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The Solubility of Intramuscular Collagen in Meat Animals of Various Ages.

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Die Löslichkeit intramuskuläres Kollagens in Fleischtieren von verschiedenen Alter

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Zusammenfassung

Der Anteil an intramuskulärem Kollagen, der durch Erhitzung eine Stunde bei 77°C. in $\frac{1}{4}$ Stärke Ringerslösung von Muskeln von 98 Rindern, 15 Schafen und 9 Schweinen löslichgemacht wurde, nahm mit steigendem chronologischem Alter ab. Eine allgemeine Zunahme in gesamtintramuskulärem Kollagenanteil mit steigendem Alter ist nicht bemerkt worden. Aus den Ergebnissen ist es möglich anzunehmen, dass die Zahl oder Stärke von Kreuzgliedern intramuskuläres Kollagens mit steigendem Alter zunehmen. Die Ergebnisse zeigen auf annehmbare Weise, warum die Gesamtanteile an intramuskulärem Kollagen ungenügend sind, die mit steigendem chronologischem Alter verbundene Zähigkeit zu erklären.

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Белград, 1965 г.

Растворимость межмускульного коллагена мяса скота различного возраста

Ф. Хилл

Сводка

Количество межмускульного коллагена в мускулах 98 штук рогатого скота, 15 овец и 9 свиней растворимого в 25% растворе Рингере при нагревании до 77°С в течении часа уменьшается с увеличением возраста скота. Указаний на общее увеличение содержания межмускульного коллагена с увеличением возраста скота не было получено. Есть основания предполагать, судя по полученным результатам, что как число так и прочность поперечных соединений межмускульного коллагена увеличивается с возрастом. Жесткость мяса, связанную с увеличением возраста нельзя приписать целиком общему количеству межмускульного коллагена, в то время как присутствие поперечных соединений может служить достаточным объяснением.

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Onzième réunion européenne des travailleurs de recherche sur la viande.

Belgrade, 1965.

La solubilité du collagen intramusculeux (protéine) en animaux de viande de divers âges.

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Résumé.

La quantité de collagen intramusculeux solubilisé par chauffage en une solution Ringer de force de 1/4 (un quart) des muscles de 98 (quatre-vingt-dix-huit) bestiaux, 15 (quinze) moutons, et 9 (neuf) cochons à 77°C pour 1 (une) heure, baissa à mesure que l'âge chronologique s'augmenta. Il n'y avait aucune indication d'une augmentation générale du contenu de collagen intramusculeux avec l'âge. Les résultats donnent du fort temoignage présumptif d'une augmentation avec l'âge du nombre ou force des liens croisés du collagen intramusculeux. Ils fournissent une explication acceptable pour ce que les quantités totales du collagen intramusculeux sont insuffisantes pour expliquer la nature coriace associée avec l'accroissement de l'âge chronologique.

The Solubility of Intramuscular Collagen in Meat Animals of Various Ages.

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SUMMARY

The amount of intramuscular collagen solubilised by heating in $\frac{1}{4}$ strength Ringer's solution, muscles from 98 cattle, 15 sheep and 9 pigs at 77°C for 1 hour decreased as chronological age increased. Total intramuscular collagen was high in very young (8 - 9 weeks) bovines and in two of the three very old (7 - 15 years) bovines studied. In the age range, 16 weeks - $4\frac{1}{2}$ years, variation in total bovine intramuscular collagen was wide. There was no indication of a systematic increase as age increased. The results provide strong presumptive evidence of an increase with age in the number or strength of the cross links of intramuscular collagen and furnish an acceptable explanation as to why total amounts of intramuscular collagen are inadequate to explain toughness associated with increasing chronological age.

Wilson, Bray and Phillips (1954) reported that the collagen content of the longissimus dorsi muscle of veal was greater than that of steers or cows and suggested that total amounts of collagen are not always adequate to explain different degrees of toughness. Loyd and Hiner (1959) however, found no significant differences between veal and beef in hydroxyproline content. Verzar (1960, 1963) found that the corium of cows' skin and also human skin when heated for 10 minutes at 65°C liberated hydroxyproline complexes and that as chronological age increased the amount liberated decreased. He explained these observations by suggesting that as an animal ages, cross linkages increase within the collagen macromolecule. Verzar (1963) postulated that as collagen has not a metabolic turnover, molecular movements eventually bring the polypeptide chains nearer to each other, thus assisting the formation of cross linkages.

Jackson and Bentley (1960) in their work on guinea pig skins found that the collagen fraction which was most extractable also incorporated carbon 14 glycine most rapidly. They proposed that a continuous spectrum of collagen aggregates of varying degrees of cross linking exists at any given time in developing connective tissue and that the longer the time that has elapsed since the syntheses of a collagen molecule the more firmly will it be bound into a collagen aggregate. They explain increase in cross linking with increasing age as follows: "The deeper in the fibre the collagen molecule is, the more firmly will it be cross linked, as with time the molecules move into more favourable steric apposition under the influence of thermal agitation". Schaub (1963) studied the ageing of collagen in the connective tissue of the rat's skeletal musculature by determining the amount of collagen solubilised in Ringer's solution in 10 minutes at 65°C. The values dropped from 30-40% in 5 week old animals to 10% in 10 month old ones, and in old age to 3-4%. Goll et al.(1963) suggested that a structural change may take place in collagen, as

an animal matures. Goll et al. (1964b. 1) studied age associated structural changes in collagenous residues isolated from the biceps femoris muscles of 11 bovines, representing 4 different age groups. They reported that the susceptibility of this material to collagenase digestion decreased as chronological age increased. Scheraga (1961) pointed out that increased cross linking of protein molecules should result in a slower rate of proteolytic hydrolysis. In an extension of their studies on the residues Goll et al. (1964a, 1964b, 11) found that as chronological age increased, less hydroxyproline was released by heating in phosphate buffers at temperatures of 60°, 65°, 70° and 100°C. The work reported in this paper deals with changes in the solubility of intramuscular collagen of meat animals as chronological age increases.

EXPERIMENTAL METHODS

The sternomandibularis was the muscle mainly used in this study. It is a uniformly straight fibred neck muscle which is exposed at decapitation. It can be excised very soon after slaughter without mutilating the carcase. It contains a large amount of collagen. In a few cases, other muscles such as the psoas major, semitendinosus and longissimus dorsi were also investigated.

The muscles were dried over silica gel in an evacuated desiccator at a temperature of 2°C overnight. The desiccated muscle was then broken up in a micro hammer mill so that it passed through a 2mm. screen. A weight of dried muscle equivalent to 5 grams of fresh tissue was put into a 50 ml. centrifuge tube (containing a rubber stopper with thermometer and a vent) and 12 mls. of $\frac{1}{4}$ strength Ringers solution added. It would appear from Verzar's work (1963) that Ringer's solution is more effective than distilled water in weakening the intermolecular forces of collagen.

The tube was put in a water bath at 77°C for 63 minutes and the contents stirred at intervals. The average time taken for the contents of the tube to reach 77°C was 3 minutes. 77°C is considered a desirable temperature for the interior of a well done beef joint when removed from the oven. (National Live Stock and Meat Board).

After centrifugation and removal of the supernatant, the residue in the tube was washed with 8 mls. of Ringer's solution and centrifugation was again performed. The supernatants were bulked and hydrolysed for 7 hours. The residue of muscle was also hydrolysed for 7 hours. Hydroxyproline was determined in the hydrolysates by the Mohler and Antonacopoulos (1957) modification of the Neumann and Logan (1950) method.

RESULTS

Table 1 shows the percentages of total collagen and of the total collagen solubilised (by heating for 1 hour at 77°C) in the sternomandibularis muscles of a total of 42 Friesian bovines (15 calves, 22 steers and 5 cows). The total collagen was high (2.21%) at 8-9 weeks and varied from 1.20% at 3½ years to 1.75% at 4-6 months. The high total collagen at 8-9 weeks is probably a reflection of poor muscle development at this age. Collagen solubility decreased with increasing age. This is illustrated in Figure 1. Table 2 shows similar data for 24 Hereford bovines. Solubility also decreased here with increasing chronological age. Figure 11 shows graphically the decrease in solubility with advancing age of the 22 Herefords and Hereford crosses listed in the top portion of Table 2. Table 3 shows a general decrease in solubility (illustrated in Fig. 111) in the sternomandibularis muscles of 11 Shorthorn cows as chronological age increases. The oldest cow (7 years) had a very high total

collagen content (2.79%) but, there was no indication of a general increase in total collagen content with age. Table 4 shows pooled data on seven 13 week old male Jersey calves and 12 old factory cows. Details of breed and age were not available for the latter, but, it is probable that each cow was older than 3 years of age. Table 5 shows that there is little variation in the solubility of collagen in muscles from different anatomical locations of the same carcase. Results are shown for a 15 year old bull and three young calves, 8-9 weeks of age. Table 6 shows data for 9 six months old Galway lambs, 6 miscellaneous ewes (each more than 5 years old), 2 Large White hogs, each 5 months old, 1 old boar and 6 old sows. It would appear that the collagen in the sternomandibularis muscles of Galway lambs becomes insoluble at a faster rate than that in Friesians or Hereford crosses. While there is no overall increase in collagen with age solubility decreases as in the bovine species. The six old factory sows and the boar had a collagen solubility of about 4% compared to about 23% for the 5 months old hogs.

DISCUSSION

The results presented in this study indicate that the intramuscular collagen in the sternomandibularis muscles of cattle, sheep and pigs undergoes subtle chemical changes as chronological age increases. The results are consistent with those of Verzar (1960, 1963), Jackson and Bentley (1960), Schaub (1963), and Goll et al (1964a,b). The ageing phenomenon associated with collagen is not confined to the sternomandibularis muscle. This is indicated by the results for 3 other muscles (taken from different anatomical locations) of the 15 year old Aberdeen Angus bull (Table 5). The work reported here can be considered as strong presumptive evidence of an increase in the number or strength of the cross linkages of intramuscular collagen in meat animals as chronological age increases.

Reed et al (1963) point out that mature collagen is almost

completely insoluble in acidic buffers even in the presence of hydrogen bond breaking reagents. This fact suggests the existence of strong types of linkages in mature collagen. Harding (1965), in his review concludes that it would appear fairly certain that ester linkages occur in collagen and that they take part in the intramolecular cross linking of the polypeptide chains. Hormann (1960) cited by Harding (1965) reported that procollagen gave rise to 0.64 moles of hydroxamic acid per 100 moles of amino acid and that mature collagen gave 1.07 moles. The hexose contents of both types of collagen were approximately equal. Hormann (1962a) cited by Verzar (1964) suggested that there are intramolecular hexose ester links in both procollagen and old collagen, and that old collagen also contains intermolecular hexose ester linkages.

Reed et al (1963) citing Hormann (1962b) state that although solubilisation of mature collagen can be achieved when ester and hexose type linkages are broken in the presence of hydrogen bond breaking and other reagents, the rate of solution does not correspond with the rate of destruction of these specific linkages. They consider that while there seems no doubt that hydroxylamine and periodate sensitive cross linkages are present in mature collagen, and so help to determine its degree of insolubility, it is likely that additional factors which depend upon the age of the tissue are involved. They point out that as collagen fibrils mature coatings of mucopolysaccharides and glycoproteins appear to make them more insoluble.

The results reported here indicate that the degree of solubility of the collagen as well as the total amount should be considered when biochemical explanations of toughness in meat are considered. During the cooking of meat from older animals, less collagen is solubilised than in meat from younger animals. The situation results in an increased sensation of

toughness when the meat of older animals is consumed.

An extension of the work on collagen solubility reported here, could provide a chemical method of evaluating carcases of different breeds and crosses for toughness. The hydroxyproline determination could be made more sensitive and accurate by using the modification of Woessner (1961). The method used in the work presented here is subject to interference by tyrosine and to partial inhibition of colour development by the presence of other amino acids. (Sharp (1963)). By raising the temperature of solubilisation to a value above 77°C (say 95°C) increased sensitivity could also be obtained.

Results from an investigation of the type suggested should indicate if the collagen in different breeds and crosses becomes insoluble at different rates. If this is so, breeding of meat animals of improved tenderness should be possible by selection.

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TABLE 1.

Percentage total collagen and percentage of the total collagen solubilised (by heating for 1 hour at 77°C) in the sternomandibularis muscles of 42 Friesian bovines.

Sex	Number of animals	Age	% Total collagen in the muscles	% of total collagen solubilised by heating for 1 hour at 77°C.
Male calves	2	8-9 weeks	2.21 ± .31	21.91 ± .57
	8	16 weeks	1.21 ± .06	24.59 ± 1.06
	5	18 weeks	1.31 ± .11	20.66 ± 1.16
Steers	5	4-6 months	1.75 ± .11	21.13 ± 1.16
	14	10 months	1.30 ± .26	11.89 ± .61
	3	22 months	1.74 ± .16	8.47 ± .95
Cows	1	2½ years	1.51	3.62
	1	3 years	1.67	3.71
	2	3½ years	1.20 ± .13	4.51 ± .41
	1	4½ years	1.25	3.72

TABLE 2.

Percentage total collagen and percentage of the total collagen solubilised (by heating for 1 hour at 77°C) in the sternomandibularis muscles of 24 Hereford and Hereford cross bovines.

Sex	Cross	Number of animals	Age	% Total collagen in the muscles	% of total collagen solubilised by heating for 1 hour at 77°C
Steers	H x H	4	4-6 months	1.27 ± .07	21.80 ± 1.39
	H x S	8	17 months	1.08 ± .06	10.70 ± .33
	Hereford Cross	7	2 years	1.48 ± .16	6.43 ± .27
Heifers	Hereford idertical twins	2	$3\frac{5}{12}$ years	1.14 ± .16	5.07 ± .78
Bull	H x H	1	12 years	1.45	1.98
Steer	A.A. x H	2	4-6 months	1.03 ± .03	16.24 ± .40

H = Hereford

S = Shorthorn

A.A. = Aberdeen Angus.

TABLE 3.

Percentage total collagen and percentage of the total collagen solubilised (by heating for 1 hour at 77°C) in the sternomandibularis muscles of 11 Shorthorn cows.

Number of cows	Age (years)	% Total collagen in the muscle	% of total collagen solubilised by heating for 1 hour at 77°C.
1	$2\frac{1}{2}$	1.19	3.82
1	3	1.58	4.61
2	$3\frac{1}{2}$	1.40 ± .002	2.74 ± .05
1	4	2.12	2.48
3	$4\frac{1}{3}$	1.53 ± .26	3.21 ± .21
2	$5\frac{1}{3}$	1.71 ± .05	2.65 ± .50
1	7	2.79	1.79

TABLE 4.

Percentages of total collagen and percentages of the total collagen solubilised (by heating for 1 hour at 77°C) in the sternomandibularis muscles of seven 13 week old male Jersey calves and 12 old factory cows.

	% Total collagen	% of total collagen solubilised
7 Jersey calves	1.52 ± .14	19.24 ± 1.06
12 old cows	1.17 ± .06	2.15 ± .23

TABLE 5.

Percentage of total collagen and percentage of the total collagen solubilised (by heating for 1 hour at 77°C) in muscles from different anatomical locations in 4 carcases.

	Muscle	% Total collagen	% of total collagen solubilised
15 years old Aberdeen Angus bull	Sternomandibularis	1.78	0.79
	Longissimus dorsi (10th - 13th ribs)	.92	Nil.
	Psoas major	.62	Nil.
	Semitendinosus	2.19	Nil.
8 weeks old Friesian calf	Sternomandibularis	2.53	21.34
	Psoas major	.39	18.37
	Semitendinosus	.87	19.72
8 weeks old Friesian calf	Psoas major	.27	18.13
	Semitendinosus	..60	18.84
9 weeks old Friesian calf	Sternomandibularis	1.90	22.48
	Psoas major	.40	17.47
	Semitendinosus	.92	16.65

TABLE 6.

Percentage total collagen and percentage of the total collagen solubilised (by heating for 1 hour at 77°C) in the sternomandibularis muscles of 15 sheep and 9 pigs.

	Number of animals	Age	% Total collagen in the muscle	% of total collagen solubilised by heating for 1 hour at 77°C.
Sheep				
Male lambs	9	6 months	1.01 ± .05	8.16 ± .57
Old ewes	6	>5 years	1.09 ± .06	2.81 ± .23
Pigs				
Large White hogs	2	5 months	1.93 ± .04	22.82 ± 5.17
Old boar	1	-	1.39	4.33
Old sows	6	-	1.65 ± .32	3.94 ± .29

Figs. I, II and III Decrease in Collagen Solu
in the Sternomandibularis Muscle with Age