THE DETERMINATION OF THE AMOUNT OF CONNECTIVE TISSUE IN MEAT.

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The desirability of any cut of meat as an article of diet depends largely upon the flavor and the toughness of its lean. While no appreciable headway has been recorded in the quantitative study of meat flavor and of the constituents of meat to which it is due, a study of the toughness of meat has been carried on for a number of years with notable success by Lehmann of the Hygienic Institute at Würzburg, Germany. In collaboration with a number of his pupils, Lehmann has shown (1) that the toughness of different cuts of meat, measured mechanically, was closely related to their content of connective tissue, and that the decrease in toughness resulting from cooking was related to the collagen of connective tissue rather than to the elastin. Under the influence of moist heat the collagen is readily changed to gelatin, thus losing its toughness. In the raw condition, white fibrous connective tissue (mainly collagen) is almost twice as tough as yellow elastic connective tissue (mainly elastin), but when cooked, the former loses most of its toughness while the latter remains practically unchanged in this respect. Physical differences in the muscle fibers themselves were not found to be appreciable factors in determining differences in toughness of muscle samples.

There is an obvious advantage in the use of a chemical measure of toughness as compared with a mechanical one, since Lehmann found that results of the mechanical test were so variable that a series of ten to twenty individual determinations should be run in order to obtain a representative average value. When only a limited amount of meat is available, therefore, it may be impossible to use the mechanical test to advantage.

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Two methods were used in determining the connective tissue content of different muscles. One was a purely mechanical method developed by Schindler (2) in which a weighed portion of meat was simply scraped parallel with the fibers with a moderately sharp knife. The muscle cells are said to be easily scraped away, leaving a white framework of connective tissue fibers, which may be weighed either in the moist or in the dry condition.

In the same laboratory, Schepilewsky (3) worked out a chemical method for determining the connective tissue in flesh. In this method the muscle tissue is removed largely by mechanical means, consisting of trituration in a mortar with water, and sieving. The last traces are removed by treatment for 15 hours with cold 5 per cent sodium hydroxide solution. The separation of collagen and elastin is accomplished by treatment with hot alkali, in which the collagen is converted to gelatin and goes into solution.

We have followed the Schepilewsky method on a number of samples of meat and on white tendon obtained from pig shanks. We have found that a satisfactory filtration of the cold alkaline solutions is difficult, and we have obtained clear indications that collagen is appreciably soluble in 5 per cent sodium hydroxide, the solution used to remove the last traces of muscle tissue. Two samples of beef rib were submitted to the Schepilewsky method and to the method developed in this laboratory, which will be described later; in both cases, the gelatin results of the Schepilewsky method were only a small fraction of the results of the new method in which no alkaline extraction is used. A sample of white tendon was obtained by freezing pig tendons and slicing them in the frozen condition into thin sections. The sliced tendon (30 gm.) was then thoroughly macerated for 90 minutes with 300 cc. of water in a small ball mill. The water treatment and the sieving, followed by the treatment with cold alkali, was done according to Schepilewsky's directions. The filtrate, however, was cloudy, and contained over 50 per cent of the total nitrogen of the tendon. Upon acidification with acetic acid, a white cloudy precipitate separated, which was filtered off. The filtrate was clear and gave a deep color with the biuret test. Although no quantitative determination of the nitrogen contained in this clear solution was made, it was clearly evident that con-

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siderable amounts of collagen nitrogen had been dissolved in the cold alkali.

It seems evident that a sharp separation of residual muscle stroma proteins from the characteristic proteins of connective tissue, collagen and elastin, is difficult if not impossible of attainment by either physical or chemical means. However, a more satisfactory separation than that accomplished by treatment with cold 5 per cent alkali would seem to be essential. We have adopted the following procedure. After the removal of most of the muscle tissue by cold water extraction and mechanical separation on a sieve, according to a method equivalent to that of Schepilewsky, the collagen is converted into gelatin by heating in the autoclave, and is removed by exhaustive hot water extraction. There is no reason to suspect that the water-insoluble proteins of the residual muscle tissue would be soluble in hot water. The residue remaining from this treatment is then digested with an alkaline trypsin solution at approximately 40°C. The muscle proteins are readily digested by trypsin while elastin is quite resistant.

In 1910, Baumstark and Cohnheim (4) showed that *in vitro* digestion of small pieces of meat (2 to 3 cm. long and 1 cm. in diameter) with pancreatic juice for 36 hours, removed the muscle fibers, leaving the connective tissue framework apparently intact. They concluded that digestion of connective tissue *in vivo* must be brought about by pepsin either in the stomach or in the upper part of the small intestine. The great resistance of elastin to tryptic digestion was amply confirmed by Abderhalden and Strauch (5) and Abderhalden and Meyer (6). In these investigations it was further shown that elastin readily adsorbs pepsin in the stomach and that the hydrolysis of elastin by this adsorbed pepsin continued throughout the length of the small intestine. Thus, elastin and possibly also collagen, are digested *in vivo* almost entirely through the action of the gastric protease, pepsin.

Trypsin does slowly attack elastin (5, 7), so that the separation of muscle proteins and elastin by means of tryptic digestion is only an approximation, the success of which will depend upon the choice of a time of reaction such that the error is least. The digestion of meat *in vitro* by trypsin in alkaline solution fully confirmed the results of preceding investigators. In 12 hours,

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the muscle tissue was obviously largely if not entirely removed, leaving the white fibers of connective tissue. In the case of a sample of lean meat from the ribs of a 3 year old steer, it was found that at the end of 16 hours digestion, 36.3 per cent of the total nitrogen was undissolved, at the end of 24 hours, 32.8 per cent remained, and at the end of 40 hours, 27.5 per cent. A digestion period of 16 hours was adopted.

The filtration of the tryptic digest presented difficulties, since the alkali could not be neutralized without bringing down a precipitate of partially hydrolyzed protein. The expedient was finally adopted of filtering through a 120 mesh sieve.

While it might be considered that the connective tissue proteins might well be determined simply by means of the trypsin digestion, since collagen as well as elastin is extremely resistant to tryptic action (8), our attempts to devise such a method were not particularly successful. Satisfactory duplicates were difficult to obtain. Furthermore, since the separation is only approximate under the best of conditions, it would seem that the error would be the less the smaller the amounts of the separable proteins to be digested.

Description of Method.

The method finally adopted for the determination of the characteristic proteins of connective tissue in meat may be described as follows: Free the meat from all visible fat and surrounding connective tissue. Grind the sample through a meat chopper using a medium cutting plate. All meat remaining in the mill should be removed and thoroughly mixed with the ground sample.

Duplicate samples varying in weight from 25 to 100 gm. each may be used. The more connective tissue there is in the sample the smaller need the sample be. Place the sample in a small ball mill with 300 cc. of distilled water and macerate for 90 minutes. Transfer the sample onto a 40 mesh sieve, rejecting the filtrate. Wash the residue by taking up in 150 to 300 cc. of cold water, stirring thoroughly in a beaker, and filtering through the 40 mesh sieve. Repeated tests have shown that seven washings are sufficient to remove practically all of the water-soluble protein and most of the finely divided granular material.

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Transfer the residue from the sieve to an 800 cc. beaker. Bring the volume up to about 400 cc., cover with a watch-glass, and heat for 2 hours in an autoclave under 16 to 18 pounds pressure. Release the pressure in the autoclave gradually and do not disturb the beakers for at least 5 minutes after the door is opened, for as long as the contents of the beakers are superheated there is danger of loss. Decant the hot supernatant liquid through a fluted filter paper, collecting the filtrate in a 1 liter volumetric flask. Wash the residue on the filter back into the beaker with 100 cc. of hot water, boil for a few minutes, and filter again. Repeat this process five times, or until the washings give only a constant faint color with the biuret test. Make the combined filtrates in the volumetric flask up to the mark and take aliquots for total nitrogen determinations.

Wash the residue on the filter back into a 400 cc. beaker with 100 cc. of cold trypsin solution.[1] Add 3 cc. of a mixture of chloroform and toluene and digest for 16 hours in the constant temperature oven at 38–40°C. Heat the solution to boiling, filter the trypsin extract through a 120 mesh sieve, and wash the residue three times with hot water, filtering each time through the sieve. Reject the filtrates. Determine total nitrogen in the residue by transferring to a Kjeldahl flask with water, digesting in the usual manner, and taking aliquots for distillation.

The nitrogen taken out by treatment in the autoclave and extraction with hot water is considered to be collagen nitrogen, and the nitrogen in the final residue, elastin nitrogen. The results are conveniently expressed in percentages of the total nitrogen of the meat.

There may be some question as to whether the results obtained for elastin may not include some of the other proteins of connective tissue. The coagulable proteins and the nucleoproteins of connective tissue, in so far as they are not removed by sieving in the initial process of the method, would presumably be dissolved during the tryptic digestion. The mucoid of connective tissue may also be dissolved at this stage, though no positive statement as to this can be made. In any case, the mucoid contamination

[1] The trypsin solution is made as follows: 1.5 gm. of powdered trypsin (we use a product put out by Fairchild Brothers and Foster of New York) and 6 gm. of sodium carbonate dissolved in 2 liters of water.

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TABLE I.

Connective Tissue Proteins in Meat.

Sample No.	Description of sample.	Total nitrogen in sample.	Collagen nitrogen in per cent of total.	Elastin nitrogen in per cent of total.	Collagen + elastin nitrogen in per cent of total.
		per cent			
1	Beef rib.	3.19	8.4	6.4	14.8
			7.9	7.2	15.1
2	" "	3.29	2.5	8.1	10.6
			3.0	8.0	11.0
3	" "	3.65	9.4	4 7	14.1
			9.1	5.1	14.2
4	" "	3.35	4.2	8.7	12.9
			4.2	8.2	12.4
5	" shank.	3.42	7.5	14.4	21.9
			6.2	12.0	18.2
6	Pork tenderloin.	3.68	3.0	1.7	4.7
			2.3	1.8	4.2
7	Chicken, composite boneless meat from 2 lb. cockerels.	3.63	19.6	5.2	24.8
8	Chicken, composite boneless meat from 2 lb. pullets.	3.48	17.8	3.7	21.5
			18.0	4.1	22.1
9	Chicken, composite boneless meat from 3 lb. pullets.	3.28	15.1	1.0	16.1
			14.4	0.6	15.0
10	Chicken, composite boneless meat from 4 lb. cockerels.	3.61	20.5	0.6	21.1
			20.8	0.5	21.3
11	Chicken, breast muscle from 3 lb. cockerel.	3.24	2.1	0.8	2.9
			1.1	0.6	1.7
12	Chicken, thigh muscle from 3 lb. cockerel.	3.21	2.4	3.7	6.1
			2.0	6.5	8.5

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TABLE I—*Concluded.*

Sample No.	Description of sample.	Total nitrogen in sample.	Collagen nitrogen in per cent of total.	Elastin nitrogen in per cent of total.	Collagen + elastin nitrogen in per cent of total.
		per cent			
13	Chicken, breast muscle from 3 lb. pullet.	4.06	1.7 3.4	 0.3	 3.7
14	Chicken, thigh muscle from 3 lb. pullet.	3.23	12.2 12.4	1.7 1.7	13.9 14.2
15	Chicken, breast muscle from 4 lb. cockerel.	4.14	6.5 6.8	1.6 1.6	8.1 8.4
16	Chicken, thigh muscle from 4 lb. cockerel.	3.69	11.9 13.5	1.8 2.4	13.8 15.9
17	Pork, white tendon.	5.50	87.7 90.7	4.8 4.1	92.5 94.8
18	Beef, " "	6.98	85.6 86.3	0.4 0.4	86.0 86.7
19	" yellow " ligamentum nuchæ.	6.97	17.9 17.4	80.0 80.0	97.9 97.4
20	Pork, water- and fat-free adipose tissue.	14.74	32.4 34.5	25.2 26.1	57.6 60.6

of elastin would probably not detract greatly from the relative significance of the results obtained.

Results Obtained by the Method.

A number of samples of animal tissue were analyzed according to the method described above. In the tabulation of these results, the duplicate determinations are given as illustrations of the agreement to be expected. All of our results to date with this method are included in Table I, though obviously a few of them in routine work would call for repetition.

The method is evidently not capable of detecting small differences in the connective tissue content of different samples of

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meat. However, it would appear to be sufficiently accurate for many practical purposes. For example, a rib of beef is a tenderer cut than a shank, and the figures in the table indicate a distinctly smaller content of connective tissue, though different cuts of the same description apparently vary greatly in this respect, probably because of differences in condition of the steers from which they are taken. Pork tenderloin also evidently ranks as a very tender cut, according to the analysis of Sample 6. A comparison of Samples 7 to 10, inclusive, indicates that the composite sample of cockerel flesh is somewhat tougher than the composite sample of pullet flesh, while a comparison of Samples 11 to 16 inclusive, shows that the thigh of a chicken carries distinctly tougher meat than the breast. The latter samples do not indicate any clear cut differences between cockerel and pullet flesh.

The results for Samples 17, 18, and 19 permit a rough comparison with the results of other methods of separation. Buerger and Gies (9) report the composition of the organic matter of white tendinous tissue from the ox as follows: 85.1 per cent of collagen, 4.4 per cent of elastin, 3.5 per cent of mucoid, 0.59 per cent of coagulable proteins, 2.8 per cent of ether-soluble matter, and 2.4 per cent of extractives and undetermined constituents (obtained by difference). Vandegrift and Gies (10) give the following as the average composition of the organic matter of the ligamentum nuchæ of the ox: 74.6 per cent of elastin, 17.0 per cent of collagen, 1.2 per cent of mucoid, 1.5 per cent of coagulable protein, 2.6 per cent of ether-soluble matter, and 1.9 per cent of undetermined material.

The results obtained on Sample 20 may not be representative of ordinary connective tissue, since the sample was analyzed in a dry, finely ground condition, permitting the possibility of appreciable loss of collagen or elastin elements through the 40 mesh sieve.

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