THE PARTIAL HYDROLYSIS OF PROTEINS.

II. ON FIBRIN-HETEROALBUMOSE.

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The study of partial cleavage has proved of great service for the interpretation of the constitution of many complex substances. The value of the same method for the elucidation of the structure of the protein molecule has been discussed by one of us some time ago. Here, again, we wish to emphasize that we are fully aware of the difficulties which one encounters when attempting to separate the individual products of partial hydrolysis of proteins, both of colloidal and crystalline nature. However, we feel confident that the classical methods of the schools of Kühne, of Chittenden, of Hofmeister, and the recently added method of Siegfried and his co-workers—though from a chemical standpoint imperfect—still remain of great service for the study of the chemical structure of the protein molecule. The different albumoses may not represent chemical individuals; but even if they are mixtures, they are mixtures similar within each fraction not only by the physical properties, but also by the chemical composition of the parts of the mixture. This can be stated with certainty in regard to the so-called primary digestion products, the hetero- and the proto-albumoses. The work of Adler and of Birchard in Siegfried's laboratory has contributed much evidence in support of this view. The work undertaken in this laboratory several years ago, and which was planned to be a systematic study of every individual fibrin-albumose, and of the products of partial hydrolysis of the

1 P. A. Levene, this Journal, i.
2 Dissertation, Leipzig, 1907.
same, was for a time interrupted. This interruption was caused principally by work on the improvement in the methods of analysis of the products of protein hydrolysis. Considerable advance in the methods of analysis have been made through the efforts of Osborne and his co-workers, of Siegfried and his pupils, and through the work done in this laboratory.

It was therefore concluded to resume the work on the products of partial hydrolysis of fibrin. The aim of the work is to find evidence which will lead to a definite solution of the following queries.

First, whether or not on partial hydrolysis the protein molecule is decomposed into large fragments—which appear in the form of albumoses.

Second, whether or not the partial hydrolysis of proteins proceeds by degrees, in a manner such that only individual amino-acids, or simple peptides are detached from the original molecule, so that by degrees the number of amino-acids contained in it decreases, and the protein is transformed through the more complex albumoses into the simpler, and finally into peptones, peptides, and amino-acids.

Third, whether or not the two processes occur simultaneously. In that case the task will arise to trace the relationship of the simpler albumoses or peptones to one or the other of the more complex substances.

The present communication is limited to the work on the preparation and on the hydrolysis of the hetero-albumose.

Preparation of the Hetero-albumose.

The method of preparation consisted in a combination of each of the methods as worked out by Kühne and Pick, and was the same as that adopted by one of us\(^1\) in a former investigation.

The details of the method are as follows:

A 10 per cent solution of Witte's peptone was carefully neutralized with dilute sulphuric acid, allowed to stand over night, and the undissolved residue filtered off. It was later found that the solution could be centrifugalized

\(^1\) P. A. Levene: this Journal, i, p. 1.
with advantage and consequent saving of considerable time. To the clear solution obtained by either method was added an equal volume of a concentrated solution of ammonium sulphate. The primary albumoses were filtered off, washed with half-saturated ammonium sulphate solution, and twice reprecipitated in half the former dilution. The hetero-albumose was separated from the proto-albumose by adding an equal volume of 95 per cent alcohol, allowing to stand two days, and filtering off the hetero-albumose by the aid of suction. The product so obtained was carefully washed with 50 per cent alcohol, dissolved in a volume of warm water equal to half of that formerly employed, and twice reprecipitated by the addition of an equal volume of 95 per cent alcohol. The final product was dissolved in warm water, a little ammonium sulphate being added to aid the solution. The product was then subjected to dialysis until no more sulphate could be detected by barium chloride. By this process a fine granular substance was obtained which was finally washed by decantation in a large excess of distilled water. This method involves a very large loss of material, but it was only in this way that a pure product could be obtained.

**Properties.**

The albumose obtained in this way was extremely insoluble in water; when suspended in 4 liters of water and after intermittent stirring for 12 hours, 100 cc. of the supernatant liquid contained only 0.0042 gm. of nitrogen or 0.025 gm. of albumose. For analysis a sample was dried by heating to constant weight under diminished pressure at the temperature of boiling water. The following results were obtained:

0.2056 gm. substance gave 0.3734 gm. CO₂ and 0.1220 gm. H₂O.
0.1628 gm. of the substance employed for a nitrogen estimation after Kjeldahl, required 19.15 cc. \( \frac{9}{19} \) H₂SO₄.

These results agree fairly well with those obtained by Adler with Pick’s hetero-albumose, as can be seen from the following table:

<table>
<thead>
<tr>
<th>Adler’s Analysis.</th>
<th>Present Analysis.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Per Cent</strong></td>
<td><strong>Per Cent</strong></td>
</tr>
<tr>
<td>C = 48.18</td>
<td>C = 49.52</td>
</tr>
<tr>
<td>H = 6.63</td>
<td>H = 6.64</td>
</tr>
<tr>
<td>N = 16.00</td>
<td>N = 16.46</td>
</tr>
</tbody>
</table>

For further identification the rotatory power of the substance dissolved in 5 per cent ammonium sulphate solution was deter-

1 Dissertation, Leipzig, 1907.
mined. For this determination very careful drying is necessary, as prolonged heating at temperatures over 100° C. tends to change the substance into a form insoluble even in salt solution. The albumose was first dried two days under diminished pressure over sulphuric acid, then for two days under diminished pressure at the temperature of boiling chloroform, and finally to constant weight at the temperature of boiling alcohol.

0.1615 gm. substance, dissolved in 25 cc. rotated −0.84° in 1.855 gm. tube at t = 20° C. Thus:

\[ \alpha_d = -70.11°. \]

This is in close agreement with the value found by Adler, −70.69° for an ammoniacal solution of the hetero-albumose.

Primary Amino Nitrogen: 0.2393 gm. albumose treated with nitrous acid gave 4.40 cc. of nitrogen gas at 21°, 756 mm.

Amino N = 1.03 per cent = 6.3 per cent of the total N.

HYDROLYSIS.

Glutaminic Acid and Esterified Acids.

One-hundred and twenty-nine grams of the hetero-albumose were hydrolyzed by boiling 15 hours, with 20 per cent hydrochloric acid. The solution was concentrated, saturated with hydrochloric acid, and left for 10 days in the refrigerator. The glutaminic hydrochloride was filtered on asbestos and recrystallized. 10.83 gms. of the pure hydrochloride, equivalent to 5.67 gms. of glutaminic acid, were obtained.

Analysis: 0.4370 gm. subst.; 23.80 cc. \( \frac{N}{10} \) Ag NO₃.

<table>
<thead>
<tr>
<th>Calculated for ( \text{C}_6\text{H}_8\text{O}_4\text{N.HCl} )</th>
<th>Found:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cl..................</td>
<td>19.31 per cent</td>
</tr>
</tbody>
</table>

The mother liquors were esterified according to Fischer's method, three crops of esters being freed with barium hydrate and extracted by the method of Levene and Van Slyke.¹ The esters were distilled, using \( \text{H}_2\text{SO}_4 \) in place of liquid air refrigeration, to absorb uncondensed vapors.² The esters boiling above 90° were not

¹ Levene and Van Slyke: this Journal, vi, p. 391. 1909.
distilled, but worked up as recently proposed by Osborne and Jones.\textsuperscript{1} The results were satisfactory.

The esters were divided into the following fractions by distillation.

\begin{center}
\begin{tabular}{|c|c|c|}
\hline
Fraction & Temperature of Vapors & Pressure & Weight of Esters \\
& degrees & mm. & gm. \\
\hline
I & to 60 & 12.0 & 27.8 \\
II & to 90 & 0.4 & 32.0 \\
III & Undistilled & . . . & 32.3 \\
\hline
Total & & & 92.1 \\
\hline
\end{tabular}
\end{center}

\textit{Fraction I} was chiefly alcohol. It yielded by crystallization 0.95 gm. of a mixture containing 14.18 per cent N, and on evaporating the mother liquors to dryness, 0.96 gm. of more soluble acids. The first crop was worked up with the alanin-valin mixtures from Fraction II. The second crop was combined with the more soluble portion of Fraction II, and extracted with absolute alcohol to remove prolin.

\textit{Leucin-Valin Sub-Fraction.}

\textit{Fraction II} yielded three fractions by crystallization, which nitrogen determinations by the nitrous acid method\textsuperscript{2} showed to be mixtures of valin and leucin, the weights and nitrogen contents being 2.96 gms., 11.07 per cent N; 4.00 gms., 11.34 per cent N; 4.12 gms., 11.71 per cent N. These fractions were combined, and the leucin, isoleucin and valin determined by precipitating the leucin isomers as lead salts, and determining the proportion of the two isomers by the rotation of their mixture in 20 per cent HCl.\textsuperscript{3} One-half of the mixture was used for the lead separation. It yielded 6.91 gms. of the lead salt of leucin, equivalent to 7.76 gms. of the leucin isomers from the entire mixture.

\textsuperscript{1} Amer. Journ. of Physiol., xxvi, p. 212, 1910.


\textsuperscript{3} Levene and Van Slyke: Analysis of the Leucin Fraction of Proteins, this Journal, vi, p. 391, 1909.
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**Analysis:** 0.2958 gm. subst.; 0.1924 gm. PbSO₄; 0.2158 gm. subst.; 22.5 cc. N at 18°, 756 mm. (nitrous acid method).

<table>
<thead>
<tr>
<th>Substance</th>
<th>Calculated</th>
<th>Found</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pb</td>
<td>44.29%</td>
<td>44.32%</td>
</tr>
<tr>
<td>N</td>
<td>6.00%</td>
<td>5.92%</td>
</tr>
</tbody>
</table>

The leucin and isoleucin were freed from their salts as described by Levene and Van Slyke, and the rotation of the mixture taken in 20 per cent HCl.

0.2632 gm. subst; 5.016 gm. solution; concentration, 5.15 per cent; rotation in 1 dm. tube, + 1.52°.

\[ \alpha^D = + 26.32° \]

Calculated from the rotation, the mixture contained 50.8 per cent of l-leucin, 49.2 d-isoleucin, or 3.94 and 3.82 gms. respectively. The mixture of the free leucin isomers gave the following figures on analysis:

0 1570 gm. subst.; 30.34 cc. N at 24°, 760 mm. (nitrous acid method).

<table>
<thead>
<tr>
<th>Substance</th>
<th>Calculated</th>
<th>Found</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>10.69%</td>
<td>10.78%</td>
</tr>
</tbody>
</table>

The filtrate from the leucin lead salts yielded 1.46 gms. of valin, equivalent to 2.92 gms. for the entire portion.

**Analysis:** 0.1141 gm. subst.; 25.30 cc. N at 30°, 756 mm. (nitrous acid method).

<table>
<thead>
<tr>
<th>Substance</th>
<th>Calculated</th>
<th>Found</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>11.96%</td>
<td>11.88%</td>
</tr>
<tr>
<td>C</td>
<td>51.24%</td>
<td>51.33%</td>
</tr>
<tr>
<td>H</td>
<td>9.47%</td>
<td>9.64%</td>
</tr>
</tbody>
</table>

**Valin-Alanin Sub-Fraction.**

The mother liquors from the above crystallization fractions were freed from prolin by extraction in the usual manner with absolute alcohol, then submitted to fractional crystallization. 1.65 gms.
more of valin was obtained, making the entire yield of pure valin 4.57 gms.

**Analysis:** 0.1340 gm. subst.; 30.5 cc. N at 32°, 756 mm. (nitrous acid method).

Calculated for

\[ \text{C}_6\text{H}_{11}\text{O}_2\text{N} : \]

<table>
<thead>
<tr>
<th>Component</th>
<th>Calculated</th>
<th>Found</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>11.96 per cent</td>
<td>12.08 per cent</td>
</tr>
</tbody>
</table>

Rotation in 20 per cent HCl: 0.2460 gm. substance; 5.114 gm. solution; concentration, 4.81 per cent; rotation in 1 dm. tube, + 1.25°.

\[ [\alpha]_b^{28} = + 23.63°. \]

3.61 gms. of recrystallized alanin were obtained from the valin mother liquors.

**Analysis:** 0.1161 gm. subst.; 34.7 cc. N at 32°, 756 mm. 0.1388 gm. subst.; 0.2054 gm. CO₂; 0.0955 gm. H₂O.

Calculated for

\[ \text{C}_5\text{H}_{10}\text{O}_2\text{N} : \]

<table>
<thead>
<tr>
<th>Component</th>
<th>Calculated</th>
<th>Found</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>15.73 per cent</td>
<td>15.85 per cent</td>
</tr>
<tr>
<td>C</td>
<td>40.42 per cent</td>
<td>40.36 per cent</td>
</tr>
<tr>
<td>H</td>
<td>7.93 per cent</td>
<td>7.69 per cent</td>
</tr>
</tbody>
</table>

1.10 gms. of a mixture of alanin and valin (C = 44.81, H = 8.58) were obtained which could not be separated. No glyccoll could be obtained by the carbamino method of Siegfried despite this method was tried out on mixtures of glyccoll and d-alanin and found to be fully as satisfactory as Siegfried claims. For example from a mixture of 0.37 gm. glyccoll and 1.00 gm. d-alanin in 50 cc. baryta water, 0.28 gm. of pure glyccoll was obtained (N = 18.90, calc. 18.67). The failure to detect glyccoll by this method therefore indicates that very little if any was present. No fractions which from analysis appeared to be mixtures containing glyccoll could be obtained on recrystallization.

**Prolin.**

The amino-acids completely soluble in cold alcohol were dissolved to 100 cc. in water, and the prolin determined by the method of Van Slyke.¹ Kjeldahls on 5 cc. samples required 35.75 — 35.65

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cc. of $\frac{1}{10}$ $\text{H}_2\text{SO}_4$ for titration, indicating 1.000 gm. of nitrogen in the entire solution. Determination of the primary amino nitrogen in 10 cc. of the solution gave 71.10 cc. of nitrogen gas at 21°, 762 mm., indicating that 0.4025 gm. of the nitrogen came from the ordinary aliphatic amino-acids, 0.5975 gm. from the prolin in the mixture. This corresponds to 4.92 gms. of prolin.

The 80 cc. of the solution, remaining after removal of the samples for the above determinations, was racemicized, boiled with CuO, and the prolin obtained as crystalline $d$-$l$ copper salt. 3.77 gms. were regained, equivalent to 60.6 per cent of the amount of prolin calculated.

Analysis: 0.3784 gm. subst.; loss at 100°, 0.0385 gm.; 11.70 cc. $\frac{N}{10}$ ammonium sulphocyanide (Volhard titration), 0.2310 gm. subst.; amino nitrogen, 4.70 cc. at 19°, 760 mm.

<table>
<thead>
<tr>
<th>Component</th>
<th>Calculated</th>
<th>Found</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\text{H}_2\text{O}$</td>
<td>10.99 per cent</td>
<td>10.72 per cent</td>
</tr>
<tr>
<td>Cu</td>
<td>19.41 per cent</td>
<td>19.72 per cent</td>
</tr>
<tr>
<td>Amino N</td>
<td>0.00 per cent</td>
<td>1.16 per cent</td>
</tr>
</tbody>
</table>

After recrystallizing three times the prolin salt was obtained almost free from amino nitrogen. The amino nitrogen test is by far the most delicate for the purity of prolin obtained from proteins.

Analysis: 0.3484 gm. subst.; 0.0375 loss at 100°; 10.70 cc. $\frac{N}{10}$ ammonium sulphocyanide. 0.1906 gm. subst.; 0.60 cc. N at 30°, 760 mm. (nitrous acid method).

<table>
<thead>
<tr>
<th>Component</th>
<th>Calculated</th>
<th>Found</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\text{H}_2\text{O}$</td>
<td>10.99 per cent</td>
<td>10.76 per cent</td>
</tr>
<tr>
<td>Cu</td>
<td>19.40 per cent</td>
<td>19.51 per cent</td>
</tr>
<tr>
<td>Amino N</td>
<td>0.00 per cent</td>
<td>0.17 per cent</td>
</tr>
</tbody>
</table>

The mother liquors from the first crop of copper salt were freed from copper by hydrogen sulphide, and the amino-acids crystallized from dilute alcohol. 1.35 gms. were thus obtained, a mixture of alanin and valin ($N = 13.72$ per cent). The mother liquors were reconverted into copper salts, and yielded 0.57 gm. more of prolin salt ($\text{H}_2\text{O} = 11.04$, Cu = 19.62, amino $N = 0.72$), making the total crystallized copper salt regained 69.7 per cent of that calculated by the nitrogen determinations.
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Phenylalanin.

Fraction III of the esters was dissolved in water and the phenylalanin ester extracted with ether in the usual manner. The ethereal extract contained practically all of the coloring matter. It was decolorized with charcoal, and the phenylalanin obtained as hydrochloride from aqueous solution after saturation with HCl. 3.85 gms. of the hydrochloride, equivalent to 3.16 gms. of phenylalanin, were obtained.

Analysis: 0.3201 gm. subst.; 16.05 cc. $\frac{N}{10}$ silver nitrate.

Calculated for $C_9H_7O_2N$: Found:

Cl........................................ 17.55% 17.76% per cent

Glutaminic and Aspartic Acids from Esters.

The esters not extracted from water solution by ether were hydrolyzed with barium hydrate, as usual, the solution was freed from barium with sulphuric acid, and the glutaminic acid crystallized as hydrochloride. 4.47 gms. equivalent to 3.59 gms. of glutaminic acid were obtained.

Analysis: 0.2970 gm. subst.; 16.30 cc. $\frac{N}{10}$ silver nitrate.

0.1430 gm. subst.; 19.80 cc. N at 25°, 760 mm. (nitrous acid method).

Calculated for $C_5H_7O_4N.HCl$: Found:

Cl........................................ 19.31 per cent 19.45 per cent

N........................................ 7.63 per cent 7.68 per cent

The mother liquors were freed from HCl by concentration in vacuo, followed by use of silver sulphate, hydrogen sulphide, and an equivalent of barium hydrate to remove SO$_4$. The solution was concentrated and mixed with several volumes of alcohol. 0.10 gms. of aspartic acid crystallized on standing in the refrigerator.

Analysis: 0.1297 gm. subst.; 22.30 cc. N at 29°, 758 mm. (nitrous acid method).

0.1272 gm. subst.; 0.1643 gm. CO$_2$; 0.0614 gm. H$_2$O.

Calculated for $C_4H_7O_4N$: Found:

N........................................ 10.54 per cent 10.55 per cent
C........................................ 36.06 per cent 36.28 per cent
H........................................ 5.30 per cent 5.40 per cent

No serin could be obtained from the mother liquors.
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Prolin, Alanin, and Glycocoll from Unextracted Ester Residues.

The barium residues left after the third extraction of esters were freed from hexone bases by phosphotungstic acid, and from sulphate, chloride, and excess phosphotungstate by barium hydrate and silver sulphate. The solution of amino-acids was concentrated, and 1 gm. of tyrosin filtered off. The other amino-acids were all converted into copper salts; the salts proved extremely soluble in water. Their solution was brought to 150 cc. and 600 cc. of alcohol added. The precipitated salts dried at 100° in vacuo weighed 15.6 gms.; the salts soluble in 80 per cent alcohol, 8.5 gms.

The insoluble fraction was changed back to free acids. They refused to be crystallized from water or dilute alcohol. An attempt was made to obtain the picrate of glycocoll by Levene's method. However, in place of the usual glycocoll picrate 1.56 gms. of a picrate differing in composition and in properties from the glycocoll compound were obtained. It was attempted to purify the substance by recrystallization from an alcoholic solution of picric acid. A great part of the substance remained in solution, as only 0.4 gm. was obtained on recrystallization. This picrate was not explosive and contained only traces of mineral impurities. Tested in the usual manner for pyrrol, it gave a very definite positive reaction, melted between 235–240° C. (corr.) with decomposition and evolution of gas.

Analysis: 0.1248 gm. subst.; 15.7 cc. N(over 50 per cent KOH) at 29°, 763 mm.

Calculated for

\[ C_6H_5NO_2.C_6H_2(NO_2)_3OH \]

Found:

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>18.42 per cent</td>
</tr>
</tbody>
</table>

Thus the substance was not glycocoll picrate. Lack of material did not permit of a detailed study of the substance at the present moment.

In the mother liquors of the first picrate on standing a second precipitate formed, about 0.80 gm. in weight with the properties of the glycocoll compound. M.p. = 190° C. (corr.) sharp.

1 P. A. Levene, this Journal, i. p. 463.
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Analysis: 0.120 gm. subst.; 20.0 cc. N (over 50 per cent KOH) at 30°, 757 mm).

Calculated for

\[ \text{N} \quad 18.42 \text{ per cent} \quad 18.80 \text{ per cent} \]

The copper salts soluble in 80 per cent alcohol were extracted with absolute alcohol. The soluble salts were reconverted into amino-acids, racemized, changed back to Cu salts, and recrystallized from water. 0.75 gm. of anhydrous prolin copper salt, equivalent to 0.59 gm. prolin, was obtained. The product was not entirely pure, but was of the characteristic violet color when dried at 100°, and gave the following analysis.

0.3490 gm. subst.; 11.10 cc. \( \text{N}_{\text{NH}} \) ammonium sulphocyanide.

0.2200 gm. subst.; 9.30 cc. N at 32°, 756 mm. (nitrous acid method).

Calculated for

\[ \text{Cu} \quad 19.40 \text{ per cent} \quad 20.21 \text{ per cent} \]

\[ \text{N} \quad 0.00 \text{ per cent} \quad 2.15 \text{ per cent} \]

The copper salts soluble in 80 per cent alcohol, but insoluble in absolute, were decomposed with \( \text{H}_2\text{S} \), and the amino-acids crystallized from dilute alcohol in the hope of obtaining serin. Instead of serin, however, 0.75 gm. of alanin was obtained.

Analysis: 0.1339 gm. subst.; 0.0958 gm. \( \text{H}_2\text{O} \).

Calculated for

\[ \text{C} \quad 40.42 \text{ per cent} \quad 40.23 \text{ per cent} \]

\[ \text{H} \quad 7.93 \text{ per cent} \quad 8.00 \text{ per cent} \]

Tyrosin.

Thirty-one grams of hetero-albumose were hydrolyzed with 20 per cent hydrochloric acid. The acid was removed by concentration in vacuo and the use of silver sulphate. The solution of amino-acids was concentrated in vacuo until crystallization began. 1.025 gms. cf tyrosin were obtained.

Analysis: 0.1586 gm. subst.; 0.3475 gm. \( \text{CO}_2 \); 0.0881 gm. \( \text{H}_2\text{O} \).

0.1235 gm. subst.; 16.35 cc. N at 21°, 768 mm. (nitrous acid method).
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Calculated for

\[ \text{C}_{280}\text{H}_{505}\text{O}_{158}\text{N}_5; \]

\begin{align*}
\text{C} & \quad \text{69.67 per cent} \\
\text{H} & \quad \text{6.06 per cent} \\
\text{N} & \quad \text{7.73 per cent}
\end{align*}

Found:

\begin{align*}
\text{C} & \quad \text{59.73 per cent} \\
\text{H} & \quad \text{6.21 per cent} \\
\text{N} & \quad \text{7.58 per cent}
\end{align*}

By further concentration a second crop of 0.053 gm. was obtained, making the total yield 1.078 gms., or 3.48 per cent.

Analysis: 0.0530 gm. substance; 0.1151 gm. CO\(_2\); 0.0300 gm. H\(_2\)O.

C, 59.23 per cent; H, 6.29 per cent.

Hexone Bases by the Osborne Modification of Kossel’s Method.

18.80 gms. of albumose were hydrolyzed; and the hexone bases precipitated by phosphotungstic acid at 2 liters dilution in the presence of 5 per cent sulphuric acid. The precipitate was freed from phosphotungstate and sulphate by means of barium hydrate, and the histidin, arginin, and lysin determined by the Kossel-Patton method as modified by Osborne, Leavenworth and Brautlecht.\(^1\)

The histidin solution was brought to 25 cc.; determinations were made of total nitrogen by the Kjeldahl method, and of primary amino nitrogen by the nitrous acid method. As has been shown,\(^2\) in pure histidin the ratio, total nitrogen: amino nitrogen, is 3:1, and consequently small amounts of histidin in pure solution can be analyzed by determining this ratio. The following results were obtained on the histidin solution:

Amino N: 10 cc. solution; 11.85 cc. N at 29°, 758 mm. Amino N in total 25 cc. is 0.01605 gm.

Total N: 15 cc. solution; 20.35 cc. \(\text{H}_2\text{SO}_4\). Total N in 25 cc. solution is 0.0476 gm.

Total N: Amino N = 2.97:1

The histidin solution was evidently pure. The total nitrogen corresponds to 0.176 gm. histidin, or 0.93 per cent.

The arginin solution was brought to 250 cc. volume. The ratio, total N: amino N, is 4:1 in the case of arginin. Determina-

\(^1\) Amer. Journ. of Physiol., xxiii, p. 180, 1908.

tion of amino and Kjeldahl nitrogens serves here to check the purity of the arginin solution. Also, it has been found that one-half of the arginin nitrogen is quantitatively evolved in the form of ammonia during 6 hours boiling with 25 per cent NaOH under a reflux. The greater part of the ammonia diffuses into standard H₂SO₄ in a Folin 3-bulb tube at the top of the condenser, the remainder being distilled off later after addition of water to the alkaline solution. The following determinations were made on the arginin solution:

- Total N: 20 cc. solution; 23.50 cc. H₂SO₄. Total N = 0.4010 gm.
- Amino N: 10 cc. solution; 9.70 cc. N at 25°, 764 mm. Amino N = 0.1353 gm.
- Arginin N: 40 cc. solution; 19.05 cc. H₂SO₄. Arginin N = 0.3330 gm.
- NH₃: 40 cc. solution; boiled with MgO; 0.00 cc. H₂SO₄. NH₃ absent.

The ratio, total N: amino N, is lower than 4:1, and the arginin determination indicates that only 83.1 per cent of the total nitrogen present was in the form of arginin. Calculated on this basis, the arginin present was 1.036 gms., or 5.50 per cent.

The solution not used in the above determinations was concentrated and treated with an equivalent of picrolonic acid in alcoholic solution. 0.9 gm. of arginin picrate-lonate were obtained.

**Analysis:**

- 0.1440 gm. subst.; 0.2297 gm. CO₂; 0.0696 gm. H₂O.

<table>
<thead>
<tr>
<th>Substance</th>
<th>Calculated</th>
<th>Found</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>43.82%</td>
<td>5.05%</td>
</tr>
<tr>
<td>H₂</td>
<td>43.50%</td>
<td>5.41%</td>
</tr>
</tbody>
</table>

From the lysin solution 2.44 gm. of lysin picrate were obtained, equivalent to 0.95 gm. of lysin, or 5.06 per cent. The picrate was recrystallized from water, and gave the following figures on analysis.

- 0.1517 gm. subst.; 20.4 cc. N at 18°, 746 mm. (nitrous acid method).

<table>
<thead>
<tr>
<th>Substance</th>
<th>Calculated</th>
<th>Found</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amino N</td>
<td>7.47%</td>
<td>7.58%</td>
</tr>
</tbody>
</table>

1 Van Slyke: *Proc. Soc. Exp. Biol. and Med.*, May 18, 1910. The decomposition of nearly one-half the arginin nitrogen into ammonia was noted by Osborne, Leavenworth and Brautlecht. The above method is quantitative.
Partial Hydrolysis of Proteins

Determination of the Hexone Bases and Nitrogen Distribution by the Method of Van Slyke.¹

2.07 gms. of albumose containing 0.3413 gm. of nitrogen, were hydrolyzed with 20 per cent hydrochloric acid, the acid removed as completely as possible by evaporation, and the ammonia distilled in vacuo with an excess of barium hydrate solution. It neutralized 20.10 cc. of $\frac{1}{10}$ H₂SO₄.

The solution was acidified with the H₂SO₄, the melanin removed by adsorption with AgCl, and determined by Kjeldahl. 19.75 cc. $\frac{1}{10}$ H₂SO₄ were neutralized.

The filtrate was brought to 100 cc. Portions of 5 cc. each were taken for determination of total and amino nitrogen.

Total Nitrogen: 10.10, 10.25 cc. $\frac{1}{10}$ H₂SO₄; average 10.18. Total N = 0.2856 gm.

To the remaining 80 cc. 4 cc. of concentrated sulphuric acid and 60 cc. of a 20 per cent solution of phosphotungstic acid in 5 per cent H₂SO₄ were added. The mixture was made up to 200 cc. volume and heated until the precipitate was nearly dissolved, then allowed to stand three days. The phosphotungstates of arginin, histidin, lysin and cystin are precipitated under these conditions. The precipitate was washed with a solution containing 5 per cent H₂SO₄ and 2 per cent phosphotungstic acid and decomposed with a slight excess of baryta water. The solution of bases was freed from Ba with CO₂ and brought to a volume of 50 cc. 10 cc. were used for Kjeldahl and amino determinations, in each case the remaining 30 cc. being used, first to determine the arginin by alkaline decomposition as already described (p. 281), and then to determine the cystin sulphur by fusion with KNO₃. The results were:

Phosphotungstate Precipitate.

Total N; 10 cc. solution: 8.25 cc. $\frac{1}{10}$ H₂SO₄. Total N = 0.0722 gm. = 21.15 per cent. of the total N.
Amino N: 10 cc. solution, 11.40 cc. N at 24°, 754 mm. Amino N = 0.0394 gm.
Arginin: 30 cc. solution; 6.80 cc. $\frac{N}{10}$ H₂SO₄. Arginin N = 0.0397 gm. = 11.62 per cent. of the total N.

The non-amino nitrogen (\(\frac{2}{3}\) of arginin N + \(\frac{3}{4}\) of histidin N) is 0.0722 - 0.0394 = 0.0328 gm. Subtracting three-fourths of the arginin N from the non-amino N, gives 0.0081 gm. as the non-amino histidin N, or 0.0046 gm. as the total histidin N, 1.34 per cent of the total nitrogen.

The difference (lysin and cystin N) between the total "basic" N and the histidin N + arginin N is 0.0279 gm. The 30 cc. of solution used for arginin and cystin determinations gave, for the latter 0.0790 gm. BaSO₄, indicating 0.0099 gm., 2.90 per cent, of cystin N. This leaves 0.0180 gm., or 5.28 per cent, for the lysin N.

By subtracting the nitrogen in the phosphotungstic precipitate from that found in the solution before precipitation, the nitrogen of the "mono-amino acid" fraction is obtained. This method avoids the difficulties of Kjeldahling solutions containing phosphotungstic acid. The total N of this fraction is 0.2134 gm. = 62.54 per cent. Of this, 0.1722 gm., or 50.48 per cent, is amino nitrogen, 0.0412 gm., or 12.06 per cent, is non-amino nitrogen, consisting of the nitrogen of prolin, oxyprolin, one-half the tryptophan and perhaps some yet unknown acid or acids.

The results are summarized as follows:

<table>
<thead>
<tr>
<th>Nitrogen Source</th>
<th>Grams</th>
<th>Per Cent of Total Nitrogen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ammonia</td>
<td>0.0281</td>
<td>8.23</td>
</tr>
<tr>
<td>Melanin</td>
<td>0.0276</td>
<td>8.08</td>
</tr>
<tr>
<td>Arginin</td>
<td>0.0397</td>
<td>11.62</td>
</tr>
<tr>
<td>Histidin</td>
<td>0.0046</td>
<td>1.35</td>
</tr>
<tr>
<td>Lysin</td>
<td>0.0180</td>
<td>5.23</td>
</tr>
<tr>
<td>Cystin</td>
<td>0.0099</td>
<td>2.90</td>
</tr>
<tr>
<td>Amino N in phosphotungstic filtrate</td>
<td>0.1722</td>
<td>50.48</td>
</tr>
<tr>
<td>Non-amino N in phosphotungstic filtrate</td>
<td>0.0412</td>
<td>12.06</td>
</tr>
</tbody>
</table>

The hexone base determinations, calculated for percentages of amino-acids in the dry albumose, compare as follows with those obtained by the older method.

<table>
<thead>
<tr>
<th>Amino-Acid</th>
<th>Kossel-Patton-Osborne Method</th>
<th>New Method</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arginin</td>
<td>5.50</td>
<td>5.96</td>
<td>5.73</td>
</tr>
<tr>
<td>Histidin</td>
<td>0.95</td>
<td>0.82</td>
<td>0.89</td>
</tr>
<tr>
<td>Lysin</td>
<td>5.06*</td>
<td>4.54</td>
<td>4.80</td>
</tr>
</tbody>
</table>

* Lysin was calculated from unrecrystallized picrate, so the result may be slightly high.
Correction for the solubility of the phosphotungstates, which is practically the same in both methods, as the concentrations at precipitation were alike, increases the average arginin to 6.35 per cent, the histidin to 1.76 per cent.

The results of the hydrolysis are summarized in the following table, expressed in grams of amino-acid from 100 gms. of albumose.

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>Grams</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glutaminic acid</td>
<td>9.51</td>
</tr>
<tr>
<td>Leucin</td>
<td>3.05</td>
</tr>
<tr>
<td>Isoleucin</td>
<td>2.96</td>
</tr>
<tr>
<td>Valin</td>
<td>3.54</td>
</tr>
<tr>
<td>Alanin</td>
<td>3.39</td>
</tr>
<tr>
<td>Valin-Alanin Mixture</td>
<td>1.86</td>
</tr>
<tr>
<td>Prolin</td>
<td>4.27</td>
</tr>
<tr>
<td>Phenylalanin</td>
<td>2.45</td>
</tr>
<tr>
<td>Aspartic acid</td>
<td>4.73</td>
</tr>
<tr>
<td>Glycocoll</td>
<td>0.15</td>
</tr>
<tr>
<td>Tyrosin</td>
<td>3.48</td>
</tr>
<tr>
<td>Arginin</td>
<td>6.35</td>
</tr>
<tr>
<td>Histidin</td>
<td>1.76</td>
</tr>
<tr>
<td>Lysin</td>
<td>4.80</td>
</tr>
<tr>
<td>Cystin</td>
<td>4.10</td>
</tr>
<tr>
<td>Ammonia</td>
<td>1.65</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>58.05</strong></td>
</tr>
</tbody>
</table>
THE PARTIAL HYDROLYSIS OF PROTEINS: II. ON FIBRIN-HETEROALBUMUMOSE
P. A. Levene, D. D. Van Slyke and F. J. Birchard

J. Biol. Chem. 1910, 8:269-284.

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