THE AMINO ACID COMPOSITION OF ANIMAL TISSUE PROTEIN*

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(Received for publication, March 12, 1943)

Recent knowledge demonstrates that the biological value of a food protein is dependent upon its amino acid composition (1-4). It is important from the practical standpoint, therefore, not only to understand the quantitative and qualitative requirements of man for essential amino acids (5-7) but also the quantitative composition of food proteins which are commonly utilized to fulfill these body needs. Although the more theoretical and academic aspects of proteins may be investigated by analyses of individual crystalline materials, the application of well established methods of amino acid analysis to the protein mixtures in the body tissues and in common foods can yield significant information provided these proteins are obtained free from carbohydrate, fat, and other contaminants and if no loss of amino acids occurs during the purification.

That analytical methods for the amino acids are not frequently applied to food proteins may be explained, in part at least, by the fact that it is difficult to free the proteins from accompanying carbohydrate and other contaminants; also, there are inherent errors in the methods for determining some of the amino acids, even when applied to crystalline proteins. Comprehensive studies of the amino acid composition of food and tissue proteins are relatively few (8-13) and since newer refinements of analytical methods have proved capable of yielding more nearly exact values it becomes increasingly difficult to compile satisfactory data from the biochemical literature relative to the amino acid distribution in foods.

The present study has been undertaken to determine the quantitative occurrence of ten amino acids in six soft organs of beef and in the muscle tissues of a variety of cold and warm-blooded animals. Data of this type may demonstrate similarities or differences in composition bearing upon such problems as: (1) the relative value of different meats, meat products, poultry, and fish, in supplying amino acids, (2) the amino acid pattern to which the growing animal must conform in the synthesis of tissue proteins, (3) the possible repetition of amino acid pattern in the muscle tissues of a

*Partial reports of the data presented in this paper have been given at the Forty-seventh annual meeting of the Michigan Academy of Science, Arts and Letters at Ann Arbor, March 14, 1942, and at the annual meeting of the American Society of Biological Chemists at Boston, April 3, 1942.
wide variety of species and classes of animals or the existence of specific species differences.

Meats, poultry, and fish furnish 20 to 30 per cent of the animal proteins in the average American dietary and contain protein which has long been known to possess superior biological value (1). Studies such as the one presented herein seem fundamental in establishing the amino acid pattern upon which protein nutritional superiority depends; therefore, data on the amino acid composition of food proteins have special significance. The analytical values for arginine, histidine, lysine, phenylalanine, tyrosine, tryptophane, serine, threonine, cystine, and methionine in meat, poultry, and fish muscle, and in beef organs are reported in the present paper.

Preparation of Protein Samples

The beef organs (brain, lung, liver, stomach, kidney, and heart) and the muscle tissues (beef shank, lamb leg, pork chops, veal steak, frog legs, and roasting chicken) were all obtained from a local market. Quick frozen salmon and codfish steaks, and shrimps were used. The reptilian flesh analyzed was that of wood and painted turtles.

In so far as possible, muscle fascia, connective and adipose tissue, and unedible portions were removed and discarded. The dissected tissues were ground in a food chopper, frozen in dry ice, and desiccated under a vacuum while frozen (cryochem apparatus). Early in the course of the work raw meats were used, but later the tissues were cooked prior to desiccation, in order to insure the highest possible recovery of proteins through the inactivation of the tissue enzymes (14). Cooking the meats for a short period was a routine procedure in the preparation of the majority of the samples. The dried tissue was ground to a fine powder in the ball mill or with a mortar.

Extraction of lipid1 was effected by letting the powdered tissue stand overnight at room temperature with an alcohol-ether mixture (3:1), approximately 15 ml. of the mixture being used per gm. of dried tissue, followed by a 20 hour continuous extraction with ether in a Soxhlet apparatus. After the material was dried, the fat-free powder was extracted with three successive portions of boiling water for 15 minutes to remove minerals, carbohydrates, and other extractives. In each extraction, 1500 ml. of water were used per 100 gm. of the dry tissue preparation. The extracted material was dried in a vacuum oven at 60°.

Except with shrimp, only 8 to 14 per cent of the total nitrogen was lost during water extraction at 100°. This loss may be considered as non-protein nitrogen. Howe (14) found that 10 to 15 per cent of the total

1 The lipid distribution in these tissues will be reported in another paper. The amino acid constituency of protein from growing tissues is being determined.
nitrogen of raw muscle was composed of nitrogen extractives. Roughly, 7 to 29 per cent of the wet weight of the fresh tissue was recovered and the purity of the protein preparations used for the amino acid analyses is evidenced by their nitrogen contents of 14 to 17 per cent, on a moisture- and ash-free basis.

**Analytical Methods**

Moisture was determined by placing samples of the desiccated, fat-free, water-extracted tissues under a vacuum in a metal desiccator at 60° until they attained constant weight, after which the tissue preparations were used to determine total ash. Nitrogen determinations were carried out by the macro-Kjeldahl method. Total sulfur was estimated gravimetrically after combustion of the samples in the Parr oxygen bomb. Inorganic sulfur was determined gravimetrically on hydrolysates of samples which had been hydrolyzed 6 hours in 20 per cent HCl.

The method of Lugg (15), modified for use with the spectrophotometer, was used to determine tyrosine\(^2\) and tryptophane.\(^3\) Phenylalanine was determined by Block's (11) modification of the Kapeller-Adler (16) method. Block's method (17), with the correction factors suggested by Tristram (18) and Gulewitsch (19), served in the analyses for histidine, arginine, and lysine.\(^4\) Cystine was determined by both the cuprous oxide method of Graff, Maculla, and Graff (20) and by Sullivan's colorimetric procedure (21), adapted to the spectrophotometer.\(^5\) Methionine was determined by a modification of the Beach and Teague\(^6\) (22) method and by the McCarthy and Sullivan (23) colorimetric method, adapted to the Cenco photometer with Cenco Filter 87309-B, maximum at 525 m\(\mu\). The methods proposed by Nicolet and Shinn (24, 25) were used to determine serine and threonine. The Sørensen-Haugaard method (26) for carbohydrate showed no appreciable carbohydrate in the amino acid solutions obtained by hydrochloric acid hydrolysis of the purified proteins.

\(^2\) Wave-length 495 m\(\mu\) was used in reading the tyrosine color.
\(^3\) Wave-length 430 m\(\mu\) was used in reading the tryptophane.
\(^4\) Silver nitrate was used as the silver salt in the separation of the bases.
\(^5\) A glycine blank was used and the colors read at wave-length 550 m\(\mu\).
\(^6\) Accuracy and reproducibility of the method may be insured by observing the following precautions. (1) Only I\(\text{I}\) which has been recently redistilled should be used. Redistillation is essential even when I\(\text{I}\) of reagent grade, which appears to contain little free iodine, is available. (2) For the determination of cystine, alone, preliminary reduction with zinc is unnecessary and may result in too high cystine values. The reducing power of the Cu\(\text{II}\)O is sufficient to reduce cystine to cysteine. (3) Hydrolysis of the samples for 6 hours is sufficient; longer hydrolysis may result in low methionine estimates.
AMINO ACIDS OF TISSUE PROTEIN

Results

The amino acid composition of prepared animal tissue proteins, corrected for moisture and ash, and calculated to 16 per cent nitrogen, is presented in

TABLE I
Per Cent Amino Acid Composition of Proteins from Prepared Muscle Tissue and Organs
Corrected for moisture and ash and calculated to 16 per cent nitrogen.

<table>
<thead>
<tr>
<th></th>
<th>Arginine*</th>
<th>Histidine</th>
<th>Lysine$</th>
<th>Phenylalanine</th>
<th>Tyrosine</th>
<th>Tryptophane</th>
<th>Serine</th>
<th>Threonine</th>
<th>Cystine</th>
<th>Sulfurian</th>
<th>Reach-Teague</th>
<th>Sulfur</th>
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<th>Organic S</th>
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<td>5.43</td>
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<td>2.75</td>
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</table>

*Arginine values are corrected for the loss through solubility of arginine silver by the factor 3.6 per 100 ml. proposed by Gulewitsch (19). For instance, in the adaptation of the Block method, a volume of 325 ml. used (which includes mother liquor and washings) gives a solubility loss of 11.7 mg. of arginine.

†Not corrected for solubility.

‡The lysine values are corrected for the constant loss of 8.38 mg. of lysine, mostly through the solubility of lysine phosphotungstate. This factor was determined by Tristram (18). It is recognized that small losses of histidine also are incurred in the procedure.

§The serine values have been corrected for the solubility of the dimedon derivative as suggested in Nicolet's method. Also the serine values are subject to the error due to this method by which not only serine but hydroxylysine in addition is determined.

||These tissues were cooked in being prepared.

Table I. Such an adjustment of the values to the same percentage of nitrogen, while purely arbitrary, possesses advantages when comparisons are to be made from one tissue to another.
Lysine, a dietary essential, occurs in muscle tissues of all types in higher concentration than do any of the other amino acids studied, 7.7 to 9.6 per cent. Arginine also is present in high concentrations, 6.3 to 7.6 per cent. Histidine, another essential amino acid, occurs in low concentration, 1.8 to 2.4 per cent. Methionine, 2.9 to 3.4 per cent by gravimetric determination and 3.1 to 4.1 per cent by colorimetric analysis, occurs at a higher concentration than does the other sulfur-containing amino acid, cystine, which was found to represent 0.6 to 1.0 per cent by colorimetric analysis and 1.1 to 1.4 per cent by the gravimetric procedure. The two hydroxy-amino acids, threonine and serine, were found at a level of 4.0 to 5.3 per cent; however, some serine values were higher, that for veal being 6.1 per cent and for frog legs, turtle, and lamb 6.3 per cent. Tyrosine and phenylalanine, aromatic amino acids, have a similar concentration, varying from 4.2 to 4.9 and 3.8 to 4.9 per cent, respectively. Tryptophane, an indispensable amino acid, occurs at a level of 1.2 to 1.4 per cent. Only one other amino acid, cystine, was found in smaller concentrations than tryptophane. Although these two amino acids are found in small concentrations in the muscle protein, comparison of the cystine and tryptophane intakes per kilo of body weight by 10 to 12 year-old boys and by breast-fed infants shows that the rapidly growing infants received in their diets greater quantities of cystine and tryptophane per unit of body weight than were provided by the diet considered adequate for the larger but slower growing children.

In general, it can be seen that muscle tissues of these different classes of animals do not differ widely in their amino acid patterns, which implies that the same amino acid composition of muscle proteins is repeated throughout the animal kingdom and indicates that, as far as these ten amino acids are concerned, the protein of one muscle is as good as that of another in supplying amino acids in the diet.

The organs, while showing some similarity to the muscle tissue in composition, differ from it in certain respects. Outstanding are their lower lysine contents, 5.8 to 7.1 per cent, which are smaller than that found in any muscle studied. In the organs, the percentages of lysine are exceeded by the percentage of arginine, 6.3 to 7.4 per cent (Table I). Brain appears to contain more histidine (2.5 per cent) than any other organ or muscle, while stomach (1.7 per cent) contains the least. With the exception of heart, the organs, particularly lung and stomach, seem to have a slightly lower methionine content than do muscles; however, the organs seem to be as good, if not a better, source of cystine. The beef organs were found to be high in serine, 5.9 to 7.3 per cent; only four of the muscles had serine contents within this range. Threonine appears in the organs in about

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7 A complete discussion of the sulfur distribution of the tissues will be included in a forthcoming publication.
the same amounts as in the muscles. Heart, liver, kidney, and brain contain more phenylalanine than do any of the muscles, 5.1 to 6.1 as contrasted with 3.8 to 4.9 per cent for muscles. The tyrosine content of the organs, 3.7 to 5.1 per cent, is more variable than that found in muscles. Brain, liver, and kidney exhibit higher tryptophane and cystine values than do muscle tissue or lung, heart, and stomach. The tryptophane values range from 1.8 per cent for kidney and liver to 0.95 per cent for stomach.

### Table II

Per Cent Contribution of Amino Acids to Total Nitrogen of Protein from Muscle Tissue and Organs

<table>
<thead>
<tr>
<th></th>
<th>Arginine*</th>
<th>Histidine</th>
<th>Lysine*</th>
<th>Phenylalanine</th>
<th>Tyrosine</th>
<th>Threonine</th>
<th>Cystine</th>
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<td>0.81</td>
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*Correction for solubility applied.
†No solubility factor applied.

According to the figures in Table I, liver, kidney, and brain are very much alike in amino acid composition but differ from stomach and lung in cystine, tryptophane, tyrosine, and phenylalanine. With reference to such significant differences in amino acid composition the functional characteristics of kidney, liver, and brain tissue should be recalled, together with the fact that they contain large amounts of nuclear material, in contrast to muscle which represents large quantities of highly specialized cytoplasm. Similarly, the functional activities of the stomach and lungs may be related to the protein mixture. Heart muscle is similar to skeletal muscle in function and amino acid composition, although it does appear
to be lower in lysine. Thus, the differences in the values for the beef organs might be accounted for by the presence in these organs of different proportions of various types of tissue (muscle, parenchymal, and connective). Each tissue may have a different amino acid composition, determined by the specific function of the organ.

Table II shows the per cent of the total nitrogen of the tissues represented by the ten amino acids. From 40 to 45 per cent of the total nitrogen is thus accounted for by ten amino acids. 30 to 39 per cent of the total nitrogen is contributed by the seven amino acids determined which are essential to animal growth and nutrition.

Fig. 1 is a graphic representation of the molecular proportionality of the ten amino acids in mammalian muscles and may be taken as representative of the amino acid pattern in muscle meats in general. The use of molecular proportionality as a basis for comparison, as contrasted with percentage composition data, has the advantage of taking into account the different molecular weights of the amino acids. It will be seen that for every 10 moles of lysine furnished by mammalian muscle tissue about 9 moles of serine, 7 of arginine and threonine, 4 of phenylalanine and tyrosine, 3.5 of methionine, 2.5 of histidine, and about 1 of tryptophane and cystine are present.

**SUMMARY**

The amino acid composition of the protein mixtures of ten edible muscle meats (beef, veal, lamb, pork, chicken, turtle, codfish, salmon, frog legs, and shrimp) and of six beef organ tissues (liver, kidney, brain, heart, stomach, and lung) is presented.
The determinations of amino acid distribution included arginine, histidine, lysine, tyrosine, tryptophane, phenylalanine, serine, threonine, cystine, and methionine, seven of which are nutritionally essential for optimal animal growth, either through a limited ability or a total inability of the body to synthesize them.

The protein mixture which makes up voluntary muscle tissues is similar in Mammalia, Aves, Amphibia, Pisces, and Crustacea, with respect to ten of the constituent amino acids. Since muscle tissues of these various classes of animals do not differ widely in their amino acid patterns, the findings support the belief that the same or closely similar amino acid composition of muscle proteins is repeated throughout the animal kingdom.

Larger differences in amino acid composition were found among the beef organs than among the muscle proteins of different species, as would be expected from their higher concentration of nuclear material and different functional activities.

In addition to an evaluation of the relative dietary values of the animal proteins in terms of ten specific amino acids, the data demonstrate the amino acid pattern to which animal muscle must conform in the synthesis of tissue protein.

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ANIMAL TISSUE PROTEIN
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