THE RELATION OF THE CONNECTIVE TISSUE CONTENT OF MEAT TO ITS PROTEIN VALUE IN NUTRITION.

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The relation of the connective tissue content of meat to its toughness has been established by the investigations of Lehmann (1), which showed that the toughness of raw meat depended largely upon its content of collagen and elastin fibers. In so far as the toughness was due to collagen fibers, it could be practically removed by cooking, resulting in the conversion of collagen to gelatin, although the rate of this conversion is evidently slow at ordinary cooking temperatures (2).

It appeared probable that the proportion of collagen and elastin in meats would also be related to their protein values in nutrition, as indicated, for example, by the biological values of their digestible nitrogen, since it is known that collagen, at least, is an incomplete protein. In so far as this relation exists, it would not be affected by cooking, since the conversion of collagen to gelatin would not presumably affect its nutritive value.

Before the investigation to be reported below was undertaken, we had at hand evidence of a circumstantial character that the more fibrous a cut of meat the lower the biological value of its nitrogen would be. Thus, it was found (3) that a cut of veal, evidently very fibrous when dried, ground, and sieved, gave a biological value of only 62, considerably lower than the values obtained with other meat samples; *i.e.*, 69 for a sample of beef (3) and 74 for a sample of pork (4). Similarly, in unpublished experiments, the biological value of the total nitrogen of a particularly tough and fibrous piece of beef, the lower round (heel) cut from a bull, was found to be only 56, not much higher than the value for white flour; *i.e.*, 52. The most plausible explanation of these variable values is the one suggested by the appearance of the

767

different samples; namely, that an increased connective tissue content will decrease the value of the nitrogen in the nutrition of maintenance and growth.

The weakness of the argument lay in the absence of quantitative data relating to the connective tissue content of the various samples of meat used. With the working out of a method for the determination of the collagen and elastin content of meat (5) sufficiently accurate for the purpose, the opportunity of putting the question to a direct test was, for the first time, at hand. The plan of the experiment here reported was to determine the biological value of the nitrogen of (a) a cut of meat of low connective tissue content, (b) a sample of connective tissue itself, and (c) a definite mixture of the two, such as would be found in the less desirable cuts of meat.

The sample of meat chosen was a pork tenderloin which was ground in a sausage mill, dried at a low temperature, reground, and extracted with ether to remove the fat. The sample of connective tissue was prepared from pork adipose tissue (fat back). Most of the fat was rendered out of this material at a temperature of approximately 100°C. The pork cracklings thus obtained were ground and extracted with ether until practically fat-free.

The pork tenderloin was found to contain collagen equivalent to 2.7 per cent of its total nitrogen and elastin equivalent to 1.8 per cent. The corresponding values for the pork cracklings were 33.5 per cent (collagen) and 25.7 per cent (elastin).

In studying the value of these two animal products, alone and in a definite combination, as sources of protein for maintenance and growth, the biological values of their total digestible nitrogen were determined according to the routine adopted in this laboratory. The products were incorporated into a ration well balanced in all respects except for the absence of protein, to such an extent that the final ration contained approximately 8 per cent of crude protein (N \times 6.25). This required 8.68 per cent of the dried ether-extracted cracklings and 8.89 per cent of the dried etherextracted pork tenderloin. Each ration also contained 4 per cent of the Osborne and Mendel (6) salt mixture, 4 per cent of Cellu Flour¹ as a source of roughage, 1 per cent of NaCl, 10 per cent of

¹ A product obtained from the Chicago Dietetic Supply House, containing 37.8 per cent crude fiber and 0.015 per cent nitrogen.

Nitrogen Metabolism Data and Biological Values.															
Rat No.	Initial weight.	Final weight.	Food intake.	N intake.	Fecal N.	Urinery N.	Biological value.	Rat No.	Initial weight,	Final weight.	Food intake.	N intako.	Fecal N.	Ucinary N,	Biological value.
Period L. Egg ration, containing 0.70 per cent N.							Period I. Egg ration, containing 0.70 per cent N.								
	gm.	gm.	$_{gm.}$	gm.	gm.	gm.	per cent		gm.	gm.	gm.	gnt.	gm.	gm.	per cent
451	50	57	7.2		9	10		461	52	59	7.5		9	12	
452	53	58	6.5		8	10		462	52	59	7.0		8	· 11	1
453	55	61	7.8		9	13		463	50	57	7.1		9	10	
454	53	59	6.9		10	13		464	54	58	6.2		8	11	
4 55	61	68	7.4		8	13		46 5	56	62	6.5		8	10	ĺ
Period II. Pork tenderloin ra- tion, containing 1.35 per cent N.							Period II. Pork crackling ration, containing 1.26 per cent N								
451	67	79	8.0	108	10	36	80	461	54	48	3.7	47	10	51	4
452	70	82		108	9	34	81	462	53	46	3.4	43	9	53	0
453	69	81		108	9	38	80	463	63	45	2.8	35	10	47	0
454	68	81	8.0	108	9	39	78	464	53	47	3.1^{1}	39	9	49	0
455	80	92	8.0	108	9	39	78	465	59	52	2.6	33	9	40	0
Period III. Pork tenderloin- Pei									erio	1 I I I	. Po	ork t	ende	rloin	
crackling ration, containing							crackling ration, containing								
1.34 per cont N.							1.34 per cent N.								
451	81	93	8.0	107	11	43	75	461	59	72	7.9	106	10	46	70
452	85	97	8.0		11	48		462	58	73	8.0		10	41	76
453	84	94	8.0		12	54	68	463	56	69	7.9		11	40	76
454	85	97	8.0	107	12	52	68	464	60	74	8.0	107	10	40	76
455	99	110	8.0	107	13	50	68	465	63	75	6.7	99	11	35	74
Por	ind I	v	Pork	079.0	Lline	r rat	ion	Pori	iod T	V I	orkt	ende		n rat	ion
Period IV. Pork crackling ration, containing 1.26 per cent N.						Period IV. Porktenderloin ration, containing 1.35 per cent N.									
451	89	; 89	8.0	101	23	85	23	4 6 1	82	90	8.0	108	10	46	77
452	96	95	8.0	101	21	84	24	462	82	92	8.0	108	- 9	44	78
453	92	87	8.0	101	23	84	28	463	80	88	8.0	108	10	48	74
454	94	92	8.0	10 1	22	89	20	464	83	96	8.0	108	8	38	82
4 55	112	113	7.8	99	21	80	29	465	87	101	8.0	108	9	39	79
							···				- '			. 1	

TABLE I.

Rat No.	Initial weight.	Final weight.	Food intake.	N intake.	Fecal N.	Urinary N.	Biological value.	Rat No.	Initial weight.	Final weight.	Food intake.	N intake.	Fecal N.	Urinary N,	Biological value.
Period V. Egg ration, containing 0.65 per cent N.						Period V. Egg ration, containing 0.65 per cent N.									
	ym.	gm.	g m.	gm.	gm.	gm.	por cent		g m.	gm.	gm.	gm.	gm.	gm.	per cent
451	95	103	8.0		10	19		46 1	89	93	8.0		11	23	
452	99	109	7.8		11	17		462	91	96	8.0		11	24	
453	91	93	6.7		10	20		463	87	93	8.0		12	24	
454	93	98	6.3		8	16	1	464	97	103	8.0		12	20	
4 55	116	117	5.3		7	15		465	104	112	8.0		12	18	

TABLE I-Concluded.

butter fat, 10 per cent of sucrose, and enough starch to complete the ration. In one of the experimental periods these two rations were combined in the ratio of 3 parts of the tenderloin ration to 1 part of the cracklings ration. Each rat received per day, besides its allotment of ration (8.0 gm.), 25 mg.² of yeast vitamin (Harris), containing from 2 to 3 mg. of nitrogen, and 1 drop of cod liver oil.

In the standardizing periods (Nos. I and V) the rats received a ration similar to the above except that it contained a small amount of dried ether-extracted whole egg, the nitrogen of which is practically completely utilized in digestion and metabolism and hence would not be found to an appreciable extent in either the feces or the urine at this low level of feeding.

Ten albino rats, averaging 50 to 60 gm. in weight, were handled in two groups of five each by two persons. The order in which the three test rations were fed was reversed for the second as compared with the first group.

The results of the nitrogen balance studies (Table I) are used in the calculation of the biological value by a method fully explained elsewhere (7). The biological value obtained is taken as a measure of the percentage of the absorbed nitrogen that is used in the body for maintenance and growth under conditions in which maximal growth is restricted by the percentage of protein in the

² Except in Period II for Rats 461 to 465. In this period the vitamin allowance was increased to 50 mg. daily in the vain attempt to induce the rats to consume an adequate amount of food.

diet. The collection periods were of 7 days duration and were preceded by preliminary feeding periods of 4 days. A summary of the biological values is given in Table II.

The digestible nitrogen of pork tenderloin, containing an extremely low proportion of connective tissue, was found to possess a relatively high average biological value, *i.e.* 79, not much lower than that consistently found for milk under similar conditions, *i.e.* 84. The average biological value for the digestible nitrogen of pork cracklings was 25 for the first group of rats. The determination for the second group of rats cannot be considered satis-

Rat No.	Sex.	Pork tenderloin.	Fork cracklings.	Mixture	
451	F.	80	23	75	
452	М.	81	24	69	
453	66	80	28	68	
454	F.	78	20	68	
455	М.	78	29	68	
461	F.	77	4	70	
462	**	78	0	76	
463	¢2	74	0	76	
464	М.	82	0	76	
465	"	79	0	74	
verage.			25*	72	

TABLE II. Summary of Biological Values.

* Excluding the unsatisfactory determinations for Rats 461 to 465.

factory, because of the inadequate intake of food, ranging from 2.6 to 3.7 gm. per day. It is probable that the protein of pork cracklings is incomplete, since even with daily food intakes of 8 gm., the attainment of nitrogen equilibrium was impossible. This conclusion is in agreement with the results of the feeding experiments of Hoagland and Snider (8) on this material.

The biological values of the mixed ration, containing 3 parts of tenderloin nitrogen to 1 part of cracklings nitrogen, averaged 72. For Rats 451 to 455, in which the consumption of food was satisfactory in all periods, the mixed ration gave an average biological value of 70, while for the other group of rats, in which the con-

772 Factors Affecting Protein Value of Meat

sumption of food in the preceding (pork cracklings) period was inadequate, the average biological value was 74. Possibly the values for the mixed ration for this group of rats were raised somewhat above the correct value by the condition of inadequate nutrition prevailing throughout the preceding period of 11 days. For nine of the ten rats, the mixed ration gave a lower value than the tenderloin ration, so that the conclusion appears to be justified that the addition of connective tissue to the muscle tissue of the tenderloin cut had depressed the biological value.

There is good reason to suppose, however, that some supplementary relation exists between the nitrogenous compounds of muscle and connective tissue. Confining attention to the complete data for Rats 451 to 455, if no supplementary relation existed between the nitrogenous compounds of the two animal foods, the biological value of the mixture should have been

$$\frac{(79 \times 3) + 25}{4} = 65$$

Since the mixture actually showed a biological value of 70, some supplementary relation, by which the amino acid deficiencies of each product were corrected to some extent by the excess amino acid proportions of the other, was apparently at work.

The proportion of collagen + elastin nitrogen in the mixture of pork tenderloin and pork cracklings was 18.2 per cent of the total nitrogen. This is not as high as the percentages found in several cuts of yeal analyzed in this laboratory. The fore shank of yeal may run as high as 25 per cent of collagen and elastin nitrogen. so that for the mature animal a still higher per cent may be found. Furthermore, in both beef and pork the proportion of collagen to elastin nitrogen is always much greater than the proportion found in the pork cracklings used in this experiment (5). Therefore, if gelatin is lower in biological value than elastin, as appears probable, it would follow that a cut of meat containing 18.2 per cent of its total nitrogen in the form of collagen and elastin would possess a lower biological value than the mixture of tenderloin and cracklings used in this experiment. Hence, it appears possible to account for the variations noted above in the biological values obtained for different cuts of meat on the basis of a variable proportion of connective tissue.

It has been our experience that different cuts of pork possess rather constant biological values, averaging close to 74, in contrast to the variable values obtained for veal and bccf. An explanation for this constancy is to be found in the comparatively constant amounts of connective tissue found in pork cuts. In Table III, the analyses for two hog carcasses are summarized, illustrating this fact.

Contrast with these series of values the variations in veal cuts ranging from 8 to 9 per cent of collagen N in the rib, round, sirloin, and tenderloin to 24 per cent in the fore shank, and from 0.5 per cent of elastin N in the tenderloin and rib to 4 or 5 per cent in the navel and the round.

TABLE III.

Connective Tissue Content of Pork Cuts.

Results expressed as percentages of collagen or elastin N on the total N of the lean of the cut.

	Нı	m,	Loin.		Pienie.		Boston.		Belly.	
	Collagen.	Elætin.	Collagen.	Elastin.	Collagen.	Elastin.	Collagen.	Elastin.	Collagen.	Elastin.
Sow Barrow	7.8 8.1	$1.0 \\ 3.2$			$\begin{array}{c} 11.3\\12.5\end{array}$	1.0 1.9	8.0 10.8			0.6 5.0

SUMMARY.

The biological value of the nitrogen of pork tenderloin, containing a minimal amount of connective tissue, was found to be 79. That of pork cracklings, consisting largely of connective tissue, was found to be 25.

When the two materials were mixed in the proportion of 3 parts of tenderloin nitrogen to 1 part of cracklings nitrogen, a distinct depression of the biological value of the tenderloin nitrogen was observed, the mixture possessing a value of 72.

Distinct indications of a supplementary relation between the nitrogenous compounds of muscle tissue and of connective tissue were noted.

Reasons are given for believing that cuts of beef varying widely in their content of connective tissue would also vary widely in the

774 Factors Affecting Protein Value of Meat

biological values of their nitrogen, so that the less desirable cuts, containing large amounts of connective tissue, would be distinctly less valuable as sources of protein for maintenance and growth. This is not so true of pork, since different cuts of pork do not seem to vary greatly in their content of connective tissue.

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