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**The Relationship between the Content
of the Connective Tissue
and the Biological Value of Proteins
in Meat**

by

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Graph 1.

Relation between the available lysine and the relation
tryptophan/hydroxyproline in beef.¹⁾

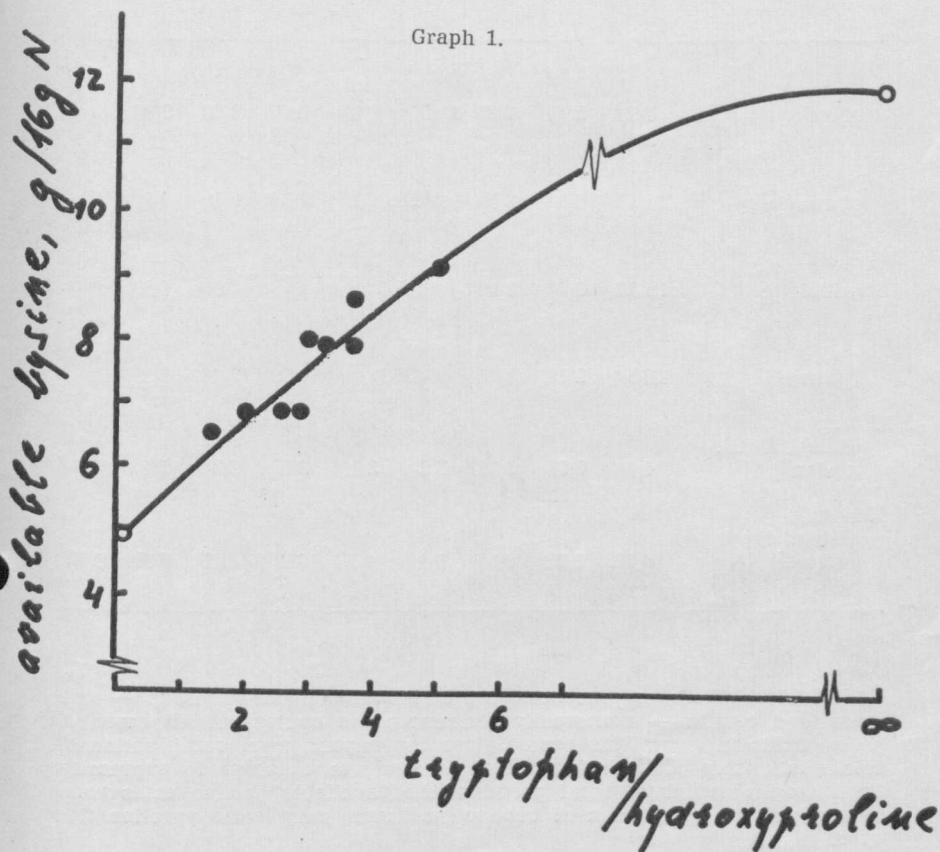
[Point on the vertical axis represents value for acid soluble collagen,
the other extreme point represents value for myosin.]

The Relationship between the Content of the Connective Tissue and the Biological Value of Proteins in Meat

INTRODUCTION

During the study of the influence of technological processes on the decrease of the biological value of meat proteins, especially on the formation of enzyme-resistant complexes of the type of amino acid-sugar the authors have found that reducing of the biological value is chiefly due to the formation of a lysine-complex. On this occasion the "available" lysine in different sorts of beef was determined.¹⁾ The amount of this amino acid in meat changes evidently according to the content of connective tissue. The dependence of lysine on connective tissue can be expressed graphically in relation to tryptophan/hydroxyproline (Graph 1).

Graph 1.



On the basis of this result the authors concluded that other essential amino acids in proteins of meat will exhibit similar relation to the connective tissue. This assumption follows from the composition of amino acids in some principal proteins present in meat, as indicated in Table I.

Table I.

Composition of amino acids in some principal meat proteins
(g of amino acid per 100 g of protein)

	Lys	Try	Met	Leu	Ile	Val	Thr	Phe	Hypro
Myogen A of rabbit ²⁾	9,54	2,31	1,17	11,5	7,87	7,40	6,50	3,06	0
Myosin of rabbit ³⁾	11,92	0,8	3,4	15,6		2,60	5,1	4,3	0
Tropomyosin of rabbit ³⁾	15,70	0	2,80	15,6		3,13	2,90	4,60	0
Myoglobin of horse ⁴⁾	15,5	2,34	1,71	16,8		4,09	3,46	5,9	0
Collagen of beef Achil. tendon ^{5), 6)}	4,9	0,01	0,84	3,5	2,1	2,8	2,3	2,4	13,4
Elastin of beef lig. nuchae ^{5), 6)}	0,40	0,01	0,03	8,4	4,3	18,4	1,15	5,7	1,84

It follows that the presence of proteins of connective tissue — collagen and elastin — necessarily influences the content of all essential amino acids in meat. This report gives the experimental confirmation of this relationship. Considering that this study is to serve to expression the biological value of meat proteins, the "available" amino acids were determined. As a criterion of connective tissue, only hydroxyproline was used.

EXPERIMENTAL

Representative samples of the following parts of beef from the slaughter-house were used for the experiments: fillet, round, loin, shoulder, ribs, neck, flank and shank (hind). Four pieces of each part of meat were well ground and dried with ethyl alcohol and ether. Equal weight parts of these pieces were then mixed and a uniform sample was used for analysis. The beef samples were chosen from animals irrespective of sex, age, etc.

Hydroxyproline was determined after total hydrolysis with 2 N HCl at 120° C in an autoclave for 5 hours according to the modified method of Neumann and Logan.⁷⁾ For the available lysine, Carpenter's method with fluorodinitrobenzene and methoxycarbonylchloride was used.⁸⁾

For the determination of the available essential amino acids a special method was developed,⁹⁾ consisting in enzymatic hydrolysis of proteins with pancreatine or papain and subsequent hydrolysis of peptides with leucine aminopeptidase and prolidase. In this manner, all proteins of meat (excl. elastin) decomposed in free amino acids. These amino acids can be taken as available as far as they are not blocked in complexes with carbonyl and/or other complexoforming compounds. Free available amino acids are determined microbiologically with *Lactobacillus arabinosus* 17/5 - ATCC 8014 (phenylalanine), *Str. faecalis* R - ATCC 9790 (threonine) and *Str. zymogenes* NCDO 592 (tryptophan, methionine, leucine, isoleucine and valine.⁹⁾

All values for amino acids are expressed in grams of the particular amino acid per 16 g of nitrogen, which was determined in the sample by conventional method.

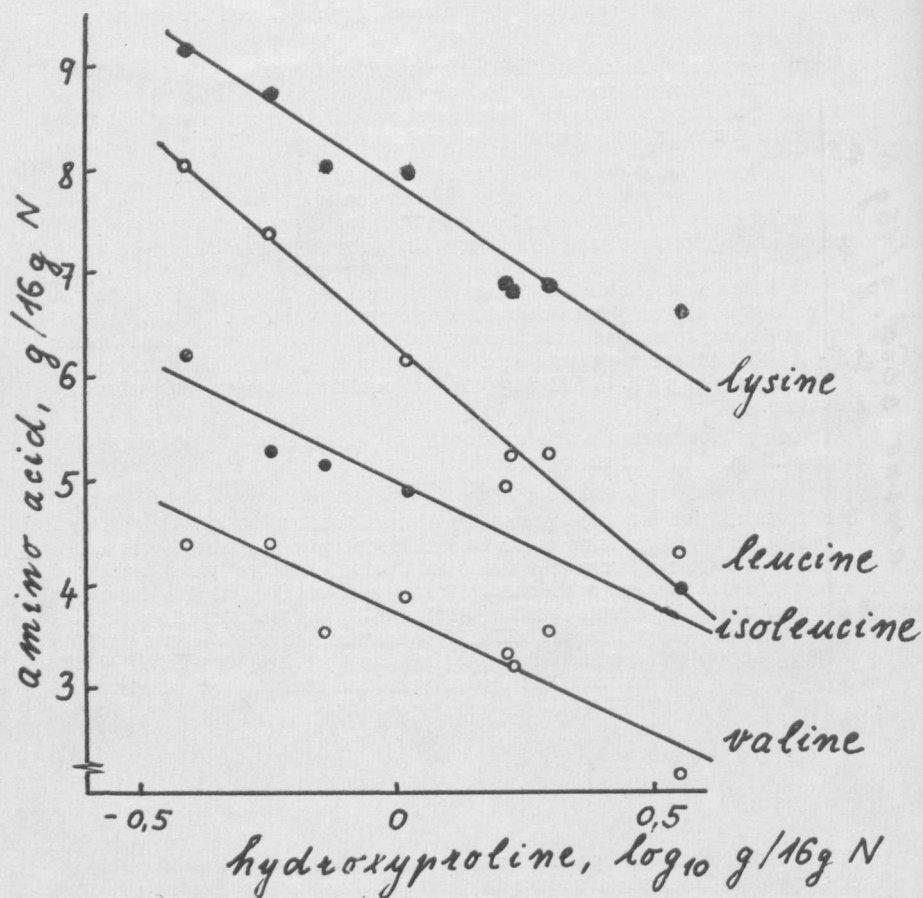
For fractionation of the connective tissue (determination of collagen and elastin) m. biceps femoris of five different pieces of beef was used. The tendrillar parts from the ends of these muscles were analysed separately. Fractionation was carried out by extraction of the tissue in a solution of NaCl, citrate buffer, 0.1 N NaOH, by transforming the insoluble collagen to gelatine at 120° C in an autoclave for 5 hours.¹⁰⁾ This collagen fraction and the insoluble elastin were both hydrolysed in 6 N HCl at 105° C for 24 hours and the hydroxyproline content was determined in them.⁷⁾

RESULTS

The dependence of particular available essential amino acids on hydroxyproline in beef is shown in graph 2. To express this relation as linear it was necessary to plot hydroxyproline in logarithmus of concentration. In this linear function, the greatest decrease is exhibited by valine, leucine and tryptophan, the lowest by threonine and methionine. The results are not significant with regard to the low number of analyses made. But it can be said that the decrease of every amino acid takes place regularly. With the increase of hydroxyproline ten times (approx. from 0.4 to 3.9 g of hydroxyproline/16 g N), the amount of every available essential amino acid decreases in logarithmic proportion.

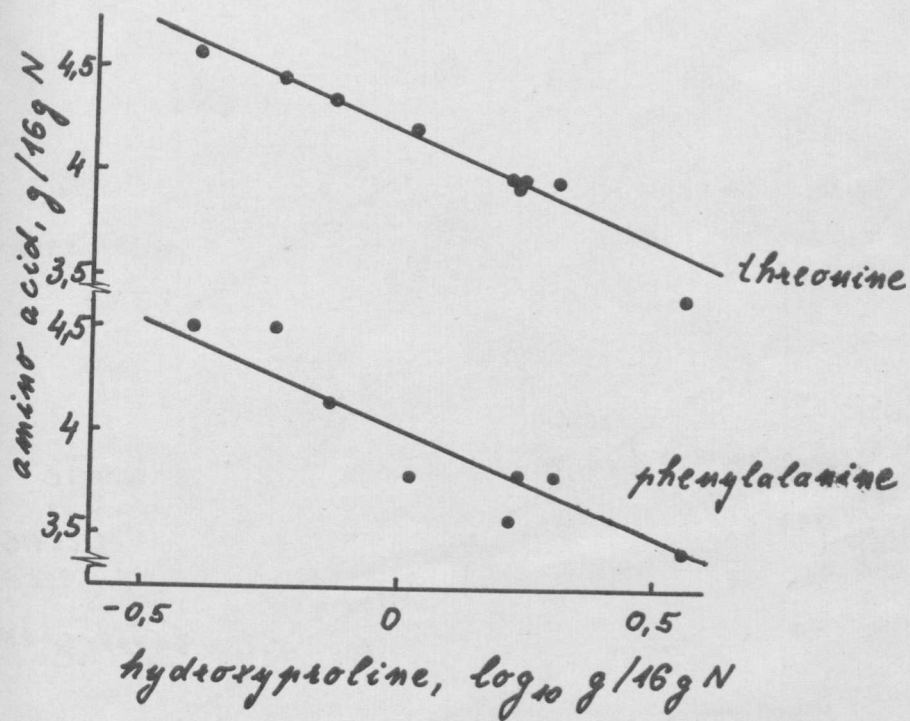
Graph 2a.

Relation between the available amino acids and hydroxyproline in beef.



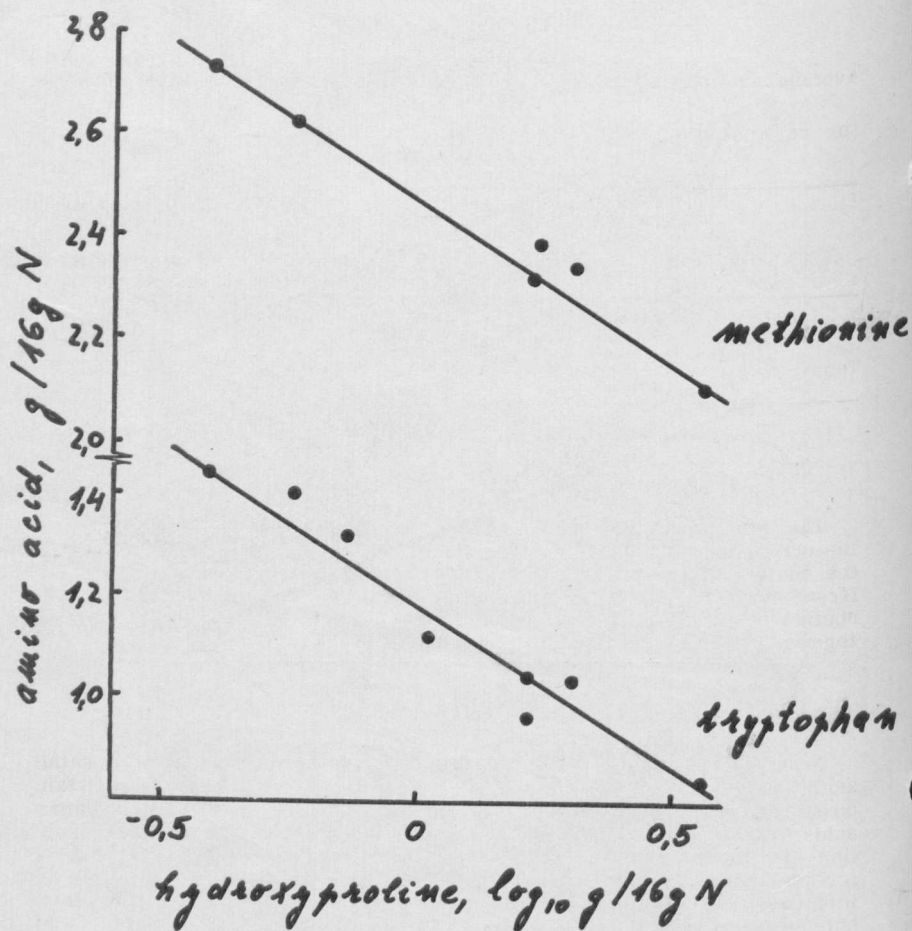
Graph 2b.

Relation between the available amino acids and hydroxyproline in beef.



Graph 2c.

Relation between the available amino acids and hydroxyproline in beef.



The content of hydroxyproline in meat is given by the amount of collagen and elastin. Considering the unequal content of hydroxyproline in these proteins the relationship between amino acids and hydroxyproline holds good only if a certain relationship between collagen and elastin is preserved. The average amount of insoluble collagen and elastin in m biceps femoris is presented in Table II.

Table II.

Average amount of insoluble collagen and elastin in m. biceps femoris
(g/16 g N)
(by determination of hydroxyproline after hydrolysis of these proteins
isolated through fractionation)

	Hydroxyproline		Collagen*)	Elastin**)
	from collagen	from elastin		
Meaty part	0,490	0,027	3,65	1,40
Tendrillar part	10,98	0,055	81,80	2,89

*) hydroxyproline multiplied by 7,46

**) hydroxyproline multiplied by 52,3

The relationship between collagen and elastin in the meaty part of the muscle is nearly 2.6:1 and changes to 57:2 in tendrillar part. In no part the content of elastin is greater than that of collagen. Since in the different sorts of beef there was determined an increasing content of connective tissue, we can say that hydroxyproline is a satisfactory criterion for the content of available essential amino acids in beef.

DISCUSSION

From the validity of the relationship between the available essential amino acids and hydroxyproline in meat it follows that under certain circumstances it is possible to establish the content of all studied amino acids in meat from the known amount of hydroxyproline, on the assumption that the relationship cited is constant. This can be said to hold good for beef. But more analyses are necessary for a definitive decision, taking into consideration the different age, sex and other factors. The relationship between the essential amino acids has not been studied in veal and pork. It is probable that, e.g. in veal, other relations will hold good, because of the higher content of the stroma proteins in veal.¹¹⁾

The relationship between the available essential amino acids and hydroxyproline may be used for the quick establishment of the biological value of the meat proteins. From the known essential amino acids we

can determine the chemical score of Mitchell and Block¹²⁾ or index of essential amino acid of Oser.¹³⁾ As the connective tissue is, to some degree, also an indicator of the meat quality, the established biological value of meat proteins can serve the same purpose. All the relations between hydroxyproline and available essential amino acids will be further studied from this aspect.

In relation to the cited facts, hydroxyproline in meat may be considered a better indicator for the quality of the raw material used in meat products than the determinations of proteins. The amount of proteins in the meat product is not satisfactory from the nutritional aspect, because it does not always indicate the constant content of the fully valuable proteins. The authors consider the possibility of using hydroxyproline for this purpose.

SUMMARY

The relationship between the content of particular available amino acids and hydroxyproline in raw beef was studied. This relationship is linear, if hydroxyproline is plotted as decadic logarithmus of its amount. Since these relationships were established for all essential amino acids, the use of hydroxyproline as a criterion for the calculation of the biological value of meat proteins and/or for the determination of the quality of meat is considered.

ZUSAMMENFASSUNG

Der Zusammenhang zwischen dem Gehalt der einzelnen ausnutzbaren essentiellen Aminosäuren und des Hydroxyprolins im rohen Rindfleisch wurde untersucht. Dieser Zusammenhang ist linear, wenn der Hydroxyprolinegehalt als dekadischer Logarithmus ausgewertet wird. Da diese Zusammenhänge für alle essentiellen Aminosäuren gefunden wurden, diskutieren die Autoren über die Möglichkeit der Ausnutzung des Hydroxyprolins als Kriterium für die Berechnung der biologischen Wertigkeit der Fleischproteine evtl. für die Auswertung der Qualität des Fleisches.

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