THE FRACTIONATION OF THE AMINO ACIDS OF LIVETIN

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(Received for publication, August 1, 1933)

Most of the schemes existing for the analysis of proteins necessitate the use of large amounts of material. The need is great for a scheme which can be used with smaller amounts. It was felt that a combination of certain of the more generally accepted methods for amino acid determination could be arranged and an economy of original material be thus effected. The following scheme was eventually decided upon as a result of trial experiments.

1. The protein is hydrolyzed and humin, ammonia, and the basic amino acids are removed and estimated by the methods of Vickery and his coworkers (1).

2. The various mother liquors so obtained are freed from interfering substances and combined. Tyrosine is removed by crystallization. The filtrate is treated with alcohol and baryta for the precipitation of the dicarboxylic acids. Aspartic and glutamic acids are removed and estimated according to the directions of Jones and Moeller (2). Hydroxyglutamic acid is removed at this stage by precipitation of its silver salt.

3. The amino acids in the filtrate and mother liquors from this procedure are converted into their copper salts and separated into three groups according to the method of Brazier (3). As eight amino acids have been removed, the scheme of Brazier becomes simplified as follows: (a) The copper salts insoluble in water include the salts of leucine and phenylalanine. The colorimetric method of Kapeller-Adler (4) for the estimation of phenylalanine facilitates the examination of this fraction. (b) The copper salts insoluble in methyl alcohol include the salts of glycine and alanine,

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with small amounts of tyrosine. Glycine is removed as its barium carbamate, which is insoluble in water at 0°. Alanine is estimated in an aliquot by the method of Fürth (5). (c) The copper salts soluble in methyl alcohol include the salts of proline, valine, and isoleucine. They are dealt with according to the recommendations of Brazier.

The advantage claimed for this scheme over the method of Brazier is that it is less involved and that the preliminary removal of the basic and dicarboxylic amino acids enables the remaining amino acids to be separated into groups more conveniently. This possibility was mentioned by Damodaran (6). The use of sulfuric acid for hydrolysis as recommended by Brazier is attended by large losses in its removal; it was therefore abandoned and 20 per cent hydrochloric acid was used.

The experiments reported were part of a general investigation into the proteins of egg yolk. The protein used was livetin.

**EXPERIMENTAL**

Livetin was prepared as described in a previous article (7). About 50 gm. of the protein were hydrolyzed with 400 cc. of 20 per cent hydrochloric acid. Boiling was discontinued after 22 hours, as it was found that the non-amino nitrogen was beginning to increase. The acid-insoluble melanin was removed by centrifuging, and nitrogen determined on weighed aliquots of the hydrolysate. For analysis, a portion of the hydrolysate was taken which represented 5603 mg. of protein nitrogen, or about 36 gm. of the dry protein.

**Basic Amino Acids**—The basic amino acids were removed according to the method of Vickery and Leavenworth (8). The solution was kept suitably diluted as recommended by Vickery and Shore (9). The purified histidine fraction was freed from cystine as recommended by Vickery and Leavenworth (10).

The purified histidine fraction, freed from cystine, contained nitrogen equivalent to 3.39 per cent of the total nitrogen. The yield of histidine diflavianate corresponded to a content of 1.62 per cent of total nitrogen in the form of histidine.

The purified arginine fraction, containing 13.45 per cent of the total nitrogen, was made up to 200 cc. Duplicate 5 cc. aliquots were taken for determination of arginine by the method of Van
Slyke as modified by Plimmer and Rosedale (11) and Koehler (12). 150 cc. were treated with flavianic acid for the precipitation of arginine. No second crop of arginine flavianate was obtained from the filtrate from the first crop even after 16 hours boiling under a reflux condenser to decompose any arginine diflavianate possibly present. The findings were as follows:

<table>
<thead>
<tr>
<th>Arginine N</th>
<th>Per cent of total N</th>
</tr>
</thead>
<tbody>
<tr>
<td>By Van Slyke procedure</td>
<td>11.0</td>
</tr>
<tr>
<td>Isolated as flavianate</td>
<td>11.6</td>
</tr>
<tr>
<td>By arginase method in previous experiment (7)</td>
<td>11.7</td>
</tr>
</tbody>
</table>

The lysine phosphotungstate was not washed, as washing was found to cause losses. From it 6.15 per cent of the total nitrogen was isolated as lysine in the form of recrystallized lysine picrate. This corresponded to 66 per cent of the total nitrogen in the purified lysine fraction.

A further precipitate of insoluble phosphotungstates, weighing about 0.5 gm., separated from the filtrate from the lysine phosphotungstate. It was examined for lysine, but no insoluble picrate could be precipitated.

**Dicarboxylic Amino Acids**—The residues from the preceding manipulations were treated as follows:

1. The main filtrate from the precipitation of lysine phosphotungstate was freed from phosphotungstic acid by shaking with a mixture of equal parts of butyl alcohol and ether. The combined alcohol-ether phases were evaporated to a syrup, taken up with water, and the phosphotungstic acid precipitated with baryta. The filtrate from this precipitation was added to the aqueous phase containing the main bulk of the amino acids.

2. The mercuric sulfate-soluble material from the purification of the histidine fraction was freed from mercury with H₂S. This material has been shown to contain aspartic acid by Vickery and Leavenworth (8). It may possibly contain hydroxyglutamic acid and isoleucine, as both these amino acids are precipitated by silver (13, 14).

3. The mother liquors from the precipitation of the flavianates of arginine and histidine were acidified with sulfuric acid and the flavianic acid shaken out with butyl alcohol.

4. The alcoholic mother liquor from the precipitation of lysine...
Picrate was evaporated to dryness and taken up with water. It was acidified with sulfuric acid and extracted with ether to remove picric acid.

The solutions obtained in procedures (1) to (4) were combined and cold saturated barium hydroxide solution was added until the reaction to Congo red began to change. The precipitated barium sulfate was removed and the solution concentrated to about 300 cc. The dicarboxylic amino acids were then precipitated twice with baryta and alcohol according to the directions of Jones and Moeller. The combined alcoholic filtrates were preserved for separation of the monoaminomonocarboxylic acids.

The precipitated barium dicarboxylates were decomposed with sulfuric acid and brought to volume for estimation of nitrogen. Sulfuric acid was then removed by quantitative addition of barium chloride solution. The solution was concentrated to about 50 cc., saturated with hydrogen chloride gas, and boiled for 4 hours under a reflux condenser. It was allowed to stand for a week in the refrigerator, and the glutamic acid hydrochloride was then filtered on asbestos and dried at room temperature. 2.932 gm. of glutamic acid hydrochloride were thus obtained, corresponding to 4.13 per cent of the total nitrogen as glutamic acid nitrogen.

The filtrate was concentrated repeatedly to a syrup under reduced pressure and taken up in about 200 cc. of water. Hot saturated silver sulfate solution was then added in slight excess, the silver chloride precipitate was removed, and the excess of silver ion removed with hydrogen sulfide. The solution was concentrated to about 100 cc. at 45° under reduced pressure. Sulfuric acid was removed with baryta and the filtrate concentrated at 45° to small bulk and allowed to evaporate to dryness at the same temperature. During the evaporation a mass of leaf-shaped crystals separated. The dry residue was triturated in a mortar with 50 cc. of glacial acetic acid at room temperature and the mixture centrifuged. This was repeated three times; the residue was treated with copper carbonate according to the directions of Jones and Moeller. 2.210 gm. of copper aspartate, dried at room temperature, were thus obtained. The salt contained 5.00 per cent of nitrogen, theoretical value 5.09 per cent. The aspartic acid nitrogen thus isolated was 2.04 per cent of the total nitrogen. The filtrate from the isolation of copper aspartate was freed from copper and combined with the monoaminomonocarboxylic acid fraction.
The material dissolved in glacial acetic acid was evaporated to dryness under reduced pressure. The residue was taken up with a few cc. of concentrated hydrochloric acid and boiled under a reflux condenser for 3 hours. 0.033 gm. of glutamic acid hydrochloride was filtered off after cooling the solution, increasing the value for glutamic acid nitrogen to 4.18 per cent.

The filtrate was freed from hydrochloric acid and made up to 100 cc. Analysis of aliquots showed a content of 162 mg. of nitrogen, 96 per cent of which was in the amino form. The main bulk of the solution was examined for hydroxyglutamic acid as follows: It was warmed to 40° and silver oxide was stirred in until the solution was neutral to litmus. Hot saturated silver sulfate solution was then added until the solution gave a deep brown spot test with a drop of barium hydroxide solution. Cold saturated barium hydroxide solution was added to a pH of about 8.2. The mixture was diluted to about 450 cc. and cooled overnight, and the precipitate centrifuged down and washed twice with water. An aliquot of the combined supernatant liquids was tested with silver nitrate and sodium hydroxide to insure complete removal of hydroxyglutamic acid; the remainder was freed from silver and combined with the monoaminomonocarboxylic acid fraction.

The washed precipitate was decomposed with hydrogen sulfide, and sulfuric acid removed with baryta. It was found to contain 25 mg. of nitrogen, 23 mg. of which were in the amino form. It was concentrated at 45° to 20 cc. and treated with silver nitrate and sodium hydroxide according to the directions of Dakin (13). A small quantity of a fine white precipitate separated. The precipitate was decomposed with hydrogen sulfide, and yielded 0.70 mg. of nitrogen, all in the amino form. Its small quantity prevented further examination to see if it were really hydroxyglutamic acid, but the finding indicated that this amino acid, if present in livetin, was there only in very small amount.

Monoaminomonocarboxylic Acids—The combined residues from the separation of the dicarboxylic amino acids were freed from alcohol by distillation at 40° under reduced pressure and the barium hydroxide removed with sulfuric acid. The solution of amino acids was concentrated to 300 cc. Tyrosine crystallized freely; it was filtered off, recrystallized, and weighed. The weight of recrystallized tyrosine was 1.030 gm. Corrected for its solubility, 0.05 per cent, in the 85 cc. of water of recrystallization, the
weight was 1.071 gm., corresponding to 1.50 per cent of the total nitrogen in the form of tyrosine nitrogen. This value was increased by subsequent colorimetric examination of the succeeding fractions as follows:

<table>
<thead>
<tr>
<th>Tyrosine N</th>
<th>Per cent of total N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Separated by crystallization</td>
<td>1.50</td>
</tr>
<tr>
<td>In leucine-phenylalanine fraction</td>
<td>0.012</td>
</tr>
<tr>
<td>“ glycine-alanine fraction</td>
<td>0.28</td>
</tr>
<tr>
<td>“ proline-valine fraction</td>
<td>0.011</td>
</tr>
<tr>
<td>Total</td>
<td>1.80</td>
</tr>
</tbody>
</table>

The remaining amino acids were converted into their copper salts by evaporating twice with excess of copper carbonate according to the method of Brazier (3). The copper salts were stirred mechanically with 100 cc. of water at room temperature for half an hour and the soluble portion separated by centrifuging. This process was carried out six times. The solution, Fraction B, consisted of copper salts soluble in water. The residue, Fraction A, consisted of copper salts insoluble in water together with excess of copper carbonate. Fraction A was suspended in about 600 cc. of water and decomposed with hydrogen sulfide at 60° with stirring. It was not found necessary to add acid. The fraction contained 556 mg. of nitrogen, including a loss of 12 mg. in the CuS precipitate. This corresponded to 10.1 per cent of the total nitrogen. Fraction A consisted of the copper salts of leucine and phenylalanine; the other amino acids of which the copper salts are insoluble in water are aspartic acid and cystine. These had already been removed. A colorimetric cystine determination was made on an aliquot by the method of Folin and Marenzi (15) and the fraction found to contain but 10 mg. of cystine. The copper salt of tyrosine is only slightly soluble in water (16), but analysis of an aliquot of Fraction A revealed the presence of only 8.6 mg. of tyrosine in the entire fraction. The main bulk of the fraction was evaporated to 30 cc. and leucine was crystallized from it in two crops. A total weight of 1.966 gm. of crude leucine was thus obtained; it contained 10.27 per cent of nitrogen. Upon recrystallization the nitrogen content was raised to 10.52 per cent (theoretical 10.69 per cent). The filtrate was made up to 250 cc., and 1 cc. aliquots taken for the estimation of phenylalanine by the method of Kapellner-Adler (4). In spite of the virtual absence of tyrosine, the violet
color produced by phenylalanine was interfered with by a yellow discoloration. Preliminary addition of permanganate and sulfuric acid in the cold did not remove the interfering color. It was found necessary to add additional permanganate to the hot solution. This procedure probably led to some loss of phenylalanine by oxidation. The procedure used was as follows: Aliquots of the solution to be tested were pipetted into 50 cc. centrifuge tubes. 0.2 cc. of 14 N H₂SO₄ was added to each, followed by sufficient decinormal potassium permanganate to produce a faint permanent rose color. The tubes were placed in a boiling water bath and a quantity of permanganate equal to that already used was added dropwise over a period of about 15 minutes. The tubes were left in the water bath until their contents were evaporated to a syrup. Simultaneously, aliquots of a standard phenylalanine solution containing 1 mg. of the amino acid per cc. were evaporated to dryness without permanganate treatment. All samples were nitrated as described by Kapeller-Adler. Each was washed into a 25 cc. volumetric flask with 7 cc. of water and cooled. 5 cc. of 15 per cent hydroxylamine hydrochloride solution were added. 8 cc. of ammonium hydroxide solution (sp. gr. 0.90) were cautiously layered on the surface. The flask was kept cool in ice water, and its contents were slowly mixed and allowed to cool. Ammonium hydroxide solution was then added to the mark with mixing. The samples were warmed at 40° for 5 minutes, cooled at 0° for 15 minutes, and read in the colorimeter. Between 2 and 4 mg. of phenylalanine gave a satisfactory color intensity.

By this means, 640 mg. of phenylalanine were found in the filtrate from the crystallization of leucine. The main bulk of the filtrate was treated with zinc hydroxide for further isolation of leucine according to the directions of Brazier.

An additional 50 mg. of phenylalanine were found present by a colorimetric examination of the mother liquors from the recrystallization of the crude leucine, bringing the total value for phenylalanine N to 1.07 per cent of the total N.

Two fractions were obtained from the zinc hydroxide treatment, consisting of soluble and insoluble zinc salts. These were freed from zinc and analyzed for nitrogen and for phenylalanine. The soluble zinc salts yielded 210 mg. of nitrogen, of which 24.5 mg. were in the form of phenylalanine nitrogen. The insoluble zinc
salts contained 120 mg. of nitrogen, 22.1 mg. of phenylalanine N. The zinc treatment was therefore ineffective, and fractional crystallization was continued for further separation of leucine. By this means 1.64 gm. of leucine were separated. The total amount of crystalline leucine corresponded to 7.29 per cent of the total nitrogen. 83 per cent of the nitrogen of the leucine-phenylalanine fraction was accounted for as leucine and phenylalanine.

The effect of the presence of leucine upon the colorimetric determination of phenylalanine was examined by submitting to colorimetric analysis mixtures of the two amino acids in the proportion of 2 or 3 parts of leucine to 1 of phenylalanine. A yellowish discoloration was produced by the leucine. It was found possible to remove this by treatment with permanganate, but it resulted in a diminution of the phenylalanine color by about 20 per cent. From this it would seem that, in order to obtain an approximately accurate colorimetric analysis of the leucine-phenylalanine fraction for phenylalanine, the comparison should be made against standards consisting of varying mixtures of the two amino acids.

The copper salts soluble in water were evaporated to dryness and ground up with acetone. They were extracted with five changes of absolute methyl alcohol, ground up with methyl alcohol, and extracted with seven more changes of methyl alcohol.

The residue, insoluble in methyl alcohol, was dissolved in about 500 cc. of water and decomposed with hydrogen sulfide. The solution was concentrated under reduced pressure. During the concentration a small amount of crystals separated; these had the appearance of glycine. The nitrogen content of the fraction was 1080 mg., corresponding to 19.6 per cent of the total nitrogen. It contained 200 mg. of tyrosine (colorimetric). For the separation of glycine from the fraction, the amino acids present were converted to their barium carbamino compounds by three successive treatments with barium hydroxide and carbon dioxide according to the method of Kingston and Schryver (17). It has been shown by Kingston and Schryver (17), Schryver, Buston, and Mukherjee (18), and Buston and Schryver (19) that the only barium carbamate compound insoluble in ice-cold water, other than that of hydroxylysine, isolated by them, is that of glycine.

The precipitated carbamino compounds were not dried but were
extracted with 400 cc. of water at 0°. This was carried out four times. The insoluble material was decomposed with steam to give the glycine fraction; the residue of barium carbonate was then washed by adding a slight excess of sulfuric acid. The watersoluble carbamates were decomposed by acidifying with sulfuric acid and boiling to give the alanine fraction. Only 14.5 mg. of nitrogen escaped precipitation as carbamate.

An aliquot was treated with phosphotungstic acid, but no precipitate suggestive of hydroxylysine separated. The remainder of the solution was esterified for the preparation of glycine ethyl ester hydrochloride. The yield of the ethyl ester hydrochloride was low in comparison with the total nitrogen of the glycine fraction, the quantitative separation of this compound, however, is often difficult. 0.08 per cent of the total protein nitrogen was separated as glycine ester hydrochloride.

The alanine fraction contained 894 mg. of nitrogen, 816 mg. of which were in the amino form. It was made up to 500 cc. and 2 cc. aliquots were submitted to the procedure of Fürth, Scholl, and Herrmann (5) for the determination of alanine. It was found necessary to modify slightly the apparatus shown in Fig. 1 of their article; the tube b was made to project below the surface of the liquid in a so as to bubble air through the liquid. In spite of this, and in spite of lengthening the time of redistillation to 1½ hours only partial recovery of alanine from standard solutions was found possible. By comparing aliquots of the alanine fraction against identically treated aliquots of a standard alanine solution, a value of 2.2 gm. was obtained for the alanine content of this fraction, corresponding to 6.3 per cent of the total nitrogen as alanine nitrogen. More than half of the total nitrogen of the alanine fraction remained unidentified; a sample of dried amino acids from it had a content of 10.5 per cent N and was apparently a mixture.

The copper salts soluble in methyl alcohol were treated as follows: The methyl alcohol was distilled off and the residue taken up in about 500 cc. of water and decomposed with hydrogen sulfide. The resultant solution of amino acids was concentrated to 200 cc. It contained 975 mg. of nitrogen, 650 mg. of which were in the amino form, and 7.8 mg. of tyrosine. The amino acids were dried and extracted with absolute alcohol according to the directions of Brazier (3). The combined alcoholic washings were
Fractionation of Amino Acids of Livetin

treated with picric acid by the method of Town (20) for the isolation of proline. 0.321 gm. of crude proline picrate was thus separated. The mother liquor was freed from picric acid by ether extraction and evaporated to small bulk. It was boiled for 15 hours with 25 per cent sulfuric acid to hydrolyze any proline peptides present and treated with cadmium chloride as described by Kapfhammer and Eck (21) and Brazier (3). 0.616 gm. of proline was obtained in the form of its cadmium chloride compound. The nitrogen in the form of proline corresponded to 1.71 per cent of the total nitrogen.

The amino acids insoluble in alcohol, containing valine and isoleucine, were dried at 100°. The mixture weighed 3.51 gm. and contained 11.05 per cent of nitrogen, 99 per cent of which was in the amino form.

DISCUSSION

The main source of loss in the method consists of the numerous rejected precipitates. These were thoroughly washed as previously described (7). The precipitation of cupric sulfide was sometimes made troublesome by the formation of a sol; this usually flocculated upon standing overnight.

It is recommended that tyrosine be removed before precipitation of the dicarboxylic amino acids, instead of afterwards as was done in this investigation. Glutamic acid and hydroxyglutamic acid in aqueous solution readily pass respectively into α-pyrrolidonecarboxylic acid and α-hydroxy-γ-pyrrolidonecarboxylic acid. This change is accelerated by heating (22, 23, 13, 2), and might take place during the removal of ammonia. It has been shown by Abderhalden and Kautsch (22) and Foreman (23) that these pyrrolidone compounds may be reconverted to their straight chain precursors by boiling with hydrochloric acid. This procedure was accordingly employed.

The barium salts of the dicarboxylic amino acids were precipitated twice, but it is recommended that three precipitations be employed, since about one-third of the nitrogen of this fraction was found to be in a form other than that of the three dicarboxylic amino acids. Tyrosine and glycine tend to contaminate this fraction.

Methods are being sought to incorporate the determination of
hydroxyproline and serine into the fractionation, and to separate valine from isoleucine. Livetin quite probably contains methionine since its total sulfur content, 1.8 per cent, is well in excess of its cystine sulfur content, 0.8 per cent.

Determination of the carbohydrate content of livetin by the method of Tillmans and Philippi (24) showed the presence of about 4 per cent of carbohydrate, expressed as glucose, while vitellin contains about 2 per cent. The investigations of Frankel and Jellinek (25) and Levene and Mori (26) indicate the carbohydrate complex of egg yolk proteins is in the form of a polymer of glucosaminodimannose. If this be the case, it is necessary to increase the above values by 50 per cent, since glucosamine produces no color in the method of Tillmans and Philippi (cf. Rimington (27)). This would provide for about 0.4 per cent of the nitrogen of livetin in the form of glucosamine.

Separate determination of the tryptophane content of livetin by the method of Folin and Marenzi (15) gave a value of 1.11 per cent of the total nitrogen as tryptophane N. Tyrosine N was 3.04 per cent of the total nitrogen by this method; this was far more than the amount isolated (1.50 per cent of total nitrogen).

Attention is called to the fact that the leucine-phenylalanine fraction plus the tyrosine represents the total ketogenic amino acids. This could be fairly easily determined on a small sample of protein.

The results of the analysis conducted in this investigation are summarized in Table I.

**SUMMARY**

1. A method is described for fractionation of the amino acids resulting from protein hydrolysis.

2. The method consists of removing the basic amino acids by the method of Vickery and Leavenworth (8). Tyrosine is then removed by crystallization and the dicarboxylic amino acids are removed as their barium salts. The monoaminomonocarboxylic acids are then converted into their copper salts and fractionated.

3. The method has been applied to livetin of hen's egg yolk. 87 per cent of the total nitrogen was recovered in the various fractions; 56 per cent of the total nitrogen was definitely allocated.
### TABLE I

**Result of Fractionation of Amino Acids of 36 Gm. of Livetin**

<table>
<thead>
<tr>
<th>Form of N</th>
<th>Form in which separated</th>
<th>Exact nature determined</th>
<th>Exact nature undetermined</th>
<th>Per cent of weight of protein (15.5 per cent N)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ammonia</td>
<td></td>
<td>8.3</td>
<td></td>
<td>1.56</td>
</tr>
<tr>
<td>Histidine</td>
<td>Flavianate</td>
<td>1.62</td>
<td></td>
<td>0.93</td>
</tr>
<tr>
<td>Arginine</td>
<td>“</td>
<td>11.6</td>
<td></td>
<td>5.6</td>
</tr>
<tr>
<td>Lysine</td>
<td>Picate</td>
<td>6.15</td>
<td></td>
<td>5.0</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>Hydrochloride</td>
<td>4.18</td>
<td></td>
<td>6.8</td>
</tr>
<tr>
<td>Aspartic</td>
<td>Copper salt</td>
<td>2.04</td>
<td></td>
<td>3.0</td>
</tr>
<tr>
<td>Hydroxyglutamic acid</td>
<td>Silver</td>
<td>Trace (?)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tyrosine</td>
<td></td>
<td>1.80</td>
<td></td>
<td>3.6*</td>
</tr>
<tr>
<td>Copper salts insoluble in water</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leucine</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>Colorimetric</td>
<td>7.29</td>
<td></td>
<td>10.6</td>
</tr>
<tr>
<td>Copper salts soluble in water but insoluble in methyl alcohol</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glycine fraction</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alanine</td>
<td>Lactic acid</td>
<td>6.3</td>
<td></td>
<td>19.7</td>
</tr>
<tr>
<td>Copper salts soluble in methyl alcohol</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proline</td>
<td>Picrate and cadmium chloride compound</td>
<td>17.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Valine + isoleucine fraction</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cystine</td>
<td>Colorimetric†</td>
<td>2.64</td>
<td></td>
<td>7.18</td>
</tr>
<tr>
<td>Tryptophane</td>
<td>‡</td>
<td>1.11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucosamine</td>
<td>Calculated</td>
<td>0.4</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Total nitrogen recovered 87 per cent; total nitrogen definitely allocated 56 per cent.

* Method of Folin and Marenzi (15) gave 3.04 per cent of N as tyrosine N and 6.1 per cent of protein as tyrosine.
† Method of Folin and Marenzi (28).
‡ Method of Folin and Marenzi (15).
§ Calculated as glucosaminodimannose.
The alanine determinations were carried out with the cooperation of Miss M. J. Lawson. The author has received advice from Professor H. B. Vickery, which is gratefully acknowledged.

BIBLIOGRAPHY
25. Frankel, S., and Jellinek, C., Biochem. Z., 185, 392 (1927).
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