

## Heat-induced Changes in Meat

### I. Electronmicroscopic Studies on Changes in Heated Bovine Muscle

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## 肉의 加熱變化에 關한 研究

### 第 1 報 : 加熱牛肉의 組織變化에 關한 전자현미경적 관찰

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#### Abstract

Ultrastructural changes in endomysial connective tissue, sarcolemma, transverse ridges and myofibrillar structures with particular attention given to Z-discs, A and I-filaments induced by heating to 80°C were observed by transmission and scanning electron microscopy. Muscle heated to 80°C produced granulation of sarcolemma and ultrastructural changes and coagulation were observed in endomysial and sarcolemmal connective tissue. The edvient changes in myofibrillar structure were an increase in coagulation compactness of the A-band portion of sarcomere and disintegration of the I-bands. Z-discs appeared to be relative resistant to heat but I-filaments were observed to be most heat labile.

#### Introduction

Heating of muscle tissue has a profound effect on the physicochemical and structural characteristics of meat. Chemical changes in muscle proteins during heating have been of importance in terms of meat tenderness and flavor. Heat-induced changes in collagen,<sup>(1-3)</sup> in sarcoplasmic proteins<sup>(4-6)</sup> and in myofibrillar proteins<sup>(6,7)</sup> have been reported. Protein denaturation commence at about 40°C and proceed until coagulation is complete at about 65°C.<sup>(8)</sup> Collagen shrinks at about 58°C<sup>(9)</sup> and begins transform

ation into gelatin around 63°C.<sup>(8)</sup> These changes give rise to characteristic texture of cooked meat.

Recent use of scanning electron microscopy(SEM) has been valuable in demonstrating structural changes in postmortem muscle<sup>(10,11)</sup> and in cooked bovine muscle<sup>(12,13)</sup>. They demonstrated that progressive changes occur in myofibrillar fragmentation at the Z-discs and in collagen denaturation with increased temper ature of 50 to 90°C. Although few studies on hea t-induced changes in beef by scanning electron microscopy have been reported,<sup>(12-14)</sup> there are still some problems not to be elucidated.

This study was designed to elucidate certain

structural changes which occur in meat as the results of cooking and to link the results with changes in chemical components during heating planned to publish in following paper. Beef psoas muscle was cooked to internal temperature of 80°C in a 168°C gas heated oven and morphological changes in myofibrillar structure, sarcolemma, endomysial connective tissue and Z-discs were observed by SEM and transmission electron microscopy (TEM).

## Materials and Methods

### Muscle preparation

Fresh bovine psoas muscle was obtained immediately after slaughter from the university abattoir. The muscle was brought to the meat laboratory where they were freed of excess fat. Cubes of psoas muscle about 4cm in diameter were cooked to internal temperature of 80°C in a 168°C gas heated oven. Upon reaching the desired internal temperature, steaks were removed from oven and allowed to room temperature for 1 hour. After temperature equilibration, most central sample were removed from steak and cut in small pieces and then used for histological evaluation.

### Scanning electron microscopy

Heated muscle strips (3×5×7mm) were fixed in 1.25% glutaraldehyde buffered with 0.07 M Na<sub>2</sub>HPO<sub>4</sub>, 0.041 M NaH<sub>2</sub>PO<sub>4</sub> and 0.043 M NaCl for 4 hrs. and postfixed in 1% osmium tetroxide for 4 hours. The fixed muscle were dehydrated first in ethanol then in amylacetate. Residual amylacetate in the sample was removed by critical point dehydration in liquid CO<sub>2</sub>. The dehydrated muscle strips were then mounted to aluminium discs with copper conductive cement, coated with gold (10nm thick) and examined with JEOL-JsM-S1 scanning electron microscope at 10KV as an accelerating voltage. Surface structural alterations during cooking and overall physical integrity of muscle fibers and associated connective tissue were observed with SEM.

### Transmission electron microscopy

Heated muscle strips (2×2×2mm) were fixed in glutaraldehyde for 2 hours and postfixed in osmium

tetroxide for 2 hours as the same manner in SEM sample preparation. The sample were dehydrated in acetone and then infiltrated with spurr. The infiltrated samples were embedded with spurr and polymerized for 48 hours at 65°C and sectioned with LKB ultramicrotome. Silver sections (approximately 60~80nm thick) were picked up on uncoated copper grids and stained with uranyl acetate<sup>(15)</sup> and lead citrate<sup>(16)</sup> and examined under RCA 3G transmission electron microscope at 100KV as an accelerating voltage.

## Results and Discussion

### SEM of cooked and uncooked bovine psoas muscle

The scanning electron micrograph (Fig. 1A) of uncooked muscle shows characteristic intact muscle fibers wrapped by fine network of endomysial connective tissue and sarcolemmal layers have been removed during preparation of the sample revealing myofibrils (arrow). Higher magnification of this area (Fig. 2A) shows detailed myofibrillar ultrastructure. Transverse ridges (TR) connecting myofibrils pass through areas where Z-discs are expected and orderly arranged along the myofibrils. It is not clear whether these ridges are Z-discs or part of the transverse tubular system although these structures have been identified as Z-line structure<sup>(17)</sup> as t-tubules<sup>(18)</sup> and as Z-discs<sup>(12,13,19,20)</sup>. Slight channels at the midpoint of adjacent sarcomeres correspond to the M-lines.

Psoas muscle cooked to an internal temperature of 80°C is shown in Fig. 1B and 2B. Endomysial connective tissue and sarcolemma exhibit severe destruction and some coagulation. Jones *et al.* observed endomysial collagenous connective tissue coagulation occurred when muscle was heated to 60°C. Sarcomeres exhibit evidence thermally induced contraction and breakage of myofibrils (Fig. 2B). Transverse ridges (TR) are relatively resistant to heating and partially destroyed but severe destructions were observed in I-band areas. Surface topography of the myofibrils appear granular, evidence of protein coagulation.

### TEM of cooked and uncooked bovine psoas

**muscle**

The transmission electron micrograph(Fig. 3A) of uncooked muscle reveals well orderly arranged sarcomere, Z-lines, M-lines, A and I-bands, and individual myofibrils are discernible, whereas, the heated sample(Fig. 3B) exhibits coagulation and considerable disappearance of I-band filaments and some coagulation of A-band filaments. Z-lines are observed to be relatively resistant to heating to 80°C. Severe destruction of I-band area connected to Z-discs suggest that filamental actin attachment to the Z-discs are broken by thermal treatment, whereas, filaments in the A-band are less affected by heating. Crespo and Ockerman<sup>(21)</sup> reported that actomyosin in avian breast and leg muscle was relatively heat stable. However, myosin when not in the actomyosin complex was very susceptible to thermally induced denaturation. They suggested that actin in the actomyosin complex may have protected myosin and resulted in the increased heat resistance of the actomyosin complex. Cheng and Parrish<sup>(7)</sup> reported that  $\alpha$ -actinin was most heat labile and become insoluble at 50°C. Next, heavy and light chains of myosin become insoluble at 55°C. Actin was insoluble between 70-80°C and tropomyosin and troponin become insoluble above 80°C. The relative insusceptibility of Z-discs to thermal treatments is not in complete agreement with the reports of most labile  $\alpha$ -actinin<sup>(7)</sup> However, it is assumed that differences existed between the intact muscle and isolated myofibrillar proteins.

**要 約**

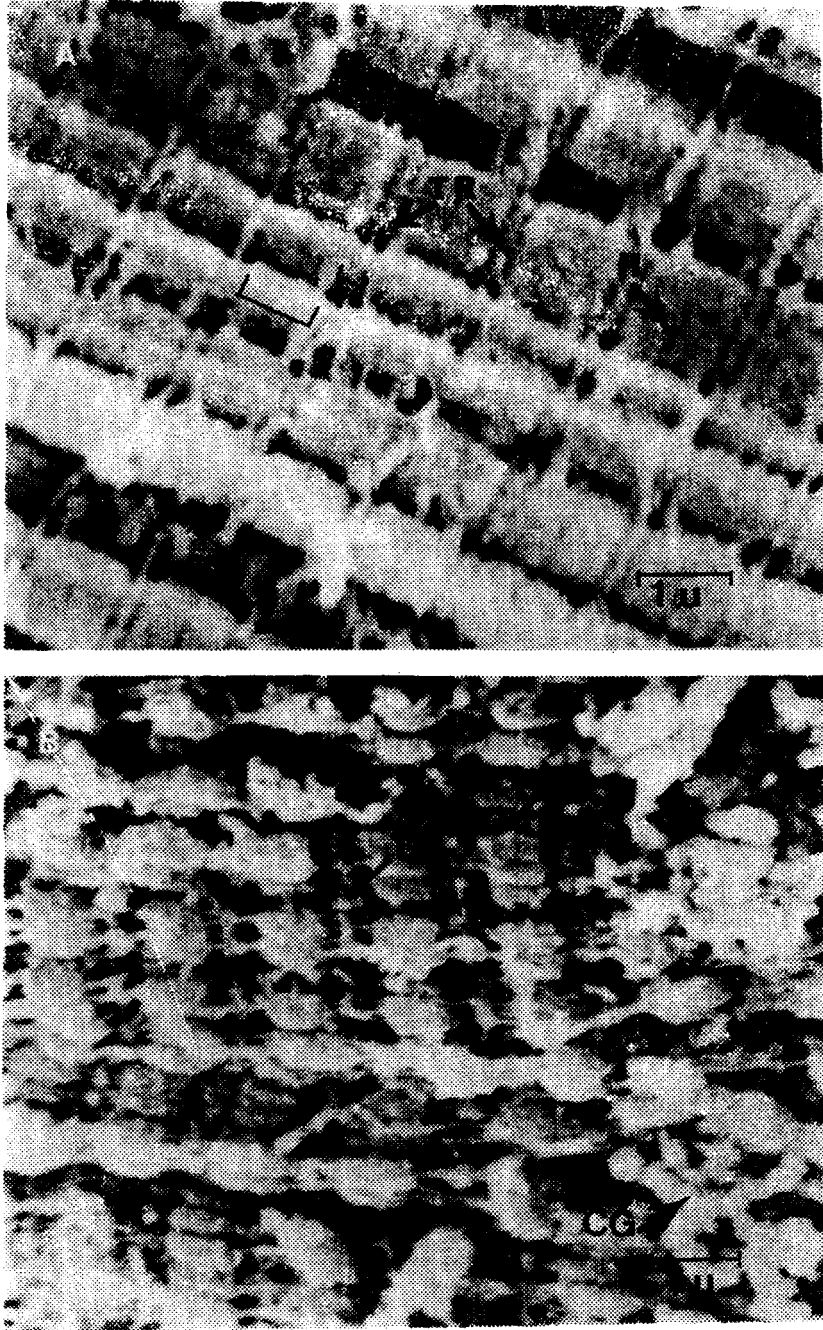
肉의 加熱時 肉組織內에 어떤 變化가 일어나는지를 관찰하기 위하여 牛肉을 80°C로 加熱한후 scanning 및 transmission 전자현미경으로 肉의 endomysial connective tissue, sarcolemma, transverse ridge, Z-disc, A 및 I-band 등을 관찰한바 80°C에의 加熱로 endomysium, sarcolemma 등이 응고됨과 아울러 심한 파괴 현상을 보였으며 근원섬유 구조에 있어서도 I-band가 심한 절단현상을 보임과 아울러 A-band에 있어서는 심한 응고현상이 관찰되었으나 Z-disc는 비교적 熱에 잘 견디는 것으로 관찰되었다.

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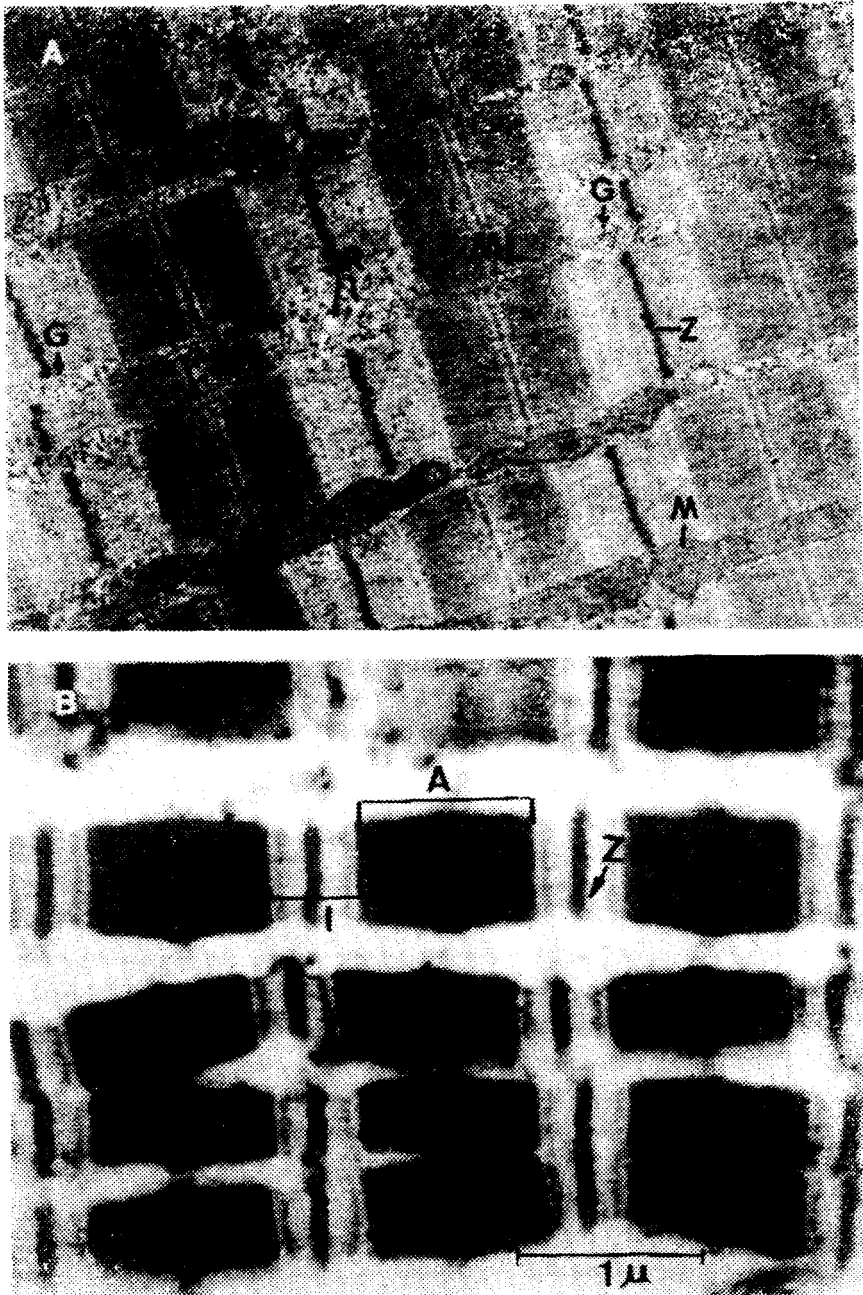
**Fig. 1. Scanning electron micrographs of unheated and heated bovine psoas muscle**  
A: unheated( $\times 1570$ ), B: heated( $\times 1570$ )  
E, endomysium; EC, endomysial collagen; MB, muscle bundle



**Fig. 2.** Scanning electron micrographs of unheated and heated bovine psoas muscle

A: unheated( $\times 11,000$ ), B: heated( $\times 11,000$ )

A, A-band; I, I-band; H, H-zone; TR, transverse ridge; CG, collagen granule



**Fig. 3. Transmission electron micrographs of unheated and heated bovine psoas muscle**

A: unheated( $\times 25,000$ ), B: Heated( $\times 25,000$ )

G, glucose granule; M, mitochondria; ML, M-line SR, sarcoplasmic reticulum;

Z, Z-line; A, A-band; I, I-band