MONOGRAPHS ON BIOCHEMISTRY

EDITED BY

R. H. ADERS PLIMMER, D.Sc., AND F. G. HOPKINS, M.A., M.B., F.R.S.

THE CHEMICAL CONSTITUTION OF THE PROTEINS

BY

R. H. ADERS PLIMMER, D.Sc.

ASSISTANT PROFESSOR OF PHYSIOLOGICAL CHEMISTRY IN, AND FELLOW OF UNIVERSITY COLLEGE, LONDON

IN TWO PARTS

PART I

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39 PATERNOSTER ROW, LONDON
NEW YORK, BOMBAY, AND CALCUTTA
1908

Dedicated

TO

EMIL FISCHER

THE MASTER OF

ORGANIC CHEMISTRY IN ITS RELATION TO BIOLOGY

GENERAL PREFACE.

THE subject of Physiological Chemistry, or Biochemistry, is enlarging its borders to such an extent at the present time, that no single text-book upon the subject, without being cumbrous, can adequately deal with it as a whole, so as to give both a general and a detailed account of its present position. It is, moreover, difficult, in the case of the larger text-books, to keep abreast of so rapidly growing a science by means of new editions, and such volumes are therefore issued when much of their contents has become obsolete.

For this reason, an attempt is being made to place this branch of science in a more accessible position by issuing a series of monographs upon the various chapters of the subject, each independent of and yet dependent upon the others, so that from time to time, as new material and the demand therefor necessitate, a new edition of each monograph can be issued without re-issuing the whole series. In this way, both the expenses of publication and the expense to the purchaser will be diminished, and by a moderate outlay it will be possible to obtain a full account of any particular subject as nearly current as possible.

The editors of these monographs have kept two objects in view: firstly, that each author should be himself working at the subject with which he deals; and, secondly, that a *Bibliography*, as complete as possible, should be included, in order to avoid cross references, which are apt to be wrongly cited, and in order that each monograph may yield

full and independent information of the work which has been done upon the subject.

It has been decided as a general scheme that the volumes first issued shall deal with the pure chemistry of physiological products and with certain general aspects of the subject. Subsequent monographs will be devoted to such questions as the chemistry of special tissues and particular aspects of metabolism. So the series, if continued, will proceed from physiological chemistry to what may be now more properly termed chemical physiology. This will depend upon the success which the first series achieves, and upon the divisions of the subject which may be of interest at the time.

R. H. A. P.
F. G. H.

PREFACE.

THE substance Protein, which constitutes the most important part of the material basis of all animal and vegetable life, has naturally attracted the attention and energy of numerous investigators throughout the past century. Progress in the study of this subject, on account of its difficulty, has been exceedingly slow, and it is only of recent years that the discovery of new methods by Emil Fischer has enabled us to increase our knowledge to its present extent. By these methods we have been able to advance from the conception of "albumin" to its actual separation into numerous units, and also to determine their arrangement in the molecule. On this account a monograph embodying the results of the most recent investigations, together with their connections with the work of the other and earlier investigators, needs no excuse for its appearance, as the subject is now being studied in every direction.

On account of the mass of material connected with the subject, this monograph has exceeded the proposed limit in length, and consequently it has become necessary to divide it into two parts:—

I. { The Chemical Composition of the Protein Molecule.
   { The Chemical Constitution of its Units.

II. The Synthesis of the Proteins.

R. H. A. P.

CONTENTS OF PART I.

THE CHEMICAL CONSTITUTION OF THE PROTEINS.

PART I.

INTRODUCTION.

THE proteins, of which we know some forty or fifty natural ones occurring in both animals and plants, are divided, according to their origin, solubility, coagulability on heating and other physical characteristics, into the following groups:—

I. Protamines, *e.g.*, salmine, sturine, clupeine, scombrine, cyclopterine, cyprinine.

II. Histones, *e.g.*, thymus histone, Lota histone, Gadus histone, histone from blood corpuscles.

III. Albumins, *e.g.*, ovalbumin, conalbumin, serum albumin, various plant albumins.

IV. Globulins, *e.g.*, serum globulin, fibrinogen and its derivative fibrin, myosinogen and its derivative myosin. Crystalline vegetable globulins: edestin, excelsin.

V. Glutelins, *e.g.*, legumin, conglutin, amandin, occurring in plants, soluble in very dilute alkali.

VI. Gliadins, *e.g.*, wheat-gliadin, hordein, zein, occurring in cereals, soluble in 70-80 per cent. alcohol.

VII. Phosphoproteins, *e.g.*, caseinogen, vitellin, ichthulin.

VIII. Scleroproteins, *e.g.*, keratin from hair, horn, feathers, egg-membrane. Collagen, gelatin, elastin. Silk-fibroin, silk-gelatin.

IX. Conjugated Proteins:—

(*a*) Nucleoproteins: nucleic acid in combination with protein, generally I., II., III.

(*b*) Chromoproteins: chromogenic substance in combination with protein, *e.g.*, hæmoglobin.

(*c*) Glucoproteins: carbohydrate in combination with protein, *e.g.*, mucin, ovomucoid.

X. Derivatives of Proteins:—

(*a*) Metaproteins, *e.g.*, acid-albumin, alkali-globulin.

(*b*) Proteoses, *e.g.*, caseose, albumose, globulose.

(*c*) Peptones, *e.g.*, fibrinpeptone.

(*d*) Polypeptides, *e.g.*, glycyl-alanine, leucyl-glutamic acid, a tetrapeptide (2 glycine + 1 alanine + 1 tyrosine).

Except the protamines, the histones and the derivatives of the proteins, all the proteins contain carbon, hydrogen, nitrogen, sulphur and oxygen, and they possess the following elementary composition:—

C	51-55	per cent.
H	7	,,
N	15-17	,,
S	0·4-2·5	,,
O	20-30	,,

from which a formula such as,

$$C_{726}H_{1174}N_{194}S_3O_{214},$$

which is that of globin, the basis of hæmoglobin, can be calculated.

The phosphoproteins and the nucleoproteins contain also the element phosphorus; in the former, probably combined directly with one of the constituents of the protein molecule; in the latter, combined with a purine base or a carbohydrate, which substances constitute nucleic acid.

Investigations upon their chemical constitution have been carried on now for nearly a century, but it is only during the last ten years that, by the work of Emil Fischer and his pupils, any clear view has really been obtained of their actual constitution. The main result of these investigations is that the protein molecule is built up of a series of amino acids, which form the basis of their composition, and of which the following have been definitely determined:—

A. Mono-aminomonocarboxylic acids.

1. Glycine, $C_2H_5NO_2$, or amino-acetic acid.
   $CH_2 . (NH_2) . COOH$
2. Alanine, $C_3H_7NO_2$, or α-aminopropionic acid.
   $CH_3 . CH(NH_2) . COOH$
3. Valine, $C_5H_{11}NO_2$, or α-aminoisovalerianic acid.
   $(CH_3)_2 > CH . CH(NH_2) . COOH$
4. Leucine, $C_6H_{13}NO_2$, or α-aminoisocaproic acid.
   $(CH_3)_2 > CH . CH_2 . CH(NH_2) . COOH$

5. Isoleucine, $C_6H_{13}NO_2$, or α-amino-β-methyl-β-ethyl-propionic acid.

6. Phenylalanine, $C_9H_{11}NO_2$, or β-phenyl-α-aminopropionic acid.

$C_6H_5 . CH_2 . CH(NH_2) . COOH$

7. Tyrosine, $C_9H_{11}NO_3$, or β-parahydroxyphenyl-α-aminopropionic acid.

$HO . C_6H_4 . CH_2 . CH(NH_2) . COOH$

8. Serine, $C_3H_7NO_3$, or β-hydroxy-α-aminopropionic acid.

$CH_2(OH) . CH(NH_2) . COOH$

9. Cystine, $C_6H_{12}N_2O_4S_2$, or dicysteine, or di-(β-thio-α-aminopropionic acid).

$HOOC . CH(NH_2) . CH_2 . S—S . CH_2 . CH(NH_2) . COOH$

B. Monoaminodicarboxylic acids.

10. Aspartic acid, $C_4H_7NO_4$, or aminosuccinic acid.

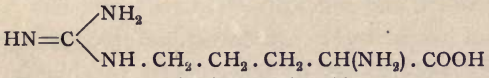
$HOOC . CH_2 . CH(NH_2) . COOH$

11. Glutamic acid, $C_5H_9NO_4$, or α-aminoglutaric acid.

$HOOC . CH_2 . CH_2 . CH(NH_2) . COOH$

C. Diaminomonocarboxylic acids.

12. Arginine, $C_6H_{14}N_4O_2$, or α-amino-δ-guanidinevalerianic acid.



13. Lysine, $C_6H_{14}N_2O_2$, or α, ϵ-diaminocaproic acid.

$H_2N . CH_2 . CH_2 . CH_2 . CH_2 . CH(NH_2) . COOH$

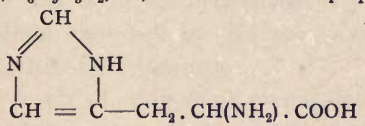
D. Diamino-oxy-monocarboxylic acid.

14. Caseinic acid, or diaminotrioxydodecanic acid.

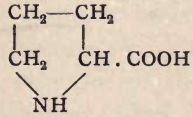
$C_{12}H_{26}N_2O_5$

E. Heterocyclic compounds.

15. Histidine, $C_6H_9N_3O_2$, or β-imidazole-α-aminopropionic acid.



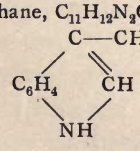
16. Proline, $C_5H_9NO_2$, or α-pyrrolidine carboxylic acid.



17. Oxyproline, or oxypyrrolidine carboxylic acid.

$C_5H_9NO_3$

18. Tryptophane, $C_{11}H_{12}N_2O_2$, or β-indole-α-aminopropionic acid.



This long list is sufficient evidence of the complexity of the protein molecule; and, as yet, it seems to be still incomplete, for several other products have been described. They are not here included, since their presence in the molecule still requires confirmation.

These amino acids are known as the foundation-stones or units of the protein molecule. Before the constitution of a protein can be determined, it is essential that both the amount of any of these units and their chemical composition be known with certainty. The combination together of these amino acids has then to be determined. Consequently the study of the chemical constitution of the protein molecule must be divided into three main sections:—

I. The Chemical Composition of the Protein Molecule.
II. The Chemical Constitution of its Units.
III. The Synthesis of the Proteins.

SECTION I.

THE CHEMICAL COMPOSITION OF THE PROTEIN MOLECULE.

THE methods which have been employed for the purpose of determining the composition of the protein molecule have been many and various. They may be classified under four headings:—

(1) Fusion with alkali.

(2) Oxidation with permanganate, chromic acid, etc.

(3) Action of halogens.

(4) Hydrolysis.

Of these, the last, that of hydrolysis, has thrown most light on the darkness of this complex problem. Hydrolysis has been effected by the action of acids, of alkalies and of the various proteoclastic enzymes which occur in plants and animals, and is practically the only method by which we have attained to our present knowledge. Proteins were first hydrolysed by acids in 1820 by Braconnot, who used dilute sulphuric acid; between 1850 and 1875 hydrochloric acid was most frequently employed as the hydrolysing agent by Ritthausen, Hlasiwetz and Habermann and others, and from 1870 to 1880 Schutzenberger employed baryta water under pressure. The action of vegetable enzymes on proteins has been studied chiefly by Schulze and his co-workers, that of animal enzymes by Kühne, Kossel, Kutscher, Drechsel and numerous other investigators.

As the result of hydrolysis a complex mixture of all, or nearly all, the previously mentioned units is obtained. These have been isolated by various methods based upon the fractional crystallisation of the compounds themselves, or of their copper, silver and other salts. Only when one or more of the amino acids occurred in somewhat large amounts was their isolation and characterisation effected; their amount seldom reached a value higher than 20 per cent. of the total quantity and the remainder was represented by uncrystallisable syrups of unknown nature. A great advance was made when Drechsel discovered that the protein molecule contained diamino acids as well as monoamino acids, and to Kossel and Kutscher we owe our chief knowledge concerning their isolation and estimation. Emil Fischer, in 1901, by his

study of the amino acids and their derivatives, introduced a new method of isolating and separating the monoamino acids, which depended upon the fractional distillation *in vacuo* of their esters, and which is now commonly known as the *ester method*. This method, though not yet really quantitative, has enabled us to obtain a knowledge of some 70 per cent. of the total products resulting by hydrolysis, and it has shown us that phenylalanine, serine and alanine, which were only known to occur in a few, were present in all proteins, and that phenylalanine in its distribution was the principal aromatic constituent, for it often exceeds in amount that of tyrosine and occurs when this latter is absent. Further, it has demonstrated the presence of two new compounds, proline and oxyproline. In their isolation and estimation the various units are therefore divided into two main groups :—

I. The Monoamino acids, including proline and oxyproline.

II. The Diamino acids, including histidine.

I. The Monoamino Acids.

The Isolation and Estimation of Tyrosine, Cystine and Diaminotrioxydodecanic Acid.

Three of the amino acids, namely, tyrosine, cystine and diaminotrioxydodecanic acid are characterised by their extremely slight solubility in neutral aqueous solutions. They are therefore easily obtained after hydrolysis by acids by neutralising and concentrating the solution, when they crystallise out.

The separation of cystine and tyrosine as they are obtained by hydrolysis with hydrochloric acid was described by Mörner in 1901. The protein—hair, keratin from horn, eggshells, etc.—was boiled with five times its quantity of 13 per cent. hydrochloric acid under a reflux condenser on a water bath for six to seven days. The solution was then decolorised with charcoal and evaporated *in vacuo*, and the residue dissolved in 60 to 70 per cent. alcohol. The two acids then crystallised out on neutralising with soda, and were separated by fractional crystallisation from ammonia; if much tyrosine was present it separated out first, but if cystine exceeded tyrosine in quantity this compound crystallised out first; the remainder was only separated with difficulty. Embden separated the mixture of the two acids by means of very dilute nitric acid, in which tyrosine is very easily soluble, but cystine with difficulty. Their separation may also be effected by precipitation with mercuric sulphate in 5 per cent. sulphuric acid solution in which the mercury compound of tyrosine is soluble (Hopkins and Cole).

Hydrolysis by sulphuric acid possesses one great advantage over that by hydrochloric acid, as it can be subsequently completely removed by baryta. This method was employed by Fischer for obtaining tyrosine and diaminotrioxydodecanic acid from proteins, such as caseinogen, which contains very little cystine. The protein was hydrolysed by boiling with five to six times its quantity of 25 per cent. sulphuric acid for twelve to fifteen hours; the solution, after filtration, was diluted with twice its volume of water and neutralised with barium carbonate, or a strong solution of baryta, the excess of which was then removed by dilute sulphuric acid. The solution, together with the water used in thoroughly washing out the precipitate of barium sulphate, was then evaporated down, until these acids crystallised out. They were separated from one another by phosphotungstic acid, which precipitated the diaminotrioxydodecanic acid, and they were estimated by weighing, after removal of the phosphotungstic acid by baryta.

On account of the insolubility of these compounds and the difficulty of filtering and completely washing out the barium sulphate precipitate, in order to abstract from it the whole of the tyrosine, Abderhalden and Teruuchi, in the case of silk, have hydrolysed the protein with hydrochloric acid, the greater part of which was then removed by evaporation *in vacuo;* the remainder of the hydrochloric acid was then estimated in a small aliquot portion, and then separated quantitatively by neutralising with the calculated amount of caustic soda. The tyrosine then crystallised out, and was purified by recrystallisation from water.

A new method of determining the presence of tyrosine by bromination was introduced by Horace Brown and employed by Adrian Brown and Millar in 1906 for estimating the rate at which tyrosine is split off from proteins by the action of trypsin. This method might be used for the estimation of tyrosine in proteins; its non-employment may be due to the fact that tryptophane and also histidine react with bromine and might thus vitiate the result for tyrosine.

The Isolation and Estimation of the other Monoamino Acids.

For the preparation and estimation of the monoamino acids, hydrolysis by hydrochloric acid is more convenient than that by sulphuric acid. It was formerly carried out in the presence of stannous chloride (Hlasiwetz and Habermann) in order that the solution should remain colourless, instead of becoming dark brown, but this addition is not essential, as was shown by Cohn, and was not used by E. Fischer in his researches. Hydrolysis by hydrochloric acid is carried out by heating the protein with three times its quantity of concentrated hydrochloric acid

of specific gravity 1·19, when it gradually passes into solution, if the flask in which it is contained be occasionally shaken and slightly warmed, and then boiling under a reflux condenser for five to ten hours, depending on the particular protein, and until the biuret reaction has completely disappeared. The solution, which may become at first violet, becomes finally of a dark brown colour and a portion of the hydrochloric acid is evolved as gas; it is boiled with charcoal, filtered from humin substances (secondary products probably arising from carbohydrate) and fatty material and consists of a solution of amino acids in 25 per cent. hydrochloric acid.

It is concentrated *in vacuo* to a small volume, and glutamic acid, if present in any large amount, is removed as its hydrochloride by saturating the solution with dry gaseous hydrochloric acid and allowing to stand at 0° C. for some days, when glutamic acid hydrochloride crystallises out. This occurs in the case of caseinogen and certain vegetable proteins, which contain from 10 to 40 per cent. of this amino acid. The glutamic acid hydrochloride is filtered off after adding an equal volume of ice-cold alcohol, redissolved in water, boiled with charcoal and again precipitated as hydrochloride by saturating the solution with gaseous hydrogen chloride, and weighed.

The solution thus freed from the greater part of the glutamic acid is again concentrated *in vacuo* at a low temperature to a thick syrup; this is dissolved in absolute alcohol (3 litres to 1 kilo. protein) and the amino acids are esterified by saturating the alcohol with dry gaseous hydrochloric acid at the ordinary temperature and then warming on the water bath for half an hour. In the process of esterification a large amount of water is formed, which prevents complete esterification; the alcohol is therefore evaporated off *in vacuo* at a temperature below 50° C., and the resulting syrup again dissolved in absolute alcohol and saturated with dry gaseous hydrochloric acid. In some cases it may be necessary to repeat this operation once more. At this stage, glycine, if it occurs in the protein, *e.g.*, in gelatin, in any considerable amount, is separated as glycine ester hydrochloride by seeding the solution with a crystal of this compound and allowing to stand for twenty-four hours at 0° C. It is filtered off while still cold, and the mother-liquor, on further concentration and saturation again with hydrochloric acid, may give another crop of glycine ester hydrochloride, so that almost the whole of the glycine may be isolated in this way. One recrystallisation from alcohol suffices to purify it and it is characterised by its melting point of 144° C. and analysis.

The filtrate containing the esters of the hydrochlorides of the other

amino acids is then concentrated to a syrup *in vacuo* at 40° C. and from this syrup the free esters are extracted as follows:—

About an equal volume of water is added to dissolve the syrup, and, if 1 kilo. of protein has been used, it is divided into four or six portions for convenience and to ensure the subsequent thorough cooling; to each portion one to two volumes of ether are added, and the mixture is thoroughly cooled in a freezing mixture of ice and salt; strong caustic soda is now added till the free hydrochloric acid is neutralised, and then a considerable excess of finely granulated potassium carbonate. The feebly basic esters of aspartic and glutamic acids, which are very sensitive to free alkali, are thus liberated and are dissolved by the ether, which is quickly poured off and replenished by a fresh quantity. The addition of 33 per cent. caustic soda in small portions at a time and of solid potassium carbonate sets free the other esters from their hydrochlorides; these are dissolved by the ether, which is frequently renewed throughout the process and thoroughly mixed with the mass of salt and potassium carbonate; sufficient caustic soda must be added to combine with the whole of the hydrochloric acid, and as much potassium carbonate as is necessary to form a pasty mass in order that the free esters, which are very easily soluble in water, are salted out and dissolved by the ether. A large amount of ether is required for this extraction, and an essential condition is that, throughout the process of extraction, the various portions be kept thoroughly cold by shaking in the freezing mixture.

The several ethereal extracts are then dried by shaking for about fifteen minutes with potassium carbonate; they are then combined together and allowed to stand for twelve hours with anhydrous sodium sulphate.

The ether is next evaporated off, preferably *in vacuo* at the ordinary temperature, as in this way the lower boiling esters do not distil with the ether and the danger of decomposing them by a higher temperature is avoided, and a brown oil, consisting of the esters of the amino acids, is obtained, which is fractionally distilled *in vacuo*.

By this method of extracting the esters from their hydrochlorides, neither that of tyrosine, which remains behind combined with alkali, nor those of the diamino acids, which are soluble with difficulty in ether, are obtained. This is advantageous for the subsequent process of separation, but the method has the disadvantage that the whole quantity of esters is not taken up by the ether on account of their destruction by the alkali. In order to avoid their loss, the mass of carbonate is treated with excess of hydrochloric acid and evaporated down, the potassium

chloride being filtered off as it separates out; the residue is extracted with alcohol and the above process of esterification is repeated.

In order to avoid this loss and to obtain the amino acids as completely as possible, another method was introduced to liberate the esters from their hydrochlorides, *i.e.*, by treatment with sodium ethylate. The hydrochloric acid is therefore removed as completely as possible by evaporation *in vacuo* and the mixture of ester hydrochlorides is dissolved in five times its quantity of absolute alcohol. The amount of chlorine is estimated in a small portion of this, and to the remainder the calculated quantity of sodium dissolved in absolute alcohol, so as to make a 3 per cent. solution, is added. The sodium chloride formed is filtered off, and the alcohol is removed by evaporation *in vacuo*. A small quantity of the lower boiling esters of the amino acids passes into the distillate with the alcohol, but is recovered by acidifying with hydrochloric acid and evaporating when the amino acid hydrochlorides are obtained. A dark brown oil again results, which is fractionally distilled *in vacuo*. Although this method prevents loss by the action of alkali, the yield of the higher boiling fractions is not so great on account of the more complex nature of the mixture of esters. The residue which does not distil contains the tyrosine, the diamino acids and other substances.

The fractional distillation of the brown oil, which is obtained by either of these methods, is carried out firstly at a pressure of 10 to 12 mm. produced by a water pump, and then at a pressure of 0·5 mm. produced by a Geryck vacuum pump, as described by Fischer and Harries.[1]

The temperatures at which the various fractions are collected are those of the vapours of the esters at 10 mm. pressure, and those of the water bath at 100° C. and of an oil bath, which replaces the water bath for the higher temperatures up to 160° C., at 0·5 mm. pressure.

The lower boiling fractions are again distilled *in vacuo* to obtain a further fractionation, but each fraction, even then, does not generally contain a single ester of an amino acid; in the case of the higher boiling fractions a second fractionation is not essential, since the esters contained in them can be separated by their varying solubility in water, ether and petroleum ether. The following table shows the fractions which are collected, and the amino acid esters which they may contain :—

[1] In this process liquid air is used for condensing the vapours in order to preserve the high vacuum; carbonic acid has been used by other investigators, and Levene and van Slyke have recently employed sulphuric acid, cooled by a freezing mixture, as an absorbent for this purpose.

Temperature.	Pressure.	Esters of
Fraction I. To 40° (vapour) .	10 mm.	Glycine, alanine.
,, II. 40-60° (vapour) .	10 mm.	Alanine, leucine, proline.
,, III. 60-90° (vapour) .	10 mm.	Valine, leucine, proline.
,, IV. 100° (water bath) .	0·5 mm.	Leucine, proline.
,, V. 100-130° (oil bath) .	0·5 mm.	Phenylalanine, aspartic acid, glutamic acid, serine.
,, VI. 130-160° (oil bath) .	0·5 mm.	Phenylalanine, glutamic acid, aspartic acid, serine.

The next operation consists in the reconversion of the esters into the amino acids. In the case of the lower boiling fractions, this reconversion is effected by boiling the esters with five to six volumes of water under a reflux condenser for six to seven hours, until the alkaline reaction has disappeared; in the case of the higher boiling fractions, which contain the esters of aspartic and glutamic acids, the reconversion is effected by boiling with baryta water for one and a half to two hours; hydrolysis by water alone only converts these esters into their acid esters. Phenylalanine ester is converted into its hydrochloride by evaporation to dryness with hydrochloric acid.

The Separation and Characterisation of the Individual Monoamino Acids.

The separation, estimation and characterisation of each constituent contained in an ester fraction has still to be carried out after the conversion into the free amino acid, and for each amino acid a somewhat special process has to be performed, which is best described for each individual product.

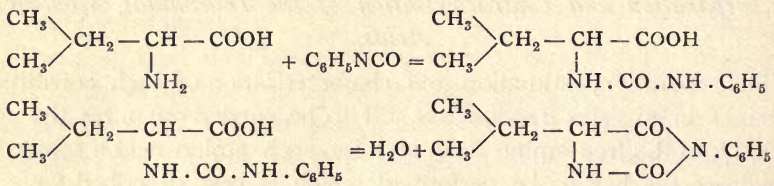
Glycine.—As previously stated, if glycine occurs in large amounts in a protein, the greater quantity of it is separated as ester hydrochloride before the esters are liberated from their hydrochlorides and fractionally distilled. The remainder is contained in the first ester fraction, from which it is obtained by again esterifying and separating as ester hydrochloride. It is identified by its melting point and by elementary analysis, and its amount determined by its yield.

Alanine.—After the glycine has been removed as above described, the alanine is obtained by crystallisation after removal of the hydrochloric acid by boiling with lead hydroxide.

When mixed with valine, leucine and proline, the two former separate in the first fractions on crystallisation when their solution is evaporated; if present in large amounts it crystallises out in an almost pure state. It is separated from proline by evaporating to dryness and boiling with

five to six times the quantity of absolute alcohol, which dissolves the proline, leaving the alanine, which is then purified by crystallisation from water. It may be characterised by its melting point, its rotation in hydrochloric acid solution, by elementary analysis, or by conversion into its benzoyl derivative, which may, however, have a rather low melting point on account of its partial racemisation. It is estimated by its yield.

Valine.—This amino acid is contained mixed with leucine in the fractions of the esters which boil between 60° and 90° C. Its isolation and separation from leucine is of extreme difficulty, since these compounds, as well as their copper salts into which they are converted by boiling with freshly precipitated cupric oxide, tend to form mixed crystals. Its isolation was only effected by these means in certain cases, and its amount is really much more than the figures represent from its yield. It is best characterised by conversion into its phenylhydantoine derivative by treatment with phenylisocyanate in alkaline solution. The phenylureido acid is first formed, and this loses a molecule of water, as shown by Mouneyrat, and is changed into its anhydride or phenylhydantoine by treatment with hydrochloric acid. The following reactions occur:—



Leucine.—The greater part of the leucine is contained in the ester fractions, which boil between 70° and 90° C. It generally occurs in considerable amounts in the protein, and is obtained by crystallisation from water, in which it is less soluble than the other amino acids which may be present. It is seldom present in its pure, optically active form, as this is easily racemised, and the various crops of crystals most probably also contain isoleucine. It is more easily isolated by completely racemising the mixture of amino acids contained in this fraction by heating in an autoclave with baryta to 160-180° C., and then, after removal of the baryta, separating it by crystallisation. The difficulty of separating it from the other amino acids, especially valine and isoleucine, makes an exact quantitative estimation almost impossible. The values which have been found are therefore minimal ones, and they will also include in many cases the yield of isoleucine.

Isoleucine.—The separation of leucine and isoleucine has been

carried out by F. Ehrlich, who makes use of the different solubility of the copper salts of these two amino acids in methyl alcohol. Levene has also employed this method.

Proline.—This is the only product of hydrolysis obtained from an ester fraction which is soluble in alcohol; it is also much more easily soluble in water than the other products with which it is present and therefore is somewhat easily separated, as it remains in the mother-liquor after these have crystallised out. The solution, in which it is contained, is evaporated to dryness and extracted with absolute alcohol; the combined alcoholic extracts from the several fractions are evaporated to dryness and taken up by absolute alcohol several times, so as to remove small amounts of the other amino acids, which, though insoluble in alcohol, are dissolved when proline is present.

As thus obtained, the proline is a mixture of the optically active and the racemic forms; these are separated by conversion into their copper salts and treatment with absolute alcohol which dissolves that of the optically active proline. Their purification is easy, and a determination of the water of crystallisation and of the copper establishes the identity of the compound. The phenylhydantoine derivative may also be used for this purpose.

Phenylalanine.—Phenylalanine is separated in the form of its ester from those of serine and glutamic and aspartic acids. The mixed esters are dissolved in water, and if a large amount of phenylalanine ester be present, it separates in the form of oily drops, but in any case the aqueous solution is extracted with ether. The ester, obtained after removal of the ether, is hydrolysed by evaporation with concentrated hydrochloric acid, and the resulting phenylalanine hydrochloride is purified by crystallisation from strong hydrochloric acid. By evaporating its aqueous solution with ammonia, treating with ice-cold water to dissolve the ammonium chloride, and precipitating it from its hot aqueous solution by alcohol, a pure preparation of phenylalanine is generally obtained, from the weight of which its percentage amount in the protein is calculated.

Glutamic Acid.—The greater part of the glutamic acid is isolated as hydrochloride before the mixture of amino acids is esterified. It is contained with aspartic acid ester in the aqueous solution after the phenylalanine ester has been extracted by ether, and it is separated from aspartic acid, after hydrolysis by baryta, by conversion into its hydrochloride; from this it is obtained by treatment with the calculated quantity of soda to combine with the hydrochloric acid and by crystallisation from water, in which it is soluble with some difficulty.

Elementary analysis of the free acid, or of its hydrochloride, determines its identity and its weight gives the amount in the protein.

Aspartic Acid.—A portion of the aspartic acid, after separation from phenylalanine ester and after hydrolysis by baryta, may separate as barium salt; this is the barium salt of racemic aspartic acid. The remainder is isolated, when the glutamic acid has been removed as hydrochloride, by boiling with lead hydroxide and treating with hydrogen sulphide to remove hydrochloric acid and lead respectively, and by crystallising from water. It may be characterised by conversion into its copper salt, or by analysis, and is estimated by its weight.

Serine.—Its ester is contained in the fractions which distil between 100° and 130° at 0·5 mm. The mixed esters contained in this fraction are treated with a small quantity of water and then with five to six volumes of petroleum ether, which precipitates serine ester as an oil; the oil is then shaken up with petroleum ether to remove admixtures as far as possible and is hydrolysed with baryta water. On removal of the baryta it crystallises when the solution is concentrated, and it is purified by treatment with alcohol, which dissolves other substances which are also present, and recrystallisation from water. Its β-naphthalene sulpho-derivative,

$$C_{10}H_7SO_2Cl + H_2N \cdot CH\begin{matrix}CH_2OH\\COOH\end{matrix} = HCl + C_{10}H_7SO_2 \cdot NH \cdot CH\begin{matrix}CH_2OH\\COOH\end{matrix}$$

obtained by shaking it in alkaline solution with β-naphthalene sulphochloride, is very suitable for its characterisation.

The Isolation of Oxyproline.

Only in a few cases has this compound been isolated from the products of hydrolysis of proteins, since its separation is extremely laborious. It can only be effected after all the other amino acids have been removed by crystallisation and by the ester method, and after the diamino acids have been precipitated by phosphotungstic acid. From the last mother-liquors it is obtained by crystallisation, and is best identified in the form of its β-naphthalene sulpho-derivative.

The Isolation and Estimation of Tryptophane.

Tryptophane is not obtained in any large amount by the hydrolysis of proteins by acids and is best prepared by the action of trypsin. According to Hopkins and Cole, the protein is digested in alkaline solution by trypsin, until the solution gives a maximal coloration when tested with bromine water; the solution is then acidified, boiled and

filtered. The clear solution (better after concentrating and filtering off tyrosine, which crystallises out) is acidified with sulphuric acid until it contains 5 per cent., and then mercuric sulphate dissolved in 5 per cent. sulphuric acid is added as long as a precipitate, which contains tryptophane, cystine and tyrosine, is formed. The precipitate is freed from tyrosine by washing with 5 per cent. sulphuric acid in which the tyrosine compound is soluble, that is, until the washings no longer react with Millon's reagent. It is then decomposed by sulphuretted hydrogen, and the solution containing cystine and tryptophane is again acidified with sulphuric acid to 5 per cent. and fractionally precipitated with the mercuric sulphate reagent. The cystine is thrown down first, and filtered off, and then the tryptophane is precipitated. The precipitate is again decomposed by hydrogen sulphide, and the solution, freed from sulphuric acid, is evaporated down, alcohol being continually added to hasten the evaporation and prevent decomposition of the tryptophane, which is estimated by weighing.

Neuberg and Popowsky, as also Abderhalden and Kempe, have introduced a few alterations in the procedure, such as evaporation *in vacuo*, and Levene and Rouiller suggested in 1906 that the tryptophane, on account of its proneness to decompose on evaporation of its solution with consequent loss, be estimated colorimetrically; the mercury sulphate precipitate is decomposed, and the solution, freed from hydrogen sulphide, is titrated with bromine water in presence of amyl alcohol. Both cystine and tyrosine react with bromine water; the latter can, however, be removed, but for the former a correction has to be made. Up to the present no values concerning the amount of tryptophane in various proteins have appeared, and it will be of interest to see if the values so obtained are very much higher than those obtained by crystallisation of the tryptophane.

II. The Diamino Acids.

The separation and estimation of the three compounds—arginine, histidine, lysine—is carried out by the method described by Kossel and Kutscher in 1900, which was slightly modified in 1903 by Kossel and Patten. It is based upon the earlier work of Drechsel, Hedin and Kossel, and depends upon the precipitation of arginine and histidine as their silver salts, and of lysine by phosphotungstic acid, and then by picric acid.

As described by Kossel, Kutscher and Patten the method is as follows:—

I. About 25 to 50 grammes of protein are hydrolysed by boiling with

a mixture of three times the weight of concentrated sulphuric acid and six times the weight of water under a reflux condenser for fourteen hours. The exact amount of protein is then estimated by making the volume up to 1 litre, and determining the nitrogen in 5 or 10 c.c. by Kjeldahl's method; from this figure the amount of protein can be calculated, if the amount of nitrogen in it be known.

II. The acid solution is treated with a hot concentrated solution of baryta until the reaction is only faintly acid and almost the whole of the sulphuric acid is precipitated as barium sulphate, which is filtered off and thoroughly washed out with boiling water. The filtrate and washings are evaporated down and again made up to 1 litre. A determination of the nitrogen in 5 or 10 c.c. of this solution gives the amount of nitrogen contained in the melanin, which is carried down by the barium sulphate. It is known as "humin nitrogen I.".

In this liquid also two determinations are made of the amount of nitrogen present as ammonia by distilling portions of 100 c.c. with magnesium oxide. From the remainder the ammonia is removed by evaporating with magnesia on the water bath. The three portions, free from ammonia, are then combined, and made alkaline with baryta. The precipitate of barium sulphate is filtered off and thoroughly washed out, the excess of barium removed by dilute sulphuric acid and the precipitate again filtered off and washed out. Filtrate and washings are combined together, evaporated down and made up to 1 litre and a Kjeldahl nitrogen determination again made. Allowing for the nitrogen given off as ammonia, the difference between this and the previous estimation gives the humin nitrogen II. contained in the alkaline barium magnesia precipitate.

III. The solution, now containing a small quantity of sulphuric acid, is placed in a 5-litre flask, made up to 3 litres and heated on the water bath. Finely powdered silver sulphate is slowly added until the solution contains sufficient to give a yellow, not a white, precipitate, when a drop is removed and tested with baryta water in a watch-glass. If, during the process, there be any undissolved silver sulphate at the bottom of the flask it is dissolved by adding more water before a fresh quantity is added, in order that a yellow precipitate be given in the test drop with baryta. As soon as sufficient silver is present to combine with all the arginine and histidine, it is allowed to cool to 40° C. and then saturated with finely powdered baryta. The precipitate which is thus formed, and which consists of the silver salts of arginine and histidine, is filtered off, stirred up with baryta, again filtered off and washed with baryta water.

IV. Separation of arginine and histidine. The above precipitate of the silver salts of these compounds is suspended in water containing sulphuric acid, and decomposed with hydrogen sulphide. The filtrate from the silver sulphide, which is thoroughly extracted with boiling water, is evaporated down to remove the hydrogen sulphide and again made up to 1 litre; a Kjeldahl nitrogen determination in 20 c.c. now gives the amount of nitrogen in the substances precipitated by silver and baryta.

The liquid is now neutralised with baryta, and barium nitrate is added, so long as a precipitate of barium sulphate is formed; this is filtered off and washed. The filtrate is concentrated to 300 c.c. and treated with silver nitrate, as before, till a test drop gives a yellow colour with baryta; when this occurs it is exactly neutralised with baryta, and from a burette small quantities of baryta are added till the silver salt of histidine is completely precipitated; this is determined by taking out a drop when the precipitate has settled and testing with ammoniacal silver solution; if a precipitate easily soluble in excess of ammonia be formed, when the two liquids come together, histidine is still present; and more baryta water must be added, until it is completely thrown out, when it is filtered off, stirred up with water, again filtered off and washed out.

The precipitate of the silver salt of histidine is then stirred up in a measured quantity of 5 per cent. sulphuric acid and decomposed with hydrogen sulphide. The filtrate and washings from the silver sulphide are concentrated so that the solution contains $2\frac{1}{2}$ per cent. sulphuric acid and then precipitated by not too large an excess of mercuric sulphate. The precipitate is allowed to stand for twelve to twenty-four hours, when it is filtered off and decomposed by sulphuretted hydrogen. A nitrogen determination in this solution by Kjeldahl's method gives the amount of nitrogen from which the amount of histidine can be calculated; the histidine itself is obtained by making alkaline with baryta, filtering off the barium sulphate, removing excess of baryta by carbon dioxide, evaporating to dryness, extracting the residue with boiling water, filtering from barium carbonate, adding hydrochloric acid and evaporating down, when histidine hydrochloride $C_6H_9N_3O_2 . 2HCl$ is obtained.

V. The filtrate containing the arginine is saturated with baryta, and the precipitate of the silver salt of arginine, so obtained, is stirred up with baryta, filtered off and washed till free from nitric acid. It is then suspended in water containing sulphuric acid and decomposed with hydrogen sulphide. The filtrate and washings from the precipitate

of silver sulphide are evaporated down and made up to 1 litre; the amount of arginine is calculated from the amount of nitrogen determined in 10 or 20 c.c. of this liquid by Kjeldahl's method. The remainder is freed from sulphuric acid by baryta, the excess of which is removed by carbon dioxide, and the arginine is determined as nitrate $C_6H_{14}N_4O_2 . HNO_3 + \frac{1}{2} H_2O$ by neutralising with nitric acid and evaporating and drying, when it is obtained as a dry white crystalline mass.

VI. The lysine is obtained from the filtrate from the precipitate of arginine and histidine. This is acidified with sulphuric acid, freed from silver by hydrogen sulphide and evaporated to 500 c.c. Sulphuric acid is then added until the content is 5 per cent. and the lysine is precipitated by phosphotungstic acid. The precipitate is filtered off and thoroughly washed, and is decomposed by baryta; the barium phosphotungstate formed is filtered off, and the filtrate, freed from baryta by carbon dioxide, is evaporated almost to dryness; the residue is dissolved in water, filtered from barium carbonate and again evaporated; it is then treated with small quantities of alcoholic picric acid, so long as a precipitate is formed; excess must be avoided as lysine picrate is soluble in excess. After some hours it is filtered off and washed with very little absolute alcohol; it is then dissolved in boiling water and evaporated, when lysine picrate $C_6H_{14}N_2O_2 . C_6H_2 (NO_3)_2 . OH$ crystallises out, and is collected on a weighed filter. The mother-liquor yields a little more lysine picrate, which is treated in the same way.

The separation and estimation of the two main groups of amino acids can be carried out in one experiment, instead of separately as described. The protein is hydrolysed by sulphuric acid, the tyrosine, cystine and diaminotrioxydodecanic acid are removed by crystallisation, and the diamino acids are precipitated by phosphotungstic acid. From this precipitate they are obtained by decomposition with baryta, and they are then separated by means of their silver compounds by Kossel, Kustcher and Patten's method. The filtrate from the phosphotungstic acid precipitate is freed from the excess of phosphotungstic acid by means of baryta, and the solution is treated by Fischer's ester method for the monoamino acids.

The combination of the two processes is generally only carried out when the amount of protein available is limited; they require very different quantities of material; thus, the diamino acids can be determined in 25 to 50 grammes of protein with considerable accuracy, whereas the monoamino acids can only be determined with fair accuracy when 250 to 500 grammes of protein can be used. On the whole, it is not

advisable to combine the processes, since the phosphotungstic acid precipitation does not effect a perfect separation of the two groups.

A very large number of proteins have now been hydrolysed and the products of hydrolysis determined by these methods by investigators not only in Germany, but also in France and America. The results are collected together in the following tables which are arranged according to the classification of the proteins.

PROTAMINES.

	Salmine. (Kossel; Abderhalden; Kossel and Dakin.)	Sturine. (Kossel and Kutscher.)	Clupeine. (Kossel and Kutscher; Kossel and Dakin.)	Scombrine. (Kossel.)	Cyclopterine. (Kossel and Kutscher; Kossel; Morkowin.)	Cyprinine I. (Kossel and Dakin.)	Cyprinine II.
Glycine	...	...	...	...	...	...	...
Alanine	...	...	+	...	...	...	...
Valine	4·3	...	+	...	...	...	+
Leucine	...	...	...	...	...	...	...
Isoleucine	...	...	...	...	...	...	...
Phenylalanine	...	...	...	...	...	...	...
Tyrosine	...	...	...	...	8·3	+ ?	+
Serine	7·8	...	+	...	...	...	...
Cystine	...	...	...	...	...	...	...
Proline	11·0	...	+	...	...	...	...
Oxyproline	...	...	...	...	...	...	...
Aspartic Acid	...	...	...	...	...	...	...
Glutamic Acid	...	...	...	...	...	...	...
Tryptophane	...	...	...	+	+	...	...
Arginine	87·4	58·2	82·2	+	62·5	4·9	+
Lysine	0	12·0	0	0	0	28·8	+
Histidine	0	12·9	0	0	0	0	0
Diaminotrioxydodecanic Acid	...	...	..	...	...	...	...
Ammonia	...	...	...	...	...	...	...
Total	110·5	83·1	82·2	...	70·8	33·7	...

HISTONES.

	Globin of Hæmoglobin from Horse's Blood. (Abderhalden.)	Globin of Hæmoglobin from Dog's Blood. (Abderhalden and Baumann.)	Thymus-Histone (Kossel and Kutscher; Kutscher; Abderhalden and Rona.)		Lota-Histone. (Ehrström.)	Gadus-Histone. (Kossel and Kutscher.)
Glycine	...	...	...	0·5	...	...
Alanine	4·19	3·0	...	3·5	...	...
Valine	...	1·0	...	...	...	...
Leucine	29·04	17·5	...	11·8	...	...
Isoleucine	...	...	...	...	...	...
Phenylalanine	4·24	5·0	...	2·2	...	...
Tyrosine	1·33	...	6·4	5·2	...	...
Serine	0·56	...	...	...	...	...
Cystine	0·31	...	...	...	...	...
Proline	2·34	4·5	...	1·5	...	...
Oxyproline	1·04	...	...	...	...	...
Aspartic Acid	4·43	2·5	...	0	...	...
Glutamic Acid	1·73	1·2	3·7	0·5	...	...
Tryptophane	+	...	...	...	...	...
Arginine	5·42	...	14·4	15·5	12·0	15·6
Lysine	4·28	...	7·7	6·9	3·2	8·3
Histidine	10·96	...	1·3	1·5	2·9	2·4
Diaminotrioxy-dodecanic Acid	...	...	...	...	...	...
Ammonia	...	...	1·7	...	0·7	0·8
Total	69·87	34·7	35·2	49·1	18·8	27·1

ALBUMINS.

	Egg-albumin. (Abderhalden and Pregl; Mörner.)	Serum-albumin. (Abderhalden; Mörner.)	Lact-albumin. (Abderhalden and Pribram.)	Bence-Jones Albumin.[1] (Abderhalden and Rostoski.)
Glycine	0	0	0	1·7
Alanine	2·1	2·7	2·5	4·5
Valine	...	...	0·9	...
Leucine	6·1	20·0	19·4	10·6
Isoleucine	...	...	...	...
Phenylalanine	4·4	3·1	2·4	1·5
Tyrosine	1·1	2·1	0·9	1·7
Serine	...	0·6	...	...
Cystine	0·3	2·5	...	...
Proline	2·3	1·0	4·0	1·9
Oxyproline	...	...	...	...
Aspartic Acid	1·5	3·1	1·0	4·5
Glutamic Acid	8·0	7·7	10·1	6·0
Tryptophane	+	+	...	...
Arginine	...	...	...	...
Lysine	...	...	...	...
Histidine	...	...	...	...
Diaminotrioxy-dodecanic Acid	...	...	...	...
Total	25·8	42·8	41·2	32·4

[1] This protein is included under this group on account of its name; no classification has yet been given to it, though from its reactions it is more closely allied to the proteoses.

GLOBULINS.

	Serum-globulin. (Abderhalden; Abderhalden and Samuely; Mörner.)		Fibrin. (Abderhalden and Voitinovici; Mörner.)
Glycine	3·5	...	3·0
Alanine	2·2	...	3·6
Valine	+	...	1·0
Leucine	18·7	...	15·0
Isoleucine	...	...	...
Phenylalanine	3·8	...	2·5
Tyrosine	2·5	...	3·5
Serine	...	...	0·8
Cystine	0·7	1·5	1·1
Proline	2·8	...	3·6
Oxyproline	...	...	...
Aspartic Acid	2·5	...	2·0
Glutamic Acid	8·5	...	10·4
Tryptophane	+	...	+
Arginine	...	...	...
Lysine	...	...	...
Histidine	...	...	...
Diaminotrioxydodecanic Acid	...	...	...
Total	45·2	...	46·5

CRYSTALLISED VEGETABLE GLOBULINS.

	Edestin from Hemp Seed. (Abderhalden; Kossel and Patten; Schulze and Winterstein.)			Edestin from Cotton Seed. (Abderhalden and Rostoski.)	Edestin from Sunflower Seed. (Abderhalden and Reinbold.)	Cryst. Globulin from Pumpkin Seed. (Abderhalden and Berghausen.)	Cryst. Globulin from Squash Seed. (Osborne and Clapp; Osborne and Gilbert.)	Excelsin from Brazil Nut. (Osborne and Clapp.)
Glycine	...	3·8	...	1·2	2·5	0·1	0·6	0·6
Alanine	...	3·6	...	4·5	4·5	+	1·9	2·3
Valine	...	+	...	+	0·6	0·7	0·3	1·5
Leucine	...	20·9	...	15·5	12·9	4·7	7·3	8·7
Isoleucine	...	...	...	...	...	...	...	...
Phenylalanine	...	2·4	...	3·9	4·0	2·6	3·3	3·5
Tyrosine	...	2·1	...	2·3	2·0	1·4	3·1	3·1
Serine	...	0·4	...	0·4	0·2	...	...	...
Cystine	...	0·3	...	...	...	...	0·3	...
Proline	...	1·7	...	2·3	2·8	1·7	2·9	3·6
Oxyproline	...	2·0	...	...	...	...	...	...
Aspartic Acid	...	4·5	...	2·9	3·2	4·5	3·3	3·8
Glutamic Acid	...	6·3	...	17·2	13·0	13·4	12·4	12·9
Tryptophane	...	+	...	+	+	...	+	+
Arginine	11·2	11·7	14·4	...	...	...	14·4	16·1
Lysine	1·6	1·0	1·7	...	...	...	2·0	1·6
Histidine	1·4	1·1	2·4	...	...	...	2·6	1·5
Diaminotrioxy-dodecanic Acid	...	...	...	...	...	...	...	...
Ammonia	...	...	...	...	...	...	1·6	1·8
Total	...	61·8	...	50·2	45·7	29·1	56·0	61·0

GLUTELINS.

	Legumin, from Pea. (Osborne and Clapp.)	Amandin, from Almond. (Osborne and Clapp; Osborne and Gilbert.)	Glycinin, from Soy Bean. (Osborne and Clapp.)	Phaseolin, from White Kidney Bean. (Osborne and Clapp.)	Protein of Maize. (Osborne and Clapp.)
Glycine	0·38	0·51	0·97	0·6	0·3
Alanine	2·08	1·40	...	1·8	...
Valine	...	0·16	0·68	1·1	...
Leucine	8·00	4·45	8·45	9·7	6·2
Isoleucine	...	...	...	...	...
Phenylalanine	3·75	2·53	3·86	3·3	1·8
Tyrosine	1·55	1·12	1·86	2·2	3·8
Serine	0·53	?	...	0·4	...
Cystine	...	...	...	...	...
Proline	3·22	2·44	3·78	2·8	5·0
Oxyproline	...	...	...	...	...
Aspartic Acid	5·30	5·42	3·89	5·3	0·7
Glutamic Acid	13·80	23·14	19·46	14·6	12·7
Tryptophane	+	+	...	...	+
Arginine	10·12	11·85	5·12	4·9	7·1
Lysine	4·29	0·70	2·71	4·0	3·0
Histidine	2·42	1·58	1·39	2·0	3·0
Diaminotrioxydodecanic Acid	...	...	...	...	...
Ammonia	1·99	3·70	2·56	2·1	2·1
Total	57·43	59·00	54·73	54·8	45·7

GLUTELINS.

	Protein, from Fir-tree Seed. (Abderhalden and Teruuchi; Schulze and Winterstein.)	Conglutin, from Lupine Seed. (Abderhalden and Herrick; Schulze and Winterstein.)	Legumin, from Bean. (Abderhalden and Babkin; Schulze and Winterstein.)	Avenin, from Oats. (Abderbalden and Hämäläinen.)	Gluten, from Wheat. (Abderhalden and Malengreau; Kossel and Kutscher.)
Glycine	0·6	0·8	1.0	1·0	0·4
Alanine	1·8	2·5	2·8	2·5	0·3
Valine	+	1·1	1·0	1·8	...
Leucine	6·2	6·8	8·2	15·0	4·1
Isoleucine	...	...	...	...	...
Phenylalanine	1·2	3·1	2·0	3·2	1·0
Tyrosine	1·7	2·1	2·8	1·5	1·9
Serine	0·1	+	...	...	...
Cystine	0·3	...	...	...	...
Proline	2·8	2·6	2·3	5·4	4·0
Oxyproline	...	...	...	...	...
Aspartic Acid	1·8	3·0	4·0	4·0	0·7
Glutamic Acid	7·8	19·5	16·3	18·4	24·0
Tryptophane	+	+	...	...	+
Arginine	10·9	6·9	4·6	...	4·4
Lysine	0·3	2·1	5·1	...	2·2
Histidine	0·7	0·7	1·1	...	1·2
Diaminotrioxydodecanic Acid	...	...	...	...	...
Ammonia	...	...	...	...	2·5
Total	36·2	51·2	51·2	52·8	46·7

GLIADINS.

	Gliadin Wheat. (Abderhalden and Samuely; Kossel and Kutscher; Kutscher.)	Gliadin, from Wheat. (Osborne and Clapp.)	Gliadin, from Rye. (Osborne and Clapp.)	Hordein, from Barley. (Osborne and Clapp.)	Hordein. (Klein-schmitt.)	Zein, from Maize. (Osborne and Clapp.)	Zein Maize. (Langstein; Kutscher; Kossel and Kutscher.)
Glycine . .	0·7	0·02	0·13	0·00	0	0·00	–
Alanine . .	2·7	2·00	1·33	0·43	1·4	2·23	0·5
Valine . .	0·4	0·21	...	0·13	1·4	0·29	+
Leucine . .	6·0	5·61	6·30	5·67	7·0	18·60	11·2
Isoleucine. .	...	...	...	...	...	...	...
Phenylalanine .	2·6	2·35	2·70	5·03	5·5	4·87	7·0
Tyrosine . .	2·4	1·20	1·19	1·67	4·0	3·55	10·1
Serine . .	0·2	0·13	0·06	...	0·1	0·57	–
Cystine . .	...	0·45	...	...	...	...	...
Proline . .	2·4	7·06	9·82	13·73	5·9	6·53	1·5
Oxyproline .	...	...	...	...	...	...	...
Aspartic Acid .	1·3	0·58	0·25	...	1·3	1·41	1·0
Glutamic Acid .	31·5	37·33	33·81	36·35	41·3	18·28	11·8
Tryptophane .	1·0	+	+	+	...	0·00	...
Arginine . .	2·8	3·16	2·22	2·16	3·2	1·16	1·9
Lysine . .	0·0	0·00	0·00	0·00	0·0	0·00	0·0
Histidine . .	1·2	0·61	0·39	1·28	0·5	0·43	0·9
Diaminotrioxy-dodecanic Acid	...	...	...	...	...	...	...
Ammonia . .	4·1	5·11	5·11	4·87	4·4	3·61	2·6
Total . .	59·3	65·81	64·31	71·32	76·0	61·53	48·5

PHOSPHOPROTEINS.

	Caseinogen, Cow's Milk. (Abderhalden; Fischer; Mörner; Fischer and Abderhalden; Hart.)	Caseinogen, Goat's Milk. (Abderhalden and Schittenhelm.)	Caseinogen, Human Milk. (Abderhalden and Schittenhelm.)	Vitellin. (Abderhalden and Hunter.)	Vitellin. (Levene and Alsberg.)	Vitellin. (Hugounenq.)
Glycine . .	0	0	...	1·1	trace	<0·5
Alanine . .	0·9	1·5	...	+	0·2	<0·5
Valine . .	1·0	...	...	2·4	...	1·5
Leucine . .	10·5	7·4	...	11·0	3·3	6·8
Isoleucine .	...	...	...	...	...	...
Phenylalanine .	3·2	2·75	...	2·8	1·0	0·7
Tyrosine . .	4·5	4·95	4·71	1·6	0·4	2·0
Serine . .	0·23	...	...	–	...	<0·5
Cystine . .	0·06	...	...	...	...	...
Proline . .	3·1	4·6	...	3·3	4·0	<0·5
Oxyproline .	0·25	...	...	...	...	...
Aspartic Acid .	1·2	1·2	...	0·5	0·6	0·7
Glutamic Acid .	11·0	12·0	...	12·2	1·0	1·0
Tryptophane .	1·5	+	...	...	...	...
Arginine . .	4·84	...	...	...	1·2	1·0
Lysine . .	5·80	...	...	...	2·4	1·2
Histidine . .	2·59	...	...	...	trace	2·1
Diaminotrioxy-dodecanic Acid	0·75	+	...	...	...	...
Ammonia . .	1·6	...	...	...	...	1·2
Total . .	53·42	34·4	...	34·9	14·1	20·2

SCLEROPROTEINS.

	Gelatin. (Fischer, Levene and Aders; Fischer, Hart, Kossel and Kutscher.)		Silk-gelatin. (Fischer and Skita; Fischer.)	Silk-fibroin. (Fischer and Skita; Fischer.)	Spider-silk-fibroin. (Fischer.)	Elastin. (Abderhalden and Schittenhelm; Schwarz; Kossel and Kutscher.)	Spongin. (Abderhalden and Strauss; Kossel and Kutscher.)	Koilin. (Knaffl-Lenz.)	Egg-membrane of Scyllium Stellare. (Pregl.)
Glycine	...	16·5	0·2	36·0	35·2	25·8	13·9	...	2·6
Alanine	...	0·8	5·0	21·0	23·4	6·6	...	...	3·2
Valine	...	1·0	...	0	...	1·0	...	...	...
Leucine	...	2·1	...	1·5	1·8	21·4	7·5	...	5·8
Isoleucine	...	–	...	...	...	...	...	...	...
Phenylalanine	...	0·4	...	1·5	...	3·9	...	...	3·3
Tyrosine	...	0	5·0	10·5	8·2	0·4	0	...	10·6
Serine	...	0·4	6·6	1·6	...	...	...	...	...
Cystine	...	–	...	...	...	...	...	...	?
Proline	...	5·2	...	+	3·7	1·7	6·3	...	4·4
Oxyproline	...	3·0	...	...	...	...	..	...	...
Aspartic Acid	...	0·6	...	+	...	+	4·7	...	2·3
Glutamic Acid	...	0·9	...	0	11·7	0·8	18·1	...	7·2
Tryptophane	...	0	...	...	...	...	...	...	+
Arginine	9·3	7·6	+	1·0	} 5·24	0·3	...	3·6	3·2
Lysine	5	2·8	...	+		...	...	1·7	3·7
Histidine	...	0·4	4·0	+		...	...	0·1	1·7
Diaminotrioxydodecanic Acid	...	–	...	...	...	...	...	...	...
Ammonia	...	0·4	...	...	1·2	...	...	...	...
Total	...	42·1	20·8	73·1	90·44	61·9	50·5	5·4	48·0

SCLEROPROTEINS.

	Keratin, from Ox Horn. (Fischer and Dörpinghaus; Mörner.)	Keratin, from Sheep's Horn. (Abderhalden and Voitinovici.)	Keratin, from Sheep's Wool. (Abderhalden and Voitinovici.)	Keratin, from Horse Hair. (Abderhalden and Wells.)	Keratin, from Goose Feathers. (Abderhalden and Le Count.)	Keratin, from Egg-membrane. (Abderhalden and Ebstein; Mörner.)	Keratin, from Egg-membrane of Testudo Græca. (Abderhalden and Strauss.)	Ichthylepidin, from Fish Scales. (Abderhalden and Voitinovici.)
Glycine	0·4	0·5	0·6	4·7	2·6	3·9	+	5·7
Alanine	1·2	1·6	4·4	1·5	1·8	3·5	+ ?	3·1
Valine	5·7	4·5	2·8	0·9	0·5	1·1	...	...
Leucine	18·3	15·3	11·5	7·1	8·0	7·4	...	15·1
Isoleucine	...	...	...	...	...	...	...	...
Phenylalanine	3·0	1·9	...	0	0	...	+ ?	...
Tyrosine	4·6	3·6	2·9	3·2	3·6	...	...	1·0
Serine	0·7	1·1	0·1	0·6	0·4	...	...	...
Cystine	6·8	7·5	7·3	{ above 10·0 }	...	7·6	...	...
Proline	3·6	3·7	4·4	3·4	3·5	4·0	11·8 ?	6·7
Oxyproline	...	...	...	...	...	...	...	...
Aspartic Acid	2·5	2·5	2·3	0·3	1·1	1·1	1·8 ?	1·2
Glutamic Acid	3·0	17·2	12·9	3·7	2·3	8·1	3·0 ?	9·2
Tryptophane	...	...	...	...	...	...	...	...
Arginine	2·3	2·7	...	...	...	...	...	...
Lysine	...	0·2	...	...	...	...	...	...
Histidine	...	...	...	...	...	...	..	...
Diaminotrioxydodecanic Acid	...	...	...	...	...	...	...	...
Total	52·1	62·3	49·2	35·4	23·8	36·7	16·6 ?	42·0

DERIVATIVES OF PROTEINS.

	Syntonin. (Abderhalden and Sasaki; Hart.)	Hetero-albumose, from Syntonin. (Hart.)	Prot-albumose, from Syntonin. (Hart.)	Deutero-albumose, from Witte's Peptone. (Haslam.)	Hetero-albumose, from Witte's Peptone. (Haslam.)	Protein in Urine. (Abderhalden and Pregl.)
Glycine	0·5	...	...	...	...	+
Alanine	4·0	...	...	...	...	+
Valine	0·9	...	...	...	...	...
Leucine	7·8	...	...	...	...	+
Isoleucine	...	...	...	...	...	...
Phenylalanine	2·5	...	...	...	...	...
Tyrosine	2·2	...	...	...	..	...
Serine	...	...	...	...	...	...
Cystine	...	...	...	...	...	...
Proline	3·3	...	...	...	...	...
Oxyproline	...	...	...	...	..	...
Aspartic Acid	0·5	...	...	...	...	+
Glutamic Acid	13·6	...	...	...	...	+
Tryptophane	...	...	...	...	...	...
Arginine	5·1	8·5	4·6	7·1	4·9	...
Lysine	3·3	7·1	3·1	6·9	3·5	...
Histidine	2·7	0·4	3·4	1·5	2·2	...
Diaminotrioxydodecanic Acid	...	...	...	...	...	...
Ammonia	0·9	1·0	0·8	1·0	0·8	...
Total	47·3	17·0	11·9	16·5	11·4	

The result of hydrolysis definitely shows that the various proteins are composed of the same units; in some cases certain are missing, and in other cases one or more units exceed the others by very large amounts.

The protamines are built up almost exclusively of diamino acids, salmine containing over 80 per cent. of arginine. Only small amounts of monoamino acids are present in them, and even these amounts may be due to impurity, for fish sperm only at maturity is made up of protamine and nucleic acid, whereas at other times histone takes the place of protamine, and histones contain less diamino acids. Kossel and Dakin's analysis appears to show us a quantitative result in the case of salmine.

The histones contain about 30 per cent. of diamino acids, and only in the case of thymus-histone has an estimation been made of the monoamino acids. They were supposed to be intermediate compounds between protamines and other proteins, and this supposition is confirmed by the results of analysis.

The protein constituent—globin—of hæmoglobin has always been regarded as a histone, but the presence of only 20 per cent. of diamino

acids is against this supposition. Further, the principal diamino acid is histidine, whereas in the other histones it is arginine. It should be noted that hæmoglobin contains a considerably greater amount of histidine than the other proteins.

Albumins contain no glycine, whereas globulins contain this amino acid. Their differentiation on physical grounds is thus borne out by chemical analysis.

No great difference is to be noted between the crystalline vegetable globulins and the vegetable proteins soluble in dilute alkali, but there is a marked difference between these proteins and the alcohol-soluble vegetable proteins. These contain scarcely any glycine, the small amount obtained is probably due to impurity; except in zein the amount of leucine in them in comparison with other proteins is small. The most distinct features of these proteins are the absence of lysine and the presence of an enormous quantity of glutamic acid (except zein); the amount of proline is also high, and the amount of arginine is low. It is curious that those amino acids which are absent in zein are present in the protein of maize, which is soluble in dilute alkali. The mixture of these proteins in the grain, therefore, gives all the amino acids present in other proteins. As the gliadins are all so much alike, it would seem that they are the same protein.

There is no striking peculiarity noticeable with the phosphoproteins; the two caseinogens examined appear to have the same composition. Vitellin, which has been hydrolysed by three sets of investigators, has given very different results; the most detailed examination is that by Hugounenq, whose values are lower than those of Abderhalden and Hunter and of Levene and Alsberg; the latter observers used a purified product, whereas the former used the commercial article. The amount of glycine is small in vitellin and it corresponds in this particular with caseinogen.

The scleroproteins, which represent a heterogeneous collection of proteins in their physical properties give on hydrolysis, as would be expected, results which support their classification.

Gelatin contains no tryptophane, cystine or tyrosine, but it contains a large amount of glycine and a considerable amount of proline. It appears to have no similarity to silk-gelatin, which contains so much serine.

Silk-fibroin is composed of practically only three amino acids, glycine, alanine and tyrosine, and is probably the simplest protein known. It resembles the fibroin of spider's silk very closely, but here the presence of so much glutamic acid is one of the characteristics.

Elastin again seems to be made up of only three or four amino acids; spongin is rather more complex.

The keratins are distinguished by containing more cystine than any other protein; in human hair it exists to the extent of about 14 per cent. (Mörner). Tyrosine also is present in fair quantities.

The presence of diamino acids in all proteins led Kossel to suppose that there was a protamine nucleus (*i.e.*, of diamino acids) in all proteins; the more recent work, especially that by Osborne and Clapp on the gliadins, where the diamino acids are present in such small amounts, though it supports the theory, yet suggests that proteins may exist in which it is not present, more especially if the view of Emil Fischer be taken that all the proteins we know, even the crystalline ones, are still mixtures of several proteins. The isolation of complexes containing only diamino acids from proteins, where they are combined together, will be the only proof of a protamine nucleus in a protein molecule.

SECTION II.

THE CHEMICAL CONSTITUTION OF ITS UNITS, OR THE DISCOVERY AND SYNTHESES OF THE AMINO ACIDS.

IN Section I. an account was given of how the units of the protein molecule are now isolated and estimated, and the results were embodied in several tables. In general, it may be said, that the amino acids were first discovered in that protein in which they occurred in largest amounts. An account will be given in this section of the discovery and of the determination of the constitution of each amino acid.

A. MONOAMINO-MONOCARBOXYLIC ACIDS.

Glycine.

This, the simplest of the products of hydrolysis of the proteins, was also the first to be discovered; it was obtained by Braconnot, in 1820, by boiling gelatin with dilute sulphuric acid, and on account of its sweet taste he called it sugar of gelatin. In 1846 Dessaignes obtained it from hippuric acid by hydrolysis, and, in 1848, Strecker showed that cholic acid (now glycocholic acid) consisted of this amino acid and cholalic acid, so that, as a constituent of substances of animal origin, it became of great importance. Its presence in elastin was demonstrated by Jeanneret, in horn by Horbaczewski, in spongin by Krukenberg, in conchiolin by Wetzel, and in silk-fibroin by Cramer; Faust and Spiro showed that it was present in globulin. It does not occur in albumin, nor in caseinogen, nor in hæmoglobin; it is present only to a small extent in the vegetable proteins, and for this reason it was not isolated until Abderhalden showed its presence in these proteins by Fischer's ester method.

In the free state, glycine was found by Chittenden in an extract of the American mussel, *Pecten irradians*, and of recent years it has been recorded as sometimes occurring in the urine.

Its elementary composition of $C_2H_5NO_2$ was first correctly determined in 1846 by Mulder and by Laurent, and in this year, after Dessaignes had pointed out the unsuitability of the name given to it of

sugar of gelatin, as there were other substances like it with a sweet taste and which were not fermentable, its name of glycocoll (γλυκυς, sweet, κολλα, glue) originated and was first used by Horsford, who made an extensive study of it and its derivatives, whilst working in Liebig's laboratory where much of the early work on proteins was carried out.

Laurent regarded glycocoll as belonging to the ammonia type of organic compounds; it was supposed by Cahours to be a derivative of acetic acid, which supposition was only proved by its synthesis from bromacetic acid and ammonia by Perkin and Duppa, and from chloracetic acid and ammonia by Cahours, both in 1858:—

$$CH_2Cl.COOH + NH_3 = HCl + CH_2.NH_2.COOH.$$

About this time the terms glycocine and glycine were used for glycocoll as it was then recognised as a homologue of alanine and leucine. The whole of this series of compounds were termed the glycines.

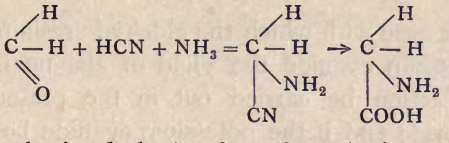
A very interesting synthesis of glycine was described by Emmerling, in 1873, by the action of hydriodic acid upon cyanogen; here the hydriodic acid acts both as a reducing agent and as a hydrolysing agent:—

$$\begin{matrix} CN \\ | \\ CN \end{matrix} + 5HI + 2H_2O = \begin{matrix} CH_2.NH_2 \\ | \\ COOH \end{matrix} + NH_4I + 2I_2,$$

and, in 1877, Wallach obtained it by the reduction of cyanoformic ester with zinc:—

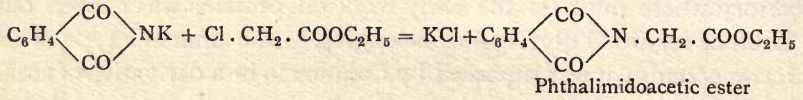
$$\begin{matrix} COOC_2H_5 \\ | \\ CN \end{matrix} + 4H = \begin{matrix} COOC_2H_5 \\ | \\ CH_2.NH_2 \end{matrix}$$

Lubavin, in 1882, stated that glycine was formed by the action of ammonium cyanide upon glyoxal, which probably first breaks down into formaldehyde and then by the cyanhydrin reaction yields glycine:—

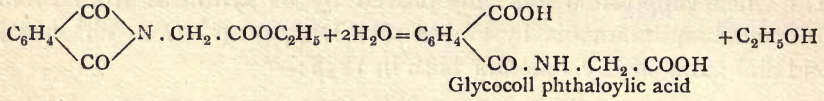


The direct synthesis of glycine from formaldehyde was only carried out in 1894 by Eschweiler. This method, as well as the method from chloracetic acid and ammonia, by which both Nencki and Mauthner and Suida by slight modifications in technique attempted to obtain larger yields, only gives about 20 per cent., but the method described by Gabriel and Kroseberg, in 1889, who made use of Gabriel's phthalimide reaction, as first shown by Goedeckemayer, gives an almost theoretical yield of glycine; this reaction takes place in the following stages:—

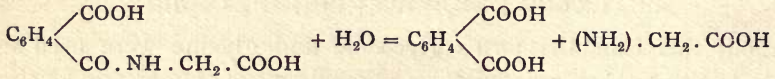
Phthalimidoacetic ester is obtained by the action of chloracetic ester upon potassium phthalimide; this is first hydrolysed by alkali to glycocoll phthaloylic acid, and then by 20 per cent. hydrochloric acid to glycocoll and phthalic acid:—



Phthalimidoacetic ester

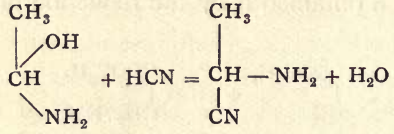


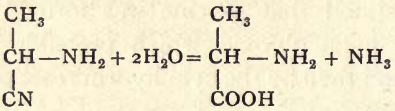
Glycocoll phthaloylic acid



Alanine.

Of the naturally occurring amino acids alanine only was prepared synthetically many years before it was discovered as a constituent of the protein molecule. Its name was given to it by its discoverer, Strecker, who prepared it in 1850 from aldehyde ammonia, which, when treated with hydrogen cyanide gives the aminocyanohydrin, and this by hydrolysis is then converted into the amino acid:—





Owing to the ease with which the aldehyde resinifies in presence of alkali and potassium cyanide the yield of alanine is very poor. If, however, the reaction be carried out in the presence of excess of ammonium chloride and if the potassium cyanide be slowly added to the aldehyde dissolved in ether, a yield of alanine amounting to 60 to 70 per cent. can be obtained, as has been recently shown by Zelinsky and Stadnikoff.

This is the first of the general methods employed in the synthesis of the amino acids.

Alanine was prepared in 1860 by Kolbe by the second general method, by the action of ammonia upon *a*-chloropropionic acid:—

$$CH_3 . CHCl . COOH + NH_3 = HCl + CH_3 . CH(NH_2) . COOH,$$

and in 1864 by Kekulé from monobromopropionic acid and alcoholic ammonia.

A synthesis of acetylalanine, from which alanine can be obtained by hydrolysis, was described in 1900 by de Jong. Pyruvic acid was neutralised with ammonium carbonate; there was a considerable rise in temperature, carbon dioxide was evolved and the ammonium salt of acetylalanine crystallised out. The explanation of this reaction is based upon Erlenmeyer and Kunlin's synthesis of phenylalanine from phenylpyruvic acid and it proceeds as follows:—

Ammonium pyruvate, which is first formed, is tautomeric with *a*-amino-oxypropionic acid, into which it changes:—

$$CH_3.CO.COOH + NH_3 = CH_3.CO.COONH_4 \rightarrow CH_3.C(OH)(NH_2)COOH$$

This compound then reacts with pyruvic acid:—

$$CH_3-C(OH)(NH_2)-COOH + CH_3.CO.COOH = CH_3-C(OH)-COOH \;\; NH \;\; CH_3-C(OH)-COOH$$

and the compound thus formed loses carbon dioxide giving the compound,

$$CH_3-C(OH)-CH \;\; NH \;\; CH_3-C(OH)-COOH$$

which possesses the group,

$$-CHOH-NH-COH-COOH.$$

By intramolecular rearrangement and loss of water this becomes

$$-C(=O)-NH-CH(-)-COOH$$

The above compound by rearrangement and loss of water is thus converted into acetylalanine:—

$$CH_3.C=O \;\; NH \;\; CH_3.C(H).COOH$$

The occurrence of alanine in proteins was first shown by Schutzenberger, who did not actually identify his product with the synthetical one; Weyl in 1881 obtained it as a decomposition product of silk and showed that his preparation was similar in properties to Strecker's synthetical alanine. He thus established it as a constituent of a protein molecule. The researches of Emil Fischer have shown that alanine is a constant constituent of all proteins. It is worthy of note that of the eighteen definitely determined units of a protein molecule, six of them, namely, isoleucine, phenylalanine, tyrosine, serine, histidine and tryptophane, are derivatives of *a*-aminopropionic acid.

Valine.

A body of the composition $C_5H_{11}NO_2$ was obtained in 1856 by v. Gorup-Besanez from an aqueous extract of pancreas, and on account of its similarity in properties to leucine he regarded it as a homologue of leucine and termed it butalanine. Schutzenberger, in 1879, also obtained a substance which had this empirical formula and properties like that of leucine.

An aminovalerianic acid was described in 1883 by Schulze and Barbieri as occurring in the seedlings of yellow lupines, and subsequently Schulze again isolated it from the extracts of other seedlings. It appeared to correspond to n-aminovalerianic acid, which had been synthesised by Lipp.

In 1899 Kossel isolated a similar substance from the protamine, clupeine, of herring milt, and since then E. Fischer and his pupils have obtained it from caseinogen, horn and other proteins. The preparation from horn, when racemised, corresponded in properties with the synthetical *a*-aminoisovalerianic acid,

$$\begin{matrix} CH_3 \\ \\ CH_3 \end{matrix}\!\!\rangle CH \cdot CH(NH_2) \cdot COOH,$$

which had been first prepared by Clark and Fittig in 1866 from the corresponding bromo-compound and later by Lipp in 1880 from isobutyraldehyde; its derivatives were identical with those of this acid which were prepared by Slimmer in 1902. The exact identity of the natural and synthetical substances was only established in 1906 when Fischer prepared d-aminoisovalerianic acid from the synthetical product, and showed that its specific rotation was the same as that of Schulze and Barbieri's natural substance. The name valine was given to this compound in 1906 by E. Fischer.

Leucine.

A substance, corresponding to our leucine, was described by Proust in 1818 under the name of oxide-caséux. Two years later, in 1820, Braconnot isolated from the products resulting by boiling meat with dilute sulphuric acid a substance which he named leucine on account of its glistening white (λευχος) appearance. Mulder, in 1839, obtained it by boiling meat with alkali and by the putrefaction of casein. Its occurrence and oxidation products were investigated by Liebig, who regarded it as one of the constituents of the protein molecule, as was proved in 1849 by Bopp, who prepared it from caseinogen, fibrin and albumin by fusion with potash, by hydrolysis with acids and by putrefaction. Hinterberger showed that it was present in horn, and Zollikofer in elastin.

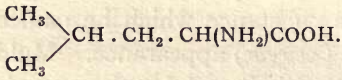
Leucine also occurs in the free state in the various organs of the animal body as pointed out by Frerichs and Städeler and many other observers.

Not only is it present in the animal proteins, but also in the vegetable ones, from which it passes by the action of enzymes into the extracts of germinating seedlings, as shown by Schulze and his co-workers. Leucine is, with the exception of arginine, the most widespread of all the amino acids which go to make up the protein molecule.

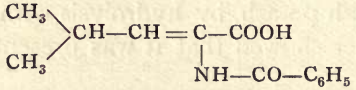
Its correct empirical formula $C_6H_{13}NO_2$ was first given to it by Laurent and Gerhardt. These observers and also Cahours showed that it belonged to the glycine series of compounds; Liebig and others showed that on oxidation it gave ammonia and valerianic acid, and also valeronitrile, and Strecker obtained leucic acid by treating it with nitrous acid. But only in 1868, when Hüfner obtained caproic acid and ammonia by reducing it with hydriodic acid, was it shown to be an *α*-aminocaproic acid. Hüfner tried to prove this by comparing the natural leucine with two synthetical leucines, (1) that prepared by the action of ammonia on bromocaproic acid obtained from the fermentation caproic acid, and (2) that prepared from isovaleraldehyde, hydrogen cyanide and ammonia which had been first synthesised by Limpricht in 1855. Neither of these two synthetical leucines corresponded exactly with natural leucine, and Hüfner, rather than regard them as isomers, regarded them as identical compounds.

The question of the constitution of leucine was again taken up in 1891 by Schulze and Likiernik. The natural product is optically active, but by heating with baryta at 160° C. it is racemised; this inactive leucine, on being compared with the leucine prepared from

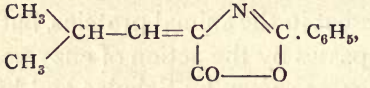
isovaleraldehyde, hydrogen cyanide and ammonia, was found to be identical with it, and further, both compounds gave d-leucine, when acted upon by the mould *Penicillium glaucum*, and the same leucic acid, when treated with nitrous acid. Leucine is therefore *a*-aminoisobutylacetic acid,



Among the syntheses of *a*-amino acids carried out by E. Erlenmeyer jun., by his method, that of leucine was described in 1901 by Erlenmeyer and Kunlin. It was prepared from *a*-benzoylamido-*β*-isopropylacrylic acid,

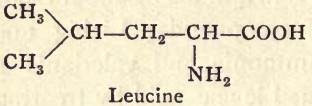


which resulted when isobutylaldehyde and hippuric acid were condensed together in the presence of acetic anhydride and the condensation product,

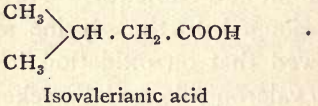


was treated with alkali.

By heating *a*-benzoylamido-*β*-isopropylacrylic acid in sealed tubes at 150-170° C., with excess of ammonia, hydrolysis occurred with the formation of leucine, isovalerianic acid and benzoic acid :—



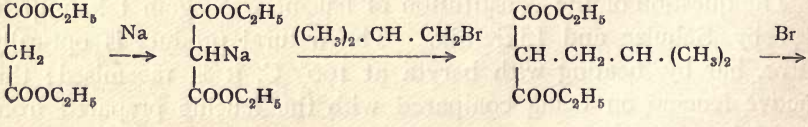
Leucine

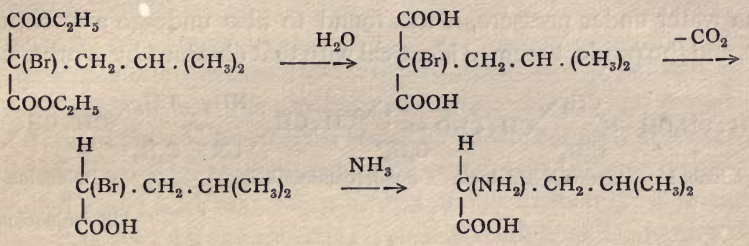


Isovalerianic acid

Bouveault and Locquin have also synthesised leucine by the reduction of *a*-oximinoisobutylacetic acid, which was prepared in a similar way to the isomeric compound from which they obtained isoleucine.

The most convenient method of preparing leucine by synthesis is that given by Fischer and Schmitz in 1906 by the action of ammonia upon the corresponding halogen derivative of isocaproic acid, which they prepared by brominating the alkylmalonic ester and heating whereby it was converted into the bromo-fatty acid. The several reactions are represented by the following scheme:—



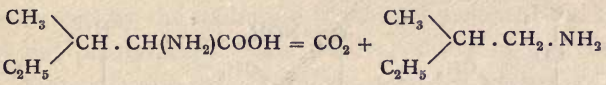


Isoleucine.

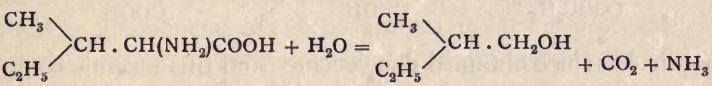
This amino acid was first obtained by F. Ehrlich in 1903 from the nitrogenous constituents of beet-sugar molasses, and was subsequently isolated by him from the decomposition products of fibrin and other proteins. Like leucine, to which it is very similar in properties, it thus appears to be a widely distributed constituent of the protein molecule.

Of the various isomeric amino-caproic acids only leucine and isoleucine occur in the protein molecule; both of them, combined with tyrosine and valine in the form of polypeptides, from which they are easily split off by enzymes, seem to form a very important part of most proteins.

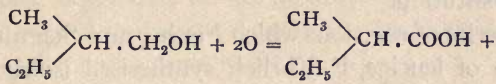
Ehrlich showed that leucine, when heated to 200° C., was converted into d-amylamine with loss of CO_2:—



and that, when fermented by yeast in the presence of cane sugar, it yielded d-amylalcohol:—



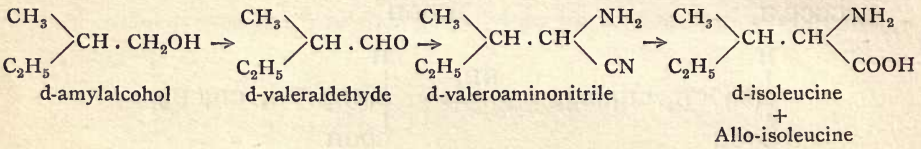
This was determined by oxidising to methylethylacetic acid:—



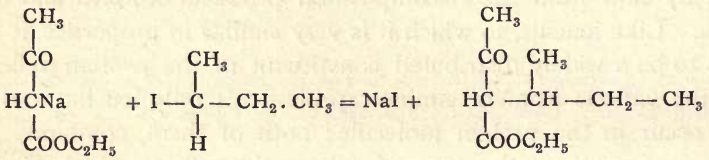
from which the constitution of isoleucine appeared to be α-amino-β-methyl-β-ethyl-propionic acid.

Ehrlich proved this by synthesising it from d-amylalcohol; this was first oxidised to valeraldehyde, which on treatment with hydrogen cyanide and ammonia gave valeroaminonitrile, and then, on hydrolysis d-isoleucine mixed with allo-isoleucine; isoleucine has two asymmetric carbon atoms, and allo-isoleucine is formed by the rearrangement of the groups attached to one of them. By heating natural isoleucine with

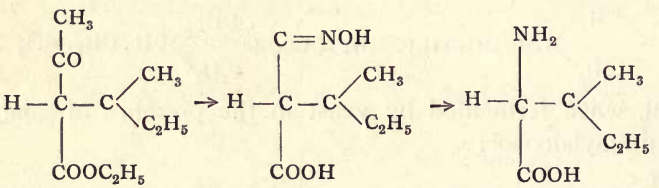
baryta water under pressure, it was found to also undergo a rearrangement, and the product seemed identical with that obtained by synthesis:—



Further proof that isoleucine has this constitution was given by Bouveault and Locquin in 1906. They synthesised it from sec. butylacetoacetic ester, which they prepared from sec. butyliodide and sodium-acetoacetic ester:—



This compound, when treated with nitrosyl sulphate was decomposed into *a*-oximino-sec-butylacetic acid, which, on reduction with zinc dust and hydrochloric acid in alcohol, gave a 60 per cent. yield of dl-isoleucine:—



Locquin has since obtained d-isoleucine from this racemic compound which was identical with Ehrlich's natural product, and this therefore has the above constitution.

By the same series of reactions which Fischer and Schmitz employed in the preparation of leucine, F. Ehrlich synthesised isoleucine in 1908 from malonic ester and secondary butyliodide, *i.e.*, according to the following scheme:—

$C_2H_5 . CHI . CH_3 + CHNa(COOC_2H_5)_2 \rightarrow C_2H_5 . CH(CH_3) . CH(COOC_2H_5)_2$
$\rightarrow C_2H_5 . CH(CH_3) . CH(COOH)_2$ $\rightarrow C_2H_5 . CH(CH_3) . CBr(COOH)_2$
$\rightarrow C_2H_5 . CH(CH_3) . CHBr . COOH$ $\rightarrow C_2H_5 . CH(CH_3) . CH(NH_2) . COOH$

The same synthesis has also been carried out by Brasch and Friedmann.

Phenylalanine.

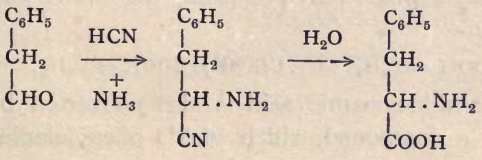
In a note published in 1879 Schulze mentioned a substance which he had obtained from the seedlings of *Lupinus luteus;* two years later he and Barbieri showed that this substance had the composition $C_9H_{11}NO_2$, and they described it as phenylamidopropionic acid, because, on oxidation, it gave benzoic acid, and, when heated, it lost carbon dioxide and gave a base, $C_8H_{11}N$. In its properties it closely resembled Tiemann's phenylaminoacetic acid, and they regarded it therefore as a homologue of this acid, though it differed from the substance described by Posen as phenyl-*a*-aminopropionic acid.

About the same time Schutzenberger obtained a substance, which he called tyroleucine, by the action of baryta on proteins; when heated it gave a sublimate of aminovalerianic acid and a base $C_8H_{11}N$, which probably had as mother-substance the same body which was isolated by Schulze and Barbieri.

Schulze, Barbieri and Bosshard next showed that their substance arose during the germination of the seed, and that it was also obtained from vegetable proteins by hydrolysis, by hydrochloric acid and zinc chloride, or by baryta. It was therefore contained in the protein molecule.

It had been known for a long time that benzaldehyde and benzoic acid were formed by the oxidation of animal proteins, and that phenylpropionic and phenylacetic acids were products of putrefaction (Salkowski); phenylalanine was therefore regarded, as suggested by Tiemann, as the constituent from which these substances arose, but the actual presence of phenylalanine in the proteins was only proved when E. Fischer commenced his investigations upon the proteins. He then found that in some proteins it exceeded in amount that of tyrosine, and that it was in fact the principal aromatic constituent. Those proteins, such as gelatin, in which its presence was demonstrated by Spiro, and which contains no tyrosine, was found to contain phenylalanine as its aromatic constituent.

The constitution of phenylalanine was determined in 1882 by Erlenmeyer and Lipp, who synthesised it by Strecker's method from phenylacetaldehyde, hydrogen cyanide and ammonia:—



This synthetical substance closely resembled Schulze and Barbieri's natural compound, and their identity was established. Posen's preparation, described under this name, was at the same time shown to be phenyl-β-aminopropionic acid.

In 1893 a new method of synthesising amino acids, starting from hippuric acid, was introduced by Erlenmeyer jun., phenylalanine being the first product to be prepared.

When benzaldehyde is condensed with hippuric acid in the presence of acetic anhydride a Perkin's reaction takes place and benzoyl-a-amidocinnamic acid is formed :—

$$C_6H_5 . CHO + CH_2\begin{<}NH . CO . C_6H_5\\COOH\end{>} = C_6H_5 . CH = C\begin{<}NH . CO . C_6H_5\\COOH\end{>} + H_2O$$

Under the influence of acetic anhydride, this is converted into the lactimide,

$$C_6H_5 . CH = C\begin{<}N—CO . C_6H_5\\CO\end{>}$$

which by hydrolysis by acids or by alkalies is reconverted into benzoyl-a-amidocinnamic acid.

Benzoyl-a-amidocinnamic acid is reduced by sodium amalgam or by zinc and hydrochloric acid to benzoyl-a-amino-β-phenyl-propionic acid :—

$$C_6H_5 . CH = C\begin{<}NH . CO . C_6H_5\\COOH\end{>} + H_2 = C_6H_5 . CH_2 . CH\begin{<}NH . COC_6H_5\\COOH\end{>}$$

from which the benzoyl group is easily removed by hydrolysis with the formation of phenylalanine :—

$$C_6H_5 . CH_2 . CH\begin{<}NH . COC_6H_5\\COOH\end{>} + H_2O = C_6H_5 . CH_2 . CH(NH_2) . COOH + C_6H_5 . COOH$$

Benzoyl-a-amidocinnamic acid is converted by the action of acids or alkalies into phenylpyruvic acid and benzamide,

$$C_6H_5 . CH = C\begin{<}NHCOC_6H_5\\COOH\end{>} + HOH = C_6H_5 . CONH_2 + C_6H_5 . CH = C\begin{<}OH\\COOH\end{>}$$

$$\downarrow$$

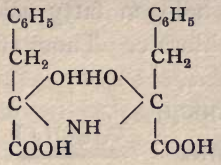
$$C_6H_5 . CH_2 . CO . COOH$$

which Erlenmeyer proved by preparing the oxime and reducing it to phenylalanine :—

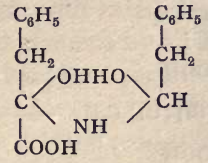
$$C_6H_5 . CH_2 . CO . COOH \rightarrow C_6H_5 . CH_2 . C(NOH) . COOH \rightarrow C_6H_5 . CH_2 . CH . NH_2 . COOH$$

Benzoyl-a-amidocinnamic acid is also converted by the action of ammonia into a compound, which yields phenylalanine on hydrolysis.

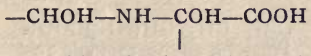
The mechanism of this reaction was explained by Erlenmeyer and Kunlin in 1899. Just as benzoyl-*a*-amidocinnamic acid is converted by alkali into phenylpyruvic acid and benzamide, so also does this reaction take place with ammonia; the phenylpyruvic acid then reacts with ammonia giving a body of the composition,



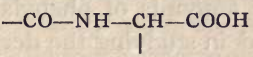
which loses carbonic acid yielding,



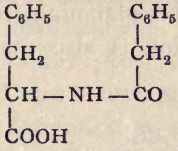
This substance contains the group,



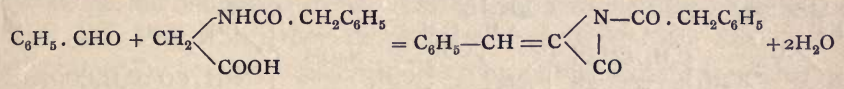
which, by rearrangement and by the loss of a molecule of water, becomes



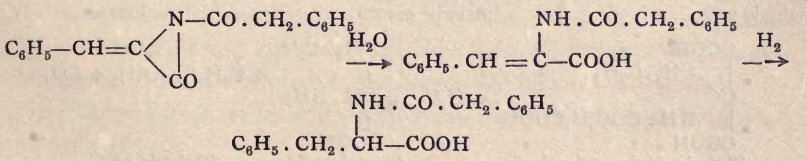
and it therefore changes into phenylacetyl-phenylalanine:—



By subsequent hydrolysis phenylalanine and phenylacetic acid result. The proof of this reaction was given by the synthesis of phenylacetyl-phenylalanine by condensing benzaldehyde with phenaceturic acid:—



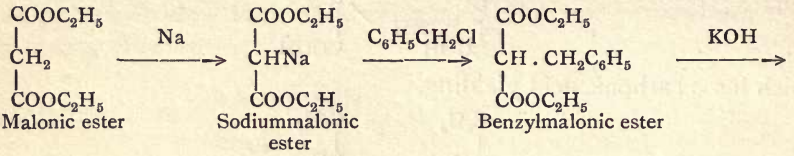
hydrolysing the resulting lactimide, just as in the case of benzoyl-*a*-amidocinnamic acid, reducing it with sodium amalgam:—

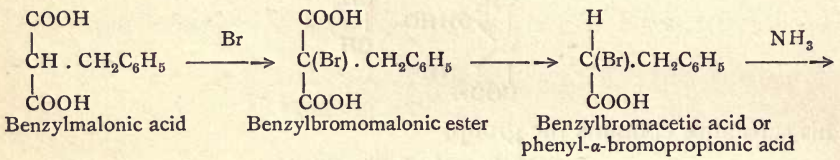


and showing the identity of the two substances.

The condensation of benzaldehyde and hippuric acid, and the formation of phenylalanine by the action of ammonia had been previously carried out by Plöchl in 1884, but he was unable to explain the various stages which took place.

E. Fischer has synthesised phenylalanine by the action of ammonia upon the corresponding halogen fatty acid, which he prepares from malonic ester and benzylchloride. There are six stages in the complete process, as follows:—

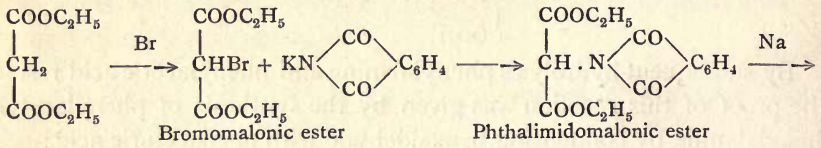


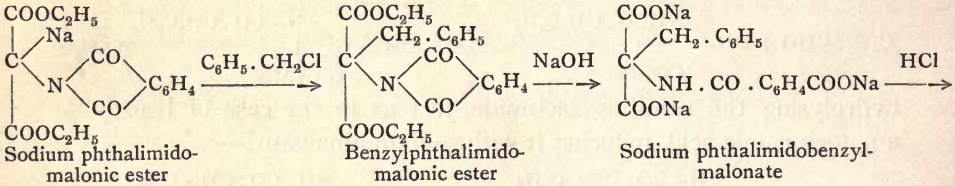


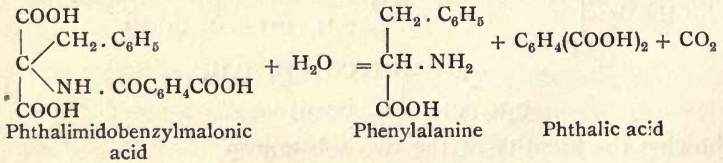
$C_6H_5 . CH_2 . CH(NH_2)COOH$
Phenyl-α-aminopropionic acid

By this means large amounts of phenylalanine can be prepared, and have been employed in studying the derivatives of phenylalanine and in the synthesis of polypeptides.

Another synthesis of phenylalanine from malonic ester, in which Gabriel's phthalimide reaction is also made use of, is described by Sörensen, namely,







Tyrosine.

By fusing cheese with caustic potash Liebig, in 1846, obtained a new compound, consisting of a mass of fine silky needles, soluble with difficulty in water; he named it tyrosine from τυρος, cheese. The same substance was isolated by Warren de la Rue from cochenille, and a year later, in 1849, Hinterberger obtained it by the hydrolysis of horn. Its presence in albumin, fibrin and caseinogen was demonstrated by Bopp.

The results of numerous investigations were published in 1860 by Städeler, who found tyrosine in silk-fibroin, mucin and various other proteins, and who also noted its occurrence in the free state in various organs, generally in conjunction with leucine. Since then, tyrosine has been constantly obtained from proteins by hydrolysis with acids and by the action of trypsin, and has long been regarded as a constituent of the protein molecule.

Its formula $C_9H_{11}NO_3$ was determined by Warren de la Rue and by Hinterberger. Strecker, in 1850, showed that it behaved like leucine and glycine, but pointed out that it did not belong to this series; and Wicke, in 1857, suggested that it stood in the same relation to the series of aromatic acids as glycine and leucine did to the fatty acids. Städeler was really the first to show that tyrosine was an aromatic compound, when he obtained chloranil (tetrachloroquinone) from it by the action of chlorine; he also found that it had a constitution similar to that of glycine and leucine. Fröhde also held this view, but Thudichum and Wanklyn, as they could not obtain picric acid from tyrosine by the action of nitric acid, considered that it was not an aromatic compound.

Städeler's discovery of the formation of chloranil from tyrosine led to the supposition that tyrosine was a derivative of salicylic acid, and on this assumption Schmidt and Nasse attempted to synthesise tyrosine from ethylamine and iodosalicylic acid, and from amidosalicylic and ethyl iodide, but did not succeed. On heating tyrosine they obtained a base $C_8H_{11}NO_2$, which they thought analogous to the one Schmidt had obtained by heating amidosalicylic acid; on this account they held to the accuracy of the theory that tyrosine was ethylamidosalicylic acid.

A great advance was made by Barth in 1865, who showed that tyrosine was not ethylamidosalicylic acid. As yet salicylic acid had never been obtained from tyrosine, and Barth, in his attempt to prepare this compound from tyrosine by oxidation, by fusion with potash, obtained para-oxybenzoic acid and acetic acid, the decomposition taking place as follows:—

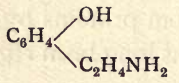
$$C_9H_{11}NO_3 + H_2O + O = C_7H_6O_3 + C_2H_4O_2 + NH_3.$$

He concluded that tyrosine was related to paracumaric acid,

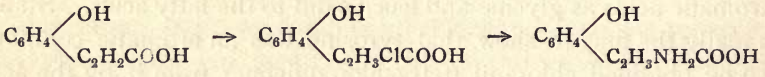
$$(OH)C_6H_4 . CH = CH\ COOH,$$

in the same way as alanine was related to acrylic acid. Ost confirmed this result of Barth's several years later, when he obtained p-oxybenzoic acid by fusing tyrosine with caustic soda.

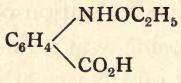
Tyrosine was now regarded as ethylamidopara-oxybenzoic acid; on reduction, therefore, it should yield ethylamine, but instead of this Hüfner, in 1868, obtained ammonia, and he supposed tyrosine to be amidophloretic acid.[1] This view was strengthened when Barth in the following year obtained p-oxybenzoic acid from phloretic acid and also from Schmidt and Nasse's base. This here garded as



and tyrosine as oxyphenylamidopropionic acid, the nitrogen being attached to the side chain and not to the benzene ring as supposed by Schmidt and Nasse. Barth's attempt to synthesise tyrosine from paracumaric acid by the following reactions



which were also put forward by Beilstein and Kuhlberg, was not sufficiently successful to prove that tyrosine had this formula, so that Ladenburg, who stated that the reactions of tyrosine could be just as well explained by the formula



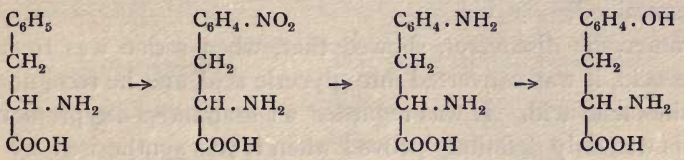
synthesised this compound. It was quite different to tyrosine, and Barth's formula was therefore correct.

The work of Baumann in 1879 upon the decomposition of tyrosine by putrefaction showed that hydroparacumaric acid or p-oxyphenylpropionic acid was the first product and that tyrosine must be p-oxyphenylaminopropionic acid. It only remained to determine the position of the NH_2 group, whether it was in the α- or β-position.

This was decided in 1882 by Erlenmeyer and Lipp who synthesised tyrosine from phenylalanine. Their first method to prepare p-sulpho-

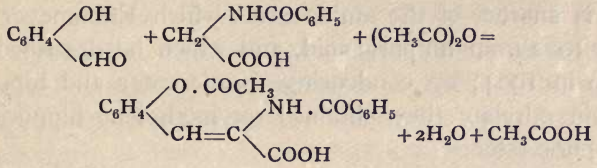
[1] Until 1900 phloretic acid had the constitution $OH . C_6H_4 . CH . COOH$, but in that year Bougault showed it to be β-oxyphenylpropionic acid $OH . C_6H_4 . CH_2 . CH_2 . COOH$.

phenyl-α-aminopropionic acid and to exchange the sulpho-group for the hydroxyl group was not successful, as in the fusion with potash the side chain also became oxidised and no tyrosine resulted. They then prepared p-nitrophenylalanine, and converted it into p-amidophenylalanine; on treating this latter compound with the calculated quantity of sodium nitrite and warming, they obtained p-oxyphenylalanine, thus,

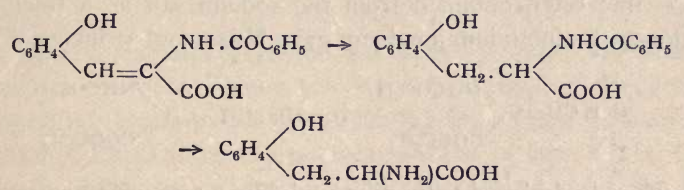


This compound had the same properties as the natural tyrosine, which was thus proved to be p-oxyphenyl-α-aminopropionic acid.

Erlenmeyer jun., and Halsey, in 1899, synthesised tyrosine by the condensation of hippuric acid with p-oxybenzaldehyde in the presence of acetic anhydride. The reactions are the same as those described by Erlenmeyer for the synthesis of phenylalanine, except that the hydroxyl group of the p-oxybenzaldehyde becomes acetylated in the process:—



The lactimide is again formed, but, on hydrolysis by alkali, the acetyl group is removed and p-oxy-α-benzoylaminocinnamic acid is obtained. On reduction it yields benzoyltyrosine, from which tyrosine is formed by hydrolysis:—

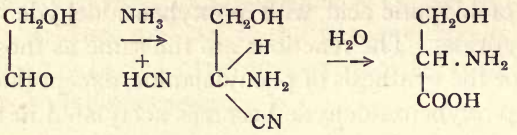


Just as in the case of phenylalanine, p-oxy-α-benzoylaminocinnamic acid when treated with ammonia yields an α-oxo acid, which reacts with ammonia, giving a complex compound; this, on hydrolysis, by heating in a sealed tube with hydrochloric acid, is converted into tyrosine.

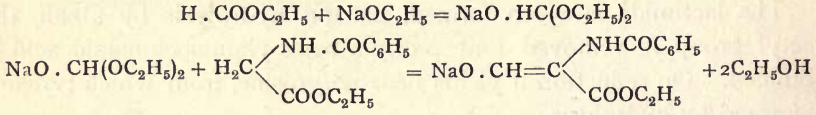
Serine.

Serine is, as yet, the only member of the oxyamino acids of the aliphatic series which has been isolated with certainty from the mixture of decomposition products of the proteins. It was first obtained in 1865 by Cramer from silk-gelatin, and was not again obtained until E. Fischer isolated it from the various proteins which he and his pupils have examined.

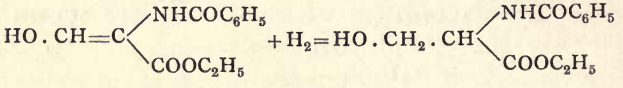
Cramer, the discoverer, showed that, when serine was treated with nitrous acid, it was converted into glyceric acid, and he recognised it as an aminolactic acid. It was regarded as α-amino-β-oxypropionic acid, but this was only definitely proved when it was synthesised by Fischer and Leuchs in 1902 from glycollic aldehyde, hydrogen cyanide and ammonia, which is the first instance of the employment of Strecker's method to build up oxyamino acids from oxyaldehydes.



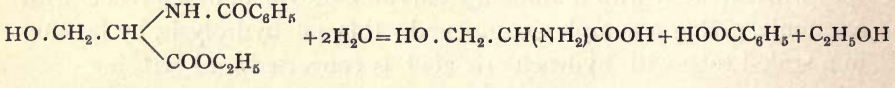
Serine is another of the amino acids which Erlenmeyer jun. synthesised in 1902 from hippuric acid, and which he described in detail with Storp in 1904; by condensing formic ester and hippuric ester with sodium ethylate they obtained oxymethylene hippuric ester or formyl hippuric ester:—



The free ester, obtained from the sodium salt as a thick oil, on reduction with aluminium amalgam gave N-benzoyl serine ester:—

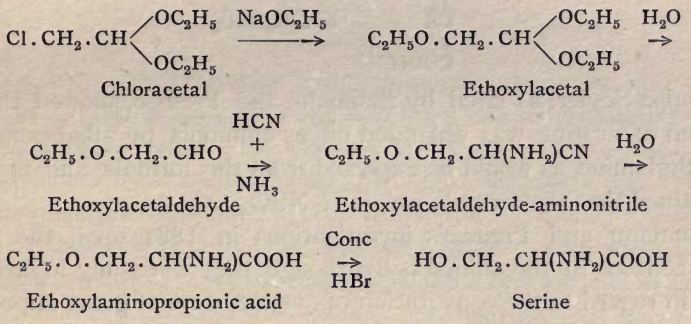


which, when hydrolysed with dilute sulphuric acid, was converted into benzoic acid and serine:—



A better method of synthesising serine was described by Leuchs

and Geiger in 1906, and was carried out as follows, starting from chloracetal :—



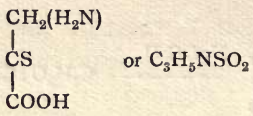
Cystine.

Under the name of cystic oxide, a new species of urinary calculus, this compound was first described by Wollaston in 1810. Lassaigne found it under the same conditions in a dog in 1823. Its presence in the kidney of an ox was shown by Cloetta in 1856, and in the following year Scherer found it in the liver of a patient, who had died of typhoid fever. The name cystine was given to it by Berzelius. Drechsel, in 1891, isolated it from horse's liver and in 1896 from a porpoise, and then first regarded it as a normal product of metabolism. In 1890 Külz obtained cystine by the digestion of fibrin with pancreas, and Emmerling, in 1894, found it mixed with tyrosine which he had prepared by the hydrolysis of horn. An attempt was made by Suter, in 1895, to obtain it from horn, but he could only obtain *a*-thiolactic acid, and not until 1899 was it shown by K. A. H. Mörner to be a product of hydrolysis of this protein, and, in 1901, of other proteins also. His results were confirmed by Embden, who was working independently, and who also obtained cysteine, which is derived from cystine as proved by Patten.

The earliest analyses of cystine are given by Prout, who overlooked the fact that cystine contained sulphur, the presence of which element was first shown by Baudrimont and Malagutis. Thaulow gave cystine the formula $C_6H_{12}N_2O_4S$, and pointed out that it was one of the few compounds made up of five elements. On account of the uneven number of atoms in its molecule, Gmelin replaced this formula by $C_3H_7NSO_2$, which formula was confirmed by Grote in 1864, and later by Külz in 1884.

The first investigations on the constitution of cystine are those of Dewar and Gamgee in 1871, who, on treating cystine with nitrous acid, obtained an acid which they thought was pyruvic acid, $CH_3 . CO . COOH$,

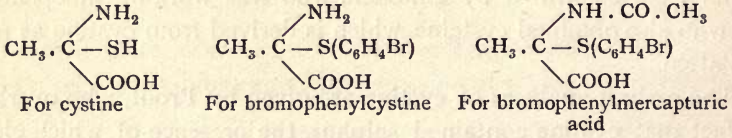
and on this account gave cystine the constitution of



or $C_3H_5NSO_2$

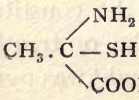
Hoppe-Seyler, as cited by Baumann and Preusse, showed that the nitrogen of cystine was separated off as ammonia by alkalies and not as methylamine, as would be expected from this formula, and moreover maintained that the formula was $C_3H_7NSO_2$.

Baumann and Preusse's investigations in 1881 upon the fate of bromobenzene in the animal body, though they were only indirect evidence in regard to the constitution of cystine, were of great importance, as they were carried out at the time when cystine was a very scarce compound and only obtainable from calculi. They found that, when bromobenzene was given to animals, it was excreted in the urine in combination with a sulphur-containing compound, which combination had the formula $C_{11}H_{12}BrSNO_3$, and in this they were confirmed by Jaffé. When boiled with hydrochloric acid, this compound was converted into acetic acid and a substance $C_9H_{10}BrNSO_2$, from the empirical formula of which Baumann and Preusse supposed that it was cystine $C_3H_7NSO_2$, in which one of the hydrogen atoms was replaced by C_6H_4Br. Their further experiments led them to the conclusion that it was really a derivative of cystine. On decomposition by alkali, this latter compound yielded bromophenylmercaptan, ammonia and another substance, which they eventually recognised must be pyruvic acid. It had been shown that Dewar and Gamgee's formula for cystine, which was also based upon the formation of pyruvic acid, was not accurate, so they proposed the following :—

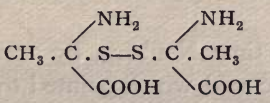


For cystine | For bromophenylcystine | For bromophenylmercapturic acid

Baumann next found that cystine, on reduction with zinc and hydrochloric acid, was converted into a new base, which he called cysteine; this gave the same products on decomposition as cystine, into which it was easily reconverted by oxidation. He therefore recognised that these compounds were related to each other, as a mercaptan is to a disulphide; consequently the formula



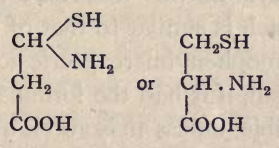
was really that of cysteine, and that of cystine was



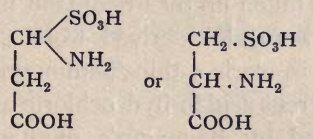
the former bromophenylcystine being bromophenylcysteine, etc.

The actual formation of pyruvic acid from various mercapturic acids upon which these formulæ for cysteine and cystine were founded, was only shown later by Baumann's pupils, Königs, Brenzinger and Schmitz, and in conjunction with Suter's observation that *a*-thiolactic acid was formed by the hydrolysis of horn, this formula for cystine was accepted. The results obtained, however, scarcely justified this formula as pointed out by Friedmann in 1902, who showed conclusively that the cystine, obtained from proteins, had not this constitution.

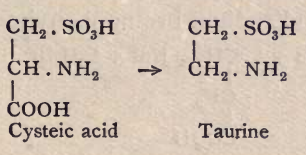
It had been found by Jochem in Hofmeister's laboratory that amino acids, when treated with nitrous acid in hydrochloric acid solution, were converted into the corresponding chloro-derivatives, and Friedmann, on applying this reaction to cystine obtained dichlorodithiopropionic acid; this when reduced gave β-thiopropionic acid, and on subsequent oxidation β-dithiopropionic acid, identical with the compound prepared from β-iodopropionic acid and potassium hydrogen sulphide. The sulphur atoms in cystine and cysteine were therefore in the β-position, and it remained to show in which position the amino group was situated, whether as



By oxidising cystine with bromine water, Friedmann obtained cysteic acid, *i.e.*, either



which, when heated, by loss of carbon dioxide, was converted into taurine, which is only explainable by the second formula



These reactions also showed how taurine might originate in the body from cystine.

At about the same time Neuberg, by treating cystine with nitric acid, obtained isethionic acid, which pointed to the correctness of Friedmann's formula; it at any rate showed that the sulphur and nitrogen atoms were attached to different carbon atoms. In the reaction the SH group was oxidised to the SO_3H group, and the NH_2 group was converted into the OH group by nitrous acid formed in the oxidation:—

$$\begin{array}{l} CH_2.SH \\ | \\ CH.NH_2 \\ | \\ COOH \end{array} \rightarrow \begin{array}{l} CH_2.SO_3H \\ | \\ CH.NH_2 \\ | \\ COOH \end{array} \rightarrow \begin{array}{l} CH_2.SO_3H \\ | \\ CH_2OH \end{array} + CO_2$$

The synthesis of cystine by Erlenmeyer jun. in 1903, which was more fully described by him and Storp in 1904, showed that Friedmann's formula was correct:—

Benzoylserine was heated with phosphorus pentasulphide, and the product after hydrolysis with hydrochloric acid, gave cysteine which was converted by oxidation into cystine:—

$$\begin{array}{l} CH_2OH \\ | \\ CH.NH.COC_6H_5 \\ | \\ COOH \end{array} \rightarrow \begin{array}{l} CH_2SH \\ | \\ CH.NH.COC_6H_5 \\ | \\ COOH \end{array} \rightarrow \begin{array}{l} CH_2SH \\ | \\ CH.NH_2 \\ | \\ COOH \end{array} \rightarrow \begin{array}{l} CH_2.S—S.CH_2 \\ | \quad\quad | \\ CH.NH_2 \quad CH.NH_2 \\ | \quad\quad | \\ COOH \quad\quad COOH \end{array}$$

Benzoylserine — Benzoylcysteine — Cysteine — Cystine

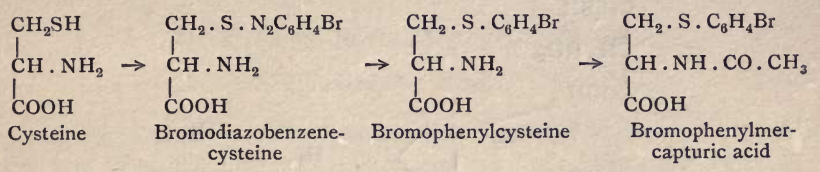
Another synthesis of cystine was described by Fischer and Raske in 1908 (see page 75) which is similar to that of Erlenmeyer.

The formation of bromophenylmercapturic acid from bromobenzene and cystine in the organism, if it had the formula given it by Baumann now seemed scarcely possible, unless an isomeric α-thio-β-aminopropionic acid were also present in the protein molecule together with the di-β-thio-α-aminopropionic acid or cystine. The investigation of their constitution was therefore taken up by Friedmann in 1904, who succeeded in showing that they were also derived from β-thio-α-aminopropionic acid and not from the isomeric α-thio-β-aminopropionic acid.

By the action of nitrous acid in hydrochloric acid solution on bromophenylcystine, prepared by Baumann's method, chlorobromophenylthiopropionic acid was obtained, which, on reduction, gave bromophenylthiolactic acid. This was identical with the substance prepared from β-iodopropionic acid and sodium bromophenylmercaptan:—

$$C_6H_4BrSNa + \begin{array}{l} I.CH_2 \\ | \\ CH_2 \\ | \\ COOH \end{array} = NaI + \begin{array}{l} C_6H_4Br.S.CH_2 \\ | \\ CH_2 \\ | \\ COOH \end{array}$$

and therefore the SH group was in the β-position. Further proof was given by Friedmann by the synthesis of bromophenylmercapturic acid from cysteine. p-Bromodiazobenzene chloride was combined with cysteine; this compound, when decomposed by dilute soda, gave bromophenylcysteine, which, on acylation, was converted into bromophenylmercapturic acid:—

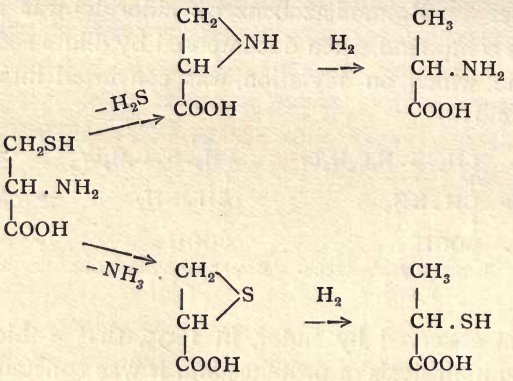


It was first observed by Suter, in 1895, that α-thiolactic acid was formed by the hydrolysis of proteins, and it was constantly obtained by Friedmann. It was always regarded as a secondary product, but its formation from cystine could not be explained, as cystine is a β-thiopropionic acid.

In 1904 Mörner found that pyruvic acid was a constant product of hydrolysis of proteins, and that this compound gave α-thiolactic acid with hydrogen sulphide. Its formation was thus explained, but it was curious that hair, which is very rich in sulphur, gave less pyruvic acid than horn, which is less rich, and that caseinogen, which contains very little sulphur, also gave it. Mörner therefore supposed that there was another sulphur-containing compound in the protein molecule, which supposition was strengthened by Neuberg and Mayer's statement that stone cystine differed from protein cystine in many of its physical properties. Mörner's subsequent work on the decomposition of stone cystine, when he obtained α-thiolactic acid, ammonia and alanine helped to support this idea; he regarded the alanine as formed from cystine and the α-thiolactic acid from the isomeric α-thio-β-aminopropionic acid, both of which he supposed were present in the stone cystine in equal quantities.

Fischer and Suzuki soon afterwards showed that Neuberg and Mayer's stone cystine contained tyrosine, and that its different behaviour to protein cystine was due to the presence of this compound. Rothera also could find no difference between stone cystine and protein cystine, and further, Gabriel's synthesis of isocysteine or α-thio-β-aminopropionic acid and isocystine, which had quite different properties to cystine, though the two were much alike in many of their reactions, proved that stone cystine and protein cystine must be identical substances. Finally, it has been shown by Friedmann that α-thiolactic acid, ammonia

and alanine can be obtained from protein cystine, which decomposition may take place according to Gabriel in the following way :—



Thus, the work of Friedmann on the constitution of cystine, its synthesis by Erlenmeyer jun. and by Fischer, definitely show that it has the composition

$$HOOC . CH(NH_2) . CH_2—S—S—CH_2 . CH(NH_2) . COOH.$$

The proof that bromophenylmercapturic acid is derived from cysteine, the formation of *a*-thiolactic acid from cystine derived either from stones or proteins, and the identity of protein cystine with stone cystine show that cystine is the only sulphur-containing compound in the protein molecule: and also that the number of sulphur atoms in the protein molecule is two or a multiple of two, instead of the variable number which had been determined by the earlier workers upon the sulphur in the protein molecule. This work was commenced by Mulder, who was the first to observe that albumin, caseinogen, etc., when heated with alkali gave off hydrogen sulphide ; in consequence of this he regarded these compounds as composed of sulphur and protein in various proportions. Fleitmann, a pupil of Liebig's, in 1847 then showed that this view of the constitution of albumin, etc., was erroneous, for he found that only a portion of the sulphur was spilt off by alkali, and that a portion still remained combined with the protein. The later investigators upon this question—Nasse, Danilewsky, Kruger, Suter, Malerba, Schulz—confirmed Fleitmann's results, and in addition they determined the ratio of total sulphur to loosely bound sulphur, as this sulphur easily split off by alkali was called. Their results varied considerably, and this was due to the different methods which they employed. In some proteins, *e.g.*, serumalbumin, the ratio of loosely bound sulphur to total sulphur was as 2 : 3, in others 1 : 2 or 5 : 3. From these values determinations were made of the molecular weight: thus serumalbumin was given a mole-

cular weight of 5,100, egg-albumin of 4,900, globulin of 4,600, edestin of 7,300. Mörner's isolation of cystine from proteins, which he found also lost only a portion of its sulphur—about 75 per cent.—when boiled with alkali, did not at once prove that cystine was the only sulphur-containing compound in the molecule of all proteins; this was really only proved by Friedmann's work.

B. Monoaminodicarboxylic Acids.

Aspartic Acid.

Asparagine, the amide of aspartic acid, was first isolated by Robiquet and Vauquelin, in 1806, from the juice of *Asparagus officinalis;* hence its name. Not only is asparagine found in asparagus, but also in the seedlings of lupines, peas, vetches, etc., from which it is best and most easily prepared.

Aspartic acid was first obtained by Plisson, in 1827, from asparagine by boiling it with lead hydroxide, and is usually prepared from this compound by hydrolysis with alkali or acid.

Only however in 1868 was the presence of aspartic acid in vegetable proteins shown by Ritthausen, who obtained it by the hydrolysis of conglutin and legumin with sulphuric acid; in the following year Kreussler obtained it in the same way from animal proteins. In 1874 Radziejewski and Salkowski found that it was a product of the tryptic digestion of proteins, and the asparagine in plants most probably arises from the aspartic acid of the protein in the seed.

Its composition, $C_4H_7NO_4$, was established in 1833 by Boutron-Charlard and Pelouze, and confirmed by Liebig. In 1848 Piria showed that aspartic acid was converted into malic acid by the action of nitrous acid, and he regarded aspartic acid and asparagine as the two amides of malic acid

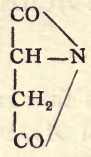
$$CONH_2 . CH_2 . CHOH . COOH \qquad CONH_2 . CH_2 . CHOH . CONH_2$$

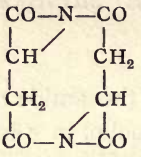
corresponding to oxamic acid and oxamide.

This idea of their constitution was proved to be erroneous by Kolbe in 1862, who showed that aspartic acid did not give off ammonia when boiled with dilute caustic alkali, and that asparagine only lost half of its nitrogen when thus treated. Aspartic acid was therefore not the amide of malic acid, but amino-succinic acid, and asparagine the amide of this compound.

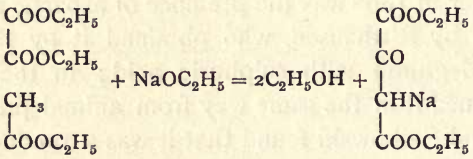
The first synthesis of aspartic acid is that by Dessaignes in 1850, who obtained a crystalline substance by heating acid ammonium malate to 160-200° C., which, when treated with hydrochloric or nitric acid, was

converted into aspartic acid. In the same way Dessaignes obtained aspartic acid from acid ammonium fumarate and maleate. At Liebig's suggestion these reactions were confirmed by Wolff. It was shown by Engel, in 1887, that aspartic acid could be obtained directly by heating maleic or fumaric acid with alcoholic ammonia to 140-150° without the formation of the intermediate substance which is fumarimide, and to which the following constitutional formulæ have been given :—

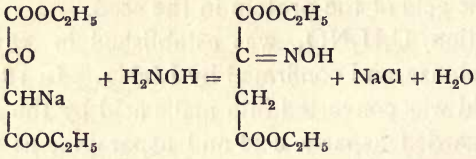




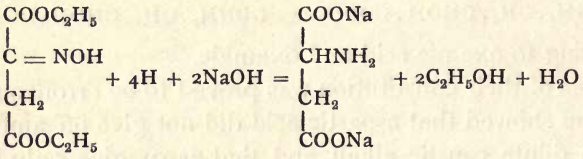
These syntheses give no indication as to the structure of aspartic acid, the constitutional formula of which is based upon Kolbe's work, that it is amino-succinic acid ; the only synthesis of aspartic acid which confirms this constitution appears to be that by Piutti in 1887. Sodium oxalacetic ester, prepared from oxalic ester and acetic ester in the presence of sodium ethylate :—



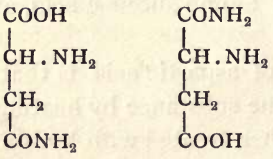
gives an oxime when treated with hydroxylamine hydrochloride :—



and this is reduced by sodium amalgam to sodium aspartate



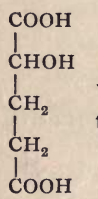
From this oxime Piutti has also prepared the two isomeric asparagines :—



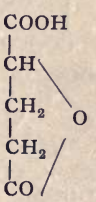
Glutamic Acid.

The presence of this amino acid in the protein molecule was shown by Ritthausen in 1866, who obtained it from wheat gluten by hydrolysis with sulphuric acid. He showed that it was an amino acid and termed it glutaminic acid, on account of its first preparation from gluten. Subsequently Ritthausen and Kreussler isolated it from the hydrolysis products of other vegetable proteins. Kreussler could not demonstrate its presence in animal proteins, in which it was afterwards shown to occur in 1873 by Hlasiwetz and Habermann. Not only is glutamic acid formed by acid hydrolysis, but also by the action of enzymes: Knieriem and Kutscher obtained it by the tryptic digestion of fibrin, and its amide, glutamine, is found in the extracts of seedlings as shown by Schulze, v. Gorup-Besanez and Scheibler.

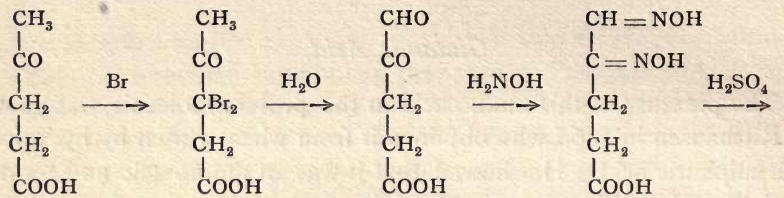
Ritthausen gave glutamic acid the empirical formula $C_5H_9NO_4$, and found that, when treated with nitrous acid, it was converted into an oxy-acid, which he termed glutanic acid and regarded as a homologue of malic acid. Dittmar again prepared glutanic acid and reduced it with hydriodic acid to an acid which was shown by Markownikoff to be what we now call glutaric acid; this was identical with the substance obtained by the hydrolysis of trimethylene cyanide, which was prepared from trimethylene bromide $CH_2Br.CH_2.CH_2Br$ and potassium cyanide. Glutanic acid differed from Simpson's β-hydroxy-glutaric acid, and Markownikoff regarded it as the α-hydroxy-glutaric acid,



which, according to Bredt, exists in the free state as the γ-lactone.

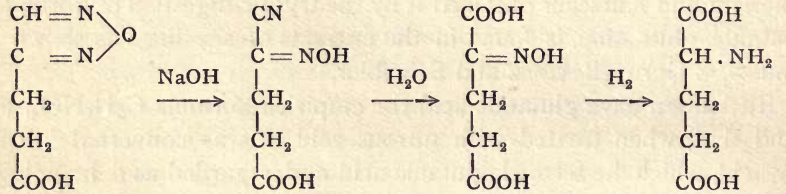


Glutamic acid would therefore be α-aminoglutaric acid. The proof for this constitution was only given in 1890 by L. Wolff who synthesised glutamic acid from laevulinic acid. Dibromolaevulinic acid is obtained by bromination, and this when boiled with water gives diacetyl and glyoxylpropionic acid; diisonitrosovalerianic acid is formed from the latter, on treatment with hydroxylamine:



Laevulinic acid — Dibromolaevulinic acid — Glyoxylpropionic acid — Diisonitrosovalerianic acid

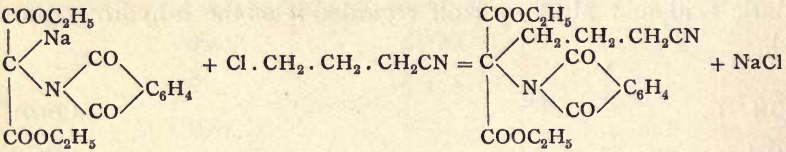
This is converted into furazanpropionic acid by sulphuric acid and then into cyanonitrosobutyric acid by caustic soda. Saponification changes the cyanonitrosobutyric acid into α-nitrosoglutaric acid from which glutamic acid is obtained by reduction:

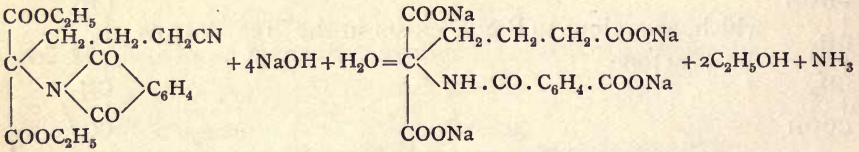


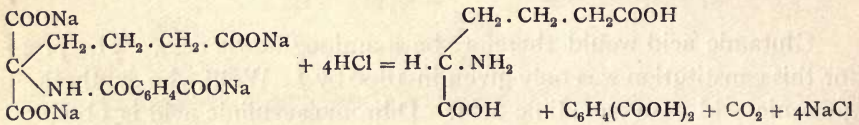
Furazanpropionic acid — Cyanonitrosobutyric acid — α-Nitrosoglutaric acid — Glutamic acid

This appears to be the only recorded synthesis of glutamic acid.

The next member of this homologous series, α-amino-adipic acid, has been prepared by Sörensen from phthalimidosodium malonic ester and chlorobutyronitrile in the following way:—







Sörensen suggests that the same reactions might be employed for the synthesis of aspartic acid and of glutamic acid, in the case of the former condensing the sodium phthalimidomalonic ester with chloracetic ester and in that of the latter with β-chloropropionic ester

$Cl.CH_2.CH_2.COOC_2H_5$. In view of our having only one synthesis of each of these two amino acids, and these syntheses being somewhat arduous, Sörensen's suggestion might with advantage be carried out.

C. Diaminomonocarboxylic Acids.

Drechsel's discovery of lysine amongst the products of hydrolysis of caseinogen in 1889 first showed that the monoamino acids were not the only constituents of the protein molecule; the substance, lysatinine, which he and his pupils also isolated a few years later from several proteins, was shown by Hedin to be a mixture of arginine and lysine, the former body having been many years previously obtained by E. Schulze and E. Steiger from germinating seedlings. Though ornithine had been discovered over ten years before lysine, its importance as a constituent of the protein molecule was not recognised until it was shown by Schulze to be a constituent of arginine. Histidine, discovered in 1896 by Kossel, was classed with the diamino acids until its constitution was determined, on account of its method of separation and its close relationship in many of its properties to arginine and lysine, the three bases having been termed by Kossel as the hexone bases and regarded as a very important portion of the protein molecule.

The synthesis of the diamino acids, in comparison with that of the monoamino acids, is very much more difficult and has only been achieved within the last few years.

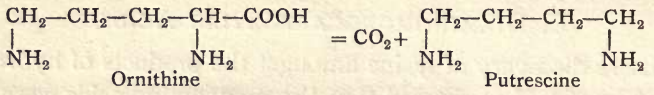
Diamino-acetic Acid.—This acid, the first member of the series, was described by Drechsel as a decomposition product of caseinogen. Its existence is extremely doubtful, its attempted synthesis by Klebs did not succeed, and Willstätter could only obtain certain of its derivatives.

Diaminopropionic Acid has not yet been described as a constituent of the proteins, but it was synthesised by Klebs in 1894 by the action of ammonia upon dibromopropionic acid.

Diaminobutyric Acid.—α-γ-Diaminobutyric acid was prepared in 1901 by E. Fischer by the same method as he employed in the synthesis of ornithine.

Ornithine or α, δ-diaminovalerianic Acid.—In 1877 Jaffé obtained from the urine of birds, which he had fed with benzoic acid, dibenzoyl ornithine or ornithuric acid, and from this substance he prepared ornithine chloride. He regarded it as a diaminovalerianic acid, the first known representative of the series of diamino acids, but only in 1898 was the position of the two amino groups definitely determined by Ellinger, who obtained putrescine from it by putrefaction; the identity of putrescine with tetramethylenediamine had been previously shown by

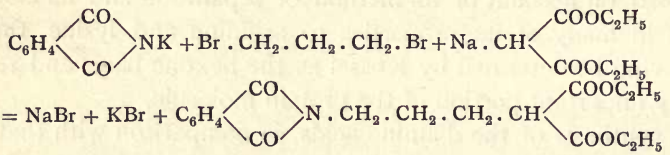
Udransky and Baumann, and ornithine was therefore *a*, δ-diaminovalerianic acid, the hydrolysis of ornithine taking place according to the equation:—



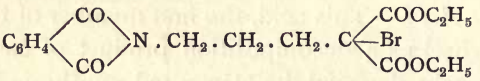
Ornithine — Putrescine

The expected synthesis of *a*-δ-diaminovalerianic acid, which was attempted by Willstätter in 1900, by the action of ammonia upon *a*-δ-dibromovalerianic acid, led to the synthesis of *a*-pyrrolidine-carboxylic acid, and only in the following year was the synthesis of this important naturally occurring diamino acid accomplished by E. Fischer. He made use of Gabriel's phthalimide method with a slight modification and obtained ornithine by the following series of reactions:—

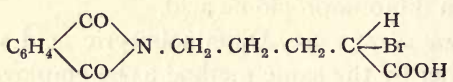
γ-phthalimidopropylmalonic ester was prepared from potassium phthalimide, propylene bromide and sodium malonic ester:



On bromination this gave phthalimidopropylbromomalonic ester,



which, on treatment with ammonia, did not give the desired result. On hydrolysis, however, and by loss of carbon dioxide, it is converted into δ-phthalimido-*a*-bromovalerianic acid,



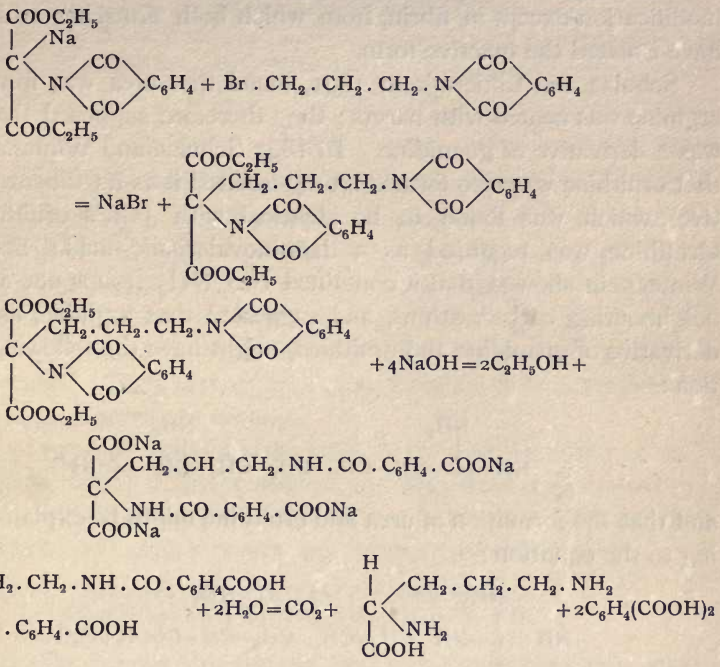
On treatment with ammonia, whereby Br is exchanged for NH_2, and on subsequent hydrolysis, this acid yielded *a*-δ-diaminovalerianic acid or ornithine,

$$H_2N \cdot CH_2 \cdot CH_2 \cdot CH_2 \cdot CH(NH_2) \cdot COOH.$$

The dibenzoyl compound only differed from Jaffé's ornithuric acid by being optically inactive.

By a very similar series of reactions Sörensen has also synthesised ornithine: he first introduces the phthalimido group into the sodium

malonic ester and then allows γ-bromopropylphthalimide to act upon this; the new substance is reduced with sodium and alcohol, and, on subsequent hydrolysis of the acid, whereby the phthalyl groups are removed, and by loss of carbon dioxide, it yields ornithine, thus:—



Arginine.

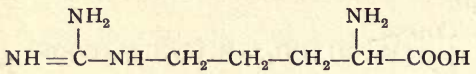
In 1886 E. Schulze and E. Steiger obtained a nitrogenous base from the extracts of the germinated cotyledons of *Lupinus*, which had the composition $C_6H_{14}N_4O_2$, and to which they gave the name arginine; it was also found in the seedlings of other plants and is contained in all the vegetable proteins.

Hedin, in 1894, isolated it from the products of hydrolysis of horn, gelatin, conglutin, vitellin, egg-albumin, blood-serum, caseinogen. He also showed that Drechsel's lysatinine consisted of a mixture of arginine and lysine. From elastin both Bergh and Hedin failed to isolate it, but its presence in this protein was demonstrated by Kossel and Kutscher. Its occurrence in the protamines was shown by Kossel in 1896, and in histone from leucocytes by Lawrow in 1899. About the same time Kutscher found that it was contained in antipeptone, obtained by the tryptic digestion of fibrin; it is also formed when protamines are digested by trypsin (Kossel and Matthews).

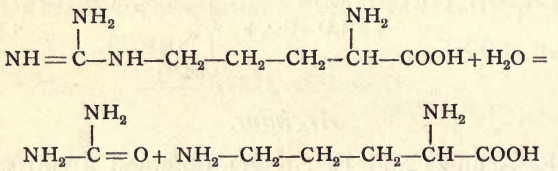
The identity of the arginine obtained from animal proteins with that from vegetable proteins was at first denied by Gulewitsch, but a little later he showed that they were identical, as also did Schulze.

The arginine, as it occurs in the proteins, is the dextro-rotatory modification except in fibrin, from which both Kutscher and Cathcart have isolated the inactive form.

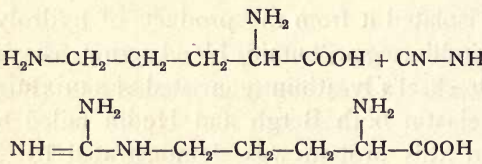
Schulze and Likiernik, in 1891, found that urea was formed when arginine was heated with baryta; they therefore supposed that arginine was a derivative of guanidine. In 1897 Schulze and Winterstein found that ornithine was also formed; they isolated it as its dibenzoyl derivative, which was found to be identical with Jaffé's ornithuric acid. Ornithine was regarded as a diaminovalerianic acid; Schulze and Winterstein showed that it contained two NH_2 groups not attached to neighbouring carbon atoms, and suggested that arginine, as it was a derivative of guanidine and ornithine, might have the following constitution :—



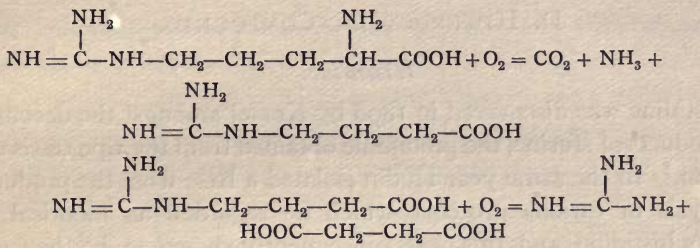
and that the formation of urea and ornithine might be explained according to the equation :—



Schulze and Winterstein, in 1899, proved that arginine was δ-guanidine-α-aminovalerianic acid by synthesis from cyanamide and ornithine :—



The presence of a guanidine group in arginine is also proved by the formation of guanidinebutyric acid and of guanidine and succinic acid by oxidation with permanganate, which probably takes place according to the following equations (Kutscher) :—



Lysine.

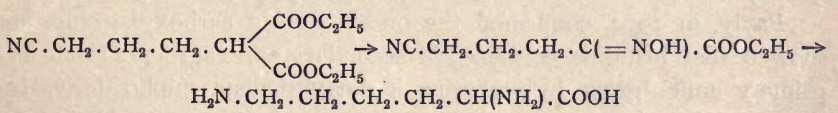
Lysine was discovered by E. Drechsel amongst the decomposition products of caseinogen in 1889, and its presence in other proteins—gelatin, egg-albumin, conglutin, fibrin—was shown by his pupils, Ernst Fischer, Siegfried and Hedin. It was found by Kutscher in antipeptone and by Kossel in the protamines. Its occurrence in germinating seedlings was demonstrated by Schulze and in vegetable proteins by Schulze and Winterstein. Thus, like arginine and histidine it is a very widely occurring constituent of the proteins.

Drechsel gave it the formula $C_6H_{14}N_2O_2$ and regarded it as a diaminocaproic acid; Ellinger proved in 1899 that it possessed this constitution, by obtaining cadaverine from it by putrefaction, which showed that the two amino groups were in the α, ϵ-positions :—

$$NH_2.CH_2.CH_2.CH_2.CH_2.CH(NH_2)COOH = CO_2 + NH_2.CH_2.CH_2.CH_2.CH_2.CH_2.CH_2.NH_2.$$

Henderson's experiments also showed that lysine must have this constitution, namely, α, ϵ-diaminocaproic acid. Its constitution was only definitely determined by synthesis by Fischer and Weigert by the following method :—

When γ-cyanopropylmalonic ester is treated with nitrous acid, it loses one of its carboxethyl groups and is converted into α-oximido-δ-cyanovalerianic acid, which on reduction with sodium amalgam yields α, ϵ-diaminocaproic acid, thus :—



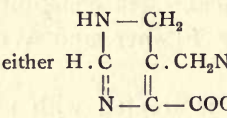
D. Heterocyclic Compounds.

Histidine.

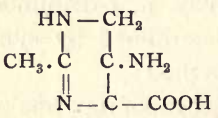
Histidine was discovered in 1896 by Kossel amongst the decomposition products of sturine, the protamine obtained from the ripe testis of the sturgeon. In the same year Hedin isolated a base from the products of hydrolysis of various proteins, which he regarded as identical with Kossel's histidine, and this was subsequently shown to be the case by Kossel and Kutscher. Kutscher also found it in antipeptone obtained by the pancreatic digestion of fibrin, and Schulze and Winterstein have shown that it occurs as a decomposition product of various vegetable proteins.

Histidine was found to possess the formula $C_6H_9N_3O_2$, but beyond the facts that it formed a dichloride, that two of its hydrogen were replaceable by metals and that it was optically active and therefore contained an asymmetric carbon atom, no experiments to determine its constitution were published until 1903. Herzog then showed that it gave the biuret reaction on warming, that it did not contain a methyl nor a methoxyl group, and that it was very resistant to oxidising reagents and in fact behaved as a saturated compound. At the same time Fränkel showed that it contained a carboxyl group and an amino group which was replaced by the hydroxyl group by the action of nitrous acid; it was therefore $(NH_2).C_5H_6N_2.COOH$. As it gave Weidel's pyrimidine reaction and did not contain a pyrrol ring nor a guanidine group, Fränkel suggested that it might be a derivative of dihydropyrimidine,

either



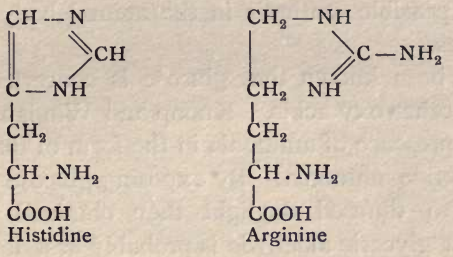
or



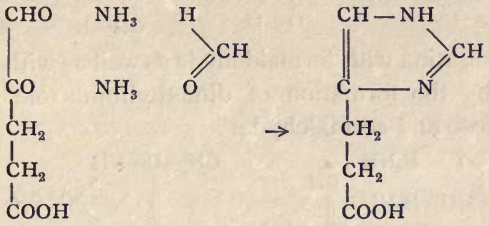
but Weigert pointed out that neither of these formulæ possessed an asymmetric carbon atom, and that histidine was optically active; consequently its formula must remain as $(NH_2).C_5H_6N_2.COOH$.

Pauly, in 1904, confirmed the presence of a carboxyl group, and showed that histidine contained a secondary amine group as well as a primary amine group by preparing a dinaphthalene sulpho derivative, the remaining nitrogen atom being probably a tertiary one. He pointed out that the resistance of histidine to oxidation and to acid permanganate, and that the formation of a di-silver compound were against the presence of a dihydropyrmidine ring in its molecule. These properties, as well as the capability which histidine possessed of forming azo-dyes with diazonium salts, pointed to the existence of a glyoxaline or imidazole ring in its composition. Pauly, therefore, gave it the constitu-

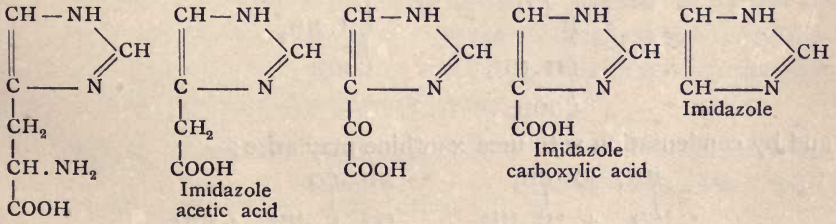
tion of imidazole-amino-propionic acid, at the same time showing its relation to arginine:—



This assumption of Pauly's was confirmed by Knoop and Windaus, who found that histidine is resistant to reduction by sodium and alcohol whereas the pyrimidine ring is very unstable towards this reagent. On reducing Fränkel's oxydesaminohistidine, which is obtained from histidine by the action of nitrous acid, they obtained β-imidazole-propionic acid.[1] This compound was identical with the synthetical product prepared from glyoxylpropionic acid, ammonia and formaldehyde:—



The presence of an imidazole ring in histidine was thus proved, and it only remained to show the position of the amino group. Fränkel urged certain objections against the presence of an imidazole ring in histidine, but Knoop and Windaus showed that these did not hold good. Knoop has since obtained imidazole-glyoxylic acid, imidazole-carboxylic acid, and imidazole from oxydesaminohistidine, and also imidazole-acetic acid. The imidazole ring is therefore in the β-position and histidine is β-imidazole-α-amino-propionic acid:



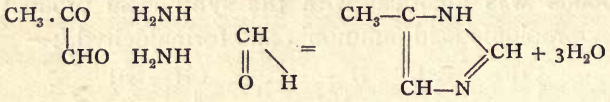
[1] Windaus and Vogt in 1908 showed that Fränkel's chlorohistidine carboxylic acid was the hydrochloride of β-imidazole-propionic acid.

In connection with histidine, the work of Windaus and Knoop on the formation of methylimidazole from glucose must be mentioned on account of the possible synthesis in the animal body of both histidine and purine bases.

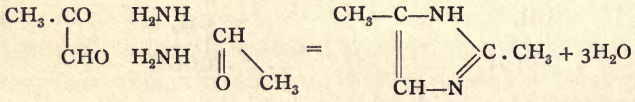
It has long been known that glucose is converted by alkalies into lactic acid and other oxy acids. Knoop and Windaus investigated this reaction in the presence of ammonia in the form of the strongly dissociated zinc hydroxide ammonia. By exposing glucose and zinc hydroxide ammonia to diffused daylight they obtained methylimidazole. From the sugar glyceric aldehyde is probably first formed, and this is converted into methyl glyoxal by loss of water, from which lactic acid may arise by the subsequent addition of water.

$$CH_2OH.CHOH.CHO \rightarrow CH_3.CO.CHO \rightarrow CH_3.CHOH.COOH$$

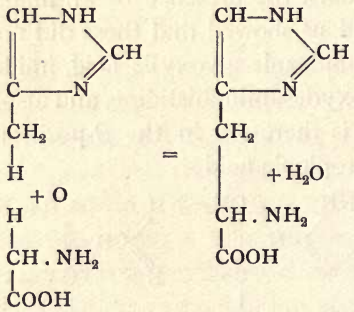
In the presence of ammonia and formaldehyde, also a product from the sugar, methylimidazole is formed as follows:—



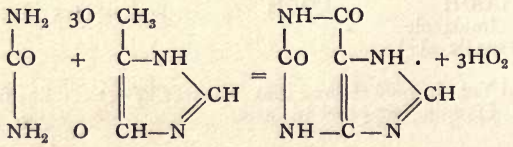
This condensation with formaldehyde as well as with methyl glyoxal is confirmed by the formation of dimethylimidazole when ammonia acts upon glucose and acetaldehyde.



From imidazole by condensation with glycocoll and simultaneous oxidation histidine may possibly be formed thus:—

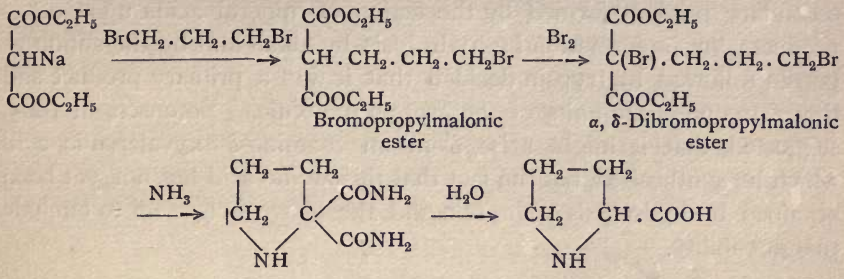


and by condensation with urea xanthine may arise:—

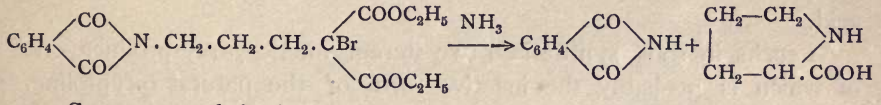


Proline.

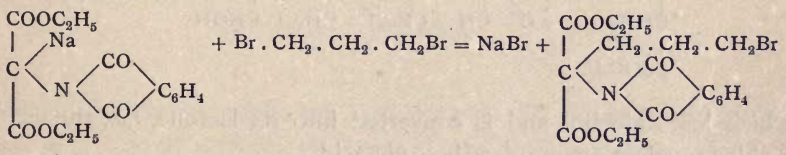
Just a year before E. Fischer obtained this compound by the hydrolysis of caseinogen, it was synthesised by Willstätter in 1900 from sodium malonic ester and trimethylene bromide by the following reactions:—



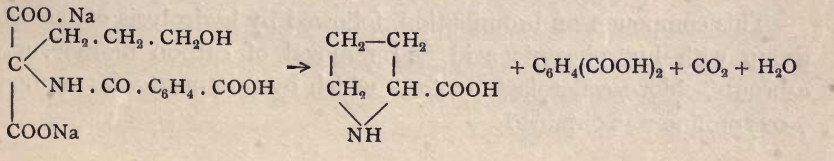
It was also synthesised by E. Fischer in 1901 from γ-phthalimidopropylmalonic ester which he employed in the preparation of ornithine. The bromine derivative of this compound when treated with ammonia gave a complex mixture of products which after hydrolysis by hydrochloric acid at 100° C. gave phthalimide and α-pyrrolidine carboxylic acid:—



Sörensen and Andersen in 1908 synthesised proline by the sodium phthalimidomalonic ester method, a yield of about 80 per cent. being obtained. Sodium phthalimidomalonic ester is condensed with trimethylene bromide,



the resulting γ-bromopropyl-phthalimidomalonic ester is heated in alcoholic solution with sodium hydroxide and the product so formed is evaporated with hydrochloric acid. Proline is obtained instead of the expected α-amino-δ-ethoxyvalerianic acid, ring formation occurring just as in the other methods of preparing proline:—



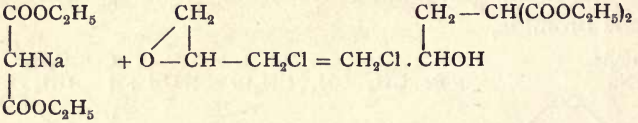
There was no difficulty in identifying the natural substance with the synthetical one, and its presence in egg-albumin, gelatin and other proteins was soon afterwards established.

The question at once arose whether this *a*-pyrrolidine carboxylic acid, or *a*-proline as Fischer termed it in 1904, was a primary product or a secondary product formed by the action of mineral acids upon other products, but its formation by hydrolysis by alkali and by the action of pepsin followed by trypsin decided that it was a primary product and therefore one of the units of the protein molecule. Sörensen, in 1905, suggested that it might arise from an *a*-amino-δ-oxyvalerianic acid which he synthesised, but the fact that this amino acid has not yet been obtained by hydrolysis of protein and the above facts seem to exclude this possibility.

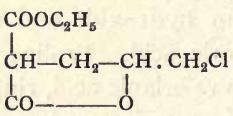
Oxyproline.

In 1902 E. Fischer isolated a compound of the empirical formula $C_5H_9O_3N$ from the hydrolysis products of gelatin. From its composition he supposed that it was an oxy-derivative of pyrrolidine carboxylic acid, and this was proved by its reduction to proline with hydriodic acid.

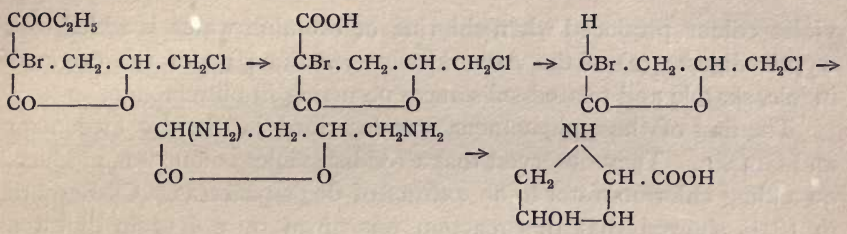
Leuchs, in 1905, synthesised two stereoisomeric-γ-oxy-prolines, one of which is probably the inactive form of the natural oxyproline. Epichlorhydrin and sodium malonic ester yield γ-chlor-β-oxy-propyl-malonic ester,



which loses alcohol and is converted into its lactone, *i.e.*, the ester of δ-chlor-γ-valerolactone-*a*-carboxylic acid,

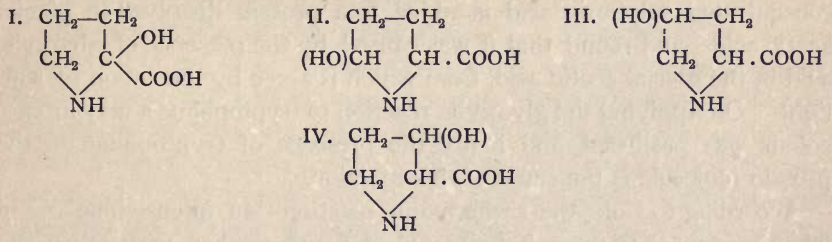


This compound on bromination, followed by hydrolysis of the ester group with hydrobromic acid and removal of carbon dioxide, gave *a*-brom-δ-chlor-γ-valerolactone, from which by treatment with ammonia γ-oxyproline was obtained :—



As this compound contains two asymmetric carbon atoms, four stereoisomeric forms are possible; by synthesis these must occur in two inactive forms. These forms Leuchs separated by crystallisation of the copper salts, the more insoluble acid being termed (*a*)-γ-oxy-proline, the other (b)-γ-oxyproline.

The constitution of these acids was confirmed in 1908 by Leuchs and Felser, who converted them, by reduction with hydriodic acid, into proline. Their attempt to determine whether natural oxyproline was the active form of one of the synthetical compounds by converting the natural substance into its racemic form by heating with baryta to 200° C. was unsuccessful, since complete racemisation did not occur. As, however, all compounds containing one asymmetric carbon atom to which a carboxyl group is attached are easily racemised, the result led to the conclusion that oxyproline contains two asymmetric carbon atoms. Of the four possible formulæ,



formula I. is therefore excluded; and formula II. is not possible on account of the great stability of the acid to baryta; consequently the natural product can only be a γ- or a β-oxyproline.

Tryptophane.

The isolation of tryptophane by Hopkins and Cole in 1902 from the mixture of products formed by the tryptic digestion of caseinogen by precipitation in sulphuric acid solution with mercuric sulphate, besides adding to our list of foundation-stones or units of the protein molecule, gave us the explanation of three phenomena long known in connection with the chemistry of the proteins, namely (1) of the reddish-

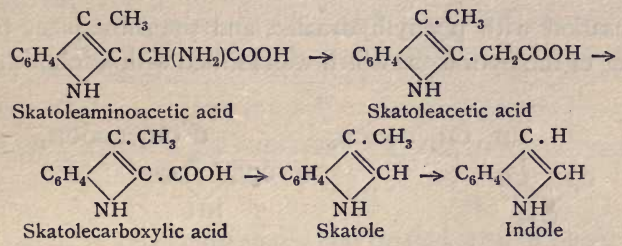
violet colour produced when chlorine or bromine water is added to a tryptic digest; (2) of the Adamkiewicz reaction; (3) of the origin of indole, skatole and related substances occurring in putrefaction.

The first of these phenomena was described in 1826 by Tiedemann and Gmelin. They observed that a reddish-violet colour was produced on adding chlorine water to an extract of dog's pancreas. Cl. Bernard, in 1856, showed that this reaction was given by a trypsin digest of caseinogen, and Kühne, in 1875, found that bromine water gave a better reaction than chlorine water, whilst iodine did not produce the colour. Kühne showed also that this reaction was given by a pure trypsin digest in presence of chloroform, *i.e.*, without the intervention of micro-organisms, and was, in fact, the first to point out the difference between soluble ferments or enzymes, as he called them, and living ferments or bacteria. Stadelmann called the then unknown substance proteino-chromogen and the coloured body proteinochrome, whereas Neumeister, who showed that the reaction was obtained with any deep-seated decomposition of protein, whether by trypsin, baryta water or dilute sulphuric acid gave the substance the name of tryptophane, which name Hopkins and Cole gave to their crystalline substance as it gave this reaction, and to whose presence in the digest the reaction is due.

Shortly before Hopkins and Cole isolated tryptophane, they studied the Adamkiewicz reaction—the production of a violet colour when concentrated sulphuric acid is added to a protein dissolved in glacial acetic acid—and found that it was caused by the presence of glyoxylic acid in the glacial acetic acid, from which it arose by the action of sunlight. On applying the glyoxylic reaction to tryptophane a very intense colour was produced, and hence the presence of tryptophane in the protein molecule is the cause of this reaction.

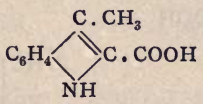
According to Cole, the Liebermann reaction—an intense blue colour when proteins are precipitated by alcohol and washed with ether and then heated with concentrated hydrochloric acid—is also due to the presence of tryptophane in the protein and to glyoxylic acid in the ether employed in washing the precipitated protein. The reddish-violet colour produced when proteins are heated with concentrated hydrochloric acid is due to tryptophane and to furfurol formed from carbohydrate in the protein; it is very marked when cane sugar or furfurol is added to a protein which does not give the reaction very strongly. Reichl's reaction again—a green to blue colour when proteins are heated with an aldehyde such as benzaldehyde, a drop of ferric chloride and concentrated hydrochloric acid—is due also to the presence of tryptophane in the protein.

The formation of indole by the putrefaction of proteins was observed by Kühne and by Nencki in 1874, that of skatole by Brieger in 1877, of skatolecarboxylic acid by E. and H. Salkowski in 1880, and of skatoleacetic acid by Nencki in 1889. Nencki regarded these substances as originating from a skatoleaminoacetic acid in the protein in a manner similar to that by which phenol, cresol, oxyphenylacetic acid and oxyphenylpropionic acid arose from tyrosine, namely:—

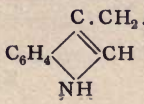


Tryptophane was found by Hopkins and Cole to have the empirical formula $C_{11}H_{12}N_2O_2$ and to yield large amounts of indole and skatole when heated, and when subjected to putrefaction by bacteria the above-mentioned four products resulted. As under anaërobic conditions a large yield of skatoleacetic acid was obtained, and as skatole was the principal product when it was fused with potash, Hopkins and Cole regarded their substance as skatoleaminoacetic acid rather than the isomeric indoleaminopropionic acid.

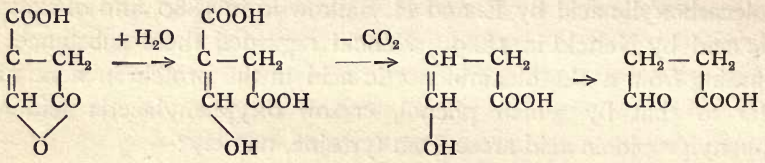
The constitution of indole and skatole had been proved by synthesis, but that of the other two compounds had not been determined, and Nencki's formulæ for them were accepted. Investigations by Ellinger and Gentzen in 1903, who found that in the large intestine indole was formed in large amounts from tryptophane, but skatole only in small amounts, and that skatole only gave traces of indole under the same conditions led Ellinger to doubt the accuracy of Nencki's formulæ for skatoleacetic and skatolecarboxylic acids, more especially as Wislicenus and Arnold's skatolecarboxylic acid, which was synthesised from propionyl formic acid phenylhydrazone had the formula



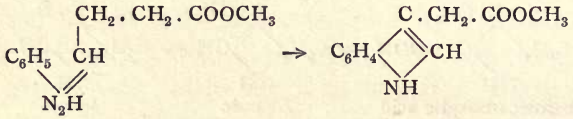
and was not identical with the putrefaction product, which might equally well possess the constitution of



The synthesis of this compound by Ellinger from β-aldehydo-propionic acid, prepared from aconic acid as follows :—

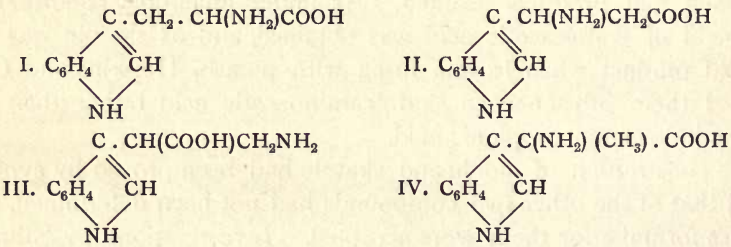


by condensation with phenylhydrazine, and the subsequent treatment of the ester of the hydrazone which was formed with alcoholic sulphuric acid,

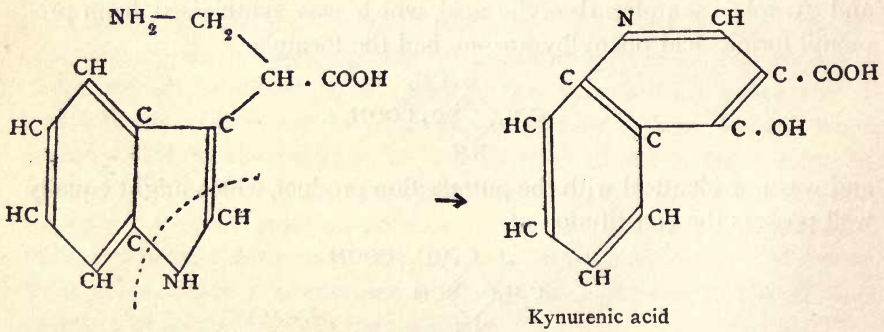


showed that it was identical with Salkowski's acid, and that it was indoleacetic acid and not skatolecarboxylic acid.

Four formulæ were therefore possible for tryptophane :—

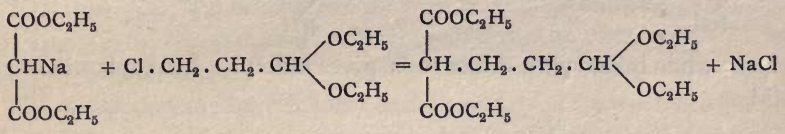


and as Ellinger had found that tryptophane when given to dogs and rabbits was converted into kynurenic acid, formula III. was regarded as the most probable for tryptophane, because it most easily explained this change :—

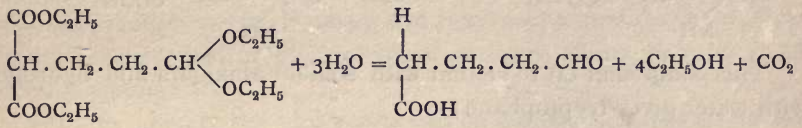


Kynurenic acid

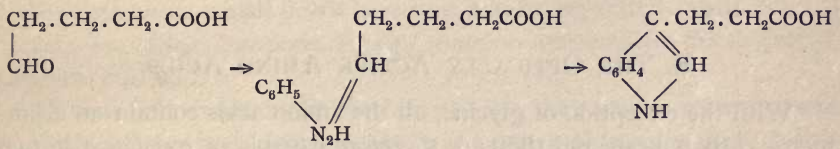
Ellinger's further work did not confirm this supposition. By condensing β-chloropropionacetal with sodium malonic ester he obtained propionacetal malonic ester,



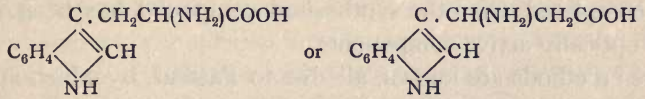
which, when heated in a sealed tube with water to 190°, was converted into γ-aldehydobutyric acid with loss of alcohol and carbon dioxide :—



The hydrazone of this compound when treated with alcoholic sulphuric acid gave the ester of indolepropionic acid :—

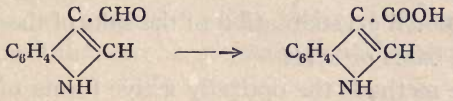


The indolepropionic acid obtained by hydrolysis was identical with Nencki's skatoleacetic acid, and tryptophane was therefore either



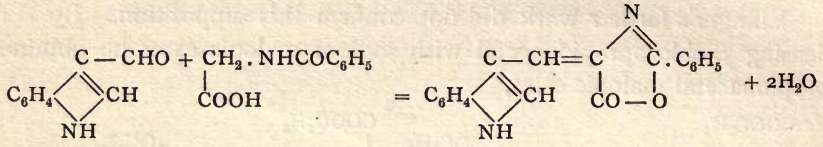
and kynurenic acid must be formed by some other reaction.

Hopkins and Cole had obtained by the oxidation of tryptophane with ferric chloride a body of the composition of C_9H_7NO; this body has been shown by Ellinger to be β-indole-aldehyde, firstly by oxidising it to β-indole-carboxylic acid,

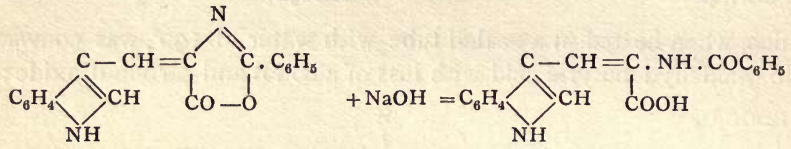


a compound synthesised by Ciamician and Zatti, and secondly by synthesis from indole and alcoholic chloroform. From this compound by the method employed by Erlenmeyer in the synthesis of phenylalanine, Ellinger and Flamand synthesised tryptophane in 1907.

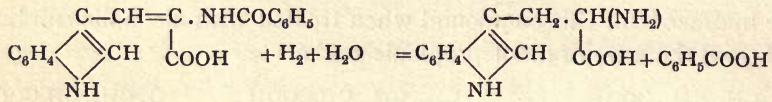
By condensing β-indolealdehyde with hippuric acid the azlactone is obtained,



which, when boiled with dilute soda, gives indolyl-*a*-benzoylaminoacrylic acid,



This compound on reduction with sodium amalgam and hydrolysis with water gives tryptophane :—



E. The Optically Active Amino Acids.

With the exception of glycine, all the amino acids contain an asymmetric carbon atom, and they are therefore capable of existence in two optically active forms. In one of these forms they are present in the protein molecule, and the synthesis of a naturally occurring amino acid is only completed when the synthesised compound has been separated into its optically active components.

Three methods are known, all due to Pasteur, by which an inactive mixture can be separated into its optically active isomers :—

1. The fractional crystallisation and mechanical separation of the two isomers.

2. The action of micro-organisms—moulds, yeasts—which destroy the one isomer more rapidly than the other. This is known as the biological method.

3. The fractional crystallisation of the salts of these compounds with optically active bases or acids.

By all these methods the optically active forms of the amino acids have been prepared.

By the first method Piutti, in 1887, obtained dextro- and lævo-aspartic acid. He fractionally crystallised natural asparagine and separated it into dextro- and lævo-asparagine, from which compounds he prepared both the dextro- and the lævo-aspartic acids. As, however, Piutti used asparagine from vetch seedlings, this separation cannot strictly be said

to be the actual synthesis of a natural compound, but there seems no reason to doubt the possibility of separating synthetical asparagine by this means.

Glutamic acid, the homologue of aspartic acid, according to Menozzi and Appiani, on recrystallisation from water, can be obtained in its two enantiomorphous forms.

By the second method Schulze and Bosshard prepared d-leucine and l-glutamic acid, and Engel prepared d-aspartic acid. Menozzi and Appiani also separated glutamic acid by this method. In all these cases the mould *Penicillium glaucum* was used to effect the separation.

From inactive cystine Neuberg and Mayer separated d-cystine, using *Aspergillus niger* instead of *Penicillium glaucum*, which gave no result with this amino acid.

Not only moulds, but also yeasts can be employed in the separation of optically active compounds as was shown by F. Ehrlich in 1906, who obtained in this way l-alanine, d-leucine, l-valine. Further, amino acids, other than those which occur in nature, can be separated by moulds and yeasts into their components, *e.g.*, n-aminocaproic acid, methylethyl-aminoacetic acid.

It was first shown by E. Fischer, in 1894, that enzymes were specific in their action; thus maltase acts only upon α-glucosides and emulsin only upon β-glucosides. Later, he found that trypsin acted "asymmetrically" upon inactive polypeptides, *e.g.*, alanyl-leucine was hydrolysed in such a way that only the compound composed of d-alanine and l-leucine, the natural isomers, was split up into its constituents, whereas the compound composed of l-alanine and d-leucine was unattacked. Again, inactive leucine ester was found by Warburg to be only partially hydrolysed by trypsin; he obtained l-leucine and d-leucine ester.

Kossel and Dakin, in 1904, found that d-arginine was hydrolysed by the enzyme arginase into d-ornithine and urea; and by using this enzyme Riesser, in 1906, separated dl-arginine, which he had prepared by heating d-arginine with sulphuric acid to 160-180° C. into l-arginine, d-ornithine and urea, the racemic compound being hydrolysed asymmetrically by the enzyme. l-Ornithine can be prepared from the l-arginine by hydrolysis with baryta.

The biological method thus only serves for the preparation of that isomer which does not occur in nature, since the mould or yeast or enzyme destroys the naturally occurring form, leaving the other isomer untouched, or according to Marckwald and Mackenzie, it acts upon the natural isomer more rapidly than upon the other. The method therefore does not lead to the synthesis of the naturally occurring amino acid.

The third method of separating optically active substances by combining them with optically active bases or acids had not been employed with any success until E. Fischer took up this question, the study of the optically active amino acids being his first work upon the chemical constitution of the proteins. The non-success of this method was in all probability due to the small affinity which the simple amino acids themselves have for combining with acids and bases; even the attempts to separate the monoaminodicarboxylic acids, which are fairly strong acids, were not successful.

Hippuric acid, or benzoylglycine, has been known for a long time, and by preparing the benzoyl derivatives of the other amino acids, Fischer found that their acidic character was greatly increased, and that they then combined with the optically active bases brucine, strychnine, cinchonine, morphine, forming stable salts. These salts were much less soluble and their power of crystallising much greater than the salts of the amino acids themselves, and consequently they were more easily isolated; further, they were easily reconverted into the amino acids.

These benzoyl derivatives were prepared by shaking the amino acid with excess of benzoyl chloride in the presence of sodium bicarbonate instead of in the presence of excess of alkali, *i.e.*, by the Schotten-Baumann method, which gave poor and varying yields of the benzoyl compound.

Alanine, aspartic acid, glutamic acid, tyrosine, leucine, phenylalanine and also *α*-amino-n-caproic acid and *α*-aminobutyric acid have in this way been separated by Fischer into their optically active isomers. To these must be added ornithine which was synthesised by Sörensen in 1903, and separated into d- and l-ornithine in 1905.

Not only can the benzoyl derivative be employed for this purpose but also the formyl derivative which is prepared by heating the amino acid with anhydrous formic acid at 100° C. These formyl derivatives also give beautifully crystalline salts with the optically active bases, and they possess one great advantage over the benzoyl derivatives, namely, that the formyl group is easily removed by hydrolysis, whereas the benzoyl group requires prolonged heating with a large excess of acid for its removal. The formyl derivative is of enormous advantage also for building up optically active polypeptides, as it admits of the preparation of large quantities of the optically active amino acids.

Fischer and his pupils have thus prepared the optically active forms of leucine, phenylalanine, and valine, and also of phenylaminoacetic acid, and Locquin has prepared, by means of the formyl derivative, d-isoleucine.

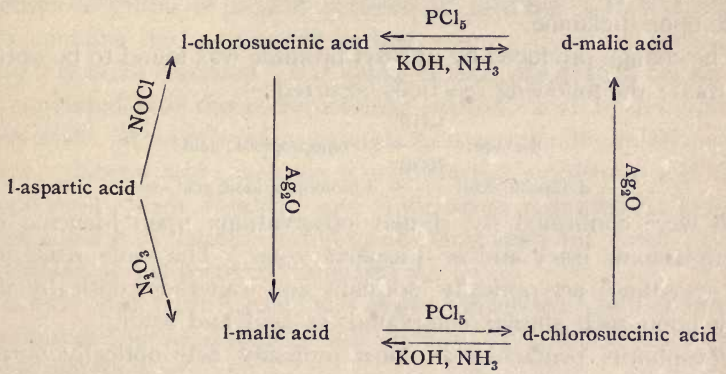
In the case of serine, no separation could be effected by means of

these derivatives, but, by making the p-nitrobenzoyl compound, Fischer and Jacobs obtained d- and l-serine. Isoserine and diaminopropionic acid must also be added to the list of optically active amino acids separated by Fischer and his pupils.

By combining *a*-aminophenylacetic acid with d-camphorsulphonic acid Betti and Mayer in 1908 separated it into its isomers. This seems to be the first case in which the basic function of an amino acid has been requisitioned for purposes of separation; in all the above cases, the acidic function, by combination with optically active bases, has been made use of.

The separation of serine into its optical antipodes was of the greatest importance, since it has enabled Fischer to correlate together the configuration of d-alanine, l-serine and l-cystine, and also to connect them with d-glucose.

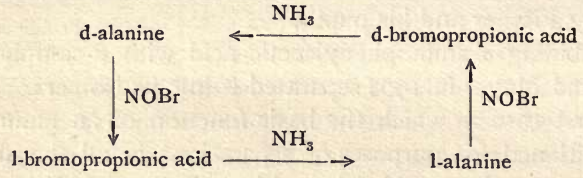
It was first observed by Walden, in 1896, that a change of configuration took place in the conversion of the malic acids into the chlorosuccinic acids and *vice versâ*. His results were collected together in 1897 and expressed in the following scheme, in which was included Tilden and Marshall's observations on aspartic acid :—



Walden concluded that potassium hydrate and phosphorus pentachloride acted optically normally, *i.e.*, without alteration of the configuration, but that silver oxide, and therefore also nitrous acid and nitrosyl chloride, acted optically abnormally, but as to which of these reactions was really the normal one he was not able to decide. The conclusion was remarkable, since the action of silver oxide takes place in aqueous solutions at a low temperature and the effect of potash in producing racemisation is well known. Still more curious is the supposition that nitrous acid and nitrosyl chloride act optically abnormally.

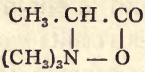
A similar change in rotation was observed in 1905 by Fischer and

Warburg in the conversion of alanine into the corresponding halogen fatty acid by nitrosyl bromide and in the reconversion of this compound into alanine by the action of ammonia :—



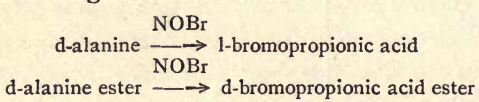
It was termed the "Walden inversion" by Fischer in 1907.

A change in configuration occurs either by the action of ammonia or by the action of nitrosyl bromide. By studying the conversion by the action of ammonia under various conditions, Fischer was able to show conclusively that this reagent behaved optically normally, which result was confirmed by a later experiment upon optically active trimethyl-*a*-propiobetaine (*a*-homobetaine),



which he prepared from trimethylamine and d-*a*-bromopropionic acid, and showed was identical with that prepared by the action of methyl iodide upon d-alanine.

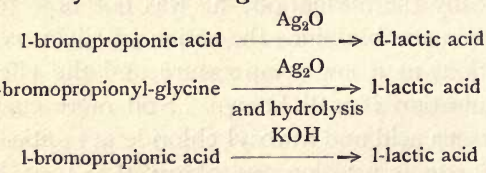
The change produced by nitrosyl bromide was found to be optically abnormal; the following reactions occurred :—



which were confirmed by similar observations upon l-leucine ester, l-phenylalanine ester and on l-aspartic ester. The same reagent can thus sometimes act optically normally and sometimes optically abnormally upon such similar compounds as acid and ester.

Phosphorus pentachloride most probably acts optically normally since it yields products having the same configuration whether it acts upon a hydroxy acid or its ester; further evidence is, however, still required.

Silver oxide behaves like nitrosyl bromide, sometimes normally, sometimes abnormally. The changes



were observed.

It may be concluded that the "Walden inversion" is limited to the reactions between nitrosyl bromide and the amino group and between silver oxide and halogen fatty acid, and is dependent upon the presence of the carboxyl group.

All amino acids would be expected to behave in the same way as leucine, etc., but valine was found by Fischer and Scheibler in 1908 to behave differently:—

$$\text{l-valine} \xrightarrow{NOBr} \text{bromovalerianic acid} \xrightarrow{NH_3} \text{l-valine}$$

that is, the same valine and not its optical antipode was obtained. Either a "Walden inversion" has occurred twice, which is very improbable, or the change into bromovalerianic acid has taken place without a change in the configuration.[1] It appears to be due to the effect of the isopropyl group. In valine,

$$(CH_3)_2 . CH . CH(NH_2) . COOH,$$

it is attached directly to the asymmetric carbon atom; in leucine,

$$(CH_3)_2 . CH . CH_2 . CH(NH_2) . COOH,$$

a methylene group is present between it and the $CH(NH_2)$ group which contains the asymmetric carbon atom.

No "Walden inversion" was found to take place when amino acids were converted into the corresponding hydroxy acid by the action of nitrous acid. It was therefore possible to determine the relationship of serine to glyceric acid: d-serine was converted by nitrous acid into a glyceric acid which Neuberg and Silbermann regarded as l-glyceric acid on account of its relationship to l-tartaric acid, but which Neuberg, a little later, stated required confirmation.

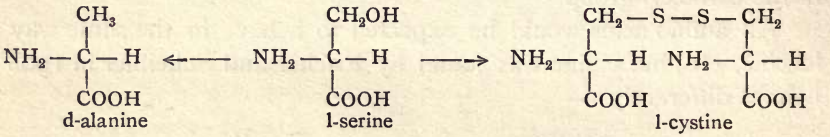
In 1907 and 1908 Fischer and Raske correlated together the configurations of l-serine, d-alanine and l-cystine by means of l-α-amino-β-chloropropionic acid,

$$CH_2Cl . CH(NH_2) . COOH,$$

which they obtained from l-serine methyl ester by the action of phosphorus pentachloride and subsequent hydrolysis with hydrochloric acid. By reducing it with sodium amalgam they obtained d-alanine, and by treating it with barium hydrosulphide and oxidising the resulting cysteine, by drawing a current of air through the solution, they obtained cystine.

[1] As this monograph is going through the press a more recent publication by Fischer and Scheibler (Ber., 1908, 41, 2891) points to the probability of the "Walden inversion" occurring twice.

If l-serine has the configuration represented, d-alanine and l-cystine will have the groups attached to the asymmetric atom arranged in the same order as in l-serine:—



The foundation for the configuration of these amino acids will be obtained if α-amino-β-chloropropionic acid can be converted into aspartic acid, the configuration of which is known from its relationship to malic acid. (d-aspartic acid is converted into d-malic acid by nitrous acid.) The configuration of malic acid can be referred to that of tartaric acid and thence to d-glucose.

Besides the above-mentioned products, several other amino acids have been described as occurring in the protein molecule. Of these, the presence of aminobutyric acid, which would complete the series of monoaminomonocarboxylic acids, was assumed by Schutzenberger, but has not been demonstrated by any of the subsequent investigators. A large number of new products were added to the list by Skraup in 1904, but he has since shown that two of them were mixtures of glycine and alanine. Another amino-oxy acid was described, as also caseanic and caseinic acids; the latter is apparently identical with Fischer and Abderhalden's diaminotrioxydodecanic acid. Another product, diamino-oxysebacic acid, was stated by Wohlgemuth to be a constituent, but its presence as well as that of those described by Skraup has not been definitely proved; they cannot therefore be regarded as units of the protein molecule.

Numerous amino acids—including diamino- and oxyamino-acids—have also been synthesised of recent years by Neuberg and his co-workers and by Sörensen. Our knowledge of these acids should render the task of identifying a new unit in the protein molecule less laborious than it has hitherto been. Their preparation was no doubt due to the possibility of the presence of other units than those above described, which possibility will not be excluded until the quantity of products isolated reaches 100 per cent.

The presence of glucosamine in the protein molecule is also a disputed question; there is no doubt that a carbohydrate containing nitrogen is contained in the glucoproteins in their prosthetic group, but it is doubtful if it is present in the protein part of the molecule, although a

carbohydrate has been obtained from carefully purified proteins containing no prosthetic group, such as crystallised egg-albumin, serum-albumin (Langstein). The fact that the yield of carbohydrate from such a protein becomes smaller the more often it is recrystallised, suggests that the presumably pure protein still contained an impurity; this impurity would be a glucoprotein, which is found in both egg white and serum from which the crystallised proteins are separated, and this would give rise to the carbohydrate.

The constitution of glucosamine, which has been synthesised by Fischer and Leuchs, therefore forms part of the subject of glucoproteins.

The constitution of all the amino acids except diaminotrioxydodecanic acid is thus known, and with the exception of histidine they have all been synthesised. The separation of the synthetical compound into its optical antipodes has still to be effected in the case of several of the amino acids.

A brief summary of the discovery of the above amino acids and by whom they were first synthesised is given in Table A. Table B contains the specific rotatory powers of the natural and synthetical substances.

TABLE A.

	Discovered		Racemic "dl" Form Synthesised		Natural Active Form Synthesised		
	by	in	by	in		by	in
Glycine	Braconnot	1820	Perkin and Duppa	1858	...	...	...
Alanine	Schutzenberger / Weyl	1888	Strecker	1850	d	Fischer	1899
Valine	v. Gorup-Besanez	1856	Fittig and Clark.	1866	d	„	1906
Leucine	Proust / Braconnot	1818 / 1820	Limpricht / Schulze and Likiernik	1855 / 1885	l	„	1900
Isoleucine	F. Ehrlich	1903	Bouveault and Locquin	1905	d	Locquin	1907
Phenylalanine	Schulze and Barbieri	1881	Erlenmeyer and Lipp	1883	l	Fischer and Schöller	1907
Tyrosine	Liebig	1846	Erlenmeyer and Lipp	1883	l	Fischer	1900
Serine	Cramer	1865	Fischer and Leuchs	1902	l	Fischer and Jacobs	1906
Cystine	Wollaston / Mörner	1810 / 1899	Erlenmeyer, jun.	1903	l	Fischer and Raske	1908
Aspartic Acid	Plisson	1827	Dessaignes	1850	l	Piutti	1887
Glutamic Acid	Ritthausen	1866	Wolff	1890	d	Fischer	1899
Ornithine	Jaffé	1877	Fischer	1900	d	Sörensen	1905
Arginine	Schulze and Steiger	1886	Schulze and Winterstein	1899	d	...	...
Lysine	Drechsel	1889	Fischer and Weigert	1902	d	...	...
Proline	Fischer	1901	Willstätter	1900	l	...	...
Oxyproline	„	1902	Leuchs (?)	...	l	...	...
Histidine	Kossel	1896	...	...	l	...	...
Tryptophane	Hopkins and Cole	1901	Ellinger and Flamand	1907	l	...	...
Diaminotrioxydodecanic Acid	Skraup / Fischer and Abderhalden	1904	...	...	...	...	...

TABLE B1.

SPECIFIC ROTATORY POWER OF THE NATURAL AMINO ACIDS.

				Observed by
d-Alanine	in water	in alkali	in HCl + 10·3°	Fischer
d-Valine	...	...	,, + 27·9°	Schulze and Winterstein
l-Leucine	...	...	,, + 16·9°	Schulze
,,	in water − 10·8°	...	in 20 % HCl + 15·7°	Ehrlich
d-Isoleucine	,, + 9·7°	...	,, + 36·8°	,,
l-Phenylalanine	,, − 35·3°	...	...	Schulze
l-Tyrosine	,,	...	in 21 % HCl − 8·5°	Schulze & Bosshard
,,	...	...	in 4 % HCl − 15·6°	,, ,,
,,	...	...	,, − 12·6°	Fischer
l-Serine	...	...	in HCl + 11·6°	,,
l-Cystine	...	...	in HCl − 223 to − 224·3°	Mörner
l-Aspartic Acid	...	in alkali − 2·4°	in HCl + 25·7°	Fischer
d-Glutamic Acid	...	...	,, + 30·5°	,,
,,	...	...	,, + 31·1°	Schulze & Bosshard
d-Ornithine	...	...	...	...
d-Arginine	...	...	in HCl + 21·2°	Gulewitsch
d-Lysine	...	...	,, + 17·5°	Lawrow
l-Histidine	...	...	...	Kossel
l-Proline	in water − 77·4°	in alkali − 83·5°	in HCl − 46·5°	Fischer
l-Oxyproline	,, − 81·1°	...	...	,,
l-Tryptophane	− 33°	...	...	Hopkins and Cole
,,	− 30° to − 40°	in $\frac{N}{1}$ alkali + 5·7°	in HCl − 13·5°	Fischer
,,	in water − 30·3°	in $\frac{N}{2}$ alkali + 6·2°	in HCl + 1·3°	Abderhalden and Baumann
l-Diaminotrioxy-dodecanic Acid	,, − 9°	...	...	Fischer and Abderhalden

TABLE B2.

SPECIFIC ROTATORY POWER OF THE SYNTHETICAL AMINO ACIDS.

				Observed by
d-Alanine	in water −	in alkali −	in HCl + 9·7°	Fischer
l-Alanine	...	...	,, − 10·3°	,,
d-Valine	in water + 6·4°	...	in 20 % HCl + 28·7°	,,
l-Valine	...	...	,, − 28·7°	,,
,,	...	...	,, − 27·4°	Ehrlich
d-Leucine	...	...	,, − 15·6°	Fischer and Warburg
l-Leucine	...	...	,, + 15·8°	,, ,,
d-Isoleucine	in water + 11·3°	...	,, + 40·6°	Locquin
l-Isoleucine	,, − 10·6°	...	,, − 31·4°	,,
d-Phenylalanine	,, + 35·1°	...	in 18 % HCl + 7·1°	Fischer and Mouneyrat
l-Phenylalanine	...	...	...	...
d-Tyrosine	...	...	in 21 % HCl + 8·6°	Fischer
l-Tyrosine	...	...	,, − 8·6°	,,
,,	...	...	in 4 % HCl − 13·2°	,,
d-Serine	in water + 6·9°	...	in HCl − 14·3°	Fischer and Jacobs
l-Serine	,, − 6·8°	...	,, + 14·5°	,, ,,
d-Cystine	...	...	...	...
l-Cystine	...	...	in HCl − 209·6°	Fischer and Raske
d-Aspartic Acid	...	...	,, − 25·5°	Fischer
l-Aspartic Acid	...	in alkali − 2·3°	...	,,
d-Glutamic Acid	...	...	in HCl + 30·8°	,,
l-Glutamic Acid	...	...	,, − 30·1°	,,
l-Arginine	...	..	in HCl − 20·5°	Riesser

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