DISTRIBUTION OF MOLECULAR SPECIES OF COLLAGEN IN MUSCLE AND THE RELATIONSHIP TO MEAT TEXTURE.

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The physical properties of collagen, both in the native and heat-denatured state, play an important role in determining the texture of meat. Significant differences in properties exist between the intramuscular collagen and the external epimysium and tendon, and these have been rationalised on the basis of the thermal stability of the intermolecular cross-links.

We have now shown that there is a distribution of genetically distinct types of collagen within the hierarchies of skeletal muscle. The tendon and epimysium are composed of Type I collagen, the perimysium Types I and III and the endomysium surrounding the single muscle fibres is composed of Types I and III in addition to the non-fibrous basement membrane. Variation in the proportion of these types of collagen and the nature of their stabilizing cross-links would result in texture variation between muscles.

DISTRIBUTION DES ESPECES MOLECULAIRES DE COLLAGENE DANS LES MUSCLES ET SON RAPPORT A LA TEXTURE DE LA VIANDE

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Les propriétés physiques du collagène dans l'état naturel et dénaturé par la chaleur, jouent un rôle important dans la détermination de la texture de la viande. Des différences importantes de propriétés existent entre le collagène intramusculaire et l'épimysium et le tendon externes, et celles-ci ont été rationalisées sur la base de la stabilité thermique de la liaison transversale intermoléculaire.

Nous avons maintenant démontré qu'il y a une distribution de types de collagène distincts sur le plan génétique au sein des hiérarchies de muscle squelettique. Le tendon et l'épimysium consistent en collagène du Type I, et en périmysium des Types I et III et l'endomysium entourant les fibres des muscles uniques consiste en des Types I et III en plus de la membrane à base non fibreuse. Des variations de la proportion de ces types de collagène et de la nature de leurs liaisons transversales stabilisatrices aboutiraient à une variation de texture entre les muscles.

DIE VERTEILUNG VON MOLEKULARTEN VON KOLLAGEN IN DER MUSKULATUR UND DAS VERHÄLTNIS ZUR FLEISCHFASERBESCHAFFENHEIT

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Die physischen Eigenschaften von Kollagen, sowohl im natürlichen als auch im hitzeverformten Zustand spielen eine wichtige Rolle bei der Bestimmung der Fleischfaserbeschaffenheit. Wesentliche Unterschiede kommen vor zwischen dem intramuskulären Kollagen und dem externen Epimysium und der Sehne, und diese wurden untersucht auf der Grundlage der thermalen Stabilität der intermolekularen Kreuzverbindungen.

Wir haben jetzt festgestellt, daß es eine Verteilung von genetisch verschiedenen Arten von Kollagen innerhalb der Hierarchie der Skelettmuskulatur gibt. Sehne und Epimysium bestehen aus Kollagen vom Typ I, Perimysium aus den Typen I und III, und Endomysium, das einzelne Muskelfasern umschließt, beseht aus den Typen I und III zusätzlich zur Nichtfasergrundmembran. Veränderungen des Anteils dieser Kollagentypen und die Art ihrer Stabilisationskreuzverbindungen würden eine Faserveränderung zwischen Muskeln bewirken.

РАСПРЕДЕЛЕНИЕ МОЛЕКУЛЯРНЫХ ВИДОВ КОЛЛАГЕНА В МЫШЦАХ И СООТНОШЕНИЕ К МЯСНОЙ ТКАНИ

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Физические свойства коллагена, и в натуральном, и в денатурированном при нагревании состоянии, имеют важное значение в определении ткани мяса. Существует большая разница в свойствах между внутримышечным коллагеном и внешним эпимизиумом и сухожилием, а разница эта рационализирована на основе тепловой устойчивости внутримолекулярного взаимодействия.

Мы доказали, что генетически разные виды коллагена распределяются по структурам скелетной мышцы. Сухожилие и эпимизиум состоят из коллагена типа I, перемизиум из типов I и III и эндомизиум, окружающий волокна одиночных мускулов, состоит из типов I и III вдобавок к безволокнистой основной оболочке. Изменение в пропорции этих типов коллагена и свойствах их стабилизирующего взаимодействия привело бы к изменению ткани между мышцами.

DISTRIBUTION OF MOLECULAR SPECIES OF COLLAGEN IN MUSCLE AND THE RELATIONSHIP TO MEAT TEXTURE

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INTRODUCTION

The physical properties of collagen, both in the native and heat-denatured state, play an important role in determining the texture of meat[1].

The collagenous connective tissue within muscle has been histologically classified as the epimysium, the perimysium and the endomysium. The epimysium forms the outer sheath of the muscle, the perimysium encloses the muscle bundles and the endomysium surrounds each individual muscle fibre[2].

Previous studies have demonstrated significant differences in the solubility between the intramuscular and tendon collagen[3,4], and this has been correlated with the chemical and thermal stability of the intermolecular cross-links present in the collagen of these two tissues[5]. However, the recent finding of genetically distinct types of collagen in embryonic human[6,7] and bovine[8,9] skin, aorta[10] and other tissues[11] suggested that the change in the stability of the cross-links may be due to a change in the genetic type of collagen present. We now report that there is indeed a distribution of the genetically different collagens throughout the muscle, and discuss their possible relationship to the texture of meat.

MATERIALS AND METHODS

Materials

Tendon and epimysium were carefully dissected from psoas major, longissimus dorsi, and sternomandibularis muscles from a two year old steer. The tissues were homogenized and washed in physiological saline (0.9% NaCl, pH 7.4). The perimysium was obtained by mild homogenization, using a Marsh-Snow blender[12], of the muscle stripped of tendon and epimysium. The endomysium was prepared from single muscle cells following dissection under a Leitz dissecting microscope. The individual myofibres were then immersed in Hasselbach-Schnieder solution, followed by water then 0.6M KI solution to remove the actomyosin. The residue of collagenous tissue was then suspended in physiological saline.

Reducible cross-links

The collagen fibres were reduced with tritiated sodium borohydride, hydrolysed in 6N hydrochloric acid, and the radioactive components separated and identified on an amino acid analyzer as previously descried[13].

Types of collagen

The various collagenous tissues were digested with pepsin at an enzyme substrate ratio of 10:1. The Type III collagen was re-precipitated from the soluble collagen solution at 1.5M NaCl and the Type I collagen at 2.5M NaCl using the technique of Chung and Miller[16].

SDS Acrylamide gel electrophoresis

Analysis of the re-precipitated collagens to confirm their identity as to type of collagen was achieved by electrophoresis. A flat-bed apparatus was employed using borate buffers at pH 8.5 as previously described[14]. The conversion of Type III γ to α components was carried out by incubation with mercaptoethanol prior to electrophoresis, the resulting α(III) having a slower mobility than α(I)[9,11].

RESULTS

The tendon and epimysium of the muscles has been shown to consist almost entirely of Type I collagen. In contrast the perimysium and endomysial collagens contained an high proportion of Type III collagen (20-30%) with the Type I. More accurate estimates of the proportion of the two different collagens are currently being determined from the analysis of cyanogen bromide peptides.

The endomysial collagen from the single myofibres was also shown to contain Types I and III in addition to the basement membrane Type IV collagen known to be present from electron microscope studies[15]. Chemical characterization of the Type IV collagen in endomysium has not yet been definitively established in view of the small quantities available.

The nature of the intermolecular cross-links similarly varied with the tissue. The major cross-link present in the endo- and perimysial collagen was found to be the stable cross-link hydroxylysino-5-keto-norleucine[16]. The presence of this cross-link accounts for the low-solubility of these collagens. The solubility of the Type III collagen is probably further decreased by the presence of disulphide bonds in addition to the lysine-derived cross-links.

The epimysial and tendon collagen were found to be similar and contain approximately equal proportions of the labile cross-link dehydrohydroxylysinonorleucine[16] and the stable 'keto' cross-link. These collagens are therefore more soluble in nondenaturing solvents and following thermal denaturation.

DISCUSSION

The variation in the nature of both the type of collagen and the stabilizing cross-links is clearly of importance in elucidating the role of collagen in the texture of meat. In the raw state these differences in properties will be minimal and the shear force values will be dependant on the total amount of collagen present. In contrast, following cooking and thermal denaturation of the collagen, the differences in thermal stability of the collagen and their cross-links becomes the controlling factor. Following denaturation the collagen becomes much weaker and elastic. If the collagen possesses thermally stable cross-links it is insoluble and has sufficient strength to maintain the binding together of the muscle bundles, whilst in contrast, if the bonds are thermally labile the collagen partially dissolves and possesses little tensile strength. This difference in stability of the bonds is clearly demonstrated on cooking similar muscles from a calf and an old cow. The collagen from the calf exudes from the meat and sets to a gel on cooling, whilst the older muscle retains its denatured collagen within the muscle. This effect is due to an increase in the proportion of thermally stable cross-links with increasing age, following the conversion of the labile aldimine bond to an as yet unknown stable cross-link[5,13]. In addition, as the collagen shrinks during denaturation a tension is generated which depends on the thermal stability of the cross-links. The contractile tension generated will be greatest in the endo- and perimysial collagens since they possess a higher proportion of thermally stable cross-links. In this case, with a hiefer or steer, the difference depends on the relative proportion of the labile aldimine and stable keto type cross-links. The role of the disulphide cross-link in the Type III collagen is not yet clear. Similarly the properties of the Type IV collagen are not yet understood, and again both disulphide and lysine-derived cross-links are also present in this non-fibrous collagen[17].

During thermal contraction of the collagenous sheath, fluid is exuded, resulting in a tighter interaction of the denatured myofilaments. The actomyosin filaments are the major proteins of muscle but possess little tensile strength or resistance to shear in the native state. On thermal denaturation the increase in shear force value will depend on the tension generated by the collagenous network which in turn depends on the proportion of stable cross-links. The greater the tension the greater the interaction of the denatured actin-myosin complex due to fluid loss, resulting in closer hydrophobic and ionic interactions, and ultimately in an increase in toughness of the meat.

The morphological organization of the collagen fibres varies considerably at the three different levels of organization. The tendon and epimysium fibres tend to lie in parallel. The collagen fibres of the perimysium are organized in lamina the direction of the fibre layers being at an angle to each other[18]. The endomysium consists of the amorphous basement membrane collagen together with the fibrous Type I and III collagen. The importance of these different orientations on the tension generated similarly remains to be elucidated.

Although the relative contribution of the various types of collagen has not yet been elucidated it is felt that these results give further confirmation to the proposal previously made that, in the absence of abnormal post-mortem factors such as cold-shortening, the thermal stability of the intermolecular cross-links of collagen determines the ultimate toughness of meat. It is clear that an understanding of the nature of these various collagens is crucial to a complete understanding of the collagen in relation to meat texture.

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Legends

Fig.1

Typical elution pattern of an acid hydrolysate of tritiated NaB^3H_4 reduced native collagen. The location of the reduced cross-linking amino acid is shown relative to the usual amino acids a) pattern typical of epimysial or tendon collagen, b) pattern typical of endomysial collagen. Reduced components are DIOH-LNL (dihydroxy-lysinonorleucine); and HHMD (Histidino-hydroxymerodesmosine).

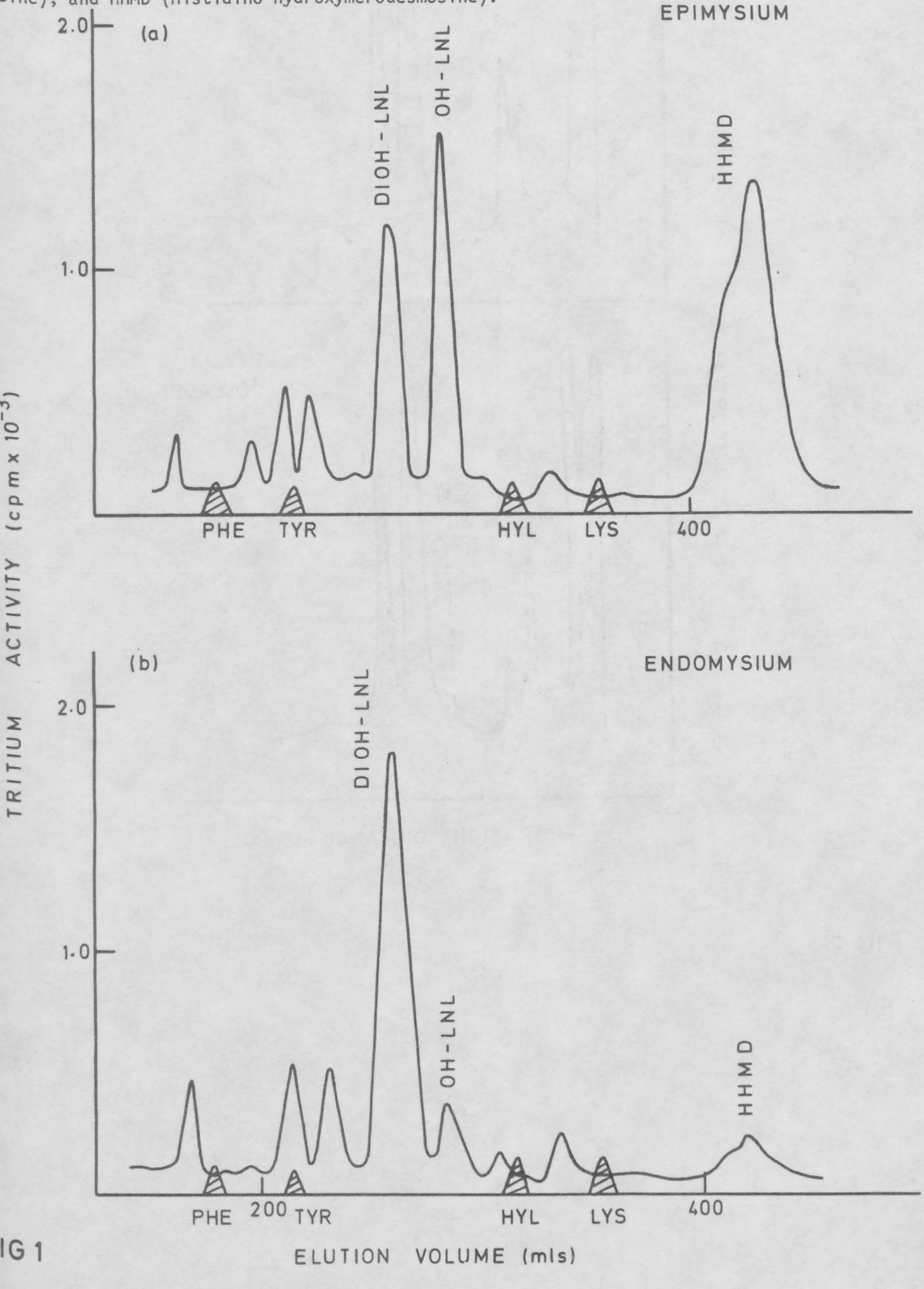

FIG 1

Fig.2

Densitometric traces of SDS polyacrylamide-gel electrophoresis patterns of pepsin solubilized material from epimysial and endomysial collagen. a) Total digest of epimysial collagen. The pattern was unaltered following pre-incubation with 2% mercaptoethanol. b) Total digest of endomysial collagen. Following pre-incubation with 2% mercaptoethanol the γIII components were converted to αIII, as shown by the appearance of the latter with a slower mobility than α1(I).

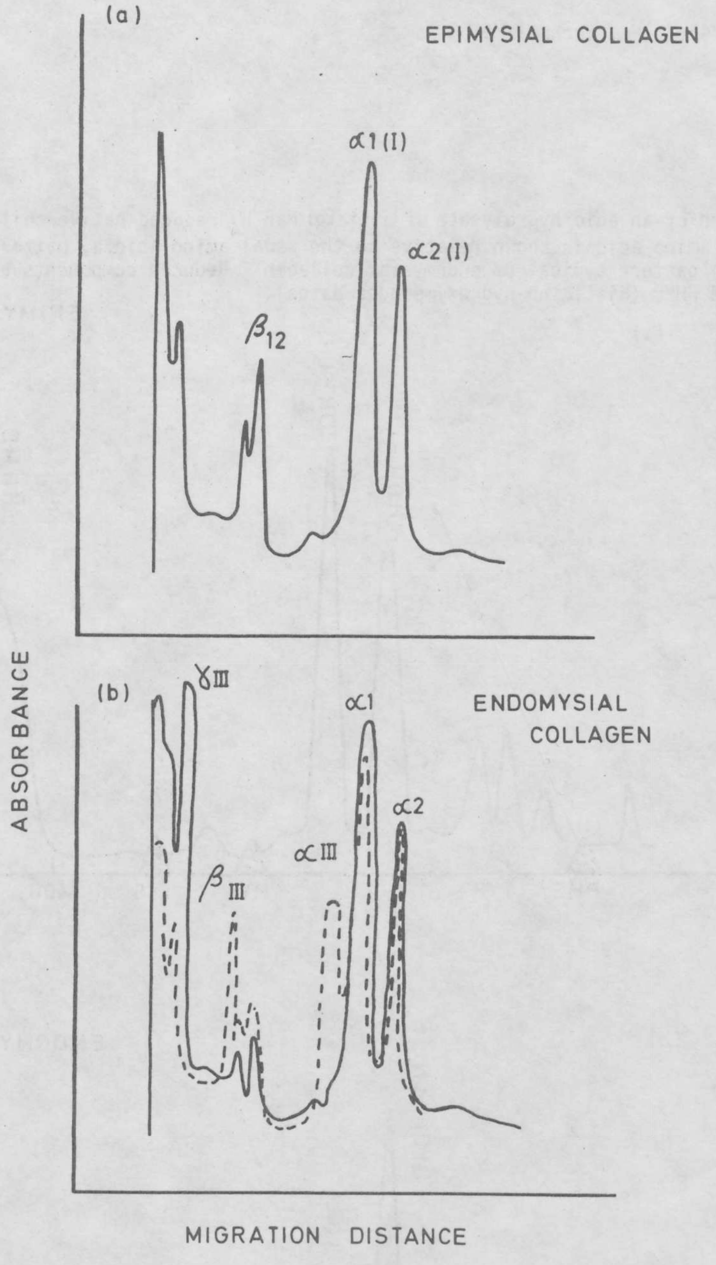

FIG 2