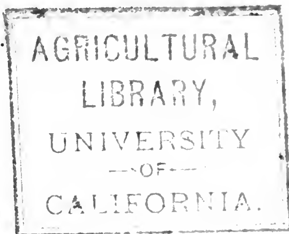
LIBRARY
OF THE
UNIVERSITY
OF
CALIFORNIA

BULLETIN NO. 54.

U. S. DEPARTMENT OF AGRICULTURE.

DIVISION OF CHEMISTRY.



AGRICULTURAL
LIBRARY,
UNIVERSITY
—OF—
CALIFORNIA.

REPORT

ON

AN INVESTIGATION OF ANALYTICAL METHODS FOR DISTINGUISHING BETWEEN THE NITROGEN OF PROTEIDS AND THAT OF THE SIMPLER AMIDS OR AMIDO-ACIDS.

BY

J. W. MALLET,

Professor of Chemistry, University of Virginia.

WITH

A CHAPTER ON THE SEPARATION OF FLESH BASES FROM PROTEID MATTERS BY MEANS OF BROMIN.

BY

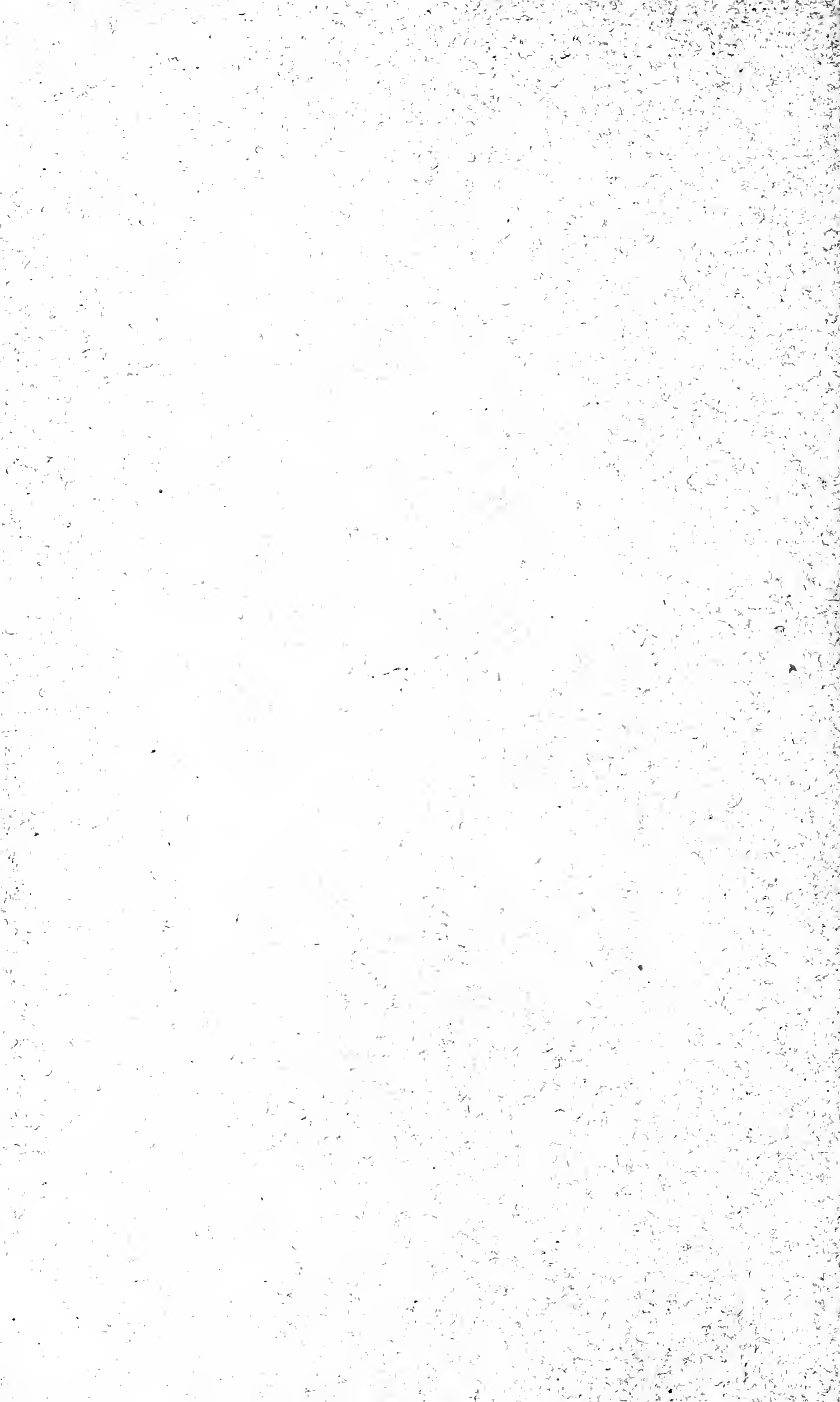
H. W. WILEY,

Chief of Division of Chemistry.

WASHINGTON:

GOVERNMENT PRINTING OFFICE.

1898.



BULLETIN No. 54.

U. S. DEPARTMENT OF AGRICULTURE.

DIVISION OF CHEMISTRY.

REPORT

ON

AN INVESTIGATION OF ANALYTICAL METHODS FOR DISTINGUISHING BETWEEN THE NITROGEN OF PROTEIDS AND THAT OF THE SIMPLER AMIDS OR AMIDO-ACIDS.

BY

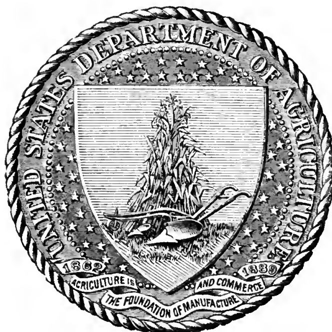
J. W. MALLET,
Professor of Chemistry, University of Virginia.

WITH

A CHAPTER ON THE SEPARATION OF FLESH BASES FROM PROTEID MATTERS BY MEANS OF BROMIN.

BY

H. W. WILEY,
Chief of Division of Chemistry.



WASHINGTON:
GOVERNMENT PRINTING OFFICE.
1898.

Digitized by the Internet Archive
in 2007 with funding from
Microsoft Corporation

http://www.archive.org/details/reportoninvestig00mallrich

LETTER OF TRANSMITTAL.

U. S. DEPARTMENT OF AGRICULTURE,
DIVISION OF CHEMISTRY,
Washington, D. C., July 9, 1898.

SIR: In accordance with your request of April 15, I have carefully read the manuscript submitted by Prof. J. W. Mallet, being a result of the investigation of analytical methods for distinguishing between the nitrogen of proteids and that of the simpler amids or amido-acids.

This investigation was undertaken by Professor Mallet at the suggestion of the Office of Experiment Stations, and under the immediate direction of the director of that office and of Prof. W. O. Atwater, special agent. The work being purely of a chemical character, it was, at your suggestion, and with the assent of Drs. True and Atwater, submitted to this office for inspection. The investigation consists in a chemical study of the methods of quantitative analysis employed in the separation of proteid and amid bodies, especially in animal products. The results are similar in their scope to the chapter on this subject contained in my work on the Principles and Practice of Agricultural Analysis, vol. 3.

Professor Mallet has stated in an admirable manner the different methods which have been proposed for the separation of proteid matters in animal products. By a happy modification of the phosphotungstic acid method he has greatly improved this process, and shown how a practical separation of the flesh bases from the other nitrogenous substances can be effected by this reagent. The flesh bases are to some extent precipitated by the new form of the reagent proposed by Professor Mallet, but they are brought into a soluble state by the addition of water and heat, so that a practically complete separation of them is effected. This process, together with the use of tannic acid for the separation of peptones, leaves little to be desired in securing a practically complete separation of the nitrogenous matters. The analytical processes proposed have been thoroughly worked out by experiment upon products of known composition, so that their reliability has been by this means more firmly established.

One of the most useful and simple methods of separating proteid matters into insoluble and gelatinoid proteids, and of separating these two classes from the flesh bases, is by the use of hot water, followed by the use of chlorin or bromin—a method not mentioned either in the Principles and Practice of Agricultural Analysis, above referred to,

nor in Professor Mallet's paper. This method, which has lately come into use, has been thoroughly worked out in this laboratory and applied in a long series of analyses of flesh products. It is so superior in every respect to the methods in ordinary use that it appears destined to entirely replace them. I have therefore added an outline of this method as an appendix to Professor Mallet's report.

I submit this manuscript with the request that it be published as Bulletin No. 54 of the Division of Chemistry.

I am, respectfully,

H. W. WILEY,
Chief of Division.

Hon. JAMES WILSON,
Secretary of Agriculture.

CONTENTS.

REPORT BY J. W. MALLET.

	Page.
Discussion of materials and conditions	7
Classes of nitrogenous constituents in food	7
Nutritive values of three classes	8
Lack of precision in chemical constitution	8
List of substances examined	9
Solutions used in experiments	10
Foods involved in the investigation	11
Methods of separating nitrogen	11
Dialysis	11
Interaction with nitrous acid	12
Interaction with potassium permanganate in presence of free acid or alkali	15
Interaction with sodium hypobromite	16
Behavior with cupric hydroxid (Stutzer's reagent)	18
Relations to alcohol as a solvent	19
Behavior with several new or little used reagents	19
Behavior with phospho-tungstic acid	19
Classification of substances examined	20
Use of hot water	21
Details of the method with phospho-tungstic acid	22
Factors for calculation of total nitrogen	24

REPORT BY H. W. WILEY.

Separation of proteid bodies from the flesh bases by means of chlorin and bromin	27
Details of method	27
Factors for calculation of total nitrogen	28
Application to commercial meat extracts	29

ANALYTICAL METHODS FOR DISTINGUISHING BETWEEN NITROGEN OF PROTEIDS AND NITROGEN OF SIMPLER AMIDS OR AMIDO-ACIDS.

DISCUSSION OF MATERIALS AND CONDITIONS.

It is admitted by all who have had experience in the chemical analysis of materials used as food that the common practice of determining the total nitrogen in such materials by multiplying the result by 6.25 and calling the product so obtained "protein," is but a crude and clumsy way of approximately representing the value of the material under examination as respects its nitrogenous constituents. Besides the substances which are properly designated as proteids there are other nitrogenous constituents of food materials which differ widely from these in nutritive value and some of which also differ greatly from these and from each other in the proportion of nitrogen which they contain.

CLASSES OF NITROGENOUS CONSTITUENTS IN FOODS.

The following classes of the nitrogenous constituents of food are commonly recognized as requiring separate consideration.

(1) Proteids proper (by some called albuminoids), and their closely related derivatives, the proteoses and peptones.

(2) Gelatinoids or collagens, and allied substances immediately derived from them, such as gelatin, chondrin, etc.

(3) Simpler amids, amido-acids, and allied substances, such as the asparagin, glutamin, etc., of vegetable materials, and the "flesh bases" (kreatin, kreatinin, etc.) of animal origin.

(4) Alkaloids, or amine-like compounds of well-determined basic character.

(5) Ammonia and its salts.

(6) Nitrates.

To these, no doubt, should be added lecithin and analogous substances containing nitrogen but closely allied to the fats.

The known and commonly used methods for determining nitrogen in the forms of ammonia and nitrates, which occur but sparingly in food materials, may be considered fairly satisfactory. Alkaloids in the commonly accepted sense of the term demand attention only in connection with such special accessories of food as tea, coffee, and similar nervous stimulants, and the chief substances of alkaloidal character admit of being separately dealt with in these special cases without serious trouble and with a fair degree of accuracy.

It is with the first three of the above-named classes of food constituents that difficulty is encountered, and for which further study of methods is desirable. The object of the work which the writer has undertaken, and to which he has devoted a good deal of time for several months past, has been to study the means of distinguishing between the first and third of these classes of constituents, the proteids and related bodies on the one hand and the simpler amids and amido-acids—sometimes grouped together as "nitrogenous extractives"—on the other. Incidentally only, some experiments have been made with representatives of the gelatinoid class.

NUTRITIVE VALUES OF THREE CLASSES.

It is commonly assumed that proteids, gelatinoids, and the simpler amids have very different nutritive values, and while all authorities would agree in assigning the highest value to the first of these there is probably no small difference of opinion as to the order in which the second and third should be rated. In considering such a question, there should be separately taken into account relative digestibility or solubility, capability of undergoing osmotic absorption, and oxidizability in order to the production of energy. At present no definite numerical statement of the relative nutritive values of nitrogenous bodies of these three classes can be made. It seems much to be desired that more extended experiments than have so far been recorded should be made upon living animals—as far as possible upon human beings—in regard to the utilization of both the gelatinoids and the simpler amids. The latter no doubt undergo oxidation to some extent in the animal body, and produce some energy in consequence. It is probably true of these simpler amidic substances that much larger quantities than analysis exhibits as constituents of the food consumed or than analysis detects among the residua of food rejected from the body without having undergone complete oxidation, may be constantly formed among the earlier products of the metabolism of the proteids, and afterwards themselves undergo further change into the simpler and more stable forms of carbon dioxid, water, and urea.

LACK OF PRECISION IN CHEMICAL CONSTITUTION.

It must be admitted that to a chemist the question of distinguishing between the proteids and the simpler amids is not one of a scientifically precise character. The proteids doubtless contain at least a part of the nitrogen in the amidic relation, and where the line is to be drawn between more complex and more simple amids is, of course, more or less a matter to be arbitrarily decided. But, of greater importance still is the doubt whether any of the so-called proteids are entitled to recognition as definite chemical substances. We usually understand by the term "a definite chemical substance" a substance of which all the molecules are exactly alike in constitution. Thus to the chemist the identity of

such a substance as pure common salt or cane sugar or caffeine depends on the absolute identity in character of all the myriad molecules of which even the smallest sensible mass is made up. But in regard to such materials as these we are able to determine the relative and absolute number of atoms of the elements of which each molecule is composed, and in many cases their order of attachment to each other, or the "structure" of the molecule. On the other hand, we have reason to believe that the so-called proteids are made up of molecules of such extreme complexity, assemblages of such large numbers—hundreds—of atoms of carbon, hydrogen, oxygen, and nitrogen, that we can make but random guesses at their arrangement, and can not even determine with any certainty their number, relative or absolute. We talk of albumin, myosin, syntonin, as if these terms stood for pure chemical substances in the same sense that attaches to ammonia, benzene, or urea. But it is by no means certain that in a specimen of the most carefully prepared albumin from blood or white of egg any hundred, or any ten, molecules are absolutely alike. It may well be that a minute specimen of such a material consists in reality of numerous more or less similar but yet in some respects different molecules, which we lump together under a single name merely because they have a general resemblance, with certain properties in common. Furthermore, in nutrition investigations we have to deal with articles of food representing complex mixtures of substances referable to the two classes of the proteids and nitrogenous extractives, with many other things besides. Hence, an answer to the question to be examined must be a limited one, and such only as may serve the limited purpose of practical application in connection with nutrition investigations. Any process of separation, to be of value in such application, must be reasonably simple, and capable of being carried out without too great consumption of time.

LIST OF SUBSTANCES EXAMINED.

A good deal of work was necessary at the outset in procuring satisfactory specimens of the several substances to be examined. Some of these have been prepared out and out in this laboratory from natural animal or vegetable sources. Some have been purchased in a more or less crude state and carefully purified. Some have been purchased, and their purity ascertained by testing.

The following representatives of the simpler amidic and imidic compounds were experimented with:

Amido-acids of the fatty series:

- Glycocin (glycocoll or amido-acetic acid).
- Alanin (*α*-amido-propionic acid).
- Leucin (amido-caproic acid).

Amido-acids of the succinic acid and allied series:

- Aspartic acid (amido-succinic acid).
- Asparagin.
- Glutamic acid (*α*-amido-oxyglutaric acid).
- Glutamin.

Amido-acid, including a benzene nucleus:
Tyrosin (oxy-phenyl-a-amido-propionic acid).

Betaine bases:
Betaine.

Guanidine bases:
Kreatin.
Kreatinin.

Bases and neutral amids related to uric acid:
Hypoxanthine (sarkine).
Carnine.
Allantoine.

As representatives of the proteids and allied substances the following were used:

Albumin, from white of egg.
Albumin, from blood.
Fibrin, from blood.
Casein, from milk.
Legumin, from peas.
Globulin (para-globulin), from serum of blood.
Vitellin, from yolk of egg.
Myosin, from muscular tissue.
Syntonin (acid-albumin), from muscular tissue.
Hæmoglobin (mainly oxy-hæmoglobin), from blood corpuscles.
Albumose, from egg albumin.
Peptone, from fibrin of blood.
Gelatin, from skin.
Chondrin, from cartilage.

SOLUTIONS USED IN EXPERIMENTS.

These were, for the experiments by precipitation, brought into solution as follows, involving, as will be seen, change of chemical constitution in some cases:

Albumin, Hæmoglobin, } dissolved in cold water.

Gelatin, Chondrin, } dissolved in hot water.

Fibrin, brought sparingly into solution by prolonged action of a 10 per cent solution of sodium chloride in water.

Fibrin, digested with a 4 per cent solution of caustic soda, and the solution afterwards acidified with acetic acid.

Casein, dissolved in 1 per cent solution of caustic soda, and the solution cautiously treated with dilute acetic acid to an extent just short of coagulation.

Globulin, dissolved in 5 per cent solution of sodium chlorid.

Vitellin, Myosin, } dissolved in 10 per cent solution of sodium chlorid.

Syntonin, dissolved in 0.1 per cent solution of hydrochloric acid.

Albumose, from the early stage of digestion of egg albumin by means of pepsin and 0.2 per cent solution of hydrochloric acid.

Peptone, from advanced stage of digestion of blood fibrin by means of trypsin and 0.25 per cent solution of sodium carbonate.

FOODS INVOLVED IN THE INVESTIGATION.

While it was especially desirable to ascertain the behavior of these several substances, of both classes, in their separate condition, it was borne in mind that in connection with nutrition investigations they have to be dealt with as constituents of ordinary food materials, principally the following:

Meats, including poultry and fish (raw and cooked), soups and stews, meat extracts, eggs, milk and cheese, bread and other farinaceous preparations such as cakes and pastry, fruits and raw table vegetables, cooked fruits and table vegetables, fruit conserves, undigested and unabsorbed residues of food.

It was assumed as probable that the same method (in detail) would not be best adapted to all cases, but would be likely to need modification in application under different conditions.

Of course, the investigation undertaken has gone over ground already well trodden, and the results recorded by Schulze, Barbieri, Sachsse, Kormann, Boemer, Huefner, Allen, Tankard, Chittenden, Osborne, Wiley, and others have been carefully examined.

The experimental work of the present investigation is reported, not altogether in the order in which it was done, but rather in logical succession, taking account first of physical differences between the classes of substances to be distinguished, then of the effects of chemical agents of decomposition, and, lastly, of relations to solution and precipitation.

METHODS OF SEPARATING NITROGEN.

A brief notice of several methods of separating nitrogen will afford means of comparison. The phospho-tungstic method which was especially investigated is treated more fully than the others.

DIALYSIS.

The attempt to separate such proteids as are soluble in water from the simpler amids intentionally mixed with them by applying Graham's method of dialysis was made with no great hope of success. It is, of course, well known that the amorphous proteids in solution exhibit as a class a very small degree of diffusive mobility, while the crystallizable, simpler amids diffuse through a porous septum, as of parchment paper, with much greater rapidity. But separation based on this difference can never be complete. Only a part of the more diffusible material can be obtained in the water on the opposite side of the diaphragm from the mixed solution, and that a large part shall be so obtained requires that the volume of water used shall be large as compared with the volume of the solution. Moreover, the absolute volume of the solution itself must be large where amids of but slight solubility, such as tyrosin, are present, in order that they may not be left behind in the undissolved state. But the time required for dialytic separation

extends to many hours, or even several days, and during such protracted exposure to the air and to common atmospheric temperature weak solutions of the proteids readily undergo chemical change. Again, it is to be remembered that the peptones, classed with the proteids, are diffusible in much higher degree than natural proteid material prior to its exposure to the action of the digestive fluids. This fact has well-known physiological importance in its bearing on the preparation of proteid food for absorption from the alimentary canal, but in analysis it tends to confound the particular group of the peptones with the simpler amids. In the recovering from weak solutions of small quantities of dissolved substances by the evaporation of large amounts of water, further chemical change of the substance recovered is likely to vitiate the results.

Nevertheless, some twenty or more experiments were carried out with dialysers made from the tubes of parchment paper which can now be bought in Germany. Cylinders of about 6 inches long were cut from these tubes, and closed at one end by thin glass, carefully tested as to freedom from holes, and immersed in water contained in ordinary glass beakers. A fairly clean separation was obtained of leucin, aspartic acid, and kreatin from solutions to which had been added egg or serum albumin, the diffusate being in each case evaporated at a moderate temperature over the water bath, and the residue weighed. But the process of diffusion was inconveniently slow, and less satisfactory results were obtained when the dialyzed solution was made more complex by the addition of other substances.

Subsequently the writer's attention was attracted by the paper of Charles J. Martin[1] on the use for a like separation of a Pasteur filter in the pores of which a film of silicic acid has been deposited, the filtration being brought about under pressure. It has been practicable to make only two or three experiments in this way. The method is decidedly promising, but it seems more likely to be useful in the purification of substances in quantity than as a process of analysis. With small quantities of material it can hardly be made available for regular laboratory work in connection with nutrition investigations. It involves the same difficulty as any other form of dialysis in cases in which the proteoses and peptones are present.

INTERACTION WITH NITROUS ACID.

It was hoped that by varying the conditions of experiment with this reagent some characteristic differences of behavior might be observed as regards the evolution of elementary nitrogen.

The most advantageous mode of producing nitrous acid in definite amount was found to consist in bringing together, along with the material to be acted upon, pure silver nitrite and a hydrochloric-acid solution of known strength. The action took place in a glass flask of

[1] Jour. Physiol., 1896, **20**, pp. 364–371.

about 100 c. c. capacity, closed by a stopper through which passed the long neck of a cylindrical funnel holding about 60 c. c., the neck having an interior diameter of but 1 mm. and separated from the wider cylindrical reservoir by a well-ground glass stopcock. There also passed through the same stopper the stem of a thermometer reading to one-tenth of 1 degree with accuracy, a small tube with stopcock for the introduction of gas to displace the air of the apparatus, and a second small tube to carry off the nitrogen evolved. The capacity of the flask was carefully gauged with the tubes and thermometer in position and the stopper inserted to a marked depth in the neck, so that after the introduction of any known volume of solid or liquid material a simple subtraction would give the volume of gaseous matter remaining included. A gas-measuring cylinder, connected with the flask by a tube of very small bore and immersed in water in a larger cylinder which could be raised or lowered, provided for collecting and measuring the evolved nitrogen. The flask was supported in a water bath, so that it could be heated or cooled at pleasure.

In order to give time for action upon the organic material under experiment, and to keep down to conveniently small limits the evolution of nitrogen dioxid, it was found important to work with the nitrous acid in a sufficiently diluted condition. With the same object in view it was found desirable to raise the temperature of the flask very gradually. In carrying out an experiment the particular amidic or proteid material was finely pulverized, weighed off, and introduced into the flask, either in the dry state or with as little recently boiled water as possible, and with the necessary amount of silver nitrite, as a fine crystalline powder. This powder was contained in a small, thin glass cylinder which could be easily upset in order to mix its contents with the other materials in the flask. A current of nitrogen gas was next run through the flask until all air was expelled. In the case of substances dissolving in water with difficulty the flask was now allowed to stand closed for some time to permit of solution taking place. Communication was established with the gas-measuring cylinder and the proper quantity of standard hydrochloric acid made by diluting very strong aqueous acid with boiled water and kept in small well-closed bottles, was gradually introduced through a funnel with capillary bore. Care was taken that no air was admitted. After all action in the cold had ceased the temperature of the flask was gradually raised by means of the water bath up to a maximum of about 96° or 97° C. As soon as all action was over and the apparatus had cooled down to atmospheric temperature the flask was nearly filled up with recently boiled water, introduced through the capillary funnel, and a small measured volume of nitrogen was run in to displace any remaining nitrogen dioxid. The gas in the measuring cylinder was then treated with oxygen in sufficient excess to dispose of all nitrogen dioxid and with caustic potash and pyrogallol to remove excess of oxygen. After standing at

rest for some time the gas was measured, due allowance being made for the nitrogen present in the flask at first and for the small additional amount afterwards introduced. Of course, the proper corrections for pressure, temperature, and tension of aqueous vapor were made in connection with all the measurements of gas, and the quantity of nitrogen obtained was divided by 2, one half being yielded by the organic substance and the other by the nitrous acid.

With a view to guard against the retention of any nitrogen in the form of di-azo derivatives from amids, a moderate excess of hydrochloric acid was used. In the case of kreatinin the results are complicated by the formation of the supposed nitroso compounds observed by Dessaignes. With several of the proteids the bright yellow color of the so-called xantho-proteic acid was well marked, as the consequence, doubtless, of the action in the flask of nitric acid produced by the breaking up of nitrous acid into nitric acid, nitrogen dioxid and water. Too little is known of the yellow substance formed, Mulder's xantho-proteic acid, to indicate how far its nitrogen comes from the proteid and how far from the nitric acid, or in what direction or to what extent its formation may influence the amount of elementary nitrogen given off. In the case of proteid solutions coagulable by heat it was manifestly important to allow the action of the hydrochloric acid on the silver nitrite to become complete below the temperature of coagulation, as otherwise silver nitrite was entangled in clots of the coagulating proteid. In some, at least, of these experiments, it seemed probable that a part of the nitrogen dioxid given off, and perhaps of the free nitrogen, was due to the reducing action of the carbon or hydrogen of the organic substance upon nitrous or nitric acid, and not solely to the normal interaction of nitrous acid and the amidogen radicle present.

The process was varied, not only in respect to the temperature applied, but also, within moderate limits, in respect to the pressure on raising or lowering the gas-measuring tube in the water. It was further varied by increasing the proportion of nitrous acid to different extents in excess of the theoretically necessary amount.

In all some fifty or sixty experiments were made. In a number of cases the simpler amidic compounds gave a fair approximation to the quantity of nitrogen theoretically to be expected from them, though even with such substances as leucin, asparagin, and aspartic acid the results were not as sharp as the claims of Sachsse and Kormann would lead one to suspect. One of the best experiments yielded for aspartic acid 9.57 per cent of nitrogen, instead of the 10.53 per cent actually present. Kreatin did not give one-half of its nitrogen, as has been heretofore recorded as the fact, but a somewhat nearer approach to one-third—10.19 per cent as against 32.06 per cent—the probability of which result is indicated by the accepted structure of the molecule. In all cases the proteids gave some nitrogen, but the proportion was

much smaller than from the simpler amids, and varied much more with the precise conditions of temperature, pressure, and strength of solution used. In one experiment serum albumen gave but 2.68 per cent, and in another but 2.92 per cent was obtained from hæmoglobin.

On the whole, the indications pointed to the simpler amids and amido-acids being most easily decomposed by nitrous acid, the proteoses and peptones perhaps next, and the proteids proper least. But no differences were observed upon which any useful analytical process ot separation or distinction could be based. Experiments made in this way are, moreover, troublesome, and require strict observance of the necessary precautions to avoid error from introduction of air into the apparatus and its action on nitrogen dioxid.

INTERACTION WITH POTASSIUM PERMANGANATE IN PRESENCE OF FREE ACID OR ALKALI.

The writer had not much hope of obtaining useful results by this method, in view of former experience gained in connection with the extended research upon the determination of organic matter in drinking water carried out many years ago for the U. S. National Board of Health, of which the results were published in the annual report of that board for 1882. Still, as the work then done was upon extremely dilute solutions, comparable in respect to the amount of organic matter present with natural potable waters, it seemed possible that results not altogether of the same sort might be obtained with solutions of greater concentration. Hence some thirty or forty experiments were made with permanganate strongly acidified with sulphuric acid, and a rather larger number with the same salt after potassium hydroxid had been added in about the same proportion as is usual for the Wanklyn, Chapman, and Smith so-called albuminoid-ammonia process. In the former set of experiments the amount of oxygen withdrawn from the permanganate, and in the latter set the amount of ammonia produced, were determined. In both cases the reactions were carried out at various temperatures up to the boiling points of the several liquids.

Some difference was observable in the results obtained with the comparatively strong solutions used, such difference being more notable for alkaline than for acid permanganate. But in the main these results only confirmed the conclusions arrived at in the earlier research. The effect of the reagent employed is imperfect, and varies much with the nature of the individual organic materials tested; much, also, with the conditions of the experiment and with the rate at which the action proceeds. No valid evidence is obtained in support of Wanklyn's view that simple and definite fractions of the total nitrogen present are evolved as ammonia on treatment with alkaline permanganate.

Some special difficulties and sources of error already known were reobserved; as, for instance, the continuous evolution of ammonia by

boiling many organic substances with alkaline permanganate until distillation has practically brought the contents of the retort to dryness, without all of the nitrogen present having been given off. And some other causes of trouble were noticed; as, for instance, the loss of oxygen given off as such from a strong and acid solution of permanganate on standing in a heated condition irrespective of action on the organic matter present.

The general tenor of the results in the case of the nitrogenous substances treated pointed to more energetic and extensive action of permanganate in alkaline than in acid solution; also to more extensive action on the simpler amidic compounds than on the proteids. But notwithstanding sundry variations of method as to strength of the reagent solutions, proportion of reagent to organic material acted on, temperature and time of action, no indication was obtained of any valid basis for distinction in analysis between the two classes of nitrogenous material studied.

INTERACTION WITH SODIUM HYPOBROMITE.

This reaction is so frequently used for the approximate determination of urea (carbamid), while its results with other amids have been so scantily examined and almost no facts bearing on its relation to the proteids have been recorded that a good deal of interest was felt in examining it somewhat extensively with the two classes of materials which were studied.

The solution of bromin was prepared with 240 grams of potassium bromid, 200 grams of free bromin, and enough water to make up a liter. The solution of caustic soda was made with 340 grams of pure sodium hydroxid to the liter. These solutions were preserved in separate bottles and equal volumes of the two were mixed just before using. When undiluted the mixture represented 0.1 gram of originally free bromin to each cubic centimeter and was in most of the experiments used of this strength; but various dilutions were also employed, being made by additions of water in definite amount.

The apparatus with which the reaction was carried out was essentially the same as that adopted for the experiments with nitrous acid, save that the small tube was omitted which was intended to introduce an inert gas to displace air from the apparatus, this precaution being unnecessary in the hypobromite experiments.

Nearly 80 experiments were made, varying the conditions as to temperature from that of the atmosphere, usually 15° to 20°, up to 96° to 98° C., as to pressure within the limits allowed by the immersion of the gas-measuring cylinder, and as to time from fifteen or twenty minutes up to five or six hours.

In some cases the results obtained were in agreement with those of the few hitherto recorded experiments—as, for instance, aspartic acid gave no nitrogen, as was found to be the fact by Allen and Tankard.

In other cases there was disagreement with some of the published statements and confirmation of others. Thus, Allen and Tankard obtained no nitrogen from glycocin,[1] and Tankard none from asparagin,[1] while Oechsner de Coninck reported both these substances as acted on (by sodium hypochlorite) when gently heated, nitrogen gas being evolved.[2] The writer obtained from glycocin 4.2 per cent of nitrogen instead of 18.67 per cent as required by the formula,[3] and from asparagin 11.12 per cent instead of 18.67 per cent, the amount calculated, taking account of the presence of a molecule of water of crystallization.

In a number of cases the quantity of nitrogen evolved from an amid or amido-acid of known constitution seemed to represent a simple fraction of the total quantity contained in the material operated on, and it might not unnaturally be suspected, as in some of the cases reported by Allen and Tankard, that one-fourth or one-half to two-thirds of the whole quantity was liberated. Thus in one experiment leucin gave 2.58 per cent of nitrogen instead of 10.69 (calculated), or about one-fourth; in another, kreatin gave 21.96 per cent instead of 32.06 (calculated), about two-thirds; and in another, hypoxanthine gave 18.80 per cent instead of 41.18 (calculated), which might mean one-half—two atoms out of four. But it is not believed there is any more real significance in these approximations to definite fractional parts of the nitrogen being evolved than in the similar approximations to which Wanklyn drew attention in connection with his so-called albuminoid-ammonia process. His conclusions have been shown to be entirely erroneous. Different figures could be obtained from the same materials acted on by varying the conditions of the experiment, and in some cases there was no really sharp ending to the reaction, traces of gas continuing to be slowly given off for hours after the main portion had been collected. Moreover, while our knowledge of the constitution of kreatin would make it not improbable that two atoms of nitrogen out of three should be liberated, or two out of four in the case of hypoxanthin, there is no similar ground for any expectation that leucin, containing but one atom of nitrogen in the molecule, should yield one-fourth; or glutamic acid, also with but one atom, should yield something like one-fourth (2.56 per cent instead of 9.52 calculated).

The researches of S. Hoogewerff and W. A. van Dorp have shown that numerous definite products other than elementary nitrogen may be formed by the action of alkalin hypobromites upon amids and amido-acids, especially those containing cyclic nuclei. In several of the writer's experiments—as, for instance, with alanin among the simpler

---

[1]A. H. Allen, Commercial Organic Analysis, 1896, Vol. III, Part III, p. 275. Possibly the solutions were not heated.

[2]Comptes rendus, 1895, **121**, 893–894. Jour. Chem. Soc. (London), (1896), Abstracts, org. chem., p. 282.

[3]In Watt's Dictionary of Chemistry, revised edition by M. M. Pattison Muir and H. F. Morley, Vol. II, p. 627, it is stated, on the authority of Denigès (Comptes rendus, **107**, 662), that with sodium hypobromite nitrogen is evolved from glycocin.

amido-acids, and with a sample of globulin among the proteids—a crystalline residue separated out in the flask on cooling. This residue was not examined further than to determine qualitatively that it contained nitrogen. The proportion borne by the nitrogen left in such fixed residual products to that collected as gas evidently varied with the conditions of the particular experiment, as well as with the nature of the substance acted on.

All the proteids and analogous materials treated gave some gaseous nitrogen. For the most part the amount was about two-fifths of the whole amount present; but occasionally a larger proportion, as in one experiment with globulin just about one-half, and in another with myosin about three fourths. In this last case the action was allowed to go on at a high temperature for a time much longer than usual. The remarks already made in regard to the simpler amidic substances, as to modification of results by variation of the conditions of experiments, fully apply also to the experiments with proteids and their congeners. The lack of sharpness of ending to the reaction was more noticeable with the latter class of materials than with the former.

Although these experiments with alkaline hypobromite were interesting, and occasionally offered points which might repay further investigation, they did not, taken altogether, afford any satisfactory basis for distinguishing in analysis between the classes of materials to be studied in contrast with each other.

BEHAVIOR WITH CUPRIC HYDROXID (STUTZER'S REAGENT).

The formation of insoluble compounds of the proteids with cupric hydroxid, while leaving the simpler amids soluble in the presence of an excess of this reagent, has been extensively adopted as the means of separation, but experiments made in this way have not given the writer much confidence in the method as a general one. In some cases, working with a proteid alone, the copper compound underwent partial solution, a blue liquid being formed, although care had been taken to avoid the presence of free alkali. Possibly this result was due to incipient decomposition of the proteid material. As Stutzer himself has pointed out, peptones are very incompletely precipitated by cupric hydroxid. A further objection is to be found in the very slight solubility of the copper salts of some of the simpler amido-acids, especially leucin and glutamic acid; in a less degree the same statement applies to aspartic acid. Even at the temperature of boiling water the copper compounds of these substances are but very sparingly soluble, and if the liquid, after digestion with cupric hydroxid, be filtered cold,[1] the compounds in question will, if present, be almost certainly left on the filter along with the proteid material.

---

[1] As directed by the Association of Official Agricultural Chemists, Bulletin No. 46 of the U. S. Department of Agriculture, Division of Chemistry (1895), p. 25.

RELATIONS TO ALCOHOL AS A SOLVENT.

It has been repeatedly proposed to use strong alcohol for the precipitation of the proteids, with a view to their quantitative determination, and this even in cases involving the simultaneous presence of some of the amidic compounds under discussion, such as the flesh bases.[1] But not only do the character and amount of the proteids so precipitated or left insoluble vary with the strength of the alcohol and the quantity of it used, but the further serious objection presents itself that nearly all the simpler amids and amido acids are either insoluble in alcohol or so slightly soluble that it is practically impossible to wash them out satisfactorily from the precipitated or coagulated proteids. A method which is not properly applicable to such important substances as asparagin among vegetable food materials, and kreatin among those of animal origin, manifestly deserves but little consideration.

BEHAVIOR WITH SEVERAL NEW OR LITTLE USED REAGENTS.

A number of miscellaneous experiments were tried with reagents which have either been but occasionally applied to materials of the kind under examination, or have not been so applied at all, so far as published records show.

A weak solution of pure phenol (carbolic acid), trichloracetic acid, formic aldehyde in aqueous solution, and hydrazoic acid (azoimide) were thus tried, but from none of these reagents were results obtained which furnished any ground for a general method of distinguishing the two classes of nitrogenous materials which were being studied.

BEHAVIOR WITH PHOSPHO-TUNGSTIC ACID.

This reagent, the discussion of which I have left to the last, has proved of much more value than any other I have tried, and its application under proper conditions affords, I believe, a fairly satisfactory practical solution of the question I have undertaken to examine. The use of phospho-tungstic acid for the precipitation in general of nitrogenous compounds, alkaloidal, amidic, and proteid, is, of course, well known and often practiced, but some of the special facts on which may be founded its application to the purpose now under discussion are believed to be new, and the particular use made of these points of behavior has not been before described. In connection with the experiments made with phospho-tungstic acid, the results obtainable from a parallel series of experiments with a strong solution of tannic acid were compared, one of these two reagents being found under special circumstances to replace the other with advantage.

The precipitant was employed not as a sodium or other salt, but as the phospho-duodeci-tungstic acid, crystallized in small cubes and dis-

[1] See Watts's Dictionary of Chemistry, revised edition. Vol. IV, p. 330, and H W Wiley's Principles and Practice of Agricultural Analysis, Vol. III, p. 453.

solved in dilute hydrochloric acid, 25 grams of real HCl to the liter. Solutions of two degrees of strength were prepared, the one containing 50 grams of the solid reagent to the liter, the other 100 grams. In the experiments with tannic acid, solutions in like manner containing 50 and 100 grams, respectively, of a remarkably good sample of the reagent, dissolving readily to a perfectly clear liquid, were made use of.

It has been assumed by Stutzer and others that the proteid and allied substances are precipitated by phospho-tungstic acid, while the simpler amids and amido acids are not so precipitated. As qualifying this general assumption, it has been stated that some of the proteid derivatives, as the peptones,[1] are incompletely precipitated, and on the other hand that the flesh bases, kreatin, kreatinin, etc., are fully precipitated. The reagent in question has been recommended as the means of separating and determining them.[2]

Account does not seem to have been taken hitherto of the fact that some of the precipitates formed by substances of amidic character with phospho-tungstic acid are to a small extent soluble in water, and that their solubility is much increased by rise of temperature.

CLASSIFICATION OF SUBSTANCES EXAMINED.

It has been found that the various substances on which these experiments have been made fall into three classes, as follows:

(*a*) Those which, even in pretty strong solutions, give no precipitate with phospho-tungstic acid.

(*b*) Those which are precipitated at any rate in strong solutions, the precipitate redissolving with more or less ease on heat being applied to the liquid or on treating the precipitate with hot water, and reappearing on cooling.

(*c*) Those which are precipitated, the precipitate not being sensibly soluble and the supernatant liquid remaining clear on being heated along with the precipitate and subsequently cooled.

Under the first head fall glycocin, alanin, leucin, aspartic acid, asparagin, glutamic acid, tyrosin, and allantoin. In the case of alanin there was a very slight turbidity, not increased by using a saturated solution, suggesting the probability of a trace of some impurity being present.

Under the second head were observed glutamin, a slight precipitate, the solution easily cleared by heating, the turbidity reappearing on cooling; betaine in strong solution, a copious white precipitate, dissolving gradually on addition of more water and heating, the precipitate reappearing on cooling; kreatin, strong precipitate, solution

---

[1]Dr. W. D. Halliburton in the article "Proteids" in Watts's Dictionary of Chemistry, revised edition, Vol. IV, p. 331. In Gamgee's Text-book of Physiological Chemistry, Vol. II, p. 139, it is stated that peptones are precipitated by phospho-tungstic and phospho-molybdic acids, and that these two reagents furnish the means of separating them. A similar unqualified statement is to be found in the Appendix (by A. Sheridan Lea) to Michael Foster's Text-book of Physiology, p. 45.

[2]Koenig and Boemer—Zeitschrift für analyt. Chemie., **34**, 560, adopted in Prof. H. W. Wiley's Principles and Practice of Agricultural Analysis, **3**, 454.

cleared by heating, becoming turbid again on cooling; kreatinin, large precipitate, disappearing on free addition of water and heating, reappearing on cooling; hypoxanthine, strong precipitate, cleared up on heating, reappearing on cooling; and carnine, well-marked precipitate, cleared by moderate addition of water and heating, reappearing on cooling. Urea also, which is not likely to occur among food materials, but possibly needs to be considered in connection with undigested residua, gave a copious white precipitate of crystalline character, cleared by heating, and the precipitate forming anew on cooling. A peptone solution gave an abundant precipitate, becoming clotted by heating and dissolving to a considerable extent, reprecipitating on cooling.

Under the third head were found egg albumin, fibrin, casein, legumin, globulin, vitellin, myosin, syntonin, hæmoglobin, albumose, gelatin, and chondrin. In nearly all these cases the precipitate formed was bulky, taking into account the strength of the solution used, and became clotted on heating, shrinking very considerably. In the case of myosin only (in 10 per cent sodium chlorid solution) was there a very slight appearance of turbidity on cooling the solution which had been heated with the precipitate.

USE OF HOT WATER.

As it was evidently important to ascertain with some degree of definiteness how far the precipitates formed by amidic substances of the second of these classes would dissolve in hot water, quantitative experiments were made with those which seemed to be least soluble. In each case the precipitate formed by phospho-tungstic acid in the cold was filtered off, washed with cold water, and dried at ordinary temperature (15° to 20°) over sulphuric acid. Stutzer advises that the phospho-tungstic acid precipitates be washed with dilute sulphuric acid, and Wiley recommends for the same purpose a solution of the precipitates. Of the precipitate formed by betaine, 1 part dissolved in 71 parts of water at 98.2°; of that produced by kreatin,[1] 1 part dissolved in 107 of water at 98.1°; of that produced by kreatinin, 1 part dissolved in 222 of water at 97.9°; of that produced by hypoxanthin, 1 part dissolved in 98 of water at 97.6°; and of that produced by carnin, 1 part required for solution 132 of water at 98.4°.

By the use of phospho-tungstic acid as a precipitant, therefore, followed by washing of the precipitate with hot water, it seems possible to effect a separation of all the simpler amidic substances from all the proteids and proteid-like bodies, except only the peptones. As regards this last group it is stated unreservedly by A. S. Lea,[2] A. Gamgee,[3]

---

[1] The phospho-tungstic acid precipitate formed by kreatin, white at first, darkened notably on exposure to light, looking after a while like silver chloride which had been in like manner exposed. The experiment on solubility was made with a sample which had been screened from light and was unaltered.

[2] The Chemical Basis of the Animal Body, an appendix to M. Foster's Text-book of Physiology (1893), p. 45.

[3] A Text-book of the Physiological Chemistry of the Animal Body (1893), **2**, 139.

and W. D. Halliburton[1] that the peptones are precipitated by tannic acid. The last-named writer says "completely precipitated." In one or two of the writer's own experiments, using tannic acid, an abundant precipitate was formed. This became clotted on heating and the clear supernatant liquid showed some little return of turbidity on cooling. The writer is inclined, however, to attribute this apparent partial re-solution of the precipitate merely to the presence of a little of a proteose formed in the earlier stages of digestion and not afterwards completely removed. Assuming this view to be correct, tannic acid furnishes the reagent needed to dispose of the one case unprovided for by phospho-tungstic acid.

DETAILS OF THE METHOD WITH PHOSPHO-TUNGSTIC ACID.

The method proposed is as given in the following paragraphs. It is stated, for the sake of simplicity, first, as applicable to meat, raw or cooked. The variations required in the examination of other classes of food materials are reserved for notice afterwards.

A carefully selected and accurately weighed sample is to be ground in a glazed porcelain mortar with as much sharp-edged siliceous sand, previously heated to redness with free exposure to air, or with as much hard glass in small, sharp splinters similarly ignited, as shall suffice to thoroughly subdivide the tissue and reduce it to the condition of a smooth pulp. Of this pulp, very carefully mixed, so as to insure uniformity, two aliquot parts are to be taken. In one the total nitrogen is to be determined by the well-known Kjeldahl process with addition of potassium sulphate, as recommended by Gunning, using a rather large proportion of sulphuric acid, so that no previous drying of the sample is needed. The other part is to be digested with cold water, filtered on a nitrogen-free filter,[2] and the residue washed on the filter with water at the same low temperature as long as it gives up soluble matter in sensible amount. Cold water is used to avoid action on and extraction of the gelatinoids. Kreatinin is quite easily dissolved, as is also sarcosine; kreatin with a very fair degree of ease. Xanthin, hypoxanthin, and carnin are less soluble.[3]

The filtrate is then to be slightly acidified with acetic acid, heated to about 90° C., and again filtered from any coagulum produced. A little more sand or pulverized glass may with advantage be stirred in before bringing it onto the filter the second time.

To this second filtrate is to be added an acidified solution of phospho-tungstic acid as long as a precipitate continues to form, avoiding any very large excess of the reagent solution. With a moderate amount of

---

[1] Watt's Dictionary of Chemistry, revised edition, 1894, **4**, 331.

[2] The ease with which filtration may be effected is much increased by the presence of the sand or crushed glass.

[3] Hypoxanthine, 1 part in 300 of water. The solubility of carnine does not seem to have been recorded till now. The writer has found it to be 1 part in 312 of water at 15.3° C.

sand or pulverized glass added, to prevent the formation of a dense clot, the liquid and precipitate are to be heated to about 90° C, filtered, and the precipitate washed thoroughly on the filter with water at about the same temperature. This third filtration may be carried out on the same filter already used for the second, but as a general rule it will be found better to use a new filter, thus avoiding possible delay due to partial drying of the previously used one and subsequent clogging of its pores.

Assuming now that nitrogen is present in the sample under examination only in the two forms of proteids and simpler amidic compounds, the three (or two) filters used and their contents are to be submitted to the Gunning-Kjeldahl process for the determination of proteid nitrogen. By subtraction of this from the total nitrogen previously determined the amount of this element present in the simpler amidic compounds will be obtained.

In cases involving the presence of ammonia or its salts, nitrates, or alkaloids, the nitrogen occurring in these forms must, of course, also be deducted from the total nitrogen before recording the residue as nitrogen of the simpler amids and amido-acids. In like manner a separation of lecithin, when present, may be effected by the use of ether as a solvent,[1] a determination of phosphorus made the basis of a calculation of lecithin nitrogen, and this in turn subtracted from the total nitrogen found.

When peptones are present, these are to be precipitated by tannic acid from the solution which has been acidified with acetic acid and heated. After this has completely cooled down, and before adding phospho-tungstic acid, the filter on which the tannic-acid precipitate is collected and washed with cold water is, with its contents, to be submitted to the modified Kjeldahl process, and the nitrogen obtained counted as part of the proteid nitrogen.

The several filters and precipitates from which the proteid nitrogen is obtained may either be treated separately by the Kjeldahl process or, preferably, may all be brought together and submitted to this process in a single operation. If the latter course be pursued, it will be well to introduce each filter with its contents as soon as washed into the strong sulphuric acid, so as to avoid any possible decomposition and loss of nitrogen as ammonia until all the filters have been brought together and the moist combustion process can be proceeded with.

When proteoses are present it may be well to make a check determination of their amount by saturation of the aqueous solution, after acidification with acetic acid, heating and subsequent cooling, with zinc sulphate,[2] and determining nitrogen in the precipitate so formed by means of the Kjeldahl process.

---

[1] Extraction with the ether alone will remove only a portion of the lecithins. A mixture of ether and alcohol should follow the ether in order to secure a complete extraction.

[2] As suggested by Boemer, Zeitschrift für analyt. Chemie, 1895, **34**, 562.

When gelatinoids are present, as may be the case with soups, stews, and meat extracts, hot water may be used at once for solution or washing the original material, and this with the advantage of facilitating the extraction of the less soluble simpler amids and amido-acids. These are, as a rule, more easily dissolved in the presence of a little free acid; hence acidification at an early stage of the treatment is advantageous. In a case in which tyrosin might be present, as in some vegetable materials, and possibly among unabsorbed residua of food, the use of hot water and the presence of free acid would greatly increase the solubility of this substance.

In food of vegetable origin where much starch is present it will be better to avoid the use of hot water at first, so that the solution may not be loaded with viscid material, rendering filtration difficult.

In all cases in which the food material to be examined is already fluid from the presence of water—as, for instance, soup, milk, and the like—filtration will of course at once be resorted to, being almost always much facilitated by the addition of sand or pulverized glass, and only such further quantity of water will be used as is required for washing the undissolved matter left upon the filter.

In the presence of fat in large quantity, it may be well first to remove this, or most of it, by extraction with ether. The simpler amidic substances are, as a rule, insoluble in ether, but by way of precaution the ethereal solution of fats might be shaken up two or three times with acidified water, and the watery fluid evaporated and tested for nitrogen.

In regard to the method of reporting results, the most important point is the separate statement of the amount of nitrogen present in the form of proteids and their more closely related congeners and in the form of the simpler amids and amido-acids. But in attempting to calculate from the nitrogen found under these heads the actual amount of the proximate nitrogenous constituents of the food material examined, the question arises, What factor should be used by which to multiply the nitrogen found in each case?

FACTORS FOR CALCULATION OF TOTAL NITROGEN.

The error noticed by Professor Wiley[1] as involved in the multiplication of the total nitrogen of a sample of meat by 6.25, and the assumption that the product represents the true quantity of nitrogenous matter, is not restricted to the use of the same factor for the proteids and flesh bases. While the multiplier should be a much smaller one for the latter, it also confounds under a single head these two classes of material, unquestionably possessing very different nutritive values.

It is evident that for each substance examined, or at any rate for each class of generally similar food materials, there should be made a qualitative investigation of the simpler amidic constituents present,

[1] Principles and Practice of Agricultural Analysis (1897), **3**, 551.

and if possible a roughly approximate estimate of the proportions in which they severally occur; also it is clear that the factor to be used in calculating the nitrogenous constituents to be reported under each analysis should be decided by such preliminary investigation. In the light of present knowledge of this kind the writer is inclined to suggest the following numbers:

For proteids and allied substances, multiply nitrogen found by 6.25, as usual at present.

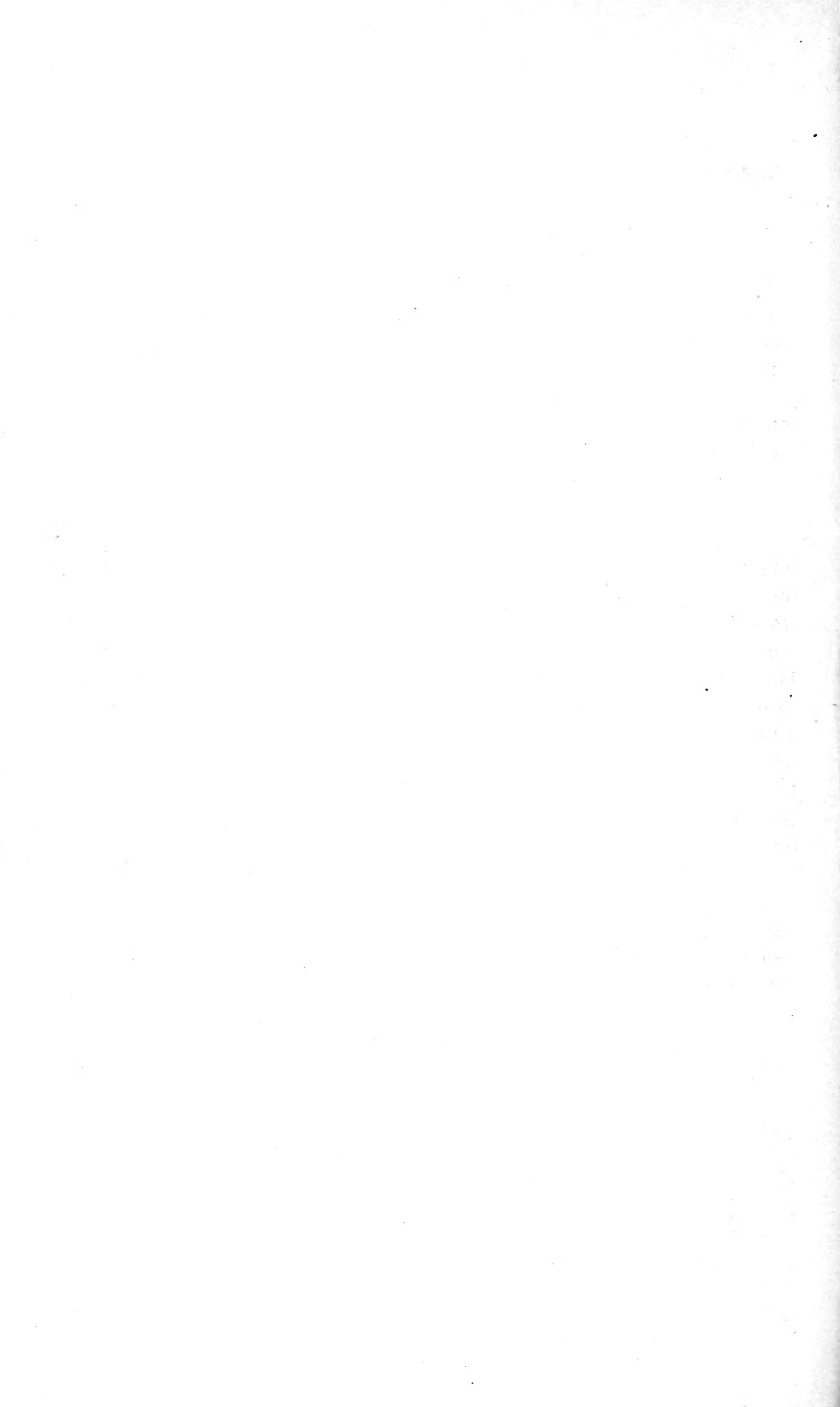
For flesh bases and simpler amids of animal origin in food materials, multiply by 3.05.

For simpler amids and amido-acids of vegetable origin in food materials, multiply by 5.15.

For mixed amidic constituents of unabsorbed solid residua in digestion experiments, multiply by 9.45.

As a matter of general practice, in all statements of the results of nutrition experiments the rule should be invariably observed to give the actual amounts of nitrogen obtained by analysis, whatever calculated conclusions be afterwards deduced therefrom; so that, with further knowledge of the nature of the proximate nitrogenous constituents present, the factor used in calculation may be changed, if such change seems to be called for, while the original experimental work still retains its value.

In concluding this report the writer wishes to express the hope that the method suggested, which seems to carry with it some improvement upon present practice and in a fairly simple and easily applied form, may be tried with yet other amids and proteids than those experimented on by him, and that any special difficulties which may be encountered with particular articles of food may be investigated. Especially is it desirable that the variations be studied which may prove to be necessary in dealing with vegetable instead of animal materials. The latter have been chiefly kept in view, in accordance with the instructions of the letter of authorization under which this investigation has been conducted.



SEPARATION OF PROTEID BODIES FROM THE FLESH BASES BY MEANS OF CHLORIN AND BROMIN.

By H. W. Wiley.

In dry, finely-ground animal matters from which the fats have been thoroughly extracted with ether, it is possible to effect an easy separation of the nitrogenous bodies into three groups. These groups, for purposes of dietetic study, are sufficiently distinct to afford a safe basis of valuation of the different nitrogenous constituents. The process which has been adopted in the laboratory of the Division of Chemistry, Department of Agriculture, for this separation is given in detail here.

DETAILS OF METHOD.

In the dry, fat-free, finely-ground animal substance the nitrogenous bodies soluble in water may be separated by first thoroughly exhausting the material with cold or lukewarm water, and afterwards with water near the boiling temperature. By this method the water-soluble constituents of the nitrogenous substances are thoroughly removed. Having determined the total percentage of nitrogen in the whole sample, the residual insoluble nitrogen is determined in the residue left after extraction. This percentage multiplied by 6.25 gives the total quantity of insoluble proteid matter contained in the animal material. In the filtrate the soluble proteid matter which has been dissolved by the water may be completely thrown out of solution by treatment with bromin in the manner about to be described.

About one-gram portions of the dry animal material are washed with ether by decantation, using from 50 to 100 cc of ether for each sample, and decanting the ether through filters which are afterwards used to receive the portion of the sample insoluble in hot water. After allowing the ether to evaporate, the samples are treated first with cold and then with hot water, this washing also being by decantation, the total amount of water used being from 300 to 400 cc. The undissolved residues are brought on to the filter with the last portions of water. The nitrogen in the residues on the filters is determined by the Gunning method.

The filtrate from the insoluble portions of the meat is received in Kjeldahl flasks and used for the separation of the soluble proteid nitrogen by bromin. The filtrate is first acidulated with two or three drops of strong hydrochloric acid and then about 2 cc of liquid bromin are added and the contents of the flask vigorously shaken. If the bromin be all taken up more is added until finally a globule of ½ cc of liquid bromin is left undissolved and the supernatant liquid is thoroughly saturated

with bromin. The mixture is then allowed to stand overnight, by which time the precipitate will have settled. The supernatant liquor is passed through the filter and the precipitate in the flask washed by decantation with water, the globule of undissolved bromin serving to saturate the wash water so that it is unnecessary to use additional bromin water for the washing. The filter containing the precipitate is returned to the same flask in which the precipitation has taken place and the nitrogen therein determined by the Gunning method. The sum of the nitrogen in the part insoluble in water and the part precipitated by bromin is subtracted from the total nitrogen determined on the original sample, and the difference gives the total nitrogen in the flesh bases.

FACTORS FOR CALCULATION OF TOTAL NITROGEN.

The factors used for calculating the total nitrogenous bodies are as follows:

For the part insoluble in water, $N \times 6.25$.

For the part soluble in water and precipitated by bromin, $N \times 6.25$.

For the flesh bases, $N \times 3.12$.

This method is based upon investigations reported by Rideal and Stewart[1] last year.

These writers recall some of the experiments made in 1876, in which it was shown that a current of chlorin gas, conducted through an aqueous solution of proteid matters, produces a precipitate which is of a quite constant composition, and one which can be collected, dried in vacuo, and weighed. They describe particularly the use of this reagent in precipitating gelatin prepared from the high-grade commercial article. They show that the total quantity of gelatin can be accounted for from the weight of the precipitate by multiplying the weight of the precipitate obtained by the factor 0.78. The authors also point out the possibility of using bromin for chlorin for the precipitation, and state that the studies of the use of bromin are under way. They call attention to the fact that as early as 1840 chlorin had been used by Mulder for the precipitation of soluble proteids, and refer to a paper of his published in Berzelius' Jahresbericht, volume 19, page 734, in which he states results on precipitation quite similar to those secured by Rideal and Stewart.

At the close of their paper Rideal and Stewart mention the work in this direction of De Vrij, Ann. Pharm. **61**, 248; Thénard, Mém. d'Arcueil, **2**, 38; Mulder, Bulletin en Néerlande, 1839, 153, and Berzelius' Jahresbericht, **19**, 729, on the same subject.

Allen and Searle,[2] acting on the suggestion of Rideal and Stewart, worked out the bromin method by applying it to various soluble proteids, including the whole range from albumin to peptone. In the

---

[1] The Analyst, 1897, **22**, 228 et seq. [2] The Analyst, 1897, **22**, 258-263.

application of this test to commercial gelatin the following process was employed.

Fifty grams of commercial gelatin were dissolved in warm water and the solution diluted to half a liter. In 10 cc of this solution, corresponding to 1 gram of the gelatin, the nitrogen is determined directly by the Gunning-Kjeldahl process. Another portion of 10 cc is treated with an excess of bromin in the following manner.

The solution is first brought to a volume of 100 cc with water and placed in a conical beaker with a sufficient quantity of hydrochloric acid to produce distinct acid reaction. A saturated solution of bromin water is added in considerable excess, and the liquid stirred vigorously for some time. The precipitate which separates is flocculent when first formed, but becomes more viscous after stirring and adheres for the most part to the sides of the beaker, which, with its contents, is allowed to stand for about half an hour, or until all the precipitate is settled. The supernatant liquor is decanted through an asbestus filter. The precipitate adhering to the beaker is washed several times with cold, distilled water and the washings poured through the filter. Occasionally, when most of the free bromin is washed out of the precipitate, the liquid does not filter clear. It is therefore advisable to keep the washings separated from the filtrate, and, if necessary, wash with sodium-sulphate solution or with bromin water. The nitrogen in the precipitate is determined by the Gunning-Kjeldahl process as follows:

The precipitate which has been collected on the asbestus filter, together with the asbestus, is returned to the beaker in which the precipitation took place. Twenty cubic centimeters of strong sulphuric acid are added, the beaker covered with a watch glass and placed on a wire gauze over a lamp. When frothing has ceased, about 10 grams of powdered potassium sulphate are added and the liquid boiled until colorless. After cooling it is distilled with water and the ammonia distilled off and determined in the usual way. The percentage of nitrogen found, when multiplied by the factor 6.33, or in the case of gelatin by 5.5, gives the amount of proteid matter precipitated by bromin. In the commercial gelatin above mentioned the nitrogen content was found to be 14.1 and 14 per cent, respectively, on two determinations. Solutions of kreatinin, asparagin, and aspartic acid were found to yield no precipitates with bromin, but bromin was found to precipitate all albumin, acid albumin, and all peptones formed by the digestion of albumin with pepsin.

APPLICATION TO COMMERCIAL MEAT EXTRACTS.

On applying the bromin method to commercial meat extracts the following results were obtained. The solutions of the Bovril preparations were not previously filtered, and therefore the figures contain the nitrogen and the fiber present.

Nitrogen in commercial meat extracts.

Substance.	Nitrogen in precipitate by bromin.	Proteids (N x 6.25).
	Per cent.	*Per cent.*
Liebig Company's extract	1.41	8.92
Seasoned bovril	1.94	12.28
Bovril for invalids	2.64	16.71

Koenig and Boemer have shown that the proteid nitrogen in meat extracts is generally much overestimated. They found a total of 1.17 per cent of proteid nitrogen in the Liebig Company's extract, which is equivalent to 7.41 per cent of total proteids, mostly albumose. The fact that bromin completely precipitates all proteid and gelatinoid matters in solution, affords a convenient means of solving certain problems which have hitherto presented considerable difficulty. For instance, in a solution which has been subjected to digestion it may be possible to precipitate all the unchanged proteids by saturation with zinc sulphate. The peptones which have been formed during digestion remain in solution and can be separated by filtration. In the filtrate the peptones can be completely precipitated by bromin, and thus the total quantity of these bodies formed during digestion can be accurately determined.

Allen and Searle applied this method to an examination of the Liebig Company's extract, 5 grams of which were dissolved in 100 cc of water and the solution saturated with zinc sulphate. After filtering, bromin water was added to the filtrate and a precipitate produced which redissolved on diluting with water and the addition of hydrochloric acid. When the filtrate from the saturated zinc sulphate was previously diluted with water and acidulated, no precipitate was formed on the addition of bromin. This reaction shows that no considerable quantities of real peptones exist in Liebig's extract.

Since this bulletin was prepared for the press, an extensive article on the halogen derivatives of albumin has been published by F. Blum and W. Vaubel[1] of Frankfurt, Germany.

[1] Ueber Halogeneiweissderivate, Journal für praktische Chemie, 1897, **56**, 393–6, and 1898, **57**, 365–396.

○

YD 18295