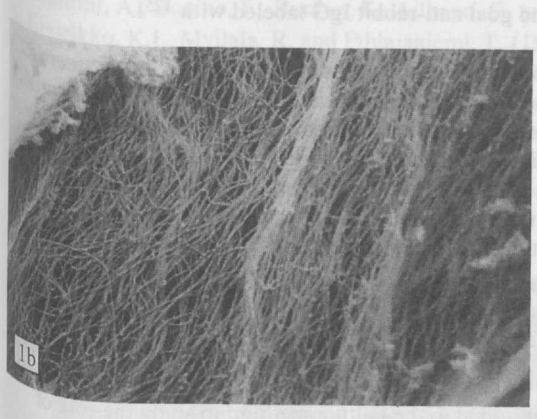
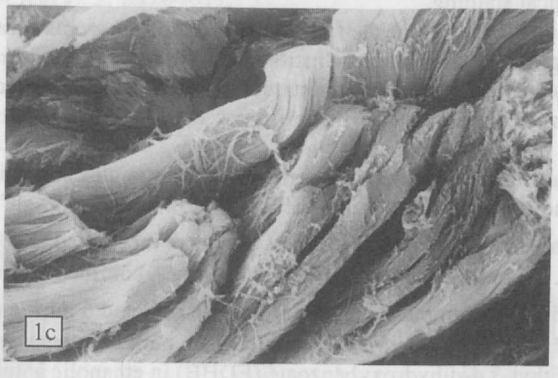


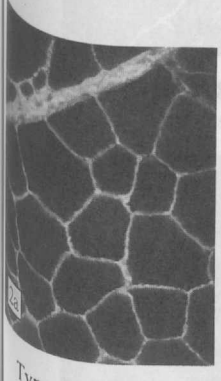
Low magnification micrograph of connective tissue. The muscle fibers were digested with the maceration. Arrows indicate the endomysium and an asterisk indicates the perimysium.



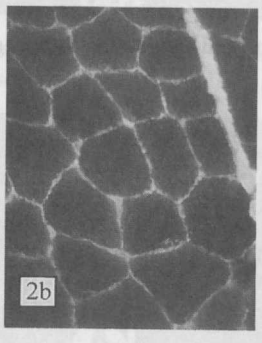
Endomysium



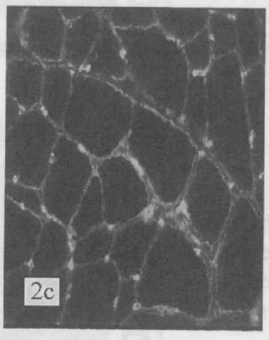
Perimysium



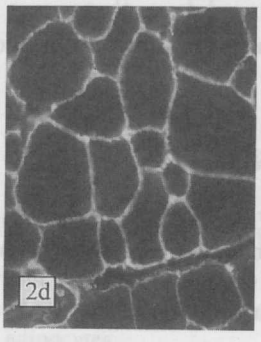
Type I collagen



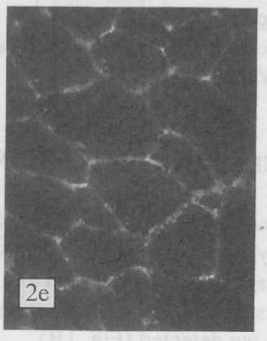
Type III collagen



Type IV collagen



Laminin



Fibronectin