becomes solid. By this means phosphorus iodide can be made in any desired quantity quickly and with safety.

HOWARD W. DOUGHTY.

BUREAU OF STANDARDS, WASHINGTON, D. C.
May 19, 1905.

# REVIEW.

## RECENT WORK IN BIOLOGICAL CHEMISTRY.

BY P. A. LEVENE.

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THE activity of biological chemists since the time of the appearance of the last review (This Journal, March, 1904) was still greater than it had ever been before. The principal problems for investigation continued to be the same as in previous years. However, in the preceding years, the progress was more marked in the knowledge of the chemical nature of substances having a biological interest. Also in the past year, considerable contributions were made in this direction, but the work was mostly a continuation of that already begun in preceding years. Noteworthy for the last year is the renewed interest in problems of metabolism and in those of enzyme action. Indeed, theories of metabolism totally contradictory to those generally accepted were adduced by many writers, and it was suggested that the existing principles of nutrition have to be revised. The study of enzyme action was very fruitful in its application to the study of physiological problems. It was attempted to explain the mechanism of many functions by enzyme action.

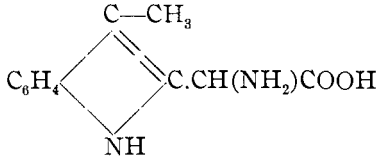
The chemical study of the tissue components again was directed principally to that of proteid. In preceding years the progress of the knowledge of the composition of the proteid molecule was achieved through the introduction of Fischer's method of isolating and of separating amino acids. By the aid of Fischer's process it was demonstrated that individual proteids differ in the nature and more so in the proportion of the monoamino acids entering into their molecules. The study of the proteid molecule has been further facilitated by the efforts of Skraup (*Z. physiol. Chem.*, **42**, 274 (1904)). This author has observed that only monoamino acids are readily esterified by treatment with alcohol and hydrochloric acid gas, and that many diamino acids, or oxyaminoacids remain unchanged by the process. Skraup has made the further observation that hydrochloric ethyl esters of amino acids are soluble in a mixture of alcohol and ether. Thus it was made possible to accomplish a separation of substances which esterify readily from those that do not. The last substances could further be separated by means of fractional precipitation with phospho-

tungstic acid. In this manner Skraup discovered among the components of casein several new substances. They belong to the following groups: (1) Diaminodicarboxylic acids; diaminoglutamic acid, $C_5H_{12}O_4N_2$, and diaminoadipic acid, $C_6H_4O_4N_2$. (2) Aminoxypolycarboxylic acids; $C_4H_7O_5N$, aminohydroxysuccinic acid, $C_8H_{16}N_2O_6$, dihydroxydiaminosuberic acid, $C_9H_{16}N_2O$, caseanic acid, $C_{12}H_{24}N_2O_5$ and caseic acid.

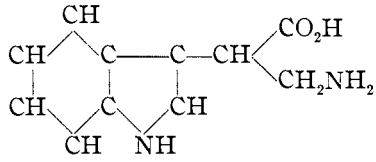
A substance closely related to Skraup's caseic acid, was discovered also by Fischer and Abderhalden. (*Z. physiol. Chem.*, **42**, 540 (1904)). It has the composition $C_{12}H_{26}N_2O_5$, and is designated by the authors diaminotrihydroxydodecanoic acid. And a substance having the composition $C_{12}H_{22}N_4O_5$ was obtained from protoalbumose by Levene (*Proc. Am. Physiol. Soc.*, *Journ. of Physiol.*, 13). Hydroxyaminosuberic acid and hydroxydiaminosebacic acids were isolated also by Wolgemuth from the nucleoproteid of the liver (*Ber.*, **37**, 4362 (1904)).

Thus it has been established that the number of components of the proteid molecule is greater than hitherto accepted.

Also in the knowledge of the chemical nature of the individual components considerable progress has been made. Thus Ellinger (*Ber.*, **37**, 1801 (1904)) has shown that the view of the constitution of tryptophane generally accepted since the work of Hopkins and Cole has to be modified. Hopkins and Cole represented tryptophane as skatolaminoacetic acid. According to Ellinger, tryptophane has to be regarded as indol-$\beta$-aminopropionic acid.




Hopkins and Cole.




Ellinger.

The finding is of considerable significance as it contains very definite evidence for the assumption that the proteid molecule is composed of amino acids of the aromatic as well as of the aliphatic series. It is also important for the interpretation of the origin of kynurenic acid in the organism. Reference to this will be made later.

The constitution of another very important component of the

proteid molecule was the subject of further investigation. It was stated in the previous review that Fränkel on the ground of his investigation arrived at the conclusion that histidine was a pyrimidine derivative. The same author has also assumed that the substance contained in its molecule a carboxyl group and one primary amino group. Pauly (*Z. physiol. Chem.*, **42,** 508 (1904)) resumed the work of Fränkel. He corroborated the assumption of Fränkel of the existence of a carboxyl group by obtaining the methyl ester of the substance. He further demonstrated that of the two remaining nitrogen atoms one was present in a secondary and the other in a tertiary amino group, as he found that histidine is capable of uniting with only two naphthalene sulphochloride groups. Further the fact that histidine is capable of combining with two atoms of silver, that it is resistant towards acids, and that it is easily decomposed by alkaline oxidizing agents seemed to Pauly to justify the conclusion, that the substance is an imidazole derivative.

The knowledge of the nitrogenous constituents of the proteid molecule was further enlarged by the finding of Ehrlich (*Ber.*, **37,** 1821) that not all the leucine has the composition of α-amino-isobutylacetic acid. The isomer is designated by the writer, isoleucine.

Considerable progress has been made, also, in the study of the radical of the proteid molecule, which contains sulphur. In the preceding review it was mentioned that through the work of Friedmann it had been shown that the old assumption of Baumann that cysteine had the composition $CH_3C\begin{cases}H_2S\\ \text{——}COOH\\ NH_2\end{cases}$ was erroneous, and that the constitution of cysteine was that of α-amino-β-thiolactic acid. Paul Meyer and Neuberg and A. Loewy and Neuberg have recently made a statement that cysteine occurring in urinary calculi differs in its constitution from that of protein cysteine, and is isomeric with the latter, being β-amino-α-thiolactic acid. They have surmised that in the proteid molecule both forms are present, all the more, that other writers had also expressed similar views. However, some workers disagreed with the conclusions of Neuberg. Gabriel (*Ber.*, **38,** 630 (1905)) succeeded in obtaining synthetically β-amino-α-thiolactic acid. This was achieved by transferring dihydrouracil into the bromine compound, and by treating the latter with potassium sulphocyanide. The sulphocyanide compound was heated in a sealed tube at 170° and in this manner isocysteine obtained. Gabriel compared a sample of Neuberg's stone cysteine with the synthetical isocysteine and found that they were not identical. Also Rotera (*J. Physiol.*, **32,** 175 (1905)) and Alsberg and Folin (*Am. J. Physiol.*, **14,** 54 (1905)) failed to corroborate the conclusions of Neuberg. In a more recent article Neuberg and Paul

Meyer (*Z. physiol. Chem.*, **44**, 472 (1905)) make the statement that not all cysteine calculi are composed of stone cysteine. They further point out in detail the differences in the chemical and in the physical properties of the two forms. Thus, it still remains probable that the proteid molecule contains two forms of cysteine, although it is not improbable that the two forms found by Neuberg in urinary calculi and in the products of hydrolysis of proteids are the two modifications of one form originally present in proteid material.

Also the more complex parts of the proteid molecule continued to be the subject of further investigation. The substances of this group deserving most interest are the protamines. It was stated in the previous review that originally Kossel regarded protamines as the nucleus of all proteid material. According to the first analysis, protamines consisted of arginine, lysine and histidine only. However, subsequently the view was modified, and very recently Kossel and Dakin reinvestigated the question of composition of various protamines (*Z. physiol. Chem.*, **40**, 565 (1904) and **44**, 342 (1905)). The authors demonstrated that various protamines differed in their composition, but that they all contained in their molecule some amino acids. In another paper Kossel discusses the origin of protamines (*Z. physiol. Chem.*, **44**, 347 (1905)) in the organism, and there adduces the theory that protamines are cleavage products of proteids, protected from further cleavage partly by their combination with nucleic acid. Thus, according to this view that part of the proteid molecule which is composed of the basic substances is characterized by greater resistance towards hydrolytic influences. In harmony with this view seems to be the finding of Levene, that gelatin-peptone is poorer in glycocol, than gelatoses (*Z. physiol. Chem.*, **41**, 8 (1904)). This view seems to find support in the work of Siegfried (*Z. physiol. Chem.*, **43**, 44 and 46). This author obtained on hydrolysis of gelatin and of casein by concentrated mineral acids substances which resembled in their composition protamines, and which were designated as kyrines. The molecule of glutokyrin derived from gelatin is according to Siegfried composed of 1 mol. of arginine, 1 mol. of lysine, 1 mol. of glutamic acid and of 2 mols. of glycocol. Caseinkyrin is composed of 1 mol. of arginine, 2 mol. of lysine, 1 mol. of glutamic acid. Both protamines and kyrines give a distinct biuret reaction. However, the biuret reaction of the proteid molecule is not due exclusively to the part composed of basic substances. This was made clear by the work of v. Fürth (Hofmeister's Beiträge, 6, 296 (1905)). This author studied the products of oxidation of proteids by means of permanganate. By precipitation with silver nitrate, lead acetate and mercuric acetate he obtained three different complex substances. They all gave the biuret reaction. The fraction precipitated by lead acetate is characterized by its

low proportion of nitrogen and high oxygen. On hydrolysis it yields glutamic acid, leucine, benzoic acid and ammonia. Thus v. Fürth arrives at the conclusion that the biuret reaction is not dependent on the integrity of the basic group of the proteid molecule.

In connection with the study of the complex components of the proteid molecule, reference should be made also to the work of Haslam (*J. Physiol.*, **32**, 267 (1905)). The author asserts that the albumoses obtained according to the process of Pick could not be regarded as individual substances. However, they can be obtained in a pure condition by repeated reprecipitation by means of sodium sulphate at 37°.

The progress in the knowledge of the composition of proteids naturally led to attempts to build up the substances synthetically. In the previous review the results of Fischer's efforts to condense various amino acids were reported. The work has been continued since then by Fischer and his co-workers. The process of Fischer is to combine halogen-substituted acid chlorides with amino acids and further substituting the halogen by an amino group. In this second communication (*Ber.*, **37**, 2982 (1904)) the author reports the synthesis of the following substances:

(1) Dipeptides: Glycylalanine, leucylleucine, glycyltyrosine, leucyl-*l*-tyrosine.

(2) Tripeptides: Diglycylglycine.

(3) Tetropeptides: Triglycylglycine, dileucylglycylglycine.

(4) Pentapeptides: Tetraglycylglycine.

The polypeptides resemble peptones in that they give the biuret reaction, are precipitated by phosphotungstic acid, and are capable of being hydrolyzed by means of trypsin. In a further communication Fischer in conjunction with Suzuki (*Ber.*, **37**, 2842 (1904)) announces the synthesis of prolinalanine. The substance was obtained by the action of alanine on the chloride of dibromvaleric acid, and by the action of ammonia on the substance resulting from the foregoing synthesis. The ornithine derivative that *a priori* could be expected to form was not isolated. Further, Fischer succeeded in introducing phenylalanine in the molecule of polypeptides (*Ber.*, **37**, 3062 (1904)). For this purpose phenyl-$\alpha$-brompropionic acid was obtained, this transformed into the chloride and acted upon by glycylglycine. Through the action of ammonia phenylalanylglycylglycine was obtained. Fischer reports in the same article on the synthesis of phenylalanylphenylalanine. In another communication from Fischer's laboratory Leuchs and Suzuki (*Ber.*, **37**, 3306 (1904)) report on the synthesis of a series of phenylalanine derivative obtained by the same process as the foregoing substances. These authors obobtained leucylphenylalanine, leucylleucylphenylalanine, alanylphenylalanine, glycylphenylalanine, leucylglycylphenylalanine diglycylphenylalanine. Further progress was attained by Fischer

(*Ber.*, **38,** 605 (1905)) in that he succeeded in preparing the hydrochlorides of amino acid chlorides. Thus there were obtained the chloride of leucyl chloride, the chloride of alanyl chloride, the chloride of $\alpha$-aminobutyryl chloride.

More complex polypeptides were obtained by Fischer by preparing chlor-substituted polypeptides. Thus he condensed $\alpha$-bromisocaproylglycylglycine with glycine ester and by treating the product with ammonia he obtained leucyldiglycylglycine. Further by the action of acetyl chloride and phosphorus pentachloride the author obtained hippuryl chloride, and by the aid of this substance he obtained benzoyldiglycylglycine ester, substances recently obtained by Curtius in a different manner.

In the same communication Fischer reports on a new process of preparing dipeptides. The method consists of the treatment of diacipiperazines at ordinary temperature with the calculated amount of alkali. The importance of this finding lies in that it makes possible the assumption of the presence in the protein molecule of diacipiperazine rings. The fact that proteids can be decomposed by the action of enzymes in slightly alkaline media into amino acids was regarded as evidence against its presence. The finding of Fischer renders the old view no longer tenable. The synthesis of polypeptides was accomplished very successfully and in a very ingenious manner by Curtius and his co-workers (*J. prakt. Chem.*, **70,** 57). The result was attained by the action of amino acids on acid azides. Acid azides ($RCON_3$) are substances obtainable without great difficulty. By this process it was possible to condense six glycine radicals. The work of Curtius is important not only because it made it possible to prepare substances composed of a long chain of amino acids, but also for the reason that the polypeptides obtained in this manner yield on hydrolysis substances different from those that served for the synthesis. By the action of ammonia or amines on acid azides under varying conditions substances either of the nature of acid amides or of that of urea derivatives are obtained. On hydrolysis of these products, substances important from the physiological standpoint are obtainable. Thus the urethane formed by the action of alcohol on hippurazide gives rise on hydrolysis to benzoic acid, ammonia, formaldehyde, carbonic acid and alcohol. In this manner glycocol gives rise to formaldehyde. The substance resulting from the action of aniline on hippurylalanine breaks down into hippuric acid, ammonia, acetaldehyde, carbonic acid and aniline. The same synthesis may also serve for the transformation of monoamino acids into the diamino derivatives. Thus the substance obtained by the action of aniline on the azide of hippurylaspartic acid gives rise among other products to diaminopropionic acid, as is seen from the following equation:

$$C_6H_5CONHCH_2CONHCHCONHC_6H_5$$
$$\qquad\qquad\qquad\qquad\qquad\qquad\qquad\quad |$$
$$\qquad\qquad\qquad\qquad\qquad\qquad\qquad CH_2NHCONHC_6H_5 + 3H_2O =$$
$$C_6H_5CONHCH_2COOH + NH_2CHCOOH + 2C_6H_5NH_2 + CO.—$$
$$\qquad\qquad\qquad\qquad\qquad\qquad\qquad\quad |$$
$$\qquad\qquad\qquad\qquad\qquad\qquad\qquad CH_2NH_2.$$

Further, the urethane derived from the action of alcohol on the azide of hippuryl-β-aminobutyric acid gives rise on hydrolysis among other products to propylenediamine.

As already mentioned, the formation of the foregoing substances from simple polypeptides is of great significance from the biological view-point. The formation of formaldehyde may serve to explain the mechanism of sugar formation from proteid. Diamino acids and diamines are obtained on hydrolysis of proteids, and their formation from derivatives of simple polypeptides is also of considerable significance.

The substances already obtained by this process are as follows: Curtius and Wüstenfeld (*J. prakt. Chem.*, **70,** 73) have obtained the benzoyl derivative of aminoacetic acid, of glycylaminoacetic acid, bisglycylaminoacetic acid, triglycylaminoacetic acid, tetra- and penta-aminoacetic acid. The acids are fairly insoluble in water, but soluble in dilute alkalies forming the corresponding salts, they are crystalline, and form a crystalline silver salt. They all give a positive biuret test.

In order to accomplish the synthesis of the complex substances each new acid had to be transformed into its hydrazide, $R.CONH.NH_2$, and that into the azide. In order to simplify the process Curtius and Levy (*J. prakt. Chem.*, **70,** 89 (1904)) attempted to condense hydrazides with azides. The results were satisfactory. Besides glycocol the condensation was accomplished with alanine (Curtius and Lambatte, *J. prakt. Chem.*, **70,** 109 (1904)); the most complex substance obtained is hippurylalanylalanylalanine. Curtius and von-der-Linden (*J. prakt. Chem.*, **70,** 137 (1904)) further succeeded in condensing α-alanine with glycine by means of benzoylalanine azide. The substance obtained in this manner was benzoylalanylglycylglycine. Another improvement in the process was introduced for the purpose of obtaining the derivatives of aspartic acid. (Curtius Th. and Curtius Hans, *J. prakt. Chem.*, **70,** 158 (1904)) hippurylaspartic acid was obtained in the usual manner. However, for further condensation, it was found necessary to dissolve the hippurylaspartic acid azide in aspartic ester. In this manner hippurylasparagylaspartic acid was obtained. It was also possible to condense hippurazide with β-amino-α-hydroxypropionic acid and with β-aminobutyric acid, also γ-butyric acid with phenylalanine (Curtius and Gumlich, *J. prakt. Chem.*, **70,** 195 (1904) and Curtius and Müller, *Ibid.*, **70,** 223 (1904)). Finally Curtius and Lenhard (*J. prakt. Chem.*, **70,**

230 (1904)) succeeded in introducing into the molecule of a polypeptide a carbamic acid radical. However, it was impossible to introduce more than one such group. The following substances of this group were obtained: Phenylcarbaminoaminoacetic acid, phenylcarbaminoglycylglycine, phenylcarbaminobisglycylglycine.

The successful condensation of amino acids naturally led to an effort to improve the process of preparing amino acids. In this direction are worthy of note the work of Sörens on (*Z. physiol. Chem.*, **44,** 448 (1905)) and that of Neuberg and Silbermann (*Z. physiol. Chem.*, **44,** 147, and **45,** 92 (1905)).

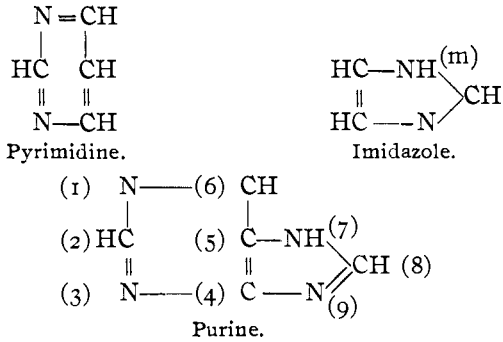
Thus the progress in the knowledge of the simple proteids was very pronounced. The study of combined proteids is not marked by the same degree of progress.

The study of nucleoproteids was extended by Wolgemuth to that of the liver (*Z. physiol. Chem.*, **37,** 475 (1903), **42,** 519 (1904), **44,** 530 (1905) and *Ber.*, **37,** 4362 (1904)). The author succeeded in demonstrating the presence of xylose in the molecule of that substance and also of the four purine bases, adenine, guanine, xanthine and hypoxanthine. Levene and Stooky (*Z. physiol. Chem.*, **41,** 404 (1904)) have made it probable that the pancreas nucleoproteid was not a derivative of guanilic acid as generally accepted, but of a more complex nucleic acid. The other points of interest in the study of nucleic acid is the observation of Inouye (*Z. physiol. Chem.*, **42,** 117 (1904) and that of Levene (*Z. physiol. Chem.*, **43,** 199 (1904)) that a carbohydrate yielding on hydrolysis levulinic acid is present in the molecule of all nucleic acids, thus far analyzed. Further progress in the knowledge of nucleic acid was attained by the work of Steudel (*Z. physiol. Chem.*, **42,** 165 (1904) and **43,** 402 (1905)) on the quantitative analysis of the thymus nucleic acid. Of considerable importance is the observation made by Steudel in the course of his work, that the yield of cleavage products depends on the nature of the acid employed for hydrolysis, the most satisfactory results being obtained by the use of sulphuric acid. On hydrolysis with that, acid thymus nucleic acid showed the following distribution of its nitrogen:

As ammonia, 5.20 per cent.; humine, 6.58 per cent.; guanine, 10.07 per cent.; adenine, 16.39 per cent.; cytosine, 11.47 per cent.; thymine, 13.11 per cent.

Some improvement in the preparation of nucleic acid was introduced by Levene (*Z. physiol. Chem.*, **45,** 370 (1905)). This made possible the quantitative analysis of spleen nucleic acid.

Most worthy of note is the effort of Burian to elucidate the manner in which the components of nucleic acid are combined within the molecule (*Ber.*, **37,** 696 and 708 (1904)). According to Burian the purine ring is regarded as a condensed pyrimidine and imidazole.



Pyrimidine. Imidazole.

Purine.

The author explains the property of purines to give the alloxan reaction by the presence in their molecule of pyrimidine, and the property of the silver salts of purine bases, by the presence of imidazole. Burian further refers to the finding of Wallach, Rung and Behrend that imidazole gives a very characteristic, colored derivative with benzenediazonium chloride. The reaction takes place also with substituted imidazoles with one exception, namely when the hydrogen in position 7 is substituted. The author then proceeded to condense nucleic acid with benzenediazonium chloride, but failed to do so. However, if a cleavage of purine bases was caused previous to treatment with the diazo compound the typical color reaction took place. On the basis of these observations, Burian adduced the theory that the condensation of the diazo compound takes place in position 7 of the purine ring, and that in nucleic acid the condensation of purines takes place also in position 7. Objection to this assumption was raised by Steudel (*Z. physiol. Chem.*, **43,** 199 (1904)) who found that thymine is also capable of condensing with benzenediazonium chloride. An attempt to elucidate the manner in which the components of nucleic acid are combined in the proteid molecule was made also by Levene (*Z. physiol. Chem.*, **40,** 370 (1904)).

Still less significant is the progress made in the chemistry of the other very important combined proteid of the blood pigment. In the preceding review mention was made of the discovery that the blood pigment is a pyrrol derivative, and as such is closely related to the bile and urinary pigments and chlorophyl. The relationship of chlorophyl to the bile pigments was further demonstrated by Marchlewski (*Z. physiol. Chem.*, **43,** 207 (1904) and **44,** 464 (1905)). The author showed that the substance isolated from feces of cattle fed on fresh grass and designated phylloerythrin had the same appearance, solubility and absorption lines as the bile pigments cholehämatin and bilipurpurin. Worthy of note is also the attempt of Buraczewski and of Marchlewski (*Z. physiol. Chem.*, **44,** 410 (1905)) to obtain synthetically hemopyrrol, which, according to Nencki and Zaleski, is methylpropyl-

pyrrol. The imide of propylmalonic acid was subjected to distillation with zinc dust. It has been stated in the previous review that hemapyrrol on exposure to air is transformed into urobilin. The substance obtained synthetically forms on exposure to air a reddish brown dye. However, the absorption line of the dye differs from that of urobilin. Still more insignificant is the progress made in the chemistry of mucoids. However, mention should be made here of the work on the presence of a carbohydrate group in the proteid molecule. This question was one of considerable discussion in recent years. The question was reopened for discussion by the work of Abderhalden, Bergell and Dörpinghause (*Z. physiol. Chem.*, 41, 530 (1904)). The authors analyzed serumglobulin, serumalbumin and ovalbumin, and noted that the quantity of carbohydrate obtainable from these proteids is very insignificant, that on reprecipitation the yield diminishes, and that serumalbumin on recrystallization may be obtained absolutely free of carbohydrates. The authors are therefore inclined to regard the carbohydrate detected by them as a mere impurity. In response to the work of Abderhalden, Bergell and Dörpinghause appeared the work of Langstein (*Wiener Monatsh.*, **25**, 453–463 and *Z. physiol. Chem.*, **42**, 171 (1904)). Langstein isolated from serumglobulin on treatment with potassium hydroxide or with barium hydroxide a polysaccharide resembling Fränkel's albamine. On hydrolysis the substance yielded glucosamine. In his theoretical discussion Langstein advances the view that the carbohydrate is present in the proteid molecule in very loose combination, analogous to that of water of crystallization. This admission is very significant, as the previous work of Langstein more than that of any other investigator rendered strong support to the assumption of the presence of a carbohydrate group in the proteid molecule, in a glucoside-like form.

It has been stated already that in the past the work that has attracted most attention was that on metabolism. It seemed from that work that both the principles of nutrition and the views on the mechanism of proteid metamorphoses in the organism will have to be revised. The old principles of nutrition were based principally on the work of v. Voit. This author formulated the requirements for the daily diet of an adult as follows: Proteid, 118 grams; fat, 56 grams; carbohydrates, 500 grams. This diet contained 2810 calories.

The investigation of Voit was largely of a statistical nature based on the observation of the diets of individuals engaged in different forms of physical work. In recent years there seems to have arisen sufficient cause for the belief that the daily diet as formulated by Voit was in excess of the actual requirement of the average individual. The investigation of the subject was undertaken by Prof. Chittenden and in collaboration with Prof. Mendel it was carried out in a very thorough and careful manner (R. H.

Chittenden, "Physiological Economy in Nutrition," New York, F. A. Stokes, 1904). The observations were made on the author himself, on Prof. Mendel and on the other members of the laboratory staff, further on several students actively engaged in college athletic work, and finally on a company of soldiers, especially commissioned for that purpose. Every one under observation was allowed a fairly liberal and varied diet. However, the amount of albuminous matter was gradually diminished, until it reached a certain minimum. The diet of the author was reduced to one consisting of 2000 calories and containing 40 grams of proteid material with 6.4 grams of nitrogen. The food of the students was reduced to 2500 calories and contained 55 grams proteid (8.8 nitrogen) per day, and that of the soldiers to 2500 to 2600 calories with 7 to 8 grams nitrogen per day. The observations extended on a very considerable period of time and every one under observation remained in good health. Loss in weight was noted only at the beginning of the experiment, during the remaining time it kept unchanged. On the ground of this work, Prof. Chittenden arrives at the conclusion that a person in good health, and under normal conditions of activity can subsist on a diet containing only one-half of the amount of albuminous matter which was regarded as an average requirement. The deduction is as important as it is new. And the work may prove very valuable not only from the theoretical but also from the practical therapeutic point of view. But it also ought to be borne in mind that every one under observation was continuing the work which he had been in the habit of doing and which he was trained to do, and further it has to be noted that no individual worked to a condition of actual fatigue, that wear of tissues in fatigue is out of proportion to the amount of work performed.

The deductions of Chittenden were strongly supported by Folin (*Am. J. Physiol.*, **13**, 117 (1905)). Folin succeeded in reducing the nitrogen elimination to a considerably lower level than that in the experiments of Chittenden. This he achieved by introducing a diet composed of soluble starch and of cream, and containing only 1 gram of nitrogen. On the new diet the nitrogen elimination was between 3 to 4 grams per day. However, most remarkable is the difference in the proportion of the nitrogenous constituents of the urine on the two different diets. It can be best seen from the following table showing the composition of the urine of the same individual on the different diet.

| | Ordinary diet. | Cream-starch diet. |
|---|---|---|
| Volume of urine ..... | 1170 cc. | 385 cc. |
| Total nitrogen..... | 16.8 gr. | 3.60 gr. |
| Urea nitrogen..... | 14.70 gr. = 87.5 per cent. | 2.20 gr. = 61.7 per cent. |
| Ammonia nitrogen..... | 0.49 gr. = 3.0 " | 0.42 gr. = 11.3 " |
| Uric acid nitrogen ..... | 0.18 gr. = 1.1 " | 0.09 gr. = 2.5 " |
| Creatinine nitrogen..... | 0.58 gr. = 3.6 " | 0.60 gr. = 17.2 " |

| | Ordinary diet. | Cream-starch diet. |
|---|---|---|
| Undetermined nitrogen | 0.85 gr. = 4.9 per cent. | 0.27 gr. = 7.3 per cent. |
| Total $SO_3$ | 3.6 gr. | 0.76 gr. |
| Inorganic $SO_3$ | 3.27 gr. = 0.90 " | 0.46 gr. = 60.5 " |
| Ethereal $SO_3$ | 0.19 gr. = 5.2 " | 0.10 gr. = 13.2 " |
| Neutral $SO_3$ | 0.18 gr. = 4.8 " | 0.20 gr. = 26.3 " |

From this table it is seen that creatinine remains unaltered in its absolute quantity under the two diets, but rises in its proportion to the other constituents. Ammonia elimination has nearly the same character.

Uric acid elimination has also the same character but is less constant in its elimination.

Undetermined nitrogen is depressed on the cream-starch diet, but its proportion to the other constituents is raised.

Urea elimination marks the greatest depression both in its absolute quantity and in its proportion to the other nitrogenous components of the urine.

Inorganic sulphates show exactly the same variations as urea.

Neutral sulphur follows exactly the curve of creatinine elimination, and ethereal sulphates follow that of uric acid elimination.

On the ground of these observations Folin accepts two forms of protein metabolism existing in the human organism. One is variable in its intensity, the other remains constant. The nitrogenous end-products of one form are principally creatinine and uric acid, ammonia, neutral and ethereal $SO_3$; those of the other are urea and inorganic sulphates. The form of metabolism characterized by creatinine elimination is designated by Folin as endogenous in distinction to the form characterized by its variability and designated as exogenous. The endogenous form represents that resulting from tissue activity, the other is that of the excessive proteid ingested with the food. Only the endogenous form is essential for maintenance of life. The nitrogen output caused by that form of metabolism represents the actual nitrogen requirement of the organism, all the nitrogen ingested in excess of it is unnecessary ballast. A nitrogen elimination of 3 to 4 grams is, according to Folin, in excess of the actual requirement of the average man. One can see that Folin advocates a diet poorer in proteid material than the diet recommended by Chittenden. Also regarding Folin's work it has to be remarked that it was done with marvelous thoroughness. However, the assumption that the most desirable form of metabolism is the one resulting in elimination of a urine composed as that on the cream-starch diet, needs further confirmation. It is worthy of note that in the advanced stages of many pathological forms the urine has the same character as the one considered by Folin the most desirable; it is worthy of note, further, that uric acid ingested in the organism is removed principally in the form of urea. Recently it was claimed by Czernecki that also ingested creatine was removed at least in part in the form

of urea (*Z. physiol. Chem.*, **44**, 294 (1905)). Furthermore, it is very probable that creatine, uric acid and ammonia, though all formed in the organism from proteid material, have their own cycle of metamorphosis regulated by a special mechanism, independent one of another, in the same manner as have carbohydrates and fat. With all that, the work of Chittenden and Folin proves that under favorable conditions the average man can subsist on a diet much poorer in albuminous matter than required by Voit's formula.

The process of proteid assimilation has also been the subject of new investigation. The manner in which foreign proteid is transformed into tissue substance has always been the subject of much speculation. In recent years it has been demonstrated that proteolytic enzymes of the gastro-intestinal tract were capable of decomposing proteid material to its simple crystalline components. It was therefore assumed that ingested albuminous matter previous to being assimilated is decomposed into amino acids, diamino acids and other simple substances. Loewy and Neuberg (*Z. physiol. Chem.*, **43**, 338 (1904)), in the course of their study on cystinuria, came to the conclusion that the foregoing assumption was erroneous. Loewy and Neuberg made the observation on their patient that amino acids or diamino acids ingested with the food were not decomposed further in the organism, and reappeared in the urine. Their conclusion was that proteid material was not decomposed in the organism as far as amino acids. Thus far, however, the work of Loewy and Neuberg has been contradicted by the investigations of Alsberg and Folin (*Am. J. Physiol.*, **14**, 54 (1905)), and of Simon (*Z. physiol. Chem.*, **45**, 357 (1905)).

On the other hand, the view of Loewy and Neuberg finds some corroboration in feeding experiments with products of proteid hydrolysis by means of enzymes or mineral acids (Abderhalden and Rona, *Z. physiol. Chem.*, **42**, 528 (1904) and **44**, 198 (1905); Henriquer and Hausen, *Z. physiol. Chem.*, **43**, 417 (1905)). Thus it has been demonstrated that it was impossible to maintain nitrogenous equilibrium by feeding animals on products of the acid hydrolysis of proteids. The equilibrium, however, was maintained, and even retention of nitrogen was possible when the animals were fed on products of tryptic digestion. This difference in the food value of the substances obtained from proteid in the two different manners may be explained by the assumption of the presence among the products of tryptic digestion of substances of the nature of polypeptides.

Mention should be made here also of the fact that the progress in the knowledge of proteid chemistry and the improvements in the methods of analysis have had their influence also on clinical chemistry. The method of isolating amino acids by means of $\beta$-naphthalene sulphochloride was employed also for urinary analy-

sis. By this process Ignatowski (*Z. physiol. Chem.*, **42**, 371 (1904)) demonstrated the presence of glycocol in the urine in different pathological conditions. The method was later improved by Abderhalden and Lewellys Barker (*Z. physiol. Chem.*, **42**, 524) and by Erben (*Z. physiol. Chem.*, **43**, 320).

It has already been mentioned that very important contributions were made to the knowledge of enzymes. Of the work on the enzymes of the gastro-intestinal tract that of Pawlow and Parastschruik deserves special attention (*Z. physiol. Chem.*, **42**, 415 (1904)). These authors come to the conclusion that the proteolytic and curdling function of digestive secretions belong to one enzyme.

Most of the work of the year was done on the tissue enzymes. The discovery of self-digesting power of animal substances was reported in the preceding review; mention was made also of the importance of the discovery for the interpretation of many physiological and pathological phenomena. The work was continued on the old lines and extended on a number of tissues by Levene (*Z. physiol. Chem.*, **41**, 393 (1904); *Am. J. Physiol.*, **11**, 437 and **12**, 276 (1904)) and by Mochizuki and Kotake (*Z. physiol. Chem.*, **43**, 165 (1904)) and by Kutscher (*Centrlbl. Physiol.*, **8** (1904); *Z. physiol. Chem.*, **43**, 93 (1904)). In these investigations attention was given primarily to crystalline end-products of self-digestion. A new contribution to the study of autolysis is found in the work of Matthes on the origin of the autolytic enzymes (*Arch. exper. Path.*, **51**, 442 (1904)). The author investigated the autolytic power of tissues of animals after the removal of the pancreatic gland. The conclusion Matthes arrived at was that they are in their origin independent of the enzymes of the digestive glands.

Most striking, however, was the discovery by Kossel and Dakin of a new enzyme ariginase and the work on nuclease by Jones and others.

It has been known since the work of Drechsel that part of the urea removed by the organism is formed by direct cleavage from proteid material. Kossel and Dakin (*Z. physiol. Chem.*, **41**, 321 and **42**, 181 (1904)) have demonstrated that this is brought about by the action of a special enzyme present in various organs. The enzyme could be isolated from liver, kidney, small intestines, thymus and lymphatic glands. In muscle and blood the presence of the enzyme is doubtful. It is absent in the suprarenals, spleen, pancreatic juice and bile. Shiga (*Z. physiol. Chem.*, **42**, 582 (1904)) demonstrated the presence of the enzyme also in yeast. The work on nuclein digestion is important for the reason that it interprets the mechanism of uric acid formation from purine bases. W. Jones (*Z. physiol. Chem.*, **42**, 35 (1905)) has first communicated his discovery of enzymes capable of transforming guanine and adenine into xanthine and hypoxanthine,

respectively. In a later publication Jones and Partridge (*Z. physiol. Chem.*, **42**, 343 (1904)) reported further results of their investigation, showing the existence of two individual enzymes. Schenk arrived at a similar conclusion (*Z. physiol. Chem.*, **43**, 406 (1904)). Independently of Jones the observation of the power of the animal organism to transform guanine and adenine into xanthine and hypoxanthine, and cytosine into uracil was made by Levene (*Am. J. Physiol.*, **12**, 276 (1904)). The results of the two investigations differed only in one point, *viz.*, according to Jones, the spleen contained only one enzyme, adenase, while other organs contained also guanase; according to Levene the action of the spleen on purine bases did not differ from that of any other organ. Nearly simultaneously with the foregoing work appeared a communication by Schittenhelm (*Z. physiol. Chem.*, **43**, 228 (1904)) on purine metabolism in which the author communicates observations very similar to those of Levene. In a later publication, Schittenhelm (*Z. physiol. Chem.*, **45**, 152 (1905)) reported his failure to find any difference in the action of the spleen and liver on the purine bases.

The discrepancy in the results were finally explained by Jones (*J. Physiol. Chem.*, **45**, 84 (1905)) who made the observation that the organs of animals of different species differed in the character of their enzymes. Very important also is the observation of Schittenhelm that three enzymes are concerned in the nuclein metabolism, one splitting nucleic acid into its components, the other a "desamidirung" ferment converting guanine and adenine into the corresponding oxypurines and finally an oxidizing enzyme, transforming the purine bases into uric acid. The oxidizing enzyme is active only in the presence of oxygen. The work of Burian (*Z. physiol. Chem.*, **43**, 497 (1905)) corroborated the observations of Schittenhelm on the formation of uric acid from purine bases.

In the same work Burian has emphasized again an older observation of Wiener, that animal organs contain also an enzyme decomposing uric acid.

In connection with guanase and adenase mention should be made also of the power of animal organs to remove the amino group from amino acids, and substitute in its place a hydroxyl group. This observation of Lang (*Hofmeister's Beiträge*, **5**, 321 (1904)) may be of considerable importance for the interpretation of the mechanism of sugar formation from proteid material.