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AGRICULTURAL ANALYSIS

By the Same Author

SOIL ANALYSIS

A HANDBOOK OF PHYSICAL AND CHEMICAL METHODS

Second edition in preparation. Thoroughly revised and with new matter.

AGRICULTURAL ANALYSIS

A HANDBOOK OF METHODS EXCLUDING THOSE FOR SOILS

BY

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PREFACE

THIS book is a laboratory manual giving the details of the methods of analysis of fertilizers, feeding stuffs, milk, milk products, insecticides and fungicides with references to the sources of information. It also contains the preparation of the indicators and standard solutions used in the methods described and the data required for calculating the results. It is intended for agricultural analysts with limited library facilities, but it is hoped that it will also be of use to research workers and advanced students.

Official Agricultural Analysts, when analysing samples taken under the Fertilisers and Feeding Stuffs Act, are obliged to adopt the prescribed methods. But for those analysts who are at liberty to choose their methods alternative ones are given, except where one method is considered to be much better than the others. It will be seen that there is a lack of uniformity in some of the conventional methods, *e.g.* those for the determination of oil or fat and fibre, and in many of the absolute determinations, *e.g.* the gravimetric determination of phosphoric acid as magnesium pyrophosphate, there is a lack of agreement as to the best procedure to adopt.

The need for such a book was felt when I was in Fiji, where I did varied analytical work with very few text-books and journals at my disposal. I decided that, if it were possible, I would fill the gap in the literature, and began the book soon after my transfer to Nigeria, but was not able to devote much time to it except during periods of leave spent in England. The rate of progress was therefore very slow, and much of what I wrote became out of date. After my retirement I completed the part on soils, which

was published as *Soil Analysis*. Its favourable reception encouraged me to finish the parts dealing with the rest of agricultural analysis.

I have pleasure in acknowledging the help I received from Dr. F. Tattersfield and Dr. J. T. Martin in writing the part on pyrethrum and derris. They suggested the methods to be dealt with, and increased my indebtedness to them by reading through and amending the typescript and later correcting the proofs of that part.

I am also indebted to Mr. W. Godden for information about the determination of chlorine in milk and for advice in choosing methods for the determination of the mineral constituents of feeding stuffs. Mr. Godden gave me the details of the methods for iron and copper before they were published by him in Technical Communication No. 9 of the Imperial Bureau of Animal Nutrition. He is not, however, responsible for the choice of all the methods in that part of the book. The methods for the determination of sulphur and iodine in Technical Communication No. 9 differ from those in this book, but most of the other methods in that publication are included in the following pages.

I am very grateful to Capt. J. Golding and Dr. W. L. Davies for information about milk analysis, and particularly to Dr. Davies for giving me the details of the Embden-Fetter method of determining phosphorus in milk. I also thank Dr. A. H. Lewis for the method of determining nitrogen in urea; Dr. H. L. Richardson for the details of the use of bromo-cresol green in the Kjeldahl titration, and Mr. G. V. Jacks for kindly supplying me with many extracts from journals.

Figs. 1, 4, 5, 6, 7 and 8 are reproductions of figures illustrating the original descriptions of pieces of apparatus. For permission to reproduce these figures from the publications named in the text I have pleasure in thanking the Editor of the Journal of the Society of Chemical Industry, the British Standards Institution, the Council of the Society of Public Analysts and the Editorial Board of the Associa-

tion of Official Agricultural Chemists. I also thank Messrs. A. Gallenkamp and Co. Ltd. for the loan of the block used for Fig. 3.

I have received great help in correcting the proofs from Mr. M. Greenwood and my nephew, Mr. R. B. Ferro, to both of whom I am very grateful.

C. H. W.

LEEDS,
15*th September*, 1937.

NOTE.—British Standard Specification No. 755 was published when this book had reached the page-proof stage. Part I gives the specifications of the apparatus, and Part II contains the procedures for the determination of fat in milk, skim-milk, cream and cheese by the Babcock method.

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AGRICULTURAL ANALYSIS

INTRODUCTION

CHEMICAL analyses are undertaken with various objects in view, but without interpretation the results of the analyses are valueless. Samples of milk are analysed by Public Analysts with the object of detecting adulteration; by technical chemists in the dairy industry to ascertain whether the milk is of average or good quality; and by agricultural chemists to find the extent of variation of some of the constituents under particular conditions. In all these cases, the results of the analyses would be meaningless without some knowledge of the average composition of milk. The interpretation of the results is beyond the scope of this book. Tabulated results of analyses are contained in the publications mentioned later, and it is hoped that these will provide the reader with the data necessary for the interpretation of his own results.

If the analysis consists of the carotene content of a sample of butter, the determination of one of the mineral constituents of a feeding stuff, the percentage of rotenone in a sample of derris or the percentages of pyrethrin I and II in pyrethrum, there may be difficulty in finding similar analyses for comparison. Sometimes these analyses can be obtained only by referring to the original papers describing the methods of analysis. With these exceptions, however, the reader will probably find the data required for the interpretation of his results in the following publications:

Manures and Manuring, Ministry of Agriculture and Fisheries, Bulletin No. 36, 7th edition, 1937.

Part I deals with farmyard and other organic manures; Part II with artificial fertilizers, and Part III with the

purchase and use of artificial fertilizers. The average composition of artificial fertilizers is included in Part II.

Sir E. J. Russell, *Artificial Fertilizers in Modern Agriculture*, Ministry of Agriculture and Fisheries, Bulletin No. 28, 2nd edition, 1932.

This bulletin presents the results of a large number of field experiments and summarizes the effects of artificial fertilizers on farm crops. It also deals with the manufacture and composition of artificial fertilizers more fully than Bulletin No. 36. It includes a table giving the average composition of phosphate rock.*

T. B. Wood, revised by H. E. Woodman, *Rations for Live Stock*, Ministry of Agriculture and Fisheries, Bulletin No. 48, 9th edition, 1936.

After a brief account of general principles, the general properties of feeding stuffs and the methods of rationing are described. Then follow tables giving the composition and nutritive value of a large number of feeding stuffs and the mineral composition of some common feeding stuffs. The latter table gives the percentages of total ash, lime, phosphoric acid, potash and chlorine.

F. B. Morrison, *Feeds and Feeding: A Handbook for the Student and Stockman*, 20th edition. Ithaca, New York: The Morrison Publishing Co.; London: Kegan Paul, Trench, Trubner & Co., Ltd., 1936.

This is the last edition of the well-known book on feeding stuffs by Henry and Morrison.† Part I deals with the fundamental principles of animal nutrition, particular attention being given to the functions of minerals and the diseases due to mineral deficiencies. Part II contains information concerning the composition, properties and uses

* The composition of American phosphate rock is more fully dealt with in the Bulletin referred to on p. 54.

† The first nine editions of *Feeds and Feeding* were written by W. A. Henry, who died in 1932. The tenth to fourteenth editions were revised by Henry assisted by Morrison. Later editions were revised and rewritten by Morrison.

of common feeding stuffs. In the Appendix are given the average composition of American feeding stuffs, digestible nutrients, mineral and fertilizing constituents and digestion coefficients. Under mineral and fertilizing constituents are given the percentages of calcium, phosphorus, nitrogen and potassium.

A. L. Winton and K. B. Winton, *The Structure and Composition of Foods*. Vol. I, *Cereals, Starch, Oil Seeds, Nuts, Oils, Forage Plants*, 1932. Vol. II, *Vegetables, Legumes, Fruits*, 1935. New York: John Wiley & Sons, Inc.; London: Chapman & Hall, Ltd.

In this work the foods are divided into the groups mentioned in the sub-titles. Each member of the group is considered separately. After brief statements of the origin, habitat and uses, the macroscopic and microscopic structures are described in considerable detail with figures from original drawings. Then follows the chemical composition, which is compiled from published analyses with references to the sources of information. The introduction to the first volume gives an outline of the chief constituents of plants. This is supplemented by the introduction to the second volume, which contains a valuable summary of the chemistry of the minor constituents of plants, including the pectins, chlorophyll, the carotenoids and vitamins.*

Variations in the Composition of Milk, Ministry of Agriculture and Fisheries, Bulletin No. 16, 2nd edition, 1935.

This bulletin contains a discussion of the average composition of milk and the circumstances affecting the composition, with references to the authors quoted.

W. L. Davies, *The Chemistry of Milk*. London: Chapman & Hall, Ltd., 1936.

This book is divided into five parts which deal respectively with the composition of milk, the constituents of milk, the

* The third volume dealing with animal products was published in September, 1937.

physical chemistry of milk, the chemistry of milk processing and the nutritive value of milk. The large number of references to the original literature, the very complete authors and subject indexes, and the many tables of physical constants and analytical data make this book of great value to the research worker and the analyst.

F. Tattersfield, article Plant Sprays (Insecticides and Fungicides), *Thorpe's Dictionary of Applied Chemistry, Supplement*, ii, 1935.

H. Martin, *The Scientific Principles of Plant Protection*, 2nd edition. London: E. Arnold & Co., 1936.

In both these publications insecticides and fungicides are dealt with from many aspects, and particular attention is paid to the constitution and the relative toxicities of the constituents of pyrethrum and derris. The many references given by both authors serve as a guide to the widely scattered literature of the subject.*

In giving the details of the analytical methods in the following pages the term concentrated hydrochloric acid is used for the saturated solution of hydrochloric acid, and the solution prepared by diluting the saturated solution with water is termed dilute hydrochloric acid. Similarly, sulphuric acid (sp. gr. 1·84) and nitric acid (sp. gr. 1·42) are designated concentrated acids, and the solutions prepared by diluting the concentrated acids are termed dilute sulphuric acid and dilute nitric acid respectively. In the case of the saturated solution of ammonia (sp. gr. 0·88) there is unfortunately no uniformity in nomenclature. It is termed strong ammonia by some authors and ammonium hydroxide by others. The word strong is now correctly used with reference to the strength of acids and bases, and should no longer be used as an alternative for concentrated. The alkalinity of a solution of ammonia in water is due to the presence of

* The agreed specifications of some of the commoner insecticides and fungicides will be found in the Bulletin of the Ministry of Agriculture and Fisheries referred to on p. 279.

hydroxyl ions; but it is doubtful whether the hydroxyl is formed by the ionization of ammonium hydroxide, $NH_3+H_2O \rightleftharpoons NH_4OH \rightleftharpoons NH_4^{\cdot}+OH'$, or by the direct action of ammonia on water, $NH_3+H_2O=NH_4^{\cdot}+OH'$ (see J. W. Mellor, *Inorganic and Theoretical Chemistry*, viii, pp. 230-231). For these reasons the saturated solution of ammonia in water is here termed concentrated ammonia, and the solution prepared by diluting the concentrated solution is termed dilute ammonia. The degree of dilution is indicated by a ratio in brackets, the first figure being the volume of liquid diluted and the second the volume of water added.

PREPARATION OF THE SAMPLE

SAMPLING consists of two operations, in the first of which a sample, as representative as possible, is taken from the whole bulk of the material to be analysed. In the second stage of the process the sample is prepared for the analysis, so that small uniform portions can be weighed out for the separate determinations. Since this book is a laboratory manual, the methods of taking the sample from bulk are not dealt with; the treatment of the sample after its arrival at the laboratory is considered here. Below are given general rules for the preparation of samples of fertilizers, feeding stuffs, plants and grasses. In the case of calcium cyanamide and some other materials, which require special treatment, the preparation of the sample for analysis is given under the same heading as the method of analysis.

Fertilizers.—The British official methods for the preparation of the sample of a fertilizer for analysis (Fertilisers and Feeding Stuffs Regulations, 1932, **11**, i) are as follows:

In the case of powdered fertilizers in a dry, or moderately dry, condition, the sample is passed through a sieve having apertures about 1 mm. square. Adventitious materials which cannot be conveniently crushed—*e.g.*, fragments of metal in basic slag—are removed and allowed for. Other substances which are dry enough to powder, but are not in a fine condition, are pulverized until the sample passes through a sieve having apertures about 1 mm. square.

Wool, hair, hoof, shoddy and similar substances are pulled apart and cut until they are in a fine condition; or, if dry, they may be passed through a shredding machine. Moist fertilizers, which do not admit of being passed through a sieve, are mixed by the most suitable means.

In the case of substances which gain or lose water during the process of pulverizing or mixing, the proportion of water is determined in the coarse and in the fine condition, and the results of the analysis of the powdered sample are calculated to the water content of the original coarse substance.

Crystalline or saline materials, such as sulphate of ammonia, nitrate of soda or potash salts, may be prepared by being well mixed and rapidly ground in a stoneware mortar, the portion finally reserved for analysis being specially finely ground.

When the sample has been passed through the sieve and thoroughly mixed, or, if not passed through the sieve, has been thoroughly mixed, a part of it which is not less than 100 gm. is placed in a stoppered bottle, and from this the portions for analysis are weighed.

The Association of Official Agricultural Chemists (*Official and Tentative Methods of Analysis of the Association of Official Agricultural Chemists*, Washington, D.C., 4th edition, 1935,* p. 18) give the following directions for the preparation of the sample: Pass the entire sample submitted for analysis through a 10-mesh sieve before subdividing it. Reduce the whole sample by quartering to a quantity sufficient for analytical purposes. Transfer the sample to a sieve having circular openings 1 mm. in diameter and sift it, breaking the lumps with a pestle. Grind the portion remaining on the sieve until all particles pass through, grinding and sifting as rapidly as possible to avoid loss or gain of moisture during the operation. Mix thoroughly and preserve in tightly stoppered bottles.

Feeding Stuffs.—The British official methods for the preparation of the sample of a feeding stuff for analysis (Fertilisers and Feeding Stuffs Regulations, 1932, **12**, i) are as follows:

If the sample is in a fine condition and passes through a sieve having apertures about 1 mm. square, it is thoroughly mixed and a portion not less than 100 gm. in weight is

* In the following pages this book is referred to as *Methods of Analysis*, 1935.

placed in a stoppered bottle. From this portion the quantities for analysis are taken.

If the sample does not wholly pass through a sieve having apertures about 1 mm. square, and wholly passes through a sieve having apertures from 2 to 3 mm. square, it is thoroughly mixed and a portion for the determination of moisture is at once taken. If the sample is in a coarse condition, as, for example, pieces of broken cake, it is pulverized until the whole passes through a sieve having apertures from 2 to 3 mm. square. It is then thoroughly mixed and a portion for the determination of moisture is at once taken. From the mixed sample which has passed through the sieve having apertures from 2 to 3 mm. square a portion not less than 100 gm. in weight is further powdered and passed through a sieve having apertures about 1 mm. square. The portion of the sample so prepared is placed in a stoppered bottle, and from it the quantities for analysis are weighed.

If the original sample is appreciably moist, or if for any reason the operations of pulverization and mixing are likely to result in loss or gain of moisture, the moisture is determined in this prepared portion, in order that the results of the analysis may be corrected to correspond with the sample in its original condition.

Materials which cannot be conveniently pulverized or passed through a sieve are thoroughly mixed by the most suitable means.

The Association of Official Agricultural Chemists (*Methods of Analysis*, 1935, p. 335) give the following directions for the preparation of samples of grain and stock feeds: Grind the sample to pass through a sieve having circular openings 1 mm. in diameter, and mix thoroughly. If the sample cannot be ground, reduce it to as fine a condition as possible.

Plants.—The Association of Official Agricultural Chemists (*Methods of Analysis*, 1935, p. 121) give the following directions for preparing samples of plants in which it is intended to determine the mineral constituents: Thoroughly

remove all foreign matter from the material, especially adhering soil or sand, avoiding excessive washing to prevent leaching. Air-dry as rapidly as possible to prevent decomposition or loss in weight by respiration. Grind and preserve in tightly stoppered bottles. If the results are to be expressed on the fresh weight basis, record the weights of the sample before and after air-drying. When determinations of copper, manganese, zinc, iron, aluminium, etc., are to be made, take precautions to prevent contamination of the sample by dust during air-drying and from the grinding and sieving machinery.

F. B. Shorland (*Trans. Roy. Soc. New Zealand*, 1934, **64**, pp. 35-50) found that in carefully cleaned samples of pasture the alumina content is less than 0·025 per cent., and thinks that alumina is the best index of soil contamination, especially that due to fine atmospheric dust which cannot be easily detected by other methods. Hence, if the alumina content of a sample of pasture, determined by the method described on p. 173, exceeds the above limit, soil contamination is indicated. In the estimation of iron the problem of soil contamination is best overcome by brushing and careful washing of the sample before ashing. The washing of samples of pasture renders them unsuitable for the determination of the major mineral constituents, since potash, lime, magnesia and phosphoric acid are so combined in the plant as to be largely extracted by water.

Grasses.—Samples of grass are sometimes dried by removing the greater part of the moisture by air-drying and completing the drying in a steam oven. When dried in this way, the grass is kept for some time at a comparatively low temperature and is liable to undergo chemical changes during the process of drying. Drying the fresh sample in a well-ventilated oven is more likely to ensure that the dry matter remains unchanged. A. W. Greenhill (*Agric. Prog.*, 1933, **10**, pp. 163-168) makes the following recommendations for the oven-drying of samples of grass: The grass should be spread out loosely in shallow trays to a depth of not more

than 1½-2 inches, and the number of trays and the ventilation of the oven should be such that evaporation can proceed readily from all the trays. The oven should operate at a temperature between 70° and 100° C., but it does not appear to be necessary to employ a temperature much above 70° C. Drying should be completed in 4-12 hours, depending mainly on the ventilation of the oven. Thick layers and close packing of the grass, and overcrowding of the oven, particularly whilst the grass is in the fresh state, should be avoided. Neglect of these precautions prolongs the time of drying and often results in loss of dry matter and the occurrence of chemical changes.

An electric oven for drying samples from field plots has been described by F. H. Garner, J. Grantham and H. G. Sanders (*J. Agric. Sci.*, 1935, **25**, pp. 315-317). Where a suitable oven is not available, an alternative method, which has been much used in the past, consists in drying the grass on a hot plate heated by gas burners. Garner, Grantham and Sanders point out that accurate control of the temperature is difficult, and the temperature over the plate is not uniform. It is therefore necessary either to run the risk of occasionally burning the sample or to dry more slowly than is desirable. Another disadvantage is that the samples are unprotected from dust during the drying.

The final weighing of the dried material should be performed as soon as the grass has been dried to constant weight at a temperature of 70°-100° C., and before the grass has absorbed any moisture. The procedure sometimes adopted of exposing the oven-dried grass to the air to take up moisture until equilibrium is reached has the disadvantage that the equilibrium point varies with different conditions, and the weights obtained are not comparable.

After being weighed, the sample of grass is finely ground; this is most easily done if the oven-dry material is not allowed to absorb an appreciable amount of water before being ground. Any change of moisture content during grinding should be allowed for in the subsequent analytical deter-

minations. The size of the particles affects the results of the determinations. Greenhill (*loc. cit.*) states that the results of the total and amide nitrogen, as well as the fibre determinations, are significantly affected, though not to a large extent; but the pepsin-digestible nitrogen is affected to a marked degree. At Jealott's Hill a Christy and Norris laboratory mill with a 1/64 inch sieve has been found to be generally satisfactory.

When samples of pasture grass are taken at regular intervals from the same plot with the object of finding the seasonal variation, it is impossible to prevent the samples being contaminated with soil. The analytical data must therefore be corrected for the small amount of soil included in the grass sample during the cutting operations. As the soil included in the sample is derived mainly from worm casts, H. E. Woodman, D. L. Blunt and J. Stewart (*J. Agric. Sci.*, 1926, **16,** pp. 205-274) took a large sample of worm casts, in which ash and silica were determined by the methods employed in the analysis of the grass. Since most of the ash of worm casts consists of silica, it is possible to calculate the soil content of the grass from a knowledge of the silica contents of the grass as cut and the soil-free grass.

If x = gm. of soil in 100 gm. of dry grass sample,
a = per cent. of silica in dry soil,
b per cent. of silica in dry soil-free grass,
and c per cent. of silica in dry grass sample,

$$\text{then } x = \frac{100\,(c - b)}{a - b}$$

Since in no estimation of silica was a percentage lower than 1·72 obtained, this figure was taken as representing the amount of silica in clean dry grass, and the corrected analyses were based on a common silica content of 1·72 per cent. The same correction for the soil content of the grass was used in subsequent work by Woodman and his co-workers (*ibid.*, 1927, **17,** pp. 209-263; 1928, **18**, pp. 266-296; and 1929, **19,** pp. 236-265).

NITROGENOUS FERTILIZERS

Organic nitrogen is determined in fertilizers and feeding stuffs by one of the many modifications of Kjeldahl's method. In all these methods a weighed quantity of the sample is heated with concentrated sulphuric acid. By this means the carbon is oxidized to carbon dioxide, and the sulphuric acid is reduced to sulphur dioxide; at the same time the nitrogen in the sample is converted into ammonium sulphate. When the oxidation is complete, the residue is dissolved in water; sodium hydroxide is added and the ammonia is distilled into a measured volume of standard acid. Either potassium sulphate or anhydrous sodium sulphate is generally added in order to raise the boiling point of the mixture, and thus to shorten the time of the digestion. Mercury, mercuric oxide or copper sulphate may also be added to hasten the oxidation. If mercury or mercuric oxide is used as a catalyst, it is necessary to precipitate the mercury as sulphide before making the solution alkaline, in order to prevent the formation of a mercuri-ammonium derivative.

All these different processes are very often termed Kjeldahl's method. It is therefore necessary to explain that in the method described by J. Kjeldahl (*Z. anal. Chem.*, 1883, **22,** pp. 366-382) the nitrogenous substance is heated with sulphuric acid alone and the oxidation is completed by means of potassium permanganate, which is cautiously added as a fine powder, in very small quantities at a time, until the solution becomes green. When cool, the residue is diluted with water, made alkaline with sodium hydroxide, and the ammonia is distilled into a measured volume of standard acid. H. Wilfarth (*Chem. Zentr.*, 1885, [3], **16,** pp. 17-19 and 113-115) observed that certain metallic oxides hasten the oxidation; and found that if mercuric oxide is added to the sulphuric acid before the digestion, the addition of potassium permanganate is unnecessary. He determined nitrogen by heating the sample

with concentrated sulphuric acid and mercuric oxide, dissolving the residue in water, adding potassium sulphide and potassium hydroxide, and distilling the ammonia into standard acid. The addition of potassium sulphate to the sulphuric acid was proposed by J. W. Gunning (*Z. anal. Chem.*, 1889, **28,** pp. 188-191). He found that with this addition the oxidation can be completed without any catalyst. C. Arnold and K. Wedemeyer (*ibid.*, 1892, **31,** pp. 525-533) carried out the determination by heating the substance to be analysed with sulphuric acid, potassium sulphate, mercuric oxide and copper sulphate. Recently M. F. Lauro (*Ind. Eng. Chem.* (*Anal.*), 1931, **3,** pp. 401-402) found that the clearing of the digested matter is accelerated when selenium is used in place of mercuric oxide and copper sulphate.

The British official method of determining organic and ammoniacal nitrogen, and the three methods adopted by the Association of Official Agricultural Chemists for the same purpose, are given on pp. 19-22. They differ in details, but it will be observed that the British official method is very similar to the A.O.A.C. Gunning method. In both these methods potassium or sodium sulphate is added to the sulphuric acid, and the use of a catalyst (copper sulphate or mercury in the former and copper sulphate in the latter) is optional. The method called the Kjeldahl method by the Association of Official Agricultural Chemists, in which mercury, mercuric oxide or copper sulphate is added to the sulphuric acid, but neither potassium nor sodium sulphate is used, is similar to Wilfarth's procedure. The Kjeldahl-Gunning-Arnold method, in which both a catalyst and potassium or sodium sulphate are used, is similar to Arnold and Wedemeyer's procedure. A method for the determination of nitrogen in urea is given on p. 22. It consists of heating the sample with sulphuric acid and an equal volume of water until the water has boiled away and then continuing the digestion after the addition of sodium sulphate and selenium.

It is now realized that the whole of the nitrogen is not converted into ammonium sulphate when the contents of the flask become clear. In the British official method the heating is continued for one hour after the colour of the liquid ceases to diminish. This is a common practice, but recent work has shown that a much longer period of heating is necessary to ensure the complete conversion of the nitrogen into ammonium sulphate. F. L. Ashton (*J. Agric. Sci.*, 1936, **26,** pp. 239-248) compared the catalytic efficiency of selenium and copper sulphate as applied to the analysis of grass. 1 gm. portions of the sample, which had passed a sieve with holes 1/64 inch in diameter, were weighed into 500 c.c. Kjeldahl flasks. Either 0·2 gm. of selenium or 1 gm. of copper sulphate was added to each flask. 7 gm. of sodium sulphate and 25 c.c. of concentrated sulphuric acid were then added to all the flasks. Digestion was carried out over burners, the full heat of which was applied directly to the flasks. The temperature during digestion was about 345° C. Refluxing took place after the first few minutes from over half-way up the necks of the flasks. Blank determinations were made by digesting the reagents together with 1 gm. of sucrose for 5 hours.

The same value for total nitrogen was eventually obtained by both methods, but with selenium it was reached earlier than with copper sulphate. Selenium not only cleared the contents of the digestion flask more rapidly than copper sulphate, but it also accelerated the conversion of the nitrogen into ammonium sulphate. Both with selenium and with copper sulphate this conversion was by no means completed as soon as clearing occurred. Maximum values for total nitrogen were obtained with selenium by heating the flasks for 2-3 hours after the contents had cleared, and with copper sulphate by heating for 90 minutes longer.

If the sample contains ammonium salts, the results obtained by any of the above methods include the ammoniacal nitrogen, but do not include nitrate nitrogen. In order

to determine the total nitrogen, including the nitrate nitrogen, the nitrates are reduced to ammonia. This is effected by adding to the sample sulphuric acid containing salicylic acid or phenol, together with sodium thiosulphate or zinc; potassium or sodium sulphate and mercury, mercuric oxide or copper sulphate can also be added to shorten the time of digestion. The British official method and the two methods adopted by the Association of Official Agricultural Chemists for the determination of total nitrogen are given on pp. 22-23. In the British official method potassium or sodium sulphate is used, but the addition of copper sulphate or mercury is optional. In the modified Kjeldahl method mercury or mercuric oxide is added, but no potassium or sodium sulphate is used. In the modified Gunning method potassium or sodium sulphate is added, but a catalyst is omitted. The latter method is therefore very similar to the British official method.

Nitrogen in ammonium salts is determined by liberating the ammonia and distilling it into a measured volume of standard acid. In the case of ammonium salts, the ammonia can be liberated by the addition of sodium hydroxide; but the action of sodium hydroxide on the organic matter in mixed fertilizers may lead to the formation of ammonia. In the British official methods, which are given on p. 23, the ammonia is liberated by an alkali (not specified) in the absence of organic matter; but in the presence of organic matter, magnesium oxide is used to liberate the ammonia. The latter procedure is similar to that adopted by the Association of Official Agricultural Chemists, which is given on p. 24. The British official method for the determination of free acid in sulphate of ammonia is given on p. 28.

The British official methods for the determination of nitrogen in nitrates are given on p. 24. Method A, in which the nitrate is reduced to ammonia by Devarda's alloy in alkaline solution, is similar to the Devarda method adopted by the Association of Official Agricultural Chemists (see

p. 25). Method B, in which the reduction is effected by reduced iron and dilute sulphuric acid, is similar to the reduced iron method, which is adopted by the Association of Official Agricultural Chemists for the determination of nitrate and ammoniacal nitrogen (see p. 26). Another method of determining nitrate nitrogen is that known as the ferrous sulphate-zinc-soda method (see p. 26).

Two methods of determining nitrate nitrogen in mixed fertilizers are given on pp. 26-28. In both methods the nitrate nitrogen is determined by difference after determining the nitrogen excluding that in the nitrates. The latter figure is obtained by heating the sample with concentrated sulphuric acid and ferrous sulphate in order to decompose the nitrates and then completing the determination as in the Kjeldahl method.

In all the methods so far considered the nitrogen in the sample is converted into ammonia, which is determined volumetrically. The Fertilisers and Feeding Stuffs Regulations give no details about the determination of the ammonia in the distillate. The Association of Official Agricultural Chemists (*Methods of Analysis*, 1935, p. 23) recommend N/2 acid for ordinary work and N/10 acid for determining very small quantities of nitrogen. They also recommend the use of N/10 sodium hydroxide solution and cochineal or methyl red as the indicator.* Bromo-cresol green has nearly the same pH range as methyl red, but the change of colour is much more marked. L. H. Bailey (*Cereal Chem.*, 1929, **6**, pp. 454-456) pointed out that it is particularly suitable as an indicator for the titrations in the Kjeldahl method. It is used for this purpose at Rothamsted Experimental Station. The acid distillate, to which 1 c.c. of 0·04 per cent. bromo-cresol green solution has been added, is titrated with standard alkali until the pH value

* For the preparation of indicators and standard solutions of hydrochloric acid, sulphuric acid and sodium hydroxide, see pp. 319-325. The preparation and standardization of other standard solutions are included in the same section.

of the solution is 4·8. The end-point is judged by matching the colour of the solution being titrated with an acetate buffer solution at pH 4·8, to which bromo-cresol green has been added. The buffer solution is prepared by placing in a flask, similar to that used for the distillate, 6 c.c. of N/5 sodium acetate solution and 4 c.c. of N/5 acetic acid solution, diluting to the same volume as the distillate (about 200 c.c.) and adding 1 c.c. of 0·04 per cent. bromo-cresol green solution. If a few drops of mercuric chloride solution are added, the standard buffer solution will last for at least a month.*

The Association of Official Agricultural Chemists direct that the weight of the sample taken shall be 0·7-3·5 gm. The calculation is greatly simplified by taking 0·7 gm. or a multiple of 0·7 gm., since 1 c.c. of N acid =0·014 gm. of nitrogen. The greatest volume of standard acid which can be conveniently used to absorb the ammonia is 50 c.c., and this volume sets the upper limit to the weight of nitrogen in the weight of the sample taken for the analysis: 50 c.c. of N acid are equivalent to 0·7 gm. of nitrogen, and 50 c.c. of N/10 acid=0·07 gm. of nitrogen. Hence, if the sample contains 20 per cent. of nitrogen, the weight of the sample must not exceed 3·5 gm. if N acid is used, and must not exceed 0·35 gm. if N/10 acid is used to absorb the ammonia. In many laboratories N/10 acid is used for the determination of nitrogen. This practice necessitates the use of rather small quantities of the sample. In the case of ammonium salts and nitrates, which are soluble in water, it is advisable to dissolve an accurately weighed quantity of the sample in water, make the solution up to a definite volume and determine nitrogen in aliquot parts of the solution.

Fox and Geldard's methods for the determination of urea alone and urea in calcium cyanamide are given on p. 28. They are based on the fact that in neutral solution urea is quantitatively converted into ammonium carbonate by the

* The author is indebted to Dr. H. L. Richardson for this information.

enzyme urease, which occurs in jack bean and soya bean. Cyanamide, dicyanodiamide and guanylurea do not affect the reaction; but calcium must be precipitated as carbonate, the excess of carbonate being removed by acidifying with hydrochloric acid and aerating before the addition of urease.

Special methods are required for the analysis of calcium cyanamide. Those employed at Rothamsted Experimental Station have been described by Richardson, and are given on pp. 29-31. The determination of total nitrogen in calcium cyanamide presents some difficulty. If the determination is carried out in the ordinary way, the results are low and discordant, owing to the evolution of gases smelling of cyanide, which are formed by the action of the concentrated sulphuric acid on the dry material. The Kjeldahl method is therefore modified by adding water so that the cyanamide nitrogen is completely hydrolysed during the first stages of the digestion. The modified procedure is given on p. 29.

Cyanamide nitrogen is determined by extracting the sample with water, precipitating silver cyanamide and determining the nitrogen in the precipitate. Silver cyanamide is precipitated by adding dilute ammonia and silver nitrate solution to the filtered extract. Ammonia is added to prevent the precipitation of the silver derivative of dicyanodiamide, which is soluble in ammonia.* Urea nitrogen is determined by a modification of Fox and Geldard's method. In mixed fertilizers the phosphate is removed by the addition of barium hydroxide, and the excess of barium is precipitated by adding sodium carbonate. The excess of carbonate is then removed by acidification and aeration. Dicyanodiamide nitrogen is determined by Garby's method, which is described on pp. 30-31. This method depends on the hydrolysis in acid solution of dicyanodiamide to guanylurea, which forms an insoluble nickel derivative.

* For further information about the chemistry of calcium cyanamide see E. M. Crowther and H. L. Richardson, *J. Agric. Sci.*, **1932, 22,** pp. 300-334, particularly pp. 302-304.

In order to arrest the hydrolysis at the right point and secure complete precipitation of the nickel compound, careful control of the conditions is necessary. Urea does not interfere with the determination, and guanylurea salts, such as might occur in superphosphate mixtures, are almost insoluble in the acetone used as a solvent.

Detection of Nitrates.—The Fertilisers and Feeding Stuffs Regulations, 1932, **11,** iii, direct that the presence or absence of nitrates shall first be ascertained, but a test for nitrates is not included in the Regulations. The Association of Official Agricultural Chemists (*Methods of Analysis*, 1935, p. 23) have adopted the following method of detecting nitrates: Mix 5 gm. of the fertilizer with 25 c.c. of hot water and filter. To 1 volume of this solution add 2 volumes of concentrated sulphuric acid (free from nitrogen) and allow the mixture to cool. Add a few drops of a concentrated solution of ferrous sulphate in such a way that the fluids will not mix. If nitrates are present, the junction shows at first a purple, afterwards a brown, colour, or, if only a minute quantity is present, a reddish colour. To another portion of the solution add 1 c.c. of 1 per cent. sodium nitrate solution and test as before to find whether sufficient sulphuric acid was added in the first test.

Organic and Ammoniacal Nitrogen.—The British official method for the determination of organic and ammoniacal nitrogen in the absence of nitrates (Fertilisers and Feeding Stuffs Regulations, 1932, **11,** iii, a) is as follows: A weighed portion of the sample is transferred to a Kjeldahl digestion flask, 25 c.c. of concentrated sulphuric acid (or more, if necessary) are added, and the flask is gently heated until frothing has ceased. 10 gm. of potassium or sodium sulphate (anhydrous) are added, and the flask is heated until the colour of the clear liquid ceases to diminish. The digestion is then continued for an hour to ensure complete oxidation of the organic matter. The operation may be accelerated by the addition of a small crystal of copper sulphate or a globule of mercury to the liquid in the digestion

flask. The quantity of ammonia present in the liquid is determined by distillation into standard acid after liberation with alkali,* and, if mercury has been used, with the addition also of sodium or potassium sulphide solution.

Regulation 11, iii, e, states that the materials used in this and the other methods for the determination of nitrogen must be examined as to their freedom from nitrogen by means of a control experiment carried out under similar conditions with the same quantities of the reagents which have been employed in the actual analysis; in the case of the above method 1 gm. of pure sugar is used in place of the weighed portion of the sample. The quantity of standard acid found to have been neutralized in the control experiments is deducted from the total quantity of acid neutralized in the distillation of the sample.

The Association of Official Agricultural Chemists (*Methods of Analysis*, 1935, pp. 23-25) have adopted three methods for the determination of organic and ammoniacal nitrogen only, the details of which are given below. In addition to standard acid, standard alkali and an indicator, there is required the following solution:

Sodium Hydroxide Solution.—Dissolve about 450 gm. of commercial sodium hydroxide, free from nitrates, in 1 litre of water. This solution should have a specific gravity of 1·43-1·48.

If mercury or mercuric oxide is used as a catalyst in the acid digestion, one of the following solutions is also required:

Potassium Sulphide Solution.—Dissolve 40 gm. of commercial potassium sulphide in 1 litre of water.

Sodium Sulphide Solution.—Dissolve 40 gm. in 1 litre of water.

Sodium Thiosulphate Solution.—Dissolve 80 gm. of the hydrated salt in 1 litre of water.

Before using the reagents they must be tested by a blank

* The alkali is not specified in the Regulations, but sodium hydroxide is generally used.

determination with sugar, which causes partial reduction of any nitrate present.

Kjeldahl Method.—Place 0·7-3·5 gm. of the sample, depending on the nitrogen content, in a Kjeldahl flask. Add about 0·7 gm. of mercuric oxide, or its equivalent weight of metallic mercury, and 20-30 c.c. of concentrated sulphuric acid (0·1-0·3 gm. of crystallized copper sulphate may be used in addition to, or in many cases in place of, the mercury). Heat gently until frothing has ceased, and then more strongly until the acid boils briskly. Digest for a time after the mixture is colourless or nearly so, or until oxidation is complete. When cool, dilute with about 200 c.c. of water and add a few pieces of granulated zinc or pumice stone to prevent bumping. Then add 25 c.c. of potassium sulphide or sodium sulphide solution followed by, or 25 c.c. of sodium thiosulphate solution mixed with, sufficient sodium hydroxide solution to make the solution strongly alkaline (50 c.c. is usually sufficient), pouring it down the side of the flask so that it does not mix at once with the acid solution. (If mercuric oxide or mercury is not used, the addition of sulphide or thiosulphate solution is unnecessary.) Connect the flask to a condenser by means of a connecting bulb. Mix the contents by shaking, and distil the ammonia into a measured volume of standard acid. Titrate the distillate (the first 150 c.c. generally contain all the ammonia) with standard alkali, using methyl red or cochineal as indicator.

Gunning Method.—Place 0·7-3·5 gm. of the sample, depending on the nitrogen content, in a Kjeldahl flask. Add 10 gm. of powdered potassium sulphate or anhydrous sodium sulphate and 15-25 c.c. of concentrated sulphuric acid (0·1-0·3 gm. of crystallized copper sulphate may also be added). Continue as directed in the Kjeldahl method, but do not add potassium sulphide, sodium sulphide or sodium thiosulphate solution. In making the solution alkaline before distilling, add litmus paper or a few drops of phenolphthalein indicator. (The pink colour of the latter is destroyed by a considerable excess of fixed alkali.)

Kjeldahl-Gunning-Arnold Method.—Place 0·7-3·5 gm. of the sample, depending on the nitrogen content, in a Kjeldahl flask. Add 15-18 gm. of potassium sulphate or anhydrous sodium sulphate, 1 gm. of copper sulphate, or about 0·7 gm. of mercuric oxide or its equivalent of metallic mercury, and 25 c.c. of concentrated sulphuric acid. Continue as directed under the Kjeldahl method, and if mercury has been used add 50 c.c. of potassium sulphide or sodium sulphide or sodium thiosulphate solution.

Nitrogen in Urea.—The following method of determining nitrogen in commercial samples of urea, which is based on the work of E. A. Werner (see *Chemistry of Urea*, London, 1923, p. 34), has been privately communicated to the author by Dr. A. H. Lewis: 1 gm. of the sample is heated with 50 c.c. of dilute sulphuric acid (1:1) until the water has boiled away. Then 7 gm. of anhydrous sodium sulphate and 0·2 gm. of selenium are added, and the mixture is digested for 1 hour. It is usually necessary to transfer the residue to a graduated flask, make up to the mark and distil an aliquot part with sodium hydroxide.

Total Nitrogen.—The British official method for the determination of nitrogen when nitrates are present (Fertilisers and Feeding Stuffs Regulations, 1932, **11**, iii, b) is as follows: A weighed portion of the sample is transferred to a Kjeldahl flask, 30 c.c. of concentrated sulphuric acid containing 1 gm. of salicylic acid or 1 gm. of phenol are added, and the flask is shaken so as to mix the contents without delay. The shaking is continued at intervals during 10 minutes, the flask being kept cool. Then 10 gm. of potassium or sodium sulphate (anhydrous) are added, together with either 5 gm. of crystalline sodium thiosulphate or 2 gm. of zinc dust. The flask is then heated until the colour of the clear liquid ceases to diminish, and for an hour afterwards. A further quantity of concentrated sulphuric acid may be added, if necessary. Copper sulphate or mercury may also be used. The ammonia is determined by distillation into standard acid after liberation with alkali

and, when mercury has been used, with the addition of sodium or potassium sulphide solution.

The Association of Official Agricultural Chemists (*Methods of Analysis*, 1935, pp. 25-26) have adopted two methods for the determination of total nitrogen, including the nitrogen of nitrates. They are as follows:

Modified Kjeldahl Method.—Place 0·7-3·5 gm. of the sample, depending on the nitrogen content, in a Kjeldahl flask. Either (i) add 30 c.c. of concentrated sulphuric acid containing 1 gm. of salicylic acid, shake until thoroughly mixed, allow to stand for at least 30 minutes with frequent shaking, then add 5 gm. of crystallized sodium thiosulphate and digest as directed below; or (ii) add 30 c.c. of concentrated sulphuric acid containing 2 gm. of salicylic acid, allow to stand at least 30 minutes with frequent shaking, then add gradually 2 gm. of zinc dust, with shaking, and digest. Heat over a low flame until all danger of frothing has passed. Then heat more strongly until the acid boils briskly, and continue the boiling until white fumes escape from the flask (5-10 minutes). Add about 0·7 gm. of mercuric oxide or its equivalent of mercury, and continue boiling until the liquid in the flask is colourless or nearly so. If necessary, add 10 c.c. more concentrated sulphuric acid. Continue as directed under the Kjeldahl method (see p. 21).

Modified Gunning Method.—Place 0·7-3·5 gm. of the sample, depending on the nitrogen content, in a Kjeldahl flask. Add 30 c.c. of concentrated sulphuric acid containing 1 gm. of salicylic acid, and shake until thoroughly mixed. Allow to stand with frequent shaking for at least 30 minutes or until solution is complete. Add 5 gm. of sodium thiosulphate and heat the solution for 5 minutes. Cool, add 10 gm. of potassium sulphate or anhydrous sodium sulphate, heat very gently until foaming ceases, and continue as directed under the Gunning method (see p. 21).

Ammoniacal Nitrogen.—The British official method for the determination of nitrogen in ammonium salts (Fertilisers and Feeding Stuffs Regulations, 1932, **11,** iii, c) is as

follows: In the case of compound fertilizers containing calcium carbonate with small quantities of ammonium salts, the portion taken for analysis must be dissolved in hydrochloric acid, and the solution used for the distillation with alkali. If organic matter is absent, a weighed portion of the sample is dissolved in water and made up to a definite volume. An aliquot part of the solution is transferred to a distillation flask, and the quantity of ammonia is determined by distillation into standard acid after liberation with alkali.* If organic matter is present, a weighed portion of the sample is transferred to a distillation flask with about 200 c.c. of water and 5 gm. of magnesium oxide, free from carbonate, and the quantity of ammonia is determined by distillation into standard acid.

The magnesium oxide method for the determination of ammoniacal nitrogen (*Methods of Analysis*, 1935, p. 26) is as follows: Place 0·7-3·5 gm. of the sample, depending on the ammonia content, in a distillation flask together with 200 c.c. of water and 2 gm. or more of magnesium oxide, free from carbonate. Connect the flask with a condenser by means of a Kjeldahl connecting bulb, and distil 100 c.c. of the liquid into a measured volume of standard acid. Titrate the distillate with standard alkali, using cochineal or methyl red as indicator.

Nitrogen in Nitrates.—The British official methods for the determination of nitrogen in nitrates (Fertilisers and Feeding Stuffs Regulations, 1932, **11,** iii, d) are as follows:

Method A (*In the Absence of Organic Matter*).—A weighed portion of the sample is dissolved in water and made up to a definite volume. An aliquot part of the solution is transferred to a flask, and a quantity of finely powdered Devarda's alloy, not less than six times the weight of the sample present in the aliquot part taken, is added. An excess of concentrated alkali* is added, and the flask is at once connected with a distillation apparatus. After standing

* The alkali is not specified in the Regulations, but sodium hydroxide is generally used.

for 30 minutes to allow the reaction to proceed, heating gently if necessary, the ammonia is distilled into standard acid.

Method B (In the Absence of Organic Matter).—10 gm. of the sample are dissolved in water and the solution is made up to 500 c.c. 50 c.c. of the solution are placed in a 500 c.c. Erlenmeyer flask, and 10 gm. of reduced iron and 20 c.c. of sulphuric acid of specific gravity 1·35 are added. The flask is closed with a rubber stopper provided with a thistle tube, the head of which is half filled with glass beads, and is allowed to stand until effervescence ceases. The liquid is then boiled for 5 minutes, the flask is removed from the flame, and any liquid that may have accumulated among the beads is rinsed back with water into the flask. The solution is boiled for 3 minutes more, and the beads are again washed with a little water. The quantity of ammonia is then determined by distillation into standard acid after liberation with alkali.

Method B (In the Presence of Organic Matter).—1 gm. of the sample, or a larger quantity if the proportion of nitrates is small, is placed in a 500 c.c. Erlenmeyer flask with 50 c.c. of water. 10 gm. of reduced iron and 20 c.c. of sulphuric acid of specific gravity 1·35 are added, and the procedure in the last paragraph is followed, except that the quantity of ammonia contained in the liquid, after treatment with reduced iron, is determined by distillation after the addition of magnesium oxide.

Nitrate and Ammoniacal Nitrogen.—The Association of Official Agricultural Chemists (*Methods of Analysis*, 1935, pp. 26-27) have adopted three methods for the determination of nitrate and ammoniacal nitrogen, which are as follows:

Devarda Method.—Place 0·5 gm. of the sample in a 600-700 c.c. flask, and add 300 c.c. of water, 3 gm. of Devarda's alloy and 5 c.c. of sodium hydroxide solution (42 per cent. by weight), pouring the latter down the side of the flask so that it does not mix with the contents at once. Connect with the condenser by means of a Davisson*

* See B. S. Davisson, *J. Ind. Eng. Chem.*, 1919, **11**, pp. 465-466.

or other suitable scrubbing bulb. Mix the contents of the distilling flask by rotating it. Heat slowly at first, then at such a rate that 250 c.c. of the distillate required pass over in 1 hour. Collect the distillate in a measured volume of standard acid, and titrate it with standard alkali, using cochineal or methyl red as indicator.

Reduced Iron Method.—Place 0·7 or 1 gm. of the sample in a 500 c.c. flask. Add about 30 c.c. of water and 2-3 gm. of reduced iron. After allowing the mixture to stand until the ammonium salts and nitrates are dissolved, add 10 c.c. of dilute sulphuric acid (1 : 1). Shake thoroughly, place a long-stemmed funnel in the neck of the flask to prevent mechanical loss, and allow to stand until the violence of the reaction has moderated. Heat the solution slowly, boil for 5 minutes and cool. Add about 100 c.c. of water, a little paraffin wax and 7-10 gm. of magnesium oxide, free from carbonate. Connect the flask by means of a connecting bulb with a condenser, and boil the contents nearly to dryness (about 40 minutes). Collect the ammonia in a measured volume of standard acid, and titrate the distillate with standard alkali, using cochineal or methyl red as indicator.

In the analysis of nitrates proceed as above, but use 25 c.c. of the nitrate solution, corresponding to 0·25 gm. of the sample, and 5 gm. of reduced iron. After boiling, add 75 c.c. of water and an excess of sodium hydroxide solution, and complete the determination as above.

Ferrous Sulphate-Zinc-Soda Method.—Place 0·35 gm., 0·5 gm., or 0·7 gm. of the sample in a 600-700 c.c. flask, and add 200 c.c. of water, 5 gm. of powdered zinc, 1-2 gm. of ferrous sulphate and 50 c.c. of sodium hydroxide solution (sp. gr. 1·33). Connect with a distilling apparatus and distil into a measured volume of N/10 sulphuric acid. Titrate the distillate with standard alkali, using cochineal or methyl red as indicator.

Nitrate Nitrogen.—The two following methods, which are adopted by the Association of Official Agricultural Chemists

(*Methods of Analysis*, 1935, p. 27), are suitable for the determination of nitrate nitrogen in mixed fertilizers.

Robertson's Method.—This method is applicable in the presence of cyanamide and urea.

(*a*) Determine total nitrogen by the modified Kjeldahl or the modified Gunning method (see p. 23).

(*b*) Place 2 gm. of the fertilizer on a filter, wash with water to nearly 200 c.c. in a graduated flask and make up to the mark. Determine nitrogen in the residue by the Kjeldahl, Gunning or Kjeldahl-Gunning-Arnold method (see pp. 21-22).

(*c*) Determine ammoniacal nitrogen in 50 c.c. of the filtrate by the magnesium oxide method (see p. 24).

(*d*) Place another 50 c.c. of the filtrate in a 500 c.c. Kjeldahl flask. Add 2 gm. of ferrous sulphate, or 5 gm. if the total nitrogen is more than 5 per cent., and 20 c.c. of concentrated sulphuric acid. Digest over a hot flame until all the water is evaporated and white fumes are evolved. Continue the digestion for at least 10 minutes to drive off the nitrate nitrogen; add 10-15 glass beads if severe bumping occurs. Add 0·65 gm. of mercury, or its equivalent of mercuric oxide, and digest until all the organic matter is oxidized. Cool, dilute and add potassium sulphide solution. Complete the determination as in the Kjeldahl method, but add a pinch of a mixture of zinc dust and granular zinc (20-mesh) to each flask before distillation to prevent bumping.

The total nitrogen found in (*a*) − the water-insoluble nitrogen found in (*b*) = the water-soluble nitrogen. The water-soluble nitrogen − the nitrogen found in (*d*) = the nitrate nitrogen.

Jones's Modification of Robertson's Method.—Place 0·5 gm. of the sample in a Kjeldahl flask. Add 50 c.c. of water and then 2 gm. of ferrous sulphate, rotating after each addition. Add 20 c.c. of concentrated sulphuric acid and digest over a hot flame. When the water is evaporated and white fumes are evolved, add 0·65 gm. of mercury and complete the determination as in the Kjeldahl method.

The total nitrogen – the nitrogen thus found = the nitrate nitrogen.

Free Acid in Sulphate of Ammonia.—The British official method for the determination of free acid in sulphate of ammonia (Fertilisers and Feeding Stuffs Regulations, 1932, **11,** vi) is as follows: 20 gm. of the sample are dissolved in about 50 c.c. of distilled water, and the solution is filtered. The filtrate is diluted to about 250 c.c., and is then titrated with N/2 sodium hydroxide solution, using as indicator 2 or 3 drops of methyl orange solution containing 0·5 gm. in 1 litre of water. The result is expressed as the percentage by weight of sulphuric acid.*

Urea.—For the determination of urea E. J. Fox and W. J. Geldard (*Ind. Eng. Chem.*, 1923, **15,** pp. 743-745) used an extract of jack bean (*Canavalia ensiformis* DC.). This bean contains 15 times as much urease as soya bean. A few grams of jack bean flour are extracted with 20 times their weight of water for 10-15 minutes, exactly neutralized with hydrochloric acid (about 1 c.c. of N/10 hydrochloric acid per gram of jack bean flour) and filtered. 10 c.c. of this extract are sufficient to convert 0·1 gm. of urea into ammonia in less than 1 hour. Fox and Geldard's procedures are as follows:

Urea Alone.—Dissolve 0·5 gm. of the sample in 250 c.c. of water. Pipette 25 c.c. into a wide-mouthed stoppered bottle of about 100 c.c. capacity. Add a few drops of methyl red indicator and bring to exact neutrality, using N/10 hydrochloric acid or sodium hydroxide. Add 10 c.c. of neutral urease solution and allow to stand for 1 hour with the mouth of the bottle stoppered. Add from a burette a measured excess of N/10 hydrochloric acid, insert in the bottle a glass tube with a bulb and fine holes, and aerate for 5-10 minutes or until all the carbon dioxide is removed. The addition of 2 or 3 drops of capryl alcohol or liquid petroleum will prevent frothing. After aerating for

* 1 c.c. of N/2 sodium hydroxide solution = 0·0245 gm. of sulphuric acid.

5 minutes, reduce the current of air and titrate the solution to exact neutrality with N/10 sodium hydroxide solution.*

Urea in Cyanamide.—Extract 2 gm. of cyanamide with 400 c.c. of water for 2 hours. Add 2 gm. of anhydrous sodium carbonate to precipitate calcium, and continue to shake for another half-hour. Filter the extract through a dry filter. Pipette 25 c.c. into a stoppered bottle, make the solution distinctly acid with dilute hydrochloric acid and aerate until all the carbon dioxide is expelled, as shown by the sharpness of the end-point in the subsequent neutralization. Then make the solution exactly neutral, using methyl red as indicator, add urease solution and determine urea as described above.

Calcium Cyanamide.—The following methods of determining total nitrogen, and nitrogen as cyanamide, urea and dicyanodiamide, in calcium cyanamide are described by H. L. Richardson (*J. Agric. Sci.*, 1932, **22**, pp. 348-357).

Preparation of the Sample.—The bulk sample is well mixed, and large lumps are crushed. From 50 to 100 gm. obtained by repeated quartering are ground to completely pass a 0·5 mm. sieve. Since calcium cyanamide contains quicklime, exposure to air during sampling and weighing should be reduced to a minimum.

Total Nitrogen.—To 0·25-0·3 gm. of the sample are added 50 c.c. of distilled water, 20 c.c. of concentrated sulphuric acid, 10 gm. of sodium sulphate (crystals) and a pea-crystal of copper sulphate. The flask is heated gently for 1-2 hours until the water has boiled off, and digestion is continued for a further 2-3 hours. The ammonia is distilled into N/10 acid (about 40 c.c.) in the usual way.

Cyanamide Nitrogen.—5 gm. of the sample are shaken with 500 c.c. of water in a shaking machine for 3 hours. The extract is filtered through a fluted filter paper, and determinations are made immediately to reduce the risk of change in the alkaline solution. 25 c.c. of the filtrate are transferred to a small beaker and to it are added 5 c.c. of

* 1 c.c. of N/10 hydrochloric acid = 0·003 gm. of urea.

dilute ammonia (1 : 1) and a slight excess of N/10 silver nitrate solution, which is several c.c. more than the number of c.c. of N/10 acid likely to be required in the final distillation. The mixture is left over-night under a clock-glass, filtered with a small funnel and filter paper, and washed with water until 175 c.c. of filtrate are collected. The funnel and contents are allowed to drain and dry apart from the filtrate, which contains ammonia. The precipitate on the paper is then transferred to a Kjeldahl flask, 50 c.c. of water are added, and the nitrogen is determined as described in the last paragraph.

Urea Nitrogen.—Urea is determined in the extract prepared in the preceding paragraph by Fox and Geldard's method (see p. 28). Calcium and phosphates, if present, are precipitated by the addition of a little powdered barium hydroxide and a small excess of anhydrous sodium carbonate, followed by shaking for half an hour. The mixture is filtered, and an aliquot part of the filtrate is acidified with concentrated hydrochloric acid, aerated to remove carbon dioxide, made exactly neutral to methyl red, and allowed to stand for 1 hour in a stoppered vessel after adding freshly prepared neutral urease solution. Excess of standard acid is added together with a few drops of capryl alcohol to prevent frothing, and, after aerating for 5-10 minutes to remove carbon dioxide, the mixture is titrated with standard alkali.

Dicyanodiamide Nitrogen.—This is determined by the nickel guanylurea method worked out by C. D. Garby (*Ind. Eng. Chem.*, 1925, **17,** pp. 266-268), which is as follows: 15-100 gm. of the sample are extracted with acetone (250 or 300 c.c.) by shaking for 2-3 hours. The solution is filtered, and the acetone is distilled off from 50 c.c. of the filtrate in a small flask. If appreciable amounts of dicyanodiamide are present, a crystalline residue remains, contaminated with oil from the calcium cyanamide; this is removed by rinsing with absolute ether, in which dicyanodiamide is insoluble. The residue is dissolved in 20 c.c. of N/4 nitric acid, transferred to a small evaporating basin, partly covered

with a watch-glass to control the rate of evaporation, and taken to dryness on a steam bath in 1¾-2¼ hours. The residue of guanylurea nitrate is dissolved in 40 c.c. of a 10 per cent. solution of mannitol saturated with nickel guanylurea; it is then treated with 3 c.c. of a solution containing 40 gm. of nickel nitrate and 20 gm. of ammonium nitrate in 100 c.c. of the mannitol-nickel guanylurea solution, and sufficient 20 per cent. sodium hydroxide solution is added drop by drop to produce a greenish yellow colour. It is important to reproduce the same colour—*i.e.*, degree of alkalinity—as nearly as possible in all determinations. These operations are conducted in small weighing bottles with ground stoppers to prevent loss of ammonia. After standing over-night in the stoppered weighing bottle, the solution is filtered through a sintered glass filter crucible of known weight, and the precipitate is washed with 100 c.c. of dilute ammonia (5 c.c. of concentrated ammonia per litre). The crucible is then dried for 1 hour at 125° C. to remove water of crystallization and weighed. Dicyanodiamide nitrogen is calculated from the weight of the nickel derivative,* the formula of which is $Ni(C_2N_4H_5O)_2$.

PHOSPHATIC FERTILIZERS

Phosphoric acid in fertilizers is generally determined by precipitation as magnesium ammonium phosphate, igniting the precipitate to constant weight, and weighing the resulting magnesium pyrophosphate. In the case of ammonium phosphate, and mixed fertilizers which are free from calcium phosphate, the phosphoric acid can be precipitated directly as magnesium ammonium phosphate by the procedure of Epperson (see p. 45) or the modified procedure of Hoffman and Lundell (see p. 46). But in the case of rock phosphate, superphosphate, basic slag and mixed fertilizers containing calcium phosphate, direct precipitation of magnesium

* This compound contains 42·96 per cent. of nitrogen.

ammonium phosphate is not possible, because calcium phosphate is soluble in hydrochloric acid, but is reprecipitated by the addition of ammonia. Attempts have been made to determine phosphoric acid in the presence of calcium by the direct precipitation of magnesium ammonium phosphate in the presence of sufficient citric acid to prevent the precipitation of calcium phosphate. The results obtained in this way are, however, not reliable. B. Dyer (article Fertilisers, *Thorpe's Dictionary of Applied Chemistry*, 1922) states that the direct precipitation of magnesium ammonium phosphate in the presence of citric acid is satisfactory for basic slag, provided certain precautions are taken, and sometimes gives good results with mineral phosphates. But the results, when accurate, are due to a balance of positive and negative errors, since the precipitate finally weighed does not consist of pure magnesium pyrophosphate.

It is therefore the usual practice, in analysing fertilizers containing calcium phosphate, to separate the phosphoric acid by precipitation as ammonium phosphomolybdate, to dissolve the yellow precipitate in dilute ammonia and to determine phosphoric acid in the resulting solution as magnesium pyrophosphate. The precipitation of ammonium phosphomolybdate is carried out by adding to the solution to be analysed a large excess of molybdate reagent and heating the mixture to 65°-70° C. W. F. Hillebrand and G. E. F. Lundell (*Applied Inorganic Analysis*, 1929, p. 561) consider that the excess should vary from 10 times the theoretical amount in a solution of phosphoric acid containing nitric acid and ammonium nitrate to a 25-fold excess in ordinary solutions and a 50- to 100-fold excess in the presence of hydrochloric acid, sulphuric acid, hydrofluoric acid and excessive quantities of the ammonium salts of these acids, all of which retard the precipitation. The details of the precipitation are described in the British official methods (see p. 40) and the A.O.A.C. gravimetric method (see p. 44). The difference in the procedures is

partly due to the fact that the molybdate reagent used in the former method is prepared with the addition of ammonium nitrate.

The precipitate of ammonium phosphomolybdate is filtered and washed, and the filtrate is rejected. The washed precipitate is dissolved in dilute ammonia, and the phosphoric acid in the resulting solution is determined as magnesium pyrophosphate. In discussing the determination of phosphoric acid, it is convenient to consider also the determination of magnesium as magnesium pyrophosphate, since both methods consist in precipitating magnesium ammonium phosphate, and the accuracy of each is dependent on the production of a pure precipitate. Miss A. W. Epperson (*J. Amer. Chem. Soc.*, 1928, **50,** pp. 321-333) is of opinion that "probably no determination in analytical chemistry has been the subject of a greater number of conflicting statements than that involving the precipitation of magnesium ammonium phosphate"; and that "the general impression of great difficulty and inaccuracy that has surrounded the determination of magnesium and phosphoric anhydride as magnesium pyrophosphate is largely the effect of the mass of conflicting publications on the subject."

It is generally agreed that the precipitate formed by adding a solution of an orthophosphate to a cold ammoniacal solution of a magnesium salt, or by adding magnesia mixture to a cold ammoniacal solution of an orthophosphate, is not pure magnesium ammonium phosphate; it may be contaminated with trimagnesium phosphate or with monomagnesium ammonium phosphate. In the British official methods the solution obtained by dissolving the ammonium phosphomolybdate precipitate in 2 per cent. ammonia solution is heated to the boiling point, and to the hot solution an excess of magnesia mixture is added, drop by drop, with stirring. After standing for at least 4 hours, the precipitate is filtered and washed with 2 per cent. ammonia solution, ignited and weighed. This procedure is very similar to those of B. Schmitz (*Z. anal. Chem.*, 1906, **45,** pp. 512-522)

and G. Jörgensen (*Analyst*, 1926, **51,** pp. 61-72). In the A.O.A.C. gravimetric method the alkaline solution obtained by dissolving the phosphomolybdate precipitate in dilute ammonia is neutralized with hydrochloric acid. To the neutral solution is added magnesia mixture, drop by drop, with stirring, after which a measured volume of concentrated ammonia is added. The precipitate, after standing, is filtered, washed with dilute ammonia (1 : 9), ignited and weighed.

H. Neubauer (*Z. angew. Chem.*, 1896, **9,** pp. 435-440), after trying different ways of determining magnesium, found that the best results were obtained by acidifying the solution to be analysed, adding a solution of sodium phosphate and then precipitating by the addition of ammonia. Phosphoric acid can be determined in a similar way by adding magnesia mixture to an acid solution of a phosphate and then adding ammonia, whilst stirring, till the solution is alkaline. The addition of the magnesia solution to an acidified solution of the phosphate, with the subsequent addition of ammonia, is recommended by Hillebrand and Lundell (*op. cit.*, pp. 563-564), and is included in Lundell and Hoffman's method of determining phosphoric acid in phosphate rock (see p. 56). This procedure has been thoroughly investigated at the United States Bureau of Standards by Miss Epperson and later by Hoffman and Lundell; and its accuracy has been proved by comparing the results obtained with those obtained by the silver phosphate method.*

* The procedure used by Miss Epperson (*loc. cit.*) is as follows: To the cool phosphate solution, very slightly acidified with nitric acid and containing not more than 0·1 gm. of phosphorus pentoxide, are added 5 c.c. of saturated sodium acetate solution and then an excess of 10 per cent. silver nitrate solution. After heating on a steam bath for about 10 minutes, the precipitate is filtered on a Gooch crucible and thoroughly washed with hot water. The precipitate is dissolved on the filter with dilute nitric acid (1 : 4), and the solution is titrated with standard potassium thiocyanate solution by the Volhard method, using ferric ammonium sulphate as indicator.

The details of the procedures of Miss Epperson and of Hoffman and Lundell for the determination of phosphoric acid are given on pp. 45 and 46. In both procedures measured volumes of concentrated hydrochloric acid and acid magnesia mixture are added to the solution of the phosphate. Concentrated ammonia is then added until the solution is neutral or slightly alkaline, after which a measured volume of concentrated ammonia is added. The advantage of using an acid magnesia mixture is that it does not become contaminated with silica when kept in glass bottles, as an alkaline magnesia mixture does.* Miss Epperson observed that the crystalline precipitate is formed in a slightly coarser and more easily filterable form, with less tendency to adhere to the sides of the beaker, when precipitation is carried out in a cool solution. In Hoffman and Lundell's procedure the solution is therefore cooled in ice-water before the addition of ammonia. After standing for at least 4 hours, the precipitate is filtered and washed with dilute ammonia. The concentration of the ammonia solution used for washing the precipitate is considered later. The washed precipitate is dissolved in dilute hydrochloric acid. To the acid solution is added a little magnesia mixture, and the second precipitate is formed under the same conditions as the first.†

Miss Epperson's work showed that double precipitation, the second precipitation being carried out under exactly defined conditions, is necessary to ensure the quantitative precipitation of magnesium ammonium phosphate. The only errors not remedied by reprecipitation are those due to (i) the addition of the precipitant to an ammoniacal solution, (ii) precipitation in hot solution, (iii) the presence of ammonium sulphate, and (iv) the presence of citric acid.

* The error introduced into gravimetric determinations by using reagents which have been kept for some time in glass bottles is not always realized. This error in the gravimetric determination of calcium is referred to on p. 89.

† The corresponding procedures for the determination of magnesium will be found on pp. 99-101.

The error due to the precipitation in hot solution is slight, and the error caused by sulphate is negligible in any but the most accurate work. The above conclusions apply to the direct precipitation of magnesium ammonium phosphate; but when phosphoric acid is precipitated first as phosphomolybdate and then as magnesium ammonium phosphate, reprecipitation of the latter precipitate is even more necessary. G. E. F. Lundell and J. I. Hoffman (*J. Assoc. Off. Agric. Chem.*, 1924, pp. 184-206) state that results obtained by solution of the phosphomolybdate precipitate and single precipitation with magnesia mixture are only correct by compensating errors, for the latter precipitate always contains molybdenum and is rarely of correct composition. Hence, double precipitation should be carried out in all accurate work.

A Gooch crucible can be used for the filtration, but a filter paper is preferable; Lundell and Hoffman use a Whatman No. 42 in their procedure for the determination of phosphorus pentoxide in phosphate rock (see p. 56). Miss Epperson found that dilute ammonia containing from 2·5 to 10 per cent. by volume of concentrated ammonia could be used for washing the precipitate with equally correct results. In her procedure, described above, dilute ammonia containing from 3 to 5 per cent. by volume of concentrated ammonia is used, and in Hoffman and Lundell's procedure dilute ammonia (1 : 19). Dilute ammonia containing 3 per cent. by volume of concentrated ammonia is dilute ammonia (1 : 32·3), and contains 0·9 per cent. by weight of ammonia. Dilute ammonia containing 5 per cent. by volume of concentrated ammonia is dilute ammonia (1 : 19), and contains 1·55 per cent. by weight of ammonia. In the British official methods the precipitate is washed with 2 per cent. ammonia solution, and in the A.O.A.C. gravimetric method with dilute ammonia (1 : 9). It will thus be seen that the dilute ammonia generally used for washing the precipitate is more concentrated than it need be. The use of a less concentrated solution, besides

saving ammonia, causes less contamination of the atmosphere, a matter of some importance in an agricultural laboratory where determinations of nitrogen and potash are carried out.

If a Gooch crucible is used for the filtration, the crucible containing the precipitate is dried in an oven, and is then placed inside a larger platinum crucible and ignited strongly. If a filter paper is used, the precipitate together with the filter paper is ignited in a platinum or a porcelain crucible. If the precipitate and filter paper are heated too strongly at first, and the latter catches fire, the ignited precipitate will not be pure white owing to the presence of particles of carbon, which are very difficult to oxidize. This difficulty will not occur if the following directions given by G. E. F. Lundell and J. I. Hoffman (*Ind. Eng. Chem.*, 1923, **15,** pp. 44-47) are carefully followed: "The chief precaution in igniting the precipitate lies in first *warming* until any water in the paper or precipitate is given off, then *slowly charring* the paper without actual flaming in an oxidizing atmosphere, then igniting at as low a temperature as possible, and with the lid placed to allow circulation of air, until the carbon has been destroyed and the residue is white, and finally at approximately 1000° C. with the crucible covered to constant weight." J. I. Hoffman and G. E. F. Lundell (*Bur. Stand. J. Res.*, 1930, **5,** pp. 279-293), using an electric muffle furnace, found that in most cases ignition at 1050°-1100° C. for 1 hour yielded residues that did not change in weight on further ignition at the same temperature range. In very few cases was it necessary to ignite for more than 2 hours at 1050°-1100° C. to obtain constant weight. The use of temperatures above 1100° C. is not advisable. Prolonged ignition above 1000° C. causes appreciable losses in weight of platinum crucibles, and it is necessary in accurate work to make allowances for these losses. Heating in a muffle at 1100° C. caused an average loss in weight of about 0·1 mgm. per hour; but the losses in weight of different crucibles vary so greatly that it is

advisable to determine the actual loss in weight of the crucible used. Hoffman and Lundell (*loc. cit.*) observed that constant weight is reached more quickly in the determination of phosphorus than in the determination of magnesium. The longer time required in the determination of magnesium is probably the result of the formation of a compound containing an excess of phosphate, such as $Mg(NH_4)_4(PO_4)_2$. On igniting this compound, magnesium metaphosphate, $Mg(PO_3)_2$, is formed and causes the result to be slightly too high. Longer ignition, or ignition at a higher temperature, probably converts this compound into the pyrophosphate: $2Mg(PO_3)_2 = Mg_2P_2O_7 + P_2O_5$.

Instead of igniting the magnesium ammonium phosphate precipitate, the phosphorus pentoxide or magnesium in it can be determined volumetrically by Handy's method, which is described on p. 47. After removing the free ammonia, the precipitate is dissolved in a measured volume of standard acid, and the excess of acid is titrated with standard alkali in the presence of methyl orange. This method was used by Handy in the routine determination of phosphorus pentoxide, in order to save time and to avoid the chance of obtaining ignited residues which were not white. It is now chiefly used in the determination of small quantities of magnesium.

Another way of determining phosphoric acid is to precipitate ammonium phosphomolybdate under specified conditions, dissolve it in an excess of standard sodium hydroxide, and titrate the excess of alkali with standard acid, using phenolphthalein as indicator. This method was described by H. Pemberton (*J. Amer. Chem. Soc.*, 1893, **15,** pp. 382-395; and 1894, **16,** pp. 278-282) and has been adopted as an official method by the Association of Official Agricultural Chemists; the details are given on p. 48. Pemberton found that when ammonium phosphomolybdate is dissolved in sodium hydroxide, the molecular ratio of P_2O_5 to NaOH is 1 : 46. Taking the atomic weight of phosphorus as 31·02, the molecular weight of phosphorus

pentoxide is 142·04. Therefore, 1 litre of N sodium hydroxide = 142·04/46 = 3·088 gm. of phosphorus pentoxide. Hence, 1 c.c. of N/10 sodium hydroxide = 0·0003088 gm. of phosphorus pentoxide. In order to simplify the calculations, the Association of Official Agricultural Chemists use a solution of sodium or potassium hydroxide of such a strength that 1 c.c. is equivalent to 1 mgm. of phosphorus pentoxide. Hillebrand and Lundell (*op. cit.*, p. 568) prefer to standardize the sodium hydroxide solution against benzoic acid or acid potassium phthalate and to calculate the equivalent weight of phosphorus pentoxide. The ratio 1 : 46 can be used in calculating the results only if the ammonium phosphomolybdate is precipitated under particular conditions. In the absence of sulphates the precipitation is carried out at 45°-50° C. But in the presence of sulphates the precipitation is carried out at 25°-30° C. with continuous shaking or stirring, because sulphates interfere with the precipitation at the higher temperature, but the interference is slight at the lower temperature.

Special methods are required for the analysis of basic slag. The determination of the fineness of grinding is described on p. 49. The British official methods include the determination of the citric-soluble phosphoric acid (see p. 42), but among them there is not any special method for the determination of the total phosphoric acid in basic slag. The A.O.A.C. methods for the analysis of basic slag are given on p. 50. These include gravimetric and volumetric methods for determining the total and the citric-soluble phosphoric acid. The A.O.A.C. method of determining the citric-soluble phosphoric acid is the one agreed to at the International Congress of Applied Chemistry held at Berlin in 1903 (see T. B. Wood, *J. Agric. Sci.*, 1905, **1**, pp. 366-373). The British official method is very similar, but is less definite; the temperature of the 2 per cent. citric acid solution and the rate of shaking are not specified.

Some basic slags, which are produced by the open-hearth process with the addition of fluorspar, contain fluorine and

have a very low citric solubility. Fluorine in such slags can be determined by the method of Warren, Gimingham and Page, which is described on p. 51. The fluorine is determined colorimetrically by the method proposed by G. Steiger (*J. Amer. Chem. Soc.*, 1908, **30,** pp. 219-225) and elaborated by H. E. Merwin (*Amer. J. Sci.*, 1909, **28,** pp. 119-125). It is based on the fact that fluorine has a powerful bleaching effect on the yellow colour which is produced when a solution of a titanium salt is treated with hydrogen peroxide. In applying this method to basic slag, vanadates, which give a brown colour with hydrogen peroxide, must be removed. Vanadates and phosphates are completely precipitated by neutralizing the solution prepared by heating the slag with fusion mixture, and then adding solid silver nitrate. In making the solution neutral, phenolphthalein is used as internal indicator, but it is necessary to use an aqueous solution because alcohol reacts with the mixed solutions of titanium sulphate and hydrogen peroxide.

It is not generally realized that the presence of fluorine interferes with the determination of phosphoric acid. Lundell and Hoffman have devised a method for the determination of phosphoric acid in rock phosphate, which might with advantage be employed for basic slags containing fluorine. This method is given fully on p. 56, and its special features are explained on p. 54.

British Official Methods.—The British official methods for the determination of phosphoric acid in fertilizers (Fertilisers and Feeding Stuffs Regulations, 1932, **11,** iv) are described below. The following reagents are required:

8 *Per Cent. Ammonia Solution.*—1 volume of ammonia solution of specific gravity 0·880 is mixed with 3 volumes of water. This solution is then adjusted by the addition of more concentrated ammonia solution or water, as required, until the specific gravity of the solution is 0·967.

2 *Per Cent. Ammonia Solution.*—1 volume of 8 per cent. ammonia solution is mixed with 3 volumes of water.

*Molybdate Solution.**—125 gm. of molybdic acid and 100 c.c. of water are placed in a litre flask, and the molybdic acid is dissolved by the addition, whilst the flask is shaken, of 300 c.c. of 8 per cent. ammonia solution. 400 gm. of ammonium nitrate are added, the solution is made up to the mark with water and the whole added to 1 litre of nitric acid (sp. gr. 1·19). The solution is maintained at about 35° C. for 24 hours and then filtered.

Magnesia Mixture.—110 gm. of crystallized magnesium chloride and 140 gm. of ammonium chloride are dissolved in 1300 c.c. of water. This solution is mixed with 700 c.c. of 8 per cent. ammonia solution. After allowing the mixture to stand for not less than 3 days, it is filtered.

Ammonium Citrate Solution.—110 gm. of pure citric acid are dissolved in water; the solution is treated with 400 c.c. of 24 per cent. ammonia solution of specific gravity 0·9135, and then diluted to 1 litre.

The procedures are as follows:

Soluble Phosphoric Acid.—20 gm. of the sample are continuously agitated for 30 minutes in a litre flask with 800 c.c. of water at room temperature. The flask is then filled to the mark and, after shaking, the contents are filtered. The phosphoric acid is determined in 50 c.c. of the filtrate by either of the methods described below. In the case of fertilizers in which the proportion of phosphoric acid soluble in water is small, a larger volume of the filtrate should be taken.

Method A.—50 c.c. of the filtrate are boiled with 20 c.c. of concentrated nitric acid, and the phosphoric acid is determined by the molybdate method described below (see p. 43).

Method B.—50 c.c. of the filtrate are boiled with 20 c.c. of concentrated nitric acid, cooled, and the excess of acid neutralized with ammonia. 50 c.c. of the ammonium citrate

* In the Regulations this is termed molybdic acid solution, but molybdate solution is considered to be a more correct description of it.

solution are added, and the mixture heated to the boiling point. Magnesia mixture is then added as described under the molybdate method.

Total Phosphoric Acid (*Method A*).—A weighed portion of the sample is heated with concentrated sulphuric acid until all organic matter is destroyed, and the phosphoric acid is completely in solution. After dilution the solution is filtered, the insoluble matter is thoroughly washed and the filtrate is made up to a definite volume. The phosphoric acid is determined by the molybdate method in an aliquot part of the solution, which is first nearly neutralized and then acidified with nitric acid. The insoluble matter is washed from the filter, re-extracted with acid, and any phosphoric acid present in the solution is added to the main quantity.

Total Phosphoric Acid (*Method B*).—A weighed portion of the sample is incinerated or otherwise treated to destroy organic matter, if present. When direct incineration is employed, the weighed portion of the sample is treated, before being heated, with a nitrate or other oxidizing agent to prevent loss of phosphoric acid during heating or subsequent treatment. The residue (or the weighed portion taken, if no organic matter is present) is dissolved in hydrochloric acid, with the addition, if necessary, of nitric acid. The solution is evaporated to dryness or, if much calcium is present, to a syrupy consistency to render the silica insoluble. The residue is boiled with nitric acid, and, if much iron is present, with hydrochloric acid also. After dilution the solution is filtered, the insoluble matter is thoroughly washed, and the filtrate made up to a definite volume. The phosphoric acid is determined in an aliquot part of the solution by the molybdate method. The insoluble matter is washed from the filter, re-extracted with acid, and any phosphoric acid present in the solution is added to the main quantity.

Citric-Soluble Phosphoric Acid.—5 gm. of the sample are transferred to a stoppered bottle of about 1 litre capacity.

10 gm. of pure crystallized citric acid are dissolved in water, the volume is made up to 500 c.c., and the solution is added to the weighed portion of the sample in the bottle. To lessen the possibility of caking, the portion of the sample in the bottle may be moistened with 5 c.c. of alcohol or methylated spirit before the citric acid solution is added; in that case the volume of the citric acid solution should be 495 c.c. The bottle is at once fitted into a mechanical shaking apparatus, and is continuously shaken for 30 minutes. The solution is then filtered through a large folded filter paper, the whole of the liquid being poured on the filter at once. If not clear, the filtrate is again poured through the same paper. The phosphoric acid in 50 c.c. of the filtrate is determined by the molybdate method.

The Molybdate Method.—To the solution, which should not contain more than 0·4 gm. of phosphorus pentoxide, and preferably from 0·1 to 0·3 gm., an excess of molybdate solution (100-150 c.c. or more) is added, and the vessel containing the solution is placed in a water bath maintained at 70° C. for 15 minutes, or until the solution has reached 70° C. It is then taken out of the bath and, when cool, the solution is filtered; the phosphomolybdate precipitate is washed several times by decantation, and finally on the paper, with 1 per cent. nitric acid solution. The filtrate and washings are mixed with more molybdate solution, and allowed to stand for some hours in a warm place in order to ascertain that the whole of the phosphoric acid has been precipitated. The precipitate is dissolved in cold 2 per cent. ammonia solution; about 100 c.c. of ammonia solution should be used for the solution and washings. The solution is heated to the boiling point, the beaker removed from the burner, and an excess of magnesia mixture (15-20 c.c. or more) is added, drop by drop, with constant stirring. The stirring is continued at intervals as long as the liquid remains very warm. After standing at least 4 hours, with occasional stirring, the precipitate is filtered off, washed with 2 per cent. ammonia solution until free from chloride,

dried and finally weighed as magnesium pyrophosphate.* The filtrate and washings should not exceed 200 c.c., and should be tested by the addition of more magnesia mixture.

A.O.A.C. Gravimetric Method.—The following is an official method of the Association of Official Agricultural Chemists (*Methods of Analysis*, 1935, pp. 19-20). The following reagents are required:

Molybdate Solution.—Dissolve 100 gm. of molybdic acid (MoO_3) in a mixture of 144 c.c. of concentrated ammonia† and 271 c.c. of water. Pour this solution slowly, with constant stirring, into a mixture of 489 c.c. of concentrated nitric acid and 1148 c.c. of water. Keep the final mixture in a warm place for several days or until a portion heated to 40° C. deposits no yellow precipitate. Decant the solution from any sediment and keep it in stoppered bottles.

Magnesia Mixture.—(*a*) Dissolve 55 gm. of crystallized magnesium chloride in water; add 140 gm. of ammonium chloride and 130·5 c.c. of concentrated ammonia, and dilute to 1 litre. Or (*b*) dissolve 55 gm. of crystallized magnesium chloride in water; add 140 gm. of ammonium chloride, and dilute to 870 c.c. Add 15 c.c. of concentrated ammonia to each 100 c.c. of the solution just before using it.‡

Preparation of the Solution for the Analysis.—Treat 2 gm. of the sample by one of the following methods: (*a*) If the sample contains a small quantity of organic matter, dissolve it in 30 c.c. of concentrated nitric acid and 3-5 c.c. of concentrated hydrochloric acid, and boil until organic matter is oxidized. (*b*) If the sample contains much iron or aluminium phosphate, dissolve it in 15-30 c.c. of concentrated hydrochloric acid and 3-10 c.c. of concentrated nitric acid.

* The correct way to ignite the precipitate so as to obtain a pure white residue is described on p. 37. The weight of magnesium pyrophosphate × 0·63787 = the weight of phosphorus pentoxide.

† In *Methods of Analysis*, 1935, a saturated solution of ammonia (sp. gr. 0·88) is termed ammonium hydroxide, but, for reasons given on pp. 4-5, it is here termed concentrated ammonia.

‡ In the latter case ammonia is added just before use to prevent the contamination of the solution by its action on glass.

In either case, cool the solution, dilute to 200 c.c. and filter.

Determination.—Pipette an aliquot part of the solution, corresponding to 0·25 gm., 0·5 gm. or 1 gm., into a 250 c.c. beaker. Add concentrated ammonia in slight excess, and then a few drops of nitric acid until the precipitate barely dissolves. If hydrochloric acid was used as a solvent, add about 15 gm. of crystalline ammonium nitrate, or a solution containing that quantity. To the hot solution add 70 c.c. of the molybdate solution for each 0·1 gm. of phosphorus pentoxide present. Digest at about 65° C. for 1 hour, and ascertain whether the phosphate has been completely precipitated by adding more molybdate solution to the clear supernatant liquid. Filter and wash the precipitate with ammonium nitrate solution (100 gm. per litre). Dissolve the precipitate on the filter with dilute ammonia (1 : 1), and wash the filter with hot water; the volume of the filtrate should not exceed 100 c.c. Neutralize with hydrochloric acid, using litmus paper or bromo-thymol blue as indicator. Cool, and whilst stirring add slowly (about 1 drop per second) from a burette 15 c.c. of magnesia mixture for each 0·1 gm. of phosphorus pentoxide present. After 15 minutes add 12 c.c. of concentrated ammonia and allow to stand until the supernatant liquid is clear (usually about 2 hours). Filter and wash the precipitate with dilute ammonia (1 : 9)* until the filtrate is practically free from chlorides. Dry the precipitate, burn it first at a low temperature, and ignite to constant weight, preferably in an electric furnace, at 950°-1000° C. Cool in a desiccator and weigh as magnesium pyrophosphate.†

Epperson's Method.—The method of determining phosphorus pentoxide described by A. W. Epperson (*J. Amer. Chem. Soc.*, 1928, **50,** pp. 321-333) is as follows: To the

* This solution is more concentrated than it need be. Hoffman and Lundell in their procedure (see p. 46) use dilute ammonia (1 : 19) for washing the precipitate.

† The weight of the ignited precipitate × 0·63787 = the weight of phosphorus pentoxide.

neutral or weakly acid solution of phosphate, containing not more than 0·1 gm. of phosphorus pentoxide, add 5 c.c. of concentrated hydrochloric acid and methyl red indicator. Dilute the solution to 150 c.c. and add 10 c.c. of acid magnesia mixture containing 50 gm. of hydrated magnesium chloride, 100 gm. of ammonium chloride and 5 c.c. of concentrated hydrochloric acid in 1 litre of water. Then add concentrated ammonia slowly, whilst stirring, to neutralization. Stir for about 5 minutes, or until the precipitate is well formed; then add 5 c.c. excess of concentrated ammonia and stir for 10 minutes. Allow to stand for at least 4 hours or preferably over-night. Filter and wash with dilute ammonia containing from 3 to 5 per cent. by volume of concentrated ammonia. Dissolve the precipitate on the filter paper with warm dilute hydrochloric acid (1 : 9). Add methyl red indicator and 1 c.c. of magnesia mixture and finish the precipitation as before, but in a volume of 100-150 c.c. In this precipitation digestion for 4 hours is sufficient. In igniting the precipitate, the wet filter paper with the precipitate should be placed in a weighed platinum crucible, charred without flaming, then ignited at a low temperature (about 500° C.) until the residue is white, and finally at about 1000° C. to constant weight.

Hoffman and Lundell's Method.—The method described by J. I. Hoffman and G. E. F. Lundell (*Bur. Stand. J. Res.*, 1930, **5,** pp. 279-293) is given below. The acid magnesia mixture is prepared as follows: Dissolve 50 gm. of hydrated magnesium chloride and 100 gm. of ammonium chloride in 500 c.c. of water. Make slightly ammoniacal, allow to stand over-night and filter. Acidify the filtrate with concentrated hydrochloric acid, add 5 c.c. in excess and dilute to 1 litre. The determination is carried out as follows: To the neutral or faintly acid solution of orthophosphate, containing 0·2 gm. or less of phosphorus pentoxide, add 5-10 c.c. of concentrated hydrochloric acid. Adjust the volume of the solution to 125-150 c.c. and add 10 c.c. of acid magnesia mixture for each 0·1 gm. of phosphorus pentoxide, but never less than

10 c.c. in the first precipitation. Cool in ice-water, and add concentrated ammonia slowly with vigorous stirring until the solution is slightly alkaline to litmus. Stir for a few minutes, or until the precipitate is well formed, and then add 5-10 c.c. of concentrated ammonia. Allow to stand for at least 4 hours, preferably over-night, filter and wash with dilute ammonia (1 : 19). Dissolve the precipitate in 50 c.c. of warm dilute hydrochloric acid (1 : 9), wash the filter paper thoroughly with hot dilute hydrochloric acid (1 : 99), dilute the solution to 125-150 c.c., add 1-3 c.c. of acid magnesia mixture, cool in ice-water and again precipitate the phosphate by the above procedure. Allow to stand for 4-24 hours, filter on a filter paper of close texture, and wash with dilute ammonia (1 : 19). Transfer the filter paper and precipitate to a weighed platinum crucible, char the filter paper without flaming, burn the carbon at a temperature below 900° C., and finally ignite to constant weight, preferably in a muffle, at 1050°-1100° C.

Handy's Method.—The procedure described by J. O. Handy (*J. Amer. Chem. Soc.*, 1900, **22,** pp. 31-39) for the titration of the magnesium ammonium phosphate precipitate is as follows: After washing the precipitate, allow it to drain. Then open the filter paper and remove as much moisture as possible by placing it on a dry filter paper and, after a few minutes, transferring it to another dry filter paper. To remove the free ammonia, dry the filter paper and precipitate for 45 minutes in the air or for 15 minutes in an air oven at 50°-60° C. Then place the filter paper and precipitate in a dry beaker; add a measured volume of N/10 sulphuric acid and a few drops of methyl orange solution. If the liquid is only faintly acid, add some more N/10 sulphuric acid. Then dilute to about 100 c.c. and titrate with N/10 sodium hydroxide solution to a clear yellow colour. The reaction which takes place is $MgNH_4PO_4 + H_2SO_4 = MgSO_4 + NH_4H_2PO_4$. Therefore, 1 c.c. of N/10 sulphuric acid = 0·003551 gm. of phosphorus pentoxide and 0·002016 gm. of magnesium oxide.

W. F. Hillebrand and G. E. F. Lundell (*Applied Inorganic Analysis*, 1929, p. 516) state that the precipitate can be dried completely, or the drying can be stopped when the filter paper has dried inwards half an inch from the margin, at which time the free ammonia will have been expelled. Gooch crucibles can be used as filters*, and drying is facilitated by washing the precipitate a few times with alcohol. Instead of methyl orange, L. A. Dean and E. Truog (*Ind. Eng. Chem.* (*Anal.*), 1935, **7**, pp. 383-385) use bromo-cresol green and titrate to pH 4·5.

A.O.A.C. Volumetric Method.—For this official method of the Association of Official Agricultural Chemists (*Methods of Analysis*, 1935, pp. 20-21) the following reagents are required:

Molybdate Solution.—Add 5 c.c. of concentrated nitric acid to 100 c.c. of the molybdate solution used in the A.O.A.C. gravimetric method (see p. 44), and filter immediately before use.

Standard Sodium or Potassium Hydroxide Solution.—Dilute 323·81 c.c. of N alkali,† free from carbonate, to 1 litre. 100 c.c. of this solution should neutralize 32·38 c.c. of N acid. 1 c.c. = 1 mgm. of phosphorus pentoxide.

Standard Acid Solution.—Prepare a solution of hydrochloric acid or nitric acid corresponding to the strength, or to half the strength, of the above solution. Standardize it by titration against that solution, using phenolphthalein as indicator.

Phenolphthalein Indicator.—Dissolve 1 gm. of phenolphthalein in 100 c.c. of 95 per cent. (by volume) alcohol.

* Jena glass crucibles with sintered glass diaphragms would probably be more convenient.

† Taking the atomic weight of phosphorus as 31·02, the molecular weight of phosphorus pentoxide is 142·04. Since $P_2O_5 = 46$ NaOH, 142·04 gm. of phosphorus pentoxide = 46 litres of N sodium hydroxide, or 1 gm. of phosphorus pentoxide = 46/142·04 = 0·32385 litres = 323·85 c.c. of N sodium hydroxide solution. This volume differs slightly from that given above; the latter figure is the volume calculated by taking the atomic weight of phosphorus as 31·03.

Preparation of the Solution for the Analysis.—Treat 2 gm. of the sample by one of the methods used in the A.O.A.C. gravimetric method (see p. 44), but preferably the first.

Determination.—(*a*) For percentages of phosphorus pentoxide up to 5, between 5 and 20, and above 20, use aliquot portions corresponding to 0·4 gm., 0·2 gm., and 0·1 gm. respectively of the sample. Add 5-10 c.c. of concentrated nitric acid and then ammonia until the precipitate that forms dissolves slowly on stirring vigorously. Dilute to 75-100 c.c. and adjust to a temperature of 25°-30° C. Add 20-25 c.c. of the molybdate solution, if the percentage is below 5; add 30-35 c.c., if the percentage is between 5 and 20; and if the percentage is over 20, add sufficient to ensure complete precipitation. Place the solution in a shaking or stirring apparatus and shake or stir for 30 minutes at room temperature. Decant *at once* through a filter and wash the precipitate twice by decantation with 25-30 c.c. portions of water, agitating thoroughly and allowing to settle. Then transfer the precipitate to the filter, and wash with cold water until the filtrate from 2 fillings of the filter yields a pink colour on the addition of phenolphthalein and 1 drop of standard alkali. Transfer the precipitate and filter to the beaker or precipitating vessel, dissolve the precipitate in a small excess of standard alkali, add a few drops of phenolphthalein and titrate with the standard acid.

(*b*) If sulphates are absent, proceed as directed above to the point where the solution is diluted to 75-100 c.c. Then heat in a water bath to 45°-50° C., add the ammonium molybdate solution at the rate of 75 c.c. for each 0·1 gm. of phosphorus pentoxide present, and allow the mixture to remain in the bath, stirring occasionally, for 30 minutes. Then decant *at once* through a filter, wash and titrate as before.

Fineness of Grinding.—The British official method of determining the fineness of grinding of basic slag, ground limestone and raw phosphate or phosphate rock (Fertilisers and Feeding Stuffs Regulations, 1932, **11,** ix) is as follows: The sample is mixed, an adequate quantity is dried at

100° C. and 20 gm. of the dried sample are transferred to a British standard test sieve, mesh No. 100, with the lower receiver attached.* The sieve is shaken for 10 minutes with occasional tapping of the sides of the sieve. At the end of 10 minutes, the material which has passed through the sieve is brushed into a suitable vessel and weighed. The receiver is replaced, and the shaking continued for another 10 minutes, after which the sifted material is removed, mixed with the first portion and weighed. The process is repeated until not more than 0·2 per cent. is sifted during 10 minutes. Soft lumps, which will crumble by the application of the fibres of a bristle brush, are broken down after each shaking period, but in such a way that the hard parts of the brush do not come in contact with the sieve. The brush must not be used to brush the particles through the sieve.

A.O.A.C. Methods for Basic Slag.—The following are the official methods of the Association of Official Agricultural Chemists (*Methods of Analysis*, 1935, pp. 35-36) for the analysis of basic slag:

Preparation of the Solution.—Treat 2 gm. of the sample with 15-30 c.c. of concentrated hydrochloric acid and 3-10 c.c. of concentrated nitric acid, or use hydrochloric acid alone. Cool the solution, dilute it to 200 c.c. and pour it on a dry filter. If hydrochloric acid alone has been used, add 3-5 c.c. of concentrated nitric acid to the aliquot portion taken for the analysis, and heat for a few minutes.

Total Phosphoric Acid.—Evaporate an aliquot portion of the solution to dryness on a water bath. Treat the residue with 5 c.c. of concentrated hydrochloric acid and 25 c.c. of hot water; digest in order to complete the solution and filter off the silica. Then proceed as directed under the A.O.A.C. gravimetric method (see p. 44), except that before precipitating with magnesia mixture add 5 c.c. of 5 per cent. sodium acetate solution. Alternatively, determine phosphoric acid in an aliquot portion of the solution by the volumetric method (see p. 48), standardizing the

* See British Standard Specification No. 410, 1931.

solutions against a standard phosphate material of about the same composition as the sample under examination.

Citric-Acid-Soluble Phosphoric Acid.—Weigh 5 gm. of the sample into a 500 c.c. Wagner flask containing 5 c.c. of 95 per cent. alcohol. Make up to the mark with 2 per cent. citric acid solution at 17·5° C. Close the flask with a rubber stopper, place it at once in a rotary shaking apparatus, and shake the flask at the rate of 30-40 r.p.m. for 30 minutes. At the end of this time remove the flask, filter the solution through a dry filter paper and analyse the solution at once.

To 50 c.c. of the clear filtrate in a beaker add 100 c.c. of molybdate solution (see p. 44), and place the beaker in a water bath. When the temperature of the contents reaches 65° C., remove the beaker and cool to room temperature. Filter and wash the yellow precipitate 4 or 5 times with 1 per cent. nitric acid. Dissolve the precipitate in 100 c c. of cold 2 per cent. ammonia solution, and nearly neutralize the solution with hydrochloric acid. Add to the solution, drop by drop, with continuous stirring, 15 c.c. of magnesia mixture and proceed as directed under the A.O.A.C. gravimetric method (see p. 44). Alternatively, determine phosphoric acid in an aliquot portion of the clear solution by the volumetric method (see p. 48).

Fluorine in Basic Slag.—The following method for the determination of fluorine in basic slag is described by R. G. Warren, C. T. Gimingham and H. J. Page (*J. Agric. Sci.*, 1925, **15,** pp. 516-528).

The titanium sulphate solution for the colorimetric determination is prepared as follows: 3·0 gm. of pure potassium titanium fluoride are treated with concentrated sulphuric acid in a platinum crucible and evaporated nearly to dryness. This process is repeated several times, the final evaporation being carried out on a sand bath. The contents of the crucible are dissolved in 200 c.c. of water, to which 50 gm. of concentrated sulphuric acid are added, and the solution is made up to 1 litre. This solution contains 1 gm. of titanium dioxide per litre.

The procedure is as follows: 1 gm. of slag is fused with 6 gm. of a mixture of equal parts of anhydrous sodium carbonate and potassium carbonate in a covered platinum crucible at a dull red heat for 1 hour. If the temperature is too high, manganese is liable to be extracted during the fusion, and the yellow colour which it gives to the final solution is difficult to remove. While still hot, the crucible and contents are placed in a 400 c.c. beaker containing 200 c.c. of water, and the contents are digested for 2-3 hours. The crucible is taken out after all adhering material has been removed, and the solution is concentrated to a volume of 70-100 c.c. When cool, 3-4 gm. of solid ammonium carbonate are added, and the whole is allowed to stand over-night. It is then filtered, and the insoluble residue is washed with 5 per cent. ammonium carbonate solution. The filtrate is evaporated to dryness on a water bath, taken up with 50 c.c. of water, filtered and again evaporated to dryness. 50 c.c. of a saturated aqueous solution of phenolphthalein are then added, and the solution is titrated with 4N sulphuric acid until the colour is nearly discharged. The titration is carried out in a beaker with a clock glass to prevent loss by effervescence, and the solution is boiled after each addition of acid to expel carbon dioxide. When the end-point is near, the solution is transferred to a 100 c.c. beaker and N/10 acid is used to complete the titration.

The solution is then evaporated to a volume of 50 c.c., and to the hot solution 0·6 gm. of powdered silver sulphate is added. The solution is well stirred and allowed to stand over-night in the dark. It is then filtered in the dark into a 100 c.c. graduated flask through two 7 cm. filter papers. The solution can be protected from light by the use of cardboard covers of the correct size surrounding the beaker, flask and funnel, the covers being temporarily removed during the transference of liquid to the funnel. The precipitate is carefully transferred to the filter paper and washed with water; 20 c.c. are sufficient for the washing, which must be done with care, as the precipitate tends to

pass through the filter paper. To the filtrate are added 10 c.c. of 64·8 per cent. sulphuric acid (sp. gr. 1·55) and 3 c.c. of hydrogen peroxide (10 volumes). If no brown colour is produced, 10 c.c. of titanium sulphate solution are added and the solution made up to 100 c.c. The tint of this solution is compared in a colorimeter with that of the standard prepared by adding the same quantities of sulphuric acid, hydrogen peroxide and titanium sulphate to about 50 c.c. of water and making up to 100 c.c. The tint of this solution remains constant for about 10 days. Incandescent gas burners form a suitable source of light for the colorimetric comparisons, but it is necessary to use a glass cell containing alum solution, otherwise the rise in temperature alters the tint and may cause decomposition of the hydrogen peroxide with the formation of bubbles of oxygen. A little methylene blue dissolved in the alum solution greatly facilitates the matching.

From the ratio between the depth of colour of the test solution and that of the standard, the quantity of fluorine can be read off from a curve (on p. 523 of the paper referred to). This curve was constructed by plotting the tint ratio obtained as described above against the weight of fluorine added as calcium fluoride to 1 gm. of fluorine-free basic slag. A. D. Mitchell and A. M. Ward (*Modern Methods in Quantitative Chemical Analysis*, 1932, p. 77) give the following table showing the weights (in grams) of fluorine added to 1 gm. of fluorine-free basic slag and the corresponding tint ratios, the figures being obtained from the curve constructed by Warren, Gimingham and Page.

Fluorine Added.	*Tint Ratio.*	*Fluorine Added.*	*Tint Ratio.*
0·0000	0·909	0·0075	0·557
0·00025	0·885	0·0100	0·510
0·0005	0·865	0·0110	0·496
0·0010	0·825	0·0130	0·472
0·0025	0·738	0·0160	0·435
0·0030	0·715	0·0180	0·415
0·0040	0·668	0·0200	0·398
0·0050	0·630	0·0230	0·387
0·0065	0·585	0·0250	0·374

PHOSPHATE ROCK

K. D. Jacob, W. L. Hill, H. L. Marshall and D. S. Reynolds (*U.S. Dept. Agric. Tech. Bull.*, 1933, No. 364, 89 pp.) have described the composition and distribution of phosphate rock with special reference to the United States,* and have given the references to the methods of analysis which they adopted. The author has pleasure in acknowledging his indebtedness to K. D. Jacob and his colleagues for this information, which has been of great value to him in compiling this section.

The presence of fluorine in phosphate rock causes difficulties in the determination of phosphoric acid and silica. Phosphoric acid is determined by Lundell and Hoffman's routine gravimetric method with single precipitation (see p. 56) or by their umpire gravimetric method with double precipitation (see p. 58). Boric acid is used in preparing the solution for the analysis because it lessens the action of hydrofluoric acid on glass, and prevents the interference of that acid in the determination of phosphoric acid. As mentioned on p. 32, hydrofluoric acid retards the precipitation of ammonium phosphomolybdate; it also tends to cause the formation of a precipitate that is more soluble in the washing solutions. The precipitate of ammonium phosphomolybdate is dissolved in an ammoniacal solution of ammonium citrate, and the filter paper is washed with dilute hydrochloric acid in order to dissolve any iron phosphate that may have been precipitated together with the ammonium phosphomolybdate. The phosphate is precipitated as magnesium ammonium phosphate and weighed as magnesium pyrophosphate, preferably after reprecipitation as in the umpire method. Lundell and Hoffman state that the results obtained by double precipitation should be accurate to $\pm 0{\cdot}05$ per cent.

Total iron, aluminium, calcium and magnesium in

* The results of the analyses are summarized by E. M. Crowther in *Applied Chemistry Reports*, 1933, **18**, pp. 542-543.

phosphate rock can be determined by the methods described by Lundell and Hoffman, the details of which are given on pp. 58-61. In the analyses made by Jacob, Hill, Marshall and Reynolds the sample was brought into solution by the method of Lundell and Hoffman. Calcium was first precipitated as the sulphate in the presence of alcohol, in order to effect its separation from magnesium, and after reprecipitation as the oxalate it was weighed as oxide.* Total aluminium was determined by the routine method (see p. 59), and the results were corrected for the presence of titanium, chromium and, when necessary, vanadium. Total iron was determined by reducing with stannous chloride and titrating with potassium dichromate, using diphenylamine as internal indicator (see p. 328). Magnesium, manganese, titanium, sodium, potassium, chromium, vanadium, copper, zinc and arsenic were determined by the methods briefly outlined by W. L. Hill, H. L. Marshall and K. D. Jacob (*Ind. Eng. Chem.*, 1932, **24,** pp. 1306-1312).

Total silica is determined by Hoffman and Lundell's modification of Berzelius's method, the details of which are given on p. 61. W. L. Hill and K. D. Jacob (*J. Assoc. Off. Agric. Chem.*, 1930, **13,** pp. 112-117) found that this method is applicable to the determination of silica in phosphate rock. Owing to the presence of fluorine, the percentages of silica obtained by the ordinary methods of rock analysis were 0·5-2·7 per cent. lower than the percentages obtained by the modified Berzelius method.

Fluorine can be determined by the method described by D. S. Reynolds, W. H. Ross and K. D. Jacob (*ibid.*, 1928, **11,** pp. 225-236). In this method the sample is heated with 98·5 per cent. sulphuric acid. The reaction flask for digesting the sample with sulphuric acid is made of Pyrex glass and is heated in an electric furnace provided with a rheostat. The evolved silicon tetrafluoride is absorbed in water, and the hydrofluosilicic acid is titrated with standard sodium

* No further details are given in the Bulletin, but the precipitation of calcium as sulphate is briefly described on p. 97.

hydroxide solution. This method accounts for an average of about 93·5 per cent. of the fluorine present in phosphate rock. The results are, therefore, calculated to 100 per cent. recovery of the fluorine on the basis of an actual recovery of 93·5 per cent. The details of the method and figures of the apparatus are given in Hillebrand and Lundell's *Applied Inorganic Analysis*, 1929, pp. 600-603, and H. W. Wiley's *Agricultural Analysis*, ii, 1931, pp. 457-462. The details are not given here because the electrically heated reaction flask, which is designed specially for the purpose, may not be available to the reader. Instead, Reynolds and Jacob's method, which has the great advantage that it can be carried out with the ordinary apparatus used in quantitative analysis, is described on p. 63. The method of Hoffman and Lundell (*Bur. Stand. J. Res.*, 1929, **3,** pp. 581-595) is not suitable for the determination of fluorine in phosphate rock. Reynolds and Jacob found in several experiments that only about half the fluorine was recovered. They therefore devised this special method for phosphate rock, which consists of fusing the sample with sodium carbonate and silica, extracting the fused mass with water and dissolving the insoluble residue in dilute nitric acid. After removing calcium and phosphoric acid, the fluorine is precipitated as lead chlorofluoride, PbClF, in which the chlorine is determined by Volhard's method.

Phosphoric Acid (Routine Method).—The following is the routine gravimetric method described by G. E. F. Lundell and J. I. Hoffman (*J. Assoc. Off. Agric. Chem.*, 1924, **8,** pp. 184-206) for the determination of phosphoric acid in phosphate rock. The following reagents are required:

Molybdate Solution.—Mix 100 gm. of pure molybdic anhydride or 118 gm. of 85 per cent. molybdic acid with 400 c.c. of water, and add 80 c.c. of concentrated ammonia. When solution is complete, filter and pour the solution slowly, with stirring, into a mixture of 400 c.c. of concentrated nitric acid and 600 c.c. of water. Allow to stand for 24 hours, and use the clear supernatant liquid.

Ammonium Nitrate Solution.—Dissolve 50 gm. of ammonium nitrate in 950 c.c. of water.

Ammoniacal Ammonium Citrate Solution.—Dissolve 25 gm. of citric acid in 700 c.c. of water, and add 350 c.c. of concentrated ammonia.

Magnesia Mixture.—Dissolve 50 gm. of crystallized magnesium chloride and 100 gm. of ammonium chloride in 500 c.c. of water. Add concentrated ammonia in slight excess, allow to stand over-night, and filter if a precipitate appears. Make just acid with hydrochloric acid, and dilute to 1 litre.

The solution for the analysis is prepared as follows: Place 2·5 gm. of the sample in a 400 c.c. beaker. Add 30 c.c. of concentrated hydrochloric acid and 10 c.c. of concentrated nitric acid, and boil to a syrupy consistency. Add 1 gm. of boric acid, and dissolve the residue, which should be nearly solid on cooling, in 5 c.c. of concentrated nitric acid and 50 c.c. of water. Heat to the boiling point, cool and filter into a 250 c.c. graduated flask. Wash the filter paper with cold water, and dilute the filtrate to the mark. This procedure eliminates nearly all the silica, but it is necessary to filter soon after the digestion in order to prevent resolution of some of it.

The determination is carried out as follows: Transfer 50 c.c. of the filtrate to a 250 or 300 c.c. beaker, add 15 c.c. of concentrated nitric acid and nearly neutralize with concentrated ammonia. Add 125 c.c. of the molybdate solution and heat to 60° C. for 30 minutes with frequent stirring. Cool by immersing in tap water for 5 minutes. Filter, keeping as much precipitate as possible in the beaker, and wash the precipitate 5 times with 5 per cent. ammonium nitrate solution. Dissolve the precipitate in the beaker in 20 c.c. of ammoniacal ammonium citrate solution. Pour this solution through the filter paper, and collect the filtrate in a 250 c.c. beaker. Wash the beaker and filter paper several times with dilute ammonia (1 : 20), next with a little hot water and finally with hot dilute hydrochloric acid

(1 : 20). The volume at this stage should be 100-150 c.c. Neutralize the solution with hydrochloric acid, using litmus as indicator, and add 1 c.c. of concentrated hydrochloric acid and 10 c.c. of magnesia mixture for each 0·1 gm. of phosphorus pentoxide. Now add concentrated ammonia drop by drop, with constant stirring, until the solution is ammoniacal. Then add 15 c.c. more of concentrated ammonia, and allow to stand for 4 hours or over-night. Transfer the precipitate to a 9 cm. Whatman No. 42 filter paper, and wash it with dilute ammonia (1 : 20). Ignite the precipitate at as low a temperature as possible. Finally ignite at 1000° C. to constant weight.

Phosphoric Acid (Umpire Method).—The following is the umpire gravimetric method of Lundell and Hoffman (*loc. cit.*) for the determination of phosphoric acid in phosphate rock: The molybdate precipitate should be allowed to stand 4 hours or preferably over-night. The precipitation with magnesia mixture should be repeated as follows: After washing the first precipitate several times with dilute ammonia (1 : 20), dissolve it on the filter paper in 25 c.c. of dilute hydrochloric acid (1 : 1), receiving the solution in the beaker containing the bulk of the precipitate. Wash the filter thoroughly with dilute hydrochloric acid (1 : 20), and dilute the solution to 100 c.c. Add 2-3 c.c. of magnesia mixture and then concentrated ammonia slowly until a crystalline precipitate appears, and finally an excess of 3-5 per cent. by volume. Allow to stand for 4-6 hours or preferably over-night. Filter on paper, ignite in platinum and weigh.

Iron, Aluminium and Calcium.—The following are the methods described by G. E. F. Lundell and J. I. Hoffman (*loc. cit.*) for the determination of total ferric oxide, alumina, lime and magnesia in phosphate rock.

Preparation of the Solution.—Transfer 2·5 gm. of the sample to a platinum dish (on account of the fluorides present) and digest for 15-30 minutes on a steam bath with 50 c.c. of dilute hydrochloric acid (1 : 1). Filter off any

insoluble matter, ignite it in platinum, fuse it with a small quantity of sodium carbonate, take up the melt with dilute hydrochloric acid and add the solution to the filtrate. Remove the silica by two evaporations in platinum with hydrochloric acid and intervening filtration. Ignite the silica and treat it with sulphuric acid and hydrofluoric acid. If any residue remains, fuse it with a little sodium carbonate, dissolve the melt in a small quantity of dilute hydrochloric acid, and add the solution to the main filtrate. Dilute the solution to exactly 250 c.c.

Ferric Oxide and Alumina.—The routine method is as follows: Transfer 50 c.c. of the prepared solution to a platinum dish, and evaporate nearly to dryness. When cool, add 15 c.c. of dilute sulphuric acid (1 : 5), and evaporate till nearly all the sulphuric acid has been driven off. When cool, add 75 c.c. of water and 10 c.c. of concentrated hydrochloric acid, and heat until the sulphates are in solution. Filter into an 800 c.c. beaker, and wash the filter paper with dilute hydrochloric acid (1 : 20). Add 100 c.c. of saturated ammonium chloride solution (to prevent the precipitation of calcium phosphate), 3-4 c.c. of 10 per cent. diammonium phosphate solution, 2 drops of methyl orange indicator and macerated paper. (One tablet of S. and S. macerated paper or one 11 cm. No. 40 Whatman filter paper shaken to a pulp furnishes about the proper amount of paper for one determination). Dilute to 500 c.c., make the solution ammoniacal and just restore the pink colour with dilute hydrochloric acid (1 : 5). Heat the solution to the boiling point and add 30 c.c. of 25 per cent. ammonium acetate solution. Boil for 5 minutes and filter immediately on a 12·5 cm. ashless filter paper. Wash with hot 5 per cent. ammonium nitrate solution until 5 c.c. of the washings show only a barely perceptible opalescence with acidified silver nitrate solution; 350-450 c.c. of the washing solution are generally sufficient. Ignite the precipitate and weigh it as $AlPO_4$+$FePO_4$.

Ferric Oxide.—For the determination of iron by titration

with potassium permanganate the following manganese solution is required: (*a*) Dissolve 200 gm. of crystallized manganese sulphate in 1 litre of water. (*b*) Pour 400 c.c. of concentrated sulphuric acid into 1300 c.c. of water and then add 300 c.c. of syrupy phosphoric acid (sp. gr. 1·71). Mix solutions (*a*) and (*b*).

The determination is carried out as follows: Transfer 50 c.c. of the prepared solution to a 250 c.c. beaker. Add 1-2 c.c. of saturated potassium permanganate solution and boil to expel chlorine. If much organic matter is present, add a few crystals of potassium chlorate as the solution is evaporated to dryness, and dissolve the residue in 30 c.c. of dilute hydrochloric acid (1 : 5). Heat the solution to the boiling point, and reduce with a small excess of stannous chloride solution, added drop by drop with stirring. Cool rapidly, add 10 c.c. of saturated mercuric chloride solution and stir vigorously for 1 minute. Pour the mixture into a large porcelain dish containing 20 c.c. of the manganese solution in about 500 c.c. of water which has been faintly tinted with potassium permanganate solution. Titrate with 3N/100 potassium permanganate solution (1 gm. per litre) to the original tint, and correct the result for the titration of the reagents alone. Calculate the percentage of ferric oxide. Calculate the weight of ferric phosphate equivalent to the weight of ferric oxide.* Subtract it from the weight of the precipitated aluminium and ferric phosphates, and calculate the weight of aluminium oxide in the remainder by multiplying by 0·418.

If preferred, the iron can be determined by titration with potassium dichromate solution, using diphenylamine as an internal indicator.†

Calcium.—To 50 c.c. of the prepared solution add sufficient hydrochloric acid to make a total of 10 c.c. of concentrated acid in the solution. Heat to about 50° C., add several drops of methyl orange, neutralize with ammonia, and add

* The weight of ferric oxide × 1·8895 = the weight of ferric phosphate.

† For the details of this method see p. 328.

1 c.c. of dilute ammonia (1:3) in excess. Just acidify the solution with 10 per cent. oxalic acid solution and then add 10 c.c. in excess. Heat the solution to the boiling point, stir vigorously and boil for 1-2 minutes. Add 50 c.c. of saturated ammonium oxalate solution, dilute to 200 c.c. with hot water, boil for 1 minute, and place on a steam bath for 1 hour. Cool to room temperature, filter and wash several times with cold ammonium oxalate-oxalic acid washing solution (2 gm. of ammonium oxalate and 1 gm. of oxalic acid per litre of water). Keep the filtrate for the determination of magnesium.

Ignite the precipitate in platinum, dissolve it in 40 c.c. of dilute hydrochloric acid (1 : 4), and filter if any silica separates. Dilute to 200-250 c.c., add 0·01 gm. of ferric chloride, make slightly ammoniacal and add 10 c.c. of bromine water. Heat just below the boiling point for 15 minutes, and then add 5 c.c. more of bromine water. Allow to stand on a steam bath for ½-1 hour, filter and wash thoroughly with hot ammonia-ammonium chloride washing solution (10 c.c. of concentrated ammonia and 10 gm. of ammonium chloride per litre). Acidify the filtrate with hydrochloric acid, add 25 c.c. of saturated ammonium oxalate solution, heat nearly to the boiling point, and then make ammoniacal, adding about 1 c.c. of concentrated ammonia in excess. Boil for 1-2 minutes, allow to stand on a steam bath for 1 hour. When cool, filter and wash with a cold 0·4 per cent. ammonium oxalate solution. Ignite the precipitate and weigh as calcium oxide.

Magnesium.—This is determined by the customary procedure* after the addition of a few grams of citric acid to keep the iron and aluminium in solution.

Silica.—J. I. Hoffman and G. E. F. Lundell (*Bur. Stand. J. Res.*, 1929, **3**, pp. 581-595) have described the following modification of Berzelius's method of determining silica: Fuse 0·5 gm. of the sample with 5 gm. of sodium or potassium carbonate or a mixture of both. Leach the cooled melt

* See pp. 99-101.

with hot water, and filter when disintegration is complete. Return the insoluble residue to the dish with a jet of hot water and add 50 c.c. of 2 per cent. sodium carbonate solution. Boil for a few minutes, filter and wash thoroughly with hot water. Reserve the residue (1) for the determination. To the combined filtrates, which should have a volume of about 300 c.c., add 1 gm. of zinc oxide dissolved in 20 c.c. of dilute nitric acid (1 : 9). Boil for a minute, filter and wash the residue thoroughly with hot water. Reserve the precipitate (2) for the determination. Add a few drops of methyl red to the filtrate, nearly neutralize with nitric acid, and evaporate to a volume of 200 c.c., taking care that the solution remains alkaline during the evaporation. Finish the neutralization of the concentrated solution by adding dilute nitric acid (1 : 9) until the colour is very faint pink. Transfer 1 gm. of zinc oxide and 2 gm. of ammonium carbonate to a small beaker, add 20 c.c. of water and 2 c.c. of concentrated ammonia, and digest on a steam bath until a clear solution is obtained. Add this ammoniacal solution of zinc carbonate to the neutralized solution, and boil in a covered platinum dish until the smell of ammonia has entirely disappeared. It is usually necessary to boil the solution until the volume has decreased to about 50 c.c. After all the ammonia is expelled, add about 50 c.c. of warm water, stir, allow to stand for a few minutes, filter, and wash the precipitate (3) with cold water.

With a jet of dilute hydrochloric acid (1:19) transfer the insoluble residue (1) and the two zinc precipitates (2 and 3) from the papers to the dish in which the last precipitation was made. Ignite all the papers used in the filtrations and add any residue so obtained to the contents of the dish. Now add 25 c.c. of concentrated hydrochloric acid, and evaporate to dryness on a steam bath. Remove from the steam bath, moisten the residue with 10 c.c. of concentrated hydrochloric acid and then add 100-150 c.c. of hot water. Digest on the steam bath for 15 minutes, filter and wash thoroughly with hot dilute hydrochloric acid (1 : 19), and

then with hot water. Return the filtrate and washings to the dish in which the evaporation was made, add 10 c.c. of concentrated sulphuric acid, and evaporate until fumes are evolved. Allow to cool, add 100-150 c.c. of warm water, digest for a few minutes, filter and wash with hot water. Place the two papers in a weighed platinum crucible, and ignite until a constant weight is obtained. Determine silica by treatment with hydrofluoric acid and sulphuric acid in the usual way.

To recover the silica left in the filtrate after the second dehydration, add about 0·05 gm. of aluminium (in the form of aluminium chloride) and 10-15 gm. of ammonium chloride, and precipitate the aluminium with dilute ammonia. Filter and dissolve the precipitate in 50 c.c. of dilute sulphuric acid (1 : 9), dehydrate, filter and add the small quantity of silica to that obtained before.

Fluorine.—The following method of determining fluorine in phosphate rock and phosphatic slags is described by D. S. Reynolds and K. D. Jacob (*Ind. Eng. Chem.* (*Anal.*), 1931, **3,** pp. 366-370). The following special reagents are required:

Silica.—Any type of anhydrous fluorine-free silica, which is finely ground, may be used.

Acid Zinc Nitrate.—Dissolve 5 gm. of zinc oxide in 100 c.c. of dilute nitric acid (1 : 9).

Ammoniacal Zinc Oxide.—Dissolve 10 gm. of ammonium carbonate in 100 c.c. of water and 10 c.c. of concentrated ammonia. Add 5 gm. of zinc oxide and heat on a steam bath until a clear solution is obtained, adding more ammonia if necessary.

Lead Nitrate.—Recrystallize from a solution containing 2 c.c. of concentrated nitric acid to 100 c.c. of water. Dry the crystals at a temperature below 100° C.

Lead Chlorofluoride Washing Solution.—(*a*) Dissolve 10 gm. of lead nitrate in 200 c.c. of water. (*b*) Dissolve 1 gm. of sodium fluoride in 100 c.c. of water and add 2 c.c. of concentrated hydrochloric acid. Mix solutions (*a*) and (*b*),

and allow the precipitate to settle. Decant the supernatant liquid, and wash the precipitate 4 or 5 times with 200 c.c. of water by decantation. Transfer the precipitate to a flask, add about 1 litre of cold water and allow to stand for 1 hour or longer with occasional stirring. Filter, and keep the filtrate as a washing solution. Save the remainder of the precipitate for the preparation of more solution.

N/10 Silver Nitrate Solution.—It is preferable to standardize this solution by precipitating and weighing the silver as silver chloride.*

N/10 Potassium Thiocyanate Solution.

Ferric Alum Indicator.—Prepare a saturated solution and add a few c.c. of concentrated nitric acid to remove the brown colour.

The procedure is as follows: Fuse 1 gm. of the sample (ground to pass a 100-mesh sieve) in a platinum crucible with 2 gm. of sodium carbonate and 0·5 gm. of silica at a temperature of 900°-950° C. for 1 hour. In the case of slags containing a considerable quantity of silicates, fuse 1 gm. with 5 gm. of sodium carbonate. Digest the fused mass in a beaker with 50-75 c.c. of water on a steam bath over-night. Filter the solution, break up the lumps, add 50 c.c. of 1-2 per cent. sodium carbonate solution and heat on a steam bath for about 15 minutes. Filter into a 400 c.c. beaker, wash the residue 5 or 6 times with hot water, and evaporate the combined filtrates to a volume of 50-75 c.c. This solution A is reserved for further treatment.

By means of a jet of warm water transfer the residue to the beaker, using a total volume of about 50 c.c. Add 3 c.c. of concentrated nitric acid and allow the mixture to stand for ½-1 hour with frequent stirring. Add 50 c.c. of 5 per cent. oxalic acid solution, and precipitate the calcium by adding 10 per cent. sodium carbonate solution drop by drop until the solution is neutral to methyl orange. Boil for 1 minute, stirring continuously to prevent bumping,

* For the preparation and standardization of N/10 silver nitrate solution see p. 330.

and after cooling filter and wash the precipitate 4 or 5 times with cold water. Make the filtrate acid to methyl orange, add 4 c.c. of concentrated nitric acid and 10 c.c. of saturated potassium permanganate solution and warm gently. When the colour disappears, add more potassium permanganate solution until a permanent pink colour or a brown precipitate is formed. Neutralize the solution by adding solid sodium carbonate until frothing ceases, and then add 2 gm. of sodium carbonate in excess. If the precipitate is light coloured, add potassium permanganate solution until it is dark brown. Heat to the boiling point, filter the hot solution through a rapid filtering 12·5 or 15 cm. filter paper and wash the precipitate 4 or 5 times with hot 1 per cent. sodium carbonate solution. Collect the filtrate in the beaker containing solution A, and adjust the volume to about 250 c.c.

Heat the solution to the boiling point, add 25 c.c. of zinc nitrate solution and boil the solution, stirring continuously to prevent bumping. Filter through a rapid filtering 15 cm. filter paper into a 600 c.c. beaker, wash to a volume of about 400 c.c. and discard the precipitate. Neutralize the solution with nitric acid, using methyl red as indicator, and heating almost to the boiling point before the end-point is reached; then add 25 c.c. of ammoniacal zinc oxide solution and evaporate to a volume of 50-75 c.c. Wash down the side of the beaker with warm water, and allow to stand until the precipitate has settled. Then filter and wash the precipitate with cold water to a volume of 250 c.c. Discard the precipitate. Add 2 drops of 0·4 per cent. bromo-phenol blue solution to the solution, make it slightly acid with nitric acid and then just alkaline with sodium hydroxide solution. Then add 3 c.c. of 10 per cent. sodium chloride solution and 2 c.c. of dilute hydrochloric acid (1 : 1). Add 5 gm. of solid lead nitrate and heat on a steam bath. When it has dissolved, add 5 gm. of solid sodium acetate, and heat on a steam bath for half an hour. Allow to stand for 4 hours or over-night, and filter through a filter paper of close

texture. Wash the precipitate, beaker and filter paper onc with cold water, then 4 or 5 times with cold saturate lead chlorofluoride solution, and finally once with cold wate Transfer the precipitate and paper to the beaker in whic the precipitation was made, and add 100 c.c. of dilute nitri acid (1 : 19). Warm until the precipitate is dissolved an then pulp the filter paper; if large quantities of lead sulphat are present, the precipitate will dissolve slowly. Add slight excess of N/10 silver nitrate solution (20 c.c. generally sufficient), heat on a steam bath for half an hou and allow to cool in a dark place. Filter and wash wit cold water. Add 5 c.c. of ferric alum indicator to th filtrate, and determine the excess of silver nitrate solutio by titration with N/10 potassium thiocyanate solutior Subtract the silver in the filtrate from the total added. Th difference is equivalent to the chlorine in the lead chlor fluoride precipitate. 1 c.c. of N/10 silver nitrate solution = 0·0019 gm. of fluorine.

POTASSIC FERTILIZERS

Potassium in fertilizers is determined gravimetricall as potassium chloroplatinate or potassium perchlorat or potassium sodium cobaltinitrite. The chloroplatinat method, being the oldest, will be considered first. Th British official chloroplatinate method and the A.O.A.(methods for the determination of potash are given o pp. 72-77. In the British official method and th A.O.A.C. Method II the determination is carried out wit a solution which is free from the iron group, the alkalin earth metals, organic matter, ammonium salts and sulphate The prepared solution, containing the chlorides of pota sium and sodium, is acidified with hydrochloric acid an evaporated in a porcelain dish with an excess of platinu chloride solution. The quantity added must be more tha sufficient to convert not only the potassium but also th

sodium into chloroplatinate; otherwise some of the sodium remains as chloride, and, being insoluble, is not removed by the alcohol used for washing the precipitate. The mixture is evaporated to a syrupy consistency; it should not be evaporated to dryness, since this will dehydrate the sodium chloroplatinate, and thus make it less soluble in alcohol. Great care should be taken to avoid contamination by ammonia during the evaporation.

When cool, the residue is treated with alcohol, in which the light red sodium chloroplatinate is readily soluble. There has been much discussion about the strength of the alcohol used for this purpose; 80 per cent. (by volume) ethyl alcohol is generally used, although potassium chloroplatinate is more soluble in alcohol of that strength than in absolute alcohol.* Since the use of absolute alcohol may lead to the decomposition of sodium chloroplatinate with the formation of sodium chloride, W. F. Hillebrand and G. E. F. Lundell (*Applied Inorganic Analysis*, New York, 1929, p. 519) recommend 80 per cent. (by volume) alcohol, and explain that its use causes very little error if the volume used for washing the precipitate does not exceed 50-75 c.c., depending on the amount of the precipitate. The solubility of potassium chloroplatinate in 80 per cent. (by volume) ethyl alcohol at 20° C. is about 10 mgm. per 100 c.c., which represents about 1 mgm. of potassium oxide per 50 c.c. But the quantity that is dissolved during an analysis is considerably less than this figure owing to the presence of chloroplatinic acid and sodium chloroplatinate in the solution, and because the alcohol used for washing is by no means saturated after its short contact with the precipitate.

* E. H. Archibald, W. G. Wilcox and B. G. Buckley (*J. Amer. Chem. Soc.*, 1908, **30**, pp. 747-760) found that the solubilities at 20° C., expressed as the weights of potassium chloroplatinate in 100 gm. of solution, are:

70	per cent.	(by weight)	ethyl	alcohol,	0·0128 gm.
80	,,	,,	,,	,,	0·0085 gm.
90	,,	,,	,,	,,	0·0025 gm.
100	,,	,,	,,	,,	0·0009 gm.

The precipitate, after being washed with alcohol, should be uniformly yellow and completely soluble in water. It is either weighed on the filter or, if it is suspected to contain any insoluble matter, it is dissolved on the filter paper in boiling water and the solution is evaporated to dryness in a weighed dish.

In the British official method sulphate, if present, is removed from the solution before the determination. The sulphate is precipitated by cautiously adding barium chloride solution, drop by drop, to the boiling solution of the fertilizer until precipitation is complete. The slight excess of barium is removed by the addition of the least possible excess of sulphuric acid. This is an operation which will cause an error in the result, if it is not very carefully performed, since both barium chloride and sulphuric acid interfere with the determination as described above. The adsorption of alkali sulphates by barium sulphate, which is referred to later, is another source of error. All these difficulties can be avoided by using the Lindo-Gladding method, which is Method I of the Association of Official Agricultural Chemists. In this method, the details of which are given on pp. 74-76, the precipitate, after being washed with 80 per cent. alcohol, is washed with a solution of ammonium chloride saturated with potassium chloroplatinate in order to remove the salts which are insoluble in alcohol, and the ammonium chloride solution is removed by again washing with alcohol. This method is based on the work of D. Lindo (*Chem. News*, 1881, **44**, pp. 129-132), who, finding that potassium chloroplatinate is very slightly soluble in a solution of ammonium chloride, used a solution of ammonium chloride saturated with potassium chloroplatinate for washing impurities out of the precipitate. In this way potassium can be determined in the presence of sulphates, phosphates and magnesium, but calcium interferes with the determination. In the A.O.A.C. method the calcium is precipitated by adding ammonia and ammonium oxalate, and the ammonia is removed by ignition

after the addition of dilute sulphuric acid. The sulphuric acid converts the potassium chloride into sulphate, and thus prevents the loss of potassium which is likely to occur when potassium chloride is subjected to prolonged ignition. The recovery of platinum and alcohol from the residues obtained in the determination of potassium as chloroplatinate is described on p. 77.

Owing to the high price of platinum, and the consequent necessity of recovering the platinum from the residues, the perchlorate method has been adopted in many laboratories.* Apart from the lower cost of the reagent, it has other advantages over the chloroplatinate method. Potassium can be determined as potassium perchlorate in the presence of barium, calcium and magnesium chlorides and sodium phosphate; but sulphates and ammonium salts interfere and must be removed from the solution to be analysed. The British official perchlorate method is given on p. 78, and it is interesting to compare this with the procedure recommended by Hillebrand and Lundell, which is given on p. 80. In both methods the solution prepared for the analysis is treated with an excess of perchloric acid, which is free from chloric acid.† The mixture is heated on a hot plate or a sand bath until white fumes of perchloric acid are evolved. The residue is dissolved in water and again concentrated to the fuming stage after adding more perchloric acid. In the British official method the residue is treated with 95 per cent. alcohol, and the precipitate, after being washed with alcohol saturated with potassium perchlorate, is dried at 100° C. and weighed. In Hillebrand and Lundell's procedure the residue after the second evaporation to dryness with perchloric acid is treated with absolute alcohol containing 0·2 per cent. of perchloric acid. The

* This method has not been adopted by the Association of Official Agricultural Chemists for the determination of potassium in fertilizers.

† There is now no difficulty in obtaining perchloric acid free from this impurity. Tests for chloric acid and other impurities in perchloric acid will be found in "*Analar*" *Standards for Laboratory Chemicals*, 1937, pp. 166-167.

insoluble portion is dissolved in water and again evaporated with perchloric acid. The residue is extracted as before, and the precipitate is washed with alcohol containing 0·2 per cent. of perchloric acid, which is saturated with potassium perchlorate. The washed precipitate is dried at 120°-130° C., heated for a few minutes to 350° C. and weighed.

In the Fertilisers and Feeding Stuffs Regulations two methods are described for the determination of potassium as perchlorate in the presence of sulphate. In Method A the sulphate is precipitated as barium sulphate; the excess of barium is not removed because it does not interfere with the determination. The difficulty of precipitating pure barium sulphate in the presence of alkali sulphates has been already referred to. R. L. Morris (*Analyst*, 1923, **48**, pp. 250-260) has pointed out that the adsorption of potassium by the barium sulphate may cause a serious error in the determination of potassium in sulphate of potash. He found that accurate results can be obtained by extracting the washed and ignited barium sulphate precipitate with boiling water, and adding the second filtrate to the main filtrate. The details of his procedure are given on p. 81.

In Method B the potassium is precipitated as potassium sodium cobaltinitrite by adding freshly prepared cobaltinitrite reagent. The washed precipitate is then dissolved in hydrochloric acid, and the solution is evaporated with an excess of perchloric acid. The perchlorates of sodium and cobalt being readily soluble in alcohol, the potassium perchlorate is obtained in a pure state.* Two modifications of this method have been recently proposed for the determination of potassium in mixed fertilizers containing ammonium salts (see p. 81). In the first of these ammonia is driven off by boiling the solution with sodium hydroxide, and the potassium is determined as perchlorate in the solution obtained by dissolving the cobaltinitrite precipitate

* This method is due to A. H. Bennett, *Analyst*, 1916, **41**, pp. 165-168.

in hydrochloric acid. In the other method potassium and ammonium are precipitated together as cobaltinitrites. The precipitate is dissolved in hydrochloric acid and the solution is kept at 45° C. The ammonium is thus decomposed by the nitrous acid, and the potassium is determined as perchlorate in the resulting solution.

Another way of determining potassium is to weigh the cobaltinitrite precipitate. In Hamid's procedure, which is described on p. 83, the solution to be analysed is evaporated with an excess of cobaltinitrite reagent. The precipitate of potassium sodium cobaltinitrite is very finely divided and tends to pass through the filter paper on continued washing. The object of evaporating to dryness is to increase the particle size and lower the solubility of the precipitate. The residue is treated with 5 per cent. acetic acid and then with cold water, and the precipitate is dried at 100° C. and weighed. The precipitate has the composition $K_2NaCo(NO_2)_6.H_2O$, and contains 17·216 per cent. of potassium. The great advantage of this method is that it can be carried out in the presence of calcium, magnesium, phosphate and sulphate; it thus affords the simplest way of determining potassium in sulphate of potash. The following analyses of pure potassium sulphate made by Hamid will show the degree of accuracy which can be attained by this method.

Number.	*Taken* gm.	*Found* gm.
1	0·2187	0·2182
2	0·1062	0·1068
3	0·1104	0·1106
4	0·0471	0·0463
5	0·0856	0·0859
6	0·1236	0·1232

In a note appended to Hamid's paper Professor F. G. Donnan states that this method has been in constant use at University College, London, since 1920; and adds that

he does not think that the value of this method is sufficiently appreciated in many laboratories.

Potassium can also be determined volumetrically by oxidizing the cobaltinitrite precipitate with standard potassium permanganate solution. This method, being suitable for the determination of small quantities of potassium, is described later in the section dealing with the mineral constituents of feeding stuffs (see p. 187). But it is referred to here because several investigators have found that the cobaltinitrite precipitate obtained from small quantities of potassium salts contains less than the theoretical quantity of potassium. It is difficult to reconcile this conclusion with the accurate results obtained by Hamid and others by the gravimetric cobaltinitrite method. The probable explanation of this discrepancy is afforded by the work of C. S. Piper (*J. Soc. Chem. Ind.*, 1934, **53,** pp. 392-396T), who has shown that the composition of the precipitate varies with the quantity of potassium that is precipitated. The formula of the precipitate is $K_xNa_yCo(NO_2)_6.aq$, where $x+y=3$, but the ratio $x : y$ increases as the weight of potassium that is precipitated increases. The molecular ratio of potassium to sodium in the precipitate is 1·66 : 1·34 when 1 mgm. of potassium oxide is precipitated, but it was calculated that it would be 2 : 1 when 93 mgm. of potassium oxide were precipitated.

The Official Chloroplatinate Method.—The official British methods for the determination of potash in fertilizers as potassium chloroplatinate (Fertilisers and Feeding Stuffs Regulations, 1932, **11,** v) are as follows:

Salts of Potash Free from Sulphates.—A weighed portion of the sample, equivalent to 1·5-2·0 gm. of potassium oxide, is dissolved in water; the solution is filtered, if necessary, and made up to 500 c.c. The potash is determined in 50 c.c. of the solution by the chloroplatinate method as described below.

Salts of Potash containing Sulphates.—A weighed portion of the sample, equivalent to 1·5-2·0 gm. of potassium oxide,

is boiled with 300 c.c. of water to which 20 c.c. of hydrochloric acid have been added. Barium chloride solution is cautiously added, drop by drop, to the boiling solution until the sulphate is completely precipitated. Any slight excess of barium is removed by the addition of the least possible excess of dilute sulphuric acid. When cool, the liquid is made up to 500 c.c. and filtered. 50 c.c. of the filtrate are evaporated to dryness, moistened with concentrated hydrochloric acid, again evaporated to dryness, treated with a little dilute hydrochloric acid and filtered, if necessary. In the filtrate potash is determined by the chloroplatinate method as described below.

Potash in Guanos and Mixed Fertilizers.—10 gm. of the sample are gently incinerated in order to char the organic matter, if present, and are then heated with 10 c.c. of concentrated hydrochloric acid for 10 minutes and finally boiled with 300 c.c. of water. The liquid is filtered, heated to the boiling point and to it is added powdered barium hydroxide until it is slightly alkaline. It is then cooled, made up to 500 c.c. and filtered. 250 c.c. of the filtrate are treated with ammonia solution and an excess of ammonium carbonate and then, whilst boiling, with a little powdered ammonium oxalate; the liquid is cooled, made up to 500 c.c. and filtered. 100 c.c. of the filtrate are evaporated to dryness in a porcelain dish.* If desired, nitric acid may be added during the evaporation after the free ammonia has been driven off. The residue is gently heated over a low flame until all ammonium salts are expelled, the temperature being carefully kept below that of low redness. The residue is moistened with concentrated hydrochloric acid, evaporated to dryness, treated with dilute hydrochloric acid and filtered. The potash is determined in the filtrate by the chloroplatinate method as described below.

The Chloroplatinate Method.—To the solution prepared

* It is advisable to use a weighed dish and to find the weight of the mixed chlorides after ignition, in order to be able to estimate the quantity of platinum chloride solution required.

for the determination by one of the above methods are added a few drops of hydrochloric acid, if none is present, and also 10 c.c. or an excess of platinum chloride solution containing 10 gm. of platinum per 100 c.c.* After evaporating to a syrupy consistency on a water bath, the contents of the basin are allowed to cool and the residue is treated with alcohol of specific gravity 0·864,† and is washed by decantation until the alcohol is colourless. The washings are passed through a weighed or counterpoised filter paper, on which the precipitate is finally collected, washed with alcohol, dried at 100° C. and weighed.‡

A.O.A.C. Methods.—The following are the official methods of the Association of Official Agricultural Chemists (*Methods of Analysis*, 1935, pp. 29-31) for the determination of potash. Method I, which is known as the Lindo-Gladding method, is preferable in the presence of sulphates. For both methods the following reagents are required:

Ammonium Chloride Solution.—Dissolve 100 gm. of ammonium chloride in 500 c.c. of water, add 5-10 gm. of pulverized potassium chloroplatinate and shake at intervals for 6-8 hours. Allow the mixture to settle over-night and filter. The residue may be used for the preparation of a fresh supply.

Platinum Chloride Solution.—A platinum chloride solution containing the equivalent of 1 gm. of metallic platinum (2·1 gm. of chloroplatinic acid) in each 10 c.c. A solution containing 0·2 gm. of platinum in each 10 c.c. is recommended for materials containing less than 15 per cent. of potash.

* 10 c.c. of platinum chloride solution contain 1 gm. of platinum, which is equivalent to 0·60 gm. of sodium chloride, 0·76 gm. of potassium chloride and 0·48 gm. of potassium oxide.

† The temperature at which the specific gravity is determined is not stated in the Regulations. The specific gravity of 80 per cent. (by volume) ethyl alcohol is 0·8638 at 60° F./60° F. (see *The Chemists' Year Book*, 1928, p. 27).

‡ The weight of potassium chloroplatinate × 0·19375 = the weight of potassium oxide.

Method I is carried out as follows:

Mixed Fertilizers.—Place 2·5 gm. of the sample in a 250 c.c. volumetric flask, and add 125 c.c. of water and 50 c.c. of saturated ammonium oxalate solution. Boil for 30 minutes and add a slight excess of ammonia. After cooling, dilute to 250 c.c. and filter through a dry filter. Evaporate nearly to dryness 25 or 50 c.c. of the filtrate, to which has been added sufficient N sodium hydroxide solution (1-2 c.c.) to prevent the formation of free phosphoric acid during ignition. Add 1 c.c. of dilute sulphuric acid (1 : 1) and 6-8 granules of granulated sugar. Evaporate to dryness and ignite at a dull red heat until the residue is white. Dissolve the residue in hot water, using at least 20 c.c. for each 0·1 gm. of potassium oxide. Add a few drops of hydrochloric acid and then an excess of platinum chloride solution, and continue as directed under the Determination.

Muriate of Potash, Sulphate of Potash and Kainit.—Dissolve 2·5 gm. of the sample in water and dilute to 250 c.c. Acidify 50 c.c. of the solution with a few drops of concentrated hydrochloric acid. In the case of muriate of potash, add 10 c.c. of platinum chloride solution and continue as directed under the Determination. In the case of sulphate of potash and kainit, use 15 c.c. of platinum chloride solution and 25 c.c. portions of the ammonium chloride solution.

Organic Substances.—In order to determine the total potash in organic substances, such as cottonseed meal, saturate 10 gm. of the sample with concentrated sulphuric acid and ignite in a muffle at a low red heat. Add a little hydrochloric acid, warm slightly in order to loosen the mass, and transfer it to a 500 c.c. volumetric flask. Add ammonia and saturated ammonium oxalate solution. Cool, dilute to 500 c.c. and filter. Treat the filtrate as directed under Mixed Fertilizers.

Plant Ash.—Boil 10 gm. of the sample with 300 c.c. of water for 30 minutes, and add to the hot solution a slight excess of ammonia and then sufficient saturated ammonium

oxalate solution to precipitate the calcium present. Cool, dilute to 500 c.c. and filter. Treat the filtrate as directed under Mixed Fertilizers.

Determination.—Evaporate on a water bath to a thick paste, avoiding exposure to ammonia. Treat the residue with about 6 c.c. of 80 per cent. alcohol and add 0·6 c.c. of concentrated hydrochloric acid. Filter on a Gooch crucible, and wash the precipitate thoroughly with 80 per cent. alcohol, both by decantation and on the filter, continuing the washing after the filtrate is colourless. Then wash 5 or 6 times with 10 c.c. portions of the ammonium chloride solution to remove impurities from the precipitate. Wash again thoroughly with 80 per cent. alcohol, dry the precipitate for 30 minutes at 100° C. and weigh. The precipitate should be completely soluble in water.

Method II.—Dissolve 2·5 gm. of the sample in water and dilute to 250 c.c. without the addition of ammonia and ammonium oxalate. Dilute 25 c.c. of the prepared solution, or 50 c.c. if less than 10 per cent. of potash is present, to 150 c.c. Heat it to 100° C. and add drop by drop, with constant stirring, a slight excess of 10 per cent. barium chloride solution. Without filtering, add in the same way an excess of saturated barium hydroxide solution. Filter whilst hot and wash until the precipitate is free from chlorides. Add to the filtrate 1 c.c. of concentrated ammonia, and then saturated ammonium carbonate solution until the excess of barium is precipitated. Heat and add, in fine powder, 0·5 gm. of pure oxalic acid or 0·75 gm. of ammonium oxalate. Filter, wash the insoluble matter free from chlorides, evaporate the filtrate to dryness in a platinum dish, and ignite carefully over a free flame below a red heat until all volatile matter is driven off. Digest the residue with hot water, and filter through a small filter paper. Acidify the solution with a few drops of hydrochloric acid, add an excess of platinum chloride solution and evaporate on a water bath to a thick paste. Treat the residue repeatedly with 80 per cent. alcohol, decanting it through a weighed

Gooch crucible or other form of filter. Transfer the precipitate to the filter, wash it thoroughly with 80 per cent. alcohol, dry it for 30 minutes at 100° C. and weigh. If there is an appearance of foreign matter in the precipitate, wash it, as in Method I, with several portions, each of 10 c.c., of the ammonium chloride solution.

Recovery of Platinum.—G. J. Hough (*Ind. Eng. Chem. (Anal.)*, 1929, **1**, p. 162) has described the following method which is used in the Bureau of Chemistry and Soils for the recovery of platinum and alcohol from the residues obtained in the determination of potassium as chloroplatinate. About 1 gm. of solid ammonium chloride is added to each 300 c.c. of filtrate. The mixture is well stirred, allowed to stand until clear and filtered. The addition of the ammonium chloride should be made soon after the determination, because the 80 per cent. alcoholic solution on standing undergoes slow decomposition with formation of platinum black and acetone. The filtered alcoholic solution can be kept until a suitable quantity for distillation has been obtained. If it is distilled to a quarter of its original volume, the distillate consists of 83-85 per cent. alcohol which is suitable for use again.

The ammonium chloroplatinate, together with the potassium chloroplatinate obtained in the potassium determinations, is dissolved in hot water, and a few c.c. of dilute hydrochloric acid (1 : 2) are added. The solution is heated and magnesium powder is added gradually until a slight excess is present. After the chloroplatinates are decomposed, concentrated hydrochloric acid is added to dissolve the excess of magnesium. The platinum black is filtered off, washed, transferred to a porcelain dish and dissolved in aqua regia. The solution is evaporated to a thick paste, which is treated 3 times with hydrochloric acid and evaporated nearly to dryness after each addition. The residue is dissolved in hot water and a few drops of hydrochloric acid. The solution is filtered and diluted to the required volume.

The Official Perchlorate Methods.—The British official methods for the determination of potash in fertilizers as potassium perchlorate (Fertilisers and Feeding Stuffs Regulations, 1932, **11,** v) are as follows:

Salts of Potash Free from Sulphates.—A weighed portion of the sample, equivalent to 1·5-2·0 gm. of potassium oxide, is dissolved in water. The solution is filtered, if necessary, and made up to 500 c.c. The potash is determined in 50 c.c. of the solution by the perchlorate method as described below.

Salts of Potash containing Sulphates (Method A).—A weighed portion of the sample, equivalent to 1·5-2·0 gm. of potassium oxide, is boiled with 300 c.c. of water, to which have been added 20 c.c. of hydrochloric acid. Barium chloride solution is cautiously added, drop by drop, to the boiling solution until the sulphate is completely precipitated. The liquid is cooled, made up to 500 c.c. and filtered. 50 c.c. of the filtrate are evaporated to dryness, moistened with concentrated hydrochloric acid, again evaporated to dryness, treated with a little dilute hydrochloric acid and filtered, if necessary. In this solution potash is determined by the perchlorate method as described below.

Salts of Potash containing Sulphates (Method B).—For this method the following cobaltinitrite reagent is required: 50 gm. of cobalt nitrate and 300 gm. of sodium nitrite are dissolved in water. 25 c.c. of glacial acetic acid are added, and the solution is diluted to 1 litre. The solution is filtered after standing 24 hours, and is then ready for use. It must be kept in the dark.

A weighed portion of the sample, equivalent to 1·5-2·0 gm. of potassium oxide, is boiled with 300 c.c. of water, cooled, made up to 500 c.c. and filtered. To 50 c.c. of the filtrate are added 30 c.c. of the cobaltinitrite reagent. The mixture is stirred, allowed to stand for not less than 2 hours and then filtered. The precipitate is washed with water containing a small amount of the cobaltinitrite reagent; it is dissolved in hot dilute hydrochloric acid, the solution being filtered into

a small porcelain dish and evaporated to dryness. The residue is dissolved in water, and the potash is determined by the perchlorate method as described below.

Potash in Guanos and Mixed Fertilizers (Method A).—10 gm. of the sample are gently incinerated in order to char organic matter, if present, and are then heated with 10 c.c. of concentrated hydrochloric acid for 10 minutes and finally boiled with 300 c.c. of water. The liquid is filtered, heated to the boiling point and to it is added powdered barium hydroxide until it is slightly alkaline. It is then cooled, made up to 500 c.c. and filtered. 250 c.c. of the filtrate are treated with ammonia solution and excess of ammonium carbonate and then, whilst boiling, with a little powdered ammonium oxalate; the liquid is cooled, made up to 500 c.c. and filtered. 100 c.c. of the filtrate are evaporated to dryness in a porcelain dish; if desired, nitric acid may be added during the evaporation after the free ammonia has been driven off. The residue is gently heated over a low flame until all ammonium salts are expelled, the temperature being kept below that of low redness. The residue is moistened with concentrated hydrochloric acid, evaporated to dryness, treated with dilute hydrochloric acid and filtered. The potash is determined in the filtrate by the perchlorate method as described below.

Potash in Guanos and Mixed Fertilizers (Method B).—10 gm. of the sample are gently incinerated in order to char organic matter, if present, and are then heated with 10 c.c. of concentrated hydrochloric acid for 10 minutes and finally boiled with 300 c.c. of water. The liquid is filtered into a 500 c.c. flask; the residue is washed, and the solution is made up to the mark. 50 c.c. of this solution are boiled with a solution of ammonium nitrite to expel ammonium salts, if present, and are evaporated to dryness. The residue is dissolved in water containing a little hydrochloric acid, and sufficient sodium citrate is added to prevent the precipitation of phosphates. The solution is then mixed with 30 c.c. of the cobaltinitrite reagent used in Method B for the

analysis of salts of potash containing sulphates, and the precipitate is treated as there described.

The Perchlorate Method.—The solution prepared for the determination by one of the methods described above is placed in a small glass or porcelain basin, and to it are added 7 c.c. of 20 per cent. solution of perchloric acid (sp. gr. 1·125), which is free from chloric acid. The basin is placed on a hot plate or a sand bath, and the contents are evaporated until white fumes are copiously evolved. The residue is dissolved in hot water, a few drops of perchloric acid solution are added and the whole concentrated again to the fuming stage. When cool, the residue in the basin is thoroughly stirred with 20 c.c. of 95-96 per cent. (by volume) alcohol. The precipitate is allowed to settle and the clear liquid is poured through a weighed or counterpoised filter paper, or through a Gooch crucible, care being taken to drain the precipitate as completely as possible from the liquid before adding the washing solution. The precipitate is washed by decantation with 95-96 per cent. (by volume) alcohol saturated with potassium perchlorate at the temperature at which it is used. The precipitate is finally collected on the filter paper or Gooch crucible, on which it is dried at 100° C. and weighed.*

The Perchlorate Method.—The details of the procedure described by W. F. Hillebrand and G. E. F. Lundell (*Applied Inorganic Analysis*, 1929, p. 534) are as follows: Two washing solutions are required. The first consists of absolute alcohol containing 0·2 per cent. of perchloric acid; and the second is a solution prepared shortly before use by shaking the first solution with solid potassium perchlorate for 5-10 minutes at room temperature, allowing to settle and pouring off the clear solution. To the concentrated solution for analysis, containing not more than 1 gm. of the chlorides of potassium and sodium and no sulphates, add 1-2 c.c. of 60 per cent. perchloric acid, and evaporate to dryness on a hot

* The weight of potassium perchlorate $\times 0{\cdot}33991$ = the weight of potassium oxide.

plate. Add 10 c.c. of water, again add 1-2 c.c. of perchloric acid, and evaporate to dryness at a temperature not exceeding 350° C. Extract the residue with 20 c.c. of the first washing solution, and allow to stand for some minutes. Filter the clear solution through a Gooch crucible, and wash the crucible and residue once or twice with the first washing solution. Dissolve the residue in water, add 1-2 c.c. of perchloric acid, evaporate to dryness and extract as before. Filter through a dried and weighed Gooch crucible, and transfer the residue to the filter by means of not more than 100 c.c. of the second washing solution. Dry the crucible and contents at 120°-130° C.; finally heat for a few minutes at 350° C. and weigh.

Removal of Sulphate.—R. L. Morris (*Analyst*, 1923, **48,** pp. 250-260) found that the presence of even small amounts of sulphates will cause the results of the perchlorate method to be untrustworthy, and has described the following procedure for the precipitation of sulphate without loss of alkalies. The solution should be neutral, and should contain 1-1·5 per cent. of total potassium and sodium salts; if much sulphate is present, it should be diluted so that not much more than 2 gm. of barium sulphate are obtained from each 100 c.c. of solution. The volume of the solution should be 40-80 c.c. Hydrochloric acid, in amount equal to a ninth of the volume of the solution, is added; the solution is heated to the boiling point and the sulphate precipitated by adding 10 per cent. barium chloride solution in slight excess. After settling, the precipitate is filtered off and well washed; it is ignited wet, then boiled for 10 minutes with 50 c.c. of water containing a few drops of hydrochloric acid. The precipitate is again filtered off, washed once only, and is rejected. The filtrate is added to the main filtrate, and is reserved for the determination of potassium.

Potassium in the Presence of Ammonium Salts.—The following are two recent methods for the determination of potassium in mixed fertilizers, depending on the precipitation of the potassium as cobaltinitrite and its conversion into

perchlorate. For both methods the following reagent is required:

Cobaltinitrite Reagent.—Add to 220 gm. of sodium nitrite in 500 c.c. of water a solution of 132 gm. of cobalt nitrate in 200 c.c. of water and 200 c.c. of glacial acetic acid. Draw air through the mixture for an hour to remove oxides of nitrogen. Allow to stand for a day and filter off any precipitate. Store in a dark place, and use within 4 weeks.

(i) The method described by K. Scharrer and H. Schornstein (*Landw. Versuchs-Stat.*, 1935, **123,** pp. 227-234) is as follows: Boil 10 gm. of mixed fertilizer in a beaker with 300 c.c. of water and 10 c.c. of concentrated hydrochloric acid for 15 minutes. Cool, filter and make up to the mark in a 500 c.c. flask. Pipette 50 c.c. of this solution into a 300 c.c. beaker and add enough 20 per cent. sodium hydroxide solution to make the solution strongly alkaline. Boil the solution for about half an hour to drive off ammonia, and add water to make up to 100 c.c. Neutralize the hot solution by adding concentrated hydrochloric acid, drop by drop, until the precipitate is dissolved and the reaction is slightly acid. Evaporate twice to dryness on a water bath. Take up the residue with a little dilute hydrochloric acid and filter off the silica. To the filtrate add 10-20 c.c. of cobaltinitrite reagent, and shake frequently to encourage precipitation, which should be complete after half an hour. Filter off the precipitate in a Jena glass filter and wash with 10 per cent. acetic acid. Transfer most of the precipitate with a little water into a Schott evaporating dish, 11 cm. in diameter. Using a Witt suction vessel, dissolve the precipitate in the filter crucible in hot dilute hydrochloric acid (2 : 1), and collect the filtrate in the dish. Three lots of about 20 c.c. of acid are necessary; allow the acid to act for 2-3 minutes before sucking it off. Wash the crucible twice with 20 c.c. of warm water. Add to the dish perchloric acid and complete the determination in the usual way.

(ii) C. Krügel and A. Retter (*Z. anal. Chem.*, 1934, **96,** pp. 314-319) have described the following method of deter-

mining potassium in mixed fertilizers: Boil 10 gm. of the sample with 300 c.c. of water and 10 c.c. of concentrated hydrochloric acid for 15 minutes in a 500 c.c. flask. Allow to cool, make up to the mark and filter. To 50 c.c. of the filtrate, or 25 c.c. if the potash content is more than 10 per cent., in a beaker add an excess (25 c.c.) of the cobaltinitrite reagent. Stir for 30 minutes and filter through a Jena glass filter crucible. First pour the supernatant liquid, which should be deep red, through the crucible and wash the precipitate 5 times by decantation with 10 per cent. acetic acid. Wash the crucible twice with acetic acid, and transfer the precipitate in the crucible back to the beaker with cold dilute hydrochloric acid (d. 1·07), made by diluting 35 c.c. of concentrated acid (d. 1·19) to 100 c.c. Place the crucible on a small suction flask and dissolve the precipitate in the crucible by repeatedly pouring on hot dilute hydrochloric acid (d. 1·07). Pour the liquid in the suction flask into the beaker, and rinse the flask with cold dilute hydrochloric acid up to a volume of 70-100 c.c. Cover the beaker with a clock glass, place it in a water bath at 45°-50° C., and allow it to stand there until solution of the precipitate is complete. Allow to simmer for 15 minutes and transfer to a dish. Add perchloric acid, evaporate and proceed in the usual way.

The Gravimetric Cobaltinitrite Method.—The following is the procedure described by M. A. Hamid (*Analyst*, 1926, **51,** pp. 450-453). The cobaltinitrite reagent* is prepared as follows: 220 gm. of sodium nitrite are dissolved in 400 c.c. of water, and 113 gm. of cobalt acetate are dissolved in 300 c.c. of water and 100 c.c. of glacial acetic acid. The two solutions are carefully mixed and gently warmed. The nitrogen peroxide is removed by evacuation with a pump, and the solution is left for a day. A slight yellow precipitate, due to the presence of potassium as an impurity in the sodium nitrite, is removed by filtration; the filtered solution is made up to 1 litre and kept in a dark place.

* This is the reagent used by R. H. Adie and T. B. Wood, *J. Chem. Soc. Trans.*, 1900, **77,** pp. 1076-1080.

The solution in which potassium is to be determined is acidified with a drop or two of glacial acetic acid, then treated with an excess of cobaltinitrite reagent and evaporated to dryness on a water bath. The residue is washed by decantation with 5 per cent. acetic acid and then with cold water, and the precipitate is transferred to a Gooch crucible,* which has been previously dried at 100° C. and weighed. The precipitate is washed with cold water till free from acid and dried at 100° C. to constant weight.†

LIME AND LIMESTONE

The British official methods for the determination of calcium oxide or hydroxide in samples of lime, and calcium carbonate in chalk and limestone, are given on p. 92. The first Regulation contains the full details of the sucrose extraction method. The result gives the total alkalinity obtained by titration with standard acid, whether it is due to calcium hydroxide or magnesium hydroxide or both; and does not include the lime in readily hydrolysable silicates, which occur in many lime products, such as the freshly burnt lime from certain paper works. For these products, however, the method is sufficiently accurate, since their neutralizing powers on acid soils are more closely related to their total alkalinities than to their contents of calcium oxide. The second Regulation states the analyses which are required, without prescribing the procedures, and gives directions for stating the results of the analyses. It will be noticed that magnesium is not mentioned in the Regulation, though its presence may be indicated by the quantity of carbon dioxide being more than sufficient to combine with the calcium oxide or by the very slow effervescence when the sample is treated with hydrochloric acid.

In the methods adopted by the Association of Official

* A Jena glass filter crucible (porosity 4) is preferable.

† The weight of the precipitate × 0·20738 = the weight of potassium oxide.

Agricultural Chemists for the analysis of liming materials, the determinations of silica, iron, aluminium, etc., calcium and magnesium are carried out by the methods which are adopted for the determination of these constituents in soils after fusion with sodium carbonate. Since soils contain much more silica, and less calcium and magnesium, than limes and limestones, the quantities of the reagents stated are not always suitable. The methods of analysis here given are mostly those described by W. F. Hillebrand and G. E. F. Lundell in *Applied Inorganic Analysis*, New York, 1929. These methods are intended for the analysis of limestones, but are applicable to the analysis of quicklime, slaked lime and lime products. Other methods, most of which were published since that book appeared, are also given.

It may be thought that some of these methods aim at a degree of accuracy beyond that which is necessary in routine analyses. It should, however, be realized that though the analysis of limestone is included in most text-books of inorganic analysis, and was probably carried out by the reader in his student days, yet the accurate determinations of calcium and magnesium in limes and limestones are problems of great difficulty. It should be remembered that it is better to attempt to reach a higher degree of accuracy than to remain satisfied with a routine method which may give concordant results, but may at the same time be subject to a considerable constant error. Though the determination of magnesium may not be a part of the routine analysis, it is often advisable to carry it out as a check on the accuracy of the other determinations, if the carbon dioxide is more than sufficient to combine with the calcium oxide or if the sum of the percentages of silica, sesquioxides, calcium oxide and carbon dioxide is far below 100. But whether magnesium is determined or not, its probable presence should be borne in mind during the course of the analysis, since its presence causes great difficulties in the determination of calcium, and may lead to erroneous results unless special precautions are taken.

The preparation of the solution for the analysis does not present any difficulty. It is the usual practice to dissolve a weighed quantity of the sample in dilute hydrochloric acid, but, in the case of certain limestones, solution in acid after strong ignition is recommended by Hillebrand and Lundell (see p. 93). The ignited residue easily dissolves in dilute hydrochloric acid; and by keeping the volume of acid as small as possible time is saved in the evaporation to dryness before the separation of silica.

Calcium can be precipitated as calcium oxalate in an acetic acid solution at pH 4·0 in the presence of iron, aluminium, titanium and phosphorus, as explained on p. 94; it can thus be determined in the filtrate from the silica. Calcium is, however, generally precipitated in an alkaline solution after separating the iron, aluminium, etc., by precipitation with ammonia, in the presence of sufficient ammonium chloride to prevent the precipitation of magnesium. The procedure recommended by Hillebrand and Lundell is given on p. 95. They state (*op. cit.*, p. 394) that the precipitation of aluminium hydroxide begins at about pH 4, and is complete between pH 6·5 and 7·5. Solution of the precipitate takes place in more alkaline solutions, and becomes appreciable at pH 10. Precipitation of the alkaline earth metals as carbonates, owing to the absorption of carbon dioxide during boiling, is not likely if the pH of the solution is 6·5-7·5, but will take place if ammonia solution containing carbonate is used. If the precipitate is washed with water, some of it passes through the filter paper in colloidal solution. To prevent this taking place, the precipitate is washed with hot 2 per cent. ammonium chloride solution.*

Hillebrand and Lundell recommend double precipitation, but R. C. Wiley (*Soil Sci.*, 1930, **29**, pp. 339-347) has shown that a single precipitation is sufficient if all the ammonia is removed from the solution by boiling, or if the solution is

* The Association of Official Agricultural Chemists (*Methods of Analysis*, 1935, p. 6) use for this purpose a hot 2·5 per cent. ammonium nitrate solution.

cooled in a stream of carbon dioxide, after the precipitation. The precipitate of sesquioxides will be contaminated with calcium carbonate if impure ammonia solution containing carbonate. is used for the precipitation or if the alkaline solution is left exposed to the air for some time before filtration. If the concentration of calcium is high, an excess of ammonia may result in the precipitation of calcium as the hydroxide.

Calcium is precipitated as oxalate in the filtrate after the separation of iron, aluminium, titanium and phosphorus. Hillebrand and Lundell (*op. cit.*, pp. 493-495) state that the precipitation is never quite complete, and that the first precipitate is seldom pure. Alkali metals and barium are removed by double precipitation, but strontium is precipitated as oxalate under the same conditions as calcium. Magnesium oxalate is occluded by calcium oxalate, the degree of occlusion being proportional to the concentration of the undissociated magnesium oxalate in the solution during the precipitation. It is also slowly adsorbed by calcium oxalate, the amount depending on the time which elapses before filtration. The concentration of undissociated magnesium oxalate is increased by adding a large excess of ammonium oxalate; it is diminished by increasing the concentration of ammonium salts or by diluting the solution. Unfortunately, these remedies tend to hinder the precipitation of calcium. Hence, in ordinary analyses the separation of calcium and magnesium is not complete.

The calcium which is not precipitated as oxalate is completely precipitated as calcium phosphate in the subsequent determination of magnesium as magnesium ammonium phosphate. This calcium can be recovered by dissolving the precipitate in dilute sulphuric acid, adding alcohol and filtering off the precipitated calcium sulphate. The calcium is determined by dissolving the precipitate in dilute hydrochloric acid and precipitating the calcium as oxalate. Since the complete separation of calcium from magnesium is not possible, Hillebrand and Lundell recommend that the calcium

is precipitated under such conditions that the calcium oxalate is not contaminated with magnesium oxalate, and that the calcium which is not precipitated as oxalate is recovered from the magnesium pyrophosphate. If calcium occurs in the presence of much magnesium, the precipitation of calcium as oxalate can be omitted. Hillebrand and Lundell's procedure for the precipitation of calcium as oxalate is described on p. 96; and their procedure for the recovery of calcium from magnesium pyrophosphate is given on p. 97.

Calcium oxalate is more soluble in hot water than in cold; it is less soluble in a dilute solution of ammonium oxalate, which is used by Hillebrand and Lundell for washing the precipitate. Dilute ammonia is sometimes used for this purpose; but H. Bassett (*J. Chem. Soc.*, 1934, pp. 1270-1275) found that calcium oxalate is more soluble in 2N ammonia than in water, this being an example of the normal enhanced solubility caused by electrolytes not yielding an ion in common with the substance dissolved. Bassett considers that the ideal washing liquid for calcium oxalate precipitates is distilled water saturated with calcium oxalate. This is prepared by placing a few grams of pure calcium oxalate on a filter paper and allowing distilled water to flow through it. The saturated solution thus obtained can be used whether the precipitate is to be subsequently weighed as oxide or sulphate or titrated with potassium permanganate. It is preferable to the 0·1 per cent. ammonium oxalate solution which is used by Hillebrand and Lundell for the gravimetric determinations only. After washing the precipitate, the determination of calcium is completed by one of the following procedures: (i) The precipitate is ignited to constant weight and weighed as calcium oxide; (ii) the ignited precipitate is either heated with a mixture of ammonium chloride and ammonium sulphate or is treated with dilute sulphuric acid and weighed as calcium sulphate; (iii) the precipitate is dissolved in dilute sulphuric acid and the solution is titrated with standard potassium permanganate solution.

Hillebrand and Lundell by devoting about 1½ pages of

their book to weighing as calcium oxide and 6 lines only to weighing as calcium sulphate show their preference for the former method. Bassett (*loc. cit.*), however, found that it was practically impossible to obtain the correct weight of the ignited lime owing to the absorption of moisture, even when using a very rapid air-damped balance. The ignited lime appeared to absorb moisture whilst cooling in a desiccator containing phosphorus pentoxide, probably owing to the adsorbed film on the glass surface being renewed every time the desiccator was opened. Ignition in a platinum crucible over a good Bunsen burner appeared to be sufficient to convert calcium carbonate or oxalate completely into oxide. The sole effect of subsequent ignition over a blow pipe seemed to be to make the surface of the lime slightly less sensitive to moisture. Bassett found that it was necessary to apply a correction for moisture absorption; 0·0005 gm. was deducted from small weights of calcium oxide and 0·0007 gm. from larger weights up to 0·1 gm.

Owing to the error due to absorption of moisture by calcium oxide a more accurate result will be obtained, when calcium is weighed as the oxide, if no attempt is made to prevent the solution of calcium oxalate during the washing. This is probably one reason why washing with dilute ammonia or water has been found to give satisfactory results. Another source of error, which also causes a high result, may arise from the use of ammonia solutions which have stood for a long time in bottles. Such solutions may contain considerable amounts of silica, a small proportion of which is carried down by calcium oxalate. In such circumstances, as much as 1 per cent. of what is regarded as calcium oxide after ignition may consist of silica. In view of all these sources of error, Bassett is of opinion that the estimation of calcium as calcium oxide should be discarded.

Weighing the calcium as sulphate is preferable to weighing as oxide, since calcium sulphate is much less hygroscopic than calcium oxide. Calcium oxalate can be converted into the sulphate by the method of Willis and MacIntire (see

p. 98), in which the ignited precipitate is heated with a mixture of ammonium chloride and ammonium sulphate until the excess of ammonium salts is volatilized.* The conversion into sulphate can be carried out more conveniently in this way than by treatment with dilute sulphuric acid, which may result in loss by spurting. The procedure used by King and Crossley in determining calcium in coal ash is given on the same page.

The determination of calcium by titration with potassium permanganate is described on p. 99. This method is sometimes regarded as unreliable, because the results so obtained are lower than those obtained by weighing the calcium as oxide. But these discrepancies can be explained as being due to the solubility of the calcium oxalate in the water used for washing the precipitate and to the absorption of moisture by the calcium oxide during the weighing. Bassett (*loc. cit.*) has shown that if the precipitate is washed with water saturated with calcium oxalate, instead of with pure water, accurate results can be obtained by titration with permanganate. In fact, he thinks that it is the most accurate and convenient method of determining calcium.

Magnesium is determined in the filtrate from the calcium determination after the removal of ammonium salts. This is best carried out by treatment with nitric acid, as described on p. 100. The conditions under which pure magnesium ammonium phosphate is precipitated, both from phosphate and magnesium solutions, have been fully discussed on pp. 33-36. It will therefore be sufficient to state here that the precipitation of magnesium as magnesium ammonium phosphate is carried out by acidifying the solution with hydrochloric acid, adding an excess of diammonium hydrogen phosphate solution and then ammonia until the solution is neutral, after which more ammonia is added. Double precipitation is necessary to ensure the quantitative precipitation of magnesium, but any calcium phosphate

* This method is referred to in *Methods of Analysis*, 1935, p. 7, but the details are not given.

which is precipitated together with the magnesium ammonium phosphate is not removed by reprecipitation; this must be allowed for as explained on p. 97. The procedures recommended by Epperson and by Hoffman and Lundell are given on p. 100. They differ slightly in the quantities of the reagents used, but more particularly in the temperatures at which precipitation takes place, the solution being cooled in ice-water in the latter procedure.* The precipitate can be ignited and weighed as magnesium pyrophosphate; or, if the highest accuracy is not required, it can be titrated with standard acid, as described on p. 47.

Two methods for the determination of carbon dioxide are given on pp. 101-105. These are Collins's and Amos's methods. The first is largely used in British laboratories, because it is very rapid; a determination can be completed in about 10 minutes, and the result is sufficiently accurate for most purposes. Amos's method was intended for the determination of carbon dioxide in soils. It was subsequently found that inaccurate results were obtained owing to decomposition of the organic matter when soil is boiled with dilute hydrochloric acid; but with limestones accurate results can be obtained. In this method two Reiset towers are necessary. Until recently these were expensive, because the metal plates were made of silver or platinum; but Reiset towers fitted with perforated porcelain discs can now be obtained at a much lower price.

A determination which is not mentioned in the British Regulations, and is not included in the A.O.A.C. methods, is the loss on ignition. In limestone this is equal to the sum of the percentages of carbon dioxide and organic matter; in slaked lime it is equal to the percentage of combined water; and in lime, which has been exposed to the air and

* A. D. Mitchell and A. M. Ward, *Modern Methods in Quantitative Chemical Analysis*, London, 1932, pp. 102-103, give a procedure for the determination of magnesium, which is partly that of Epperson and partly that of Hoffman and Lundell. The author thinks that it is better to give the two procedures separately, so that the reader can see for himself the differences between them.

consists of a mixture of oxide, hydroxide and carbonate, it is equal to the sum of the percentages of combined water and carbon dioxide. This determination, which is easily carried out by heating a known weight of the sample to 1100°-1200° C. for 10-15 minutes, is often very useful as a check on the accuracy of the determinations of the other constituents, when the sum of their percentages does not approximate to 100.

British Official Methods.—The British official method for the determination of calcium oxide in "burnt lime" and calcium hydroxide (Fertilisers and Feeding Stuffs Regulations, 1932, **11,** vii) is as follows: A portion of the sample is rapidly ground and passed through a sieve with apertures about 0·2 mm. square,* and from this prepared portion quantities for the determination are weighed. About 5 gm., accurately weighed, are transferred to a stoppered bottle of about 1 litre capacity, and are moistened with 10 c.c. of alcohol (neutral to phenolphthalein) to lessen the possibility of caking. 490 c.c. of 10 per cent. cane sugar solution (made neutral to phenolphthalein) are added, and the bottle is at once fitted into a shaking apparatus and shaken for not less than 4 hours. The solution is then filtered through a dry filter paper into a dry vessel, and 50 c.c. of the filtrate are titrated with N/2 hydrochloric acid, using phenolphthalein as indicator. The method gives the total amount of lime present in the sample as quicklime and slaked lime, and the result may be calculated to either calcium oxide or calcium hydroxide.†

The British official methods for the determination of calcium carbonate in ground chalk, ground limestone and dried carbonate of lime (Fertilisers and Feeding Stuffs Regulations, 1932, **11,** viii) are as follows:

* The I.M.M. sieve No. 70, which is used in mechanical analysis of soils for separating the coarse sand (particles 2·0·2 mm. in diameter), has square holes of the dimensions stated.

† 1 c.c. of N/2 hydrochloric acid = 0·01402 gm. of calcium oxide or 0·018524 gm. of calcium hydroxide.

(*a*) A weighed quantity of the finely ground sample is treated with dilute hydrochloric acid until effervescence ceases. The solution is filtered and the insoluble matter is washed. The calcium in the filtrate is precipitated as oxalate and weighed as oxide. Steps are taken to exclude from the oxalate precipitate iron, aluminium and other interfering substances.

(*b*) The amount of carbon dioxide evolved on treatment of a weighed quantity of the finely ground sample with dilute acid is determined in a suitable apparatus.

(*c*) The amount of calcium oxide determined under (*a*) is calculated to calcium carbonate, provided that the necessary equivalent of carbon dioxide is present in the sample. If less than the equivalent of carbon dioxide is present in the sample, the quantity of carbon dioxide determined under (*b*) is calculated to calcium carbonate.*

Preparation of the Solution for the Analysis.—The following methods are recommended by W. F. Hillebrand and G. E. F. Lundell (*Applied Inorganic Analysis*, 1929, pp. 826-829) for the preparation of the solution for the analysis of limestones and the determination of silica in them: 1 gm. of the limestone is moistened with water and dissolved in dilute hydrochloric acid in a covered beaker till all effervescence ceases. It is necessary to heat gently if the effervescence is so weak as to indicate a magnesian limestone. The solution is filtered through a 7 cm. filter paper, and the residue is washed with hot water. The wet filter paper with its contents is ignited in a platinum crucible and weighed. Solution in acid after strong ignition is the best method to employ if the silica does not exceed 15 per cent. and the oxides of iron, aluminium and titanium together do not exceed 6 per cent. Limestones, in which the above percentages are not exceeded, can be converted in 10-15 minutes into a product that is entirely soluble in hydrochloric acid,

* The weight of calcium oxide $\times 1{\cdot}78477$ = the equivalent weight of calcium carbonate. The weight of carbon dioxide $\times 2{\cdot}27426$ = the equivalent weight of calcium carbonate.

provided the sample is first ground to a very fine powder. 1 gm. of the powder is heated in a covered platinum crucible at 1100°-1200° C. for 10-15 minutes. Magnesian limestones must be heated with caution, because their temperatures of decomposition are far below those of limestones. The ignited material is transferred to a beaker or evaporating dish, and moistened with water. The crucible is then cleaned with dilute hydrochloric acid (1 : 1), and the contents are poured into the beaker or dish. By heating gently, and pressing cautiously with a glass rod, the lumps are disintegrated and pass into solution. A certain amount of silica may remain undissolved in a flocculent state. When all gritty particles have disappeared, the liquid, if it is in a beaker, is washed into a dish and evaporated to dryness. If the solution was made in a dish, the volume need not exceed a few c.c., and evaporation takes a very short time.

When dry, or nearly so, the dish is placed in an air oven and heated at a temperature not exceeding 110° C. for 1 hour. The residue is treated with 10 c.c. of concentrated hydrochloric acid and 100 c.c. of water. The dish is covered and placed on a water bath for 10 minutes. The silica is then separated by filtration, thoroughly washed with dilute hydrochloric acid (1 : 99) and then twice with hot water. In accurate work the filtrate is again evaporated to dryness, and the residue is extracted for a few minutes with half the quantity of acid and water used before. The solution is then filtered through a second smaller filter paper, and the residue is washed first with cold dilute hydrochloric acid (1 : 99) and then with hot water. The two filter papers and their contents are slowly dried, ignited and weighed.

Precipitation of Calcium in Presence of Iron, Aluminium, etc.—H. D. Chapman (*Soil Sci.*, 1928, **26**, pp. 479-486) showed that it is possible to precipitate calcium as calcium oxalate in an acetic acid solution at pH 4·0 in the presence of iron, aluminium, titanium, manganese, magnesium and phosphoric acid. To the solution is added enough ammonium chloride to ensure the presence of at least 6 gm., the amount

of free hydrochloric acid being roughly calculated. To the solution are also added 1 gm. of oxalic acid in solution, 10 c.c. of 1·76N acetic acid and 10 drops of 0·04 per cent. bromo-cresol green solution. After diluting the solution to 150-200 c.c. and heating it nearly to the boiling point, dilute ammonia is slowly added until the colour changes from yellowish green to the first pure green. The solution is then boiled gently for 5-10 minutes and allowed to stand on a steam bath for at least 3 hours to ensure complete precipitation. In determining calcium by titration with potassium permanganate, the precipitate should be washed as few times as possible with distilled water, since calcium oxalate is slightly soluble in water. Using a 9 cm. S. and S. No. 589 filter paper, Chapman found that, for all ordinary amounts of calcium oxalate, rinsing the beaker 3 times and washing the precipitate 5 times removes all excess of oxalate.

Iron, Aluminium, Titanium and Phosphorus.—The procedure recommended by W. F. Hillebrand and G. E. F. Lundell (*Applied Inorganic Analysis*, 1929, p. 831) for the precipitation of iron, aluminium, titanium and phosphorus is as follows: The filtrate from the silica is boiled in a beaker, after adding a few drops of bromine water or 2-3 drops of concentrated nitric acid, until all bromine or chlorine is driven off. Then hydrochloric acid is added, if not already present, in amount sufficient to prevent the precipitation of magnesium when the solution is made alkaline with ammonia. A few drops of methyl red solution are added, and the solution (100-200 c.c. in volume) is heated just to the boiling point. To the solution are added first concentrated ammonia and afterwards dilute ammonia until the colour of the solution changes to a distinct yellow. The solution is then boiled for 1 or 2 minutes, allowed to settle for a short time and filtered. The precipitate is at once washed 2 or 3 times with hot 2 per cent. ammonium chloride solution, and is then redissolved in hot hydrochloric acid. After boiling the solution, precipitation is carried out exactly as described above. Macerated filter paper is added before

the last precipitation, if the amount of the oxides is large. The second precipitate is washed with the ammonium chloride solution, ignited in a platinum crucible and weighed.

Calcium.—The precipitation of calcium as calcium oxalate is carried out in the whole, or an aliquot part, of the filtrate from the determination of iron, aluminium, titanium and phosphorus. The procedure described by W. F. Hillebrand and G. E. F. Lundell (*op. cit.*, pp. 497-499) is as follows: The solution for the determination should have a volume of 100-400 c.c. and should not contain more than the equivalent of 1 mgm. of calcium oxide per c.c. The solution is made slightly ammoniacal, if it is not so; it is heated to the boiling point, and, whilst stirring, sufficient hot 4 per cent. ammonium oxalate solution is added to precipitate all the calcium and leave an excess of 1 gm. per 100 c.c. of solution. The solution is boiled for 1-2 minutes, heated on a steam bath for half an hour and allowed to cool for 2 hours. The solution is then filtered, and the precipitate is washed with 5 portions, 10 c.c. each, of a cold neutral 0·1 per cent. ammonium oxalate solution. The filtrate and washings are reserved, if a determination of magnesium or a recovery of unprecipitated calcium is to be made. The washed precipitate is then dissolved in 50 c.c. of dilute hydrochloric acid (1 : 4), and the solution is diluted. Oxalic acid is added, and the calcium is reprecipitated. The precipitate is filtered and washed as before, if the calcium is to be weighed as calcium oxide or calcium sulphate; but if the precipitate is to be dissolved in dilute sulphuric acid and the resulting solution titrated with potassium permanganate solution, the precipitate is finally washed with warm water until all ammonium oxalate is removed.* The filtrate and washings from the second precipitation are mixed with those from the first precipitation.

* Since calcium oxalate is slightly soluble in warm water, excessive washing should be avoided. More accurate results will be obtained by washing the precipitate with a saturated solution of calcium oxalate (see p. 88).

Recovery of Calcium from Magnesium Pyrophosphate.—The method devised by Hillebrand (W. F. Hillebrand and G. E. F. Lundell, *op. cit.*, pp. 487-488 and 513-514) for the recovery from the magnesium pyrophosphate precipitate of the small amount of calcium which escapes precipitation as oxalate is as follows: Magnesium is precipitated as magnesium ammonium phosphate in the filtrate from the calcium determination. The calcium which has not been precipitated as oxalate is then precipitated as calcium phosphate. The weighed precipitate of magnesium pyrophosphate is transferred to a small beaker, and is dissolved in a little dilute sulphuric acid, avoiding an excess of more than 0·5 c.c. If the precipitate dissolves with difficulty, it is boiled with nitric acid and evaporated until copious fumes of sulphuric acid appear. 100 c.c. of 75 per cent. (by volume) alcohol for every 0·3 gm. of pyrophosphate originally present are added, and the mixture is allowed to stand for several hours or preferably over-night. The precipitated calcium sulphate is filtered off, washed with 75 per cent. alcohol, dried and dissolved in dilute hydrochloric acid. In this solution calcium is precipitated as oxalate and weighed as oxide. If magnesium is not to be determined, the precipitated phosphate can be dissolved in acid without igniting and weighing it. If both calcium and magnesium are to be determined, the weight of calcium oxide recovered from the ignited and weighed magnesium pyrophosphate is added to that already found; and its equivalent as tricalcium orthophosphate is subtracted from the weight of the ignited magnesium pyrophosphate in order to obtain a more correct result for magnesium.

Weighing as Calcium Oxide.—The following are the details given by W. F. Hillebrand and G. E. F. Lundell (*op. cit.*, pp. 499-500): The wet precipitate in the filter paper is placed in a weighed platinum crucible with a tightly fitting lid, and is heated so as to char, but not inflame, the filter paper. When the filter paper is charred, the flame is increased; and when the carbon is burnt, the crucible is placed upright,

covered and heated at about 1200° C. for 5 minutes. If a blast flame is adjusted so that the gases do not enter the crucible, ignition for 5 minutes is sufficient for the quantities of lime generally dealt with; instead a Teclu or a Meker burner can be used. The lid is removed for a moment to allow the carbon dioxide to escape. The covered crucible is placed in a desiccator containing concentrated sulphuric acid or phosphorus pentoxide (but not calcium chloride), and is weighed as soon as it is cool. If left long in the desiccator, the calcium oxide may gain appreciably in weight. The first weighing is a preliminary one; it is followed by a short ignition and a second weighing, in which the weights are placed on the scale pan and the rider is quickly adjusted.

Weighing as Calcium Sulphate.—In the method due to L. G. Willis and W. H. MacIntire (*J. Ind. Eng. Chem.*, 1917, **9,** pp. 1114-1116) the precipitate of calcium oxalate is ignited in a crucible until the filter paper is completely incinerated. For each 0·2 gm. of calcium carbonate is added enough of a finely ground mixture of equal parts of ammonium sulphate and ammonium chloride to ensure an excess of about 0·3 gm. of the sulphate, then thoroughly mixed with the lime in the crucible by means of a small glass rod. The volatilization of the ammonium salts is carried out by inserting the crucible in a circular opening cut in a piece of asbestos board, so that the upper half of the crucible is above the asbestos board, and directing the flame of a small Bunsen burner across the crucible, so that the side of the crucible nearest the flame is strongly heated. If duplicates differ by a few tenths of a milligram, the ignited calcium sulphate may be moistened with a few drops of dilute sulphuric acid, evaporated to dryness and again ignited at a dull red heat.

The procedure adopted by J. G. King and H. E. Crossley (*Dept. Sci. Ind. Res., Fuel Res., Survey Paper*, 1933, **28,** p. 10) for converting the oxide into the sulphate is as follows: To the calcium oxide, when cool, 5 drops of water are cautiously added, followed by 3 drops of concentrated

sulphuric acid. In each case the drops should be allowed to trickle down the inside of the tilted crucible and should not be dropped directly on to the contents. The crucible is heated very gently at first, and when the contents are dry it is ignited at a dull red heat. After cooling, the addition of concentrated sulphuric acid followed by gentle ignition is repeated. The residue is weighed as calcium sulphate after cooling in a desiccator.*

Titration With Potassium Permanganate Solution.—The procedure recommended by W. F. Hillebrand and G. E. F. Lundell (*Applied Inorganic Analysis*, 1929, pp. 501-502) is as follows: The filter paper with the precipitate of calcium oxalate, which has been finally washed with water, is removed from the funnel and spread out on the inside of a beaker containing 100 c.c. of warm dilute sulphuric acid (1 : 10). The precipitate is washed off the filter paper with a little warm water. The solution is gently heated until decomposition is complete, and is then titrated at about 70° C. with a standard solution of potassium permanganate. As soon as a permanent end-point is obtained, the filter paper is dropped into the solution, the sides of the beaker are washed with water and the titration is quickly completed. The potassium permanganate solution should be standardized with pure sodium oxalate or with Iceland spar of known purity which has been dissolved in hydrochloric acid and treated in the same way as the sample.†

Magnesium.—Magnesium is precipitated as magnesium ammonium phosphate in the whole, or an aliquot part, of the filtrate from the calcium determination after removing ammonium salts either by acidifying the solution, evaporating to dryness and igniting, or by treatment with nitric acid. The latter method is recommended by Hillebrand and Lundell (*op. cit.*, p. 119) as being more satisfactory and convenient.

* The weight of calcium sulphate $\times 0{\cdot}41193$ = the equivalent weight of calcium oxide.

† 1 c.c. N/10 potassium permanganate solution = 0·002804 gm. of calcium oxide.

It is carried out as follows: The solution is made slightly acid with hydrochloric acid, and is concentrated in a beaker. To it are added about 3 gm. of nitric acid for every gram of ammonium chloride; an excess does no harm. The beaker is covered and warmed until vigorous evolution of gas ceases. The solution is then evaporated to dryness.

The following is the procedure for the determination of magnesium described by A. W. Epperson (*J. Amer. Chem. Soc.*, 1928, **50,** pp. 321-333): To the neutral or slightly acid solution, containing not more than 0·1 gm. of magnesium oxide, add 5 c.c. of concentrated hydrochloric acid and a few drops of methyl red as indicator. Dilute the solution to 150 c.c., and add 10 c.c. of saturated diammonium hydrogen phosphate solution; then add concentrated ammonia slowly, whilst stirring, until the solution is neutral. Stir for about 5 minutes, add 5 c.c. more of concentrated ammonia and stir for 10 minutes. Allow to stand for at least 4 hours or preferably over-night; then filter and wash the precipitate with dilute ammonia containing 3-5 per cent. by volume of concentrated ammonia. Dissolve the precipitate on the filter with warm dilute hydrochloric acid (1 : 9). Add methyl red and about 1 c.c. of diammonium hydrogen phosphate solution, and carry out the precipitation as before, but in a volume of 100-150 c.c. Allow to stand for 4 hours, which is sufficient for this precipitation. Wash the precipitate, and place the wet filter paper and precipitate in a weighed platinum crucible. Char the filter paper without flaming, then ignite at a low temperature (about 500° C.) with the lid partly open until the residue is white, and finally at about 1000° C. to constant weight.

Epperson's procedure has been modified by J. I. Hoffman and G. E. F. Lundell (*Bur. Stand. J. Res.*, 1930, **5,** pp. 279-293). Their modified procedure is as follows: To the nearly neutral solution, containing 0·1 gm. or less of magnesium oxide, add 5-10 c.c. of concentrated hydrochloric acid, and adjust the volume of the solution to 125-150 c.c. Cool the solution in ice-water, add 10 c.c. of freshly prepared

diammonium hydrogen phosphate solution (25 gm. of the salt per 100 c.c. of water) and then concentrated ammonia slowly and with stirring until the solution is alkaline to litmus. Stir for a few minutes, then add 5-10 c.c. of concentrated ammonia, and stir for a few minutes more. Allow the solution to stand for at least 4 hours or preferably over-night; then filter on a filter paper of close texture and wash with dilute ammonia (1 : 19). Dissolve the precipitate in 50 c.c. of warm dilute hydrochloric acid (1 : 9) and wash the filter paper thoroughly with dilute hydrochloric acid (1 : 99). Dilute the solution to 125-150 c.c., add 0·5-1 c.c. of the solution of diammonium hydrogen phosphate, cool in ice-water, and again precipitate the magnesium by the above procedure. Allow to stand for 4-24 hours, filter on a filter paper of close texture, and wash with dilute ammonia (1 : 19) as before. Transfer the precipitate and filter paper to a weighed platinum crucible, char the filter paper without flaming, burn the carbon at a temperature below 900° C. and finally ignite to constant weight, preferably in a muffle at 1100° C.*

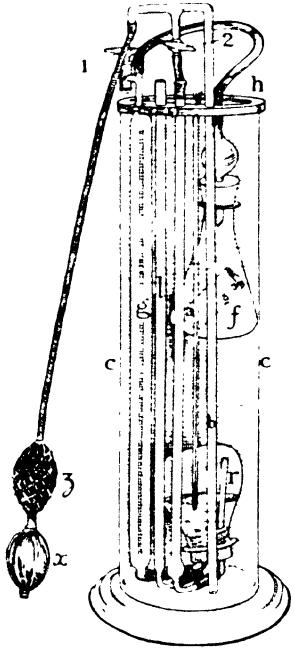


FIG. 1.

Collins's Calcimeter.— The calcimeter designed by S. H. Collins (*J. Soc. Chem. Ind.*, 1906, **25,** pp. 518-522) is shown in Fig. 1. It consists of a water jacket in which the various working parts are enclosed, and is provided with a measuring tube graduated from 0 to 50 c.c. in tenths of a c.c. and a levelling tube with enamelled back for easy reading. The

* The weight of the precipitate × 0·36213 = the equivalent weight of magnesium oxide.

level is adjusted by means of a special air flask and bellows, thus doing away with the usual raising and lowering of a separate reservoir. The same bellows are used for agitating the water to keep a uniform temperature. The gas is generated in a flask outside the jacket, but provision is made for immersing the flask in the water on the completion of the reaction. Breakage of parts is guarded against by a screen of stout copper gauze which is not shown in the figure. The limestone is weighed and placed in flask *f*, and the acid (1 of hydrochloric acid to 3 of water, or weaker) in tube *a*. Taps 1 and 2 are opened, and the flask is closed with the rubber stopper, plunged under water in the cooler *c*, and kept there by means of a hook *h*, engaging with a projection on the collar. Tap 2 is closed, and air is blown through the tube *b*, so as to stir up the water in the glass cylinder *c*, and thus obtain a uniform temperature. Tap 2 is opened, and the rubber bulb *z* is gently squeezed until the water in the burette *g* stands at zero, tap 1 being closed with the other hand. On releasing the bulb *z*, the level in tube *l* sinks to the bottom. The flask *f* is then removed from the water, the acid in the graduated celluloid tube is tilted out, and the flask shaken violently and returned to the water. After adjusting the level by squeezing the bulb *z* and closing tap 2, the volume of gas, *g*, is measured. The process of stirring, shaking and adjusting the level should be continued until a constant reading has been obtained. The temperature of the water is taken with a thermometer graduated from 0° to 40° C. in fifths of a degree. If the temperature should vary by one or two fifths of a degree during the experiment, a correction can easily be made; a rise of one mark on the thermometer means that one mark on the burette must be subtracted, since 136 c.c. of air expand 0·1 c.c. for a rise of 0·2° C. With 14 c.c. of acid the amount of air left in a 150 c.c. flask is 136 c.c. The amount of acid used need never vary from this figure sufficiently to make any appreciable error in the correction used. It is important to see that the reservoir *r* is

half full of water, and that bulb z is half full of air, before levelling.

If the volume of the flask is 150 c.c., the volume of the acid used in c.c. is a, the temperature in degrees Centigrade is t, and the height of the barometer in mm. is B, the weight in mgm. of 1 c.c. of the gas can be found from the following table. The two following examples will explain the use of the table: If $a=20$, $t=22$, $B=750$ and $g=10$, 1 c.c. of gas weighs $1{\cdot}96-0{\cdot}03+0{\cdot}01=1{\cdot}94$ mgm. Also, if $a=30$, $t=32$, $B=770$ and $g=40$, 1 c.c. of gas weighs $1{\cdot}89+0{\cdot}02-0{\cdot}05=1{\cdot}86$ mgm.

	Bar. = 760 *mm. Gas measured* (*g*) 20 *c.c.*							*B* (*mm.*).	
	Acid (*a*) *in c.c.*							750 −	770 + 740 − 780 +
° *C.*	10	15	20	25	30	35	40		
12	1·98	2·06	2 14	2·22	2·30	2 39	2·49	0·03	0·06
14	1·96	2·03	2·10	2·18	2·26	2·34	2·44	0 03	0·05
16	1·93	2·00	2·06	2·13	2·21	2·29	2·38	0·03	0·05
18	1·91	1·97	2·03	2·09	2·16	2 24	2·33	0·03	0·05
20	1·88	1·94	2·00	2·06	2·12	2·20	2·28	0·03	0·05
22	1·86	1·91	1·96	2·02	2·08	2·15	2·23	0·03	0·05
24	1·83	1·89	1·93	1·99	2·04	2·10	2·18	0·03	0·05
26	1·81	1·86	1·90	1·95	2·00	2·06	2·13	0·03	0·05
28	1·78	1·83	1·87	1·92	1·96	2·01	2·08	0·03	0·05
30	1·76	1·81	1·84	1·89	1·92	1·97	2·03	0·03	0·05
32	1·73	1·78	1·81	1·85	1·89	1·93	1·98	0·02	0·05
34	1·71	1·75	1·78	1·82	1·85	1·89	1·94	0 02	0·05
36	1·68	1·72	1·75	1·79	1·82	1·85	1·90	0·02	0 05
38	1·66	1·69	1·72	1·76	1·79	1·82	1·86	0·02	0·05
g									
0 + 40 −	0·01	0·02	0·03	0·04	0·05	0·06	0·08	Corrections.	
10 + 30 −	0·00	0·01	0·01	0·02	0·02	0·03	0·04		

The calculation can be carried out much more quickly by means of a special slide rule.* The reading of the barometer

* This slide rule and Collins's calcimeter are made by Messrs. Brady and Martin, Ltd., Northumberland Road, Newcastle-upon-Tyne.

is set opposite the reading of the thermometer; then opposite the reading of the number of c.c. of acid used will be found the number of mgm. of carbon dioxide and the number of mgm. of calcium carbonate which represent the weight equal to 100 c.c. of gas measured in the burette.

Amos's Method.—In the method due to A. Amos (*J. Agric. Sci.*, 1905, **1**, pp. 322-326) the carbon dioxide is liberated from the carbonate by hydrochloric acid and is absorbed by sodium hydroxide solution, which is then titrated with standard acid, using successively phenolphthalein and methyl orange as indicators. The apparatus consists of:

A, a Reiset apparatus containing 100 c.c. of 4 per cent. sodium hydroxide solution for removing carbon dioxide from the air.

B, a flask fitted with side tube and tap funnel.

C, a second Reiset apparatus containing 100 c.c. of 4 per cent. sodium hydroxide solution for absorbing the carbon dioxide liberated in B.

D, a small reflux condenser between B and C, in order that the condensed water (containing hydrochloric acid) may flow into flask B.

A weighed quantity of limestone, not containing more than 0·5 gm. of calcium carbonate, is placed in flask B, and 75 c.c. of water free from carbon dioxide are added. The Reiset apparatus C is connected with a filter pump, and a steady stream of air is drawn through the apparatus. 20 c.c. of concentrated hydrochloric acid are run into B by means of the tap funnel, and the contents of the flask are gradually raised to the boiling point; the boiling is continued for 20 minutes to ensure that all the carbon dioxide is swept into the Reiset apparatus C. The titration is carried out in the lower part of the Reiset apparatus, into which the contents of the absorption tube have been washed. Phenolphthalein is added, and N hydrochloric acid solution is run in until the pink colour begins to fade, and then N/10 hydrochloric acid solution until the colour is completely discharged. The reading of the burette is now taken; methyl orange is

added, and the titration is continued until the liquid shows an acid reaction. The amount of acid used in the methyl orange titration is that required for the equation $NaHCO_3 + HCl = NaCl + H_2O + CO_2$. The weight of carbon dioxide or calcium carbonate can thus be calculated from the volume of acid used in the second titration.

The object of using N hydrochloric acid in the first part of the titration is to prevent unnecessary dilution, but the liquid must be kept in motion so that the acid is never in excess at any point, with consequent evolution of carbon dioxide. A preliminary blank experiment must be carried out to allow for the carbonate in the sodium hydroxide solution and the carbon dioxide originally present in the Reiset apparatus C.

ORGANIC CONSTITUENTS OF FEEDING STUFFS

The conventional analyses of feeding stuffs comprise the percentages of the following constituents:

(i) Moisture or water;

(ii) Oil, fat, crude fat or ether extract;

(iii) Protein, crude protein or albuminoids;

(iv) Carbohydrates, soluble carbohydrates, nitrogen-free extract or nitrogen-free extractives (by difference);

(v) Fibre, crude fibre or woody fibre;

(vi) Ash or mineral matter.

Items (iii) and (v) are determined by conventional methods. It is very desirable that these conventions should be strictly adhered to, so that the results obtained by different analysts may be comparable. Though there is some agreement as to the methods of analysis, there is unfortunately no uniformity in the statement of the results. Some English authors name each constituent by the first term used above and arrange them in that order, though items (ii) and (iii) are often transposed. H. E. Woodman and his co-workers in a series of papers on the nutritive

value of pasture, for the last of which see *J. Agric. Sci.*, 1934, **24,** pp. 574-596, state the composition of samples of dry pasture as the percentages of crude protein, ether extract, nitrogen-free extractives, crude fibre and ash, and arrange these constituents in that order. In their investigations of intensive grassland management S. J. Watson and his co-workers (see No. XI in *J. Agric. Sci.*, 1932, **22,** pp. 257-290) state the percentages of ether extract, fibre, crude protein, ash, nitrogen-free extractives and moisture in the order here given.

In the Fertilisers and Feeding Stuffs Regulations, 1932 (section 12, iv, and Form B) the nitrogenous constituents of feeding stuffs are termed " albuminoids (protein) "; and in *Methods of Analysis*, 1935, p. 338, the nitrogen in these constituents is termed albuminoid nitrogen. It is, however, advisable to avoid the use of the word albuminoid as an alternative for protein, because albuminoid is correctly used in a restricted sense for a member of a particular group of proteins. The albuminoids, which are also known as the scleroproteins, occur in the skeletal and connective tissue of animals; they include collagen in gelatin and keratin in hair, skin, nails and horns (see *Chem. Soc. Ann. Reports*, 1908, **5,** p. 213, and S. W. Cole, *Practical Physiological Chemistry*, 1933, p. 75).

The Fertilisers and Feeding Stuffs Regulations, 1932, **12,** ii, give one method for the determination of moisture, which is drying a weighed quantity of the sample at 100° C. The percentage of moisture, however, is not included in the certificate of analysis (Form B). The Association of Official Agricultural Chemists have adopted four methods of determining moisture, which are given on pp. 117-119. The first of these is drying to constant weight at the boiling point of water. For samples which contain a " drying " oil, such as linseed cake, this method is not suitable, because the increase in weight of the sample due to the absorption of oxygen by the " drying " oil counterbalances the loss in weight due to the vaporization of the water. The

moisture in such samples can be determined by heating the sample to constant weight in a current of hydrogen or coal-gas, or more conveniently by drying the sample over concentrated sulphuric acid in a vacuum desiccator as in the second method. The third method, which consists in heating the sample for 2 hours in an electric oven at 135° C., is suitable for many samples which do not contain either a "drying" oil or an essential oil. The fourth method consists in measuring the water obtained by distilling a weighed quantity of the sample with toluene in Dean and Stark's apparatus. Jones and McLachlan found that with jam, honey and malt extract this method gives more consistent results than any other. It will probably be very suitable for the determination of moisture in feeding stuffs containing molasses, which cannot be satisfactorily dried to constant weight in an oven.

Oil, fat or ether extract is determined by one of the methods described on pp. 119-120. In the British official method a weighed quantity of the sample, without being previously dried, is extracted with petroleum spirit for 3 or 4 hours, after which it is finely ground and again extracted for another hour. In the A.O.A.C. method the sample, after being dried by one of the first three methods referred to above, is extracted with anhydrous ether for 16 hours. The ether extract is either weighed directly or it is found indirectly by weighing the sample before and after the extraction. If the sample contains sugars, it must be extracted with water and dried before extraction with petroleum spirit or anhydrous ether.

The percentage of protein or crude protein is determined by multiplying the percentage of organic nitrogen by 6·25 on the assumption that all proteins contain 16 per cent. of nitrogen. In the Fertilisers and Feeding Stuffs Regulations the prescribed method for the determination of organic nitrogen in feeding stuffs is the same as that used for the determination of organic and ammoniacal nitrogen in fertilizers which do not contain nitrates (see p. 19). Instead

of this, the Kjeldahl method (see p. 21), the Gunning method (see p. 21) or the Kjeldahl-Gunning-Arnold method (see p. 22) can be used. If one of the modified Kjeldahl methods which include nitrate nitrogen is employed, higher results will be obtained with some samples, such as young fodder crops, roots and tubers, which contain nitrates. Ashton has shown that the nitrate nitrogen in grass can be determined colorimetrically by the phenoldisulphonic acid method in water extracts which have been clarified and decolorized as described on p. 120.

The amide or amido nitrogen is determined by subtracting from the organic nitrogen the protein nitrogen. The latter is determined by precipitating the proteins with a mixture of cupric hydroxide and glycerol (Stutzer's reagent), as described on p. 122. After washing the precipitate to remove the amides and other soluble nitrogenous compounds, nitrogen is determined in the precipitate by the same method as that used for the determination of organic nitrogen. The protein nitrogen multiplied by 6·25 gives the true protein, and the amide nitrogen multiplied by 6·25 gives the "amides." The sum of the true protein and the "amides" is therefore equal to the crude protein. These two items can be included in the conventional analysis in place of crude protein; but Woodman (*loc. cit.*) states the true protein and "amides" separately after the conventional analysis, which includes the crude protein. The results can also be expressed by stating, in addition to the crude protein, the true protein and the ratio of true protein to crude protein.

Another way of showing differences between the crude protein in different feeding stuffs is by determining the pepsin-digestible nitrogen, as described on p. 123. The sample is treated with a hydrochloric acid solution of pepsin under specified conditions and the nitrogen is determined in the undigested residue. This nitrogen subtracted from the organic nitrogen in the original sample gives the pepsin-digestible nitrogen.

The fibre or crude fibre is the organic matter (loss on ignition) left after treating 2-3 gm. of the sample with 200 c.c. of boiling dilute sulphuric acid (1·25 gm. per 100 c.c.) for 30 minutes and then with 200 c.c. of boiling sodium hydroxide solution (1·25 gm. per 100 c.c.) for the same time. The concentrations of the acid and alkali solutions and the times of treatment are those agreed to at the International Congress of Applied Chemistry at Berlin in 1903 (see T. B. Wood, *J. Agric. Sci.*, 1905, **1**, pp. 366-373).* The British official method and the A.O.A.C. method are given on pp. 123-126; it will be seen that they differ considerably in the details of the procedure. In the British method 2 or 3 gm. of the sample, after being extracted with petroleum spirit without further grinding, are digested with boiling dilute sulphuric acid in a 1000 c.c. conical flask under such conditions that the original volume is maintained. After the acid digestion the liquid is filtered with suction through a filter paper in a Buchner funnel. After the alkali digestion the liquid is filtered through an ordinary filter paper, and the residue is washed with boiling water, dilute hydrochloric acid, again with boiling water and then with alcohol and finally with ether. The washed residue is then dried and weighed, and after ignition weighed again. In the A.O.A.C. method 2 gm. of the sample, which have been extracted with anhydrous ether, together with 0·5 gm. of asbestos, are digested with the boiling dilute sulphuric acid in an Erlenmeyer flask fitted with a reflux condenser. After the acid digestion the liquid is filtered through filtering

* A. G. Norman (*J. Agric. Sci.*, 1935, **25**, pp. 529-540) has shown that crude fibre is a variable mixture of cellulose and lignin, neither of which is a constant fraction of the quantity present in the original sample. During the acid digestion starch is hydrolysed, but cellulose and lignin are very slightly changed. During the digestion with alkali cellulose is slightly changed, lignin is attacked to a considerable but variable extent, and the proteins are almost completely removed. Norman thinks that any empirical method should include the whole of the lignin, and suggests as a possible alternative acid hydrolysis with a correction for the proteins.

cloth or linen. The residue after being washed with boiling water is washed back into the digestion flask with 200 c.c. of boiling sodium hydroxide solution by means of a wash bottle marked to deliver 200 c.c. The residue after the alkali digestion is washed in a Gooch crucible with boiling water and alcohol; it is then dried and weighed, ignited and weighed again in order to find the loss on ignition.

E. A. Fisher and R. H. Carter (*J. Soc. Chem. Ind.*, 1934, **53,** pp. 313-317T) point out that comparatively slight departures from the precise instructions laid down in the Fertilisers and Feeding Stuffs Regulations result in considerable variations in apparent fibre content. The actual limits of experimental error were carefully worked out by a committee convened by the Government Chemist in 1926. The same sample of soya cotton cake was subdivided and sent to 10 different laboratories where determinations of fibre were made by 35 different workers. Altogether 358 determinations were made on the one sample, and the results were submitted to mathematical analysis. With strict adherence to the instructions the range of experimental error may be taken as 0·6 per cent. It is desirable that all fibre determinations should be made in duplicate.

With most samples there is no difficulty in obtaining a white or greyish-white ash by ignition at a dull red heat; but sometimes difficulty is encountered because the particles of carbon are surrounded by a layer of fused ash which prevents their further oxidation. In such cases, it is advisable to extract the ash with hot water, ignite the insoluble portion and evaporate the soluble portion to dryness in the same dish, as explained on p. 126.

The percentage of carbohydrates, nitrogen-free extract or nitrogen-free extractives is found by subtracting from 100 the sum of the percentages of the other five constituents. In analyses of feeding stuffs it is usual to state the percentage of silica or sand. The determination of this constituent is described on p. 126. The determinations of the other

mineral constituents of feeding stuffs are described in the next section.

The methods of analysis which have been discussed so far in this section are conventional. They give results which can be compared with those obtained with other samples of the same kind or with published analyses. But a truer estimate of the feeding value of a feeding stuff is often obtained by determining some of the chemical substances occurring in it, particularly the sugars and other carbohydrates, which are included in the nitrogen-free extractives. The British official method for the determination of sugar in feeding stuffs is given on p. 127. The solution for the analysis is prepared by extracting the sample with water and clarifying the solution, if it is necessary, with basic lead acetate followed by sodium sulphate or alumina cream. After clarification the solution is filtered and the sugars in an aliquot part of the filtrate are inverted or hydrolysed by adding hydrochloric acid and heating the mixture to 69° C. for 7-7½ minutes. The solution is at once cooled, neutralized, made up to a definite volume and filtered. In the filtrate the reducing sugar is determined by a gravimetric or a volumetric method, the total copper reducing power being calculated as sucrose. The regulation does not give the details of the determination nor does it prescribe any particular method.

It is interesting to compare the regulation just considered with Eynon and Lane's procedure for the analysis of molasses, which is given on p. 128. It will be seen that clarification of the solution is effected by the addition of normal lead acetate and potassium oxalate. Basic lead acetate is not used for this purpose, because a considerable proportion of reducing sugar is carried down with the lead precipitate. Normal lead acetate causes no precipitation of reducing sugar; it removes some reducing non-sugar and effects sufficient decolorization of the solution for an accurate volumetric determination to be made. Potassium oxalate serves the double purpose of removing the excess of lead

and precipitating soluble calcium salts. The trace of lead left in the solution after the addition of potassium oxalate does not interfere with the determination of the sugars; but in the presence of soluble calcium salts the results are lower than the true values. Molasses generally contains considerable quantities of calcium, which must be removed from the solution in order to obtain accurate results. In Eynon and Lane's procedure hydrolysis is carried out much more easily than in the British official method by adding to the sugar solution a measured volume of N hydrochloric acid and boiling the mixture for 2 minutes. The invert sugar is determined before and after hydrolysis by Lane and Eynon's volumetric method. The results are expressed as the percentages of invert sugar and sucrose.

In the A.O.A.C. method of determining sugars in grain and stock feeds, which is fully described on p. 129, the sample, together with some calcium carbonate if it is acid, is extracted with boiling 50 per cent. alcohol and, when cool, the mixture is made up to a definite volume with 95 per cent. alcohol. The object of using alcohol is to inhibit enzyme action and prevent the growth of fungi which cause fermentations in dilute aqueous solutions of sugars. A measured volume of the clear alcoholic extract, after removal of the alcohol by evaporation, is clarified by the addition of neutral lead acetate followed by either sodium carbonate or potassium oxalate. If calcium carbonate has been added to the alcohol to neutralize the acidity, it would be advisable to clarify the solution with normal lead acetate and potassium oxalate, as recommended by Eynon and Lane, and thus precipitate the calcium which would vitiate the results. The reducing sugars are determined in the clarified solution either by Munson and Walker's method or by Allihn's method, the result being expressed either as dextrose or as invert sugar. The reducing sugars are again determined after inversion, which is effected by adding concentrated hydrochloric acid to the sugar solution and allowing the mixture to stand at room temperature.

The accurate determination of starch is a difficult problem.* Two methods adopted by the Association of Official Agricultural Chemists are given on p. 130. In the first method, which is intended only for such materials as raw starch, potatoes, etc., the sample is boiled with dilute hydrochloric acid in order to convert the starch into dextrose. After neutralization the dextrose is determined by Munson and Walker's method or by Allihn's method, and the result thus obtained is multiplied by 0·90 to give the equivalent weight of starch. This method is not very accurate because pentosans and other carbohydrates, which are converted into reducing sugars on boiling with dilute hydrochloric acid, are reckoned as starch. In the second method, which is more accurate, the sample is heated with water until the starch is gelatinized and is then treated with malt extract until starch is no longer detected. The maltose in the filtered solution is converted by boiling dilute hydrochloric acid into dextrose, which is determined by one of the methods mentioned above.

It is now necessary to consider the methods of determining sugars. Lane and Eynon's volumetric method is described on p. 132. In this method the sugar solution to be analysed is added to 10 or 25 c.c. of Fehling's solution under specified conditions until reduction is complete. Methylene blue is added towards the end of the titration and serves as an internal indicator. So long as the slightest trace of unreduced copper remains in the solution, the methylene blue retains its colour; but as soon as all the copper is reduced, the methylene blue is decolorized at the boiling temperature. A preliminary titration is carried out by the incremental method in order to ascertain the approximate volume of sugar solution required. Another titration is then carried out by the standard method, in which almost the whole volume of sugar solution required

* For a critical review of the methods of estimating starch, with a copious bibliography, see J. T. Sullivan, *Ind. Eng. Chem.* (*Anal*), 1935, **7**, pp. 311-314.

to reduce the Fehling's solution is added before heating and the titration is completed in 3 minutes after the liquid begins to boil. The Fehling's solution used in the titrations is standardized against a standard solution of invert sugar under the same conditions as those in the standard method. In this way Lane and Eynon have found that the highest degree of accuracy is attainable. They claim that their method is at least as accurate as the gravimetric methods, and is far quicker and more convenient in manipulation. Another volumetric method is Cole's ferricyanide-methylene-blue method, which is described on p. 137. This method depends on the reduction of potassium ferricyanide when a reducing sugar is added to it in a boiling alkaline solution. Methylene blue is added towards the end of the titration, and is not decolorized until the whole of the ferricyanide has been reduced.

Munson and Walker's method and Allihn's method, which have been referred to above, are both gravimetric methods. In these methods, the working details of which are given on pp. 144 and 145, a measured volume of Fehling's solution, diluted with water, is boiled under specified conditions for a definite time with a measured volume of the sugar solution which is not sufficient to completely reduce the Fehling's solution, after which the mixture is rapidly filtered. If the sugar solution is free from impurities, the cuprous oxide is dried and weighed; but if the sugar solution contains mineral or organic impurities, including sucrose, the copper in the cuprous oxide is determined by titration with sodium thiosulphate solution or by electrolysis, as described on p. 147.

The determination of pentoses and pentosans in feeding stuffs is described on p. 148. The method consists in distilling the sample with dilute hydrochloric acid, precipitating the furfural in the distillate with phloroglucinol and weighing the insoluble precipitate. The presence of hydrocyanic acid in some beans renders them unfit for use as feeding stuffs. The formation of hydrocyanic acid in beans

is due to the hydrolysis of phaseolunatin and other cyanogenetic glucosides occurring in beans. The quantity of hydrocyanic acid which is thus formed can be determined by the methods described on p. 150. In Henry and Auld's method the sample is extracted with alcohol; the alcoholic extract is distilled with dilute acid and the hydrocyanic acid in the distillate is determined by titration with standard iodine solution. In the A.O.A.C. methods the sample is macerated with water and then distilled. The hydrocyanic acid in the distillate is determined by titration with N/50 silver nitrate solution in either acid or alkaline solution.

Since farm animals are dependent on the carotene in their food as a source of vitamin A, and since cows fed on a diet containing a plentiful supply of carotene yield milk and butter rich in carotene and vitamin A, the determination of carotene in feeding stuffs is a matter of great importance. Carotene and the closely related carotenoid, xanthophyll, are most accurately determined by spectrophotometric methods. These methods are not described in detail here, because they require the use of expensive optical apparatus, which is not available in agricultural laboratories. They depend on the fact that each of these substances exhibits a group of absorption bands in the visible region of the spectrum, the principal one occurring at 463 mμ for carotene and 455 mμ for xanthophyll, and the amount of either carotenoid in a solution containing only one of them is proportional to the intensity of its particular absorption band.*

A much simpler method is that due to Ferguson, who constructed curves connecting the concentration of carotene solutions with the Lovibond tintometer readings and also with colorimeter readings using a potassium dichromate solution as standard. The total carotenoids, and xanthophyll after its separation from carotene, can be approximately

* See A. E. Gillam, I. M. Heilbron, R. A. Morton, G. Bishop and J. C. Drummond, *Biochem. J* 1933, **27**, pp. 878-888, and A. E. Gillam, *ibid.*, 1934, **28**, pp. 79-83.

determined from the same curves. Ferguson and Bishop's procedure is described on p. 151. It consists of boiling the feeding stuff with potassium hydroxide solution, filtering and washing the residue first with ether-saturated water and then with acetone. The total carotenoids in the filtrate are extracted with ether, and the extract is matched in a Lovibond tintometer or a colorimeter. The separation of carotene and xanthophyll is effected by distilling off the ether in a stream of nitrogen, dissolving the residue in petroleum spirit and extracting the solution with 92 per cent. methyl alcohol, which removes the xanthophyll. The petroleum spirit solution and the methyl alcohol extract are each matched in a Lovibond tintometer or a colorimeter. From the ratio of carotene to xanthophyll thus obtained the carotene content of the original extract is calculated. The reason for applying this ratio to the total carotenoids, rather than calculating the carotene content from the reading of the petroleum spirit extract, is that during the separation there is a loss, which is usually about 5 per cent., but may amount to about 10 per cent. of the total carotenoids.

The conventional methods of analysing feeding stuffs do not give a true indication of the nutritive value of silage. Special methods for the analysis of silage have therefore been devised. Some of those used by Watson and Ferguson are described on pp. 154-157. The determination of moisture is carried out by drying the sample to constant weight in a steam oven; but, owing to the presence of volatile acids and bases, it is necessary to apply corrections for the losses of these constituents during the drying process. The determinations of the total acidity, volatile bases, amino acids, volatile acids and residual acidity by the methods of Foreman, as modified by Woodman, are of great value for comparative purposes. The figure for the volatile bases is a measure of the extent of the protein breakdown; the amino acid figure gives an indication of the intermediate changes, and the determination of the total volatile acids gives an index of the degree of change which the non-nitrogenous constituents

have undergone. The determination of the pH value is of great importance because the type of fermentation taking place in a silo is controlled by the reaction of the fermenting material.

Moisture. — The Association of Official Agricultural Chemists (*Methods of Analysis*, 1935, pp. 335-336) have adopted the following four methods for the determination of moisture in grain and stock feeds:

Drying at the Boiling Point of Water.—Dry a weighed quantity of the sample, representing about 2 gm. of the dry material, to constant weight at 95°-100° C. under a pressure not exceeding 100 mm. of mercury. Use a covered aluminium dish at least 5 cm. in diameter and not more than 4 cm. deep.

Drying over Sulphuric Acid.—Weigh 2-5 gm. of the sample in a metal dish 5-10 cm. in diameter with a tightly fitting cover. If the fat is to be determined, a fat extraction cone may be used. Place in a vacuum desiccator 200 c.c. of concentrated sulphuric acid, which has been boiled in a large Kjeldahl flask for 4 hours and cooled in the same flask after the mouth has been closed with a stopper carrying a calcium chloride tube. Place the dish, uncovered, in the desiccator, and exhaust by means of a vacuum pump to a pressure of not more than 10 mm. of mercury. Gently rotate the desiccator 4 or 5 times during the first 12 hours. At the end of 24 hours open the desiccator, passing the incoming air through sulphuric acid; place the cover on the dish and weigh it. After weighing, place the dish in a desiccator containing fresh sulphuric acid and exhaust as before. Rotate the desiccator several times and weigh the dish again after a suitable period of drying. Repeat the process until the weight is constant.

Electric Oven Method.—Adjust an electric oven to 135°± 2° C. Weigh about 2 gm. of the sample in a shallow covered aluminium dish, and shake until the contents are evenly distributed. Remove the cover, place the dish and cover in the oven and leave them there for 2 hours. Then cover the dish, cool in a desiccator and weigh.

Distillation with Toluene.—This consists in measuring the water obtained by distilling the sample with toluene in an apparatus designed by E. W. Dean and D. D. Stark (*J. Ind. Eng. Chem.*, 1920, **12,** pp. 486-490). The apparatus is shown in Fig. 2. A weighed quantity of the sample together with a little sand is placed in the flask A, and to it is added toluene. The flask is heated and the mixed vapours are condensed in the reflux condenser B. The condensed liquid falls into the graduated receiver C. The water settles out at the bottom of the graduated receiver and the excess of toluene flows into the flask. The distillation is continued until there is no further change in the volume of the water in the graduated receiver. In Fig. 2 is shown the British standard graduated receiver, type 2, which is graduated from 0·2 to 7·5 ml. in intervals of 0·1 ml. Type 1 is graduated from 1 to 10 ml. in intervals of 0·1 ml. and has a stopcock at the bottom, so that water can be withdrawn, if necessary, during the course of the distillation (see British Standard Specification No. 614, 1936).

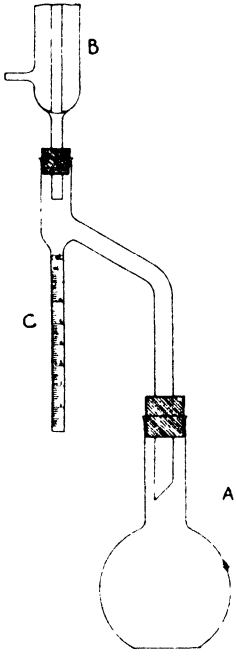


FIG. 2.

The A.O.A.C. procedure (*Methods of Analysis*, 1935, pp. 335-336) is as follows: If the liquid is likely to bump, place in the flask enough dry sand to cover the bottom. Add about 75 c.c. of toluene, introduce into the toluene a weighed quantity of the sample sufficient to give 2-5 c.c. of water and connect together the apparatus. Fill the graduated receiver with toluene by pouring it through the top of the condenser. Heat the toluene to the boiling point and distil slowly, about 2 drops per second, until most of the water has passed over. Then increase the rate of distillation to about 4 drops per second. When all the water has

apparently passed over, wash down the condenser by pouring in toluene and continue the distillation for a short time to ascertain whether any more water distils over; if so, repeat the washing down process. If any water remains in the condenser, remove it by brushing with a tube brush attached to a copper wire and washing down the condenser at the same time. The entire process is usually completed within an hour. Allow the graduated receiver to come to room temperature, read the volume of the water and calculate the percentage.

J. M. Jones and T. McLachlan (*Analyst*, 1927, **52**, pp. 383-387) heated the flask in a paraffin bath to prevent charring the sample. In order to make the small drops of water coalesce and fall to the bottom of the graduated receiver, they used a long thin copper spiral, fitting inside the condenser and graduated receiver, which was moved up and down during the distillation. They found that the time required to complete the distillation varied with the substance under examination. With malt extract 90 per cent., and with butter nearly the whole, of the water distilled in 1 hour.

Oil, Crude Fat or Ether Extract.—The British official method for the determination of oil in feeding stuffs (Fertilisers and Feeding Stuffs Regulations, 1932, **12**, iii) is as follows: A weighed quantity of the sample is placed in an extraction thimble, which is placed in an extraction apparatus and is extracted with petroleum spirit (b.p. 40°-60° C.). At the end of 3-4 hours the thimble is removed from the apparatus and dried. The contents are finely ground, preferably with sand, in a small mortar previously rinsed with petroleum spirit. The material is then returned to the thimble, the mortar being washed out with petroleum spirit, and the extraction is continued for another hour. The extract should be free from suspended matter. After evaporation of the solvent, the oil is dried at 100° C. and weighed. In the case of samples containing saccharine matter, the weighed portion in the thimble is washed with water and then dried before the extraction.

The methods adopted by the Association of Official Agricultural Chemists for the determination of crude fat or ether extract (*Methods of Analysis*, 1935, p. 339) are as follows:

The Direct Method.—Large quantities of soluble carbohydrates may interfere with the extraction of the fat. In such cases, the material is extracted with water before proceeding with the determination. About 2 gm. of the sample are dried by one of the three A.O.A.C. methods described on p. 117, and are then extracted with anhydrous ether for 16 hours. The extract is dried at the temperature of boiling water for 30 minutes, cooled in a desiccator and weighed. This alternate drying and weighing is continued, at intervals of 30 minutes, until the weight is constant. For most feeding stuffs a period of 1-1½ hours is required.

The Indirect Method.—Moisture is determined by one of the three A.O.A.C. methods described on p. 117. The dried material is extracted with anhydrous ether for 16 hours and dried again. The loss in weight is reported as ether extract.

Anhydrous Ether.—Commercial ether is washed with 2 or 3 successive portions of water. Solid sodium or potassium hydroxide is added, and the mixture is allowed to stand until most of the water has been removed from the ether. The ether is then decanted into a dry bottle; small pieces of carefully cleaned metallic sodium are added, and the mixture is allowed to stand until there is no further evolution of hydrogen. The ether, thus dehydrated, is kept over metallic sodium in loosely stoppered bottles.

Nitrate Nitrogen.—The following colorimetric method of determining nitrate nitrogen in grass has been described by F. L. Ashton (*J. Soc. Chem. Ind.*, 1935, **54,** pp. 389-390T). The following reagents are required:

Lead Subacetate Solution.—430 gm. of normal lead acetate and 130 gm. of litharge are boiled in 1000 c.c. of water for half an hour. The mixture is allowed to cool and settle, and is then diluted to a density of 1·25 with recently distilled water.

Hydrogen Peroxide (100 *vols.*), free from nitrate. The M.A.R. reagent supplied by the British Drug Houses Ltd. is most satisfactory.

Phenoldisulphonic Acid Solution.—Dissolve 25 gm. of pure white phenol in 150 c.c. of concentrated sulphuric acid, add 75 c.c. of fuming sulphuric acid (containing 13-15 per cent. of sulphur trioxide) and heat at 100° C. for 2 hours.*

Standard Nitrate Solution.—0·722 gm. of potassium nitrate is dissolved in 1 litre of water, and 50 c.c. of this solution are diluted to 500 c.c. 10 c.c. then contain 0·0001 gm. of nitrate nitrogen.

Procedure.—1 gm. of finely ground grass is mixed in a beaker with 1 gm. of magnesium oxide and 75 c.c. of water. The mixture is well stirred, boiled for 5 minutes to expel free ammonia, and allowed to cool. When cold, 30 c.c. of saturated potash alum solution are added, followed by 6 c.c. of lead subacetate solution and 15 c.c. of saturated silver sulphate solution to precipitate the chlorides. After 2 hours it is filtered into a 250 c.c. graduated flask, washed with boiling water until the flask is nearly full, and when cold made up to the mark.

10 c.c. of this solution are pipetted into a porcelain basin and 0·1 c.c. of hydrogen peroxide (100 vols.) is added. The basin is placed on a water bath and is kept there for half an hour after the contents appear to be dry. Since hydrogen peroxide gives a brown colour with phenoldisulphonic acid, it is advisable to leave the dish on the water bath half an hour after the residue appears to be dry, to ensure that the peroxide is completely dissociated. 1 c.c. of phenoldisulphonic acid is added to the residue and the mixture is well stirred with a small glass rod. About 20 c.c. of water are added, and the mixture is stirred until solution is complete. After the addition of 10 c.c. of dilute ammonia (1 : 2), the solution is washed into a 50 c.c. graduated flask and made up to the mark. The colour of the solution is

* *Methods of Analysis*, 1935, p. 506. The preparation of this reagent is not described by Ashton.

matched against a suitable quantity of the standard nitrate solution, which has been evaporated to dryness before the addition of phenoldisulphonic acid. The matching is carried out in Nessler cylinders or in a colorimeter.

The addition of 2 drops of 0·1 per cent. methylene blue solution to both solutions makes matching easier, but the solutions must be of approximately the same strength before it is added. When the matching is done in Nessler tubes, the best way is to pour off small portions of the stronger of the two until a match is obtained. The volume of the discarded liquid may then be determined and the relative concentrations of the two solutions calculated.

Protein and Amide Nitrogen.—The method adopted by the Association of Official Agricultural Chemists (*Methods of Analysis*, 1935, p. 338) for the determination of protein nitrogen is as follows: Dissolve 100 gm. of crystallized copper sulphate in 5 litres of water. Add 2·5 c.c. of glycerol and then 10 per cent. sodium hydroxide solution until the liquid is slightly alkaline. Filter and rub the precipitate in a mortar with water containing 5 c.c. of glycerol per litre. Wash by decantation or filtration until the washings are no longer alkaline. Again rub the precipitate in a mortar with water containing 10 per cent. of glycerol, thus preparing a uniform gelatinous mass which can be measured with a pipette. Determine approximately the quantity of cupric hydroxide in 5 c.c. by diluting to 50 c.c. with water, filtering, washing, igniting and weighing the cupric oxide.*

Place 0·7 gm. of the sample in a beaker and add 100 c.c. of water. Heat to the boiling point, or heat on a steam bath for 10 minutes if the sample contains much starch, and add a quantity of the cupric hydroxide reagent that contains 0·5 gm. of the hydroxide. Stir thoroughly, filter when cold and wash the precipitate with cold water. Without removing the precipitate from the filter paper, determine nitrogen by the Kjeldahl, Gunning or Kjeldahl-

* The weight of cupric oxide × 1·2264 = the weight of cupric hydroxide.

Gunning-Arnold method, using sufficient potassium sulphide solution to precipitate all the copper and mercury. In the case of seeds, seed residue or oil cake, which are rich in alkaline phosphates, add 1-2 c.c. of 10 per cent. solution of soda alum (free from ammonia) to decompose the alkaline phosphates. Then add the cupric hydroxide reagent and mix well by stirring. Subtract the percentage of protein nitrogen from the percentage of total nitrogen to obtain the amide nitrogen.

Pepsin-Digestible Nitrogen.—This determination is generally carried out by the following method described by H. W. Wiley (*Agricultural Analysis*, vol. iii, 1914, p. 681). The pepsin solution is prepared by dissolving 1 gm. of best scale pepsin in 1 litre of 0·33 per cent. hydrochloric acid. 2 gm. of the sample in fine powder are suspended in 100 c.c. of the pepsin solution and kept, with frequent shaking, at 40° C. for 12 hours. The contents of the flask are poured on a wet filter paper. The residue on the filter paper is well washed with water at a temperature not exceeding 40° C. The filter paper together with the washed residue is transferred to a Kjeldahl flask, and the organic nitrogen is determined. The percentage thus obtained is subtracted from the percentage of organic nitrogen in the original sample to give the pepsin-digestible nitrogen.

Fibre or Crude Fibre.—The following solutions, which should be accurately checked by titration, are required for both of the following methods:

Sulphuric Acid Solution, containing 1·25 gm. of sulphuric acid per 100 c.c. This solution can be prepared by diluting 50·98 c.c. of N sulphuric acid to 200 c.c.

Sodium Hydroxide Solution, containing 1·25 gm. of sodium hydroxide per 100 c.c. and free, or nearly free, from sodium carbonate. This solution can be prepared by diluting 62·49 c.c. of N sodium hydroxide solution to 200 c.c.

For the A.O.A.C. method there is also required asbestos which is prepared as follows: Digest asbestos on a steam bath for at least 8 hours with an approximately 5 per cent.

sodium hydroxide solution, and thoroughly wash with hot water. Then digest in a similar way for 8 hours with dilute hydrochloric acid (1 : 3) and again thoroughly wash with water. Dry and ignite at a bright red heat.

The British official method for the determination of fibre (Fertilisers and Feeding Stuffs Regulations, 1932, **12,** vi) is as follows: 2 or 3 gm. of the sample, accurately weighed, are extracted with petroleum spirit (b.p. 40°-60° C.) in an extraction apparatus or by stirring, settling and decanting at least 3 times. The material must not be further ground during the extraction. The dry residue is transferred to a 1000 c.c. conical flask, and to it are added 200 c.c. of the sulphuric acid solution, which is heated to the boiling point. The contents of the flask are heated and must reach the boiling point within 1 minute; the boiling must be gentle and continuous for exactly 30 minutes, the original volume being maintained. The flask is rotated every few minutes in order to mix the contents and remove the particles from the sides. At the end of 30 minutes the flask is removed and the contents are poured at once into the shallow layer of hot water remaining in a funnel fitted with a pump-plate or alternatively into the similar layer remaining in a Buchner funnel. The funnel is prepared by cutting a piece of cotton cloth or filter paper to cover the holes, so as to serve as a support for a disc of ordinary filter paper; boiling water is poured into the funnel and is allowed to remain until the funnel is hot, when suction is applied. The experiment is discarded if the time of filtration of the 200 c.c. of liquid exceeds 10 minutes. The residue is washed with boiling water until the washings are free from acid. The residue is then washed from the filter paper back into the flask with 200 c.c. of the sodium hydroxide solution, which is heated to the boiling point. The contents of the flask are boiled for exactly 30 minutes, the precautions given for the treatment with acid being observed. At the end of 30 minutes the flask is removed and the contents are immediately filtered through an ordinary filter paper. The residue on

the filter paper is washed with boiling water, then with 1 per cent. solution of hydrochloric acid, again with boiling water until free from acid, then twice with 95 per cent. alcohol and 3 times with ether. The washed residue is then transferred to a dried weighed filter paper, dried at about 100° C. in an oven and weighed in a weighing bottle until constant in weight. The ash of the paper and contents is determined by incineration at a dull red heat. The weight of the ash is subtracted from the increase in weight of the filter paper, and the difference is the fibre.

The method of determining crude fibre adopted by the Association of Official Agricultural Chemists (*Methods of Analysis*, 1935, pp. 340-341) is as follows: Extract 2 gm. of the dry material with ether, or use the residue from the ether extract determination, and transfer the residue together with about 0·5 gm. of asbestos to the digestion flask (a 700-750 c.c. Erlenmeyer flask). Add 200 c.c. of the boiling sulphuric acid solution, immediately connect with a reflux condenser and heat; the contents of the flask should begin to boil within 1 minute. Continue boiling for exactly 30 minutes, rotating the flask every 5 minutes in order to mix the contents and prevent the material from remaining on the sides of the flask. At the end of 30 minutes remove the flask, and immediately filter the contents through linen (with about 45 threads to the inch) in a fluted funnel; wash the residue with boiling water until the washings are no longer acid. Heat a quantity of the sodium hydroxide solution to the boiling point and keep it at that temperature under a reflux condenser until it is used. Wash the residue back into the digestion flask with 200 c.c. of the boiling sodium hydroxide solution, using a wash bottle marked to deliver 200 c.c. (The boiling sodium hydroxide solution is conveniently transferred to the 200 c.c. wash bottle by means of a bent tube, through which the liquid is forced by blowing into a tube connected with the top of the reflux condenser.) Then connect the digestion flask with the reflux condenser and boil for exactly 30 minutes.

Then remove the flask and immediately filter the contents through a Gooch crucible with an asbestos mat, an alundum crucible or filtering cloth in a fluted funnel. If filtering cloth is used, thoroughly wash the residue with boiling water and then transfer it to a Gooch crucible with a thin but close layer of ignited asbestos. After washing with boiling water, wash the residue with about 15 c.c. of 95 per cent. alcohol, and dry the crucible and the contents at 110° C. to constant weight. Cool in a desiccator and weigh. Incinerate the contents of the crucible in an electric muffle or over a Meker burner at a dull red heat. Cool in a desiccator and weigh. The loss in weight is the crude fibre.

Ash.—The method adopted by the Association of Official Agricultural Chemists (*Methods of Analysis*, 1935, p. 336) for the determination of the ash in grain and stock feeds is as follows: Weigh a quantity of the sample, representing about 2 gm. of the dry material, and incinerate it at a low temperature, not exceeding dull redness, until the carbon is burnt. If a carbon-free ash cannot be obtained in this way, extract the charred mass with hot water, collect the insoluble residue on an ashless filter paper, and ignite the filter paper and residue until a white, or nearly white, ash is obtained. Add the filtrate, evaporate to dryness and heat at dull redness until the ash is white or greyish white. Cool in a desiccator and weigh.

Silica.—The British official method for the determination of sand, siliceous matter or other insoluble mineral matter in feeding stuffs (Fertilisers and Feeding Stuffs Regulations, 1932, **12,** ix) is as follows : A weighed quantity of the sample, from 2 to 5 gm., is incinerated and the ash is weighed. The ash is moistened with hydrochloric acid and evaporated to dryness. The residue is repeatedly extracted with hot dilute hydrochloric acid (1 : 4) and the solution is filtered. The insoluble matter is washed, ignited and weighed. The quantity obtained is taken as sand and siliceous matter. If the quantity of sand and silica-free ash is so high as to raise a presumption that mineral matter has been added,

the nature and quantity of such added substances should, if possible, be determined.

Sugar.—The British official method for the determination of sugar in feeding stuffs (Fertilisers and Feeding Stuffs Regulations, 1932, **12,** vii) is as follows: If the sample is in a solid form, about 10 gm. of the sample, or a larger quantity if the percentage of sugar is low, are accurately weighed, ground with water in a mortar and transferred to a 250 c.c. flask, using in all about 200 c.c. of cold water. The flask is shaken at intervals during 30 minutes. If it is necessary to use a clearing agent, basic lead acetate followed by sodium sulphate, or alumina cream free from ammonia, is used. The liquid in the flask is then made up to 250 c.c. and filtered. The sugar is determined in 50 c.c. of the filtrate after the sugar has been inverted as described below.

If the sample is in liquid form, the prepared portion of the sample is thoroughly mixed immediately before weighing out the quantity for the determination. About 10 gm. of the sample are accurately weighed and washed into a 250 c.c. flask with about 200 c.c. of water. The solution is cleared, if necessary, with basic lead acetate followed by sodium sulphate or alumina cream free from ammonia. The liquid in the flask is then made up to 250 c.c. and filtered. The sugar is determined in 25 c.c. of the filtrate after the sugar has been inverted as described below.

The aliquot part of the filtrate is measured into a 100 c.c. flask; 5 c.c. of 38·3 per cent. hydrochloric acid* are added and the flask is placed in a water bath maintained at 70° C. The solution in the flask should reach the temperature of 67°-69° C. in 2½-3 minutes. It is maintained at 69° C. for 7-7½ minutes, the total period of heating being 10 minutes. The solution is then cooled at once, neutralized, made up to 100 c.c. and filtered. The total reducing sugar in the filtrate is determined by a gravimetric or a volumetric

* The specific gravity of a solution containing 38·3 per cent. by weight of hydrochloric acid is 1·1955 at 15° C./4° C.

method, the total copper-reducing power being calculated in terms of sucrose.*

Molasses.—The procedure recommended by L. Eynon and J. H. Lane (*J. Soc. Chem. Ind.*, 1923, **42,** pp. 463-466T) for the determination of invert sugar and sucrose in molasses and similar low-grade products is as follows: 12·5 gm. of the sample are dissolved in water, treated with 25 c.c. of 10 per cent. normal lead acetate solution and a little alumina cream, if necessary, and made up to 250 c.c., the whole being thoroughly shaken and filtered. 100 c.c. of the filtrate are treated with 10 c.c. of 10 per cent. potassium oxalate solution, made up to 500 c.c., shaken and filtered. The clarified solution, which is free from lead and calcium, representing a 1 per cent. solution of the sample, is used for the determination of invert sugar and sucrose as described in (*a*) and (*b*) below.

(*a*) The solution is titrated against 10 c.c. of Fehling's solution with methylene blue as internal indicator, as described on p. 132. The result thus obtained does not represent the invert sugar only, but includes the non-fermentable reducing substances, which may amount to 4 per cent. or more in terms of invert sugar. The separate determination of these substances, which involves a fermentation experiment, is not generally made in commercial analyses. (*b*) A known volume of the solution is hydrolysed with hydrochloric acid, neutralized with sodium hydroxide and made up to double the original volume. This solution, representing a 0·5 per cent. solution of the sample, is titrated against 25 c.c. of Fehling's solution, with methylene blue as internal indicator.

The hydrolysis is carried out as follows: 100 c.c. of the solution are treated with 15 c.c. of N hydrochloric acid and diluted to about 150 c.c. The solution is then heated to the boiling point and kept boiling for 2 minutes, after which it is cooled, neutralized with sodium hydroxide and made up to 200 c.c. The sucrose content is calculated by multi-

* The weight of invert sugar $\times$ 0·95 = the weight of sucrose.

plying the difference between the invert sugar before and after hydrolysis by 0·95.

Sugars.—The methods adopted by the Association of Official Agricultural Chemists (*Methods of Analysis*, 1935, pp. 341-342) for the determination of reducing sugars and sucrose in grain and stock feeds are as follows: Place 10 gm. of the sample in a 250 c.c. volumetric flask, and add 1-3 gm. of calcium carbonate if the substance has an acid reaction. Add 125 c.c. of 50 per cent. (by volume) alcohol, mix thoroughly and boil on a steam bath for 1 hour, using a small funnel in the neck of the flask to condense the vapour. Cool and allow the mixture to stand for several hours, preferably over-night. Make up to the mark with neutral 95 per cent. alcohol, mix thoroughly and allow to settle. Pipette 200 c.c. of the supernatant solution into a beaker, and evaporate it on a steam bath to a volume of 20-30 c.c. Transfer the contents of the beaker to a 100 c.c. volumetric flask, rinse the beaker with water and add the rinsings to the flask. Add enough saturated neutral lead acetate solution to produce a flocculent precipitate, shake and allow to stand for 15 minutes. Dilute to the mark with water, shake and filter through a dry filter paper. Add sufficient anhydrous sodium carbonate or potassium oxalate to precipitate the lead, again filter through a dry filter paper, and test the filtrate with a little anhydrous sodium carbonate or potassium oxalate to make sure that all the lead has been removed.

Reducing Sugars.—Determine reducing sugars by Munson and Walker's method (see p. 144) or by Allihn's method (see p. 145), using 25 c.c. of the prepared solution, representing 2 gm. of the sample. Express the result as dextrose or invert sugar.

Sucrose.—Place 50 c.c. of the prepared solution in a 100 c.c. volumetric flask, add a piece of litmus paper, and neutralize with hydrochloric acid. Add 5 c.c. of concentrated hydrochloric acid, and allow the flask to stand for 24 hours at a temperature not below 20° C., or for 10 hours if the

temperature is above 25° C., so that inversion may take place at room temperature. When inversion is complete, transfer the solution to a beaker, and neutralize it with sodium carbonate. Return the solution to the 100 c.c. flask, dilute with water to the mark and filter, if necessary. Determine reducing sugars in 50 c.c. of the solution, representing 2 gm. of the sample, by Munson and Walker's method or by Allihn's method. Express the result as invert sugar. Subtract the percentage of reducing sugars before inversion from the percentage of total sugar after inversion, both being expressed as invert sugar, and multiply the difference by 0·95 to obtain the percentage of sucrose.

Since the insoluble part of the sample occupies some space in the flask, it is necessary to apply a correction for this volume. The results of a large number of determinations have shown that the average volume of 10 gm. of material is 7·5 c.c., and therefore to obtain the true quantities of sugars present all results must be multiplied by the factor 0·97.

Starch.—The Association of Official Agricultural Chemists (*Methods of Analysis*, 1935, p. 342) have adopted three methods for the determination of starch in grain and stock feeds. One of these methods is intended for the determination of starch in the presence of interfering polysaccharides, such as occur in linseed meal; it is very complicated, and is not given here. The other two methods are as follows:

Acid Hydrolysis.—Place a weighed quantity of the sample, corresponding to 2·5-3 gm. of the dry material, in a beaker, add 50 c.c. of cold water and stir for 1 hour. Transfer to a filter and wash with 250 c.c. of cold water. Heat the insoluble residue with 200 c.c. of water and 20 c.c. of hydrochloric acid (sp. gr. 1·125) in a flask provided with a reflux condenser for 2½ hours. Cool, and nearly neutralize with sodium hydroxide. Dilute to 250 c.c., filter and determine the dextrose in an aliquot part of the filtrate by Munson and Walker's method (see p. 144) or by Allihn's method (see p. 145). The weight of dextrose thus obtained multiplied by 0·90 gives the weight of starch.

Diastase with Subsequent Acid Hydrolysis.—The malt extract is prepared as follows: Use clean new barley malt of known efficacy. Prepare an infusion of the freshly ground malt just before it is to be used. For every 80 c.c. of malt extract required digest 5 gm. of the ground malt with 100 c.c. of water at room temperature for 2 hours. Filter to obtain a clear extract; if necessary, return the first portion of the filtrate to the filter.

Extract a convenient quantity of the finely ground sample, corresponding to 4-5 gm. of the dry material, on a hardened filter paper with 5 successive portions of 10 c.c. of ether. Wash with 150 c.c. of 10 per cent. (by volume) alcohol, and then with a few c.c. of 95 per cent. alcohol. Place the residue in a beaker with 50 c.c. of water, immerse the beaker in boiling water and stir constantly for 15 minutes, or until all the starch is gelatinized. Cool to 55° C., add 20 c.c. of the malt extract and keep at this temperature for 1 hour. Heat to the boiling point for a few minutes, cool to 55° C., add 20 c.c. of the malt extract and keep at this temperature for 1 hour, or until the residue treated with iodine solution shows no blue colour on microscopical examination. Cool, make up to 250 c.c. and filter. Place 200 c.c. of the filtrate in a flask, add 20 c.c. of hydrochloric acid (sp. gr. 1·125), connect with a reflux condenser and heat in a boiling water bath for 2½ hours. Cool, nearly neutralize with 10 per cent. sodium hydroxide solution, finish the neutralization with sodium carbonate and dilute to 500 c.c. Filter and determine dextrose in an aliquot part of the filtrate by Munson and Walker's method or by Allihn's method. Carry out a blank determination with the same volume of malt extract and correct the weight of dextrose accordingly. The weight of dextrose obtained multiplied by 0·90 gives the weight of starch.

Fehling's Solution.—Soxhlet's modification of Fehling's solution is prepared by mixing, immediately before use, equal volumes of the following solutions:

(*A*) *Copper Sulphate Solution.*—Dissolve 34·639 gm. of

crystallized copper sulphate in water and dilute the solution to 500 c.c.

(*B*) *Alkaline Tartrate Solution.*—Dissolve 173 gm. of sodium potassium tartrate and 50 gm. of sodium hydroxide in water and dilute the solution to 500 c.c.

This modification of Fehling's solution is used for Lane and Eynon's method and for Munson and Walker's method, but for Allihn's method (see p. 145) a different alkaline tartrate solution is used.

Lane and Eynon's Method.—This volumetric method of determining reducing sugars by means of Fehling's solution with methylene blue as internal indicator was devised by J. H. Lane and L. Eynon (*J. Soc. Chem. Ind.*, 1923, **42,** pp. 32-37T), who have published other papers relating to this method and its applications (*ibid.*, 1923, **42,** pp. 143-146T and 463-466T; 1925, **44,** pp. 150-152T). Lane and Eynon have given the full working details of their method together with all the tables as originally published in a pamphlet, *Determination of Reducing Sugars by Fehling's Solution with Methylene Blue Indicator*, London, 1934, from which most of the following details were obtained.

The following solutions are required:

Methylene Blue Solution.—1 per cent. aqueous solution of pure methylene blue. This solution will keep for months without change.

Fehling's Solution.—The Fehling's solution used is Soxhlet's modification (see p. 131). In the gravimetric methods of determining reducing sugars an excess of Fehling's solution is used, and slight variations in the copper content do not affect the results. But in this method, which consists in determining the volume of sugar solution required to reduce a given volume of Fehling's solution, the copper content of the latter must be exactly adjusted to correspond with the tables used. Ordinary crystallized copper sulphate, though generally free from more than traces of other metallic salts, usually contains more water than corresponds with the formula, $CuSO_4,5H_2O$. In preparing Fehling's solution

for this method it is preferable to weigh 34·64 gm. of crystallized copper sulphate for solution A and titrate the Fehling's solution against a standard solution of invert sugar; any small adjustment of the copper solution to correspond with the tables can then be made by the addition of copper sulphate or water.

Standard Solution of Invert Sugar.—This is prepared as follows: A solution of 9·5 gm. of pure sucrose is treated with 5 c.c. of hydrochloric acid (sp. gr. 1·19), made up to about 100 c.c., left at room temperature for some days (about a week at 12°-15° C. or 3 days at 20°-25° C.) and then made up to 1 litre. The acid 1 per cent. solution of invert sugar thus obtained is very stable, retaining its titre unchanged for months. Slight mycelial growth may develop in the course of 3-4 months, but this does not affect the titre for several months after its first appearance. Still more stable solutions are obtained by diluting the hydrolysed solutions of sucrose to 500 or 250 c.c., giving 2 or 4 per cent. solutions of invert sugar. A known volume of the standard solution is neutralized with sodium hydroxide and suitably diluted immediately before use. With a neutralized solution containing 0·5 gm. of invert sugar per 100 c.c., and using the standard method of titration, which is described below, 25 c.c. of Fehling's solution should require 24·8 c.c. of the sugar solution. The table on p. 135 shows that this volume of sugar solution contains 124·0 mgm. of invert sugar.

Preparation of the Sugar Solution.—The final sugar solution to be titrated must be of such concentration that not less than 15 c.c. and not more than 50 c.c. of the solution will completely reduce 10 or 25 c.c. of Fehling's solution. Before the titration highly coloured solutions should be clarified as described on p. 128.

The Standard Method.—This is carried out as follows: 10 or 25 c.c. of Fehling's solution are accurately measured with a pipette into a 300-400 c.c. flask of the ordinary round form with flattened base. A 50 c.c. burette, with a pinchcock instead of a glass tap, is filled with the sugar

LANE AND EYNON'S METHOD

Milligrams of sugars corresponding to 10 c.c. of Fehling's solution.

c.c. of Sugar Solution Required.	*Invert Sugar, when Sucrose per 100 c.c of Solution is*					*Dextrose.*	*Anhydrous Lactose.*
	0 gm.	1 gm.	5 gm.	10 gm.	25 gm.		
15	50·5	49·9	47·6	46·1	43·4	49·1	64·9
16	50·6	50·0	47·6	46·1	43·4	49·2	64·8
17	50·7	50·1	47·6	46·1	43·4	49·3	64·8
18	50·8	50·1	47·6	46·1	43·3	49·3	64·7
19	50·8	50·2	47·6	46·1	43·3	49·4	64·7
20	50·9	50·2	47·6	46·1	43·2	49·5	64·6
21	51·0	50·2	47·6	46·1	43·2	49·5	64·6
22	51·0	50·3	47·6	46·1	43·1	49·6	64·6
23	51·1	50·3	47·6	46·1	43·0	49·7	64·5
24	51·2	50·3	47·6	46·1	42·9	49·8	64·5
25	51·2	50·4	47·6	46·0	42·8	49·8	64·5
26	51·3	50·4	47·6	46·0	42·8	49·9	64·5
27	51·4	50·4	47·6	46·0	42·7	49·9	64·4
28	51·4	50·5	47·7	46·0	42·7	50·0	64·4
29	51·5	50·5	47·7	46·0	42·6	50·0	64·4
30	51·5	50·5	47·7	46·0	42·5	50·1	64·4
31	51·6	50·6	47·7	45·9	42·5	50·2	64·4
32	51·6	50·6	47·7	45·9	42·4	50·2	64·4
33	51·7	50·6	47·7	45·9	42·3	50·3	64·4
34	51·7	50·6	47·7	45·8	42·2	50·3	64·5
35	51·8	50·7	47·7	45·8	42·2	50·4	64·5
36	51·8	50·7	47·7	45·8	42·1	50·4	64·5
37	51·9	50·7	47·7	45·7	42·0	50·5	64·5
38	51·9	50·7	47·7	45·7	42·0	50·5	64·5
39	52·0	50·8	47·7	45·7	41·9	50·6	64·5
40	52·0	50·8	47·7	45·6	41·8	50·6	64·5
41	52·1	50·8	47·7	45·6	41·8	50·7	64·6
42	52·1	50·8	47·7	45·6	41·7	50·7	64·6
43	52·2	50·8	47·7	45·5	41·6	50·8	64·6
44	52·2	50·9	47·7	45·5	41·5	50·8	64·6
45	52·3	50·9	47·7	45·4	41·4	50·9	64·7
46	52·3	50·9	47·7	45·4	41·4	50·9	64·7
47	52·4	50·9	47·7	45·3	41·3	51·0	64·8
48	52·4	50·9	47·7	45·3	41·2	51·0	64·8
49	52·5	51·0	47·7	45·2	41·1	51·0	64·8
50	52·5	51·0	47·7	45·2	41·0	51·1	64·9

LANE AND EYNON'S METHOD

Milligrams of sugars corresponding to 25 c.c. of Fehling's solution.

c.c. of Sugar Solution Required.	*Invert Sugar, when Sucrose per 100 c.c. of Solution is*		*Dextrose.*	*Anhydrous Lactose.*
	0 gm.	1 gm.		
15	123·6	122·6	120·2	163·9
16	123·6	122·7	120·2	163·5
17	123·6	122·7	120·2	163·1
18	123·7	122·7	120·2	162·8
19	123·7	122·8	120·3	162·5
20	123·8	122·8	120·3	162·3
21	123·8	122·8	120·3	162·0
22	123·9	122·9	120·4	161·8
23	123·9	122·9	120·4	161·6
24	124·0	122·9	120·5	161·5
25	124·0	123·0	120·5	161·4
26	124·1	123·0	120·6	161·2
27	124·1	123·0	120·6	161·0
28	124·2	123·1	120·7	160·8
29	124·2	123·1	120·7	160·7
30	124·3	123·1	120·8	160·6
31	124·3	123·2	120·8	160·5
32	124·4	123·2	120·8	160·4
33	124·4	123·2	120·9	160·2
34	124·5	123·3	120·9	160·1
35	124·5	123·3	121·0	160·0
36	124·6	123·3	121·0	159·8
37	124·6	123·4	121·1	159·7
38	124·7	123·4	121·2	159·6
39	124·7	123·4	121·2	159·5
40	124·8	123·4	121·2	159·4
41	124·8	123·5	121·3	159·3
42	124·9	123·5	121·4	159·2
43	124·9	123·5	121·4	159·2
44	125·0	123·6	121·5	159·1
45	125·0	123·6	121·5	159·0
46	125·1	123·6	121·6	159·0
47	125·1	123·7	121·6	158·9
48	125·2	123·7	121·7	158·8
49	125·2	123·7	121·7	158·8
50	125·3	123·8	121·8	158·7

solution to be analysed, and almost the whole volume required to reduce the Fehling's solution is run into the flask, so that only about 0·5-1 c.c. (but not less than 0·5 c.c.) is required to complete the titration. The contents of the flask are well mixed, and are then heated to the boiling point on a tripod stand covered with a plain wire gauze over a Bunsen burner. The liquid is kept in moderate ebullition for 2 minutes, and then 3-4 drops of the methylene blue solution are added, preferably without touching the sides of the flask. The titration is completed in 1 minute by the addition of the sugar solution, 2-3 drops at a time at intervals of about 10 seconds, until the colour of the indicator is completely discharged and the boiling liquid resumes the bright orange colour which it had before the indicator was added. The titration is thus completed in 3 minutes from the beginning of the ebullition, and during the whole time the flask should remain on the gauze. During the additions of the sugar solution to the boiling liquid the burette is held in the hand. Duplicate titrations by this method should agree to within 0·1 c.c. of the volume of the sugar solution required. In the titration of dextrose or invert sugar in the absence of much sucrose the results are not appreciably affected if the total period of boiling is reduced to 2 minutes or prolonged to 4-5 minutes; but with lactose and invert sugar in the presence of a large excess of sucrose, it is advisable to adhere to the total boiling period of 3 minutes.

In the tables on pp. 134 and 135 are given the weights of various sugars in milligrams corresponding to 10 and 25 c.c. of Fehling's solution. The tables strictly relate to the standard method, but with the incremental method they give results sufficiently accurate for many purposes. The effect of sucrose on the determination of invert sugar is shown in the tables, the first of which gives the values for invert sugar in the presence of 0, 1, 5, 10 and 25 gm. of sucrose per 100 c.c. of solution.

The Incremental Method.—This is carried out as follows: 10 or 25 c.c. of Fehling's solution are measured into a flask

as in the standard method. 15 c.c. of the sugar solution, or a larger volume which is known to be insufficient to reduce the Fehling's solution, are run into the flask, and the mixture is heated to the boiling point as in the standard method. If after the liquid has been boiling for 10-15 seconds its colour shows that the Fehling's solution is not completely reduced, further additions of the sugar solution are made, 10 c.c. or 5 c.c. at a time, with a few seconds' actual boiling after each, until the end-point is nearly reached. Then 3-4 drops of the methylene blue solution are added, and the addition of the sugar solution is continued, 1 c.c. or less at intervals of about 10 seconds, until the indicator is completely decolorized.

This method of titration is necessary for solutions of unknown titre, but it does not lend itself to rigorous standardization, and the results vary slightly according to the volumes of the successive increments of the sugar solution. When the highest accuracy is required, an incremental titration should be followed by a standard titration, but for many purposes single titrations by the incremental method will give results which are sufficiently accurate. Usually the total volume of sugar solution required by the incremental method is rather less than that required by the standard method, but the difference rarely amounts to 1 c.c.; and, if the conditions of the standard method are approached, the difference may be 0·5 c.c. or less, especially for volumes less than 30 c.c.

Cole's Ferricyanide - Methylene - Blue Method. — In this method, which has been devised by S. W. Cole (*Biochem. J.*, 1933, **27,** pp. 723-726), the sugar solution is added to a boiling alkaline solution of potassium ferricyanide. When the yellow colour has nearly disappeared, a drop of methylene blue solution is added, and the titration is continued until the colour is discharged. Both methylene blue and potassium ferricyanide are reduced by sugars in hot alkaline solution, but the indicator is not affected until the whole of the ferricyanide has been reduced.

The following solutions are required:

Potassium Ferricyanide Solution.—1 per cent. solution. This should be stored in a dark bottle and kept in a dark cupboard when not in use.

2·5N *Sodium Hydroxide Solution.*—This is best prepared from 45 per cent. solution that has been allowed to stand until the deposit has settled. It is diluted to about 11 per cent., and 10 c.c. are titrated with N hydrochloric acid, using methyl red as indicator. The bulk is then diluted so that 10 c.c. of the sodium hydroxide solution require 25 c.c. of N hydrochloric acid.

Methylene Blue Solution.—1 per cent. solution in water.

Apparatus.—100 c.c. flasks with rather long necks are required; also graduated 2 c.c. and 5 c.c. pipettes. By holding the pipette nearly horizontally it will be found that deliveries of about 0·02 c.c. can be made, the condensing steam washing the drop off the end of the pipette. It is necessary to add a pinch of clean dry porcelain chips, the size of small glass beads, to ensure smooth boiling.

Rough Titration.—To 20 c.c. of the ferricyanide solution and 5 c.c. of the sodium hydroxide solution in a 100 c.c. flask is added a pinch of broken porcelain. The mixture is heated to the boiling point on a wire gauze over a Bunsen flame. The sugar solution is added slowly until the yellow colour has appreciably decreased. After adding a small drop of methylene blue solution, the sugar solution is added at intervals of a few seconds until the mixture is decolorized.

Final Titration.—To 20 c.c. of the ferricyanide solution and 5 c.c. of the sodium hydroxide solution are added a pinch of broken porcelain and one small drop of methylene blue solution. To the cold mixture is added all but about 0·2 c.c. of the volume of the sugar solution which is thought to be necessary from the preliminary trial. The mixture is heated to the boiling point in about $1\frac{3}{4}$ minutes. The flame is then lowered a little, so that only gentle boiling takes place. After 1 minute the remaining sugar solution is added, a drop at a time at intervals of 10 or 15 seconds,

until the end-point is reached. The total boiling time should be more than 2 minutes, but must not exceed 3 minutes. If less than 2 c.c. of the sugar solution are required, the solution should be diluted so that between 3 and 5 c.c. are necessary. If more than 10 c.c. are necessary, it is advisable to use a burette with a pinchcock.

The quantity of sugar required to reduce 20 c.c. of the ferricyanide solution increases with the dilution. If x= the volume in c.c. of the sugar solution required to reduce the ferricyanide, the weights in mgm. of glucose and lactose in that volume are as follows:

Glucose	.. $20{\cdot}12+0{\cdot}035x$
Anhydrous lactose	.. $23{\cdot}6\ +0{\cdot}1x$

For example, if a c.c. of a glucose solution are required in the final titration, then since a c.c. of the solution contain $20{\cdot}12+0{\cdot}035a$ mgm. of glucose, 100 c.c. of the solution will contain

$$\frac{2{\cdot}012}{a}+0{\cdot}0035 \text{ gm. of glucose.}$$

Sucrose.—The sucrose is inverted by adding 10 c.c. of N/10 hydrochloric acid to 25 c.c. of the solution, boiling gently for 10 minutes in a Kjeldahl flask with a long neck, cooling, adding 10 c.c. of N/10 sodium hydroxide solution and making up to 50 or 100 c.c. The factor for the sucrose originally present is $19{\cdot}2+0{\cdot}065x$ mgm.

Lactose in Milk.—To 25 c.c. of water in a 100 c.c. flask add 5 c.c. of milk. Measure 5 c.c. of 5 per cent. " colloidal iron " in a pipette, and add it to the diluted milk, mixing by gentle agitation during the addition. Close the flask, shake and filter the contents through a dry filter paper. Determine lactose in the filtrate as described above. Most samples of cow's milk require 3-4 c.c. If x c.c. are required, then, since the volume before filtration is 7 times the volume of the milk, the anhydrous lactose in grams per 100 c.c. of milk=

$$\frac{7(23{\cdot}6+0{\cdot}1x)}{10x}=\frac{16{\cdot}52}{x}+0{\cdot}07$$

MUNSON AND WALKER'S METHOD

Expressed in milligrams.

Cuprous Oxide.	*Copper.*	*Dextrose.*	*Invert Sugar.*	*Invert Sugar if Total Sugars per 50 c.c. of Solution are*		*Hydrated Lactose.*
				0·4 *gm.*	2 *gm.*	
10	8·9	4·0	4·5	1·6	—	6·3
12	10·7	4·9	5·4	2·5	—	7·5
14	12·4	5·7	6·3	3·4	—	8·8
16	14·2	6·6	7·2	4·3	—	10·0
18	16·0	7·5	8·1	5·2	—	11·3
20	17·8	8·3	8·9	6·1	—	12·5
22	19·5	9·2	9·8	7·0	—	13·8
24	21·3	10·0	10·7	7·9	—	15·0
26	23·1	10·9	11·6	8·8	—	16·3
28	24·9	11·8	12·5	9·7	—	17·6
30	26·6	12·6	13·4	10·7	4·3	18·8
32	28·4	13·5	14·3	11·6	5·2	20·1
34	30·2	14·3	15·2	12·5	6·1	21·4
36	32·0	15·2	16·1	13·4	7·0	22·8
38	33·8	16·1	16·9	14·3	7·9	24·2
40	35·5	16·9	17·8	15·2	8·8	25·5
42	37·3	17·8	18·7	16·1	9·7	26·9
44	39·1	18·7	19·6	17·0	10·7	28·3
46	40·9	19·6	20·5	17·9	11·6	29·6
48	42·6	20·4	21·4	18·8	12·5	31·0
50	44·4	21·3	22·3	19·7	13·4	32·3
52	46.2	22·2	23·2	20·7	14·3	33·7
54	48·0	23·0	24·1	21·6	15·2	35·1
56	49·7	23·9	25·0	22·5	16·2	36·4
58	51·5	24·8	25·9	23·4	17·1	37·8
60	53·3	25·6	26·8	24·3	18·0	39·2
62	55·1	26·5	27·7	25·2	18·9	40·5
64	56·8	27·4	28·6	26·2	19·8	41·9
66	58·6	28·3	29·5	27·1	20·8	43·3
68	60·4	29·2	30·4	28·0	21·7	44·7
70	62·2	30·0	31·3	28·9	22·6	46·0
72	64·0	30·9	32·3	29·8	23·5	47·4
74	65·7	31·8	33·2	30·8	24·5	48·8
76	67·5	32·7	34·1	31·7	25·4	50·1
78	69·3	33·6	35·0	32·6	26·3	51·5
80	71·1	34·4	35·9	33·5	27·3	52·9
82	72·8	35·3	36·8	34·5	28·2	54·2
84	74·6	36 2	37·7	35·4	29·1	55·6
86	76·4	37·1	38·6	36·3	30·0	57·0
88	78·2	38·0	39·5	37·2	31·0	58·4

MUNSON AND WALKER'S METHOD—*Continued*

Expressed in milligrams.

Cuprous Oxide.	*Copper.*	*Dextrose.*	*Invert Sugar.*	*Invert Sugar if Total Sugars per 50 c.c. of Solution are*		*Hydrated Lactose.*
				0·4 *gm.*	2 *gm.*	
90	79·9	38·9	40·4	38·2	31·9	59·7
92	81·7	39·8	41·4	39·1	32·8	61·1
94	83·5	40·6	42·3	40·0	33·8	62·5
96	85·3	41·5	43·2	41·0	34·7	63·8
98	87·1	42·4	44·1	41·9	35·6	65·2
100	88·8	43·3	45·0	42·8	36·6	66·6
102	90·6	44·2	46·0	43·8	37·5	68·0
104	92·4	45·1	46·9	44·7	38·5	69·3
106	94·2	46·0	47·8	45·6	39·4	70·7
108	95·9	46·9	48·7	46·6	40·3	72·1
110	97·7	47·8	49·6	47·5	41·3	73·5
112	99·5	48·7	50·6	48·4	42·2	74·8
114	101·3	49·6	51·5	49·4	43·2	76·2
116	103·0	50·5	52·4	50·3	44·1	77·6
118	104·8	51·4	53·3	51·2	45·0	79·0
120	106·6	52·3	54·3	52·2	46·0	80·3
122	108·4	53·2	55·2	53·1	46·9	81·7
124	110·1	54·1	56·1	54·1	47·9	83·1
126	111·9	55·0	57·0	55·0	48·8	84·5
128	113·7	55·9	58·0	55·9	49·8	85·8
130	115·5	56·8	58·9	56·9	50·7	87·2
132	117·3	57·7	59·8	57·8	51·7	88·6
134	119·0	58·6	60·8	58·8	52·6	90·0
136	120·8	59·5	61·7	59·7	53·6	91·3
138	122·6	60·4	62·6	60·7	54·5	92·7
140	124·4	61·3	63·6	61·6	55·5	94·1
142	126·1	62·2	64·5	62·6	56·4	95·5
144	127·9	63·1	65·4	63·5	57·4	96·8
146	129·7	64·0	66·4	64·5	58·3	98·2
148	131·5	65·0	67·3	65·4	59·3	99·6
150	133·2	65·9	68·3	66·4	60·2	101·0
152	135·0	66·8	69·2	67·3	61·2	102·3
154	136·8	67·7	70·1	68·3	62·1	103·7
156	138·6	68·6	71·1	69·2	63·1	105·1
158	140·3	69·5	72·0	70·2	64·1	106·5
160	142·1	70·4	73·0	71·2	65·0	107·9
162	143·9	71·4	73·9	72·1	66·0	109·2
164	145·7	72·3	74·9	73·1	66·9	110·6
166	147·5	73·2	75·8	74·0	67·9	112·0
168	149·2	74·1	76·8	75·0	68·9	113·4

MUNSON AND WALKER'S METHOD—*Continued*

Expressed in milligrams.

Cuprous Oxide.	*Copper.*	*Dextrose.*	*Invert Sugar.*	*Invert Sugar if Total Sugars per 50 c.c. of Solution are*		*Hydrated Lactose.*
				0·4 *gm.*	2 *gm.*	
170	151·0	75·1	77·7	76·0	69·8	114·8
172	152·8	76·0	78·7	76·9	70·8	116·1
174	154·6	76·9	79·6	77·9	71·7	117·5
176	156·3	77·8	80·6	78·8	72·7	118·9
178	158·1	78·8	81·5	79·8	73·7	120·3
180	159·9	79·7	82·5	80·8	74·6	121·6
182	161·7	80·6	83·4	81·7	75·6	123·1
184	163·4	81·5	84·4	82·7	76·6	124·3
186	165·2	82·5	85·3	83·7	77·6	125·8
188	167·0	83·4	86·3	84·6	78·5	127·2
190	168·8	84·3	87·2	85·6	79·5	128·5
192	170·5	85·3	88·2	86·6	80·5	129·9
194	172·3	86·2	89·2	87·6	81·4	131·3
196	174·1	87·1	90·1	88·5	82·4	132·7
198	175·9	88·1	91·1	89·5	83·4	134·1
200	177·7	89·0	92·0	90·5	84·4	135·4
202	179·4	89·9	93·0	91·4	85·3	136·8
204	181·2	90·9	94·0	92·4	86·3	138·2
206	183·0	91·8	94·9	93·4	87·3	139·6
208	184·8	92·8	95·9	94·4	88·3	141·0
210	186·5	93·7	96·9	95·4	89·2	142·3
212	188·3	94·6	97·8	96·3	90·2	143·7
214	190·1	95·6	98·8	97·3	91·2	145·1
216	191·9	96·5	99·8	98·3	92·2	146·5
218	193·6	97·5	100·8	99·3	93·2	147·9
220	195·4	98·4	101·7	100·3	94·2	149·3
222	197·2	99·4	102·7	101·2	95·1	150·7
224	199·0	100·3	103·7	102·2	96·1	152·0
226	200·7	101·3	104·6	103·2	97·1	153·4
228	202·5	102·2	105·6	104·2	98·1	154·8
230	204·3	103·2	106·6	105·2	99·1	156·2
232	206·1	104·1	107·6	106·2	100·1	157·6
234	207·9	105·1	108·6	107·2	101·1	159·0
236	209·6	106·0	109·5	108·2	102·1	160·3
238	211·4	107·0	110·5	109·2	103·1	161·7
240	213·2	108·0	111·5	110·1	104·0	163·1
242	215·0	108·9	112·5	111·1	105·0	164·5
244	216·7	109·9	113·5	112·1	106·0	165·9
246	218·5	110·8	114·5	113·1	107·0	167·3
248	220·3	111·8	115·4	114·1	108·0	168.7

MUNSON AND WALKER'S METHOD—*Continued*

Expressed in milligrams.

Cuprous Oxide.	*Copper.*	*Dextrose.*	*Invert Sugar.*	*Invert Sugar if Total Sugars per 50 c.c. of Solution are*		*Hydrated Lactose.*
				0·4 *gm.*	2 *gm.*	
250	222·1	112·8	116·4	115·1	109·0	170·1
252	223·8	113·7	117·4	116·1	110·0	171·5
254	225·6	114·7	118·4	117·1	111·0	172·8
256	227·4	115·7	119·4	118·1	112·0	174·2
258	229·2	116·6	120·4	119·1	113·0	175·6
260	231·0	117·6	121·4	120·1	114·0	177·0
262	232·7	118·6	122·4	121·1	115·0	178·4
264	234·5	119·5	123·4	122·1	116·0	179·8
266	236·3	120·5	124·4	123·1	117·0	181·2
268	238·1	121·5	125·4	124·1	118·0	182·6
270	239·8	122·5	126·4	125·1	119·0	184·0
272	241·6	123·4	127·4	126·2	120·0	185·3
274	243·4	124·4	128·4	127·2	121·1	186·7
276	245·2	125·4	129·4	128·2	122·1	188·1
278	246·9	126·4	130·4	129·2	123·1	189·5
280	248·7	127·3	131·4	130·2	124·1	190·9
282	250·5	128·3	132·4	131·2	125·1	192·3
284	252·3	129·3	133·4	132·2	126·1	193·7
286	254·0	130·3	134·4	133·2	127·1	195·1
288	255·8	131·3	135·4	134·3	128·1	196·5
290	257·6	132·3	136·4	135·3	129·2	197·8
292	259·4	133·2	137·4	136·3	130·2	199·2
294	261·2	134·2	138·4	137·3	131·2	200·6
296	262·9	135·2	139·4	138·3	132·2	202·0
298	264·7	136·2	140·5	139·4	133·2	203·4
300	266·5	137·2	141·5	140·4	134·2	204·8
302	268·3	138·2	142·5	141·4	135·3	206·2
304	270·0	139·2	143·5	142·4	136·3	207·6
306	271·8	140·2	144·5	143·4	137·3	209·0
308	273·6	141·2	145·5	144·5	138·3	210·4
310	275·4	142·2	146·6	145·5	139·4	211·8
312	277·1	143·2	147·6	146·5	140·4	213·2
314	278·9	144·2	148·6	147·6	141·4	214·6
316	280·7	145·2	149·6	148·6	142·4	216·0
318	282·5	146·2	150·7	149·6	143·5	217·3
320	284·2	147·2	151·7	150·7	144·5	218·7
322	286·0	148·2	152·7	151·7	145·5	220·1
324	287·8	149·2	153·7	152·7	146·6	221·5
326	289·6	150·2	154·8	153·8	147·6	222·9
328	291·4	151·2	155·8	154·8	148·6	224·3

Munson and Walker's Method.—The method of Munson and Walker (*Methods of Analysis*, 1935, p. 479 and tables on pp. 626-629) is an official method of the Association of Official Agricultural Chemists. The asbestos for the filter is prepared as follows: Digest the asbestos with dilute hydrochloric acid (1 : 3) for 2-3 days. Wash it free from acid, and digest for the same period with 10 per cent. sodium hydroxide solution, and then treat for a few hours with hot alkaline tartrate solution (solutions which have stood for some time may be used for this purpose). Wash the asbestos free from alkali; digest for several hours with dilute nitric acid (1 : 3), and, after washing free from acid, shake with water into a fine pulp. The Gooch crucible used for the filtration is prepared by making a layer of asbestos $\frac{1}{4}$ inch thick, and washing it thoroughly with water to remove the fine particles. It is important that the directions as to the times of heating should be strictly observed. It is advisable to make preliminary tests, using 50 c.c. of Fehling's solution and 50 c.c. of water, and to regulate the burner before carrying out the actual determinations.

The procedure is as follows: Place 25 c.c. of copper sulphate solution and 25 c.c. of alkaline tartrate solution (see p. 131) in a 400 c.c. beaker and add a measured volume of the sugar solution; if the volume added is less than 50 c.c., add water to make the final volume 100 c.c. Cover the beaker with a clock glass, and heat it on an asbestos gauze over a Bunsen burner, regulating the flame so that boiling begins in 4 minutes. Boil for exactly 2 minutes, and filter the solution at once through an asbestos mat in a porcelain Gooch crucible, using suction. Wash the precipitate with water at about 60° C., and either (i) wash the precipitate with 10 c.c. of alcohol, then with 10 c.c. of ether, dry for 30 minutes at 100° C., cool in a desiccator and weigh as cuprous oxide, or (ii) determine the copper in the cuprous oxide by one of the methods described on p. 147. Carry out a blank determination, using 50 c.c. of Fehling's solution and 50 c.c. of water. If the weight of cuprous oxide exceeds

0·5 mgm., correct the result of the determination accordingly. The alkaline tartrate solution deteriorates on standing, and the quantity of cuprous oxide obtained in the blank increases.

The weights of dextrose, invert sugar and lactose are obtained from the tables on pp. 140-143. In the third column are given the weights of dextrose corresponding to the weights of cuprous oxide and copper in the first and second columns. The weight of invert sugar corresponding to each weight of cuprous oxide or copper depends on the presence or absence of sucrose. In the fourth column are given the weights of invert sugar in the absence of sucrose; and in the fifth and sixth columns are given the weights of invert sugar when the total weights of invert sugar and sucrose in 50 c.c. of the solution are 0·4 and 2 gm. respectively. In the last column are given the weights of hydrated lactose.

Allihn's Method.—For Allihn's gravimetric method (*Methods of Analysis*, 1935, pp. 482-483) the following solutions are required:

Copper Sulphate Solution.—Dissolve 34·639 gm. of crystallized copper sulphate in water and dilute the solution to 500 c.c.

Alkaline Tartrate Solution.—Dissolve 173 gm. of sodium potassium tartrate and 125 gm. of potassium hydroxide in water and dilute the solution to 500 c.c.

The determination is carried out as follows: Place in a beaker 30 c.c. of copper sulphate solution, 30 c.c. of alkaline tartrate solution and 60 c.c. of water. Heat the mixture to the boiling point, add 25 c.c of the sugar solution to be analysed, which should not contain more than 0·14 gm. of dextrose, and boil for exactly 2 minutes, keeping the beaker covered. Filter immediately through asbestos, and determine the weight of copper in the cuprous oxide by one of the methods described on p. 147. Obtain the corresponding weight of dextrose from the table on p. 146. In order to obtain the weight of invert sugar, multiply the weight of dextrose by 1·044.

ALLIHN'S METHOD

Expressed in milligrams.

Copper.	*Dextrose.*	*Copper.*	*Dextrose.*	*Copper.*	*Dextrose.*
12	7·1	102	51·9	192	98·4
14	8·1	104	52·9	194	99·4
16	9·0	106	54·0	196	100·5
18	10·0	108	55·0	198	101·5
20	11·0	110	56·0	200	102·6
22	12·0	112	57·0	202	103·7
24	13·0	114	58·0	204	104·7
26	14·0	116	59·1	206	105·8
28	15·0	118	60·1	208	106·8
30	16·0	120	61·1	210	107·9
32	17·0	122	62·1	212	109·0
34	18·0	124	63·1	214	110·0
36	18·9	126	64·2	216	111·1
38	19·9	128	65·2	218	112·1
40	20·9	130	66·2	220	113·2
42	21·9	132	67·2	222	114·3
44	22·9	134	68·2	224	115·3
46	23·9	136	69·3	226	116·4
48	24·9	138	70·3	228	117·4
50	25·9	140	71·3	230	118·5
52	26·9	142	72·3	232	119·6
54	27·9	144	73·4	234	120·7
56	28·8	146	74·4	236	121·7
58	29·8	148	75·5	238	122·8
60	30·8	150	76·5	240	123·9
62	31·8	152	77·5	242	125·0
64	32·8	154	78·6	244	126·0
66	33·8	156	79·6	246	127·1
68	34·8	158	80·7	248	128·1
70	35·8	160	81·7	250	129·2
72	36·8	162	82·6	252	130·3
74	37·8	164	83·7	254	131·4
76	38·8	166	84·8	256	132·4
78	39·8	168	85·9	258	133·5
80	40·8	170	86·9	260	134·6
82	41·8	172	87·9	262	135·7
84	42·8	174	89·0	264	136·8
86	43·9	176	90·0	266	137·8
88	44·9	178	91·1	268	138·9
90	45·9	180	92·1	270	140·0
92	46·9	182	93·1	272	141·1
94	47·9	184	94·2	274	142·2
96	48·9	186	95·2	276	143·3
98	49·9	188	96·3	278	144·4
100	50·9	190	97·3	280	145·5

Copper in Cuprous Oxide.—The following are two of the three methods adopted by the Association of Official Agricultural Chemists (*Methods of Analysis*, 1935, pp. 480-481) for the determination of copper in the cuprous oxide formed by the reduction of Fehling's solution.

Volumetric Thiosulphate Method.—Prepare a solution containing 39 gm. of crystallized sodium thiosulphate per litre. Weigh accurately 0·2-0·4 gm. of pure copper and transfer it to a 250 c.c. Erlenmeyer flask roughly graduated by marks at 20 c.c. intervals. Dissolve the copper in 5 c.c. of dilute nitric acid (1 : 1), dilute to 20-30 c.c. and boil to expel the red fumes. Then add a slight excess of bromine water and boil until the bromine is driven off. Cool and add sodium hydroxide solution, with shaking, until a slight precipitate of cupric hydroxide is formed. Dissolve the precipitate with a few drops of acetic acid and add 2 drops in excess. Prepare a solution of potassium iodide containing 42 gm. per 100 c.c. and make it very slightly alkaline to prevent the formation of hydriodic acid.

Observe the volume of the copper solution and add 1 c.c. of potassium iodide solution for each 10 c.c. of the solution. Titrate at once with the thiosulphate solution until the brown colour becomes faint. Again observe the volume of the copper solution and estimate the copper content from the volume of thiosulphate solution added. Then add more potassium iodide solution, so that at the end of the titration 4·2-5 gm. of potassium iodide will have been added for each 100 c.c. of total solution or a proportionately greater quantity if the solution contains more than 0·32 gm. of copper. Add sufficient starch indicator to produce a marked blue colour. Continue the titration cautiously until the colour changes to a faint lilac. As the end-point is approached, add the thiosulphate solution in fractions of drops, allowing the precipitate to settle after each addition. 1 c.c. of the thiosulphate solution = about 0·01 gm. of copper.

After washing the cuprous oxide, as directed on p. 144, cover the Gooch crucible with a watch glass, and dissolve

the cuprous oxide in 5 c.c. of warm dilute nitric acid (1 : 1) added from a pipette under the watch glass. Collect the filtrate in a 250 c.c. flask which is roughly graduated at 20 c.c. intervals, and wash the crucible and watch glass. Boil the solution to expel red fumes, add a slight excess of bromine water and complete the determination by following the procedure in the standardization of the thiosulphate solution.

Electrolytic Deposition of Copper.—Transfer the layer of asbestos from the Gooch crucible to a beaker by means of a glass rod, and rinse the crucible with about 30 c.c. of a boiling solution containing 65 c.c. of concentrated sulphuric acid and 50 c.c. of concentrated nitric acid per litre. Heat until solution is complete and the oxides of nitrogen have been driven off. Filter into a weighed platinum dish and dilute the solution to about 100 c.c. Deposit the copper by electrolysis at 20°-30° C. with a potential difference of about 2·5 volts and a current density of about 0·5 amp. per sq. cm. Cover the dish with a split watch glass, and continue the electrolysis for 14 hours or over-night. Then wash the dish with water; break the current, wash with alcohol, then with ether, dry at about 50° C. and weigh. If preferred, the electrolysis can be carried out in a beaker, the copper being deposited on a weighed platinum foil or gauze.*

Pentoses and Pentosans.—The method adopted by the Association of Official Agricultural Chemists (*Methods of Analysis*, 1935, pp. 344-345) for the determination of pentoses and pentosans in grain and stock feeds is given below. For this method the following reagents are required:

Dilute Hydrochloric Acid, containing 12 per cent. by weight of hydrochloric acid. To 1 volume of the concentrated acid add 2 volumes of water. Determine the percentage of hydrochloric acid by titration with standard alkali, and adjust to the correct concentration by addition of water or concentrated acid.

* It is interesting to compare this method with the determination of copper in copper sulphate by electrolysis, which is described on p. 294.

Phloroglucinol, free from diresorcinol.

Place 2-5 gm. of the sample, or a quantity which will not give more than 0·3 gm. of furfural phloroglucide, in a 300 c.c. distilling flask, together with 100 c.c. of the dilute hydrochloric acid and several pieces of recently ignited pumice stone. Place the flask on a wire gauze, connect with a condenser and heat gently at first. Then regulate the flame so as to distil over 30 c.c. in about 10 minutes. Pass the distillate through a filter paper. Replace the 30 c.c. distilled by the same quantity of the dilute acid, added by means of a separating funnel so as to wash down the particles adhering to the sides of the flask, and continue the process until the volume of the distillate amounts to 360 c.c. To the total distillate add gradually a quantity of phloroglucinol dissolved in the dilute hydrochloric acid and stir the resulting mixture. The quantity of phloroglucinol used should be about double that of the furfural expected. The solution turns yellow, then green, and very soon there appears an amorphous greenish precipitate which rapidly darkens, becoming almost black. Make the solution up to 400 c.c. with the dilute hydrochloric acid, and allow it to stand over-night. Collect the amorphous black precipitate of furfural phloroglucide in a weighed Gooch crucible having an asbestos mat. Wash it with 150 c.c. of water, so that the water is not entirely removed from the crucible until the end. Dry the crucible for 4 hours at the temperature of boiling water, cool and weigh.

The equivalent weights of furfural, pentoses and pentosans are calculated from the formulae given below, in which a=the weight of the precipitate in grams, and 0·0052 is the weight in grams of furfural phloroglucide dissolved by 400 c.c. of the acid solution.

If a is less than 0·03,

$$\text{furfural} = 0{\cdot}5170\ (a + 0{\cdot}0052),$$
$$\text{pentoses} = 1{\cdot}0170\ (a + 0{\cdot}0052),$$
$$\text{and pentosans} = 0{\cdot}8949\ (a + 0{\cdot}0052).$$

If a is between 0·03 and 0·3,

furfural = 0·5185 (a + 0·0052),
pentoses = 1·0075 (a + 0·0052),
and pentosans = 0·8866 (a + 0·0052).

If a is greater than 0·3,

furfural = 0·5180 (a + 0·0052),
pentoses = 1·0026 (a + 0·0052),
and pentosans = 0·8824 (a + 0·0052).

Hydrocyanic Acid.—The following method of determining the hydrocyanic acid formed by the hydrolysis of glucosides in beans is described by T. A. Henry and S. J. M. Auld (*J. Soc. Chem. Ind.*, 1908, **27**, pp. 428-432): A convenient quantity of the sample, which has been ground as rapidly as possible, is placed in a Soxhlet extractor and is extracted with hot alcohol, in which cyanogenetic glucosides are soluble. The alcohol is distilled off, and to the residue are added about 50 c.c. of water to which 10 c.c. of hydrochloric acid or sulphuric acid have been added. The mixture is distilled in a current of steam until hydrocyanic acid is no longer found in the distillate. Hydrocyanic acid is estimated by adding to the distillate a slight excess of sodium bicarbonate and titrating with standard iodine solution until the solution is faintly yellow owing to an excess of iodine. The quantity of hydrocyanic acid is calculated from the equation $HCN + I_2 = HI + CNI$.

Three tentative methods for the determination of hydrocyanic acid formed by the hydrolysis of glucosides in beans are given by the Association of Official Agricultural Chemists (*Methods of Analysis*, 1935, p. 348). Two of them are as follows:

Acid Titration Method.—Introduce 10-20 gm. of the sample, ground to pass a 20-mesh sieve, into an 800 c.c. Kjeldahl flask. Add 100 c.c. of water and macerate at room temperature for 2 hours. Then add 100 c.c. of water and distil with steam. Collect the distillate in 20 c.c. of N/50 silver nitrate solution acidified with 1 c.c. of concentrated nitric acid, and adjust the apparatus so that the tip

of the condenser is below the surface of the liquid in the receiver. When 150 c.c. has passed over, filter the distillate through a Gooch crucible, and titrate the excess of silver nitrate in the combined filtrate and washings with N/50 potassium thiocyanate solution, using ferric ammonium alum as indicator. 1 c.c. of N/50 silver nitrate solution = 0·00054 gm. of hydrocyanic acid.

Alkaline Titration Method.—Place 10-20 gm. of the sample, ground to pass a 20-mesh sieve, in an 800 c.c. Kjeldahl flask and add about 200 c.c. of water. Distil with steam and collect 150-160 c.c. of the distillate in sodium hydroxide solution (0·5 gm. in 20 c.c. of water). Dilute the distillate to 250 c.c. To 100 c.c. add 8 c.c. of 6N ammonia solution and 2 c.c. of 5 per cent. potassium iodide solution, and titrate with N/50 silver nitrate solution, using a micro-burette, until there is a faint but permanent turbidity, which can be easily recognized against a black background. 1 c.c. of N/50 silver nitrate solution = 0·00108 gm. of hydrocyanic acid.

Carotene.—The following method of determining carotene in grasses and fodders is described by W. S. Ferguson and G. Bishop (*Analyst*, 1936, **61,** pp. 515-518): The material is finely chopped and a suitable quantity is weighed into a 300 c.c. flat-bottomed flask. The quantity used depends on the amount of carotene and moisture in the sample. With hay, fresh grass and other fresh green crops, 10 gm. are taken, but with dried material moderately rich in carotene, such as dried grass, 2 gm. are sufficient. A suitable quantity is weighed out at the same time for the determination of moisture.

To the sample in the 300 c.c. flask are added 50 c.c. of 20 per cent. aqueous potassium hydroxide solution, and the mixture is boiled gently for 2 hours under reflux. With dried material boiling for 1 hour is sufficient. The flask is cooled, and the contents are filtered under reduced pressure through a Hirsch funnel previously packed with moistened cotton wool. The residue is washed 3 or 4 times with

ether-saturated water, the last two washings being carried out by transferring the residue to a beaker, stirring it vigorously with ether-saturated water and filtering. When the washings are free from colour, the residue is again transferred to the beaker and similarly extracted with small quantities of pure acetone until they are free from colour; about 30-40 c.c. are usually necessary for this extraction. A considerable quantity of yellow pigment is extracted by the acetone; this consists of a mixture of carotene and xanthophyll. The total aqueous and acetone extracts are transferred to a 500 c.c. separating funnel and are extracted with ether; 3 or 4 extractions are usually sufficient. The combined extracts, amounting to 150-200 c.c., are washed 4 times with water. The ethereal solution is transferred to a measuring cylinder, and the volume is noted. The solution is then ready for colour matching, but as the colour is generally too dense to match directly, suitable dilutions are made. A Lovibond tintometer (B.D.H. pattern), with a 1 cm. cell, is used for matching, and 2 or 3 comparisons are made between 2 and 5 yellow units. To obtain perfect matching, it is necessary to use 0·2 or 0·3 red units in conjunction with the yellow units. The total carotenoids, expressed as carotene, are found from a curve drawn from the following figures obtained by Ferguson (*ibid.*, 1935, **60,** pp. 680-683).

Carotene per 100 *c.c. mgm.*	*Tintometer Reading (Yellow Units.)*
0·0609	0·95
0·0812	1·30
0·1015	1·60
0·1218	2·05
0·1421	2·30
0·1624	2·80
0·1827	3·20
0·2030	3·70
0·2233	4·05
0·2436	4·55

Carotene per 100 c.c. mgm.	*Tintometer Reading (Yellow Units.)*
0·2639	5·00
0·2842	5·70
0·3045	6·10
0·3248	6·90
0·3451	7·35
0·3654	8·05
0·3857	8·75

If a Lovibond tintometer is not available, the colour of the carotene solution can be compared with 0·1 per cent. potassium dichromate solution in a Klett colorimeter. The carotene solutions are set at 20 on the scale and the matching is done by varying the height of the column of the potassium dichromate solution. The matching is easier and more accurate at the lower concentrations. The following are the results obtained by Ferguson (*loc. cit.*):

Carotene per 100 c.c. mgm.	*Depth of Column of Solution mm.*
0·0483	1·60
0·0676	2·26
0·0966	3·21
0·1256	4·19
0·1546	5·16
0·1800	6·04
0·2029	6·85
0·2318	7·65
0·2705	8·79
0·3280	10·50

To effect the separation of carotene and xanthophyll, 100 c.c. of the total carotenoid solution are transferred to a 250 c.c. distilling flask, and the ether is removed in a stream of nitrogen on a water bath at not more than 40° C. The residue is washed out of the flask into a 200 c.c. separating funnel with 100 c.c. of petroleum spirit. If the material does not dissolve readily, a few c.c. of ether can be used,

followed by 92 per cent. methyl alcohol. The petroleum spirit solution is extracted exhaustively with 92 per cent. methyl alcohol, which removes the xanthophyll. If the petroleum spirit fraction is cloudy, it is clarified by adding a few drops of ethyl alcohol, and the carotene is then determined by matching it in a Lovibond tintometer or a colorimeter after the necessary dilution. Similarly the xanthophyll is estimated in the methyl alcohol extract. From the two values obtained the ratio of carotene to xanthophyll is determined; and from this ratio the carotene content of the original extract is calculated.

Silage.—The following are some of the methods for the evaluation of silage described by S. J. Watson and W. S. Ferguson (*J. Agric. Sci.*, 1937, **27,** pp. 1-42).

Preparation of the Sample.—A sample of 2-3 lb. in weight is cut into short lengths by means of a laboratory chaff-cutter and thoroughly mixed. The requisite sub-samples are drawn from the well-mixed mass.

Moisture.—Two samples of 200 gm. each are dried at 98° C. in a steam oven with an efficient circulation of air. The samples are weighed after being in the oven for 3-3½ hours, replaced and weighed at intervals of ½-¾ hour until the weights are constant. To obtain the true weight of the dry matter it is necessary to make corrections for the volatile acids and bases lost during the drying. The determination of the total volatile acids (see below) before and after drying samples of silage at 98° C. showed that the losses of volatile acids ranged from 50 to 92 per cent. The results are as follows:

No. of Samples Examined	*Volatile Acids of Fresh Silage (as acetic acid) per cent.*	*Loss of Volatile Acids per cent.*	
		Mean	*Range*
10	0 -0·49	68·1	50·1-78·4
31	0·50-0·99	77·7	55·2-92·2
21	1·00-1·49	75·9	59·5-91·3
5	1·50-1·99	84·2	73·6-90·2

Crude Protein.—Nitrogen is determined on the dry material by the Kjeldahl method, using selenium as catalyst (see p. 14), and the percentage of nitrogen is multiplied by 6·25. The crude protein is corrected for the nitrogenous substances lost during the drying. The usual measure of protein breakdown is the ratio of true to crude protein. The loss of volatile bases decreases the figure for crude protein and raises the proportion of true protein in the crude protein. In the following table are given the losses of volatile bases on drying samples of silage at 98° C.

No. of Samples Examined	*Volatile Bases of Fresh Silage (as crude protein) per cent.*	*Loss of Volatile Bases per cent.*	
		Mean	*Range*
26	0 -0·49	32·2	0 -65·5
32	0·50-0·99	56·9	15·0-90·8
9	1·00-2·09	80·6	61·9-93·7

These figures are also used to correct the dry matter content.

Preparation of the Extract.—To 100 gm. of finely chopped silage are added 500 c.c. of distilled water in a litre cylinder. The extraction is then carried out either by shaking in an end-over-end shaker for 4 hours or by allowing to stand for 24 hours with occasional shaking. The liquid is then poured through a coarse cloth and the silage is pressed by hand. The total extract is stirred thoroughly and then filtered, the filtrate being discarded until it comes through perfectly clear. 60 c.c. of the extract are placed in a 200 c.c. measuring flask and made up to the mark with neutral alcohol. The solution is filtered and used for the following determinations.

Total Acidity.—To 10 c.c. of the filtered alcoholic extract are added 50 c.c. of neutral alcohol. The solution is titrated with N/10 sodium hydroxide solution, using phenolphthalein as indicator.

Volatile Bases.—To 50 c.c. of the alcoholic extract in a 250 c.c. distilling flask is added an amount of N/10 sodium

hydroxide solution sufficient to react with all the acidic groups present (total acidity value $\times$ 5). The solution is then vigorously distilled in steam for 7 minutes, the distillate being led into a known volume of N/10 sulphuric acid. The excess of acid is then titrated with N/10 sodium hydroxide solution, using alizarin red as indicator. The difference is the amount which has combined with the volatile bases.

Amino Acids.—The residue in the flask develops alkalinity due to the release of the basic amino groups on the removal of the alcohol. The titration of this alkalinity with N/10 sulphuric acid, using phenolphthalein as indicator, is a measure of the amino acids.

Volatile Acids.—To the neutral residue in the flask is added sufficient N/10 sulphuric acid to release all the acids present. The total acid added, including that used for neutralizing the amino groups, should be equivalent to the amount of N/10 sodium hydroxide solution added before the removal of the volatile bases. The solution is then distilled in steam, the distillate being collected in quantities of 250 c.c. When the quantity of volatile acids is low or medium, 500 c.c. of distillate are collected; but when the volatile acid content is high, the collection of 750 c.c. is advisable. The volatile acids are titrated with N/10 sodium hydroxide solution, using phenolphthalein as indicator.

Calculation of Results.—Assuming that the weight of liquids present in the silage is equivalent to an equal weight of water, the total volume of original extract available from 100 gm. of fresh silage is 500+(100−dry matter content) c.c. Suppose this volume is V c.c. Then if the total acidity titration figure is x, the total acidity as a percentage of the fresh silage is $20x\mathrm{V}/60$. For the other values the calculation is $4y\mathrm{V}/60$, where y is the titration figure.

Residual Acidity.—The acidity due to non-volatile acidic bodies, other than amino acids, is obtained by subtracting the sum of the amino acids and volatile acids from the total acidity. This value approximates to the lactic acid content

except when the silage is made by the addition of mineral acids.

Hydrogen-ion Concentration.—The pH value of the juice expressed from the fresh silage is determined electrometrically, using a quinhydrone electrode and a calomel half cell.

THE MINERAL CONSTITUENTS OF FEEDING STUFFS

In the determination of the mineral constituents of feeding stuffs the organic matter is first removed and a solution for the analysis is prepared. The organic matter can be oxidized by digestion with sulphuric acid or a mixture of sulphuric and nitric acids, but it is the usual practice to incinerate the sample and extract the ash with hydrochloric acid. The Imperial Bureau of Animal Nutrition (*Tech. Comm.* 9, 1937, edited by W. Godden) recommend that, for the sake of uniformity and also to conform with the generally accepted mode of expression in human nutrition investigations, results should be expressed as the percentages of the elements in the oven-dry material. If the moisture in the original material has been determined, the results can, if desired, be calculated back to the original moisture content.

Some of the A.O.A.C. methods for the analysis of plants are given on p. 166. They include the determination of silica, iron, aluminium, calcium and magnesium in the same quantity of the sample. Calcium is precipitated as calcium oxalate at pH 5·0 and magnesium is precipitated as magnesium ammonium phosphate. Iron and aluminium are precipitated as phosphates; iron is determined by titration with potassium permanganate in the weighed precipitate and aluminium is found by difference. This method does not admit of any great accuracy with the very small quantities of iron and aluminium normally occurring in feeding stuffs. Iron is more accurately determined by the colori-

metric method depending on the formation of the red-coloured thiocyanate and its extraction with amyl alcohol, as described on p. 169. A very accurate way of determining small quantities of aluminium is the colorimetric method depending on the formation of a red-coloured lake of aluminium hydroxide and aurin tricarboxylic acid. This method was thoroughly investigated by Lampitt and Sylvester, and their procedure has been applied by Shorland to the determination of aluminium in pasture grass. Both procedures are given fully on pp. 170-175. Owing to the interference of iron, it is necessary to separate this metal from the aluminium. This is most conveniently carried out by precipitating the iron and aluminium together as hydroxides by means of ammonia and then treating the mixed hydroxides with sodium hydroxide. Lampitt and Sylvester found that it is very important to carry out the precipitation of the mixed hydroxides in an almost neutral solution. A slight excess of ammonia or a deficiency caused by prolonged boiling gives low results owing to the solubility of the aluminium hydroxide. If the aurin tricarboxylic acid lake is produced under strictly controlled conditions, the colour can be measured by means of the Lovibond tintometer. Lampitt and Sylvester showed that the red component of the colour is directly proportional to the aluminium present up to 0·06 mgm. in 50 c.c. of the test solution.

Very small quantities of manganese can be accurately determined by the method of Willard and Greathouse, in which the manganese is oxidized by sodium or potassium periodate to permanganate and the colour of the resulting solution is compared with a solution prepared in the same way from a known weight of manganese. This method was applied to the determination of manganese in grass and milk by Richards, whose procedures are given on p. 175.

The most sensitive reagent for the colorimetric determination of copper is sodium diethyldithiocarbamate, which was introduced by T. Callan and J. A. R. Henderson (*Analyst*, 1929, **54,** pp. 650-653). McFarlane found that the golden

brown copper salt is much more soluble in amyl alcohol than in water, and can be quantitatively extracted from an aqueous solution by amyl alcohol. It is thus possible to use the method over a much wider range of copper concentrations. McFarlane's procedure is given on p. 178.

Zinc is determined in feeding stuffs by the oxalate-ferrocyanide method, which is based on the fact that the ferrocyanides of iron, lead, manganese, arsenic and calcium are soluble in 7-8 per cent. oxalic acid solution which also contains enough hydrochloric acid to make it half normal in free acid, but the ferrocyanides of zinc and copper are insoluble in the same mixture. In Hibbard's procedure, which is described on p. 179, the copper is precipitated as cupric sulphide. After filtering, the zinc is precipitated as the ferrocyanide in the presence of oxalic and hydrochloric acids. The zinc ferrocyanide is converted into zinc sulphide and the zinc is determined nephelometrically. Stare and Elvehjem's method of determining cobalt in foodstuffs is given on p. 180. It depends on the formation of a red dye when a cobalt salt is treated with nitroso-R-salt in a faintly alkaline solution. The red colour formed is very stable and is not destroyed by heating with acids. Iron, copper and zinc form coloured dyes with nitroso-R-salt, but these are destroyed by boiling with nitric acid.

The determination of potassium and sodium in feeding stuffs was formerly carried out by dissolving the ash in hydrochloric acid and precipitating the iron group with ammonia, the sulphate with barium hydroxide, the excess of barium and the calcium and magnesium with ammonium carbonate and ammonium oxalate, then removing the ammonium salts by volatilization and weighing the alkali chlorides.* This process, involving so many precipitations and filtrations, is not only very laborious but is subject to considerable errors. Unless special care is taken the mixed

* For the full details see *Methods of Analysis*, 1935, p. 125, and Hillebrand and Lundell, *Applied Inorganic Analysis*, 1929, pp. 794-795.

chlorides will contain some magnesium as the basic chloride, while losses of potassium and sodium may take place if the chlorides are ignited at too high a temperature. Husband and Godden have shortened the process, and at the same time made it more accurate, by using an alcoholic solution of ammonium carbonate to precipitate the alkaline earth metals. This solution, which is due to F. A. Gooch and E. A. Eddy (*Z. anorg. Chem.*, 1908, **58,** pp. 427-432), completely precipitates the magnesium. Husband and Godden's procedure, which is given in full on p. 181, involves only three precipitations: the first with barium chloride, the second with ammonia and the third with the alcoholic solution of ammonium carbonate. An aliquot part of the filtrate from the last precipitate is evaporated to dryness; the residue is heated with concentrated sulphuric acid and the sulphates of potassium and sodium are weighed.

Potassium can be determined in the mixed sulphates by the volumetric cobaltinitrite method or by the perchlorate method after precipitating the potassium as potassium sodium cobaltinitrite (see p. 78). The sodium is generally found by difference but, if an excess of potassium is not present, it can be determined directly in the nitric acid extract of the ash, as described on p. 238. The volumetric cobaltinitrite method, which is due to W. A. Drushel (*Amer. J. Sci.*, 1907, 4th Ser., **24,** pp. 433-438), has undergone many modifications. Green's procedure, which is essentially that of Drushel, is given on p. 182. It consists in precipitating the potassium as potassium sodium cobaltinitrite, which is filtered off and washed. In order to increase the size of the particles and lower their solubility, the solution is evaporated to a pasty condition with an excess of cobaltinitrite reagent. The precipitate is oxidized by adding it to a measured volume of standard potassium permanganate solution acidified with sulphuric acid. Standard oxalic acid solution is added, and the excess of that solution is titrated with the potassium permanganate solution. In the titration of the precipitate the cobalt is reduced from

the cobaltic to the cobaltous state on the addition of oxalic acid, and is not reoxidized on the subsequent addition of potassium permanganate. Hence, the quantity of permanganate required is eleven-twelfths of that required for the oxidation of the nitrite. Assuming that the formula of the precipitate is $K_2NaCo(NO_2)_6,H_2O$, and taking the molecular weight of potassium oxide as 94·192, 1 litre of N potassium permanganate solution = 94·192/11 = 8·563 gm. of potassium oxide, or 1 c.c. of N/10 potassium permanganate solution = 0·0008563 gm. of potassium oxide. It will be explained later that the ratio K : Na in the precipitate is not 2 : 1. Hence, if accurate results are obtained by using the theoretical factor, they are due to compensating errors. More reliable results will be obtained by standardizing the permanganate solution with a solution containing a known weight of a pure potassium salt.

In the method of Lewis and Marmoy, which is described on p. 184, the potassium is precipitated by adding cobaltinitrite reagent drop by drop with shaking. The precipitate is separated by centrifuging and is washed with 70 per cent. alcohol, in which it is less soluble than in dilute acetic acid. The potassium is determined indirectly by determining the cobalt in the precipitate. The cobalt is determined colorimetrically by the thiocyanate or the choline-ferrocyanide method; the former method is considered by Lewis and Marmoy to be slightly preferable to the latter, because blue solutions are easier to match than green ones. Although the precipitate is constant in composition, it contains less than the theoretical quantity of potassium and more of the other constituents. Hence, instead of using a solution of cobalt of known concentration in preparing the colour standards, the cobalt solution used as the standard is standardized against the cobalt contained in the cobaltinitrite precipitate obtained from a potassium solution of known concentration. Potassium can also be determined by Kramer and Tisdall's method (see p. 187). This consists in centrifuging the precipitate, washing it with water, and then

oxidizing it with standard potassium permanganate solution, as in Drushel's method. Owing to compensation between positive and negative errors, the recovery of potassium was found by Lewis and Marmoy to be theoretical; but variations in technique affect the permanganate conversion factor to a considerable extent.

Piper made an exhaustive examination of the volumetric cobaltinitrite method, and has shown how the variation in the composition of the precipitate can be allowed for in calculating the results. His procedure is given on p. 187. Preliminary experiments showed that the ratio of potassium to sodium increases as the quantity of potassium precipitated increases. The composition of the precipitate also varies with the quantity of cobaltinitrite reagent added, the rate at which it is added and the temperature at the time of the precipitation; but the effect of temperature is less when precipitation is brought about by the successive additions of acetic acid, sodium chloride, sodium nitrite and cobalt nitrate. This method of precipitating the potassium was therefore adopted; it has the additional advantage that the separate solutions are stable, whereas a solution of sodium cobaltinitrite will not keep for any length of time. The precipitate is washed with a saturated solution of potassium sodium cobaltinitrite. Closely agreeing results were obtained by washing the precipitate with 35 per cent. alcohol and removing the last traces of alcohol by washing three times with very small quantities of water. The washed precipitate is oxidized by adding it to cold acidified permanganate solution and then heating the solution to the boiling point, thus avoiding the loss of nitrous acid which may occur when the precipitate is added to a boiling acid permanganate solution. The determination is then completed exactly as in Drushel's method.

Determinations carried out in this way with measured volumes of pure potassium chloride solution, containing amounts corresponding to 0·1-50 mgm. of potassium oxide, showed that the volume of standard permanganate solu-

tion required to oxidize the precipitate is not proportional to the weight of potassium oxide taken. When these values were plotted, a smooth curve was obtained, which can be expressed by the equation $W=0{\cdot}354V+0{\cdot}00034V^2$, where W=the weight of potassium oxide (in mgm.) and V=the volume of N/20 potassium permanganate solution required to oxidize the precipitate (in c.c.). The quantity of potassium can thus be calculated, although the composition of the precipitate is not constant. Piper calculated that the ratio K : Na in the precipitate obtained from 1 mgm. of potassium oxide is 1·66 : 1·34, and the ratio is not 2 : 1 until 93 mgm. of potassium oxide are precipitated.

In the British official method of determining phosphorus in feeding stuffs the organic matter is oxidized by heating with concentrated sulphuric acid, and the phosphorus is precipitated first as ammonium phosphomolybdate, then as magnesium ammonium phosphate, which is ignited and weighed as magnesium pyrophosphate. The procedure is given on p. 189, and is immediately followed by the procedure used by Ashton, which serves to supplement the scanty details of the official method.

Other methods of determining phosphorus are Pemberton's method (see p. 38) and Richards and Godden's modification of Neumann's method, in both of which the weight of phosphorus in the precipitated ammonium phosphomolybdate is ascertained by titration. In Richards and Godden's method, which is fully described on p. 190, the organic matter is oxidized by heating the sample with a mixture of concentrated sulphuric and nitric acids, and the precipitated ammonium phosphomolybdate is washed first with dilute nitric acid, then with ammonium nitrate solution and finally with water. The washed precipitate is boiled with an excess of N/2 sodium hydroxide solution and the hot solution is titrated with N/2 sulphuric acid until it is acid. After again boiling and cooling, the excess of acid is titrated with N/2 sodium hydroxide solution. Under these conditions the molecular ratio of P_2O_5 to NaOH is 1:52, or

1 c.c. of N/2 sodium hydroxide solution = 0·001365 gm. of phosphorus pentoxide.

It has been explained on p. 38 that in Pemberton's method the molecular ratio of P_2O_5 to NaOH is 1 : 46. It is therefore necessary to explain why the molecular ratios in the two methods are different. Both methods are based on the work of F. Hundeshagen (*Z. anal. Chem.*, 1889, **28,** pp. 141-172), who showed that the composition of the yellow phosphomolybdate precipitate obtained in the presence of nitric acid and washed with dilute nitric acid is $(NH_4)_3PO_4,12MoO_3,2HNO_3$. The nitric acid is very loosely combined and may be removed by heating to 130° C. or by washing with ammonium nitrate solution. Pemberton concluded that the composition of the washed precipitate is $(NH_4)_3PO_4,12MoO_3$, and calculated his factor from the following equation:

$$2(NH_4)_3PO_4,24MoO_3+46NaOH=2(NH_4)_2HPO_4+(NH_4)_2MoO_4+23Na_2MoO_4+22H_2O.$$

This represents the reaction which takes place when the washed precipitate is treated with sodium hydroxide at the ordinary temperature. But when the washed precipitate is boiled with sodium hydroxide solution, as in Richards and Godden's method, the ammonium in the precipitate is replaced by sodium with the liberation of ammonia, as shown in the following equation:

$$2(NH_4)_3PO_4,24MoO_3+52NaOH=24Na_2MoO_4+2Na_2HPO_4+28H_2O+6NH_3.$$

Phosphorus can also be determined by one of the colorimetric methods depending on the blue colour produced by the reduction of ammonium molybdate in the presence of sulphuric acid and phosphoric acid. In Fiske and Subbarow's method the reducing agent is aminonaphtholsulphonic acid. Greenhill and Pollard have applied this method to the determination of phosphorus in dried grass. Their procedure is given on p. 192. If the organic matter in a feeding stuff is oxidized by incineration, there is always

a possibility that phosphoric acid in organic combination may be lost during the process and that some of the phosphoric acid may be converted into meta- and pyro-phosphates which cannot be extracted from the ash. These losses can be avoided either by oxidizing the organic matter by digestion with a mixture of concentrated sulphuric and nitric acids, as in Richards and Godden's method, or by adding magnesium nitrate to the sample before incineration, as in Greenhill and Pollard's procedure. F. L. Ashton (*J. Soc. Chem. Ind.*, 1936, **55,** pp. 106-108T) determined the percentage of phosphorus pentoxide in grass by the wet combustion method described on p. 190 and compared the results with those obtained by incinerating the samples at different temperatures, with and without the addition of magnesium nitrate. He found that the addition of magnesium nitrate is not necessary if the grass is ashed at a temperature below 600° C. At 800° C. losses of phosphate occur in the absence of magnesium nitrate, and at 1000° C. considerable losses take place whether magnesium nitrate is added or not.

Total sulphur in feeding stuffs is determined by oxidizing the sulphur to sulphate and determining the latter by precipitation as barium sulphate. In the A.O.A.C. official method, which is given on p. 193, the oxidation is effected by heating the sample with sodium peroxide in a nickel crucible. The heating requires very careful attention, because if the sample catches fire the determination is spoilt. A quicker and more certain way of oxidizing the sulphur is to use a Hodsman bomb instead of a nickel crucible. This method, which was introduced by Woodman and Evans, is described on p. 194. In Aitken's method, which is given on p. 196, the sample is treated with Benedict-Denis reagent, which contains copper nitrate. The mixture is evaporated to dryness and then ignited, when the copper oxide formed from the copper nitrate oxidizes the sulphur in the sample. Owing to the reduction of sulphates and oxidation of organic sulphur during ignition of the sample,

sulphate sulphur is determined by extracting sulphates with dilute hydrochloric acid from the finely ground sample, as described on pp. 196 and 197. The organic sulphur is found by subtracting the sulphate sulphur from the total sulphur.

When a foodstuff is incinerated at a dull red heat there is a considerable loss of chlorine. To prevent this loss in the determination of chlorine, the sample is mixed with either lime or sodium carbonate before ignition. The ash is treated with nitric acid, and chlorine is determined in the filtered solution either volumetrically or gravimetrically. Three procedures are given on p. 198. Leitch and Henderson's method of determining iodine is described on p. 199. The sample is strongly heated with potassium hydroxide and the ash is extracted with alcohol. After removing the alcohol and acidifying the solution, the iodide is oxidized to iodate by boiling it with bromine water. Potassium iodide is then added, and the liberated iodine is titrated with N/500 sodium thiosulphate solution. Although the iodine formed by the reaction between the iodate and iodide is six times the quantity originally present in the sample, it is still very small in amount. A 0·1 c.c. serum pipette graduated in thousandths of a c.c. is therefore used in the titration.

Silica, Iron, Aluminium, Calcium and Magnesium.—The following are methods adopted by the Association of Official Agricultural Chemists (*Methods of Analysis*, 1935, pp. 121-124) for the analysis of plant ash:

Sand and Silica.—Ignite 10-50 gm. of the sample in a flat-bottomed platinum dish in a muffle, at a temperature not exceeding dull redness, until the ash is white or nearly white. Moisten with 5 c.c. of concentrated hydrochloric acid, evaporate to dryness and heat on a water bath for 3 hours to render the silica insoluble. Moisten the residue with 5 c.c. of concentrated hydrochloric acid, add about 50 c.c. of water, heat on a water bath for a few minutes, filter through a hardened filter paper and wash thoroughly. To this filtrate add the filtrate and washings from the alkali-

soluble silica determination and dilute to 200 c.c. This solution is referred to as solution A.

Wash the residue from the filter into a platinum dish and boil it with about 20 c.c. of saturated sodium carbonate solution for about 5 minutes; then add a few drops of 10 per cent. sodium hydroxide solution, allow the mixture to settle and decant through an ignited and weighed Gooch crucible. Boil the residue in the dish with another 20 c.c. of the sodium carbonate solution and decant as before. Repeat the process. Transfer the residue to the Gooch crucible and wash it thoroughly, first with hot water, then with a little dilute hydrochloric acid (1 : 4), and finally with hot water until free from chlorides. Dry the filter and contents, ignite and weigh as sand.

Mix the alkaline filtrate and washings, acidify with hydrochloric acid, evaporate to dryness. Add 5 c.c. of concentrated hydrochloric acid, again evaporate and dehydrate by heating to 110°-120° C. for 2 hours. Moisten the residue with 5-10 c.c. of concentrated hydrochloric acid, add about 50 c.c. of water and heat on a water bath for 10-15 minutes. Filter through an ashless filter paper or an ignited and weighed Gooch crucible. Wash the silica with hot water, ignite it and weigh as alkali-soluble silica.

Iron and Aluminium.—In an aliquot part of solution A, containing about 40 mgm. of iron and aluminium phosphates, oxidize the iron. If the solution does not contain an excess of phosphate, add to the solution 0·5 gm. of diammonium hydrogen phosphate, stir until it is dissolved and dilute the solution to 50 c.c. with distilled water. Add a few drops of thymol blue and then ammonia until the solution just turns yellow. Add 0·5 c.c. of concentrated hydrochloric acid; then add 25 c.c. of 25 per cent. ammonium acetate solution and stir. Allow to stand at room temperature until the precipitate settles (about 1 hour). Then filter and wash the precipitate 10 times with hot 5 per cent. ammonium nitrate solution. Ignite and weigh as iron and aluminium phosphates.

Fuse the ignited precipitate in a platinum crucible with about 4 gm. of a mixture of equal parts of anhydrous sodium carbonate and potassium carbonate. When the fusion is complete, allow the crucible to cool. Then add 5 c.c. of concentrated sulphuric acid, and heat until copious fumes of sulphur trioxide are given off. When cool, transfer the residue to a flask with water, and digest until the solution is clear. Reduce the iron with zinc and, when cool, titrate the solution with N/10 potassium permanganate solution.* Calculate the iron to phosphate and subtract it from the mixed phosphates. The difference is aluminium phosphate.†

Calcium.—Transfer an aliquot part of solution A to a 200 c.c. beaker and add water, if necessary, to make the volume 50 c.c. Heat to the boiling point and add 10 c.c. of saturated ammonium oxalate solution and a drop of methyl red indicator. Almost neutralize with ammonia and boil until the precipitate is coarsely granular. Cool, add dilute ammonia (1 : 4) until the colour is a faint pink (pH 5·0) and allow to stand at least 4 hours. Filter and wash with water at room temperature until the filtrate is free from oxalate. Break the point of the filter with a platinum wire and wash the precipitate into the beaker in which the calcium was precipitated with hot dilute sulphuric acid (1 : 4) and hot water. Add about 10 c.c. of dilute sulphuric acid (1 : 4) and heat to about 90° C. Add about 50 c.c. of hot water and titrate with N/20 potassium permanganate solution. Finally add the filter paper to the solution and complete the titration.‡

Magnesium.—To the combined filtrate and washings from the calcium determination add 30 c.c. of concentrated

* 1 c.c. of N/10 potassium permanganate solution = 0·005584 gm. of iron and 0·015086 gm. of ferric phosphate.

† The weight of aluminium phosphate × 0·22108 = the weight of aluminium.

‡ 1 c.c. of N/20 potassium permanganate solution = 0·0010 gm. of calcium.

nitric acid and evaporate to dryness to decompose the ammonium salts. Take up the residue with 5 c.c. of concentrated hydrochloric acid and dilute with water to about 100 c.c. Add 5 c.c. of 10 per cent. sodium citrate solution and 10 c.c. of 10 per cent. diammonium hydrogen phosphate solution or enough to precipitate all the magnesium. Add dilute ammonia (1:4), with constant stirring, until the solution is faintly alkaline; then add about 5 c.c. of concentrated ammonia, stir vigorously and allow to stand in a cool place over-night. Filter and wash the precipitate with dilute ammonia (1:10). Ignite and weigh as magnesium pyrophosphate.*

Iron.—The following method of determining iron in foodstuffs, which is based on that of W. D. McFarlane (*Biochem. J.*, 1932, **26,** pp. 1034-1049), has been taken from Technical Communication No. 9, Imperial Bureau of Animal Nutrition. The following reagents are required:

Hydrochloric Acid, 6N.

Nitric Acid, concentrated and free from oxides of nitrogen.

Amyl Alcohol, Analar.

Potassium Thiocyanate Solution, 20 per cent.

Standard Iron Solution.—3·514 gm. of ferrous ammonium sulphate are dissolved in the minimum quantity of distilled water. To this solution 100 c.c. of 10 per cent. sulphuric acid are added and mixed. Then 17 c.c. of pure concentrated nitric acid are added to convert the iron to the ferric state. The mixture is allowed to stand over-night and then made up to 1 litre with water. This solution keeps indefinitely without the formation of any precipitate. 1 c.c.=0·5 mgm. of iron. For actual matching 25 c.c. of this solution are diluted to 250 c.c. 0·2 c.c. of the diluted solution=0·01 mgm. of iron.

* The weight of magnesium pyrophosphate × 0·21843 = the weight of magnesium. If preferred, the precipitate after removal of the free ammonia can be dissolved in standard acid, the excess of which is titrated with standard alkali, as described on p. 47.

The purest obtainable reagents must be used and must be tested for the presence of iron. Water redistilled from glass must be used throughout.

Preparation of the Extract.—About 7 gm. of the sample, the amount varying with the class of material analysed, are ashed in a vitreosil basin at a low temperature, cooled, moistened with about 1 c.c. of nitric acid, dried on a hot plate and again ashed. This is repeated. The ash is then cooled, moistened with hydrochloric acid, dried on a hot plate and ashed. This also is repeated. The residual ash is now taken up with about 14 c.c. of 6N hydrochloric acid and 50 c.c. of water, and left on a hot plate for at least 1 hour. It is then cooled, diluted and washed into a 100 c.c. flask and made up to the mark. This solution is also used for the determination of copper (see p. 178).

Determination.—An aliquot part of the extract, usually 1 c.c., is pipetted into a 50 c.c. flask and diluted; 1 drop of concentrated nitric acid is added and the solution is allowed to stand for half an hour. To it are added 10 c.c. of amyl alcohol (carefully measured) and then 5 c.c. of potassium thiocyanate solution. The mixture is shaken, made up to the mark, and the amyl alcohol layer pipetted into the colorimeter cup for comparison. If necessary, the amyl alcohol may be filtered prior to matching. To prepare the standard for comparison, 0·2 c.c. of the diluted standard iron solution is pipetted into a 50 c.c. flask and diluted. To it are added 1 drop of concentrated nitric acid and 0·1 c.c. of 6N hydrochloric acid. After standing half an hour, 10 c.c. of amyl alcohol and 5 c.c. of potassium thiocyanate solution are added; the mixture is shaken and made up to the mark. The amyl alcohol layer is pipetted off and used for the colour comparison against the unknown. It is advisable to adjust the amount of the unknown so that the readings on both sides of the colorimeter are nearly equal.

Aluminium.—L. H. Lampitt and N. D. Sylvester (*Analyst*, 1932, **57**, pp. 418-428) found that, for small amounts of

aluminium, the colour obtained with aurin tricarboxylic acid is orange; and, as larger amounts are taken, the red colour increases and the yellow decreases until the colour is pure red. For still larger amounts the colour is bluish red, the red and the blue increasing as the amount of aluminium is increased. It is therefore impossible to use a colorimeter of the Duboscq type for the colour measurements unless the amounts of aluminium in the solutions to be compared are nearly the same. The use of Nessler tubes would involve the preparation of a range of standards for every determination. These difficulties were overcome by using the Lovibond tintometer and measuring the colour values of solutions containing known amounts of aluminium. From the following table it will be seen that the red colour value (series No. 200) is directly proportional to the amount of aluminium up to 0·06 mgm., and that the yellow colour is of no value as a factor in the determination. If the red units and the weights of aluminium given in the table are plotted, the unknown aluminium content of other solutions can be found from the graph.

Mgm. of Aluminium.	*Colour Values of the Solutions in Tintometer Units.*		
	Red (*No.* 200).	*Yellow* (*No.* 510).	*Blue* (*No.* 1180).
nil	0·55	0·80	—
0·01	1·35	0·70	—
0·02	2·35	0·55	—
0·03	3·20	0·40	—
0·04	4·10	0·30	—
0·05	4·90	0·25	—
0·06	5·80	0·15	—
0·07	6·95	0·10	—
0·08	8·70	—	—
0·09	11·00	—	0·10
0·10	13·70	—	0·40

For preparing the colour standards, and for determining the aluminium content of the solution to be analysed, the following solutions are required:

Standard Aluminium Solutions.—1·757 gm. of crystallized potassium alum are dissolved in water containing 25 c.c. of 5N hydrochloric acid and made up to 1000 c.c. 1 c.c. of this solution = 0·0001 gm. of aluminium. This solution is diluted 5 times to give a standard solution, 1 c.c. of which = 0·02 mgm. of aluminium.

Aurin Tricarboxylic Acid Solution.—2 gm. of aurin tricarboxylic acid are dissolved in water containing a slight excess of ammonia. The solution is boiled to expel the excess of ammonia and is then made up to 1 litre with distilled water.

5N Ammonium Acetate Solution.—A solution containing 386 gm. of the salt per litre.

Glycerol Solution.—A solution containing equal volumes of glycerol and water.

Ammonium Hydroxide-Carbonate Solution.—Equal volumes of 10N ammonium hydroxide and 2N ammonium carbonate solutions are mixed. The solution should be kept in a stoppered bottle and examined occasionally to find if it has deteriorated by evaporation.

The following details must be strictly observed: 5 c.c. of a neutral solution containing aluminium are placed in a 100 c.c. conical flask. Exactly 2 c.c. of 5N hydrochloric acid, 5 c.c. of 5N ammonium acetate solution, 20 c.c. of glycerol solution and 5 c.c. of aurin tricarboxylic acid solution are added. The contents of the flask are well mixed, and the flask is immersed in boiling water for 5 minutes and then cooled in a mixture of ice and water for at least 5 minutes. The contents of the flask are washed into a 50 c.c. graduated flask containing exactly 3 c.c. of ammonium hydroxide-carbonate solution and mixed. After making up to the mark with water and again thoroughly mixing, the colour of the solution is measured in a $\frac{1}{2}$-inch cell in a Lovibond tintometer exactly 5 minutes after neutralization with the ammonium hydroxide-carbonate solution.

The determination of aluminium in food materials is carried out as follows: 10 gm. of foodstuff are heated in a silica flask with nitric acid and sulphuric acid until a colourless residue is obtained. When cool, this is diluted with 50 c.c. of water, care being taken to ensure the complete solution of the aluminium sulphate. A slight excess of ammonia is then added and the solution is boiled until it no longer smells of ammonia. The excess of ammonia must be removed, otherwise an appreciable loss of aluminium will occur. But if the boiling is unduly prolonged, the solution becomes acid and the precipitated aluminium is dissolved. When the operation is correctly carried out, the solution is neutral to methyl orange. After boiling off the ammonia, the solution is filtered and the filter paper is washed with 10 c.c. of cold water; the filtrate should be tested with methyl orange. The precipitate is dissolved on the filter paper with 15 c.c. of hot dilute hydrochloric acid (5 c.c. of 5N acid and 10 c.c. of water) and the filter paper is washed with 10 c.c. of hot water. The acid and washings are collected in the silica flask, and 10 c.c. of 5N sodium hydroxide solution are added. After boiling and cooling, the solution is filtered through a filter paper and the filtrate is collected in a 50 c.c. graduated flask. The silica flask and filter paper are washed with 15-20 c.c. of cold water and, after cooling, the contents of the flask are made up to the mark with water. An aliquot part of this solution is taken for the determination and is placed in a 100 c.c. conical flask. For an aluminium content from 5 to 50 p.p.m., 5 c.c. is a suitable aliquot portion. If a smaller aliquot part is taken the volume must be made up to 5 c.c. with water. Sufficient 5N hydrochloric acid is added to neutralize the sodium hydroxide present—*i.e.*, one-tenth of the aliquot volume—and then 2 c.c. in excess followed by the other reagents. The colour value of the solution is then measured by the method already described.

Aluminium.—F. B. Shorland (*Trans. Roy. Soc. New Zealand*, 1934, **64**, pp. 35-50) used a modification of Lampitt and Sylvester's method for the determination of aluminium

in pastures. Since the method of wet digestion appeared to offer no advantages over the ashing method, the air-dry pasture was ashed in a porcelain dish at a temperature not exceeding dull red heat, and the residue was taken to dryness twice with hydrochloric acid to render the silica insoluble. Next the mineral constituents were taken up with hydrochloric acid and allowed to stand for 24 hours, after which the crude silica was filtered off. The latter was then ignited and again extracted with hydrochloric acid for 24 hours, after which it was filtered off, the filtrate being combined with the first filtrate.

For the determination of aluminium in the hydrochloric acid extract the solutions used by Lampitt and Sylvester are required. There is also required a colorimetric reagent, which is prepared immediately before each series of determinations by mixing 1 part by volume of 5N ammonium acetate with 4 parts of glycerol solution (1 : 1) and finally adding 1 part of 0·002 per cent. solution of aurin tricarboxylic acid exactly neutralized with ammonia. Shorland's procedure is as follows: Measure a suitable aliquot part of the hydrochloric acid extract (enough to give from 1·8 to 6·0 red units) into a 100 c.c. beaker. Add 1 drop of methyl orange indicator and dilute to 15 c.c. Exactly neutralize this solution with 5 per cent. ammonia, boiling the solution to remove any slight excess of ammonia. Allow the solution to stand for at least an hour and then filter through a Whatman No. 42 filter paper, washing the precipitate with cold water. Dissolve the precipitate back into the precipitation beaker with hot 25 per cent. hydrochloric acid. Evaporate the solution just to dryness on a water bath. Add 2 c.c. of 5N hydrochloric acid and 5 c.c. of water. Warm to dissolve the precipitate. Add 30 c.c. of the colorimetric reagent, mix thoroughly and place the beaker in a water bath for 5 minutes. Cool the solution in running water for at least 5 minutes. Place 3 c.c. of ammonium hydroxide-carbonate solution in a 50 c.c. graduated flask. Wash the solution into the flask and make up to the mark with distilled water,

mixing the contents thoroughly. Transfer 30 c.c. of the solution from the flask to the Lovibond tintometer tube. Match the colour, and read the red units on the Lovibond scale exactly 5 minutes after neutralization of the solution with the ammonium hydroxide-carbonate solution. The amount of aluminium is then ascertained from a graph constructed from measurements made with known amounts of the standard aluminium solution.

Manganese.—M. B. Richards (*Analyst*, 1930, **55,** pp. 554-560) found that the periodate method of H. H. Willard and L. H. Greathouse (*J. Amer. Chem. Soc.*, 1917, **39,** pp. 2366-2377) can be applied to the estimation of very small amounts of manganese, such as occur in biological materials, if chlorides are removed before oxidation and if the concentration of sulphuric acid does not exceed 15 c.c. per 100 c.c. of solution. It is recommended, however, that the acidity should be about 5-6 per cent., unless very considerable quantities of manganese are present. The weight of material taken for the analysis depends on the manganese content; 2 gm. of grass, 10 gm. of tapioca and 100 c.c. of milk were used. The details of the method adopted by Richards are as follows: The weighed quantity of material is incinerated as completely as possible in a silica basin at a low red heat, and evaporated to dryness with a little concentrated hydrochloric acid. A few c.c. of 33 per cent. (by volume) sulphuric acid and 3-4 drops of concentrated nitric acid are added; the mixture is evaporated to dryness on a water bath and a sand bath, and is finally gently ignited over a Bunsen flame. To the residue are added 2-2·5 c.c. of 33 per cent. sulphuric acid and a little water, and the mixture is evaporated to the fuming stage, thus removing chlorides. After cooling and diluting, the solution is filtered into a small (50 c.c.) flask. To the solution are added one or two small pieces of pumice stone, which have been previously purified by boiling with 5 per cent. sulphuric acid and a little periodate. The solution is evaporated to about 10 c.c., when the concentration of sulphuric acid will be about 5-6 per cent., and the solution is

then ready for oxidation. 0·3 gm. of sodium (or potassium) periodate is added, and a loosely fitting pear-shaped glass stopper is inserted in the neck of the flask. The solution is heated to the boiling point and immersed in boiling water for 30 minutes, the flasks being held in position in the bath with wooden test tube holders. If the whole solution is required for the colour comparison, it is cooled and transferred to the colorimetric tube described below. If the solution needs dilution, it is either diluted to the appropriate volume with 5 per cent. sulphuric acid, which has been boiled with a little periodate, or it is diluted with water nearly to the required volume and heated for 15 minutes longer in the bath. In either case, the solution is transferred to a graduated flask and made up to the mark.

The tubes used for the colour comparison are 10 c.c. cylinders of uniform bore (capacity 12-13 c.c. and length 12-13 cm.) graduated in tenths of a c.c. and without the usual flange at the bottom, so that the tubes can be held closely together. With a standard which contains 0·001 mgm. of manganese per c.c., 7-10 c.c. give a very convenient depth of tint for matching, but a detectable pink colour is obtained with as little as 1 c.c. and differences of 0·2 c.c. give distinctly recognizable differences in tint. A colorimeter may be used, but in many cases the amount of manganese in organic materials is so small that it would require an excessive amount of material to give sufficient depth of colour. Hence it is preferable to work always with the above tubes, and usually with the above standard, bringing the unknown solutions approximately to this strength by choosing suitable amounts of material for oxidation and by suitable dilution.

The stock solution of manganese sulphate is prepared by dissolving 0·144 gm. of potassium permanganate in 100 c.c. of water and reducing with sulphur dioxide, which is generated in the solution by the action of sulphuric acid on sodium sulphite or bisulphite. The solution is heated till the reaction takes place, boiled to drive off the excess of sulphur

dioxide, cooled and diluted to 1 litre. 1 c.c. of this solution =0·05 mgm. of manganese. The standard solution for comparison is prepared by oxidizing 20 c.c. of stock solution with periodate in the usual way and making up the solution with 5 per cent sulphuric acid, which has been previously boiled with periodate. 1 c.c. of this solution=0·001 mgm. of manganese. Willard and Greathouse state that a remarkable feature of the solutions oxidized by periodate is their great stability when a slight excess of the reagent is present. Such a solution, kept for 3 months in a stoppered flask, when compared with a similar freshly oxidized solution, showed no change. Richards found that even the very dilute standard described above showed the same stability.

For substances containing much calcium—*e.g.*, milk—a slight modification of the procedure is necessary. Unless most of the calcium is removed, it is difficult to avoid loss by spurting in evaporating to dryness with sulphuric acid. There is also danger of the minute amount of manganese present in milk being carried down with the calcium sulphate if the ash is treated with sulphuric acid in the usual way. The procedure adopted for the determination of manganese in milk is as follows: 100 c.c. of milk in a silica basin are evaporated to dryness on a water bath and incinerated. The ash is evaporated to dryness with hydrochloric acid, then moistened with a little water and broken up as finely as possible. 25 c.c. of boiling 33 per cent. (by volume) sulphuric acid are added with careful stirring. After cooling (overnight, if convenient), the solution is filtered into another silica basin and washed 5-6 times with 5 c.c. of 33 per cent. sulphuric acid. It is necessary to test the filter paper to see whether it will withstand acid of this strength. If not, 20-25 per cent. sulphuric acid may be used, as this will remove sufficient calcium sulphate to permit subsequent evaporation to dryness. 10 per cent. sulphuric acid does not remove calcium sufficiently. The filtrate is evaporated to dryness and the determination is continued as described above except that it may be necessary, when filtering at the final

stage before oxidation, to re-filter several times before washing, in order to obtain a perfectly clear solution.

Copper.—The following method of determining copper in foodstuffs, which is based on that of W. D. McFarlane (*Biochem. J.*, 1932, **26,** pp. 1022-1033), has been taken from Technical Communication No. 9, Imperial Bureau of Animal Nutrition. The following reagents are required:

Sodium Pyrophosphate Solution, saturated.

Concentrated Ammonia, sp. gr. 0·880.

Sodium Hydroxide Solution, 40 per cent.

Sodium Diethyldithiocarbamate Solution, 2 per cent. in water. This solution keeps fairly well when stored in a dark-coloured bottle. A precipitate forms in a few weeks, but after filtering the reagent may still be used.

Standard Copper Solution.—0·3928 gm. of pure crystallized copper sulphate is dissolved in water, and a small quantity of dilute sulphuric acid is added to prevent the formation of basic copper sulphate by hydrolysis. The solution is diluted to 1 litre. 1 c.c. of this solution = 0·1 mgm. of copper. In most cases, 25 c.c. of this solution diluted to 250 c.c., giving a solution 1 c.c. of which = 0·01 mgm. of copper, is a suitable standard.

The hydrochloric acid and amyl alcohol are the same as those used for the determination of iron (see p. 169). All reagents must be tested for the absence of copper. Water redistilled from glass must be used throughout. The vitreosil basins must be cleaned as follows: To each dish add an alcoholic solution of sodium acetate containing 1 gm. of the salt, evaporate to dryness and ignite. Then let the dishes stand for several days in dilute hydrochloric acid (1 : 1), and keep them in this acid when not in use.*

The solution used for the analysis is the extract prepared for the determination of iron, as described on p. 170. To an aliquot part of the extract in a 25 c.c. flask add 1-5 c.c. of

* This method of removing traces of copper from the dishes is that used by C. A. Elvehjem and C. W. Lindow (*J. Biol. Chem.*, 1929, **81,** pp. 435-443).

sodium pyrophosphate solution and concentrated ammonia from a burette until the solution is just alkaline to litmus. Place the flask in a water bath at 80° C. for 15 minutes. Cool, dilute, add 1 drop of sodium hydroxide solution, 5 c.c. of amyl alcohol (carefully measured) and 0·5 c.c. of sodium diethyldithiocarbamate solution and make up to 25 c.c. Shake the mixture vigorously for about 1 minute and allow it to stand for half an hour. Pipette off the amyl alcohol when clear, and compare with a standard prepared in the same way. If the amyl alcohol is pure, the colour is stable for about 12 hours. If, after standing, the amyl alcohol is still cloudy, it may be filtered, or transferred to a small test tube having a pellet of filter paper at the bottom and centrifuged. For the colour comparison, a series of standards are prepared by taking 0·5, 0·6, etc., up to 1·0 c.c. of the diluted copper sulphate solution in 25 c.c. flasks, adding 0·2 c.c. of hydrochloric acid to each, and then the same amount of sodium pyrophosphate solution as used in the unknown, and continuing the method as above.

Zinc.—The following method of determining zinc in plant material is described by P. L. Hibbard (*Ind. Eng. Chem.* [*Anal.*], 1934, **6,** pp. 423-425). All apparatus, chemicals and water should be tested for zinc. Jena and some other glassware give up zinc to dilute acids. Pyrex glass is satisfactory. Ordinary glass bottles and good porcelain are free from zinc. Most rubber stoppers and tubing give up zinc. Plant material should be pulverized to pass a 1 mm. sieve, using an ordinary iron grinding mill and a nickel wire sieve.

Separation of Zinc Sulphide.—Ash 2 gm. of the sample in a porcelain basin in a muffle at a low red heat to avoid fusion of the ash, and extract the soluble material with about 3 c.c. of 3N hydrochloric acid and 10 c.c. of water. After half the liquid has evaporated on a steam bath add 10-11 c.c. of 10 per cent. oxalic acid solution. When further evaporation has reduced the volume to about 20 c.c., remove from the steam bath and, if copper is present, pass in hydrogen sulphide for a few minutes and allow to stand till cold or

over-night. Filter off the insoluble residue and wash with a little 5 per cent. oxalic acid solution. To the clear filtrate add 10 c.c. of 10 per cent. oxalic acid solution and 1 c.c. of 4 per cent. potassium ferrocyanide solution and mix. A white cloud indicates the presence of zinc. After 10-15 minutes filter off the zinc ferrocyanide; addition of 0·02 gm. of powdered talc aids in obtaining a clear filtrate. Wash the filter twice with 5 per cent. oxalic acid solution. Decompose the precipitate on the filter with 5-10 c.c. of N sodium sulphide solution and wash 3 times with some of the same solution diluted with 3 volumes of water. Lastly, wash once with water. If zinc was present, zinc sulphide now remains on the filter.

Determination of Zinc.—Dissolve the zinc sulphide on the filter in N hydrochloric acid, returning the filtrate to the filter till it is clear, and collect the filtrate in a 20 c.c. flat-bottomed specimen tube. To the zinc solution add 2 c.c. of 5M sodium hydroxide solution, and fill to the mark with N hydrochloric acid. Add 2 drops of 2 per cent. potassium ferrocyanide solution and mix quickly. After standing about 15 minutes, the tube is compared with similar tubes containing known amounts of zinc precipitated in the same way. For comparison, place the tubes on a dead black surface where the light strikes them at right angles to their length. Tubes containing 0·02, 0·04, 0·06, 0·08 and 0·10 mgm. of zinc will usually be sufficient. The suspension of zinc sulphide should be pure white and should not settle perceptibly in 1 hour. Greater precision is attainable by the use of a photoelectric cell in making the comparisons.*

Cobalt.—The following method of determining cobalt in foodstuffs is described by F. J. Stare and C. A. Elvehjem (*J. Biol. Chem.*, 1933, **99,** pp. 473-483).

The following solutions are required:

* N. Strafford (*Analyst*, 1936, **61,** pp. 170-176) states that the error of the nephelometric determination of zinc as ferrocyanide by the visual method is about ±25 per cent. By using the photoelectric equipment the error is reduced to about ±4 per cent.

Indicator Solution.—1 gm. of nitroso-R-salt (sodium 2:3:6-β-naphtholdisulphonate) made up to 100 c.c. with redistilled water.

Potassium Hydroxide Solution.—Saturated aqueous solution.

Standard Cobalt Solution.—0·2017 gm. of pure cobalt chloride, $CoCl_2,6H_2O$, dissolved in redistilled water and made up to 500 c.c. 1 c.c. of this solution = 0·1 mgm. of cobalt.

The determination is carried out as follows: A suitable amount of material, containing 0·05-0·5 mgm. of cobalt, is incinerated at a dull red heat and the ash is extracted with a little dilute hydrochloric acid (1:1). To this solution, the volume of which should not exceed 20-25 c.c., are added 2 drops of phenolphthalein solution, 2 c.c. of indicator solution and about 2 gm. of sodium acetate. The mixture is warmed to about 70° C. and thoroughly stirred. While still stirring, saturated potassium hydroxide solution is added drop by drop until the reaction is just alkaline. The contents of the beaker are then heated to the boiling point; concentrated nitric acid is added drop by drop until there is a distinct excess of acid, and the boiling is continued for 1-2 minutes. A permanent change in colour towards red indicates the presence of cobalt. The solution is allowed to cool and made up to 50 c.c. in a volumetric flask with water. The solution is compared in a colorimeter with a standard cobalt solution containing a similar quantity of cobalt and prepared in the same way.

Potassium and Sodium.—The method devised by A. D. Husband and W. Godden (*Analyst*, 1927, **52,** pp. 72-75) for the determination of potassium and sodium in foodstuffs is described below. In this method the magnesium is completely precipitated by an alcoholic solution of ammonium carbonate, which is prepared as follows: Dilute 180 c.c. of concentrated ammonia (sp. gr. 0·88) with water to 1 litre, and mix it with 1 litre of 90 per cent. alcohol. Dissolve in this mixture 250 gm. of ammonium carbonate by warming. When cold, filter the solution.

The procedure is as follows: A weighed quantity of the foodstuff, which will give a final weight of 0·1-0·15 gm. of mixed sulphates, is incinerated at a low temperature, and the ash is extracted twice with hot N hydrochloric acid, about 100 c.c. being used. The residue is washed with hot water, and the filtrate and washings are made up to 200 c.c. in a graduated flask. 100 c.c. of this extract are heated to the boiling point, and 5 c.c. of 10 per cent. barium chloride solution are added. The mixture is evaporated to about 25 c.c., made alkaline with ammonia whilst hot, and allowed to cool. When cold, the precipitate is filtered off and washed with 2 per cent. ammonia. The filtrate and washings are evaporated just to dryness in a 250 c.c. beaker; when cold, 30-35 c.c. of the alcoholic solution of ammonium carbonate are added. The contents of the beaker are vigorously stirred for about 5 minutes, and are then transferred to a 100 c.c. graduated flask. The beaker is rinsed with more alcoholic solution of ammonium carbonate and, after shaking, the contents of the flask are made up to the mark with the same solution. The mixture is allowed to stand for at least 2 hours, or preferably over-night, and is then filtered into a dry flask. 50 c.c. of the filtrate are evaporated to dryness in a weighed vitreosil basin, and the cold residue is treated with 5 c.c. of redistilled sulphuric acid. The basin is heated on a sand bath to drive off the excess of acid, and then over a naked flame until the residue is quite dry. During this stage it is advisable to scatter on the residue a little powdered ammonium carbonate, which facilitates the removal of the last traces of sulphuric acid. The dish is then heated in a muffle furnace at a bright red heat for 10 minutes, cooled and weighed. In the mixed sulphates the potassium is determined by the volumetric cobaltinitrite method. The percentage of sodium is then calculated from the weight of the mixed sulphates.*

Potassium (Green's Method).—H. H. Green (*Biochem. J.*, 1912, **6**, pp. 69-75) determined potassium in urine as follows:

* The necessary gravimetric factors will be found on p. 334.

The ash of 25 c.c. of urine is dissolved in dilute hydrochloric acid. The solution is neutralized with sodium hydroxide, acidified with acetic acid and evaporated to a volume of 5-10 c.c. After adding 1 c.c. of glacial acetic acid and 10 c.c. of Adie and Wood's reagent,* the solution is evaporated until the syrupy liquid is of such consistency that it will set to a hard mass on cooling. When cool, the residue is stirred with 50 c.c. of 10 per cent. acetic acid and allowed to stand, with occasional stirring, until it is disintegrated. The yellow precipitate is washed by decantation with dilute acetic acid, the washings being filtered through a Gooch crucible with an asbestos pad.† Finally the precipitate is transferred to the filter, and is washed by suction with cold water. Generally 6 washings by decantation are necessary, using in all about 300 c.c. of washing liquid.

Whilst the precipitate is being filtered, about 300 c.c. of water are heated to the boiling point in a beaker, and from 30 to 50 c.c. of N/5 potassium permanganate solution are run in from a burette. The pad of asbestos with the precipitate is dropped into the hot permanganate solution and stirred up. After a few minutes 20 c.c. of 25 per cent. sulphuric acid are added. The contents of the beaker are again stirred and kept near the boiling point until the yellow precipitate is completely decomposed with the formation of manganese hydroxide. After 5 minutes standard oxalic acid solution is added from a burette,‡ with stirring, until the manganese hydroxide is dissolved. The excess of oxalic acid is then titrated with N/5 potassium permanganate solution; the end-point is sharply marked. The difference

* This is the reagent used in the gravimetric cobaltinitrite method of determining potassium (see p. 83).

† A Jena sintered glass crucible (porosity No. 4) is preferable. The crucible together with the washed precipitate can be placed in the beaker containing the permanganate solution and left there until the end of the titration, after which it is easily cleaned.

‡ The normality of this solution is not stated by Green, but a solution of the same normality as the potassium permanganate is generally used.

between the volumes of N/5 potassium permanganate solution and N/5 oxalic acid solution gives the volume of potassium permanganate solution reduced by the cobaltinitrite precipitate.* It is preferable to standardize the potassium permanganate solution with a solution of pure potassium chloride. The calculations are simplified if the permanganate solution is made up so that 1 c.c. = 0·001 or 0·002 gm. of potassium oxide.

Potassium (Lewis and Marmoy's Method).—The method of determining potassium described by A. H. Lewis and F. B. Marmoy (*J. Soc. Chem. Ind.*, 1933, **52,** pp. 177-182T) consists in the colorimetric determination of the cobalt in the washed cobaltinitrite precipitate. The precipitate is separated and washed by centrifuging in the same way as in the volumetric method of B. Kramer and F. F. Tisdall (*J. Biol. Chem.*, 1921, **46,** pp. 339-349). The details of that method are therefore included in the following description of Lewis and Marmoy's method.

Preparation of the Solution for Analysis.—About 0·5 gm. of finely ground plant material is gently incinerated in a silica or platinum dish and, after adding 1 c.c. of concentrated hydrochloric acid, is evaporated to dryness and ignited. About 20 c.c. of hot water are added, and the solution is decanted through a filter into a 100 c.c. flask. The extraction is repeated, and the dish and filter are washed with about 20 c.c. of hot water. The solution is allowed to cool and made up to the mark.

Sodium Cobaltinitrite Reagent.—Solution A is prepared by dissolving 25 gm. of cobaltous nitrate crystals in 50 c.c. of water and adding 12·5 c.c. of glacial acetic acid. Solution B is prepared by dissolving 120 gm. of sodium nitrite (free from potassium) in 180 c.c. of water; this gives a total volume of about 220 c.c. The cobaltinitrite reagent is prepared by adding 210 c.c. of solution B to the whole of solution A and drawing air through the mixture until all

* 1 c.c. of N/5 potassium permanganate solution = 0·00142 gm. of potassium or 0·003168 gm. of potassium sulphate.

the nitric oxide has been removed. The reagent is kept as cool as possible, and is filtered each time before use. It will keep for at least a month.

Precipitation.—2 c.c. of the solution, the potassium content of which is to be determined, are placed in a centrifuge tube of 15 c.c. capacity. 1 c.c. of sodium cobaltinitrite reagent is added from a burette, drop by drop, with mixing after each addition. The mixing is best accomplished by rotating the tube between the palms of the hands. The tubes are then lightly corked and kept at room temperature for 45 minutes. 1 c.c. of water is then added, the precipitate stirred with a glass rod, and the rod and the sides of the tube are washed down with a further 1 c.c. of water. After mixing the contents, the tubes are centrifuged at a moderate speed (2,000 r.p.m.) for 15 minutes. The supernatant liquid is siphoned off to within about 0·5 c.c. by means of a small siphon tube with a fine tip bent upwards, so as not to disturb the precipitate. If care is taken to "bed" the precipitate by centrifuging, siphoning can be dispensed with and the liquid poured off without any loss of precipitate.

Washing the Precipitate.—If cobalt is to be determined colorimetrically, 1 c.c. of 70 per cent. (by volume) alcohol is added, and the precipitate is stirred with a glass rod. The rod is washed with a further 1 c.c. of 70 per cent. alcohol, and the contents of the tube are mixed. The tube is then centrifuged for 5 minutes at a moderate speed (2,000 r.p.m.), the alcohol is poured off, and the washing is repeated 3 times more. If it is intended to carry out Kramer and Tisdall's volumetric method, 5 c.c. of water are added, the precipitate being disturbed as little as possible. After a few minutes the tube is centrifuged for 5 minutes at 2,000 r.p.m. and the supernatant liquid is removed by siphoning or by pouring off to within 0·5 c.c. The washing is repeated 3 times.

Colorimetric Determination of Cobalt.—After the last alcohol washings have been poured off, 1 c.c. of water is

added, and the precipitate is stirred with a glass rod. The rod is washed with a further 1 c.c. of water, and the contents of the tube are mixed by shaking. The tube is then placed in boiling water until the precipitate is dissolved.

(i) *Thiocyanate Method.*—The volume is made up to 4 c.c. with water and then to 10 c.c. with ammonium thiocyanate reagent. The contents are thoroughly mixed; the blue colour develops immediately. The solution of cobaltous nitrate to be used as the colour standard is standardized against the cobalt in the potassium sodium cobaltinitrite precipitate obtained from a potassium solution of known concentration. The thiocyanate reagent is prepared by adding 400 c.c. of pure acetone to 100 c.c. of an aqueous solution of ammonium thiocyanate of density 1·1; this solution must be freshly prepared each day.

(ii) *Choline-Ferrocyanide Method.*—After being allowed to cool, 1 c.c. of 1 per cent. choline hydrochloride solution and 1 c.c. of 2 per cent. sodium or potassium ferrocyanide solution are added. Water is added up to the 6 c.c. mark, and the contents are mixed. The green colour reaches full intensity in about a minute, and is compared with that developed in a solution of cobaltous nitrate or sulphate, to which are added 1 c.c. of choline hydrochloride solution, 1 c.c. of sodium or potassium ferrocyanide solution and water up to the 6 c.c. mark.

Standard Cobalt Solution.—This solution is made up by dissolving approximately 0·67 gm. of cobaltous nitrate (hexahydrate) or 0·647 gm. of cobaltous sulphate (heptahydrate) in water and making up to 1 litre. The solution is standardized against the cobalt contained in the potassium sodium cobaltinitrite precipitate obtained from a standard potassium solution. This is conveniently done by using 2 c.c. of standard potassium sulphate solution containing 0·2 mgm. of potassium per c.c. for the precipitation, and comparing the colour with that developed in 2 c.c. of the standard cobalt solution. By varying the volume of cobalt solution used from 1 to 4 c.c., the range of potassium covered

is about 0·2 to 0·8 mgm. A more dilute cobalt solution may be used for comparison with smaller amounts of potassium, but below the equivalent of about 0·1 mgm. of potassium the intensity of the colour developed is not sufficient for accurate comparison. Above the equivalent of about 0·7 mgm. of potassium the coloured solutions become turbid. With a little practice it is relatively easy to estimate from the size of the precipitate, and the depth of colour developed, the volume of cobalt solution for comparison. For accurate comparison the ratio of the standard cobalt solution to the unknown should be between 0·8 and 1·2. If the ratio is outside this range, either solution may be diluted with water and the comparison made again.

Kramer and Tisdall's Volumetric Method.—After the fourth washings with water have been poured off, an excess of N/50 potassium permanganate solution is run into the centrifuge tube and 1 c.c. of 6N sulphuric acid is added. The tube is then placed in boiling water for 1 minute—*i.e.*, until the decomposition of the precipitate is complete. Excess of N/50 oxalic acid solution is then run in, and the excess is titrated with N/50 potassium permanganate solution. The permanganate solution should be standardized with N/50 oxalic acid solution by the same technique as that used in the determination. 1 c.c. of N/50 potassium permanganate solution=0·000171 gm. of potassium oxide.

Potassium (Piper's Method).—For the volumetric cobaltinitrite method described by C. S. Piper (*J. Soc. Chem. Ind.*, 1934, **53,** pp. 392-396T) the following reagents are required:

Glacial Acetic Acid.

Sodium Chloride Solution.—A filtered saturated solution.

Sodium Nitrite Solution.—35 gm. of sodium nitrite are dissolved in water, made up to 100 c.c. and filtered.

Cobalt Nitrate Solution.—20 gm. of cobalt nitrate, $Co(NO_3)_2,6H_2O$, are dissolved in water, made up to 100 c.c. and filtered.

There is also required for washing the precipitate a saturated solution of potassium sodium cobaltinitrite. This is

prepared by adding about 0·3 gm. of potassium sodium cobaltinitrite to a litre of distilled water, shaking at intervals for 1 hour, and then filtering through an 11 cm. Buchner funnel of unglazed porcelain. The filtrate must be used as soon as possible after filtration and always within ½-1 hour. It is not very stable; its colour decreases noticeably in 1 hour and disappears completely within 2-3 hours. A supply of potassium sodium cobaltinitrite can be prepared by precipitating a potassium chloride solution with sodium cobaltinitrite in the presence of sodium chloride. The precipitate is collected, washed several times with water and dried by washing with alcohol and ether. Instead of this solution, 35 per cent. alcohol can be used for washing the precipitate.

Procedure.—The solution containing the potassium salt is evaporated to dryness in a Pyrex glass basin. If other substances are present, the solution is made just acid with a few drops of hydrochloric acid before evaporation. When cold, 1·5 c.c. of glacial acetic acid and 10 c.c. of saturated sodium chloride solution are added in that order and, after 5-10 minutes, 5 c.c. of sodium nitrite solution are added. The contents of the basin are stirred until all soluble substances are dissolved. After a further 5-10 minutes, but not longer, 5 c.c. of cobalt nitrate solution are added, the addition being made very rapidly, with constant stirring, from a pipette with an extra large jet. After stirring for 40-60 seconds, the basin is covered and left over-night in a cool place.

The supernatant liquid is then decanted through a 10 c.c. Gooch crucible charged with asbestos. The asbestos is previously digested with acidified permanganate and then with an excess of oxalic acid; it is then thoroughly washed with hot water and can be used repeatedly. Sintered glass crucibles (G4) and unglazed porcelain crucibles are also satisfactory, but filtration is somewhat slower. The precipitate is transferred to the crucible, using a rubber-tipped stirring rod, and is washed 5 times with 10 c.c. portions of freshly prepared saturated solution of potassium sodium

cobaltinitrite. If the precipitate is washed with 35 per cent. alcohol, the alcohol is removed by washing 3 times with very small portions of water.

A measured volume of standard potassium permanganate solution, either N/20 or N/50, about 5 c.c. in excess of the quantity required, is pipetted into a beaker, diluted to about 150 c.c., and 5 c.c. of concentrated sulphuric acid are added. The crucible and precipitate are then added, and the solution is stirred and heated just to the boiling point. It is then removed from the flame, and after 5 minutes a small excess of standard oxalic acid solution is added. The beaker and contents are then reheated nearly to the boiling point, and the titration is completed by adding standard potassium permanganate solution until a stable pink colour first appears. Blank determinations must be carried out to correct for the small amounts of potassium invariably present in the reagents used. The potassium is calculated from the equation:

$$K_2O \text{ in mgm.} = \text{permanganate value} \times 0{\cdot}354 + (\text{permanganate value})^2 \times 0{\cdot}00034,$$

the permanganate value being the amount of potassium permanganate, expressed as c.c. of N/20 solution, required to oxidize the precipitate. This equation takes into consideration the change in composition of the precipitate as the quantity of potassium increases.

Piper (*ibid.*, 1935, **54,** pp. 157-158T) has pointed out that the presence of ammonium as an impurity in the cobalt nitrate used in the determination leads to serious error. If satisfactory reagents are used throughout, the blank titration should not exceed 0·5 c.c. of N/20 potassium permanganate solution. If it is higher, one or more of the reagents should be suspected.

Phosphorus (as Magnesium Pyrophosphate).—The British official method for the determination of phosphoric acid in feeding stuffs (Fertilisers and Feeding Stuffs Regulations, 1932, **12,v**) is as follows : A weighed portion of the sample

is heated with concentrated sulphuric acid until all organic matter is oxidized and the phosphoric acid is completely dissolved. After dilution, the solution is filtered; the insoluble matter is thoroughly washed and the filtrate is made up to a definite volume. Phosphoric acid is determined by the molybdate method (see p. 43) in an aliquot part of the solution, which has been first nearly neutralized and then acidified with nitric acid.

F. L. Ashton (*J. Soc. Chem. Ind.*, 1936, **55,** pp. 106-108T) determined phosphorus in grass as follows: 3 gm. of the sample are digested in a Kjeldahl flask with 10 c.c. of concentrated sulphuric acid and 10 c.c. of concentrated nitric acid until brown fumes cease to be evolved. Another 10 c.c. of nitric acid are added and the flask is heated as before. This process is repeated until the contents of the flask are clear. After dilution, the solution is filtered and neutralized with ammonia; it is then treated with 10 c.c. of concentrated nitric acid and 10 gm. of ammonium nitrate and boiled. The phosphorus is precipitated at 72° C. with neutral ammonium molybdate solution. The precipitate is filtered off and dissolved in ammonia. The phosphorus is reprecipitated as magnesium ammonium phosphate and weighed, after ignition, as magnesium pyrophosphate.

Phosphorus (Richards and Godden's Method).—The following method of determining phosphorus in fæces is described by M. B. Richards and W. Godden (*Analyst*, 1924, **49,** pp. 565-572) and has been used by W. Godden (*J. Agric. Sci.*, 1926, **16,** pp. 78-88) for the analysis of pasture grass. The ammonium molybdate solution used for precipitating the phosphoric acid is prepared by dissolving 121 gm. of powdered ammonium molybdate in 355 c.c. of water, adding 60 c.c. of concentrated ammonia and pouring the solution into a mixture of 489 c.c. of concentrated nitric acid (sp. gr. 1·42) and 1149 c.c. of water.

The procedure is as follows: From 1·5 to 2 gm. of fæces are transferred to a 500 c.c. round-bottomed flask and to it are added 10 c.c. of concentrated sulphuric acid and 10 c.c. of

concentrated nitric acid. The mixture is digested over a low flame until brown fumes cease to be evolved and the flask is full of white fumes. The mixture is allowed to cool, 5 c.c. more of nitric acid are added, and the digestion is continued until the liquid in the flask is quite clear and colourless. When cold, the solution is diluted to about 200 c.c., made just alkaline to litmus with concentrated ammonia, and then just acid with nitric acid. After adding 30 c.c. of 50 per cent. ammonium nitrate solution, the liquid is heated at 70°-75° C., 30 c.c. of the ammonium molybdate solution (to which 1·5 c.c. of concentrated nitric acid have been added) are run in and the mixture is well shaken. The precipitate is allowed to settle until the liquid is cold (usually 1 hour or over-night). It is filtered by suction on a disc of hardened filter paper in a Hirsch funnel, and is washed twice with 10 per cent. nitric acid, 3 or 4 times with 2 per cent. ammonium nitrate solution and twice with cold water. The precipitate and filter paper are washed into the precipitation flask with cold water, and dissolved in a known volume of N/2 sodium hydroxide solution, about 1 c.c. in excess being used. The solution is diluted to about 250 c.c. and boiled for 20 minutes. While still warm it is titrated with N/2 sulphuric acid, using phenolphthalein as indicator, and an excess of 1-2 c.c. of acid is run in. The solution is again boiled for 15 minutes and, after cooling, the excess of acid is accurately titrated with N/2 sodium hydroxide solution until the first definite pink tint is obtained. The colour will be found to fade fairly rapidly. The difference between the total alkali and the total acid used gives the volume of alkali equivalent to the phosphorus pentoxide in the weight of sample analysed. 1 c.c. of N/2 sodium hydroxide solution = 0·001365 gm. of phosphorus pentoxide. 1 c.c. of the same solution = 0·000596 gm. of phosphorus. It is desirable that the amount of phosphorus pentoxide present should not exceed 22 mgm., or there may be some difficulty in completely removing nitric acid and ammonium nitrate during the washing of the precipitate.

Phosphorus (Colorimetric Method).—The following application of Fiske and Subbarow's colorimetric method to the determination of phosphorus in grass and similar materials is described by A. W. Greenhill and N. Pollard (*J. Soc. Chem. Ind.*, 1935, **54,** pp. 404-406T). The following reagents are required:

Magnesium Nitrate Solution, N/4.

10*N Sulphuric Acid.*—450 c.c. of concentrated sulphuric acid are added to 1300 c.c. of water.*

Ammonium Molybdate Solution.—2·5 per cent. solution in water. This solution should be discarded as soon as any considerable amount of sediment has appeared.*

Aminonaphtholsulphonic Acid Reagent.—Dissolve 0·5 gm. of 1:2:4-aminonaphtholsulphonic acid in 195 c.c. of 15 per cent. sodium bisulphite solution, add 5 c.c. of 20 per cent. sodium sulphite solution (200 gm. of $Na_2SO_3.7H_2O$ in 380 c.c. of water), stopper and shake until dissolved. If the bisulphite solution is old, more than 5 c.c. of sulphite solution will be needed. In that case, add more sulphite, shaking after each addition, until solution is complete.*

Standard Phosphate Solution.—Dissolve 0·1917 gm. of monopotassium phosphate in water. Add a drop of chloroform, and make the solution up to 1 litre. 1 c.c. of this solution = 0·0001 gm. of phosphorus pentoxide.

Preparation of the Extract.—0·5 gm. of the dried and finely ground material is weighed out into a porcelain evaporating dish (Royal Worcester, deep form, 44 c.c. capacity). To it are added 4 c.c. of the magnesium nitrate solution, and the whole is stirred into a thick paste by means of a small glass rod. The glass rod is washed down with a few drops of water, and the mixture is evaporated to dryness on a sand bath (about 15 minutes), and then ignited to a light greyish ash at about 500° C. for 15 minutes in a muffle furnace. Care should be taken to ensure complete ignition, otherwise a

* C. H. Fiske and Y. Subbarow, *J. Biol. Chem.*, 1925, **66,** pp. 375-400. Greenhill and Pollard mention these three solutions, but do not describe their preparation.

brownish coloured extract will be obtained later. On cooling, 10 c.c. of 10N sulphuric acid are added, and the residue is digested gently on a sand bath for 15 minutes, and thoroughly broken up during digestion by stirring with a small glass rod. The dish is allowed to cool, and the contents are diluted with water and filtered through a 9 cm. filter paper into a 100 c.c. graduated flask. After washing the paper thoroughly with hot water, the filtrate is cooled and made up to the mark. The extract should be clear and colourless.

Determination.—An aliquot part of the extract (20-25 c.c.) is pipetted into a 100 c.c. graduated flask, and sufficient 10N sulphuric acid is added from a burette to bring the total amount of 10N sulphuric acid present to 5 c.c. After adding water to about 75 c.c., 10 c.c. of ammonium molybdate solution are added; the flask is shaken and 4 c.c. of amino-naphtholsulphonic acid reagent are added; the flask is again shaken and the solution made up to the mark with water. The standard is prepared at the same time by taking 10 c.c. of the standard phosphate solution, adding 5 c.c. of 10N sulphuric acid, diluting to about 75 c.c. with water and adding the reagents as above. After keeping for about 10 minutes, the colour comparison is made in a colorimeter in the usual way.

In working with materials in which the phosphorus pentoxide content is low (about 0·5 per cent.), it is advisable to take 25 c.c. of the extract and develop the colour in a final volume of 50 c.c. In this case, the 25 c.c. of extract contain the correct amount of 10N sulphuric acid. 5 c.c. of ammonium molybdate solution and 2 c.c. of aminonaph-tholsulphonic acid reagent are added, and the standard is prepared by taking 5 c.c. of standard phosphate solution, adding 2·5 c.c. of 10N sulphuric acid and the reagents accordingly. It should be noted that the colour is always developed in a final concentration of N/2 sulphuric acid.

Sulphur (Sodium Peroxide Method).—The method adopted by the Association of Official Agricultural Chemists (*Methods of Analysis*, 1935, p. 129) for the determination of

sulphur in plants is as follows: Place 1·5-2·5 gm. of the sample in a nickel crucible of about 100 c.c. capacity and add 5 gm. of anhydrous sodium carbonate. Mix thoroughly, using a nickel or platinum rod, and moisten with about 2 c.c. of water. Add sodium peroxide, about 0·5 gm. at a time, mixing thoroughly after each addition, until the mixture becomes nearly dry and quite granular (about 5 gm. are generally required). Heat the crucible carefully over a sulphur-free flame or an electric hot plate, with occasional stirring, until the contents are fused. Allow the crucible to cool and cover the hardened mass with more sodium peroxide to a depth of about 0·5 cm. Heat gradually and finally with the full flame until fusion again takes place, rotating the crucible from time to time. Continue heating for 10 minutes after fusion is complete. After cooling somewhat, place the warm crucible and contents in a 600 c.c. beaker and carefully add about 100 c.c. of water. After the initial violent action has ceased, wash the material out of the crucible and make the solution slightly acid with hydrochloric acid. Transfer the contents of the beaker to a 500 c.c. flask, cool and dilute to the mark. Filter and determine sulphate in an aliquot part of the filtrate as directed below.

Dilute the filtrate to about 200 c.c. and add sufficient concentrated hydrochloric acid to make the free acid present 0·5 c.c. Heat the solution to the boiling point, and add 10 c.c. of 10 per cent. barium chloride solution drop by drop with constant stirring. Continue boiling for about 5 minutes and allow to stand for 5 hours or longer in a warm place. Decant the liquid through an ashless filter paper or an ignited and weighed Gooch crucible. Treat the precipitate with 15-20 c.c. of boiling water, transfer it to the filter and wash with boiling water until the filtrate is free from chlorides. Dry the precipitate and filter, ignite and weigh as barium sulphate.*

Sulphur (Woodman and Evans's Method).—H. E. Wood-

* The weight of barium sulphate × 0·13735 = the weight of sulphur.

man and R. E. Evans (*J. Agric. Sci.*, 1933, **23**, pp. 459-462) have described the following methods of determining total sulphur and sulphate sulphur in feeding stuffs. For the determination of total sulphur there is required a bomb designed by H. J. Hodsman (*J. Soc. Chem. Ind.*, 1921, **40**, p. 74T) for the determination of sulphur in coal.

Total Sulphur.—The Hodsman bomb consists of an inner steel cartridge with a tight-fitting cap. In this cartridge is placed 1 gm. of the feeding stuff intimately mixed with sodium peroxide. A blank experiment should be carried out with this material to ensure that it is free from sulphate and other forms of sulphur. The cap is fitted on and the cartridge is placed in an outer steel cylinder, the upper part of which screws on to the lower part. A screw passing through the top of the outer cylinder is then tightened so as to keep the cap of the inner cylinder firmly in position. The bomb is clamped in an inclined position, and its base is heated with a Bunsen burner for a few minutes until firing takes place. When cool, the bomb is unscrewed and the cap is removed from the cartridge, which is then placed in a dish containing distilled water and is covered with a clock glass to prevent loss by spurting. The solution, which measures about 500 c.c., is transferred to a beaker, acidified with concentrated hydrochloric acid and evaporated to about 200 c.c. Ammonia is added to precipitate the iron, the precipitate being filtered off and washed with hot water. The filtrate is acidified with hydrochloric acid and heated to the boiling point. The sulphate is then precipitated by means of barium chloride.* The beaker is allowed to stand over-night on a steam oven and, when cool, the contents are filtered through an ignited and weighed Gooch crucible. The precipitate is washed with boiling water and, after being dried, is ignited and weighed.†

* The details of the precipitation are not given. Hillebrand and Lundell's procedure will be found on p. 197.

† The weight of barium sulphate $\times 0{\cdot}13735$ = the weight of sulphur.

Sulphate Sulphur.—In a 500 c.c. flask are placed 8 gm. of the finely powdered feeding stuff, and to it are added 200 c.c. of dilute hydrochloric acid (1:3). The flask is kept at about 80° C. for 24 hours with occasional shaking. The contents are then filtered into a graduated flask and the residue is washed with hot water up to the 500 c.c. mark. An aliquot part, usually 300 c.c., is transferred to a beaker and heated to the boiling point. The sulphate is precipitated by adding, drop by drop, 10 c.c. of 25 per cent. barium chloride solution. The contents of the beaker are evaporated slowly to about 200 c.c. and, after standing over-night, are filtered through an ignited and weighed Gooch crucible. The precipitate is dried, ignited and weighed. Before ignition the precipitate is dark coloured owing to the presence of a little pigmented material, but this disappears on ignition, leaving a white residue of barium sulphate. If the total sulphur and sulphate sulphur are determined in the same sample, the organic sulphur can be found by difference.

Sulphur (Aitken's Method).—H. A. A. Aitken (*Biochem. J.*, 1930, **24,** pp. 250-256) has described the following methods of determining total sulphur and sulphate sulphur in grass:

Total Sulphur.—1-1·5 gm. of powdered grass are transferred from a weighing bottle to a 4 in. porcelain dish. To it are added 50 c.c. of Benedict-Denis reagent (125 gm. of crystallized copper nitrate, 125 gm. of sodium chloride, 50 gm. of ammonium nitrate and 500 c.c. of water, all free from sulphate), and the mixture is evaporated to dryness on a curved asbestos gauze. It is then brought to a dull red heat, when the copper oxide formed completely oxidizes the grass. The product is taken up with 50 c.c. of 2N hydrochloric acid, gently evaporated to dryness, and finally extracted by warming with successive portions of 2N hydrochloric acid, 100 c.c. being used for the purpose. The extracts and washings are made up to 250 c.c. and filtered by suction. Sulphate is determined in portions of 100 c.c. by precipitation with barium chloride. By carrying out the precipitation at the boiling point in 3 per

cent. hydrochloric acid and using a large excess of barium chloride, easy filtration is ensured. The solution will become clear in less than 3 hours and the precipitate is crystalline.

Sulphate Sulphur.—2-3 gm. of powdered grass are transferred from a weighing bottle to a 600 c.c. beaker and boiled gently for half an hour with 100 c.c. of 2N sodium hydroxide solution. 200 c.c. of 2N hydrochloric acid are then added and the mixture is boiled for another half-hour. 2 gm. of decolorizing charcoal, previously freed from sulphate by repeated extraction with boiling hydrochloric acid, are added and the boiling is continued for about 20 minutes. Finally, the product is filtered through a 4 in. Buchner filter, and the precipitate is washed several times with dilute hydrochloric acid. The extract is made up to 500 c.c., and sulphate is determined in 200 c.c. portions by precipitation with barium chloride.

Precipitation of Sulphate as Barium Sulphate.—The following procedure is recommended by W. F. Hillebrand and G. E. F. Lundell (*Applied Inorganic Analysis*, 1929, p. 580) for the determination of sulphate in solutions containing moderate amounts of sulphates in the presence of alkali and ammonium salts: Prepare a water or hydrochloric acid solution containing not more than 0·025 gm. of sulphur as sulphate per 100 c.c. and preferably no other salts than those of sodium, potassium and ammonium. Add methyl orange and neutralize the solution; then add 1 c.c. of hydrochloric acid for each 100 c.c. of solution and heat to incipient boiling. Take enough of 10 per cent. barium chloride solution to provide an excess of 7-8 c.c. over that required to precipitate the sulphate; dilute it to 100 c.c., heat it to boiling point and quickly pour it into the hot sulphate solution, which is meanwhile vigorously stirred. Allow the solution to stand at the side of a steam bath for 30 minutes, and then decant the clear solution through a filter. Wash the precipitate 3 times by decantation with hot water, transfer it to the filter paper and continue washing until a test for chloride gives no more than a faint opalescence. Place

the wet filter paper in a weighed crucible, heat carefully until it is dry, char the paper without inflaming, carefully burn the carbon under good oxidizing conditions and finally heat to about 900° C. to constant weight. The precipitate can also be collected in a Gooch or Munroe crucible, and the heating can be done in a muffle furnace.

Chlorine.—A. D. Husband and W. Godden (*Analyst*, 1927, **52,** pp. 72-75) determine chlorine in foodstuffs as follows: A weighed quantity of the sample is mixed with 10-25 per cent. of its weight of calcium oxide. The mixture is made into a paste with water and incinerated at a low temperature. The ash is treated with nitric acid, and chlorine is determined in the filtered solution by Volhard's method, as in the A.O.A.C. volumetric method given below.

The British official method for the determination of salt in feeding stuffs (Fertilisers and Feeding Stuffs Regulations, 1932, **12,** viii) is as follows: 5 gm. of the sample are mixed with pure lime and heated until the organic matter is completely charred. The residue is extracted with water, the volume is made up to 250 c.c. and the solution is filtered. Chlorine is determined in an aliquot portion of the filtrate, and the result is expressed in terms of sodium chloride.

The Association of Official Agricultural Chemists (*Methods of Analysis*, 1935, p. 131) have adopted the following methods for the determination of chlorine in plants.

Preparation of the Solution.—Moisten 5 gm. of the sample in a platinum dish with 20 c.c. of 5 per cent. sodium carbonate solution. Evaporate to dryness, and ignite as thoroughly as possible at a temperature not exceeding dull redness. Extract with hot water, filter and wash. Return the residue to the dish and ignite. Dissolve the ash in dilute nitric acid (1:4), filter, wash the residue thoroughly and add this solution to the water extract.

Volumetric Method.—To the prepared solution add a known volume of N/10 silver nitrate solution, which is more than sufficient to precipitate the chlorine. Stir well, filter and wash the precipitate. To the combined filtrate

and washings add 5 c.c. of saturated ferric ammonium alum solution and a few c.c. of nitric acid. Titrate the excess of silver nitrate with N/10 ammonium or potassium thiocyanate solution until a permanent light brown colour appears. 1 c.c. of N/10 silver nitrate solution = 0·00355 gm. of chlorine.

Gravimetric Method.—To the solution of the ash add 10 per cent. silver nitrate solution, avoiding more than a slight excess. Heat to the boiling point, protect from light and allow to stand until the precipitate is granular. Filter on a weighed Gooch crucible, which has been previously heated to 140°-150° C., and wash with hot water, testing the filtrate for an excess of silver nitrate. Dry the crucible and silver chloride at 140°-150° C., cool and weigh.*

Iodine.—I. Leitch and J. M. Henderson (*Biochem. J.*, 1926, **20,** pp. 1003-1007) have described the following method of determining iodine in blood, milk and pasture grass. The following reagents are required:

N/500 Sodium Thiosulphate Solution.—This solution is prepared by diluting the N/10 solution.

Potassium Iodide Solution.—This is made up just before the titration by dissolving a small crystal in about 20 c.c. of water.

Starch Solution.—A pinch of soluble starch is added to 50 c.c. of water and the mixture is boiled for 1-2 minutes. A fresh solution should be prepared every third day.

Pumice Stone.—Small chips of pumice stone, about the size of rice grains, are boiled with dilute nitric acid, washed with water, dried and then strongly heated in a Davis crucible furnace. After the estimation they are recovered, re-treated and used again.

The procedure is as follows: A suitable quantity of the substance to be analysed is placed in a nickel crucible (6 cm. in diameter), 1 gm. of iodine-free stick potassium hydroxide, dissolved in water in the case of dry substances, is added, and the contents of the crucible are stirred with a nickel stirring rod. The crucible is heated gently over a

* The weight of silver chloride × 0·24737 = the weight of chlorine.

Bunsen burner till all bubbling ceases and then more strongly till no more fumes are evolved. Heating is continued in a Davis crucible furnace with a Teclu burner till the ash is dark grey, but the crucible must not be allowed to glow. When cool, the ash is moistened with water and carefully heated over a low flame till dry. The crucible is again heated in the crucible furnace till no further change appears to take place (about 10 minutes). After cooling, about 5 c.c. of water are added, and the contents of the crucible are filtered through a No. 40 Whatman ash-free filter paper into a small beaker. It is necessary to apply iodine-free vaseline to the lip of the crucible. The filter paper is washed 3 times with about 5 c.c. of water. The filtrate should be clear and only very faintly yellow.

The filter paper is returned to the nickel crucible, dried and then heated in the crucible furnace till a white ash is obtained. When the crucible is cool, the filtrate is added and evaporated to dryness with great care to avoid loss by spurting. The crucible is again heated in the furnace for about a minute and, when cool, about 3 c.c. of water are added. This thick solution is gently evaporated over a low Bunsen flame until a skin begins to form at the surface. When cool, it is ready for the extraction.

The ash is extracted 3 times with 3 c.c. of 95 per cent. alcohol (prepared by mixing 95 c.c. of absolute alcohol and 5 c.c. of water), and the extract is filtered through a No. 40 Whatman filter paper into a nickel crucible (5 cm. in diameter). The ash should form a smooth paste; if it is gritty, 1 or 2 drops of water should be added. After the third extraction the paste is poured on to the filter and allowed to drain. The filter paper is rejected; the funnel is washed with a little water, and the filtrate is evaporated to dryness on a water bath. The thin film of salt on the bottom of the crucible should be practically free from carbon. To get rid of any small particles which may be present, the crucible is heated for a few seconds till the bottom glows and the salt film just melts.

When the crucible is cool, the contents are dissolved in water and transferred to a 50 c.c. flask. To it are added 2 drops of 0·05 per cent. aqueous solution of methyl orange. The solution is neutralized by the addition of 2N sulphuric acid, drop by drop. After the solution is neutral, 1 drop is added; in all about 3 drops are generally required. A few drops of freshly prepared bromine water are then added. About 1 c.c. of bromine water is added in excess until the solution is distinctly yellow. After adding 3 or 4 pieces of pumice stone, the solution is boiled over a medium flame until the volume is reduced to between 1 and 2 c.c. When cool, 2 drops of potassium iodide solution and 2 drops of starch indicator are added, and the titration is carried out with N/500 sodium thiosulphate solution, using a 0·1 c.c. serum pipette. By holding the flask at an angle and tapping the bottom of the flask with the point of the pipette, it is possible to run in as little as 0·001 c.c. at a time. The end-point is perfectly definite, and after a little practice may be determined to about 0·002 c.c. The weight of iodine in the portion of the sample analysed is one-sixth of that equivalent to the volume of thiosulphate used in the titration.

MILK

The freezing point of milk is of fundamental importance and, since it varies within such narrow limits, its determination affords a valuable means of detecting and estimating added water.* The apparatus designed by G. W. Monier-Williams (*Analyst*, 1915, **40**, pp. 258-262) for the determination of the freezing point of milk gives results of the highest accuracy; but it is very complicated and is not suitable for rapid work. In this apparatus the milk is cooled by the evaporation of ether contained in a Dewar flask and is

* The reader is referred to a very valuable paper on the freezing point of milk and its applications by G. D. Elsdon and J. R. Stubbs in *J. Soc. Chem. Ind.*, 1931, **50**, pp. 135-141T. This paper contains a comprehensive bibliography of the subject.

continuously stirred by a mechanical stirrer. J. Hortvet (*J. Ind. Eng. Chem.*, 1921, **13,** pp. 198-208) designed a much simpler apparatus based on that of Monier-Williams, which is suitable for routine work. Hortvet's method is adopted by the Association of Official Agricultural Chemists, and their instructions for making the determination are given fully on p. 210. The recommendations of Elsdon and Stubbs for the more accurate standardization of the thermometer are given on p. 214. The Hortvet cryoscope gives results which are reproducible and are strictly comparable, but they are not the true freezing points. Suggestions have been made that the results should be corrected so as to represent more nearly the true freezing points of milk; but if different observers apply different corrections, which may or may not be correct, the results will not be comparable. To avoid confusion owing to the publication of data obtained by different methods, the Council of the Society of Public Analysts (*Analyst*, 1933, **58,** pp. 318-319) have recommended that for administrative purposes the freezing point of milk should be determined by Hortvet's method, without applying any corrections other than those mentioned in the A.O.A.C. instructions; and that the result should be recorded as the freezing point (Hortvet).

The acidity of milk may be considered in terms of titratable acidity or hydrogen ion concentration. The titratable acidity is determined by titration with standard sodium hydroxide, as described on p. 214. The result is expressed as the degrees of acidity or the percentage of lactic acid by volume or by weight, although lactic acid does not occur in fresh milk.* L. R. Parks and C. R. Barnes (*Ind. Eng. Chem.* (*Anal.*), 1935, **7,** pp. 71-72) determined the pH values of whole milk, cream and butter serum (the lower aqueous

* For the explanation of the titratable acidity of fresh milk see H. T. S. Britton, *Hydrogen Ions*, 1932, pp. 489-490. The relation between titratable acidity and pH value was determined by P. F. Sharp and T. J. McInerney (*J. Biol. Chem.*, 1927, **75,** pp. 177-184), and their results are given by W. L. Davies in *Chemistry of Milk*, 1936, p. 302.

layer formed on melting butter) in several ways; they found that the values obtained by the glass electrode and the quinhydrone electrode agreed with those obtained by the hydrogen electrode within the limits of experimental error. The antimony electrode gave results which were 0·31-0·65 pH higher than those obtained by the glass electrode. These erroneous values are probably due to citrate and lactate ions forming complexes with antimony at the surface of the electrode. The quinhydrone electrodes were made of platinum foil coated with gold; a decided drift occurred when platinum electrodes were used. The procedure adopted by Moir in determining the pH values of fresh and coagulated milk with the quinhydrone electrode is described on p. 215. He used three gold electrodes so that if one was poisoned, the poisoning would be shown by the reading of that electrode differing from those of the other two.

The pH values of whole milk, skim milk and whey can be determined colorimetrically by the dilution method of Sharp and McInerney, which is described on p. 217. In this method the sample is diluted to 20 times its original volume, and the pH value of the diluted sample is determined colorimetrically. The value so obtained is too high, owing to the dilution; but the pH value of the original sample can be obtained from a table giving the pH values of the diluted and the undiluted samples. Of greater practical importance than the accurate determination of the pH value is the detection of abnormal milks having high pH values. The method of Baker and Van Slyke is described on p. 217. It consists in adding to the milk to be tested a saturated aqueous solution of bromo-cresol purple, and comparing the colour thus produced with colour standards prepared from fresh milk of average acidity to which have been added different measured volumes of N/10 sodium hydroxide solution and the same quantity of bromo-cresol purple solution. Eight colour standards are prepared in this way, and the milks under examination are divided into eight groups, each of a limited pH range, three of which include

the normal milks and the other five the abnormal ones with high pH values.

The specific gravity of milk should not be determined until it has reached its maximum value (see p. 219). It is usually determined with a lactometer as the first stage in finding the total solids by calculation. The percentage of total solids can be calculated with a fair degree of accuracy from the specific gravity at 60° F. and the percentage of fat by Richmond's formula, which is given on p. 220. The calculation can be greatly simplified by using one of the special slide rules which are described there. The percentage of total solids is determined directly by drying a weighed quantity of milk to constant weight. When milk is evaporated, a skin is formed which hinders the escape of water on further heating. Various methods have been proposed to overcome this difficulty. The skin can be broken with a needle or the formation of the skin can be prevented by the addition of a drop of acetic acid to the milk or the milk can be mixed with clean sand or asbestos. These methods of hastening the drying are, however, unnecessary if a small quantity of milk is dried in a flat-bottomed dish, as described on p. 221. The great disadvantage of flat-bottomed dishes is that they take up so much space in the ovens and desiccators. Golding has improved the procedure by using an air-damped balance, aluminium milk-bottle caps in place of dishes and a syringe to deliver 1 gm. of milk. In this way the time taken in weighing and drying is shortened, and the space occupied in the ovens and desiccators is economized without any loss of accuracy. The details of this method are given on p. 222.

In Great Britain and on the continent of Europe the fat in milk is generally determined by the Gerber method, which is an improvement of the Leffmann-Beam method.* The apparatus for the Gerber method and the procedure have

* For the details of this method see H. Leffmann and W. Beam, *Food Analysis*, London, 1901, pp. 206-207.

recently been standardized by the British Standards Institution, and it is hoped that this procedure, which is given on p. 223, will be generally followed. The instructions given in some books are to add the sulphuric acid first, then the amyl alcohol and the milk last. These were Gerber's original instructions; but he found that the sulphuric acid and amyl alcohol could not be left long in contact without vitiating the results and afterwards recommended that the acid should be added first, next the milk and finally the amyl alcohol. It will be noticed that this is the order in which these reagents are added in the British standard method. Some samples of amyl alcohol give results with the Gerber method which are too high. The specifications for amyl alcohol are referred to on p. 224. They include a test for oily impurities, which consists of comparing the results of the Gerber method with those obtained by the Röse-Gottlieb method, when the latter method is carried out as described on p. 227. The Röse-Gottlieb method consists in treating the milk with ammonia in order to dissolve the proteins, extracting the fat with a mixture of alcohol, petroleum spirit and ether, transferring the solution of fat to a weighed flask, evaporating the solvent and drying the fat to constant weight. Various forms of apparatus have been used for this process; but it is most convenient to use wash-bottle tubes for transferring the fat solution to the weighed flask and to use for the extraction a vessel, such as that in Fig. 4, in which the milk can be weighed. Another volumetric method of determining fat in milk is the Babcock method, which is described on p. 229. This method is largely used in the United States of America and is an official method of the Association of Official Agricultural Chemists.

The colour of milk is chiefly due to carotene, which can be determined by extracting the milk fat and finding the carotene content of a petroleum spirit solution of the fat by means of a Lovibond tintometer or a colorimeter. This method is due to Ferguson and Bishop, whose procedure

is given on p. 230. Owing to the formation of emulsions the extraction of the fat is not quantitative. But Ferguson and Bishop think that the extracted fat is representative of the whole of the fat in the milk, since carotene occurs in solution in the milk fat and it is unlikely that any preferential extraction will take place.

The total proteins in milk are determined by multiplying the total nitrogen by 6·38. Some of the procedures which have been found suitable for the determination of nitrogen in milk are given on p. 231. The individual milk proteins are determined by precipitating them under particular conditions and determining the nitrogen in the filtered and washed precipitates. Owing to doubt as to the values of the conversion factors of the milk proteins, the results are expressed as the percentages of albumin nitrogen, globulin nitrogen, etc., in the whole milk, or the percentage of nitrogen in each protein is expressed as a percentage of the total nitrogen. Casein was formerly precipitated at pH 4·2 by adding 1·5 c.c. of 10 per cent. acetic acid to 10 c.c. of milk diluted with 90 c.c. of water. Moir has modified the procedure by adding to diluted milk first acetic acid and then a solution of sodium acetate so as to bring the mixture to pH 4·6, which is the isoelectric point of casein. His procedure is given on p. 231. The casein is determined by multiplying the casein nitrogen by 6·38 or by the formol titration, which consists of titrating the acidity produced by the action of formaldehyde on the casein, as described on p. 233. Moir has also devised methods for the precipitation of albumin together with globulin and casein together with globulin, which are described on p. 233. If casein nitrogen and the total protein nitrogen are determined in the same sample, albumin nitrogen and globulin nitrogen can be found by difference.

Lactose can be directly determined in milk by Lane and Eynon's volumetric method (see p. 132) after suitable dilution and without any previous clarification. The turbidity and the presence of proteins do not interfere with

the reduction of the methylene blue used as the internal indicator. Another way of determining lactose is Cole's ferricyanide-methylene-blue method. This volumetric method affords a very convenient means of determining lactose in milk. The titration is carried out with the filtrate after precipitating the proteins with colloidal iron, as explained on p. 139. The two methods adopted by the Association of Official Agricultural Chemists for the determination of lactose in milk are given on p. 235. The first is an optical one, in which the proteins are precipitated by acid mercuric nitrate solution and the clear filtrate is polarized. The second method consists in precipitating the proteins with cupric hydroxide and determining the lactose in the filtered solution by Munson and Walker's gravimetric method. It is usual in milk analysis to express the results as the percentages of anhydrous lactose.

The determination of citric acid in milk has been for some time a problem of great difficulty. Recently Lampitt and Rooke have evolved the procedure given on p. 236. The citric acid in the milk serum is oxidized by means of potassium permanganate to acetone dicarboxylic acid, which is converted by the action of bromine into pentabromoacetone. The latter compound is filtered, washed and weighed, an allowance being made for its solubility in the washing liquid. In this way citric acid up to 0·11 gm. can be determined in the presence of lactose to within 2 mgm.

The determination of the total ash of milk requires great care because milk contains alkali chlorides which are volatile at high temperatures. For the determination 20-25 gm. of milk are evaporated to dryness in a platinum or silica dish and the milk solids are ignited at a temperature below redness until a white or greyish white ash is obtained. The Association of Official Agricultural Chemists (*Methods of Analysis*, 1935, p. 265) determine the total ash by adding 6 c.c. of nitric acid to 20 c.c. of milk, evaporating to dryness and igniting below redness until the ash is free from carbon. The addition of nitric acid is not recommended by H. Leff-

mann and J. Golding (*Allen's Commercial Organic Analysis*, ix, 1932, p. 76) for the determination of the total ash, but it affords a very convenient way of preparing ash for the determination of the mineral constituents.

The chlorine content of milk can be determined by Volhard's method after ashing the milk mixed with sodium carbonate or lime to prevent loss of chlorine. But the determination can be carried out more easily without incineration. In Husband and Godden's method (see p. 237) the proteins are precipitated by the addition of picric acid and the chlorine is determined in the filtrate by Volhard's method. In Davies's method the milk is heated with potassium permanganate and nitric acid, and the chlorine is determined in the same way.

Calcium, magnesium, and potassium can be determined in milk by the same methods as those used for the determination of these constituents in feeding stuffs. A special procedure for manganese in milk will be found on p. 177. In the method for the determination of sodium in milk, which is given on p. 238, the milk is mixed with nitric acid, evaporated to dryness and ignited. The ash is dissolved in nitric acid and phosphates are removed by adding an alcoholic solution of zinc acetate with zinc hydroxide. The sodium in the filtrate is determined by a modification of Barber and Kolthoff's gravimetric method, the sodium uranyl zinc acetate being precipitated in the Jena glass filter in which it is weighed. Phosphorus in milk can be determined in the nitric acid solution of the ash by the A.O.A.C. volumetric method (see p. 48). Dr. W. L. Davies recommends the Embden-Fetter method, which is described on p. 240. The phosphoric acid is precipitated as strychnine phosphomolybdate, which when filtered off and dried contains 1·12 per cent. of phosphorus.

Since 1st January, 1937, the bacteriological grading of milk by the plate count test has been superseded in the case of Tuberculin Tested and Accredited milks by the methylene blue reduction test, which was recommended by G. S.

Wilson and his co-workers (*Medical Research Council, Special Report Series*, No. 206, 1935). This test, the details of which are given on p. 241, is a simple test with a small experimental error and requires little equipment. It gives a useful index of the cleanliness and keeping qualities of the milk; but in order to obtain reliable results strict attention to the following details is essential: careful control of the temperature, the maintenance of a homogeneous suspension of fat globules and bacteria and the exclusion of light from the water bath.

Pasteurized milk is defined in The Milk (Special Designations) Order, 1936, as milk which has been retained at a temperature of not less than 145° F. and not more than 150° F. for at least 30 minutes and is then immediately cooled to a temperature of not more than 55° F. It is possible by means of the phosphatase test to find out whether a sample of milk has been subjected to this treatment. The test depends on the fact that phosphatase, an enzyme which is present in all samples of raw milk, is almost completely destroyed by pasteurization as defined above, but is not completely destroyed if the milk is heated at a lower temperature than 145° F. or for a shorter period than 30 minutes. Phosphatase hydrolyses phosphoric esters, and its relative quantity in the milk under examination is shown by applying a delicate test for one of the products of hydrolysis. In Kay and Graham's test, which is described on p. 243, the phosphoric ester used is disodium phenyl phosphate and the phenol produced by the active enzyme is estimated by using Folin and Ciocalteu's reagent. The blue colour produced is either measured by means of a Lovibond tintometer or is compared with standard glasses in a comparator. The test is carried out in two ways. The first test takes about 30 minutes to carry out and shows whether the milk has been heated or not. But if the colour developed is close to the standard, or if there is any reason to suspect minor errors of pasteurization which might not be revealed by the first test, the sample should be tested by the second more delicate test, which takes longer because

it includes heating the sample at 37°-38° C. for 24 hours. As a result of their experience of the test, Anderson, Herschdörfer and Neave have published some additional details relating to the reagents and apparatus, which are given on p. 246. They have also pointed out that satisfactory results can be obtained only by following closely Kay and Graham's technique.

Freezing Point.—The Hortvet cryoscope and the method of using it for the determination of the freezing point of milk are described in *Methods of Analysis*, 1935, pp. 270-274, from which the following brief description of the apparatus and the details of the procedure are taken. The apparatus, which is shown in Fig. 3, consists of a cylindrical Dewar flask surrounded by a metal casing and enclosing two concentric test tubes. The inner glass tube containing the milk under examination is surrounded by a metal tube containing alcohol, and this is immersed in the ether contained in the vacuum flask. Evaporation of the ether is brought about by blowing a current of dry air through it.

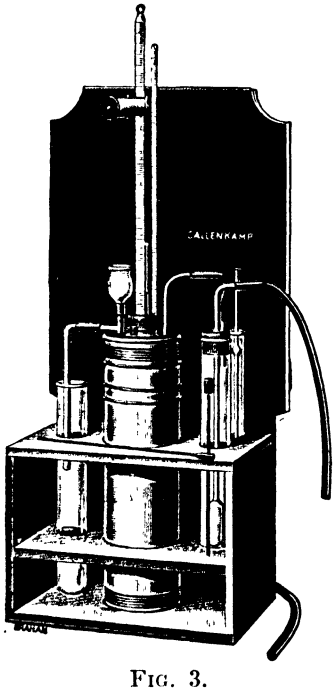


FIG. 3.

A control thermometer with a range from +20° C. to – 30° C. is provided for regulating the temperature of the ether bath. The inner glass freezing tube is fitted with a rubber stopper bored with three holes. Through the centre one passes an accurate thermometer with a range from +1° C. to – 2° C., each degree being subdivided into tenths and hundredths. Through the hole on the right passes a stirrer, the lower end of which extends nearly to the bottom of the freezing tube.

Through the hole on the left is inserted the freezing starter, which consists of a metal rod with an opening for a small piece of ice.

The determination is carried out as follows: Insert the funnel tube in the vertical portion of the T-tube on the left of the apparatus, and pour in 400 c.c. of ether previously cooled to 10° C. or lower. Close the vertical tube by means of a small cork and connect the pressure pump to the inlet tube of the air-drying attachment. Adjust the pump so as to pass air through the apparatus at a moderate rate, which can be judged by the agitation of the sulphuric acid in the drying tube. Continuous vaporization of the ether will cause a lowering of the temperature in the flask from the ordinary temperature to 0° C. in 5-10 minutes. Continue the cooling until the control thermometer registers nearly – 3° C. At this stage, by lowering the gauge tube into the ether bath, then closing the top with the forefinger and raising it to a suitable height, it is possible to estimate the quantity of ether which must be poured in to restore the volume to 400 c.c. When the volume of ether has been adjusted to 400 c.c., an additional 10-15 c.c. is generally sufficient for each succeeding determination. Pour into the freezing tube sufficient water (30-35 c.c.), boiled and cooled to 10° C. or lower, to submerge the thermometer bulb. Insert the thermometer together with the stirrer, and lower the freezing tube into the larger tube. A small quantity of alcohol, sufficient to fill the lower space between the two test tubes, serves as a conducting medium between the freezing bath and the liquid to be tested. Keep the stirrer in steady up-and-down motion at a rate of about one stroke in each 1 or 2 seconds, or at a slower rate if the cooling proceeds satisfactorily. Maintain a current of air through the apparatus until the temperature of the cooling bath reaches – 2·5° C., at which time the top of the mercury in the thermometer usually recedes to a position near the freezing point of water. Maintain the temperature of the cooling bath at – 2·5° C. and continue the manipulation of

the stirrer until a supercooling of the sample of 1·0-1·2° is observed. As a rule, the liquid at this time will begin to freeze, as shown by the rapid rise of the mercury. Manipulate the stirrer slowly and carefully 3 or 4 times as the mercury approaches the highest point. By means of a suitable cork mallet tap the upper end of the thermometer cautiously until the top of the mercury column remains stationary for at least 1 minute. Observe the exact reading on the thermometer scale with a lens, taking precautions to avoid parallax, and estimate to 0·001°. Make a duplicate determination; then remove the thermometer and stirrer, and empty the water from the freezing tube.

Rinse the tube with about 25 c.c. of the sample of milk, which has been cooled to 10° C. or lower. Pour into the tube 30-35 c.c. of milk or sufficient to submerge the thermometer bulb, and insert the tube into the apparatus. Maintain the temperature of the cooling bath at 2·5° below the probable freezing point of the sample. Make the determination on the milk, following the same procedure as that employed in determining the freezing point of water. As a rule, it is necessary to start the freezing of the milk by inserting the freezing starter, which has been kept in contact with ice for several minutes and in the open end of which has been wedged a fragment of ice; this is inserted at the time when the mercury column has receded to 1·0-1·2° below the probable freezing point. A rapid rise of the mercury takes place almost immediately. Remove the freezing starter and manipulate the stirrer slowly and carefully 2 or 3 times whilst the mercury approaches the highest point. Complete the adjustment of the mercury column in the same way as in the preceding determination; then, avoiding parallax, observe the exact reading on the thermometer scale, and estimate to 0·001°. The algebraic difference between the average readings obtained with water and the reading obtained with the sample of milk represents the freezing point depression of the milk. Apply the necessary correction to the result as explained below.

Make 3 freezing point determinations by the procedure described above with each of the following:

(*a*) Recently boiled distilled water.

(*b*) Sucrose solution prepared by dissolving 7 gm. of pure sucrose in water and making the solution up to 100 c.c. at 20° C.

(*c*) Sucrose solution prepared by dissolving 10 gm. of pure sucrose in water and making the solution up to 100 c.c. at 20° C.

Express the results as degrees of freezing point depression below the average of the observed freezing points obtained with the sample of pure water, which may be above or below the zero mark on the scale. Obtain each freezing point depression of the sucrose solutions by the algebraic subtraction of the average of the freezing point readings of pure water from each observed freezing point. Apply the average of the freezing point depressions obtained with the standard sucrose solutions for the purpose of correcting the thermometer readings obtained with the samples of milk. Check the thermometer at frequent intervals, once a week or as often as may be necessary, in order to keep an accurate record of any changes that may occur.

Two Bureau of Standards tested thermometers gave intervals of 0·199° and 0·200° between the freezing point depression readings of the two sucrose solutions. One thermometer gave freezing point depressions of −0·422° and −0·621° for the two sucrose solutions, while the other gave −0·422° and −0·622° respectively.

Laboratory thermometer No. 2 gave the following readings: water, +0·056°; sucrose solution (7 gm. per 100 c.c.), −0·425°; sucrose solution (10 gm. per 100 c.c.), −0·621°. Interval = 0·196. Correction = 0·199 / 0·196 = 1·015.

Laboratory thermometer No. 24 gave the following readings: water, 0·000°; sucrose solution (7 gm. per 100 c.c.), −0·420°; sucrose solution (10 gm. per 100 c.c.), −0·625°. Interval = 0·205. Correction = 0·199 / 0·205 = 0·971.

Using laboratory thermometer No. 24, the freezing point

depression of a sample of milk was 0·548. (0·548 – 0·420) × 0·971 = 0·124. Therefore the corrected depression = 0·422 + 0·124 = 0·546.

Standardization of the Thermometer.—G. D. Elsdon and J. R. Stubbs (*Analyst*, 1934, **59**, pp. 585-593) have pointed out that the Association of Official Agricultural Chemists assume that the stem correction of the thermometer is uniform between – 0·422° and – 0·621°; and suggest that the thermometer should be checked at intermediate temperatures. The freezing points of solutions of sucrose containing the following weights of sucrose per 100 c.c. of solution at 20° C. are:

Grams.				° *C.*
7·0	..	..	..	– 0·422
7·5	..	..	..	– 0·455
8·0	..	..	..	– 0·488
8·5	..	..	..	– 0·521
9·0	..	..	..	– 0·555
9·5	..	..	..	– 0·588
10·0	..	..	..	– 0·621

It will thus be seen that solutions of sucrose containing 8·5 gm. and 9·0 gm. per 100 c.c. are very suitable for checking the thermometer at about the freezing point of normal milk. The Association of Official Agricultural Chemists specify recently boiled distilled water for the determination of the zero. Elsdon and Stubbs have carried out experiments which show that the use of boiled or unboiled water makes no appreciable difference to the results obtained. They consider that the range of the control thermometer is unnecessarily large; and have found a solid stem instrument about 58 cm. long and 6·5-7·0 mm. in diameter, having a range of +5° to – 5° C. divided into fifths of a degree, more satisfactory.

Titratable Acidity.—The method adopted by H. D. Richmond (*Dairy Chemistry*, 1920, p. 179, and *Dairy Analysis*, 1925, pp. 15-16) for the determination of the acidity of milk is as follows: Pipette 10 c.c. of milk into a porcelain

dish, add 1 c.c. of phenolphthalein solution (0·5 gm. in 100 c.c. of 50 per cent. alcohol) and titrate with N/10 sodium hydroxide solution until a faint pink colour, which does not disappear on stirring, is produced. The pink colour at the end of the titration should be equal to that given by adding 1 drop of 0·01 per cent. solution of rosaniline acetate in 96 per cent. alcohol to 11 c.c. of the same milk. The ratio of milk to phenolphthalein should always be the same as that stated above. The acidity is expressed as the percentage of lactic acid by volume or as degrees of acidity. Each tenth of a c.c. of N/10 sodium hydroxide solution used in the titration represents 1 degree of acidity. The degrees of acidity multiplied by 0·009 give the acidity as the weight of lactic acid per 100 c.c. of milk.

The calculations are greatly simplified by using N/9 sodium hydroxide solution, 1 c.c. of which=0·01 gm. of lactic acid. Using 10 c.c. of milk, the volume of N/9 sodium hydroxide solution in c.c. divided by 10 gives the percentage of lactic acid by volume. The Association of Official Agricultural Chemists (*Methods of Analysis*, 1935, p. 264) measure the milk in a Babcock pipette, which holds 17·6 c.c. (18 gm.) of milk, add an equal volume of boiled water and titrate with N/10 sodium hydroxide solution, using phenolphthalein as indicator. The result is expressed as the percentage of lactic acid by weight, which is obtained by dividing the number of c.c. of N/10 sodium hydroxide solution used in the titration by 20.

Quinhydrone Electrode.—For the determination of the pH values of fresh and coagulated milks G. M. Moir (*Analyst*, 1931, **56**, pp. 445-448) used the quinhydrone electrode. His apparatus was very similar to that described by E. Biilmann and S. Tovborg-Jensen (*Trans. 2nd Comm. Internat. Soc. Soil Sci., Groningen*, 1927, B, pp. 236-274). This apparatus consists of a Veibel's standard quinhydrone electrode, a vessel containing 3·5M potassium chloride solution, a **U**-shaped glass tube filled with a solution of potassium chloride in agar, and a test tube containing the liquid to

be tested, in which is immersed a platinum electrode. The capillary end of the standard quinhydrone electrode is placed below the surface of the 3·5M potassium chloride solution, and connection between the latter and the liquid to be tested is made by means of the agar tube.*

Instead of one platinum electrode in the liquid to be tested, Moir used three gold electrodes. Poisoning of one electrode was shown by one electrode giving a different reading from the other two. Poisoning does not often occur with clear solutions, but liquids like milk, especially after the proteins have been coagulated, give more trouble. In such cases, washing the electrodes first under the tap and then with distilled water does not suffice; heating is often necessary after each determination. The gold electrodes were made of gold foil about $1{\cdot}0 \times 0{\cdot}5 \times 0{\cdot}025$ cm. and were attached to gold wire (S.W.G. 21) by first hammering and then heating just to redness. The gold wire was then attached in the same way to a platinum wire, which was fused into a glass tube. The electrodes must be heated in the flame of an alcohol lamp (not a coal gas burner), and care must be taken not to heat them beyond the first signs of redness.

Before measuring an unknown pH, a solution of known pH is tested. For this purpose N/10 potassium phthalate solution, which has a pH value of 3·97, is used, or standard acetate mixture, which is N/10 with respect to both acetic acid and sodium acetate, and has a pH value of 4·626. A few c.c. of the liquid to be tested are placed in the test tube, the agar bridge is inserted and about 0·1 gm. of quinhydrone added to the liquid. The electrodes are at once inserted and used to stir the liquid. The potentiometer reading is taken as soon as possible, because a drift takes place in some solutions. The pH is calculated from the formula:

$$\mathrm{pH} = 2{\cdot}03 + \frac{\mathrm{E}}{0{\cdot}0577 + 0{\cdot}0002\,(t - 18)}$$

* The apparatus is fully described in the author's *Soil Analysis*, 1934, pp. 56-59.

where E is the observed e.m.f. in volts and t is the temperature in degrees Centigrade.

Colorimetric Determination of pH Value.—The method described by P. F. Sharp and T. J. McInerney (*J. Biol. Chem.*, 1926, **70,** pp. 729-758) is as follows: One volume of whole milk, skim-milk or whey is mixed with 19 volumes of distilled water. The pH value of the diluted sample is determined colorimetrically in a test tube comparator in the usual way, using phenol red, bromo-cresol purple, chlorophenol red, bromo-cresol green or bromo-phenol blue as indicator and Clark and Lubs's buffer solutions as standards.* From the pH value of the diluted sample thus obtained, the pH value of the original sample can be found from the table on the next page.

Colorimetric Detection of Abnormal Milks.—A colorimetric method of finding the approximate pH values of milks, and thus detecting the abnormal ones, has been described by J. C. Baker and L. L. Van Slyke (*J. Biol. Chem.*, 1919, **40,** pp. 357-371). The following are the details of the method: To 3 c.c. of the milk to be tested is added 1 drop (0·05 c.c.) of a saturated aqueous solution of bromo-cresol purple (0·1 gm. per 100 c.c.). Normal milk gives a greyish-blue colour, but as the pH value increases the colour becomes more and more purple. The approximate pH value of the milk can be found by comparing the colour with colour standards which are prepared as follows: Into each of 8 test tubes are measured 10 c.c. of fresh milk known to have a normal reaction; the milk should not have an acidity greater than that equivalent to 18 c.c. of N/10 sodium hydroxide solution per 100 c.c. of milk. To the first tube nothing is added; to the others are added 0·2, 0·4, 0·6, 0·8, 1·0, 1·2 and 1·4 c.c. of N/10 sodium hydroxide solution, and the test tubes are well shaken. To 3 c.c. of milk from each of these tubes is added 1 drop of the bromo-

* It would be more convenient to use a Hellige or a Lovibond comparator, both of which are provided with glass colour standards mounted on rotating discs.

Colorimetric Reading (pH).	*Whole Milk and Skim-Milk Undiluted (pH).*	*Whey Undiluted (pH).*	*Indicator.*
7·4	6·86	—	Phenol red
7·3	6·76	—	
7·2	6·66	6·94	
7·1	6·56	6·84	
7·0	6·46	6·74	
6·9	6·36	6·64	
6·8	6·26	6·54	
6·7	6·16	6·44	
6·6	6·06	6·34	Bromo-cresol purple
6·5	5·96	6·24	
6·4	5·86	6·15	
6·3	5·76	6·05	
6·2	5·66	5·96	Chloro-phenol red
6·1	5·57	5·87	
6·0	5·48	5·77	
5·9	5·40	5·68	
5·8	5·32	5·58	
5·7	5·24	5·48	
5·6	5·17	5·38	
5·5	5·11	5·28	
5·4	5·05	5·18	
5·3	4·99	5·08	
5·2	4·92	4·98	
5·1	4·86	4·88	
5·0	4·78	4·78	
4·9	4·71	4·68	Bromo-cresol green
4·8	4·62	4·58	
4·7	4·53	4·48	
4·6	4·44	4·38	
4·5	4·34	4·28	
4·4	4·25	4·18	Bromo-phenol blue
4·3	4·15	4·08	
4·2 (?)	4·05	3·98	
4·1 (?)	3·94	3·88	
4·0 (?)	3·84	3·78	

cresol purple solution, thus giving a series of colour standards, which divide milks into 8 groups with the pH values given in the following table. The milks included in groups 1-3 are regarded as normal.

Group.	*c.c. of N/10 NaOH.*	*pH Values.*
1	0·0	6·5 -6·6
2	0·2	6·6 -6·67
3	0·4	6·67-6·75
4	0·6	6·75-6·82
5	0·8	6·82-6·90
6	1·0	6·90-6·98
7	1·2	6·98-7·05
8	1·4	7·05-7·13

In testing the milk from Shorthorn and Guernsey cattle by this method, F. Procter and A. T. R. Mattick (*J. Agric. Sci.*, 1926, **16,** pp. 145-148) found that it was necessary to prepare two series of colour standards, one for Shorthorn and the other for Guernsey milks, since, owing to their higher fat content and yellow colour, the latter always gave a lighter blue colour than the former.

Specific Gravity.—The specific gravity of milk increases gradually for some time after it has been drawn. The maximum value is reached in about 5 hours, if the temperature of the milk is below 15° C.; but at higher temperatures 12-24 hours may elapse before the maximum value is reached. The maximum value is taken as the correct figure. The results are stated as the specific gravities at 60° F./60° F.

The specific gravity can be determined with a Sprengel tube, a specific gravity bottle or a Westphal balance. It is, however, usually determined with a lactometer, which is an accurately graduated hydrometer with a range from 1·000 to 1·040. The specific gravities at 60° F. can be found from the observed specific gravities at other temperatures by using Richmond's milk scale or the dairy scale.

Total Solids by Calculation.—If the specific gravity at 60° F. (S) and the percentage of fat (F) are known, the

percentage of total solids (T.S.) and the percentage of solids not fat (S.N.F.) can be calculated from Richmond's formulae, which are:

$$\text{T.S.} = \frac{\text{G}}{4} + 1{\cdot}2\ \text{F} + 0{\cdot}14$$

$$\text{and} \quad \text{S.N.F.} = \frac{\text{G}}{4} + \frac{\text{F}}{5} + 0{\cdot}14,$$

where $\text{G} = 1000\ (\text{S} - 1)$; it is termed the degrees of gravity or the lactometer reading.

The calculation can be made with Richmond's milk scale, which is a slide rule designed for this purpose. On one side is marked total solids (1 inch = 1 per cent.) and on the other fat (1·2 inch = 1 per cent.). On the slide is marked specific gravity ($\frac{1}{4}$ inch = 1 degree) and an arrow is placed 0·14 inch from the end of the scale. If the arrow be placed against the fat, the specific gravity lies against the total solids; and conversely, if the specific gravity be placed against the total solids, the arrow will point to the fat. If the temperature at which the specific gravity was determined is not 60° F., the corrected reading can be obtained by placing the line showing the observed lactometer reading against 60° on the temperature scale, and then noting the lactometer reading opposite the temperature at which the specific gravity was determined.

Richmond's dairy scale, which is an improved milk scale, can be used to calculate either the total solids or the solids not fat. If the arrow on the upper scale be adjusted to the percentage of fat, the total solids can be read off against the specific gravity; and by adjusting the arrow on the slide to the "fat for S.N.F." scale, the solids not fat correspond to the specific gravity, the reading in each case being facilitated by the cursor provided. On the rule is a scale of degrees of gravity and on the slide are scales of temperature in both Fahrenheit and Centigrade degrees, the ranges being 33°-85° F. and 0°-30° C. An arrow is placed at 60° F. on one scale and at 15·5° C. on the other. If the arrow is placed

against the degrees of gravity found, the temperature corresponds to the specific gravity at 60° F. (H. D. Richmond, *Dairy Analysis*, 1925, pp. 44-46).

The percentage of solids not fat can be read directly on Collins's milk scale when the percentage of fat and the specific gravity at any temperature from 40° to 74° F. are known. To do this, the reading of the lactometer is set opposite the temperature at which the specific gravity was taken; the percentage of solids not fat will then be found opposite the percentage of fat.*

Total Solids (Gravimetric Method).—The percentage of total solids is determined by drying to constant weight a small quantity of milk in a flat-bottomed dish. The dish may be of any material which does not change in weight when heated to the temperature of boiling water. H. D. Richmond (*Dairy Analysis*, 1925, pp. 11-12) adopted the following procedure: A platinum, fused silica or porcelain

* The percentage of total solids can also be calculated from the percentage of fat and the density at 20° C. British Standard Specification No. 734, 1937, gives the specifications of density hydrometers for use in milk, corrections to be applied to hydrometer readings taken at temperatures other than 20° C. to obtain the density of the milk at 20° C. and tables giving the percentages of total solids and non-fatty solids corresponding to given fat contents and densities. Density has been adopted in preference to specific gravity because it affords a more satisfactory basis for hydrometry than specific gravity. The temperature 20° C. has been adopted because this temperature is being widely used as the standard temperature of adjustment for scientific apparatus. For hydrometers for use in milk it has a distinct advantage over 60° F. because the density of milk determined at temperatures below 20° C. (68° F.) is dependent on the temperature to which the milk has been subjected previously. It is therefore recommended that milk samples should be warmed to 40° C., kept at that temperature for about 5 minutes and the density taken when the sample has cooled to about 20° C. The total solids in the tables were calculated from the following formula, which is derived from Richmond's formula given above:

$$T = 0{\cdot}25[1000(d-1)] + 1{\cdot}2125F + 0{\cdot}6525.$$

where T = the percentage of total solids, d = the density at 20° C. and F = the percentage of fat.

dish, preferably $2\frac{3}{4}$ in. wide and flat-bottomed, is weighed. Into it are pipetted 5 c.c. of milk. The dish is weighed again; the weighing should be rapid, but the exactitude need not be more than 2 mgm. The dish is placed on a water bath and the skin is broken from time to time with a needle. When apparently dry, the dish is placed in a water oven and is left there for 4 hours, after which it is cooled in a desiccator and weighed. It is then replaced in the oven for periods of 1 hour each, cooled and weighed, until the loss in weight in 1 hour is less than 1 mgm.

The method recommended by H. Leffmann and J. Golding (*Allen's Commercial Organic Analysis*, ix, 5th edn., 1932, pp. 75-76) is as follows: A nickel dish, 9 cm. in diameter with sides at least 1 cm. high, is weighed. 3-5 gm. of milk are added and the dish is rapidly weighed again. The dish with its contents is heated for half an hour on a water bath and is then dried in a water oven at 100° C. for 2 hours, after which it is cooled in a desiccator and weighed. It is then dried in the oven for periods of half an hour until it ceases to lose weight.

Total Solids (with Air-Damped Balance).—For the gravimetric determination of total solids J. Golding (*Analyst*, 1934, **59,** pp. 468-474) uses aluminium milk-bottle caps in place of nickel dishes, a syringe to deliver 1 gm. of milk and an air-damped balance with which the weighings are made to 0·1 mgm.*

The milk-bottle caps are 44 mm. in diameter, 7·4 mm. deep and 0·18 mm. thick; they vary in weight from 1·1 to 1·3 gm. Before use the caps are rinsed with alcohol, followed by a fat solvent (Röse-Gottlieb distillates) to remove the lubricant used in their manufacture; they lose about 1 mgm. by this treatment. They are easily cleaned and change very little in weight if they are carefully cleaned and freed

* The most satisfactory results are obtained with a balance which is air-damped at both ends of the beam and the graticule of which is graduated over its whole length (see W. N. Bond, *ibid.*, 1936, **61,** pp. 85-90).

from traces of fat by means of alcohol and ether. The oven used by Golding measures $14\frac{1}{2}$ by $14\frac{3}{4}$ by $15\frac{1}{2}$ in. and is fitted with 5 staggered copper trays. A supply of heated air is admitted at the 4 corners of the floor of the oven through 4 metal tubes which pass through the boiling water in the detachable water bath under the oven.

The procedure is as follows: The clean dry bottle caps are taken from a desiccator and weighed. While still on the balance pan, a syringeful of milk is quickly introduced into each bottle cap, which is then weighed again. The syringe is adjusted to deliver just over 1 gm. of milk; it is delivered very rapidly with a variation of not more than 3 mgm. After delivering each sample, the syringe is quickly cleaned by rinsing it with 2 or 3 fillings of milk. The weighed bottle caps with the milk are placed in rows on one of the copper trays of the oven and are covered with a sheet of glass. When a batch of milks has been weighed out, the glass plate is removed, and the tray is placed on a steam bath for 15 minutes. After the milk has evaporated, the tray is wiped underneath and is pushed into the oven. After 2 or 3 hours the bottle caps are placed in desiccators holding 10 each, allowed to cool for 5 minutes and weighed. A second weighing is made after another hour in the oven.

The Gerber Method.—The following description of the British standard method of determining fat in milk by the Gerber method is abstracted by permission from British Standard Specification No. 696, Part 2, 1936, pp. 9-14. The specifications of the necessary apparatus are given in B.S.S. No. 696, Part 1, 1936.* The following apparatus and chemicals are required:

(i) Standard butyrometers for testing milk.

(ii) Double-ended rubber stoppers for the butyrometers.

(iii) Standard 11 ml. milk pipette.

* Copies of these publications can be obtained from the British Standards Institution, 28 Victoria Street, London, S.W.1, price 3s. 8d. each post free.

(iv) Standard 10 ml. acid pipette or automatic measure to deliver 10 ml.

(v) Standard 1 ml. amyl alcohol pipette or automatic measure to deliver 1 ml. If a large number of samples has to be examined, it is preferable to use automatic measures. The use of the 1 ml. pipette may cause injurious effects owing to the inhalation of amyl alcohol.

(vi) Centrifuge having a working speed of 1100 r.p.m and a diameter of 18-20 in.

(vii) Water bath, preferably with thermostatic control.

(viii) Sulphuric acid, having a density of $1{\cdot}815 \pm 0{\cdot}002$ at 20° C., corresponding to a specific gravity of 1·820-1·825 at 60° F./60° F. It should be colourless or not darker than pale amber.

(ix) Amyl alcohol, which should comply with the requirements in B.S.S. No. 696, Part 2, pp. 46-49. When used for the determination of fat in milk in a standard butyrometer, it should give a result which does not exceed by more than 0·05 per cent. that obtained in a determination of fat by the Röse-Gottlieb method as described on p. 227.

Successful results can be obtained by the Gerber method only if reliable apparatus is used. It is therefore strongly recommended that apparatus, particularly butyrometers, approved by the National Physical Laboratory as conforming with the specifications in B.S.S. No. 696, Part 1, should be employed.

The procedure is as follows: 10 ml. of sulphuric acid are measured into a butyrometer by means of the acid pipette or the automatic measure, care being taken not to wet the neck. If the sample is fresh and shows no appreciable separation of cream, it is warmed to 20° C. and mixed thoroughly, but not shaken so vigorously as to cause froth or churning. Any sample in which there is a marked separation of cream may be warmed to 38°-40° C. and thoroughly mixed. The sample should then be cooled to 20° C. with occasional slight agitation. All samples should be left to stand for 3 or 4 minutes after mixing to allow air

bubbles to rise. The sample should be inverted 3 or 4 times immediately before taking 11 ml. of milk for the determination. 11 ml. of milk at about 20° C. are measured by means of the standard pipette. The milk is sucked into the pipette until it is a short distance above the graduation mark, when the top of the pipette is closed by pressing a finger on it. The pipette is then withdrawn from the sample and the outside of the delivery jet is wiped free from milk with a cloth. Milk is then run out gradually, with the pipette held vertically at eye level, until the lowest point of the milk meniscus is on the graduation mark. Any drop of milk adhering to the outside of the jet is removed by touching it against the surface of the remainder of the sample. The pipette is then held with the tip of the jet just below the bottom of the neck of the butyrometer and the finger is removed from the top of the pipette; care should be taken not to wet the inside of the neck. When the meniscus comes to rest slightly above the jet, the jet is stroked against the base of the neck to remove any drop of milk adhering to the outside of the jet. The interval between the time when the meniscus comes to rest and the removal of the jet from contact with the butyrometer should be as short as convenient; a few seconds suffice, but small variations in this time will not cause significant variations in the volume of the milk delivered. This method of emptying leaves a small quantity of milk inside the jet, and the pipettes are calibrated to deliver the correct volume of milk when so emptied. 1 ml. of amyl alcohol is measured into the butyrometer by means of the standard pipette or the automatic measure, care being taken not to wet the neck.

The neck of the butyrometer is closed with a double-ended rubber stopper, which is pushed in until the widest part of the stopper is below the top of the neck. The butyrometer is carefully shaken until the contents are thoroughly mixed and the curd is dissolved. Where a number of samples are being examined, the number of butyrometers

which the centrifuge will hold can be placed in a shaking stand and shaken together after having been marked.* The butyrometer is placed with the rubber stopper downwards in a water bath at 68° C. and is kept there until the number of butyrometers to be placed in the centrifuge have been filled and placed in the water bath. The water level in the bath must be above the level of the liquid in the butyrometers.

The butyrometers are transferred from the water bath to the centrifuge and are centrifuged at 1100 r.p.m. for 4-5 minutes. Before placing a butyrometer in the centrifuge, it is desirable to adjust the stopper so that the fat column will be on the scale after centrifuging. The butyrometers must be placed symmetrically in the centrifuge or balance must be secured by placing on the disc an additional stoppered butyrometer filled with water. The butyrometers are then placed with rubber stoppers downwards in the water bath at 68° C. and left there for 2-3 minutes. If on removing from the centrifuge the fat column in any butyrometer is not on the scale, the rubber stopper must be adjusted so as to bring the fat column on the scale before placing the butyrometer in the water bath. The scale readings corresponding to the lowest point of the fat meniscus and to the surface of separation of the fat and acid are noted; the difference between the readings gives the percentage of fat. Before taking a reading, the fat column is adjusted so as to bring the lower end on to a main graduation mark; this is done by slightly withdrawing the stopper and not by forcing it further into the neck. The butyrometers are replaced in the water bath for another 2-3 minutes and a check reading of the percentage of fat is taken as rapidly as possible after the removal of each from the bath.

If there is a fluffy layer at the base of the fat column, the test should be rejected, but the rubber stopper should be examined to see that it is in good condition and more care

* The standard butyrometer is provided with a small patch of matt surface for this purpose.

should be taken to see that the curd is dissolved. If the fat column is so dark as to make the reading difficult, the test should be rejected and the density of the sulphuric acid determined.

After use the butyrometers are rinsed free from acid and visible fat, emptied and then brushed out with warm 1 per cent. caustic soda solution; this solution should not be allowed to come into contact with the operator's hands. The butyrometers are then rinsed with clean hot water and drained with the open ends downwards.

The Röse-Gottlieb Method.—In the specification for the amyl alcohol used for the Gerber test the oily impurities are determined by comparing the results of the determination of fat in a Gerber butyrometer with those obtained by the Röse-Gottlieb method, using the following procedure, which is abstracted by permission from British Standard Specification No. 696, Part 2, 1936, pp. 50-52. The following reagents are required:

Concentrated Ammonia.—Density $0{\cdot}888 \pm 0{\cdot}003$ at 20° C.

Alcohol.—Rectified spirit containing 95 per cent. by volume of ethyl alcohol.

Petroleum Spirit.—A petroleum fraction with b.p. 40°-60° C.

Washed Ether.—Prepared by shaking equal volumes of technical ether (B.S.S. No. 579, 1934) and distilled water, and separating the layer of washed ether. It is important that the washed ether should comply with the peroxide clause of the specification.

Mixed Solvent.—Prepared by mixing equal volumes of petroleum spirit and washed ether, and decanting the clear mixed solvent from any separated water. Alternatively, distillates recovered from previous fat determinations may be used.

There is also required the separator and syphoning apparatus shown in Fig. 4. The tube terminating in a hook shape at its lower end must be a sliding fit in the cork, and must be long enough for the opening at its lower end to be

40 mm. from the bottom of the inside of the extraction vessel.

The procedure is as follows: Weigh accurately 10-11 gm. of the same sample of well mixed milk as that used in the Gerber test into the apparatus in Fig. 4 with the perforated cork and syphoning tubes replaced by a well fitting solid

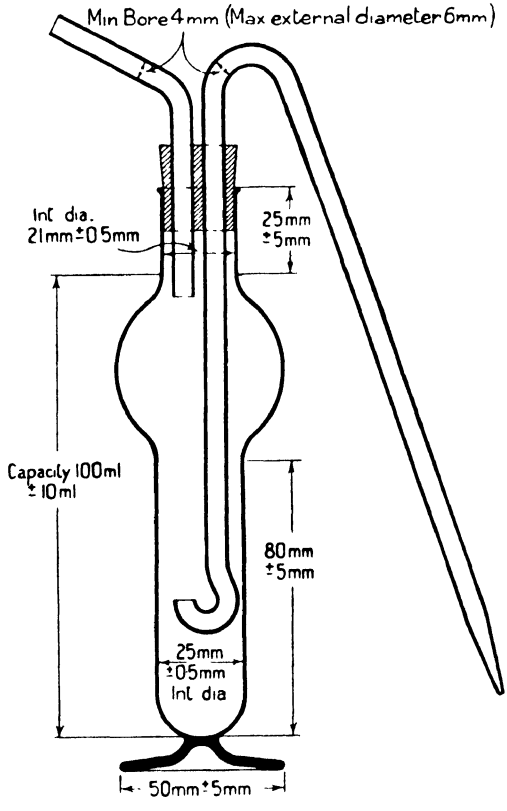


FIG. 4.

cork, which is wetted before use. Add 1 c.c. of concentrated ammonia; mix thoroughly, then add 10 c.c. of alcohol and again mix thoroughly. Add 25 c.c. of washed ether, close the apparatus with the wetted cork and shake well for 30 seconds. Then add 25 c.c. of petroleum spirit and again shake for 30 seconds. Allow the vessel to stand for about

20 minutes or until the upper layer is clear. Then wash the cork with a little of the mixed solvent and transfer as much as possible of the clear fat solution to a weighed flask which has been counterpoised with a similar one during the weighing. Wash the jet, cork and tubes of the syphoning apparatus with 5 c.c. of the mixed solvent and transfer the washings to the weighed flask. Repeat the extraction with 15 c.c. of each solvent, shaking well for 15 seconds after each addition, and transfer the ethereal solution to the weighed flask as before, washing the corks, jet and syphon tube as already described. Carry out a third extraction exactly as before. Evaporate the combined solutions of fat slowly on a water bath and dry the residue in a boiling water oven to constant weight.

Remove the fat completely with petroleum spirit, dry the residue to constant weight in a boiling water oven and deduct the weight of this residue, if any, from the weight of the fat previously obtained. At the same time carry out a blank test on the reagents used and make any allowance that may be necessary. Calculate the corrected weight of fat to a percentage of the weight of milk taken.

The Babcock Method.—This method is an official method of the Association of Official Agricultural Chemists. The following details of the procedure are taken from *Methods of Analysis*, 1935, pp. 268-269: Transfer 18 gm. of milk to a Babcock test bottle by means of a special pipette holding 17·6 c.c. of water at 20° C. Add 17·5 c.c. of sulphuric acid (sp. gr. 1·82-1·83 at 20° C.), preferably not all at one time, pouring it down the side of the neck of the test bottle in such a way as to wash all the milk into the bulb. The temperature of the acid should be about 15°-20° C. Shake until all traces of curd have disappeared; then transfer the test bottle to the centrifuge, counterpoise it, and, after the proper speed has been attained, centrifuge for 5 minutes. Add water at 60° C. or above until the bulb of the test bottle is filled, and centrifuge for 2 minutes. Add hot water until the liquid column approaches the top graduation of the

scale, and centrifuge for 1 minute longer at a temperature of 55°-60° C. Transfer the test bottle to a water bath maintained at 55°-60° C., immerse it to the level of the top of the fat column, and leave it there until the column is in equilibrium and the lower fat surface has assumed a final form. Remove the test bottle from the bath, wipe it and measure the fat column with the aid of dividers or callipers.

The centrifuge should be provided with a speed indicator. The proper rate of rotation may be ascertained by reference to the table below. By " diameter of wheel " is meant the distance between the inside bottoms of opposite cups measured through the centre of rotation of the centrifuge wheel while the cups are extended horizontally.

Diameter of wheel (inches)	10	12	14	16	18	20	22	24
Revolutions per minute	1074	980	909	848	800	759	724	693

Carotene.—The following method of determining carotene in milk is described by W. S. Ferguson and G. Bishop (*Analyst*, 1936, **61,** pp. 515-518): To 200 c.c. of milk in a 500 c.c. separating funnel are added 20 c.c. of 25 per cent. aqueous potassium hydroxide solution. 100 c.c. of ethyl alcohol and 100 c.c. of petroleum spirit (b.p. below 40° C.) are added separately with careful shaking after each addition. If the shaking has been done with care, the emulsion breaks in a short time and the petroleum spirit layer separates. The aqueous solution is run off, and the petroleum spirit extract is transferred to a round-bottomed flask by pouring the extract from the top of the funnel through cotton wool. The petroleum spirit is distilled off in a stream of nitrogen on a water bath at a temperature not exceeding 40° C., and the last traces of the solvent are removed under reduced pressure. To 2 c.c. of the butter fat contained in a 10 c.c. measuring cylinder are added varying amounts of petroleum spirit and the colours of the solutions are compared with the yellow glasses of a Lovibond

tintometer. The equivalent of the Lovibond units in terms of carotene is read off from a curve drawn from the figures on p. 152. In calculating the percentage of carotene in the butter fat, the specific gravity of butter fat is taken as 0·9. The result can be expressed in terms of the original milk by determining the fat content and making the necessary calculation.

Nitrogen.—The Association of Official Agricultural Chemists (*Methods of Analysis*, 1935, p. 265) determine total nitrogen in milk by the Kjeldahl, the Gunning or the Kjeldahl-Gunning-Arnold method, using 5 gm. of the sample. The procedure described by H. D. Richmond (*Dairy Chemistry*, 1920, pp. 171-172) is as follows: 5 gm. of milk, 20 c.c. of sulphuric acid, 10 gm. of potassium bisulphate or sodium sulphate and a small drop of mercury are placed in a Kjeldahl flask and heated until the contents are colourless. After the digestion is complete, about 100 c.c. of 30 per cent. sodium hydroxide solution and 10 c.c. of 10 per cent. potassium sulphide solution are added, and the ammonia is distilled into 50 c.c. of N/10 acid. Copper sulphate can be used as the catalyst instead of mercury, and is preferable because its use does not necessitate the addition of potassium or sodium sulphide before the distillation. G. D. Elsdon (*Allen's Commercial Organic Analysis*, ix, 1932, p. 35) uses 10 gm. of milk, 25 c.c. of sulphuric acid, 10 gm. of potassium sulphate and a small crystal of copper sulphate. H. Leffmann and J. Golding (*op. cit.*, p. 77) use 5 gm. of milk, 20 c.c. of sulphuric acid, 10 gm. of potassium sulphate and 0·05-0·1 gm. of copper sulphate. For further details see pp. 19-21.

Casein (Moir's Method).—The method of determining casein in milk described by G. M. Moir (*Analyst*, 1931, **56**, pp. 147-149) is as follows: Into a weighed covered beaker are pipetted 10 c.c. of milk, and the beaker is weighed again quickly. The milk is diluted with about 50 c.c. of water which has been warmed to 40°-42° C. 1·5 c.c. of 10 per cent. acetic acid are added at once and the mixture is stirred

gently by rotating the stirring rod 4 times; excessive stirring should be avoided. After allowing the mixture to stand for about 20 minutes, 4·5 c.c. of N/4 sodium acetate solution are added; the mixture is gently stirred and left for 1 hour.* The solution is filtered through a 9 cm. No. 42 Whatman filter paper, which is folded in the fluted way to facilitate filtration. The precipitate is washed 3 times with water by decantation, followed by 2 further washings in which the precipitate is broken up and transferred to the paper. The rim of the filter paper is then rinsed with a jet of water. The filtration and washings should be carried out without interruption, and subsequently the casein adhering to the beaker and stirring-rod should not be allowed to dry before being washed out with sulphuric acid used for the Kjeldahl digestion. For this purpose about 20 c.c. of water are placed in the beaker, and about 5-7 c.c. of concentrated sulphuric acid are carefully poured down the side. The casein can be completely removed by 3 such treatments. The filter paper and casein are added to the Kjeldahl flask, into which the washings from the beaker have been poured. The usual quantity of sodium or potassium sulphate and a trace of copper sulphate are added before heating the Kjeldahl flask. It is desirable to evaporate the water with a small flame, as frothing is liable to occur at the end of the evaporation. During digestion some of the acids from the fat condense upon the neck of the flask, and may subsequently cause frothing during the distillation. These can be decomposed if the flask is allowed to cool, when the digestion is nearly complete, and about 50 c.c. of water are carefully added and mixed with the contents. The fatty material is washed down by the condensation of steam during the evaporation of the water. The determination is completed in the usual way, the ammonia being distilled

* F. H. McDowall and A. K. R. McDowell (*Analyst*, 1936, **61**, pp. 824-828) think that Moir's time intervals of 20 minutes and 1 hour are unnecessarily long, and state that the results are not affected by adopting shorter intervals of 5 and 15 minutes respectively.

into 40 c.c. of N/10 sulphuric acid. The results are stated as percentages of casein nitrogen, because there is some doubt about the conversion factor.*

Casein (Formol Titration).—F. H. McDowall and A. K. R. McDowell (*Analyst*, 1936, **61,** pp. 824-828) have described the following method of determining casein in milk by the formol titration. 20 c.c. of milk are diluted with 100 c.c. of water at 42° C. in a 150 c.c. beaker, and the casein is precipitated as in Moir's method, except that the shorter time intervals are adopted. The mixture is decanted through a filter paper under gentle suction on a Buchner funnel 6 cm. in diameter. The precipitate is washed with water and allowed to settle. The liquid is again removed by decantation and the pump is disconnected as soon as the liquid has passed through. The washing and decantation are repeated a second time and finally all the precipitate is transferred to the funnel. Suction should cease as soon as the funnel is free from liquid and before the precipitate is dry. The filter paper and precipitate are returned to the original beaker. The funnel is inverted and washed with a little water to remove adhering particles of casein, and 4-5 c.c. of N/10 sodium hydroxide solution are added; the total volume should now be about 20 c.c. The beaker is placed in a boiling water bath for 5 minutes, with occasional shaking, until the casein is dissolved. The milky solution is cooled to 21°-24° C., 1 c.c. of 1 per cent. phenolphthalein solution is added and N/10 sodium hydroxide solution is run in until the end-point matches 20 c.c. of milk (preferably the milk under examination) tinted with a few drops of 0·01 per cent. aqueous rosaniline solution. 4 c.c. of formalin (40 per cent.) are then added and the titration with N/10 sodium hydroxide solution is continued to the same end-point. The percentage of casein = the number of c.c. of N/10 sodium hydroxide solution used in the formol titration × 0·92.

Albumin and Globulin.—The methods recommended by

* The conversion factor generally used is 6·38.

G. M. Moir (*Analyst*, 1931, **56**, pp. 228-234) for the determination of albumin and globulin are as follows:

Albumin together with Globulin.—To the filtrate obtained after the precipitation of casein (see p. 232) sufficient trichloroacetic acid is added to make the final concentration about 4 per cent. The mixture is heated for half an hour on a boiling water bath, and, after standing until cool, it is filtered and washed with 1 per cent. trichloroacetic acid solution. The nitrogen content of the precipitate is estimated by Kjeldahl's method.

Casein together with Globulin.—10 c.c. of milk, weighed as in the determination of casein, are neutralized and mixed with at least 90 c.c. of saturated sodium or magnesium sulphate solution, and sufficient extra salt to saturate 10 c.c. of water is added. The protein precipitate is filtered off, washed with saturated salt solution, and its nitrogen content determined by Kjeldahl's method. The albumin remaining in the filtrate may be separated by adding 3 c.c. of 10 per cent. acetic acid and heating on a boiling water bath for at least half an hour. After cooling, the precipitate is filtered off and washed, preferably with the saturated salt solution. The beaker in which the precipitation was made is rinsed with a little soda, which is poured into the Kjeldahl flask. Gentle heating during the early stages of the digestion is essential in order to avoid frothing.

Casein, Albumin and Globulin.—The following scheme is suggested:

A. Casein by precipitation at the isoelectric point.

B. Casein and globulin by neutral saturated magnesium or sodium sulphate solution.

C. Total protein by warm 4 per cent. trichloroacetic acid.

From A and B globulin can be obtained by difference; and from B and C albumin can be obtained by difference. Alternatively, albumin and globulin can be determined by treating the filtrate from A with trichloroacetic acid, and albumin by subtracting from the result the globulin obtained

from A and B. The results are stated as the percentages of albumin nitrogen and globulin nitrogen.

Lactose.—The following methods of determining lactose in milk are those adopted by the Association of Official Agricultural Chemists (*Methods of Analysis*, 1935, pp. 266-267).

Optical Method.—Determine the specific gravity of the milk. The volume of the sample to be taken for the determination varies with the specific gravity and must be measured at the temperature at which the specific gravity is taken. The volume to be measured is given in the following table, which is based on twice the normal weight of lactose (32·9 gm. per 100 c.c.) for the Ventzke sugar scale.

Sp. Gr. of Milk.	*Volume of Milk.*
1·024	64·25
1·025	64·20
1·026	64·15
1·027	64·05
1·028	64·00
1·029	63·95
1·030	63·90
1·031	63·80
1·032	63·75
1·033	63·70
1·034	63·65
1·035	63·55
1·036	63·50

Place the measured volume of milk in a flask graduated at 102·6 c.c., and add 1 c.c. of acid mercuric nitrate solution, prepared by dissolving mercury in twice its weight of concentrated nitric acid and diluting the solution with an equal volume of water. Fill the flask to the mark, shake, filter through a dry filter paper and polarize. If a 200 mm. tube is used, divide the polariscope reading by 2 in order to obtain the percentage of lactose in the sample.

Gravimetric Method.—Dilute 25 gm. of the sample with 400 c.c. of water in a 500 c.c. graduated flask, and add

10 c.c. of the copper sulphate solution used for the preparation of Soxhlet's modification of Fehling's solution (see p. 131). Then add about 7·5 c.c. of a potassium hydroxide solution of such a strength that 1 c.c. is just sufficient to precipitate the copper in 1 c.c. of the copper sulphate solution. Instead of this solution, 8·8 c.c. of N/2 sodium hydroxide solution may be used. After the addition of the alkaline solution, the mixture must still have an acid reaction and contain copper in solution. Fill the flask to the 500 c.c. mark, shake and filter through a dry filter paper. Determine lactose in an aliquot part of the filtrate by Munson and Walker's method.*

Citric Acid.—The following modification of the pentabromoacetone method of determining citric acid is described by L. H. Lampitt and H. S. Rooke (*Analyst*, 1936, **61**, pp. 654-665): To 150 gm. of milk heated to 50°-60° C. in a 250 c.c. graduated flask are added 25 c.c. of 2 per cent. potassium oxalate solution and the mixture is shaken. Then 20 c.c. of dilute sulphuric acid (1 : 1) are added and the mixture is again shaken. After cooling, 10 c.c. of phosphotungstic acid solution are added and the contents are made up to 250 c.c. After vigorous shaking, the contents are allowed to settle for 5 minutes and filtered.

To 50 c.c. of the filtrate are added 5 c.c. of potassium bromide solution (37·5 gm. per 100 c.c.). Potassium permanganate solution (5 gm. per 100 c.c.) is then added from a burette with constant shaking until a brown precipitate persists. The mixture is allowed to stand at room temperature for 1 hour, further addition of potassium permanganate solution being made if the brown precipitate disappears. Ferrous sulphate solution (20 gm. of crystals in 100 c.c. of 1 per cent. sulphuric acid) is added until a pale yellow solution containing a white precipitate is obtained, and the

* See p. 144. In the tables on pp. 140-143 are given the weights of hydrated lactose corresponding to the weights of cuprous oxide and copper in the first two columns. The weight of anhydrous lactose can be found by multiplying the weight of hydrated lactose by 0·95.

mixture is cooled in an ice chest for 18 hours. The precipitate is separated by filtration through a sintered glass crucible (10 G 4), the reaction flask being washed out with the filtrate and the washings passed through the crucible. The precipitate is then washed with 10, 10 and 5 c.c. portions of cold water. The crucible is dried to constant weight in a vacuum desiccator (about 16 hours). The precipitate is dissolved in industrial spirit followed by 20, 10 and 10 c.c. portions of ether. The crucible is again dried in a vacuum desiccator and weighed, the loss in weight being taken as pentabromoacetone. The weight of anhydrous citric acid $=0{\cdot}424\ (W+0{\cdot}00005\ V)$, where W=the weight of pentabromoacetone, and V=the original volume of the filtrate from the reaction mixture less the total volume of washings.

Chlorine (Husband and Godden's Method).—The following method of determining the chlorine content of milk is described by A. D. Husband and W. Godden (*Biochem. J.*, 1927, **21,** pp. 259-261): To 20 c.c. of milk in a small flask are added 40 c.c. of 1·2 per cent. picric acid solution containing 2 c.c. of glacial acetic acid per litre, and after 10 minutes the curd is filtered off on a chlorine-free filter paper. 30 c.c. of the clear filtrate are then treated with 10 c.c. of N/10 silver nitrate solution. The mixture is shaken, again filtered and 20 c.c. of the clear filtrate are titrated with standard ammonium thiocyanate solution, as in Volhard's method.

W. Godden (*Priv. Comm.*, 1934) has modified the procedure as follows: To 20 c.c. of milk are added 80 c.c of a saturated solution of picric acid containing 2 c.c. of glacial acetic acid per litre. 50 c.c. of the clear filtrate are treated with 10 c.c. of N/10 silver nitrate solution and, after filtering, 30 c.c. of the filtrate are titrated with standard ammonium thiocyanate solution.*

Chlorine (Davies's Method).—The following method of determining chlorine in milk is described by W. L. Davies (*Analyst*, 1932, **57,** pp. 79-85): 10 c.c. of milk are pipetted into a 250 c.c flask, and 10 c.c. of N/20 silver nitrate solution

* 1 c.c. of N/10 silver nitrate solution $=0{\cdot}003546$ gm. of chlorine.

are mixed with it. 2 c.c. of saturated potassium permanganate solution (about 6 per cent.) and 10 c.c. of pure concentrated nitric acid are added, and the contents of the flask are boiled over a gauze in a draught chamber until the liquid is clear except for the small amount of precipitate, and reddish-brown fumes are copiously evolved. This takes from 3 to 4 minutes, and the volume of nitrous fumes varies directly with the lactose content of the milk. A pinch of urea (0·25 gm.) is added to the hot solution, and the contents of the flask are diluted to about 100 c.c. 6 c.c. of acetone* and 1 c.c. of saturated solution of iron alum in 10 per cent. nitric acid are added, and the excess of silver nitrate is titrated with N/20 potassium thiocyanate, which has been standardized against the N/20 silver nitrate solution by means of standard potassium chloride solution. A blank determination for reagents is also made when standardizing the silver nitrate solution.†

Working with 10 c.c. of milk, 10 c.c. of N/20 silver nitrate solution are equivalent to 177·2 mgm. of chlorine per 100 c.c. If the chlorine in a sample of milk exceeds this value, it is necessary to repeat the determination and add a larger volume, 15 or 20 c.c., of N/20 silver nitrate solution. Further addition of silver nitrate after the acid digestion is useless.

Sodium.—The following method of determining sodium in milk by the triple acetate gravimetric method is described by T. S. G. Jones and W. L. Davies (*Biochem. J.*, 1935, **29,** pp. 978-981). The following reagents, which were used by R. A. McCance and H. L. Shipp (*ibid.*, 1931, **25,** pp. 449-456) for the colorimetric determination of sodium, are required for the determination:

Alcoholic Zinc Acetate with Zinc Hydroxide.—To a hot concentrated solution of A. R. zinc sulphate add a slight excess of concentrated ammonia. Filter on a Buchner funnel, wash thoroughly with hot water and finally suck

* Acetone has a protective effect in preserving the colour of ferric thiocyanate, and is added in order to obtain a sharper end-point.

† 1 c.c. of N/20 silver nitrate solution = 0·00177 gm. of chlorine.

as dry as possible. To 12·5 c.c. of glacial acetic acid add zinc hydroxide prepared as above in small amounts in slight excess. Filter and wash; make up the combined filtrate and washings to 100 c.c. Add 3 c.c. of concentrated ammonia and 300 c.c. of 95 per cent. alcohol.

Uranyl Zinc Acetate Reagent.—(*a*) Dissolve 10 gm. of uranyl acetate in 50 c.c. of boiling water containing 2 c.c. of glacial acetic acid. (*b*) Dissolve 30 gm. of zinc acetate in 50 c.c. of boiling water containing 1 c.c. of acetic acid. Mix both solutions while boiling, raise the temperature again to the boiling point, allow to stand over-night and filter. This solution is kept saturated at working temperature with sodium uranyl zinc acetate, prepared by mixing some of the reagent with an alcoholic solution of sodium chloride, and is filtered before use.

95 *per cent. Alcohol saturated with the Triple Acetate.*—The alcohol is kept in contact with the triple acetate at working temperature and is filtered before use.

The determination is carried out as follows: 25 c.c. of milk (or 50 c.c. if it contains less than 80 mgm. of sodium per 100 c.c.) are measured into a 150 c.c. silica flask. After adding 10 c.c. of concentrated nitric acid, the mixture is heated on a sand bath. When the solution has been almost reduced to dryness, the mass chars and the carbon ignites and burns in the nitric fumes. By the cautious addition of nitric acid it is possible to obtain a white ash in a few minutes. The ash is dissolved in a small amount of dilute nitric acid and transferred with the aid of 10 c.c. of water to a 25 c.c. graduated flask. To it are added 15 c.c. of alcoholic zinc acetate with zinc hydroxide in order to remove phosphate. The mixture is shaken and, after standing 24 hours, is made up to the mark and filtered.

The sodium in 2 c.c. of the filtrate is precipitated as the triple acetate by the procedure described by J. P. Peters and D. D. Van Slyke (*Quantitative Clinical Chemistry*, 1932, vol. ii, p. 733). This consists in precipitating the triple acetate in the Jena glass crucible in which it is weighed.

A solid rubber stopper is fitted into the bottom of a Jena glass filter (10 G 4), which has been dried and weighed on an ordinary analytical balance. Into the filter standing on the rubber stopper are poured 10 c.c. of uranyl zinc acetate reagent and then 2 c.c. of the filtrate are pipetted into the crucible. The mixture is vigorously stirred by means of a short thin glass rod, which is washed with two successive portions of 1 c.c. of the reagent. The crucible is then covered and set aside for 1 hour. The stopper is then removed and the fluid contents are sucked through by means of a pump. The precipitate is washed 5 times with 2 c.c. portions of 95 per cent. alcohol saturated with the triple acetate and twice with 5 c.c. portions of ether. The outside of the filter is then wiped dry. The filter is placed in a desiccator for 1 hour and weighed. The weight of the precipitate $\times 0{\cdot}01495$ = the weight of sodium.

Phosphorus.—W. L. Davies has communicated to the author the following procedure for the determination of phosphorus in milk by the gravimetric strychnine phosphomolybdate method.* The two following solutions are required for the determination: (A), 50 gm. of ammonium molybdate dissolved in 150 c.c. of water are poured into 450 c.c. of dilute nitric acid (2 : 1). (B), 3 gm. of strychnine nitrate (very finely powdered) are dissolved in 200 c.c. of water. For the precipitation 3 volumes of A are mixed with 1 volume of B. The mixed solutions will keep for at least 2 months.

An aliquot portion of the nitric acid solution of the ash from 25 gm. of milk is diluted 5 times (*e.g.*, from 20 c.c. to 100 c.c.) and two 25 c.c. portions (equivalent to 0·625 gm. of milk) are pipetted into 150 c.c. beakers and neutralized with N sodium hydroxide solution. 25 c.c. of the mixed solutions A and B are added and the whole is well mixed.

* This method, also known as the Embden-Fetter method, is not as well known as it deserves to be. For further details the reader is referred to J. P. Peters and D. D. Van Slyke, *Quantitative Clinical Chemistry*, 1932, vol. ii, pp. 873-874.

A buff flocculent precipitate is formed which becomes granular on standing for 3 hours. The precipitate is filtered through a tared Gooch crucible or a Jena glass crucible (10 G 4) by gentle suction, washed with an ice-cold mixture of solutions A and B diluted 5 times with water and finally with ice-cold water until the washings are no longer acid. The crucible is dried at 110° C. for 2 hours, or at 100° C. for 3 hours, and weighed. The increase in weight $\times 0{\cdot}0112 =$ the weight of phosphorus. The quantity of the mixed solutions A and B used above can precipitate 2·5 mgm. of phosphorus. If more phosphorus is present, the composition of the precipitate is variable and cannot be used for calculating the phosphorus content.

Methylene Blue Reduction Test.—The following apparatus and materials are required to carry out the methylene blue reduction test as described in Memo. 139/Foods (Jan., 1937) issued by the Ministry of Health:

Methylene Blue Solution.—One specially prepared standard methylene blue tablet is dissolved in 200 c.c. of cold sterile glass-distilled water in a sterile flask, and the solution is diluted to 800 c.c. with cold sterile glass-distilled water. The pipette used for transferring the methylene blue solution to the tubes of milk should not be introduced into the stock bottle. The amount of solution required for a day's work should be poured off from the stock bottle into a suitable glass container. The stock solution should be stored in the dark, but should not be used after it is more than 2 months old.

Test Tubes, conforming with British Standard Specification 152/16, 6 by $\frac{5}{8}$ in. with an internal diameter of $13{\cdot}5 \pm 0{\cdot}5$ mm. and graduated at 10 c.c. The test tubes are plugged with cotton wool and are sterilized in an autoclave at 15 pounds per square inch for 20 minutes or in an air oven at 160° C. for 2 hours.

Rubber Stoppers, to fit the test tubes. These are sterilized by immersion in boiling water for 5 minutes immediately before use.

Water Bath, fitted with a cover to exclude light and containing a test tube rack to hold the test tubes. The water in the bath must be kept above the level of the milk in the test tubes and its temperature must be maintained at 37°-38° C. by means of an automatic thermo-regulator.

Pipettes.—Straight-sided blow-out delivery pipettes having an overall length of 300 mm. with a 1 c.c. graduation mark between 140 and 180 mm. from the tip are required. The pipettes are plugged with cotton wool at the upper end and are sterilized in a hot air oven at 160° C. for 2 hours. The same pipette can be used for adding the methylene blue solution to successive tubes.

The test is carried out as follows: The sample of milk is thoroughly mixed by inverting and shaking the sample bottle. The stopper or cap of the bottle is removed with aseptic precautions; the lip of the bottle is flamed; the cotton wool plug of the test tube is removed; the mouth of the test tube is flamed and the milk is rapidly poured into the tube up to the 10 c.c. mark, care being taken to leave one side of the interior unwetted with milk. 1 c.c. of the methylene blue solution is then added to the tube by holding the tip of the pipette during the delivery against the dry side of the tube, expelling the methylene blue solution by blowing with the mouth and after the lapse of 3 seconds blowing out the solution remaining in the tip of the pipette. The tube is then closed with a sterile rubber stopper held by sterile forceps or the tips of the fingers on the upper end. The tube is then inverted slowly once or twice and within 5 minutes is placed in the water bath. With each batch of tests two control tubes are prepared. One tube contains 10 c.c. of mixed milk and 1 c.c. of tap water. The other contains 10 c.c. of mixed milk and 1 c.c. of the methylene blue solution. Both control tubes are fitted with stoppers and are immersed in boiling water for 3 minutes to destroy the natural reducing system in the milk. These tubes serve as comparisons to show when decolorization of the tests is beginning and when it is complete. The

tubes in the bath are inspected every 30 minutes. Any tube that has reached the end-point is removed; any tube that shows commencing decolorization is left until the end-point is reached, and all the other tubes are inverted once. The tubes should not be exposed to direct sunlight during the half-hourly inspections.

Decolorization is regarded as complete when the whole column of milk is completely decolorized or is decolorized up to within 5 mm. of the surface. A sample of Tuberculin Tested or Accredited milk satisfies the test if it does not decolorize the methylene blue within $4\frac{1}{2}$ hours if the sample is taken at any time from 1st May to 31st October; or within $5\frac{1}{2}$ hours if the sample is taken at any time from 1st November to 30th April.

Phosphatase Test.—The phosphatase test for pasteurized milk is due to H. D. Kay and W. R. Graham (*J. Dairy Res.*, 1935, **6,** pp. 191-203). The following reagents are required:

Buffer Substrate Solution.—Dissolve 1·09 gm. of disodium phenyl phosphate and 11·54 gm. of sodium diethyl barbiturate (sodium veronal) in water saturated with chloroform and make up to 1 litre. Add a few drops of chloroform to prevent the growth of micro-organisms and keep in a refrigerator. This reagent should be made up every 3 days.*

Folin and Ciocalteu's Phenol Reagent.—Dissolve 100 gm. of sodium tungstate, $Na_2WO_4,2H_2O$, and 25 gm. of sodium molybdate, $Na_2MoO_4,2H_2O$, in 700 c.c. of water in a 1500 c.c. flask connected by a ground glass joint with a reflux condenser. Add 50 c.c. of syrupy (85 per cent.) phosphoric acid and 100 c.c. of concentrated hydrochloric acid, and reflux the mixture gently for 10 hours. Then cool and add 150 gm. of pure lithium sulphate, 50 c.c. of water and a few (usually 4-6) drops of bromine. Boil the mixture under a hood without the condenser for 15 minutes to boil off the excess of bromine. Cool, dilute to 1 litre and filter. The finished

* This solution can be prepared from tablets which are supplied by the British Drug Houses, Ltd. One of these tablets is dissolved in 50 c.c. of water.

reagent should have a golden yellow colour with no greenish tint. Keep well protected from dust. Dilute 1 volume of this stock solution with 2 volumes of water before use. Folin and Ciocalteu's phenol reagent is obtainable from the British Drug Houses, Ltd.

Sodium Carbonate Solution.—14 per cent. solution of pure anhydrous sodium carbonate.

The following special apparatus is required:

(1) A Lovibond tintometer or, for most purposes, a simple comparator which is described later.

(2) Whatman No. 30 filter papers, 9 cm., or acid-washed filter papers of similar quality.

(3) A water bath which can be kept at 47°±2° C. for 10 minutes.

(4) An incubator maintained at 37°-38° C. This is used in test B.

Test A.—Run 10 c.c. of buffer substrate solution into each of four test tubes of 20-25 c.c. capacity. To two tubes (control tubes) add 4·5 c.c. of diluted Folin's reagent. To all four tubes add 0·5 c.c. of the milk to be tested and mix well. Incubate the two tubes which do not contain Folin's reagent in a water bath at 47° ±2° C. for 10 minutes. Then remove the tubes from the water bath, cool and add 4·5 c.c. of diluted Folin's reagent. After keeping for 3 minutes, filter the contents of the four tubes. To 10 c.c. of the filtrate add 2·0 c.c. of the sodium carbonate solution and mix. Place in a boiling water bath for 5 minutes and then filter.

If only a faint blue colour develops in all four tubes, the milk has been heated, but not necessarily properly pasteurized. If the controls show more than a trace of blue colour (more than 1·5 Lovibond blue units), the reagents should be examined. If these are free from phenol, it is likely that a phenol-producing organism is present in the milk. This does not occur in pasteurized milk which has been kept at a satisfactorily low temperature since pasteurization. With some experience, it is possible to omit

the control tubes for most of the samples submitted to this test. A colour greater than 2·3 Lovibond blue units shows that the milk has not been properly pasteurized. If the colour is close to the standard, the following test, which is more delicate, should be carried out.

Test B.—Run 10 c.c. of buffer substrate solution into each of four test tubes of 20-25 c.c. capacity. (If the controls in test A have been negative, test B controls need not be used, and two instead of four tubes are required for test B.) To two tubes (control tubes) add 4·5 c.c. of diluted Folin's reagent. To all four tubes add 0·5 c.c. of milk to be tested and mix well. Add 2 drops of chloroform to each of the two tubes in which the milk has not been precipitated, stopper, warm to 37°-38° C. and keep at this temperature for 24 hours, preferably in a bacteriological incubator. At the end of this time, remove from the incubator, add 4·5 c.c. of diluted Folin's reagent to each tube. Allow to stand for 3 minutes, and filter the contents of all four tubes. Add 2·0 c.c. of the sodium carbonate solution to 10 c.c. of the filtrate. Mix, place in a boiling water bath for 5 minutes and then filter. If the controls show only a very faint blue colour, and if the blue colour developed in the tubes after incubation exceeds 2·3 Lovibond blue units, then the milk has not been properly pasteurized.

Comparator.—A simple box type comparator suitable for routine tests has been designed by Z. Herschdörfer and F. K. Neave (*J. Soc. Chem. Ind.*, 1937, **56,** p. 78T). It consists of a small box containing two glass tubes marked at 25 mm. from the bottom and a disc of permanent coloured glass standards. Attached to the box is a white opal reflector. The disc is fitted either with one colour standard corresponding to 2·3 Lovibond blue units or with three colour standards corresponding to 1·5, 2·3 and 6·0 Lovibond blue units. One tube is filled to the mark with the filtrate obtained in the test and is placed in the right-hand compartment; the other tube containing distilled water is placed in the left-hand compartment underneath the colour disc.

Alternatively the B.D.H. Lovibond Nessleriser, fitted with the special Nessler tubes graduated at 25 mm. from the bottom and the colour disc containing the appropriate colour standards, can be used.

The colour of the filtrate from control tests should not exceed the 1·5 standard. Milks giving colours below the 2·3 standard may be considered as properly pasteurized; those giving colours between the 2·3 and the 6·0 standards should be considered as improperly pasteurized; and those giving colours greater than the 6·0 standard show evidence of serious errors in pasteurization technique.

Reagents and Apparatus.—E. B. Anderson, Z. Herschdörfer and F. K. Neave (*Analyst*, 1937, **62,** pp. 86-93) have made the following observations and recommendations with regard to the phosphatase test. There was no apparent hydrolysis when the buffer substrate solution was kept in a refrigerator for 9 weeks. If Folin's reagent is correctly prepared it is entirely free from any greenish tinge. The reagent did not show any sign of decomposition in 4 months. The diluted Folin's reagent undergoes no change in one week. Since the intensity of the blue colour depends to a large extent on the concentration of the sodium carbonate solution, it is advisable to control the concentration by titration. It is also advisable to add to the solution a few drops of chloroform. Since any errors in the measurement of the 0·5 c.c. of milk will greatly affect the result, N.P.L. Grade A pipettes should be used. A separate clean pipette must be used for each sample because an admixture of as little as 0·1 per cent. of raw milk with a properly pasteurized milk may be sufficient to give a colour exceeding 2·3 Lovibond blue units. Accurate pipettes should also be used for delivering 2·0 c.c. of sodium carbonate solution. Whatman No. 42 (9 cm.) filter papers give more satisfactory results than Whatman No. 30 filter papers. If satisfactory results are to be obtained, the technique of Kay and Graham must be closely followed.

MILK PRODUCTS

The milk products considered here are the products and the by-products of the dairy and the manufactured milk products used as food. The first group comprises cream, separated milk, butter, buttermilk, cheese and whey. The second group includes condensed milk, dried milk, dried skim-milk, dried cream, dried buttermilk and dried whey. Butter and cheese require special methods of analysis, which are considered later, but the other milk products are analysed in the same way as milk or by modifications of the methods employed in the analysis of milk.

The acidity of the liquid milk products is determined in the same way as the acidity of milk (see p. 214). The acidity of the solid milk products is determined by reconstituting a suitable weight of the milk product in water and titrating the liquid with N/10 sodium hydroxide solution, using phenolphthalein as indicator. The titratable acidity is reported as lactic acid.

The total solids of the liquid milk products are determined by evaporating a known weight to dryness and drying the solid residue to constant weight, as in the determination of the total solids in milk (see p. 221). The total solids in condensed milk are determined in a similar way, but the determination presents some difficulty. The procedures for sweetened and unsweetened condensed milk recommended by the Milk Products Sub-Committee of the Society of Public Analysts are given on p. 253. The water content of cream can be determined in the same way as the water content of butter by heating a weighed quantity over a naked flame until frothing ceases, as explained on p. 266. The water in dried milk and other solid products is determined by drying a weighed quantity of the sample to constant weight. The procedure recommended by the Milk Products Sub-Committee of the Society of Public Analysts for the determination of water in dried milk is given on p. 254.

Fat can be determined in some milk products by the Gerber method with special butyrometers. The British standard procedures for the determination of fat in separated milk, skim-milk, buttermilk, cream and dried milk are given on pp. 254-256. These procedures are modifications of the Gerber method of determining fat in milk, which is fully described on p. 223; and since the details of the manipulation are given fully in that procedure, they are not all repeated in the procedures for milk products. The fat content of the solid milk products can be determined by extraction with a volatile solvent as in the determination of oil or fat in feeding stuffs (see p. 119) or more accurately by the Röse-Gottlieb method. The procedures recommended by the Milk Products Sub-Committee of the Society of Public Analysts for the determination of fat in dried milk and condensed milk are given on pp. 257 and 259.

The proteins in separated milk, skim-milk, buttermilk and whey can be determined by Moir's methods (see p. 234). These methods are not applicable to dried milk products because the proteins in these products have been denatured by heat. The true proteins in dried milk products can be determined by precipitation with trichloroacetic acid, as described on p. 260. When albumin and globulin are denatured by heat, they are precipitated together with casein at pH 4·6, and it is not possible to determine these proteins separately in dried milk. In whey there is no casein; but Davies found that when the proteins in dried whey are precipitated at pH 4·6, the precipitate is difficult to filter and wash. This difficulty is overcome by adding separated milk to the dried whey reconstituted in water and precipitating the denatured proteins together with casein at pH 4·6, as described on p. 261. The precipitate is then easily separated and washed.

Lactose is most conveniently determined in milk products by Lane and Eynon's volumetric method (see p. 132) after suitable dilution and without any clarification. The presence of sucrose in sweetened condensed milk interferes with the

titration, but it is possible to add to the burette reading a correction which is proportional to the ratio of sucrose to lactose. These corrections are given in the table on p. 262. Sucrose in condensed milk can also be determined by Lane and Eynon's method. The Milk Products Sub-Committee of the Society of Public Analysts in their report on the determination of sucrose in sweetened condensed milk (*Analyst*, 1930, **55,** pp. 111-124) state that considerable success was achieved by the use of this method, but are of opinion that for the requisite degree of accuracy it would be necessary for each analyst to prepare his own tables. They recommend the polarimetric method, which is given on p. 261. The analysis of sweetened condensed milk in which sucrose has altered during storage is dealt with in a later report of the same sub-committee (*ibid.*, 1932, **57,** pp. 630-652), but the recommended methods are not given here.

Chlorine can be determined in liquid milk products by Davies's method (see p. 237) and in solid milk products by a modification of that method, which is described on p. 265. The determinations of the other mineral constituents are carried out by the same methods as those used for milk.

The analysis of butter involves not only the proximate analysis of the whole sample but also the examination of the butter fat. The water in butter is difficult to determine because when butter is heated it separates into two layers and the upper fatty layer prevents the evaporation of water from the lower aqueous layer. Water can be determined by finding the loss in weight when a weighed quantity of butter is heated over a small flame with constant stirring until frothing ceases, as described on p. 266. Water can, however, be determined by drying in an oven if the evaporation of the water is assisted by tilting the weighing bottles on one side and shaking round the contents at intervals, as in Bolton's routine procedure. Jones and McLachlan found that distillation with toluene in a Dean and Stark's apparatus (see p. 118) is a very satisfactory way of

determining water in butter and is more rapid than drying in an oven. The curd and salt are determined by the procedure given on p. 266. The salt can be determined separately by Davies's method, as described on p. 265, or by melting the butter and extracting the salt with hot water, as described on p. 267.

The constants which most clearly show the peculiarities in the composition of butter fat, and those which are suitable for investigating the influence of season and food on the composition, are the saponification value, the Reichert-Meissl value, the Polenske value, the Kirschner value and the iodine value. The saponification value of an oil or fat is the number of mgm. of potassium hydroxide required to saponify 1 gm. of the oil or fat. The Reichert-Meissl value is the number of c.c. of decinormal alkali required to neutralize the water-soluble volatile fatty acids distilled from 5 gm. of a fat under prescribed conditions. The Polenske value is the number of c.c. of decinormal alkali required to neutralize the water-insoluble volatile fatty acids distilled from 5 gm. of a fat under specified conditions in a standard apparatus. The Kirschner value is the number of c.c. of decinormal alkali required to neutralize the butyric acid distilled from 5 gm. of fat in the same apparatus. The method is based on the fact that silver butyrate is soluble in water but the silver salts of the other volatile fatty acids are insoluble. The iodine value is the percentage of iodine absorbed by an oil or fat, and its numerical value gives an indication of the relative proportion of unsaturated fatty acids contained in the oil or fat.

The saponification value is determined by saponifying a weighed quantity of an oil or fat with alcoholic potassium hydroxide solution and finding, by titration with standard hydrochloric acid, the amount of potassium hydroxide left over after saponification. The procedure which has been prepared by the International Commission for the Study of Fats is given on p. 267. The method of determining the Reichert-Meissl value has undergone many modifications;

and since those due to Meissl and Wollny are now obsolete, the Analytical Methods Committee of the Society of Public Analysts think that Reichert should be used alone, in place of the hyphenated forms, when applied to the soluble volatile acid value. A procedure agreed to by the Government Chemist and the Society of Public Analysts (*Analyst*, 1900, **25,** pp. 309-312) was for some time largely used. This consists of saponifying 5 gm. of fat by heating it with alcoholic sodium hydroxide solution, distilling off the alcohol, dissolving the soap in water, adding dilute sulphuric acid and distilling the volatile fatty acids in an apparatus of standard dimensions with an inclined condenser. But now a mixture of glycerol and sodium hydroxide is used for the saponification and, after acidifying the soap solution, the mixture is distilled in a Polenske apparatus with a vertical condenser, the same apparatus being used for the determination of the Polenske and the Kirschner values. Unfortunately there is no uniformity in the details of the procedure or the dimensions of the apparatus, as can be seen by comparing the figures of the apparatus and the procedures given in well known text-books. This lack of uniformity was considered by the Analytical Methods Committee of the Society of Public Analysts, who have recommended specifications of the standard apparatus and procedures for the determination of the Reichert, Polenske and Kirschner values, which are given on p. 268. The dimensions of the apparatus agree with those prescribed by Polenske and the tolerances in dimensions are in accordance with apparatus in common use. The procedure does not introduce any new feature which would invalidate results already obtained, but several details likely to cause differences in the results, such as the size of the particles of pumice added to ensure regular boiling, have been specified. The iodine value can be determined by three methods, which differ in the special reagent used as the source of iodine. In all three methods a known weight of the oil or fat is treated with a measured volume of the special reagent, and when absorption is com-

plete the unabsorbed iodine is titrated with standard sodium thiosulphate solution. In Wijs's and Hanus's methods the absorption of iodine takes place much more rapidly than in Hübl's method. The International Commission for the Study of Fats recognize the three methods, but recommend the first two. The agreed procedures are given on p. 273. When stating results the method used should be mentioned.

The natural yellow colour of butter fat is mainly due to carotene, the intensity of the colour being positively correlated with the carotene content of the butter fat and also with the vitamin A content. The relative carotene content of butter, and the change in colour when cows are turned out to pasture in the spring, can be measured in Lovibond yellow units. S. J. Watson, G. Bishop, J. C. Drummond, A. E. Gillam and I. M. Heilbron (*Biochem. J.*, 1934, **28,** pp. 1076-1085) stated the results of the colour measurements as the Lovibond yellow units corresponding to a 1 cm. thickness of the fat. For the measurement the fat was diluted with 3 times its volume of petroleum ether and the actual reading was multiplied by 4. Since the publication of that paper, Ferguson has shown that the readings of the Lovibond tintometer can be expressed as the carotene content of the butter fat. This forms part of the determination of carotene in milk which is given on p. 230. If butters contain added colouring matters, any method of determining carotene depending on the measurement of the yellow colour will give faulty results. The A.O.A.C. methods for detecting added colouring matters in butter are given on p. 275.

The determination of water in cheese is difficult because the melted fat interferes with the evaporation of the water. But if the dish is tilted, the cheese can be dried in an oven, as described on p. 276. Fat in cheese can be determined by a modification of the Gerber method, using a special butyrometer. The standard British procedure is given on p. 276. Fat can also be determined gravimetrically by a modification of the Röse-Gottlieb method, which is described

on p. 277. Cheese for the determination of the ash should be incinerated at as low a temperature as possible to prevent loss of sodium chloride by volatilization. Owing to possible loss, chlorine is best determined by Davies's method (see p. 265). The procedure for the determination of the products of ripening is given on p. 278. It consists of extracting the cheese with hot water and determining the soluble organic matter in the filtrate. An aliquot part of the filtrate can be used for the determination of the acidity.

Total Solids in Condensed Milk.—The following methods of determining total solids in sweetened and unsweetened condensed milk are recommended by the Milk Products Sub-Committee of the Society of Public Analysts (*Analyst*, 1927, **52**, pp. 402-408).

Preparation of the Sample.—The sample should be well mixed. This is best done by using a spoon with an up and down rotatory movement, in such a way that the top layers and the contents of the lower corners of the containing vessel are moved and mixed. Care should be taken that any separated crystals in the original sample are first ground and incorporated in the bulk. It is important that frothing and the formation of air bubbles should be avoided.

For the determination the following are required:

Sand.—Heat a convenient quantity of sand, which passes a 30-mesh and is retained by a 90-mesh sieve, with concentrated hydrochloric acid. Decant and repeat the digestion until the acid liquid is nearly colourless. Wash once with dilute hydrochloric acid and then thoroughly with distilled water. Dry and ignite.

Dishes.—These should be of metal (aluminium or nickel is suitable) with easily removable but close-fitting lids. A suitable size is about 3 in. in diameter and about 1 in. in depth.

Sweetened Condensed Milk.—Place 25 gm. of the prepared sand and a short glass stirring rod in a dish and dry to constant weight in an oven at 98°-100° C., the lid being removed whilst drying and replaced before removing the dish from the oven. Allow the dish to remain for 45 minutes in

the desiccator and weigh. Tilt the sand to one side of the dish; place on the clear space about 1·5 gm. of the well mixed sample and weigh rapidly. Add 5 c.c. of water to the milk and mix these; then mix the diluted milk with the sand by means of the rod. Place the dish on a rapidly boiling water bath for 20 minutes, carefully stirring during the earlier period. Transfer the dish, with rod and cover, to the oven at 98°-100° C. After 1½ hours cover the dish, place it in the desiccator for 45 minutes and weigh. Return the dish to the oven, heat for 1 hour with the lid removed and weigh as before. Repeat this process until the loss in weight between successive weighings does not exceed 0·0005 gm. This is generally found to be the case between the second and third weighings.

Unsweetened Condensed Milk.—Weigh out 3 gm. of condensed milk and use 3 c.c. of water. Otherwise proceed as above.

Water in Dried Milk.—The following method of determining water in dried milk is that recommended by the Milk Products Sub-Committee of the Society of Public Analysts (*Analyst*, 1936, **61,** pp. 105-111). For this determination metal dishes with close-fitting but easily removable lids are required. Aluminium dishes about 2 in. in diameter and about 1 in. in depth are suitable. Place the dish and lid in an electric oven at 102°-103° C. for 1 hour. Place the lid on the dish; remove from the oven, cool in a desiccator for 30 minutes and weigh. Transfer about 1 gm. of the well mixed sample to the dish, cover with the lid and weigh quickly. Remove the lid; place the dish and lid in the oven at 102°-103° C. for 2 hours or 3 hours, if the moisture content of the sample is high. Replace the lid; remove from the oven, cool in the desiccator for 30 minutes and weigh. Heat again in the oven for 1 hour, and repeat this process until the loss in weight between successive weighings does not exceed 0·0005 gm. Drying is generally complete at the end of the first 2 hours.

Fat in Separated Milk.—The following description of the British standard method of determining fat in separated

milk, skim-milk and buttermilk by the Gerber method is abstracted by permission from British Standard Specification No. 696, Part 2, 1936, pp. 15-19.* Exactly the same chemicals and apparatus are required as those for the determination of fat in milk (see p. 223), except that the standard butyrometer for testing skim-milk, separated milk and buttermilk is used in place of the standard butyrometer for testing milk.

The procedure is exactly the same as that in the determination of fat in milk up to the point at which the butyrometers are centrifuged at 1100 r.p.m. for 4-5 minutes. They are then removed from the centrifuge and placed in the water bath at 68° C.; the last to be placed in the bath should remain there for 1-2 minutes. They are again centrifuged for a further 4-5 minutes and are then placed in the water bath for 2-3 minutes. After adjusting the stoppers, the fat columns are read. The butyrometers are replaced in the water bath for a further 2-3 minutes and check readings are taken as rapidly as possible. The percentage of fat as read on the scale is corrected by the following amounts: Add 0·05 per cent. if the reading is less than 0·10 per cent.; add 0·02 per cent. if the reading is 0·10-0·25 per cent.; no correction is required if the reading is more than 0·25 per cent.

Fat in Cream.—The following description of the British standard method of determining fat in cream by the Gerber method is abstracted by permission from British Standard Specification No. 696, Part 2, 1936, pp. 20-23. The same chemicals and apparatus are required as those for the determination of fat in milk (see p. 223), except that the 11 ml. pipette is not required and the standard butyrometer for testing cream is used in place of the standard butyrometer for testing milk.†

* This publication can be obtained from the British Standards Institution, 28 Victoria Street, London, S.W.1, price 3s. 8d. post free.

† An alternative method of determining fat in cream, using the butyrometer for testing milk, is described in B.S.S. No. 696, Part 2, pp. 23-26. This method requires the use of tables which are not given here.

Immediately before weighing the portion for the analysis, the sample should be thoroughly mixed without causing undue froth or churning. If the cream is very thick, it should be warmed to between 38° and 50° C. to facilitate mixing. 5 gm. of the cream (tolerance $\pm 0{\cdot}005$ gm.) are weighed into the butyrometer, using any suitable form of support for the butyrometer. About 6 c.c. of warm water at 30°-40° C. are added to the butyrometer, followed by 10 ml. of sulphuric acid and 1 ml. of amyl alcohol. If the butyrometer is not filled up to about 5 mm. below the shoulder, warm water is added up to this level. Up to this stage there is an alternative procedure, in which 5 gm. of cream are weighed in a stoppered funnel (B.S.S. No. 696, Part 1, p. 42). After withdrawing the stopper, the cream is washed into the butyrometer with 6 c.c. of water at 30°-40° C. Then 10 ml. of sulphuric acid and 1 ml. of amyl alcohol are added, followed by warm water up to about 5 mm. below the shoulder.

The butyrometer is closed with a stopper and is carefully inverted several times until the contents are thoroughly mixed and the curd is dissolved. It is then placed with the rubber stopper downwards in the water bath at 68° C. and is left there until the other butyrometers to be placed in the centrifuge have been filled and placed in the water bath. The butyrometers are transferred from the water bath to the centrifuge as quickly as possible and are centrifuged at 1100 r.p.m. for 4-5 minutes. After adjusting the stoppers, the butyrometers are placed in the water bath for 2-3 minutes. If there is not a sharp line between the fat and the acid or if the acid layer is not clear, the centrifuging and placing in the water bath are repeated. After adjusting the stoppers, the fat columns are read. The butyrometers are again placed in the water bath for another 2-3 minutes and check readings are taken as quickly as possible after removal from the bath.

Fat in Dried Milk (Gerber Method).—The following description of the British standard method of determining

fat in dried milk by the Gerber method is abstracted by permission from British Standard Specification No. 696, Part 2, 1936, pp. 41-43. This method is applicable to cream powders, milk powders and buttermilk powders containing not less than 10 per cent. of fat. The same chemicals and apparatus as those for the determination of fat in milk (see p. 223) are required, except the 11 ml. pipette. There is also required a stemless glass funnel (B.S.S. No. 696, Part 1, p. 44) which is used to transfer the powder to the butyrometer.

After thoroughly mixing the sample, 1·69 gm. (tolerance $\pm 0{\cdot}003$ gm.) are weighed in a counterpoised scoop. 10 ml. of sulphuric acid are measured into the butyrometer by means of the standard pipette or an automatic measure, care being taken not to wet the neck. By means of a wash bottle cold water is added, the jet being directed on to the wall of the butyrometer, so as to form a layer of water about 6 mm. deep on the top of the acid. The stemless glass funnel is inserted into the neck and the contents of the scoop are transferred to the butyrometer by means of a glass rod and a camel-hair brush. 1 ml. of amyl alcohol is added by means of the standard pipette or an automatic measure. Hot water at 80° C. is then added from a wash bottle until the butyrometer is filled to the shoulder after allowing all entrained air in the powder to escape. The butyrometer is closed with a double-ended rubber stopper and is shaken, carefully at first and then more vigorously, until the contents are thoroughly mixed and no solid particles can be seen in the liquid. From this point the procedure is exactly the same as that in the determination of fat in milk (see p. 223). The scale reading is multiplied by the factor 20/3 to obtain the percentage of fat in the dried milk.

Fat in Dried Milk (Gravimetric Method).—The following method of determining fat in dried milk is recommended by the Milk Products Sub-Committee of the Society of Public Analysts (*Analyst*, 1936, **61**, pp. 105-111). For this deter-

mination the apparatus shown in Fig. 5 is used. It consists of a hard glass boiling tube, 8 in. by 1 in., with a funnelled mouth. In the mouth is fitted a cork for the extractions and another cork fitted with wash bottle tubes, as shown in the figure, for the transference of the ethereal layer to a flask. Sound well fitting corks should be used; rubber stoppers are not suitable. The funnelled mouth facilitates the introduction of the sample into the tube and has the additional advantage that any of the solution which may pass the cork when it is released is retained by the funnel and can be washed back into the tube. The cork should be moistened with water before insertion for each extraction. In order to avoid spurting of the solvent, the tube should be cooled before removing the cork.

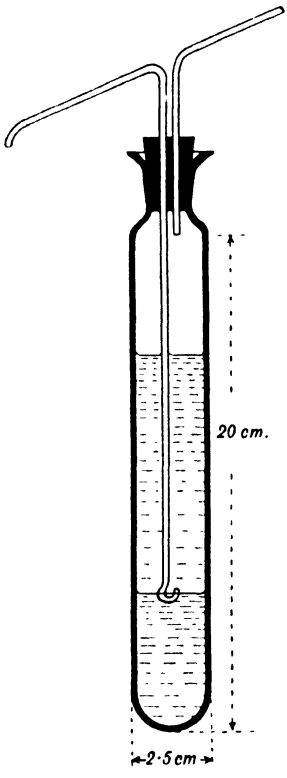


FIG. 5.

The procedure is as follows: Transfer to the boiling tube 1 gm., accurately weighed, of the well mixed sample. Add 8 c.c. of water and 2 drops of concentrated ammonia. Gently boil the mixture until all lumps are disintegrated. Add 10 c.c. of concentrated hydrochloric acid and heat in a Bunsen flame with gentle agitation; after the liquid begins to boil, continue gentle boiling for 3 minutes. Cool, add 10 c.c. of 95 per cent. (by volume) alcohol and mix well. Add 25 c.c. of ether; close the tube with the moistened cork and shake for 15 seconds. Cool and remove the cork; wash the cork and the neck of the tube with petroleum spirit (b.p. 40°-60° C.) and add, including the washings, 25 c.c. of petroleum spirit. Replace the moistened cork;

shake vigorously for 30 seconds, and either allow the tube to stand or centrifuge it until the two layers are separated. Transfer the ethereal layer as completely as possible to a suitable flask by means of the wash bottle fitting. Wash the tip of the tube with ether and wash down the inside of the boiling tube with 5 c.c. of ether. Without further shaking, transfer it to the flask and wash the tip as before. Add 15 c.c. of ether, using this ether to wash the cork and wash bottle tube before its removal. Replace the freshly moistened cork and shake for 15 seconds. Add 15 c.c. of petroleum spirit and shake for 15 seconds, taking the same precautions as to washing the neck and cork as before. When the ethereal layer has separated, transfer it to the flask as before. Repeat the extraction with 15 c.c. of ether and 15 c.c. of petroleum spirit and the transference of the ethereal layer to the flask, and wash the tip of the tube.

Cautiously distil the solvents from the flask and dry the residual fat at 102°-103° C. for 1 hour. Remove the solvent vapour from the flask by blowing air gently into the flask. Cool and weigh. Repeat the heating until there is no loss in weight. Completely extract the fat from the flask by repeated washings with petroleum spirit, allowing any sediment to settle, and washing off any fat which may have crept over the edge of the flask during the removal of the fatty solutions. Dry the flask at 102°-103° C. with removal of the solvent vapour. Cool and weigh as before.

The difference in weight before and after the petroleum spirit extraction is the uncorrected weight of the fat contained in the weight of dried milk taken for the analysis. Make a blank determination, using the specified quantities of reagents and distilled water, and deduct the weight found, if any, from the weight of fat obtained.

Fat in Condensed Milk.—The following method of determining fat in sweetened and unsweetened condensed milk is recommended by the Milk Products Sub-Committee of the Society of Public Analysts (*Analyst*, 1927, **52**, pp. 402-408):

Transfer to a suitable apparatus* 2-2·5 gm., accurately weighed, of the sample prepared as described on p. 253. Add 8 c.c. of warm water and mix well. Cool, add 1 c.c. of concentrated ammonia and mix. Add 10 c.c. of 95 per cent. (by volume) alcohol and mix again. Add 25 c.c. of ether and shake vigorously for 1 minute. Add 25 c.c. of petroleum spirit (b.p. 40°-60° C.) and shake vigorously for 30 seconds. Allow the liquids to stand until the ethereal layer is perfectly clear or centrifuge at a low speed. Transfer the ethereal layer to a suitable flask. To the milk residue add 5 c.c. of ether and transfer without further shaking. Repeat this operation with a further 5 c.c. of ether. Add 0·5 c.c. of alcohol, and repeat the extraction with 25 c.c. of ether and 25 c.c. of petroleum spirit, as before, shaking vigorously for 1 minute after the addition of the ether and for 30 seconds after the addition of the petroleum spirit. Allow the ethereal layer to separate completely and transfer it to the flask. Repeat the extraction once more with alcohol, ether and petroleum spirit in the same way. Cautiously distil the solvents from the flask and dry the residual fat at 98°-100° C. to constant weight, taking the ordinary precautions to remove all traces of volatile solvent. Completely extract the fat from the flask by repeated washings with petroleum spirit, allowing any sediment to settle before each decantation. Finally dry the flask at 98°-100° C. The difference in weight before and after the petroleum ether extractions is the weight of fat contained in the weight of condensed milk taken. Make a blank determination, using the specified quantities of the reagents and distilled water in place of the milk, and deduct the weight found, if any, from the weight of fat obtained.

Proteins in Dried Whey.—W. L. Davies (*J. Soc. Chem. Ind.*, 1935, **54,** pp. 338-341T) determined the proteins in dried whey as follows:

True Proteins.—These are determined by reconstituting

* The apparatus shown in Fig. 4 on p. 228 or that shown in Fig. 5 on p. 258 is suitable.

the dried whey with hot water at 70° C. and precipitating with trichloroacetic acid with a final concentration of 4 per cent. The nitrogen in the precipitate multiplied by 6·38 gives the true proteins.

Denatured Proteins.—To 2 gm. of dried whey, reconstituted in 20 c.c. of water, are added 10 c.c. of separated milk. The casein of the milk and the denatured proteins are precipitated together by Moir's method (see p. 231), using 1·15 c.c. of 10 per cent. acetic acid and 4·5 c.c. of N/4 sodium acetate solution. The precipitate is separated and washed. The excess of nitrogen in the precipitate over that found as casein nitrogen in the separated milk represents the nitrogen of the denatured proteins. The latter figure multiplied by 6·38 gives the denatured proteins.

Lactose.—Lactose can be determined in milk products by Lane and Eynon's volumetric method, which is fully described on p. 132. In the analysis of sweetened condensed milk the determination of lactose is affected by the sucrose present. The effect of the sucrose is to reduce the volume of lactose solution required, and it may be allowed for by adding the requisite correction to the burette reading. The corrections (in c.c.) to be added to the burette readings in the titration of lactose solutions containing 3 or 6 times as much sucrose as lactose are given in the table on p. 262 (J. H. Lane and L. Eynon, *Determination of Reducing Sugars by Fehling's Solution with Methylene Blue Indicator*, 1934, p. 7):

At any given part of the table the correction is practically proportional to the sucrose to lactose ratio, and this proportionality holds up to a ratio of about 10:1.

Sucrose in Sweetened Condensed Milk.—The following method of determining sucrose in sweetened condensed milk is that recommended by the Milk Products Sub-Committee of the Society of Public Analysts (*Analyst*, 1930, **55**, pp. 111-124). The following reagents and apparatus are required:

Zinc Acetate Solution.—Dissolve 21·9 gm. of crystallized

zinc acetate in water, add 3 c.c. of glacial acetic acid and make up to 100 c.c.

Potassium Ferrocyanide Solution.—Dissolve 10·6 gm. of crystallized potassium ferrocyanide in water and make up to 100 c.c.

6·34*N Hydrochloric Acid.*

Dilute Ammonia Solution.—10 c.c. of concentrated ammonia diluted with water to 100 c.c.

Dilute Acetic Acid.—Approximately equivalent to the dilute ammonia solution.

Apparatus.—There is required a polarimeter or a saccharimeter. The source of light for the former is sodium light or the green line of the mercury spectrum separated by means of a prism or by the use of a special Wratten screen No. 77a; and for the latter white light from an incandescent electric lamp after passing through 15 mm. of 6 per cent. potassium dichromate solution. Tubes of not less than 2 dm., exactly calibrated for length, and a standardized thermometer reading to 0·1° C. are also required.

c.c. of Sugar Solution Required.	10 *c.c. of Fehling's Solution.*		25 *c.c. of Fehling's Solution.*	
	Ratio of Sucrose to Lactose.		*Ratio of Sucrose to Lactose.*	
	3:1	6:1	3:1	6:1
15	0·15	0·30	0·30	0·60
20	0·25	0·50	0·30	0·60
25	0·30	0·60	0·35	0·65
30	0·35	0·70	0·35	0·70
35	0·40	0·80	0·40	0·80
40	0·45	0·90	0·45	0·90
45	0·50	0·95	0·55	1·10
50	0·55	1·05	0·60	1·20

Procedure.—Transfer to a 100 c.c. beaker an accurately

weighed quantity, about 40 gm., of the well mixed sample prepared as described on p. 253. Add 50 c.c. of water at 80°-90° C. Mix and transfer to a 200 c.c. measuring flask, washing with successive quantities of water at 60° C. until the total volume is 120-150 c.c. Mix, cool to air temperature and then add 5 c.c. of the dilute ammonia solution. Mix again and allow to stand for 15 minutes. Add a sufficient quantity of the dilute acetic acid to neutralize the ammonia added (the exact equivalent is determined by titration) and mix again. Add 12·5 c.c. of zinc acetate solution and then 12·5 c.c. of potassium ferrocyanide solution, mixing after each addition. Bring the contents of the flask to 20° C. and add water at 20° C. up to the 200 c.c. mark. To avoid the formation of air bubbles all the mixings should be made by rotating the flask rather than shaking. If bubbles are present before diluting to 200 c.c., their removal can be assisted by attaching the flask to a vacuum pump and rotating it. Close the flask with a dry stopper and mix thoroughly by shaking. Allow to stand for a few minutes and then filter through a dry filter paper and reject the first 25 c.c. of the filtrate.

Determine the rotation of the filtrate at 20° C. Pipette 40 c.c. of the filtrate into a 50 c.c. flask and add 6 c.c. of 6·34N hydrochloric acid. Immerse the entire bulb of the flask in a water bath at 60° C. for 12 minutes, mixing by rotation during the first 3 minutes, in which time the contents of the flask should have attained the temperature of the bath. Cool, dilute to 50 c.c. at 20° C. with water and mix. After allowing to stand for 1 hour, determine the rotation at 20° C.

Inversion Divisor Factor.—The change in the specific rotation of sucrose on inversion divided by 100 is known as the inversion divisor factor. Since the filtrates prepared as described above contain both the salts of the milk and those resulting from the clarification process, the Milk Products Sub-Committee determined the actual change in rotation when sucrose is inverted under these conditions. The fol-

lowing are the values of the inversion divisor factor at 20° C. obtained:

Sodium light	(i) 0·8825
Mercury green line (prism or special Wratten screen No. 77a)	(ii) 1·0392
International sugar scale (*j*) light ..	(iii) 2·549

If the concentration of the sugars in the filtrate does not approximate to that usually present, a correction must be applied to these inversion factors; but only in exceptional circumstances need this correction be applied. A variation of ±1° C. makes little significant difference in the direct reading, but in the case of the reading after inversion a variation of ±0·2° C. requires a correction for temperature. The following are the corrected values of the inversion divisor factors:

(i) $0{\cdot}8825+0{\cdot}0006(C-9)-0{\cdot}0033(T-20)$
(ii) $1{\cdot}0392+0{\cdot}0007(C-9)-0{\cdot}0039(T-20)$
(iii) $2{\cdot}549+0{\cdot}0017(C-9)-0{\cdot}0095(T-20)$

where C is the percentage of total sugars in the inverted solution as polarized and T is the temperature in degrees Centigrade between 18° and 22° C.

Calculation :

Let W = the weight (in gm.) of the sample taken,
F = the percentage of fat in the sample,
P = the percentage of protein (N × 6·38) in the sample,
V = the volume to which the sample is diluted before filtration,
v = the correction (in c.c.) for the volume of the precipitate produced during clarification,
D = the observed direct polarimeter reading,
I = the observed invert polarimeter reading,
l = the length (in dm.) of the polarimeter tube,
and Q = the inversion divisor factor.

The specific volume of the fat is 1·08 and the apparent volume of the precipitate due to 1 gm. of protein associated

with the ferrocyanide is 1·55 c.c. Therefore $v = W(1{\cdot}08F + 1{\cdot}55P)/100$, and the percentage of sucrose in the sample =

$$\frac{D - 1{\cdot}25\,I}{Q} \times \frac{V - v}{V} \times \frac{V}{lW}.$$

Chlorine.—Davies's method of determining chlorine in milk, which is fully described on p. 237, is applicable to butter, cheese, dried milk and other solids. The procedure for solids described by W. L. Davies (*Analyst*, 1932, **57,** pp. 79-85) is as follows: A weight of material containing 0·15-0·30 gm. of combined chlorine is weighed into a 250 c.c. conical flask and is well wetted or soaked with water, warming if necessary. 25 c.c. of N/20 silver nitrate solution are then added. After thoroughly shaking, 10 c.c. of saturated potassium permanganate solution and 25 c.c. of concentrated nitric acid are added, and the contents of the flask are boiled over a gauze. Starchy foods disintegrate quickly, yielding a yellow solution, but foods rich in protein, especially those containing fibrous protein, take longer to dissolve. If the amount of chlorine is large, or if there is a large volume of unattacked fibrous material, fat or sand, the digest is cooled, diluted to 100 c.c. and filtered by suction, the precipitate being washed repeatedly with 5 per cent. nitric acid. But when the amount of solid material is small, the excess of silver nitrate may be determined at once. The filtrate is made up to 200 c.c. and the excess of silver nitrate is determined in aliquot portions or in the whole liquid by titrating with N/20 potassium thiocyanate solution, using 1 c.c. of saturated iron alum solution in 10 per cent. nitric acid as indicator, after adding acetone until the solution contains 5 per cent. In determinations in which excess of silver nitrate was not added at the beginning, it is necessary to repeat the determination with a fresh quantity of material; further addition of silver nitrate after the acid digestion is useless.*

* 1 c.c. of N/20 silver nitrate solution = 0·00177 gm. of chlorine and 0·00292 gm. of sodium chloride.

Water in Butter.—The Association of Official Agricultural Chemists (*Methods of Analysis*, 1935, p. 288) give the following directions for the preparation of samples of butter: Soften the entire sample in a closed vessel at as low a temperature as possible. Shake vigorously until a perfectly homogeneous semi-solid mass is obtained. Weigh the portions for the analysis at once.

H. D. Richmond (*Dairy Analysis*, 1925, pp. 72-73) gives the following method of determining water in butter: Weigh a small basin about 3 in. in diameter containing a small glass rod. Place in the basin 5-10 gm. of butter and weigh again. Heat the basin over a small flame of such a size that the butter takes at least a minute to melt and stir constantly until frothing ceases. Cool and weigh again. E. R. Bolton (*Oils, Fats and Fatty Foods*, 1928, p. 112) weighs the sample in a platinum basin, which is heated about 6 in. above the flame of an argand or small Bunsen burner and is shaken until hissing ceases. If the process is properly carried out, there will be no discoloration of the curd.

For routine determinations E. R. Bolton (*op. cit.*, pp. 111-112) adopts the following method: Weighing bottles 2 in. deep and $1\frac{1}{2}$ in. wide without shoulders are dried and weighed. About 3-5 gm. of each sample are rapidly poured in from the sample bottles, after the contents have been shaken vigorously to the consistency of cream. The weighing bottles, having been reweighed, are placed in a water oven and the contents are thoroughly shaken round at intervals until visible water has disappeared. The bottles may then be tilted on one side and turned round from time to time until the weights are constant. If the shaking is skilfully done, the whole operation will take 2-3 hours.

Curd and Salt in Butter.—To the dry butter obtained in the determination of water is added ether, petroleum ether or hot amyl alcohol. After stirring and allowing to stand, the clear solution of fat is decanted. These operations are repeated until all the fat has been removed. The basin or weighing bottle is then placed in an oven and reweighed.

The weight of fat is found by difference and the weight of curd and salt directly. The salt is extracted from the curd by adding hot water, filtering the solution and washing the basin or weighing bottle and the filter paper. The filtrate is allowed to cool and the whole or an aliquot part is titrated with standard silver nitrate solution, using potassium chromate as indicator. The curd is generally determined by difference; but it can be determined directly by filtering the solution of salt through a tared filter paper, transferring the whole of the curd to the filter paper, washing thoroughly and reweighing the filter paper when dry.

Salt in Butter.—The Association of Official Agricultural Chemists (*Methods of Analysis*, 1935, p. 289) have adopted the following method of determining salt in butter: Weigh in a counterpoised beaker 5-10 gm. of the sample. Add about 20 c.c. of hot water and, after the butter has melted, transfer the whole to a separating funnel. Insert the stopper and shake for a few minutes. Allow to stand till all the fat has risen to the top; then draw off the water into a flask. Again add hot water, rinsing the beaker, and repeat the extraction 10 or 15 times, using 10-20 c.c. of water each time. Determine the sodium chloride in the whole or an aliquot part of the liquid by titration with standard silver nitrate solution, using potassium chromate as indicator.

Preparation of Butter Fat.—The following method of preparing butter fat for analysis is recommended by the Analytical Methods Committee of the Society of Public Analysts (*Analyst*, 1936, **61,** p. 404): Heat a portion of the sample of butter in a beaker until the fat separates from the water and curd. To facilitate separation and filtration, it is advisable that the temperature should not be above 50° C. Filter the fatty layer through a dry filter paper into a dry vessel, and, if necessary, refilter the fat until it is clear and free from water. Melt the fat completely before taking samples for analysis. Exposure of the warm fat to the air should be as short as possible.

Saponification Value.—The following procedure is ab-

stracted by permission from *Standard Methods for the Analysis of Fats* (*Internationally Agreed*), British Standards Institution, No. 684, 1936, p. 16. The following solutions are required:

N/2 Hydrochloric Acid.

Approximately N/2 Solution of Potassium Hydroxide in 95 per cent. Alcohol.—After leaving to stand, decant this solution into a brown or yellow glass bottle fitted with a rubber stopper.

Weigh accurately about 2 gm. of the fat into a flask of about 200 c.c. capacity made of alkali-resisting glass. Add 25 c.c. of the alcoholic potassium hydroxide solution, accurately measured, and heat to the boiling point under a reflux condenser. Then boil for half an hour, stirring occasionally. Titrate the soap solution whilst hot with N/2 hydrochloric acid in the presence of phenolphthalein. Carry out a blank test under the same conditions to standardize the potassium hydroxide solution.

Taking b and a as the numbers of c.c. of N/2 hydrochloric acid used in the test with the fat and in the blank test, and w as the weight of the fat in grams, the saponification value $=28{\cdot}05\ (a - b)/w$.

Reichert, Polenske and Kirschner Values.—Below are given the procedures recommended by the Analytical Methods Committee of the Society of Public Analysts (*Analyst*, 1936, **61**, pp. 404-408). The following reagents are required:

Glycerol.

50 per cent. (by weight) Sodium Hydroxide Solution.—Sodium hydroxide is dissolved in an equal weight of water, and the solution is stored in a bottle protected from carbon dioxide. The clear solution free from deposit is used.

Dilute Sulphuric Acid.—About 25 c.c. of concentrated sulphuric acid are diluted to 1 litre, and adjusted until 40 c.c. neutralize 2 c.c. of the 50 per cent. sodium hydroxide solution.

Pumice Powder.—Ground pumice passing through a sieve

B.S. No. 50, and remaining on a sieve B.S. No. 90 (see British Standard Specification for Test Sieves, No. 410, 1931).

Phenolphthalein Solution.—0·5 gm. of phenolphthalein dissolved in 100 c.c. of industrial methylated spirit.

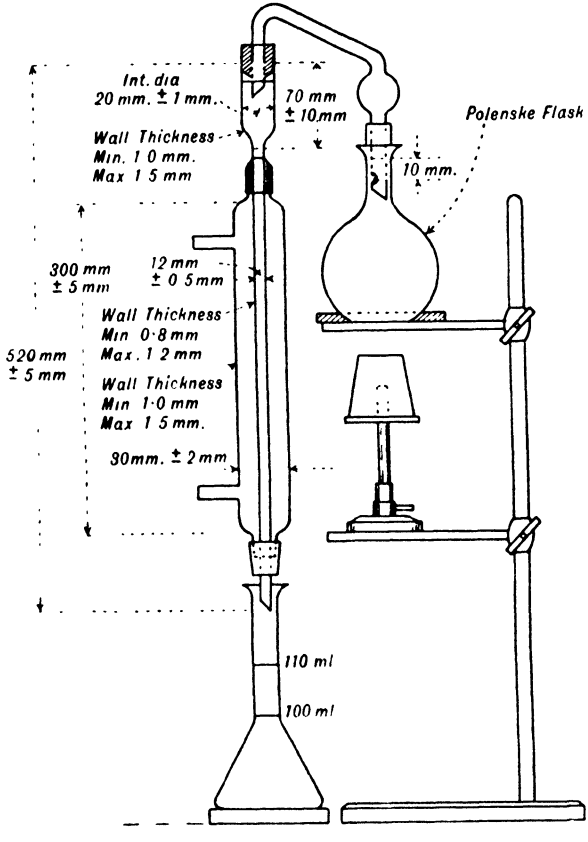


FIG. 6.

Alcohol.—Industrial methylated spirit made neutral to phenolphthalein immediately before use.

N/10 *Sodium Hydroxide Solution.*

N/10 *Barium Hydroxide Solution.*

Silver Sulphate.—Powdered.

Apparatus.—The apparatus for the distillation is shown in Fig. 6. The flat-bottomed boiling flask, termed the

Polenske flask, is made of resistance glass and conforms to the following details: Volume contained to bottom of neck, 310±10 c.c.; length of neck, 75±5 mm.; internal diameter of neck, 21±1·0 mm.; overall height, 160±5 mm.; diameter of base, 45±5 mm. The still head is made of glass and conforms with the dimensions given in Fig. 7. The condenser is made of glass and conforms with the dimensions given in Fig. 6. The receiver is a flask with two graduation

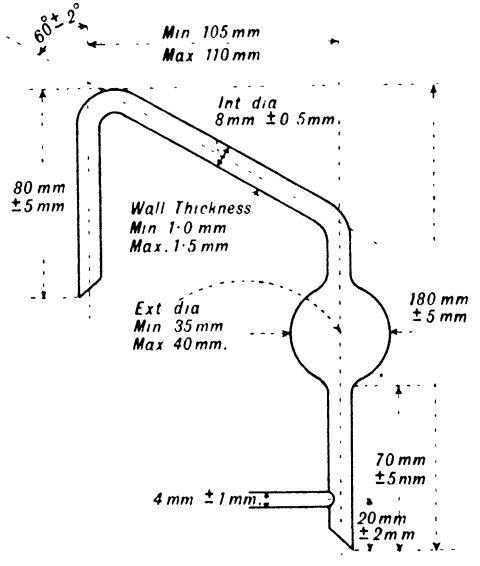


FIG. 7.

marks on the neck, one at 100 c.c. and the other at 110 c.c., conforming with the British Specification for Sugar Flasks, No. 675, 1936. The Polenske flask stands on an asbestos board, 120 mm. in diameter, 6 mm. in thickness, with a circular hole about 65 mm. in diameter. During the distillation the flask must fit snugly into the hole in the asbestos board, so as to prevent the flame from impinging on the surface of the flask above the hole. A new asbestos board can be conveniently prepared by bevelling the edge of the hole, soaking in water, moulding the edge with a flask and drying.

Saponification and Distillation.—Weigh 5 gm. (tolerance not exceeding 0·01 gm.) of butter fat, prepared as described on p. 267, into a Polenske flask. Add 20 gm. of glycerol and 2 c.c. of the 50 per cent. sodium hydroxide solution. Heat over a naked flame, with continuous mixing, until the fat, including the drops adhering to the upper part of the flask, is saponified and the liquid becomes perfectly clear. Cover the mouth of the flask with a watch glass. Make a blank test without fat, using the same quantities of the reagents and following the same procedure. Avoid over-heating, which is indicated by darkening of the solution.

Measure 93 c.c. of boiling distilled water, which has been vigorously boiled for 15 minutes, into a 100 c.c. graduated cylinder. When the soap is cool enough to allow the addition of water without loss, but before the soap has solidified, add the water to the flask, draining the cylinder for 5 seconds, and dissolve the soap. If the solution is not clear, indicating incomplete saponification, or is darker than light yellow, indicating over-heating, the saponification must be repeated with another portion of the fat.

Add 0·1 gm. of powdered pumice, followed by 50 c.c. of dilute sulphuric acid, and at once connect the flask with the distilling apparatus. Heat the flask, without boiling, until the insoluble acids are completely melted; then increase the flame and distil 110 c.c. in 19-21 minutes. The flow of water must be sufficient to keep the temperature of the issuing distillate between 18° and 23° C. When the distillate reaches the 110 c.c. mark, remove the flame, and replace the 110 c.c. flask by a 25 c.c. cylinder to catch the drainings. Close the 110 c.c. flask with a stopper, and, without mixing, place it in water at 15° C. for 10 minutes so as to immerse the 110 c.c. mark. Remove the flask from the water, dry the outside and invert the flask carefully, avoiding wetting the stopper with the insoluble acids. Mix the distillate by 4 or 5 double inversions without violent shaking. Filter through a dry 9 cm. Whatman No. 4 filter paper. Reject the first runnings

and collect 100 c.c. in a dry flask. Cork the flask, and keep the filtrate for the determination of the Reichert value.

Detach the still-head and wash the condenser with 3 successive 15 c.c. portions of cold distilled water, passing each washing separately through the cylinder, the 110 c.c. flask and the filter; nearly fill the paper each time, and drain each washing before filtering the next. Discard the washings. Dissolve the insoluble acids by 3 similar washings of the condenser, the cylinder and the filter with 15 c.c. of neutralized alcohol, collecting the solution in the 110 c.c. flask and draining the alcohol after each washing. Cork the flask and keep the solution for the determination of the Polenske value.

Reichert Value.—Pour 100 c.c. of the filtrate containing the soluble volatile acids into a titration flask, add 0·1 c.c. of the phenolphthalein solution and titrate with N/10 barium hydroxide solution, or N/10 sodium hydroxide solution if the Kirschner value is not required, until the liquid becomes pink. Rinse the 100 c.c. flask with the nearly neutralized liquid towards the end of the titration. If the Kirschner value is to be determined, the titration flask must be dry before use. Note the volume of the barium hydroxide solution used; drain the 100 c.c. flask into the titration flask, close with a cork and keep for the determination of the Kirschner value. If the volumes of N/10 barium (or sodium) hydroxide solution used for the titration of the sample and the blank are x and x_b c.c. respectively, the Reichert value $= 110\ (x - x_b)/100$.

Polenske Value.—Titrate the alcoholic solution of the insoluble volatile acids after the addition of 0·25 c.c. of the phenolphthalein solution with N/10 barium (or sodium) hydroxide solution until the solution becomes pink. If the volumes of N/10 barium (or sodium) hydroxide solution used for the titration of the sample and the blank are y and y_b c.c. respectively, the Polenske value $= y - y_b$.

Kirschner Value.—Add 0·5 gm. of finely powdered silver sulphate to the neutralized filtrate obtained in the deter-

mination of the Reichert value. Allow the flask to stand in the dark for 1 hour, with occasional shaking, and filter the contents through a dry filter. Transfer 100 c.c. of the filtrate to a dry Polenske flask, add 35 c.c. of cold distilled water (recently boiled for 15 minutes), 10 c.c. of dilute sulphuric acid and a loosely wound 5 mm. coil of 30 cm. of aluminium wire (about 1 mm. thick or about 18-20 S.W.G.) or 0·1 gm. of pumice powder. Connect the flask with the standard apparatus and repeat the process—*i.e.*, the distillation of 110 c.c. in 19-21 minutes, the mixing (but without cooling for 10 minutes), the filtration and the titration of 100 c.c. of the filtrate with N/10 barium hydroxide solution. If the volumes of N/10 barium hydroxide solution used for the titration of the fat and the blank are z and z_b c.c. respectively, the Kirschner value =

$$(z - z_b) \times \frac{(100+a) \times 121}{10000}$$

where a represents the actual volume in c.c. of barium hydroxide solution used in the titration for the determination of the Reichert value.

Iodine Value.—The following alternative methods of determining the iodine value are abstracted by permission from *Standard Methods for the Analysis of Fats (Internationally Agreed)*, British Standards Institution, No. 684, 1936, pp. 17-19. In the three methods the quantity of the reagent must be sufficient to ensure that at the end of the operation the excess of halogen remaining shall be at least equal to the quantity of halogen absorbed. For the 25 c.c. of reagent used, the weights of fat should be of the order of 32/I.V. In Hanus's method it is recommended that weights of about 25/I.V. be taken.* The operations should be carried out at about 20° C. and away from direct light.

Wijs's Method.—The necessary reagent is prepared by dissolving separately 9·8 gm. of iodine trichloride and 10·2

* The iodine value of butter fat is 25·7-37·9 (W. L. Davies, *The Chemistry of Milk*, 1936, p. 91).

gm. of iodine in glacial acetic acid of not less than 99 per cent. strength and free from alcohol. The two solutions are mixed and made up to 1000 c.c. with acetic acid. The solution, which has a deep colour due to an excess of iodine is kept in a yellow glass stoppered bottle.

The fat is weighed in a small glass dish of 1-2 c.c. capacity and is placed in a dry 200-300 c.c. stoppered bottle. The fat is then dissolved in 10 c.c. of chloroform or carbon tetrachloride, and 25 c.c. of the reagent, very accurately measured, are added by means of a pipette. The bottle is stoppered, shaken and left in the dark for 1 hour in the case of fats with iodine values below 150 and for 3 hours in the case of fats with iodine values above 150. At the end of this time 20 c.c. of 10 per cent. potassium iodide solution, free from iodine and iodate, and about 100 c.c. of water are added. The excess of iodine is titrated with N/10 or N/20 sodium thiosulphate solution. The thiosulphate solution is added gradually, with shaking, until the mixture is slightly yellow. After adding 5 c.c. of freshly prepared starch solution as indicator, the titration is continued with vigorous shaking until the colour disappears. The reagent is standardized under identical conditions in a blank test without any fat. Taking b and a as the volumes in c.c. of N/10 sodium thiosulphate solution used in the test with the fat and in the blank test, and w as the weight of the fat in grams, the iodine value $= 1{\cdot}269\ (a - b)/w$.

Hanus's Method.—10 gm. of iodine monochloride are dissolved in 500 c.c. of pure glacial acetic acid, free from alcohol, in a glass stoppered bottle, preferably of yellow glass. A weighed quantity of the fat is placed in a wide-mouthed 200 c.c. stoppered bottle, which has been previously washed and dried. The fat is dissolved in 10 c.c. of chloroform or carbon tetrachloride, and 25 c.c. of the reagent, accurately measured, are added. The bottle is stoppered, shaken and left for 1 hour in the dark. Then 20 c.c. of 10 per cent. potassium iodide solution, free from iodine and iodate, and about 100 c.c. of water are added, and the free iodine

is titrated with N/10 sodium thiosulphate solution. A corresponding blank test is carried out at the same time. The titration and calculation are carried out in the same way as in Wijs's method.

Hübl's Method.—The necessary reagent is prepared by dissolving separately 25 gm. of iodine in 500 c.c. of 96 per cent. alcohol, and 30 gm. of mercuric chloride in the same quantity of alcohol. The latter solution is filtered, if necessary. The two solutions are mixed about 24 hours before use. The mixture must not be used after being kept for more than 48 hours.

A weighed quantity of the fat is placed in a stoppered bottle of 500-800 c.c. capacity. The fat is dissolved in 10 c.c. of chloroform or carbon tetrachloride, and 25 c.c. of the reagent, accurately measured, are added. The bottle is stoppered, shaken and left in the dark for 12-24 hours, according to the expected iodine value. At the end of this time 20 c.c. of 10 per cent. potassium iodide solution, free from iodine and iodate, are added. If a precipitate of mercuric iodide is formed, more potassium iodide is added. About 300 c.c. of water are then added, and the free iodine is titrated with N/10 sodium thiosulphate solution. A corresponding blank test is carried out at the same time. The titration and calculation are carried out as in the preceding methods.

Colouring Matters.—The following are the methods adopted by the Association of Official Agricultural Chemists (*Methods of Analysis*, 1935, p. 290) for the detection of added colouring matters in butter: Pour about 2 gm. of filtered butter fat dissolved in ether into each of two test tubes. Into one of the tubes pour 1-2 c.c. of dilute hydrochloric acid (1:2), and into the other pour about the same volume of 10 per cent. sodium hydroxide solution. Shake the tubes well and allow them to stand. In the presence of azo dyes the test tube to which the acid has been added will show a pink to red colour, while the alkaline solution in the other tube will show no colour. If, however, annatto or other

vegetable colouring matter is present, the alkaline solution will be coloured yellow, while no colour will be apparent in the acid solution.

Water in Cheese.—E. R. Ling (*Dairy Chemistry*, 1930, p. 191) prepares the sample as follows: Wedge-shaped samples are cut from the material supplied or samples are taken with a borer. In either case the rind and about half an inch of the cheese adhering to it are discarded. The remainder is cut into small pieces and well mixed.

H. D. Richmond (*Dairy Analysis*, 1925, p. 95) gives the following method of determining water in cheese: Place 2-3 gm. of cheese cut into small pieces in a small flat-bottomed basin, and keep it in a water oven for 6 hours. Incline the basin so that the fat runs off the drying cheese. Weigh the basin and replace it in the oven. Weigh at intervals of 1 hour until the loss is less than 1 mgm. per hour.

Fat in Cheese (Gerber Method).—The following description of the British standard method of determining fat in cheese by the Gerber method is abstracted by permission from British Standard Specification No. 696, Part 2, 1936, pp. 33-36. This method is applicable to dry cheeses, but is not suitable for soft cheeses such as cream cheeses. The same chemicals and apparatus are required as those for the determination of fat in milk (see p. 223), except that the 11 ml. pipette is not required and the standard butyrometer for testing cheese is used in place of the standard butyrometer for testing milk.* A stoppered funnel for weighing the cheese (B.S.S. No. 696, Part 1, p. 42) can also be used, but this is not required if the cheese is weighed in the butyrometer.

The weighing funnel, with its stopper inserted in the neck, is weighed. Thin shavings from the sample, as representative as possible of the whole, are cut with a sharp knife or

* An alternative method of determining fat in cheese, using the butyrometer for testing milk, is described in B.S.S. No. 696, Part 2, pp. 36-40. This method requires the use of tables which are not given here.

razor. Immediately 3 gm. (tolerance $\pm 0 : 01$ gm.) of the shavings are weighed into the weighing funnel. For cheeses containing more than 40 per cent. of fat, 1·5 gm. of cheese is taken for the test and the butyrometer reading is multiplied by 2. 10 ml. of sulphuric acid are measured into the butyrometer by means of the standard pipette or an automatic measure, care being taken not to wet the neck. By means of a wash bottle water at 30°-40° C. is added, the jet being directed on to the wall of the butyrometer, so as to form a layer of water about 6 mm. deep on the top of the acid; about 3 c.c. are required. The weighing funnel containing the weighed quantity of cheese is inserted into the neck of the butyrometer. The stopper is withdrawn and the cheese is transferred to the butyrometer by means of a small camel-hair brush. 1 ml. of amyl alcohol is measured into the butyrometer by means of the standard pipette or an automatic measure, care being taken not to wet the neck. Water at 30°-40° C. is added from a wash bottle until the butyrometer is filled to the shoulder; about 6·5 c.c. are required. The butyrometer is closed with a double-ended rubber stopper and is shaken until the contents are thoroughly mixed and the curd is dissolved. It is then placed with the rubber stopper downwards in the water bath at 68° C. and is left there until the other butyrometers have been filled and placed in the water bath. The butyrometers are transferred as quickly as possible to the centrifuge and are centrifuged at 1100 r.p.m. for 4-5 minutes. After adjusting the stoppers, the butyrometers are placed in the water bath at 68° C. for 2-3 minutes. If there is not a sharp line between the fat and the acid or if the acid layer is not clear, the centrifuging and placing in the water bath are repeated. After adjusting the stoppers, the fat columns are read. The butyrometers are again placed in the water bath for another 2-3 minutes and check readings are taken as quickly as possible after removal from the bath.

Fat in Cheese (Gravimetric Method). — The following gravimetric method is adopted by the Association of Official

Agricultural Chemists (*Methods of Analysis*, 1935, pp. 291-292): Rub by means of a glass rod 1 gm. of the prepared sample with 9 c.c. of water and 1 c.c. of concentrated ammonia in a narrow 100-125 c.c. beaker. Digest the mixture at a low temperature until the casein is softened. Neutralize with hydrochloric acid, using litmus as indicator, and then add 10 c.c of concentrated hydrochloric acid. Add about 0·5 gm. of sand, previously digested with hydrochloric acid, to prevent bumping and boil gently for 5 minutes, keeping the beaker covered with a clock glass. Cool the solution and transfer it to a Röhrig tube or similar apparatus.* Rinse the beaker with 25 c.c. of ether, transfer the rinsings to the Röhrig tube and shake thoroughly. Add 25 c.c. of petroleum ether (b.p. below 65° C.), shake thoroughly and allow the mixture to separate. Complete the determination as in the Röse-Gottlieb method (see p. 227).

Products of Ripening.—The procedure described by H. D. Richmond (*Dairy Analysis*, 1925, pp. 95-96) is as follows: Place 10 gm. of cheese in a small mortar, add 25 c.c. of boiling water and grind the cheese with a pestle. Pour off the solution through a filter and collect the filtrate in a 250 c.c. flask. Repeat the treatment with 25 c.c. of boiling water till 9 portions have been used. Cool the filtrate, make up to 250 c.c. and mix well. Evaporate 50 c.c. in a weighed basin and weigh the solid residue after drying till the loss is less than 1 mgm. per hour. Ignite the residue and weigh the ash. The weight of the solid residue less that of the ash represents the products of ripening.†

* A Röhrig tube is a stoppered graduated tube with a tap at some distance from the bottom. The apparatus shown in Fig. 4 on p. 228 will be found much more convenient.

† In other aliquot portions of the filtrate the acidity, the total nitrogen and the total protein nitrogen can be determined.

INSECTICIDES AND FUNGICIDES

Bulletin No. 82 of the Ministry of Agriculture and Fisheries (1934) is devoted to specifications and methods of analysis of lead arsenate, lime-sulphur solutions, nicotine and nicotine sulphate, copper sulphate, Bordeaux powder, Burgundy powder, Cheshunt compound, soft soaps, sodium, potassium and calcium cyanides, and formaldehyde. These specifications and methods of analysis have been prepared by the Association of British Insecticide Manufacturers, and have been accepted by the Government Chemist, the National Farmers' Union and the Ministry of Agriculture. The specifications are beyond the scope of this book. The methods of analysis are given in the following pages in the same order as they are in the Bulletin, and for convenience they are termed the agreed methods. The full details of the agreed methods are included in the Bulletin, except in the case of lime-sulphur solutions and cyanides. But in those cases, the methods which are cited in the Bulletin are fully described in the following pages.

The methods of determining moisture, acidity, lead oxide, total and water-soluble arsenic in lead arsenate powder and paste will be found on pp. 287-288. On pp. 289-291 are given the A. O. A. C. methods for the determination of total sulphur, thiosulphate sulphur, sulphide sulphur, sulphate sulphur and total lime in lime-sulphur solutions. The agreed method for the determination of polysulphide sulphur in spray materials is that of Goodwin and Martin, which is described on p. 291. This is a modification of Chapin's method, in which the polysulphide sulphur is treated with an excess of sodium sulphite, and the resulting thiosulphate is titrated with standard iodine solution.

The agreed method for the determination of nicotine consists in precipitating the alkaloid with silicotungstic acid, igniting the precipitate and weighing the inorganic residue. The procedure is described on p. 293, and is followed by the A.O.A.C. method, which is similar but contains many

more analytical details. There are several silicotungstic acids (see J. W. Mellor, *Inorganic and Theoretical Chemistry*, vi, 1925, pp. 871-875). The one which precipitates nicotine and some other alkaloids is isohydro-silicododecatungstic acid, in which the ratio Si: W is 1: 12. There is some doubt about the number of molecules of water of crystallization in the molecule. Mellor gives the formula as (SiO_2,$4H_2O$), 12 WO_3,$2OH_2O$ or $H_8SiW_{12}O_{42}$,$2OH_2O$. The composition of the precipitate formed by the addition of this acid to a solution of an alkaloid is $4A,SiO_2,12WO_3,nH_2O$, where A is the formula of the alkaloid.

Copper in copper sulphate can be determined by electrolysis, as described on p. 294, or it may be determined volumetrically. In the usual procedure, potassium iodide is added to a solution of the copper salt in presence of acetic acid, and the liberated iodine is titrated with standard sodium thiosulphate solution. Under these conditions, the quantity of iodine liberated is less than the theoretical quantity; and in order to obtain accurate results it is necessary to standardize the sodium thiosulphate solution against pure copper or a pure copper salt under the same conditions as those in the determination. It has recently been shown by Foote and Vance that if the titration is carried out in the presence of sulphuric acid with the addition of ammonium thiocyanate towards the end of the titration, the reaction takes place in stoichiometric proportions. In this way copper can be accurately determined by using sodium thiosulphate solution, which has been standardized with resublimed iodine. Foote and Vance's procedure is given on p. 295. The agreed methods for the determination of copper in Bordeaux powder, Burgundy powder and Cheshunt compound will be found on p. 296. In these methods the copper is determined by titration with standard sodium thiosulphate solution, which has been standardized against pure copper sulphate under conditions similar to those in the determination.

The agreed methods for the analysis of soft soap are given

on p. 297. They include a test for solubility and the determinations of caustic alkali, alkali carbonate, total alkali, potash, total acids and resin acids. The agreed method of determining alkali carbonate is based on the assumption that potassium carbonate is insoluble in alcohol. A Sub-Committee of the Analytical Methods Committee of the Society of Public Analysts have recommended methods for the determination of free alkali in soft soaps, which are given on p. 299. In these methods the total free alkali is determined by adding an excess of standard acid and titrating back with standard alkali; the free caustic alkali is determined by titration after the addition of barium chloride; and the carbonate is found by difference or by a direct determination of carbon dioxide. The addition of barium chloride to a neutral alcoholic soap solution causes the reaction to become slightly acid. Hence the results obtained in this way tend to be too low, but the error may be reduced by increasing the weight of soap and by keeping the quantity of barium chloride as small as possible. The agreed methods do not include the determination of moisture. This is best carried out by Dean and Stark's method, as described on p. 300.

The agreed methods for the determination of the cyanide content of sodium, potassium and calcium cyanides are those adopted by the Association of Official Agricultural Chemists, which are given in full on p. 301. In these methods the solution of the cyanide is titrated with standard silver nitrate solution, the least excess of which above that required to form the double cyanide causes an opalescence owing to the formation of silver cyanide. The agreed method for the determination of formaldehyde is the well-known one depending on the oxidation of formaldehyde by hydrogen peroxide to formic acid, which is titrated with standard alkali. The procedure is given on p. 302.

The methods devised by Hart for the analysis of sodium fluoride, bifluoride and silicofluoride, either alone or in mixtures, are given on p. 302. Sodium bifluoride and sodium

silicofluoride, being acid in reaction, can be titrated with standard sodium hydroxide. In another experiment a solution of the sample is treated with potassium chloride and alcohol, cooled in ice and titrated with standard sodium hydroxide. The potassium silicofluoride thus formed is insoluble in alcohol at 0° C. The acidity therefore corresponds to the bifluoride, and the silicofluoride can be found by difference. The fluoride is determined by finding the total fluorine in the sample, and deducting from the value thus obtained the fluorine in the bifluoride and silicofluoride.

The active principles of pyrethrum (*Chrysanthemum cinerariaefolium*) are pyrethrin I and pyrethrin II. Pyrethrin I is the ester of a monocarboxylic acid which is volatile in steam and pyrethrin II is the ester of a dicarboxylic acid which is non-volatile. In both pyrethrins these acids, which are termed the chrysanthemum or pyrethrin acids, are combined with the ketonic alcohol, pyrethrolone.* The methods proposed for the determination of the total pyrethrin content depend either on the presence of the ketonic group in pyrethrolone—*e.g.*, the formation of semicarbazones, or on the reducing properties associated with the pyrethrolone portion of the molecule. In Gnadinger and Corl's method the copper-reducing power is measured by a colorimetric method based on that employed by Folin for the determination of dextrose in blood.† In the acid method the two pyrethrins are determined separately. Tattersfield, Hobson and Gimingham's method, Seil's method and Ripert's method are modifications and improvements of the original method of Staudinger and Harder.

The acid method consists of the quantitative separation

* For the structural formulae of pyrethrin I and II see F. Tattersfield, article Plant Sprays (Insecticides and Fungicides), *Thorpe's Dictionary of Applied Chemistry, Supplement*, vol. ii, and J. T. Martin, *J. Soc. Chem. Ind.*, 1937, **56**, pp. 85-91 T.

† For the details of Gnadinger and Corl's method see *J. Amer. Chem. Soc.*, 1929, **51**, pp. 3054-3064, or H. W. Wiley, *Agricultural Analysis*, ii, 1931, pp. 573-576.

of the volatile and non-volatile acids and the titration of each with standard sodium hydroxide. The percentages of pyrethrin I and pyrethrin II are calculated from the results of the titrations. In Tattersfield, Hobson and Gimingham's method, which is described on p. 304, the sample is extracted with petroleum ether and the extract is saponified with methyl alcoholic potassium hydroxide. The soaps are dissolved in water; the solution is acidified with dilute sulphuric acid and distilled in steam. The whole of the monocarboxylic acid is contained in the first 50 c.c. of the distillate, but a further 50 c.c. of distillate is collected because this leads to an aggregation of resinous particles in the distillation flask and facilitates the subsequent filtration. The monocarboxylic acid is extracted from the first portion of the distillate with petroleum ether and is titrated with N/50 sodium hydroxide solution. In order to clear the liquid in the distillation flask calcium sulphate is added and the mixture is allowed to stand over-night. The dicarboxylic acid is extracted from the filtered liquid with ether and is titrated with N/50 sodium hydroxide solution.

In Seil's method, which is described on p. 306, the petroleum ether extract is saponified with ethyl alcoholic sodium hydroxide. The alkaline solution is diluted with water, boiled to drive off the alcohol and, after the addition of barium chloride, is made up to a definite volume. The barium salts of the chrysanthemum acids are soluble in water, but the barium salts of fatty and resin acids are precipitated. After filtering off the precipitate, an aliquot part of the filtrate is acidified with sulphuric acid, in order to liberate the chrysanthemum acids, and is then distilled in steam. The monocarboxylic acid is determined in the distillate, and the dicarboxylic acid in the filtered liquid in the distillation flask, by extraction and titration.

Seil's method is more rapid and requires less attention than the older method of Tattersfield, Hobson and Gimingham. Both methods, however, tend to give high results owing to the presence of fatty acids, which are present in pyrethrum

extracts both in the free state and in combination.* This source of error was pointed out by J. Ripert (*Ann. Falsif.*, 1931, **24,** pp. 325-341), who has devised a modification of the acid method in which the interfering effects of the fatty acids are eliminated. In this method, the details of which are given on p. 308, the extract is saponified with alcoholic potassium hydroxide and the alcohol is distilled off. To the solution of the soaps are added sodium chloride and barium chloride, and the solution containing the salts of the chrysanthemum acids is filtered. These salts are decomposed with hydrochloric acid, and the acids are extracted with ether. The ether is evaporated and the acids are titrated with standard potassium hydroxide. In the same flask the acids are liberated by adding an excess of sulphuric acid, and the acid liquid is distilled in steam. In the distillate the monocarboxylic acid is determined in the usual way. The aqueous portion of the distillate after extraction with petroleum ether together with the washing solution is also titrated with N/50 sodium hydroxide solution. The dicarboxylic acid is determined indirectly by subtracting the volume of N/50 sodium hydroxide solution required to neutralize the monocarboxylic acid together with that required to neutralize the aqueous portion of the distillate from the total volume required to neutralize the acids before the distillation.

Haller and Acree have proposed a method of determining pyrethrin II which does not involve the separation of the volatile acid. Owing to the difficulty of determining pyrethrin II by the acid method, an entirely different method of determining that constituent is of great value. Haller and Acree's method is based on the fact that pyrethrin II is a methyl ester and yields on treatment with hydriodic acid the theoretical quantity of methyl iodide. The methyl iodide is absorbed in an acetic acid solution of potassium

* The free fatty acids can be removed by shaking the petroleum ether extract with dilute sodium hydroxide solution, as explained in Note 1 on p. 306.

acetate to which bromine has been added. The following reactions take place:

$$CH_3I+Br_2=CH_3Br+IBr$$
$$IBr+2Br_2+3H_2O=HIO_3+5HBr.$$

The solution containing the iodic acid is treated with formic acid to remove the excess of bromine. Potassium iodide is added, the solution is acidified with dilute sulphuric acid and the liberated iodine is titrated with standard sodium thiosulphate solution. The determination is carried out in a semi-micro apparatus designed by Clark for the determination of methoxyl content; his procedure is described on p. 311. Haller and Acree's procedure is given on p. 313.

The crystalline compounds derived from derris root are rotenone, deguelin, tephrosin and toxicarol.* Rotenone is by far the most toxic of these. With carbon tetrachloride it forms a crystalline complex, $C_{23}H_{22}O_6$, CCl_4, which contains 71·9 per cent. of rotenone. The method of determining rotenone due to H. A. Jones (*Ind. Eng. Chem.* (*Anal.*), 1933, **5,** pp. 23-26), which forms the basis of the other methods, consists of weighing the separated complex and assuming that it contains the theoretical quantity of rotenone. The sample is extracted with carbon tetrachloride; the extract is concentrated to a small volume and, after standing 18-24 hours, the complex is filtered off, washed with ice-cold carbon tetrachloride, dried and weighed. The results thus obtained are too high, because the complex separated in this way is by no means pure. C. D. V. Georgi and G. L. Teik (*Bull. Dept. Agric. Straits Settlements and Fed. Malay States*, 1933, No. 12) purified the rotenone by crystallization from alcohol, in which rotenone is only slightly soluble at 20° C. (0·2 gm. per 100 c.c.), but is much more soluble at higher temperatures. They extracted the sample with ether, evaporated the

* For the constitution of these compounds and a very valuable survey of the chemical evaluation of derris root see J. T. Martin, *J. Soc. Chem. Ind.*, 1937, **56,** pp. 85-91T.

extract almost to dryness and dissolved the residue in carbon tetrachloride. The solution was concentrated and left to crystallize. The rotenone-carbon tetrachloride complex was dissolved in boiling alcohol and the solution was allowed to stand over-night. The crystals of rotenone were separated by filtration, washed with cold alcohol, dried and weighed, a correction being applied for the rotenone dissolved by the alcohol. In Cahn and Boam's method, which is described on p. 314, the rotenone is extracted with trichloroethylene and is converted into the carbon tetrachloride complex. The rotenone is purified by triturating the complex with alcohol saturated with rotenone. After keeping over-night, the crystals are washed, dried and weighed.

The methods adopted by Tattersfield and Martin for the evaluation of rotenone-containing plants are given on p. 315. Georgi and Teik, also Cahn and Boam, called attention to the difficulty of obtaining a representative sample of derris root for the analysis owing to the tendency towards separation into finer and coarser particles. The same difficulty was encountered by Tattersfield and Martin, who recommend that the sample should be as finely ground as possible and thoroughly mixed before abstracting a portion for the analysis. The procedure employed by Tattersfield and Martin for the determination of rotenone is very similar to that of Georgi and Teik. The results obtained in this way agreed closely with those obtained by Cahn and Boam's method. The other determinations described by them are the ether extract and the methoxyl content, both of which are important in evaluating the sample.* Tattersfield and Martin (*Ann. Appl. Biol.*, 1935, **22,** pp. 578-605), however, explain that the determination of rotenone, the ether extract or the methoxyl content cannot separately be relied upon to give correct evaluations of all samples of derris root; but for samples of the same species of *Derris* examined by

* These two values appear to be closely correlated; the mean ratio of the methoxyl content to the ether extract was 0·141 in the samples examined by Tattersfield and Martin.

them any one of these determinations does give close comparative values of their insecticidal potency.

Cahn and Boam observed that some derris roots with a high toxicarol content, which they termed the Sumatra type, yielded either no rotenone or a very small proportion of the whole when treated in the ordinary way. In order to detect the "hidden" rotenone, they recommended that pure rotenone should be added when the rotenone content of the resin is below 17 per cent. Worsley, whose method of determining rotenone is described on p. 316, adds a weighed quantity of pure rotenone to the extracted resins so as to bring the content up to 40 per cent. in all cases. The sample is first mixed with charcoal and is then extracted by percolation with hot ethyl acetate. The addition of charcoal gives lighter coloured and purer extracts and thus increases the purity of the carbon tetrachloride complex, which in Worsley's experiments was from 91 and 96 per cent. with a mean value of about 94 per cent. After treatment with alcohol the purity of the complex was on the average 99·2 per cent. with a range of 89·9 to 99·6 per cent. except in a few cases.

Lead Arsenate.—The agreed methods for the analysis of lead arsenate (*Min. Agric., Bull.* **82,** pp. 5-6) are as follows:

Moisture.—Dry 5 gm. of lead arsenate powder or 10 gm. of lead arsenate paste at 105°-110° C. to constant weight, and express the loss in weight as the percentage of the original weight.

Acidity.—Mix 25 gm. of lead arsenate powder, or a quantity of paste containing 25 gm. of lead arsenate, with about 800 c.c. of recently boiled and cooled distilled water, and shake thoroughly for 15 minutes. Dilute the mixture to 1 litre with similar water, filter and titrate 100 c.c. of the filtrate with N/100 sodium hydroxide solution, using phenolphthalein as indicator. Neutrality should be obtained with not more than 7·5 c.c.

Lead Oxide.—Digest 1 gm. of lead arsenate powder or 2 gm. of lead arsenate paste with 25 c.c. of dilute nitric acid (1 : 3)

until solution is apparently complete. Filter off any insoluble matter, and wash the filter paper with water until free from acid. Add 5 c.c. of concentrated sulphuric acid to the filtrate, and evaporate on a sand bath until white fumes are evolved. Cool, dilute with 10 c.c. of water, and again evaporate until white fumes are evolved. Cool, dilute with 50 c.c. of a mixture of equal parts of 95 per cent. alcohol (or industrial methylated spirits) and water; allow to stand for 30 minutes, filter and wash with 95 per cent. alcohol (or industrial methylated spirits) until free from acid. Dry the precipitate of lead sulphate to constant weight. The weight of lead sulphate $\times 0{\cdot}7360$ = the weight of lead oxide (PbO).

Total Arsenic.—Heat to boiling point for 15 minutes 1 gm. of lead arsenate powder, or 2 gm. of lead arsenate paste, with 25 c.c. of dilute sulphuric acid (1 : 4). Dilute with 25 c.c. of water, filter and wash the filter paper with water until free from acid. Cool and dilute the filtrate with water to 250 c.c. Reduce the arsenic acid in 50 c.c. of the filtrate by passing sulphur dioxide through the solution until it is saturated. Complete the reduction by heating the liquid in a suitably stoppered glass vessel in a boiling water bath for 15 minutes. Transfer the liquid to a flask, and boil off the excess of sulphur dioxide. Cool, add solid sodium bicarbonate until the solution is strongly alkaline, and titrate with N/10 iodine solution, using starch as indicator. 1 c.c. of N/10 iodine solution = 0·00575 gm. of arsenic pentoxide.

Water-Soluble Arsenic.—Mix 10 gm. of lead arsenate powder, or 20 gm. of lead arsenate paste, with 1 litre of recently boiled and cooled distilled water in a stoppered vessel of about 1500 c.c. capacity. Place it in a warm place at a temperature of about 30° C., and shake at frequent intervals during 4 hours. Cool and filter through a dry filter paper. Reduce the arsenic in 50 c.c. of the filtrate and proceed as described in the method for total arsenic, but titrate with N/100 iodine solution, using starch as indicator.

Lime-Sulphur Solutions.—The following are the official methods of the Association of Official Agricultural Chemists (*Methods of Analysis*, 1935, pp. 62-64) for the determination of total sulphur, thiosulphate sulphur, sulphide sulphur, sulphate sulphur and total lime in lime-sulphur solutions. For some of these determinations the following reagent is required:

Ammoniacal Zinc Chloride Solution.—Dissolve 50 gm. of pure zinc chloride in about 500 c.c. of water. Add 125 c.c. of concentrated ammonia and 50 gm. of ammonium chloride, and dilute to 1 litre.

Preparation of the Sample.—Weigh about 10 gm. of the sample, transfer it to a 250 c.c. volumetric flask, and immediately dilute to the mark with recently boiled and cooled water. Mix the solution thoroughly, and transfer it to a number of small bottles, filling them completely and avoiding contact of the solution with the air as much as possible. Stopper the bottles, seal them with paraffin and keep them in a dark cool place. For each determination transfer the measured volume of the diluted lime-sulphur solution with a pipette, and keep the tip of it just under the surface of the liquid until it is raised for drainage at the end.

Total Sulphur.—Dissolve 2-3 gm. of sodium peroxide in 50 c.c. of water in a 250 c.c. beaker, and to this solution transfer 10 c.c. of the diluted lime-sulphur solution. Cover the beaker with a clock glass, and heat it on a steam bath with occasional stirring until all the sulphur is oxidized to sulphate, as shown by the disappearance of the yellow colour. Wash the clock glass and sides of the beaker, acidify with dilute hydrochloric acid (1:4) and evaporate to dryness. Treat the residue with water acidified with hydrochloric acid, boil and filter off the silica. Dilute the filtrate to 300 c.c. and add 50 c.c. of concentrated hydrochloric acid. Heat the solution to the boiling point, and add from a burette 10 per cent. barium chloride solution, with constant stirring, at such a rate that about 4 minutes is required for running in the necessary quantity (11 c.c. for 1 gm. of barium sulphate).

Evaporate to dryness on a steam bath, take up with water and filter through a quantitative filter. Wash the precipitate until it is free from chlorides, ignite carefully and heat it to constant weight.*

Thiosulphate Sulphur.—To 50 c.c. of water in a 200 c.c. volumetric flask add 50 c.c. of the diluted lime-sulphur solution. Add a slight excess of ammoniacal zinc chloride solution, and dilute to the mark. Shake thoroughly and filter through a dry filter paper. To 100 c.c. of the filtrate add a few drops of methyl orange or methyl red indicator, and exactly neutralize with N/10 hydrochloric acid. Titrate the neutral solution with N/20 iodine solution, using a few drops of starch indicator. From the volume of the iodine solution used calculate the percentage of thiosulphate sulphur present.†

Sulphide Sulphur.—To 10-15 c.c. of water in a small beaker add 10 c.c. of the diluted lime-sulphur solution. Calculate the quantity of ammoniacal zinc chloride solution necessary to precipitate all the sulphur, and add a slight excess. Stir, filter, wash the precipitate twice with cold water, and transfer the filter paper and precipitate to the beaker in which the precipitation was made. Add water, disintegrate the filter paper with a glass rod, and add about 3 gm. of sodium peroxide, keeping the beaker covered with a clock glass. Warm the beaker on a steam bath, with frequent shaking, until all the sulphur is oxidized to sulphate; add more sodium peroxide, if necessary. Make the solution slightly acid with dilute hydrochloric acid (1:4), filter to remove the shreds of filter paper, and wash thoroughly with hot water. Determine sulphur in the filtrate as directed under Total Sulphur.

Sulphate Sulphur.—To the solution remaining after the determination of thiosulphate sulphur add dilute hydrochloric acid (1:4) until the solution is slightly acid. Heat to the

* The weight of barium sulphate × 0·13735 = the weight of sulphur.

† 1 c.c. of N/20 iodine solution = 0·0032 gm. of sulphur as thiosulphate.

boiling point and add slowly, with constant stirring, a slight excess of 10 per cent. barium chloride solution. Boil for 30 minutes, allow to stand over-night, and filter. Calculate the sulphur from the weight of barium sulphate.*

Total Lime.—To 25 c.c of the diluted lime-sulphur solution add 10 c.c. of concentrated hydrochloric acid, and evaporate to dryness on a steam bath. Treat the residue with water and a few c.c. of dilute hydrochloric acid (1:4), warm until all the calcium chloride is dissolved, and filter to remove sulphur and any silica that may be present. Dilute the filtrate to 200-250 c.c., heat to the boiling point, add a few c.c. of concentrated ammonia in excess, and then an excess of saturated ammonium oxalate solution. Continue the boiling until the precipitated calcium oxalate assumes a granular form, and allow it to stand for an hour. Wash the precipitate a few times with hot water, ignite it in a platinum crucible to constant weight, and weigh it as calcium oxide.

Polysulphide Sulphur in Spray Materials.—The following method of determining polysulphide sulphur in lime-sulphur solutions and in liver of sulphur is described by W. Goodwin and H. Martin (*J. Agric. Sci.*, 1925, **15,** pp. 96-105). In this method, which is a modification of that devised by R. M. Chapin (*J. Ind. Eng. Chem.*, 1916, **8,** pp. 339-341), the sulphide is precipitated by means of ammoniacal zinc chloride solution, and the polysulphide sulphur is converted into thiosulphate by the action of sodium sulphite, the excess of which is precipitated with strontium chloride. After filtering and making the filtrate slightly acid with tartaric acid, the thiosulphate sulphur in the solution is determined by titration with N/20 iodine solution. The value thus obtained is corrected for the thiosulphate originally present in the sample. This is determined by a modification of the A.O.A.C. method (see p. 290), in which tartaric acid is used in place of hydrochloric acid. The following solutions are required:

* The weight of barium sulphate × 0·13735 = the weight of sulphur.

Ammoniacal Zinc Chloride Solution.—Prepared as described on p. 289.

Sodium Sulphite Solution, containing 10 per cent. of crystallized sodium sulphite ($Na_2SO_3,7H_2O$). This solution must be freshly prepared as it undergoes change on standing.

Strontium Chloride Solution, containing 10 per cent. of crystallized strontium chloride.

Tartaric Acid Solution.—10 per cent. solution.

The determination is carried out as follows: Add 25 c.c. of the diluted spray material (1:25 for liquids and 1:50 for solids) to a mixture of 10 c.c. of ammoniacal zinc chloride solution and 35 c.c. of sodium sulphite solution in a 350 c.c. conical flask. Place the flask on a steam bath at full heat for 45 minutes, and at 10, 20, 30, and 40 minutes shake the flask well, washing down the sides with boiling water from a wash bottle. At 45 minutes remove from the steam bath, and add 30 c.c. of strontium chloride solution. Allow to stand for 5 minutes and filter into a 250 c.c. volumetric flask, using a good grade filter paper of not more than 12·5 cm. diameter. Wash well with hot water till at least 200 c.c. have passed through the filter. Then cool, make up to the mark and shake. To 50 c.c. portions of the filtrate add 1 drop of methyl red solution (0·2 per cent. in 95 per cent. alcohol) and then tartaric acid solution from a burette until the solution is slightly acid. Titrate with N/20 iodine solution, using starch as indicator. The figure thus obtained must be corrected for the thiosulphate sulphur originally present, and this is determined as follows: Add 50 c.c. of the diluted spray material to 50 c.c. of water in a 200 c.c. volumetric flask. Then add 20 c.c. of ammoniacal zinc chloride solution and dilute to the mark. Shake thoroughly and filter through a dry filter paper. To 100 c.c. of the filtrate add a few drops of methyl red, and exactly neutralize with tartaric acid solution. Titrate with N/20 iodine solution.

If x=the number of c.c. used in the first titration and y=the number of c.c. used in the second titration, then,

since the weight of sample taken in the second titration is 5 times that taken in the first titration, the true polysulphide titration figure is $x - \frac{y}{5}$. If this figure is less than the number of c.c. of 10 per cent. sodium sulphite solution added (*i.e.*, 35), it shows that sufficient sodium sulphite has been added.*

Nicotine.—The agreed methods for the determination of nicotine in commercial nicotine and nicotine sulphate (*Min. Agric., Bull.* 82, p. 6) are as follows:

(*a*) *When the Sample gives a Clear Solution in Water.*—Weigh, by difference, a quantity of the sample containing 0·5-1 gm. of nicotine, and dilute with water to 500 c.c. To 50 c.c. of the solution add 10 c.c. of 2N hydrochloric acid and an excess of silicotungstic acid solution (12 gm. per 100 c.c.); between 10 and 15 c.c. may be required. Stir until the precipitate crystallizes, and allow to stand for 4 hours. Filter and wash the precipitate with dilute hydrochloric acid (about 1 in 1000) until the washings are free from silicotungstic acid, and then with a little water. Dry and ignite the precipitate. The weight of the residue $\times 0{\cdot}114$ = the weight of nicotine.

(*b*) *When the Sample does not give a Clear Solution in Water.*—Weigh, by difference, a quantity of the sample containing 1-2 gm. of nicotine, and transfer it to a steam distillation apparatus with about 100 c.c. of water. Add 10 c.c. of sodium hydroxide solution (10 gm. per 100 c.c.); distil with steam until the distillate is free from nicotine, and dilute the distillate to 1 litre. To 50 c.c. of the solution add 10 c.c. of 2N hydrochloric acid and precipitate the nicotine with silicotungstic acid as in (*a*).

The A.O.A.C. method (*Methods of Analysis*, 1935, pp. 60-61) is as follows: Weigh a quantity of the sample which will

* 1 c.c. of N/20 iodine solution = 0·0032 gm. of thiosulphate sulphur. But since half the sulphur in the thiosulphate in the first titration is derived from the polysulphides and half from the sodium sulphite added, 1 c.c. of N/20 iodine solution = 0·0016 gm. of polysulphide sulphur.

contain 0·1-1·0 gm. of nicotine. Wash it with water into a 500 c.c. round-bottomed distillation flask; add a little paraffin to prevent frothing, a few pieces of pumice and a slight excess of sodium hydroxide solution, using phenolphthalein as indicator. Distil rapidly in a current of steam through a condenser connected by means of an adapter with a flask containing 10 c.c. of dilute hydrochloric acid (1:4). When distillation has started, heat the distillation flask in order to reduce the volume as far as possible without bumping. Distil until a few c.c. of the distillate show no precipitate or opalescence when treated with a drop of silicotungstic acid and a drop of dilute hydrochloric acid (1:4). Confirm the alkalinity of the residue in the distillation flask with phenolphthalein. Make the distillate, which may amount to 1000-1500 c.c., up to a convenient volume, mix well and pass it through a large dry filter, if it is not clear. Pipette an aliquot part containing 0·1 gm. of nicotine, or as little as 0·01 gm. if the sample contains a very small quantity of nicotine, into a beaker. Add to each 100 c.c. of liquid 3 c.c. of dilute hydrochloric acid (1:4) and 1 c.c. of silicotungstic acid solution (12 gm. per 100 c.c.) for each 0·01 gm. of nicotine supposed to be present. Stir well and allow to stand over-night. Before filtering, stir the precipitate to see whether it settles quickly and is in crystalline form. Filter on an ashless filter paper, and wash with cold dilute hydrochloric acid (1:1000). Transfer the filter paper and precipitate to a weighed platinum crucible, dry carefully and ignite until all the carbon is oxidized. Then heat over a Teclu or Meker burner for not more than 10 minutes. The weight of the residue $\times 0{\cdot}1140$ = the weight of nicotine in the aliquot part.

Copper (Electrolytic Method).—The agreed method for the determination of copper in copper sulphate (*Min. Agric., Bull.* 82, pp. 6-7) is as follows: Weigh about 2 gm. of the sample, coarsely crushed if necessary, by difference from a stoppered weighing bottle, and dissolve it in 200 c.c. of water. Add 3 c.c. of concentrated nitric acid, and electrolyse the

solution at room temperature, using a weighed rotating platinum cathode (a gauze cylinder about 2½ cm. long and 2½ cm. in diameter is suitable) and a current density of about 0·06 amperes per sq. cm. Continue the electrolysis for half an hour after the solution appears colourless. Wash the cathode with water without stopping the current; disconnect, wash with alcohol or acetone, dry at 100° C. and weigh. The weight of copper deposited × 3·9282* = the weight of crystallized copper sulphate ($CuSO_4,5H_2O$). The complete deposition of copper should be confirmed by passing hydrogen sulphide through the solution; no appreciable darkening should occur.

Copper (Volumetric Methods).—The following method of finding the purity of copper sulphate is given in "*Analar*" *Standards for Laboratory Chemicals*, 1937, p. 85: Dissolve 1 gm. in 25 c.c. of water, add 5 c.c. of 5N acetic acid and 3 gm. of potassium iodide. Titrate the liberated iodine with N/10 sodium thiosulphate solution, using starch as indicator. 1 c.c. of N/10 sodium thiosulphate solution = 0·02497 gm. of crystallized copper sulphate. The reaction on which this method depends, $2CuI_2 = 2CuI + I_2$, is reversible and is not complete when the iodine is titrated. Also some of the iodine is adsorbed by the cuprous iodide which is coloured at the end-point. Hence, in accurate work the sodium thiosulphate solution is standardized against pure copper under definite conditions, as in the determination of copper in cuprous oxide on p. 147.

H. W. Foote and J. E. Vance (*J. Amer. Chem. Soc.*, 1935, **57**, pp. 845-847) have proposed a modification of the method which consists in adding ammonium thiocyanate near the end of the titration. This causes a further liberation of iodine; the precipitate becomes white because the cuprous iodide is converted, at least on the surface, into the more insoluble thiocyanate and the adsorbed iodine is liberated. Under these conditions, the end-point is very sharp and the

* This is the factor calculated from 1925 International atomic weights. The value calculated from 1937 atomic weights is 3·92808.

reaction takes place in stoichiometric proportions. Their procedure is as follows: A weighed quantity of copper is dissolved in nitric acid. 5 c.c. of 6N sulphuric acid are added and the nitric acid is removed by evaporation. The residue is dissolved in 20 c.c. of water, 2-3 gm. of potassium iodide are added, and the titration is carried out in the usual way until most of the iodine has reacted with the thiosulphate. After adding starch solution, the titration is continued nearly to the usual end-point. At this stage 2 gm. of ammonium thiocyanate are added and dissolved by stirring. The colour of the starch iodide immediately deepens. The titration is then completed. The sodium thiosulphate solution is standardized by means of resublimed iodine, and the equivalent weight of copper is calculated.

Bordeaux Powder, Burgundy Powder and Cheshunt Compound.—The agreed methods for the determination of copper in Bordeaux powder, Burgundy powder and Cheshunt compound,* and for the determination of the alkalinity of Burgundy powder (*Min. Agric., Bull.* 82, pp. 7-8) are as follows:

Copper.—Weigh 5 gm. of the well-mixed powder, add about 100 c.c. of water, and stir to break down aggregates. Add 5 c.c. of glacial acetic acid, or until effervescence ceases. Heat to boiling point, add 5 c.c. of 10 per cent. disodium phosphate solution to precipitate any ferric iron present; cool and dilute to 250 c.c. To 50 c.c. of the solution add 4 gm. of potassium iodide and titrate with N/10 sodium thiosulphate solution, adding a little starch solution when nearing the end of the titration. The thiosulphate solution should be standardized by titrating a solution containing 0·5 gm. of pure recrystallized copper sulphate, about 0·6 gm. of pure sodium carbonate crystals and 1 c.c. of

* Bordeaux powder is composed of copper sulphate and quicklime or slaked lime. Burgundy powder is a mixture of copper sulphate and sodium carbonate. Cheshunt compound consists of a mixture of 2 parts by weight of copper sulphate and 11 parts by weight of ammonium carbonate.

glacial acetic acid in 50 c.c. of water, to which 4 gm. of potassium iodide have been added. If X=the number of c.c. used in the titration of 0·5 gm. of pure copper sulphate, and Y=the number of c.c. used in the titration of 1 gm. of the sample, then the percentage of copper in the sample=

$$\frac{Y \times 0{\cdot}1273 \times 100}{X}.$$

Alkalinity of Burgundy Powder.—Mix 10 gm. of the powder with about 10 c.c. of water to break down aggregates. Add about 100 c.c. of water, and stir until any apparent action ceases. Dilute with water to 250 c.c.; a drop or two of ether may be used to remove any froth at the surface when adjusting the volume. Shake well and filter. Boil 50 c.c. of the filtrate for 2-3 minutes, filter rapidly and wash any precipitate with a small quantity of hot, recently boiled, water. Cool the filtrate and titrate it with N/10 hydrochloric acid or sulphuric acid, using methyl orange as indicator. 1 c.c. of N/10 acid=0·0053 gm. of sodium carbonate.

Soft Soap.—The agreed methods for the analysis of soft soap (*Min. Agric., Bull.* **82,** pp. 8-9) are as follows:

Solubility.—Dissolve 2 gm. of the sample in 200 c.c. of hot distilled water and cool the solution.

Caustic Alkali and Alkali Carbonate.—Dissolve 20 gm. of the sample in about 75 c.c. of 95 per cent. alcohol, previously neutralized to phenolphthalein, by boiling under a reflux condenser. Filter the solution whilst hot, and wash the filter thoroughly with hot neutralized 95 per cent. alcohol. Cool the filtrate, and titrate it with N sulphuric acid, using phenolphthalein as indicator. 1 c.c. of N sulphuric acid= 0·056 gm. of potassium hydroxide. Wash the residue from the filter paper with hot water; cool and titrate with N sulphuric acid, using methyl orange as indicator. 1 c.c. of N sulphuric acid=0·069 gm. of potassium carbonate.

Total Alkali.—Dissolve 10 gm. of the sample in 100 c.c. of water. Titrate the solution with N hydrochloric acid,

using methyl orange as indicator. 1 c.c. of N hydrochloric acid=0·0471 gm. of potassium oxide.

Potash.—Add an excess of hydrochloric acid to the solution used for the determination of total alkali, and boil gently until the fatty acids separate as a clear layer. Whilst boiling, add a little barium chloride solution, sufficient to precipitate any sulphate. Filter whilst hot through a wet filter paper into a 250 c.c. graduated flask, and thoroughly wash the fatty acids on the filter paper with boiling water. Cool the filtrate and dilute to 250 c.c. Transfer an aliquot part of the solution, containing about 0·15 gm. of potassium oxide, to a small glass or platinum dish, and evaporate it to dryness on a water bath. Place the dish in an air oven at 130°-150° C. for 1-2 hours, and then heat on a hot plate below red heat until the glycerin has volatilized. Dissolve the residue in a little water, and filter, if necessary, into a small glass dish or beaker. Add about 7 c.c. of 20 per cent. solution of perchloric acid (sp. gr. 1·125), and evaporate on a hot plate until white fumes are copiously evolved. Dissolve the precipitate in a little hot water, add a few more drops of perchloric acid and again evaporate to the fuming stage. Cool and stir the residue with 20 c.c. of 95 per cent. alcohol. Allow the precipitate to settle, and pour the clear liquid through a tared Gooch crucible, draining the precipitate as completely as possible from the liquid before adding the washing solution. Wash the precipitate by decantation with 95 per cent. alcohol which has been saturated with potassium perchlorate at the temperature at which it is used, and pour the washings through the Gooch crucible in which the whole of the precipitate is finally collected. Dry the crucible at 100° C. and weigh the potassium perchlorate.* The weight of potassium perchlorate $\times 0{\cdot}34$ = the weight of potassium oxide.

Total Acids.—Dissolve 20 gm. of the sample in about 200 c.c. of water, and transfer the solution to a separating

* The perchlorate method of determining potassium is more fully described on p. 80.

funnel. Make the solution alkaline with potassium hydroxide solution, using phenolphthalein as indicator, and extract 3 times with 50 c.c. portions of ether. Wash the mixed ether extracts first with 10 per cent. potassium hydroxide solution and then with water. Unite the washings with the main alkaline solution, and acidify it with dilute sulphuric acid. Extract the separated acids 3 times with 50 c.c. portions of ether, and wash the mixed ether extracts with water. Filter the ether extracts into a tared flask, and distil off the ether. Heat the residue on a steam bath until the smell of ether has disappeared, dry at 100° C. and weigh the fatty and resin acids.

Resin Acids.—Dissolve 3 gm. of the mixed fatty and resin acids obtained above in 30 c.c. of 99 per cent. alcohol. Pass a current of dry hydrochloric acid through the solution, which is kept cool by immersion in a cold water bath. Continue the passage of the gas until unabsorbed gas freely escapes; this usually takes 45-60 minutes. Allow the solution to stand for 1 hour to ensure complete esterification of the fatty acids; the esters and the resin acids separate as an oily layer. Wash the mixture into a separating funnel with about 150 c.c. of water and 75 c.c. of ether, and shake vigorously. Run off the lower layer and wash the ether layer with distilled water until hydrochloric acid is removed. To the ether add about 50 c.c. of neutral alcohol, and titrate the resin acids with N potassium hydroxide solution, using phenolphthalein as indicator. 1 c.c. of N potassium hydroxide solution = 0·346 gm. of resin acids.

Free Alkali in Potassium Soaps.—The methods recommended by a Sub-Committee of the Analytical Methods Committee of the Society of Public Analysts (*Analyst*, 1937, **62**, pp. 36-41) for the determination of free alkali in potassium soaps* are as follows:

Total Free Alkali.—Boil 100 c.c. of redistilled industrial methylated spirit (66 o.p.—*i.e.*, 94·7 per cent. by volume)

* A different method of determining free caustic alkali in sodium soaps is recommended.

in a 400 c.c. flask. Add 0·5 c.c. of 0·5 per cent. alcoholic phenolphthalein solution, allow to cool to 70° C. and neutralize at that temperature with N/10 alcoholic potassium hydroxide solution. Add 10 gm. of the soap and dissolve it as quickly as possible by heating. Immediately the soap is dissolved, add 3 c.c. of N sulphuric acid and boil on a water bath for at least 10 minutes to ensure complete removal of carbon dioxide. Cool to 70° C. and titrate with N sodium hydroxide solution until the pink colour reappears. If after boiling with acid the pink colour returns, a further quantity of N sulphuric acid must be added and the boiling repeated. The excess of sulphuric acid titrated should not be less than 1 c.c. From the amount of standard acid neutralized calculate the total free alkali as a percentage of potassium oxide.*

Free Caustic Alkali.—Dissolve 10 gm. of soap in 100 c.c. of neutral industrial alcohol containing 0·5 c.c. of 0·5 per cent. phenolphthalein solution. Add 5 c.c. of hot neutral 10 per cent. barium chloride solution in a thin stream. Mix thoroughly and titrate with N/10 hydrochloric acid at 70° C. until the pink colour disappears. State the result as the percentage of potassium oxide.

Carbonate Alkali.—This is found by difference or by a determination of the carbon dioxide by any of the recognized methods.

Moisture in Soap.—The following method of determining moisture in soap is adopted by the Association of Official Agricultural Chemists (*Methods of Analysis*, 1935, p. 58): Weigh about 20 gm. of the sample into a 300-500 c.c. flask. Add 50 c.c. of xylene, and to prevent foaming add about 10 gm. of lump rosin (powdered rosin contains an appreciable quantity of moisture). Connect the flask to a graduated receiver and a condenser as in Fig. 2 on p. 118. Heat the flask and continue the distillation until no more water collects in the graduated receiver. Allow the contents of the graduated receiver to cool to room temperature; read the

* 1 c.c. of N sulphuric acid = 0·0471 gm. of potassium oxide.

volume of water and from this volume calculate the percentage of moisture in the sample.

Cyanides.—The Association of Official Agricultural Chemists (*Methods of Analysis*, 1935, pp. 56-57) have adopted the following methods for the determination of cyanogen in sodium, potassium and calcium cyanides. N/10 silver nitrate solution is required for the three determinations. For the determination of cyanogen in calcium cyanide there is also required the following reagent:

Soda-lead Reagent.—Dissolve 20 gm. of lead acetate in water, dilute to 1 litre, and add 200 gm. of sodium carbonate, free from chloride.

Sodium and Potassium Cyanides.—Break the sample into small lumps in a mortar, but do not grind. Weigh quickly about 5 gm. in a weighing bottle, and wash it into a 500 c.c. volumetric flask containing about 200 c.c. of water. Add a little lead carbonate to precipitate any sulphides that may be present, dilute to the mark, mix thoroughly and filter through a dry filter paper. Transfer 50 c.c. of the filtrate to a 400 c.c. beaker. Add 200 c.c. of water, 5 c.c. of sodium hydroxide solution (100 gm. per litre) and a few crystals, or 10 drops of saturated solution, of potassium iodide. Place the beaker on a black surface, and titrate the solution with N/10 silver nitrate solution until there is a faint opalescence.*

Calcium Cyanide.—Place about 200 c.c. of water in a 500 c.c. volumetric flask, and carefully dry the neck of the flask. Weigh about 5 gm. of the sample in a weighing bottle and transfer it to the flask with the least possible exposure to the air. Wash the mixture down into the flask, and mix by whirling until solution is complete and the small quantity of calcium carbide has been decomposed. Then add 25 c.c. of the soda-lead reagent, or a quantity sufficient to remove sulphides. Close the flask with a rubber stopper and shake thoroughly, preferably for half an hour. Dilute to the

* 1 c.c. of N/10 silver nitrate solution = 0·005204 gm. of cyanogen. 1 c.c. of the same solution also = 0·009803 gm. of sodium cyanide and 0·013023 gm. of potassium cyanide.

mark, mix and filter through a dry filter paper. Transfer 50 c.c. of the filtrate to a 400 c.c. beaker, add 200 c.c. of water and proceed as directed in the last paragraph.*

Formaldehyde.—The agreed method for the determination of formaldehyde (*Min. Agric., Bull.* **82,** p. 10) is as follows: Add 3 c.c. of the sample to 50 c.c. of hydrogen peroxide (10 volumes)† and 50 c.c. of N sodium hydroxide solution, and warm until effervescence ceases. Titrate the excess of alkali with N sulphuric acid, using phenolphthalein as indicator. Repeat the operation without the solution of formaldehyde. The difference between the number of c.c. used in the two titrations is the volume of N sodium hydroxide solution required to neutralize the formic acid produced by the oxidation of the formaldehyde. 1 c.c. of N sodium hydroxide solution = 0·030 gm. of formaldehyde. Since 3 c.c. of the sample are taken for the analysis, each c.c. difference between the titrations corresponds to 1 gm. of formaldehyde per 100 c.c. of solution.

Sodium Fluoride, Bifluoride and Silicofluoride.—The following methods for the analysis of sodium fluoride, sodium bifluoride and sodium silicofluoride, alone or in mixtures, are described by L. Hart (*Ind. Eng. Chem.* (*Anal.*), 1929, **1,** pp. 133-135). Sodium bifluoride and sodium silicofluoride, when titrated with standard sodium hydroxide solution, are converted into sodium fluoride, according to the equations:

$$NaHF_2 + NaOH = 2NaF + H_2O$$
$$Na_2SiF_6 + 4NaOH = 6NaF + Si(OH)_4.$$

* 1 c.c. of N/10 silver nitrate solution = 0·009212 gm. of calcium cyanide.

† Solutions of hydrogen peroxide decompose on keeping, and it is therefore advisable to find the concentration of the hydrogen peroxide before beginning the determination. The following method is given in "*Analar*" *Standards for Laboratory Chemicals*, 1937, p. 120: Dilute 2 c.c. with 20 c.c. of water, add dilute sulphuric acid and titrate with N/10 potassium permanganate solution, 1 c.c. of which = 0·0017 gm. of hydrogen peroxide. Not less than 3 per cent. should be indicated.

Total Acidity.—Dissolve 0·5-1 gm. of the sample in 25 c.c. of cold water in a 100 c.c. platinum dish. Titrate with N/5 or N/10 sodium hydroxide solution (free from carbonate and silica), using phenolphthalein as indicator. When the pink colour fades slowly, heat to the boiling point, and continue the titration to a permanent pink colour. The titration is recorded as total acidity due to bifluoride and silicofluoride. After the titration is complete, transfer the solution to a 200 c.c. volumetric flask, dilute to the mark and keep for the determination of total fluorine.

Bifluoride.—Weigh 0·5 gm. of the sample into a 100 c.c. platinum dish, add 1 gm. of solid potassium chloride and dissolve in 25 c.c. of water. Add an equal volume of neutral alcohol and cool in an ice bath to 0° C. Titrate with N/5 or N/10 sodium hydroxide solution, using phenolphthalein as indicator, until the red colour remains for 1 minute. Keep the platinum dish in the ice bath during the titration and, to ensure accuracy, titrate slowly so that the temperature does not rise appreciably. If more than 15 c.c. of standard sodium hydroxide solution are required, repeat the titration, using either a smaller quantity of the sample or a more concentrated standard sodium hydroxide solution. This titration is calculated to sodium bifluoride.*

Silicofluoride.—Deduct the equivalent quantity of sodium hydroxide solution due to bifluoride, as determined above, from the titration recorded as total acidity. The result is the quantity of standard sodium hydroxide solution equivalent to the silicofluoride present.†

Total Fluorine.—Pipette into a 250 c.c. beaker an aliquot part containing the equivalent of about 0·25 gm. of sodium fluoride from the 200 c.c. volumetric flask containing the solution kept for the determination of total fluorine. Dilute to 100 c.c. with water, add about 0·1 gm. of sodium car-

* 1 c.c. of N/10 sodium hydroxide solution = 0·0062 gm. of sodium bifluoride.

† 1 c.c. of N/10 sodium hydroxide solution = 0·0047 gm. of sodium silicofluoride.

bonate, heat to the boiling point and add slowly an excess of 10 per cent. calcium chloride solution. Allow the precipitate to settle, filter and wash once with a few c.c. of hot water. Dry the precipitate and filter paper in a silica dish, and ignite at a dull red heat. After cooling, add 20 c.c. of 20 per cent. acetic acid and evaporate to dryness on a steam bath. Repeat the process, breaking up any lumps with a glass pestle. Take up the residue with a little hot water to which 2-3 c.c. of 20 per cent. acetic acid have been added. Filter and wash with small portions of hot water. The residue is dried and ignited as before and weighed. The result obtained can be confirmed by evaporating to dryness with concentrated sulphuric acid, which converts the calcium fluoride into calcium sulphate. Heat the residue with a little ammonium carbonate, ignite and weigh as calcium sulphate. The weight of calcium fluoride $\times 1{\cdot}0759$ = the weight of sodium fluoride. The weight of calcium sulphate $\times 0{\cdot}5735$ = the weight of calcium fluoride. Deduct the fluorine equivalent to the bifluoride and silicofluoride present, and calculate the remaining fluorine to sodium fluoride.

Pyrethrin I and II (Tattersfield, Hobson and Gimingham's Method).—The method described by F. Tattersfield, R. P. Hobson and C. T. Gimingham (*J. Agric. Sci.*, 1929, **19,** pp. 266-296) for the determination of pyrethrin I and II in pyrethrum has been modified by J. T. Martin and F. Tattersfield (*ibid.*, 1931, **21,** pp. 118-119, and 1934, **24,** p. 603). Below is a description of the modified procedure, in which are incorporated further details kindly communicated to the author by Drs. Tattersfield and Martin.

The weight of the sample taken for the analysis is dependent on the total pyrethrin content, being 10 gm. for samples containing less than 0·7 per cent., 5 gm. for samples containing from 0·7 to 1·5 per cent. and 2·5 gm. for samples containing more than 1·5 per cent. of total pyrethrins. The weighed portion of the sample is extracted with petroleum ether (b.p. below 40° C.) in a Soxhlet extractor heated over a carbon filament lamp. When extraction is complete

(Note 1), the petroleum ether is evaporated to a small bulk in a rapid stream of carbon dioxide with very gentle warming, and the evaporation is completed in a vacuum desiccator. The residue is extracted with 4 lots of 2·5 each of gently warmed purified methyl alcohol, each of which is cooled and filtered into a 100 c.c. Kjeldahl flask through a pad of cotton wool. A final washing with 2·5 c.c. of cold methyl alcohol is made, a few drops of phenolphthalein in methyl alcohol are added and then, drop by drop, till alkaline N potassium hydroxide solution in methyl alcohol. A further 5 c.c. are added, and the mixture is refluxed for a few hours. The methyl alcohol is taken off in partial vacuum with gentle warming, the temperature not being allowed to rise above 25° C. The residue is dissolved in about 20 c.c. of water; the solution is acidified with 6-7 c.c. of N sulphuric acid and the volatile acid is distilled off in steam. The volume in the distilling flask should not be allowed to exceed 30 c.c. (Note 2). Two lots of 50 c.c. are distilled off and the acids in the first distillate are extracted with 2 lots of 50 c.c. of petroleum ether. For complete extraction vigorous shaking is necessary (Note 3). Each extract is washed with 20 c.c. of water. The 2 extracts are combined, evaporated on a water bath after the addition of 20 c.c. of water, and the residue is titrated whilst it is warm with N/50 sodium hydroxide solution, the sides of the flask being washed down towards the end of the titration with a little neutral ethyl alcohol (Note 4). 1 c.c. of N/50 sodium hydroxide solution = 0·0066 gm. of pyrethrin I. The second distillate of 50 c.c. on extraction with petroleum ether should not show more than a trace of titratable acid.

The hot aqueous residue in the distillation flask is treated with 0·2 gm. of calcium sulphate and, after standing overnight, is filtered through a plug of cotton wool, the flask and plug being washed with water. The non-volatile acid is extracted with 3 successive portions of 50 c.c. each of sodium-treated ether in a separating funnel, each ether extract being washed with a little water. The ether extracts

are combined, 20 c.c. of water are added and the ether is evaporated. The aqueous layer is cooled and filtered through a plug of cotton wool. The filtrate, after heating to the boiling point, is titrated with N/50 sodium hydroxide solution, 1 c.c. of which=0·00374 gm. of pyrethrin II.

Note 1.—Free acids may occur in immature and badly stored commercial samples. These can be removed as follows: After extracting the sample with petroleum ether, the petroleum ether is transferred to a separating funnel. To it are added 20 c.c. of water, a little phenolphthalein and sufficient N/10 sodium hydroxide solution (from a burette) to make the aqueous layer alkaline after shaking. The aqueous layer is run off and the petroleum ether is washed with water. The petroleum ether layer is then treated as described above.

Note 2.—The distillation should be vigorous, and the liquid in the distillation flask should be kept as small as possible; at the end it should not exceed 20-30 c.c. After distillation the condenser should be washed with a little neutral alcohol which is added to the second 50 c.c. of the distillate.

Note 3.—If an emulsion forms, a little common salt can be added to break the emulsion.

Note 4.—It is unnecessary to distil off the petroleum ether used for extracting the volatile acid from the distillate before titrating. The petroleum ether is run off into a flask and, after adding 20 c.c. of neutral water and a little phenolphthalein, the titration is carried out, with vigorous shaking, until the aqueous layer is pink.

Pyrethrin I and II (Seil's Method).—The following method of determining pyrethrin I and II in pyrethrum flowers is described by H. A. Seil (*Soap*, 1934, No. 5, pp. 89, 91 and 111): Extract 12·5 gm. of powdered flowers in a Soxhlet extractor with low boiling point petroleum ether. After extraction is complete, recover the petroleum ether on a water bath. Add 10-15 c.c. of N/2 ethyl alcoholic sodium hydroxide and reflux the mixture for 1-2 hours. Transfer the alkaline solution to a 600 c.c. beaker; wash the flask with water, and add suffi-

cient water to bring the volume of the liquid in the beaker to 200 c.c. Add a few glass beads and remove the alcohol by boiling, taking care to avoid loss by frothing. When the volume is reduced to 150 c.c., cool the solution and transfer it to a 250 c.c. graduated flask, to which about 1 gm. of Filter-cel* has been previously added. Shake the solution to distribute the Filter-cel; then add 10 c.c. of 10 per cent. barium chloride solution and make the solution up to the mark with water. After the precipitate has settled, filter the solution through a fluted filter paper. Transfer 200 c.c. of the clear filtrate to a 500 c.c. Erlenmeyer flask and add 1 c.c. of concentrated sulphuric acid to precipitate the excess of barium and liberate the chrysanthemum acids. Distil with steam, using a distillation trap and efficient condenser. Receive the distillate in a 500 c.c. Squibb separating funnel, and distil until the liquid in the flask is 15-20 c.c. The volume in the separating funnel is usually 250 c.c. Allow the flask containing the dicarboxylic acid to cool.

To the separating funnel add 50 c.c. of neutral petroleum ether and shake thoroughly for 1 minute. After the liquids have separated, draw off the aqueous layer into a second 500 c.c. separating funnel, to which a second 50 c.c. of neutral petroleum ether has been added. Shake for 1 minute and, after the liquids have separated, discard the aqueous layer. Wash the petroleum ether in the first separating funnel with 10 c.c. of water and use the same wash water for the second funnel. Repeat with a second wash water of 10 c.c. as before. Combine the petroleum ether extracts. Neutralize 15 c.c. of water containing a drop of phenolphthalein solution with N/50 sodium hydroxide solution, and add it to the combined petroleum ether extracts. Titrate with N/50 sodium hydroxide solution, shaking after each addition, until the aqueous layer is just pink. 1 c.c. of N/50 sodium hydroxide solution=0·0066 gm. of pyre-

* Filter-cel is a proprietary preparation of infusorial earth used in filtering (W. Gardner, *Chemical Synonyms and Trade Names.* London, 1936, p. 140).

thrin I. The 200 c.c. aliquot portion taken corresponds to 10 gm. of the sample. Therefore, the number of c.c. used in the titration $\times 0{\cdot}066$ = the percentage of pyrethrin I.

Filter the solution containing the dicarboxylic acid through a Gooch crucible. Make the clear filtrate alkaline with sodium bicarbonate, and transfer it to a separating funnel. Wash it twice with chloroform.* Wash the first chloroform extract with water, and use the same wash water for the second chloroform extract. Combine the aqueous solutions. Acidify strongly with hydrochloric acid, saturate with salt and extract with 50 c.c. of ether, shaking for about 1 minute. Repeat the extraction with three more portions of ether, using 50 c.c. for the second and 25 c.c. for each of the third and fourth extractions. Wash the ether of the first extraction with 10 c.c. of water. Combine the ether solutions. Repeat with a second wash of 10 c.c. of water. Run off any separated water, and filter into a flask. Recover the ether on a water bath, and dry the residue at 100° C. for 10 minutes. Add 2 c.c. of neutral alcohol, warm gently and then add 20 c.c. of distilled water. Heat to dissolve the acid; if any remains undissolved, cool and filter through a Gooch crucible. Add a drop of phenolphthalein, and titrate with N/50 sodium hydroxide solution. 1 c.c. = 0·00374 gm. of pyrethrin II.

Pyrethrin I and II (Ripert's Method).—The method described by J. Ripert (*Ann. Falsif.*, 1935, **28**, pp. 27-38) for the determination of pyrethrin I and II is as follows: 40 gm. of pyrethrum flowers are extracted with petroleum ether. The extract, which is generally acid, is shaken with N potassium hydroxide solution till it is neutral. If an emulsion forms, it can be broken by adding a few c.c. of barium chloride solution and sodium chloride solution. The aqueous layer is discarded and the petroleum ether is filtered. The petroleum ether is distilled off and the residue is saponified

* Drs. Tattersfield and Martin (*Priv. Comm.*) use relatively small amounts of chloroform (about 15 c.c.) and wash these with a nearly equal quantity of water.

by heating it with 20 c.c. of N alcoholic potassium hydroxide solution for 1½ hours on a boiling water bath, after which the alcohol is evaporated. The residue is dissolved in 50 c.c. of hot water, and the solution is transferred to a 500 c.c. separating funnel. The flask is rinsed twice with 50 c.c. of ether to dissolve the residue, which is insoluble in water. If any remains, the flask is rinsed with 10 c.c. of alcohol, which is used later. The ether is poured into the separating funnel, which is shaken vigorously, and then 50 c.c. of 25 per cent. sodium chloride solution are added. Usually the two layers separate well; if not, the alcohol used to rinse the flask is added. The aqueous layer is run into another separating funnel. The ether layer is washed twice with 20 c.c. of water to which 25 c.c. of sodium chloride solution have been added after shaking. To the mixed aqueous layers are added 10 c.c. of saturated barium chloride solution, with shaking, and 100 c.c. of ether. When the precipitate has collected at the junction of the two layers, the aqueous layer is filtered through a quick filter, which may be provided with a little filtering powder, such as kieselguhr. The ether is washed with 25 c.c. of water, which is used to wash the filter.

The resulting solution, containing the sodium salts of the chrysanthemum acids, is acidified with 4N hydrochloric acid. The acids are extracted 3 times with 200 c.c. of ether.* The ether is washed 3 times with 10 c.c. portions of saturated sodium chloride solution in order to remove any hydrochloric acid. The ether is transferred to a 125 c.c. flask, which fits a steam distillation apparatus, and the ether is distilled off. To the residue are added 5 c.c. of neutral 95 per cent. alcohol and a little phenolphthalein, and the acids are titrated with N/5 alcoholic potassium hydroxide solution; the volume used should be at least 10 c.c. Suppose it is a c.c., which corresponds to $10a$ c.c. of N/50 potassium hydroxide solution. The neutral solution in the flask is acidified with N sulphuric acid, the

* It is not clear from Ripert's account whether he uses 200 c.c. for each of the three extractions or 200 c.c. in all.

volume added being not less than half the volume of N/5 potassium hydroxide solution required for the neutralization. The flask is then connected with the steam distillation apparatus and two distillates of 100 c.c. each are collected. Though not essential, superheated steam is recommended, because it shortens the time of the distillation.

The first distillate is poured into a 250 c.c. separating funnel. The flask which contained the distillate is rinsed with 100 c.c. of neutral petroleum ether, which is then poured into the separating funnel and vigorously shaken. The aqueous layer is run into the same flask, and the petroleum ether is washed with 25 c.c. of sodium chloride solution. The water and the 25 c.c. of sodium chloride solution run off are poured into another separating funnel and reserved. The washed petroleum ether is poured into a conical flask containing 25 c.c. of water and a little phenolphthalein to which sufficient N/50 sodium hydroxide solution has been added to turn it pink. The mixture is titrated with N/50 sodium hydroxide solution, with vigorous shaking after each addition, until the aqueous layer is permanently pink. Suppose the volume of N/50 sodium hydroxide solution used is *b* c.c. The petroleum ether is poured off into the separating funnel containing the water of the first distillate and the washing solution. After shaking, the water is run into a conical flask.

The second distillate is poured into the separating funnel, and the flask is rinsed with 25 c.c. of petroleum ether. After shaking, the water is run into the conical flask. The petroleum ether is washed with 25 c.c. of sodium chloride solution, which is run into the same flask. The petroleum ether is poured into the flask used for the last titration, the water in which should still be pink, and the mixture is titrated with N/50 sodium hydroxide solution till the aqueous layer is permanently pink. Suppose the volume used is *c* c.c. Finally the water from both distillates together with the washing solutions which are contained in the conical flask are titrated with N/50 sodium hydroxide solution. Suppose the volume

used is d c.c. If the weight of the sample taken for the analysis is w gm., the percentage of pyrethrin I $= \frac{0{\cdot}66\ (b+c)}{w}$; and the percentage of pyrethrin II $= \frac{0{\cdot}374\ [10a-(b+c+d)]}{w}$.

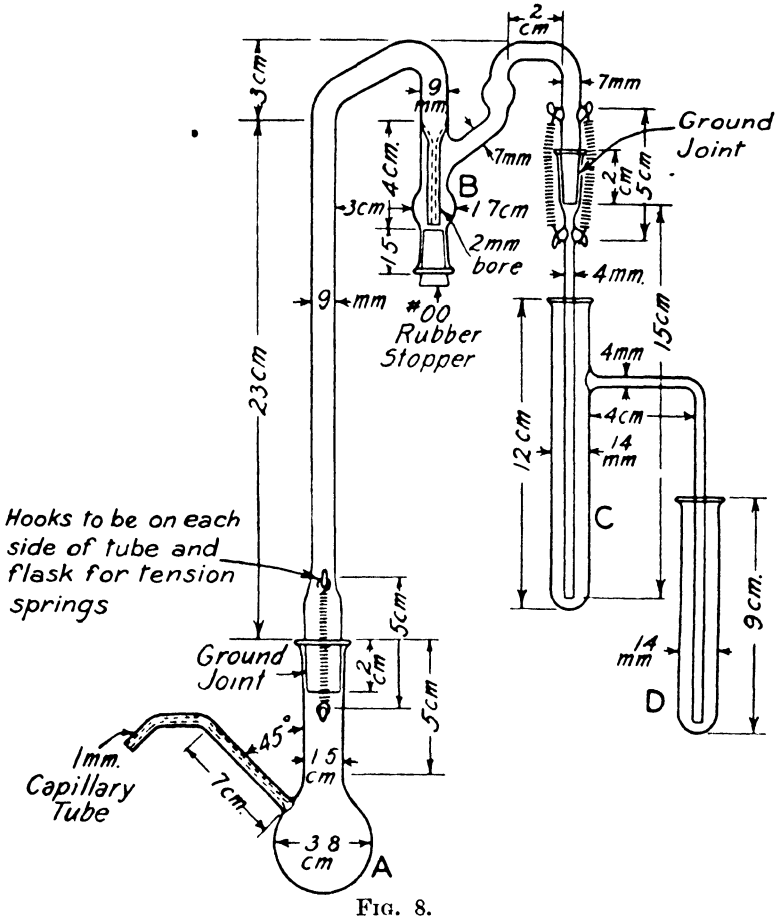


FIG. 8.

Methoxyl Content. — The special semi-micro apparatus shown in Fig. 8 is required for the determination of the methoxyl content by the method described by E. P. Clark (*J. Assoc. Off. Agric. Chem.*, 1932, **15**, pp. 136-140). There is also required a boiling rod. This is a glass tube about 60 mm. long, 3·5 mm. outside diameter with a 1 mm. bore.

It is sealed at one end and is closed about 10 mm. from the other. The open end is smoothed in a flame. When placed in the flask with the open end downwards, it causes uniform boiling as long as sufficient heat is applied to the liquid. The determination is carried out as follows: 20-30 mgm. of the substance is weighed on a counterpoised piece of cigarette paper 2 by 3 cm., using an analytical balance sensitive to 0·1 mgm. The paper containing the substance is placed in the bottom of flask A. A boiling rod, 2·5 c.c. of pure melted phenol and 5 c.c. of constant-boiling hydriodic acid are added. The flask is then connected with the rest of the apparatus, which consists of a scrubber B, containing a little water, and the receivers C and D. The receivers contain 10 c.c. of 10 per cent. glacial acetic acid solution of potassium acetate to which 6 drops of bromine have been added. About 6 c.c. of the solution are placed in C and 4 c.c. in D.

A slow uniform stream of carbon dioxide is passed through the capillary side arm of the boiling flask, and the liquid is gently heated by means of a mantled micro-burner at such a rate that the vapours of the boiling liquid rise half-way up the condenser. For most substances 30 minutes is sufficient to complete the reaction. The contents of both receivers are then washed into a 250 c.c. Erlenmeyer flask which contains 5 c.c. of 25 per cent. aqueous sodium acetate solution. The volume of the liquid is then adjusted to 125 c.c., and 6 drops of 90 per cent. formic acid are added. The flask is then rotated until the colour of the bromine is discharged. Then 12 more drops of formic acid are added and the solution is allowed to stand for 1-2 minutes. 1 gm. of potassium iodide and a few c.c. of 10 per cent. sulphuric acid are added, and the free iodine is titrated with N/10 sodium thiosulphate solution. A blank experiment should be made with all the reagents; this usually amounts to 0·06 c.c. of N/10 sodium thiosulphate solution. In this reaction, 6 atoms of iodine are liberated for each mole of methoxyl (OCH_3).*

* 1 c.c. of N/10 sodium thiosulphate solution = 0·000517 gm. of methoxyl.

If several determinations are to be made at one time, the same charge of hydriodic acid and phenol may be used for all of them, but a fresh boiling rod should be used for each determination. The blank due to the phenol should be subtracted only from the first, as in this determination the material responsible for the blank will have been destroyed.

Pyrethrin II.—The following method of determining pyrethrin II, which is described by H. L. Haller and F. Acree (*Ind. Eng. Chem.* (*Anal.*), 1935, **7**, pp. 343-344), consists in extracting the sample with petroleum ether and determining the methoxyl content of the extract by the method of Clark, which is described above. For the determination Haller and Acree used constant boiling point hydriodic acid (sp. gr. 1·70), which had been treated with hypophosphorous acid to remove free iodine. In order to reduce the blank, a stream of carbon dioxide was passed through the boiling solution under a reflux condenser for 2-3 hours.

Preparation of the Extract.—5 gm. of finely ground pyrethrum flowers are extracted for 7 hours with petroleum ether (b.p. 30°-60° C.) in either a Soxhlet or a Butt extractor.* For the Soxhlet extraction a 100 c.c. extractor fitted with an extra long condenser was used. The Butt extractor used was 18 cm. long and 2·5 cm. in diameter. It was fitted with an Allihn condenser and provided with a ground-glass joint in order that the same flask could be used for the extraction and the methoxyl determination, thus making the transfer from one flask to another unnecessary. The sample for the Soxhlet extractor was handled in the usual way; that for the Butt extractor was wrapped in filter paper.

Determination.—After the extraction most of the solvent is removed by heating in a water bath at 70° C. When the extract is prepared in a Soxhlet extractor, the residue is quantitatively transferred to the flask used for the methoxyl determination by means of 20 c.c. of chloroform used in

* See C. A. Butt, *J. Ind. Eng. Chem.*, 1915, **7**, pp. 130-131.

small portions. A small boiling rod is then placed in the flask, and the chloroform is removed by heating in a water bath, the last traces being removed under reduced pressure. 2·5 c.c. of melted pure phenol and 5 c.c. of constant-boiling hydriodic acid are added, and the flask is connected with the remainder of the apparatus. The determination is carried out by Clark's procedure with the following modifications:

(i) In the receivers C and D are placed 10 c.c. of 10 per cent. glacial acetic acid solution of potassium acetate to which 20 drops of bromine have been added. Unless a large excess of bromine is used, low results are obtained.

(ii) The contents of flask A are heated for 1½ hours to complete the reaction.

(iii) After adding 5 c.c. of sodium acetate solution and adjusting the volume of the solution to 125 c.c., 20 drops of 90 per cent. formic acid are added to remove the excess of bromine.

(iv) The liberated iodine is titrated with N/20 sodium thiosulphate solution, 1 c.c. of which = 0·00311 gm. of pyrethrin II.

Rotenone (Cahn and Boam's Method).—The following method of determining rotenone in derris root is described by R. S. Cahn and J. J. Boam (*J. Soc. Chem. Ind.*, 1935, **54**, pp. 37-42T): The root should pass entirely through a 50-mesh sieve before it is sampled. The root should be dried before extraction in a vacuum desiccator until it contains not more than 5 per cent. of moisture. Drying at 100° C. causes decomposition and low results. A quantity of root sufficient to give 5-10 gm. of extract is extracted in a Soxhlet extractor for 8 hours with trichloroethylene, which is vigorously boiled.* The solvent is then changed and the extraction is continued for a further 4 hours. If the second solution acquires more than a very pale yellow colour, extraction is repeated with a fresh quantity of solvent for a further 4 hours. The united extracts (filtered if necessary) are

* Trichloroethylene boils at 86·7° C. and is not inflammable.

evaporated on a gauze and finally in a 50 c.c. conical flask until the extract becomes thick and begins to bubble. A gentle current of air is then blown into the flask, which is rotated over a naked flame until the odour of the solvent is replaced by the typical odour of hot derris resin. At this stage the flask may be weighed to determine the approximate amount of resin. The resin is then rapidly dissolved in warm carbon tetrachloride saturated with rotenone (2 c.c. of carbon tetrachloride for each gram of resin), cooled, seeded if necessary, and kept over-night. The crystals are collected on a disc of filter paper (Whatman No. 1) in a Gooch crucible connected with a filter pump, washed with carbon tetrachloride saturated with rotenone until the filtrate is nearly colourless, and dried to constant weight in air below 50° C. The weight of the complex $\times 0{\cdot}72$ gives the weight of crude rotenone.

The crude rotenone is purified by triturating 1 part of the complex with 5 parts of alcohol saturated with rotenone and keeping over-night. The rotenone is then collected in a Gooch crucible, washed with more of the same solvent, dried at 100° C. and weighed.

Derris (Tattersfield and Martin's Methods).—The following are the methods described by F. Tattersfield and J. T. Martin (*Ann. Appl. Biol.*, 1935, **22,** pp. 578-605) for the evaluation of rotenone-containing plants:

Preparation of the Sample.—Unless the sample is very finely ground, there is a tendency towards segregation into rich and poor fractions. Impalpable powders of derris root can only be obtained with difficulty under laboratory conditions, and it is therefore necessary to mix the sample thoroughly immediately before abstracting a portion for analysis. All samples prior to extraction are mixed with purified sand.

Moisture.—This is determined by heating in an electric oven at 100° C. until constant weight is reached.

Ether Extract.—This is determined by extracting 5 gm. mixed with acid-washed sand in a Soxhlet apparatus and

drying the extracted matter to constant weight in an electric oven at 100° C.

Rotenone.—Crude rotenone is determined by extracting 50 gm. with ether, the solvent being taken off in carbon dioxide and finally in a partial vacuum. The residue is dissolved in carbon tetrachloride and, if necessary, filtered through cotton wool to separate small quantities of insoluble matter. After concentrating to 25 c.c., the cooled solution is seeded and placed in an ice chest over-night. The precipitated rotenone-carbon tetrachloride complex is separated, washed with the smallest possible quantity of ice-cold carbon tetrachloride, allowed to stand over-night and weighed. The percentage of crude rotenone is calculated from the weight of the carbon tetrachloride complex. The crude product is dissolved in a measured quantity of hot alcohol (usually 50 c.c.), cooled in an ice chest and then kept at 20° C. for some hours. The crystals are filtered off at the pump in a weighed Gooch crucible, washed with a little ice-cold alcohol, dried in a vacuum desiccator over calcium chloride and weighed as rotenone; the product contains only a trace of chlorine.* The percentage of purified rotenone is calculated, after making an allowance of 0·2 gm. of rotenone per 100 c.c. for the rotenone dissolved by the alcohol.

Methoxyl Content.—This is determined on the extract from 2·5 gm. obtained by the use of sodium-dried ether. After evaporation of the ether in an electric oven at 100° C., the methoxyl content is determined by the method of Clark (see p. 311).

Rotenone (Worsley's Method).—The following method of determining rotenone in derris root and *Mundulea* bark is due to R. R. Le G. Worsley (*J. Soc. Chem. Ind.*, 1936, **55,** pp. 349-357T). For this method the following are required:

Charcoal.—The most suitable is " decolorizing charcoal " obtainable from the British Drug Houses Ltd.

* Dr. Tattersfield (*Priv. Comm.*) states that the crystals should be practically colourless and their melting point should be 159°-163° C.

Percolation Tubes.—The following three sizes are required: diameter $\frac{3}{4}$ in. for quantities of 10-20 gm., diameter 1 in. for 20-40 gm. and diameter $1\frac{1}{2}$ in. for 40-100 gm. Each tube is about 18 in. long. The tubes are drawn out at the lower ends and pass through rubber stoppers into filter flasks, which are immersed in cold water so as to reduce the loss of solvent. In each of the two larger tubes a perforated Gooch crucible disc is placed in the lower end, and on this a disc of filter paper followed by a wad of cotton wool. In the smallest tube no perforated disc is used. The three tubes slide inside other tubes, preferably of thin metal, and should fit fairly closely to ensure rapid heating of their contents. These outer tubes are permanently fixed in a water-jacket, through which a slow stream of water heated to the required temperature is passed.

Determination.—Sufficient of the air-dried and ground sample to give about 1 gm. of rotenone is weighed and mixed with 5 per cent. of charcoal in the case of derris and 10 per cent. of charcoal in the case of *Mundulea*. The mixed material is transferred to the appropriate percolation tube. The powder must be packed evenly; the tube is therefore tapped during the filling to prevent any channels forming. The tube is lowered into the constant temperature bath, which is maintained at a few degrees below the boiling point of ethyl acetate,* and the lower end is pushed through the rubber stopper in the neck of the filter flask. Suction is applied, when the column of powder falls 1 or 2 inches and thus removes any possibility of channels in the powder. The calculated amount of ethyl acetate is then heated on a water bath almost to the boiling point and is poured into the percolation tube. The amount required is about 20 c.c. per gm. when 10-20 gm. of the sample are used, 15 c.c. per gm. when 25-40 gm. are used, 500 c.c. for 50 gm. and 800 c.c. for 100 gm. of the sample.

As soon as the solvent appears at the bottom of the percolation tube, suction is stopped and the pressure is adjusted

* The boiling point of ethyl acetate is 77·1° C.

so that the rate of percolation is about 2 drops per second. When slightly more than half the solvent has run through, the rate of percolation is increased to about 4 drops per second; and when nearly all the solvent has run through, full suction is applied. At the end of the percolation the tip of the tube is washed with hot ethyl acetate to remove any adhering resins. The time taken is about 1 minute for every c.c. of solvent—*i.e.*, from $\frac{3}{4}$-$1\frac{1}{2}$ hours. If air is drawn by means of a pump through the exhausted powder for 15-20 minutes whilst it is still in the bath, the solvent is removed and the powder is easily shaken out. The wad of cotton wool at the bottom need not be disturbed.

The solution is filtered into a distilling flask and nearly all the ethyl acetate is distilled off. The resins are poured into a small beaker (25, 30 or 50 c.c. according to the quantity), and the flask is washed out with successive small quantities of solvent which are poured into the beaker. The ethyl acetate is then removed on a water bath. When most of it has evaporated, a small funnel, the diameter of which is slightly less than the beaker, is connected to a pump, inverted and clamped just inside the top of the beaker and suction is applied for 15-20 minutes. The beaker is then weighed, without further heating, to determine the amount of resins.

Sufficient rotenone, ground and sifted through a 40-mesh sieve, to bring its proportion in the resins up to at least 40 per cent. is weighed to the nearest cgm.; in any case at least 1 gm. should be added. It is stirred into the heated resins to form a syrup and then 2 c.c. of carbon tetrachloride saturated with rotenone for every gram of resins plus rotenone are added, and the mixture is warmed until solution is complete; if any appreciable amount of carbon tetrachloride evaporates, an equal volume of the pure liquid must be added. The beaker is placed in a desiccator which contains a dish of carbon tetrachloride and is left there until the next morning. The crystals are broken up and filtered through a Gooch crucible on a disc of Whatman

No. 1 filter paper. Before washing, as much as possible of the liquid is removed by pressure, using the flat end of a small sample tube. The crystals are washed with carbon tetrachloride saturated with rotenone until no further colour is removed and are dried at about 40° C. for about 6 hours. The weight obtained $\times 0{\cdot}719$ gives the amount of crude rotenone. Results of sufficient accuracy for routine determinations are obtained by assuming the purity of the complex to be 94 per cent., and calculating the pure rotenone on this basis.

The rotenone is more accurately determined by triturating the complex with absolute alcohol saturated with rotenone, using 5 c.c. for every gram of complex, and leaving overnight in a desiccator containing a dish of alcohol. The rotenone is collected in a Gooch crucible, as much liquid as possible being removed by pressure, and then washed with 30-40 c.c. of the solvent and dried at 100° C. for 6 hours. From the weight of rotenone thus obtained, weighed to the nearest cgm., is deducted the weight added. The purity of the rotenone may be taken as 99·2 per cent. Therefore for rotenone contents above 6 per cent. a correction of $-0{\cdot}1$ per cent. is made, but for contents below 6 per cent. no correction is necessary.

SOLUTIONS FOR VOLUMETRIC ANALYSIS

The indicators commonly used in volumetric analysis are prepared as follows:

Bromo-cresol Green.—Grind 0·1 gm. of bromo-cresol green in an agate mortar with 1·43 c.c. of N/10 sodium hydroxide solution and, when solution is complete, dilute with distilled water to 250 c.c. This gives a 0·04 per cent. solution.

Bromo-phenol Blue.—Grind 0·1 gm. of bromo-phenol blue in an agate mortar with 1·5 c.c. of N/10 sodium hydroxide solution and, when solution is complete, dilute with distilled water to 250 c.c.

Bromo-thymol Blue.—Grind 0·1 gm. of bromo-thymol blue in an agate mortar with 1·6 c.c. of N/10 sodium hydroxide solution and, when solution is complete, dilute with distilled water to 250 c.c.

Cochineal.—Digest 3 gm. of pulverized cochineal in a mixture of 50 c.c. of 95 per cent. alcohol and 200 c.c. of water for 1 or 2 days at the ordinary temperature, with frequent shaking, and then filter.

Methyl Orange.—Dissolve 0·1 gm. of methyl orange in a small quantity of alcohol, and dilute to 100 c.c. with 50 per cent. alcohol.

Methyl Red.—Grind 0·1 gm. of methyl red in an agate mortar with 3·7 c.c. of N/10 sodium hydroxide solution and, when solution is complete, dilute to 100 c.c. with distilled water. A solution can be more easily prepared by dissolving 0·1 gm. of methyl red in 100 c.c. of 95 per cent. alcohol.

Phenolphthalein.—Dissolve 0·5 gm. of phenolphthalein in 50 c.c. of alcohol and dilute, with shaking, to 100 c.c. with distilled water. Or dissolve 1 gm. of phenolphthalein in 100 c.c. of 95 per cent. alcohol.

Starch.—Shake about 1 gm. of starch with cold water in a test tube, and pour the mixture into about 100 c.c. of boiling water. Continue the boiling for a few minutes and allow the liquid to stand until it is cold.

Standard solutions are prepared and standardized as follows:

Hydrochloric Acid.—Concentrated hydrochloric acid generally has a specific gravity of 1·18; and acid of that specific gravity contains 418 gm. of hydrochloric acid per litre. Hence approximately N hydrochloric acid solution can be prepared by diluting $36{\cdot}465 \times 1000/418 = 87{\cdot}2$ c.c. of the concentrated acid to 1 litre with distilled water. If the specific gravity of the concentrated acid is first determined, the more exact volume can be calculated from the following table (*Chemists' Year Book*, 1933, p. 52), which gives the specific gravities of solutions of hydrochloric acid

at 15° C., when water at 4° C.=1, and the weights of hydrochloric acid in 1 litre of solution.

Specific Gravity.	*Gm. per Litre.*
1·170	392
1·175	404
1·180	418
1·185	430
1·190	443

The solution so prepared can be standardized by titration with anhydrous sodium carbonate. Separate quantities of sodium carbonate between 1·325 and 2·65 gm., which require between 25 and 50 c.c. of N hydrochloric acid, are dried to constant weight, dissolved in water and titrated with the solution to be standardized, using either methyl orange or bromo-phenol blue as indicator. 1 c.c. of N hydrochloric acid=0·05300 gm. of sodium carbonate.

Standard solutions of hydrochloric acid can also be standardized with sodium oxalate, which is ignited to convert it into sodium carbonate. A weighed quantity of pure sodium oxalate is heated in a platinum crucible at first gently until the salt is decomposed and then more strongly, with the cover half removed, until the carbon is burnt. To the residue, when cool, water is added, and the solution is titrated with the solution to be standardized. 1 c.c. of N hydrochloric acid=0·06701 gm. of sodium oxalate.

N/10 hydrochloric acid can also be standardized by neutralizing a measured volume of it with an excess of calcium carbonate (finely ground calcite is suitable for this purpose), adding a few drops of 10 per cent. potassium chromate solution and titrating with N/10 silver nitrate solution until a faint permanent pink colour is obtained. The Association of Official Agricultural Chemists (*Methods of Analysis*, 1935, p. 23) regard this as a preliminary test, and carry out the final determination as follows: To a measured volume of the acid to be standardized add from

a burette 1 drop in excess of the required quantity of N/10 silver nitrate solution, as determined by the preliminary test. Heat to the boiling point, protect from light and allow to stand until the precipitate is granular. Filter on a Gooch crucible, which has been heated to 140°-150° C. and weighed. Wash the precipitate with hot water and test the filtrate for an excess of silver nitrate. Dry the silver chloride at 140°-150° C. and weigh.

Sulphuric Acid.—It is not possible to find the percentage of sulphuric acid in the concentrated acid by determining its specific gravity, because the specific gravity rises with increasing concentration till it reaches a maximum at about 97 per cent. and then decreases. And, even when the percentage is known, it is extremely difficult to weigh out a definite weight of the concentrated acid. To overcome these difficulties A. Marshall (*J. Soc. Chem. Ind.*, 1899, **18,** pp. 4-6 and p. 1091) devised the following method of preparing standard solutions of sulphuric acid: The pure concentrated acid is diluted with about half its volume of water. The dilute acid is cooled and its specific gravity is determined with a Sprengel or Nicol tube at 15°, 15·5° or 18° C. The specific gravities are in each case compared with water at the same temperature and the weights are not corrected for the air displaced. If P=the percentage by weight of sulphuric acid, then for values of P between 66 and 81,

$$P = 86\ S_{15} - 69{\cdot}00$$
$$P = 86\ S_{15{\cdot}5} - 68{\cdot}97$$
$$P = 86\ S_{18} - 68{\cdot}82,$$

where S_{15}, $S_{15{\cdot}5}$ and S_{18} are the specific gravities at 15°, 15·5° and 18° C. respectively. If S=the specific gravity of the diluted acid at temperature T° C. compared with water at t° C.

$$P = S\ (85{\cdot}87 + 0{\cdot}05T - 0{\cdot}0004t^2) - 69{\cdot}82.$$

The above formula can be used for diluted acids containing from 62 to 82 per cent. of sulphuric acid at temperatures

between 0° and 40° C. The weight of diluted acid required for the preparation of n litres of N/10 solution =

$$4{\cdot}9038n \times \frac{100}{P} \text{ gm.}$$

This weight of acid is diluted nearly to n litres and after cooling is made up to the required volume. This acid can be standardized with weighed quantities of anhydrous sodium carbonate or sodium oxalate in the same way as solutions of hydrochloric acid. In addition to methyl orange and bromo-phenol blue, phenolphthalein can also be used as an indicator, but, if the latter indicator is used, the solution of sodium carbonate must be kept boiling during the titration in order to drive off the carbon dioxide.

The Association of Official Agricultural Chemists (*Methods of Analysis*, 1935, p. 23) adopt the following gravimetric method of standardizing solutions of sulphuric acid: Dilute a measured volume of the acid to be standardized to about 100 c.c. Heat to the boiling point and add, drop by drop, 10 per cent. barium chloride solution until no further precipitate is formed. Continue the boiling for about 5 minutes and allow to stand for 5 hours or longer in a warm place. Then pour the supernatant liquid on a weighed Gooch crucible or an ashless filter paper. Wash the precipitate with 25-30 c.c. of boiling water, transfer the precipitate to the filter and wash it with boiling water until the filtrate is free from chloride. Dry and ignite the precipitate and weigh as barium sulphate.

Sodium Hydroxide.—Normal sodium hydroxide solution contains 40·0048 gm. of sodium hydroxide per litre, and should be free from sodium carbonate. An approximately normal solution can be prepared by weighing some of the purest sodium hydroxide obtainable as quickly as possible, dissolving it in water and diluting the solution to the required volume with the least possible exposure to the air. A much purer solution is prepared by dissolving a weighed quantity of sodium hydroxide in an equal weight of water,

and allowing the solution to stand in a flask or test tube, which is closed with a cork covered with tin foil, until the supernatant liquid is clear. Sodium carbonate is insoluble in a concentrated solution of sodium hydroxide and sinks to the bottom, leaving a clear solution free from carbonate. The density of a 50 per cent. solution of sodium hydroxide is 1·529 at 15° C. Therefore 1 litre of the solution weighs 1529 gm. and, since it contains half its weight of sodium hydroxide, the normality of the solution is $1529\text{N}/(2\times40)=$ approximately 19N. This solution serves as a stock solution from which standard solutions are prepared as required. The normality of the solution is found by diluting 5 c.c. to 1 litre with distilled water, which has been boiled to free it from carbon dioxide, and then titrating measured volumes of N/10 acid with the resulting solution. When the normality of the concentrated solution is known, standard solutions are prepared by diluting the calculated volumes to the required volumes with recently boiled distilled water. These solutions can be standardized by titration with standard solutions of hydrochloric acid or sulphuric acid, which have been standardized against sodium carbonate or sodium oxalate. The titrations are best carried out with the indicator which will be used in the determinations for which these solutions are required.

Solutions of sodium hydroxide can also be standardized by titrating weighed quantities of certain organic acids and acid salts which can be purchased in a state of great purity. Benzoic acid, potassium hydrogen phthalate and potassium hydrogen tartrate are the ones generally used. Of these, potassium hydrogen phthalate is to be preferred on account of its greater solubility and its higher molecular weight. The following details of the assay of these chemicals, which are given in "*Analar*" *Standards for Laboratory Chemicals*, 1937, pp. 53, 199 and 200, will show how the titrations are carried out. In the case of benzoic acid, 2 gm. are dissolved in 10 c.c. of alcohol, 30 c.c. of water are added and the solution is titrated with N sodium hydroxide, using phenol

red as indicator.* 1 c.c. of N sodium hydroxide solution = 0·12212 gm. of benzoic acid. Or 9 gm. of potassium hydrogen phthalate, which have been previously dried at 110° C. for 1 hour, are dissolved in 100 c.c. of water, and the solution is titrated with N sodium hydroxide solution, using phenolphthalein as indicator. 1 c.c. = 0·20422 gm. of potassium hydrogen phthalate. Alternatively, 8 gm. of potassium hydrogen tartrate, which have been dried at 110° C. for 1 hour, are suspended in 200 c.c. of hot water and titrated with N sodium hydroxide solution, using phenolphthalein as indicator and boiling the solution towards the end of the titration. 1 c.c. = 0·18818 gm. of potassium hydrogen tartrate.

Standard acids can be kept for some time without appreciable change in titre, if they are stored in dark cupboards where they are not subjected to great changes of temperature. But standard solutions of sodium hydroxide change much more rapidly and should therefore be checked at frequent intervals.

Potassium Permanganate.—The molecular weight of potassium permanganate is 158·026. In the presence of sulphuric acid, $2KMnO_4 = 5\,O$. Since 1 litre of the normal solution is equivalent to 8 gm. of oxygen, 10 litres of normal solution contain $2 \times 158{\cdot}026$ gm., and 1 litre of decinormal solution contains $2 \times 158{\cdot}026/(10 \times 10) = 3{\cdot}1605$ gm. of potassium permanganate. A decinormal solution is not prepared by weighing this exact quantity of pure potassium permanganate, because the distilled water and the apparatus used in preparing the solution contain oxidizable impurities which reduce the permanganate. It is prepared by dissolving 3·2 gm. of potassium permanganate in distilled water and diluting the solution to 1 litre; solution is hastened by grinding the potassium permanganate in a glass mortar with water and pouring off the supernatant liquid into the graduated flask. The standardization of the solution is

* Phenolphthalein is generally used as the indicator in this titration.

carried out after oxidation is complete. The solution is either made up to the mark and allowed to stand for several days in the dark with occasional shaking or it is boiled for 10-15 minutes to hasten oxidation, left to cool over-night and then made up to the mark. In either case, it is advisable to filter the solution through asbestos or a sintered glass diaphragm before standardization. A solution prepared in this way, and stored in a dark cupboard, can be kept for some time without any appreciable change in the normality.

The filtered solution is standardized with sodium oxalate or ferrous ammonium sulphate, both of which can be purchased in a pure state. For the more dilute solutions the latter is preferable on account of its high molecular weight. Separate weighed quantities of sodium oxalate, from 0·15 to 0·3 gm. in weight, which require from about 25 to 50 c.c. of N/10 solution, are dissolved in water, acidified with dilute sulphuric acid, heated to about 70° C. and titrated with the approximately decinormal solution until a permanent faint pink colour is obtained. 1 c.c. of N/10 potassium permanganate solution=0·006701 gm. of sodium oxalate. If ferrous ammonium sulphate is used, separate weighed quantities between 1 and 2 gm., which require between 25 and 50 c.c. of N/10 solution, are dissolved in water, acidified with dilute sulphuric acid and immediately titrated at the ordinary temperature with the approximately decinormal solution until a faint pink colour remains. 1 c.c. of N/10 potassium permanganate solution=0·039213 gm. of ferrous ammonium sulphate.

If the standard solution of potassium permanganate is required for the determination of calcium or iron only, there is no need to find its normality; the calculations are simplified by finding the weights of calcium oxide and ferric oxide equivalent to 1 c.c. of the solution. This can be done by multiplying the weight of sodium oxalate equal to 1 c.c. of the solution by 28·04/67·01=0·4184 to obtain the equivalent weight of calcium oxide, and by 79·84/67·01=1·1915

to obtain the equivalent weight of ferric oxide. The corresponding factors to convert the weight of ferrous ammonium sulphate into the equivalent weights of calcium oxide and ferric oxide are 0·0715 and 0·2036 respectively.

Oxalic Acid.—Standard solutions of oxalic acid are required for the volumetric determination of potassium (see p. 160). A decinormal solution contains 6·3033 gm. of crystallized oxalic acid per litre. N/10 and N/20 solutions can be prepared by weighing the calculated quantities of the acid and diluting to the required volumes. Owing to the uncertainty of the water content of the crystals, the solutions should be standardized by titration with the standard potassium permanganate solutions which will be used in the determinations of potassium. Oxalic acid solutions are unstable and require checking at frequent intervals. With the addition of sulphuric acid (50 c.c. of concentrated acid per litre) they are much more stable.

Potassium Dichromate.—The molecular weight of potassium dichromate is 294·212. In the presence of hydrochloric acid, $K_2Cr_2O_7 = 3O$. Since 1 litre of the normal solution is equivalent to 8 gm. of oxygen, 6 litres of normal solution contain 294·212 gm., and 1 litre of decinormal solution contains $294{\cdot}212/(6 \times 10) = 4{\cdot}9035$ gm. of potassium dichromate. A decinormal solution can be prepared by weighing this exact weight of pure potassium dichromate, dissolving it in distilled water and diluting the solution to 1 litre. The potassium dichromate should be finely ground and placed in a desiccator over concentrated sulphuric acid for some days before it is weighed.

Standard potassium dichromate solution can be used for the determination of iron in phosphate rock by titrating aliquot portions of the hydrochloric acid solution after reduction of the ferric iron with stannous chloride. The titration is continued until a drop of the solution being titrated does not give a blue colour when brought into contact with a drop of potassium ferricyanide solution, which is placed on a white porcelain tile. The solution of potassium

ferricyanide used as the external indicator must be free from ferrocyanide and should be very dilute. It is best prepared by shaking a small crystal about the size of a pea with water in a test tube until about half of it is dissolved, pouring away the resulting solution and dissolving the remainder in water for immediate use. If many drops are removed during the early stages of the titration, an accurate result cannot be obtained. Hence, after preliminary titrations, a final one should be made in which only a few drops are removed towards the end of the titration.

Instead of using potassium ferricyanide as external indicator, diphenylamine can be used as internal indicator as proposed by J. Knop (*J. Amer. Chem. Soc.*, 1924, **46,** pp. 263-269). To the iron solution in hydrochloric acid, after reduction with stannous chloride, are added 15 c.c. of phosphoric acid mixture (prepared by mixing 150 c.c. of concentrated sulphuric acid with 150 c.c. of phosphoric acid and diluting with water to 1 litre) and 3 drops of diphenylamine solution (prepared by dissolving 1 gm. of diphenylamine in 100 c.c. of concentrated sulphuric acid). The solution is then diluted and titrated with N/10 potassium dichromate solution until a drop causes a change from blue-green to an intense blue-violet, which remains unchanged on shaking. From the volume of the dichromate solution used 0·05 c.c. is subtracted to allow for the oxidation of the diphenylamine.

Sodium Thiosulphate.—A decinormal solution of sodium thiosulphate contains 24·8192 gm. of the hydrated salt per litre. It is difficult to prepare a solution which is exactly decinormal owing to the presence of extraneous water held by the crystals. But an approximately decinormal solution is easily prepared by dissolving 25 gm. of crystallized sodium thiosulphate in water and making the solution up to 1 litre. Its normality can be found by titrating weighed quantities of resublimed iodine, using starch as indicator, or more simply by titrating the iodine liberated from potassium iodide by the action of potassium dichromate, potassium

permanganate or potassium iodate in acid solution. 1 c.c. of N/10 potassium dichromate solution in the presence of hydrochloric acid, and 1 c.c. of N/10 potassium permanganate solution in the presence of sulphuric acid, liberate 0·012692 gm. of iodine from an excess of potassium iodide. Accurate results can only be obtained with these solutions under certain conditions (see F. Sutton, *Volumetric Analysis*, 12th edition, 1935, revised by A. D. Mitchell, pp. 130-131). In the case of potassium dichromate the reaction is not instantaneous, and the hydriodic acid formed by the action of the acid on the iodide is readily oxidized in the presence of chromium salts. It is therefore necessary to pass a current of carbon dioxide through the flask during the reaction and to allow 5 minutes for its completion. After diluting the solution, the sodium thiosulphate solution to be standardized is run in from a burette until most of the iodine has taken part in the reaction, when starch is added and the addition of the thiosulphate solution is continued until the colour of the solution changes from blue to green. With potassium permanganate the change of colour is from blue to pale pink; the end-point is therefore much more distinct, but the results are not reliable unless the solutions are dilute and contain a very slight excess of sulphuric acid.

When potassium iodate reacts with potassium iodide in the presence of sulphuric acid, $KIO_3 = 6I$. The molecular weight of potassium iodate is 214·016. Therefore a solution 1 c.c. of which = 0·012692 gm. of iodine contains $214{\cdot}016/(6 \times 10) = 3{\cdot}5669$ gm. of potassium iodate per litre, and is M/60. To standardize a solution of sodium thiosulphate, a measured volume of M/60 potassium iodate solution is treated with an excess of potassium iodide, the solution is acidified with sulphuric acid and the liberated iodine is titrated with the solution to be standardized, using starch as indicator.

Standard solutions of sodium thiosulphate undergo a slow decomposition with the formation of free sulphur. The chief

cause of this change is bacterial action. C. Mayr and E. Kerschbaum (*Z. anal. Chem.*, 1928, **73,** pp. 321-352) have recommended the addition of 1 per cent. (by volume) of amyl alcohol in order to keep the solution sterile and thus prevent the change in titre.

Iodine.—Decinormal iodine solution contains 12·692 gm. of iodine per litre. An approximately N/10 solution is prepared by weighing 12·7 gm. of iodine in a stoppered weighing tube, dissolving the iodine in a solution of potassium iodide containing about 18 gm. of that salt in 30-50 c.c. of water and, when the iodine is completely dissolved, diluting the solution to 1 litre. The solution is standardized by titration with N/10 sodium thiosulphate solution, which has been recently standardized by one of the methods described above, or by titrating weighed quantities of pure arsenious oxide. The latter titrations are carried out as follows: Dissolve 2 gm. of pure arsenious oxide in dilute sodium hydroxide solution, make the solution faintly acid with hydrochloric acid, add about 2 gm. of sodium bicarbonate and titrate the solution with the approximately N/10 iodine solution to be standardized, using starch as indicator. 1 c.c. of N/10 iodine solution = 0·004946 gm. of arsenious oxide.

Silver Nitrate.—The molecular weight of silver nitrate is 169·888. Therefore a decinormal solution contains 16·9888 gm. per litre. Since it is not possible to weigh out this exact weight of silver nitrate, an approximately decinormal solution is prepared, and its normality is found by titration with N/10 sodium chloride solution, which is prepared by dissolving 5·8454 gm. of pure sodium chloride in water and diluting to 1 litre.

The titration is carried out by placing a measured volume of N/10 sodium chloride solution in a porcelain dish and diluting with water to about 100 c.c. After adding a few drops of 10 per cent. potassium chromate solution, the silver nitrate solution is added from a burette, with constant stirring, until a permanent pink tint is obtained. Too much

silver nitrate will probably be added in the first titration. It is therefore advisable to add sufficient sodium chloride to remove the reddish tint and to use the dish for comparison with the liquid in another titration.

A solution standardized in this way will be exact enough for most purposes. Reynolds and Jacob recommend that the N/10 silver nitrate solution used for the determination of fluorine in phosphate rock (see p. 63) be standardized by precipitating and weighing the silver as silver chloride. This is carried out as follows: To a measured volume of the silver nitrate solution add a slight excess of hydrochloric acid. Heat to the boiling point, protect from the light and allow to stand until the precipitate is granular. Filter on a Gooch crucible which has been heated to 140°-150° C. and weighed. Wash the precipitate with hot water, dry it at 140°-150° C. and weigh.

Ammonium and Potassium Thiocyanates.—In the determination of fluorine in phosphate rock (see p. 63) the fluorine is precipitated as lead chlorofluoride, and the chlorine in the precipitate is determined by Volhard's method. Chlorine is also determined in feeding stuffs (see p. 198) and in milk (see p. 237) by Volhard's method. This consists in adding to the chloride in nitric acid solution a measured volume of standard silver nitrate solution, which is more than sufficient to precipitate the whole of the chloride. After filtering and washing the silver chloride, the excess of silver in the filtrate is determined by titration with standard ammonium or potassium thiocyanate, using ferric ammonium alum as indicator. During the titration the silver is precipitated as the thiocyanate, and any ferric thiocyanate which is formed locally is decomposed on shaking until the whole of the silver has been precipitated, when the slightest excess of thiocyanate produces a permanent brown colour.

Decinormal solutions of the thiocyanates contain 7·61 gm. of the ammonium salt and 9·72 gm. of the potassium salt per litre. These exact quantities cannot be weighed out,

because both salts are deliquescent. Approximately N/10 or N/20 solutions are therefore standardized against accurately standardized N/10 or N/20 silver nitrate solutions, using either weighed quantities of pure sodium or potassium chloride or measured volumes of standard sodium chloride solution.

APPENDIXES

INTERNATIONAL ATOMIC WEIGHTS
GRAVIMETRIC FACTORS AND THEIR LOGARITHMS
VOLUMETRIC FACTORS AND THEIR LOGARITHMS

INTERNATIONAL ATOMIC WEIGHTS, 1937.

Ber., 1937, 70, A, 43-46.

Aluminium	26·97
Arsenic	74·91
Barium	137·36
Calcium	40·08
Carbon	12·01
Chlorine	35·457
Chromium	52·01
Cobalt	58·94
Copper	63·57
Fluorine	19·00
Hydrogen	1·0078
Iodine	126·92
Iron	55·84
Lead	207·21
Magnesium	24·32
Manganese	54·93
Nickel	58·69
Nitrogen	14·008
Oxygen	16·0000
Phosphorus	31·02
Platinum	195·23
Potassium	39·096
Silicon	28·06
Silver	107·880
Sodium	22·997
Sulphur	32·06
Uranium	238·07
Zinc	65·38

GRAVIMETRIC FACTORS AND THEIR LOGARITHMS CALCULATED FROM INTERNATIONAL ATOMIC WEIGHTS, 1937.

	Weighed as	*Required.*	*Factor.*	*Logarithm.*
Aluminium	$AlPO_4$	Al	0·22108	$\bar{1}$·34455
	$AlPO_4$	Al_2O_3	0·41782	$\bar{1}$·62099
Calcium	$CaSO_4$	CaO	0·41193	$\bar{1}$·61482
	CaO	$CaCO_3$	1·78477	0·25158
Carbon	CO_2	$CaCO_3$	2·27426	0·35684
Chlorine	AgCl	Cl	0·24737	$\bar{1}$·39335
Copper	Cu	$CuSO_4,5H_2O$	3·92808	0·59418
Iron	Fe_2O_3	$FePO_4$	1·88953	0·27635
Lead	$PbSO_4$	PbO	0·73601	$\bar{1}$·86688
Magnesium	$Mg_2P_2O_7$	Mg	0·21843	$\bar{1}$·33931
	$Mg_2P_2O_7$	MgO	0·36213	$\bar{1}$·55886
Phosphorus	$Mg_2P_2O_7$	P_2O_5	0·63787	$\bar{1}$·80473
	P_2O_5	$Ca_3(PO_4)_2$	2·18446	0·33934
Potassium	K_2PtCl_6	K_2O	0·19375	$\bar{1}$·28724
	$KClO_4$	K_2O	0·33991	$\bar{1}$·53136
	$K_2NaCo(NO_2)_6,H_2O$	K_2O	0·20738	$\bar{1}$·31677
	K_2O	KCl	1·58300	0·19948
	K_2O	K_2SO_4	1·84997	0·26716
Sodium	Na_2SO_4	Na	0·32378	$\bar{1}$·51025
	$Na(UO_2)_3Zn(C_2H_3O_2)_9.6H_2O$	Na	0·01495	$\bar{2}$·17464
Sulphur	$BaSO_4$	S	0·13735	$\bar{1}$·13783

Volumetric Factors and their Logarithms.

	Factor.	*Logarithm.*
1 ml. N acid	=0·05300 gm. Na_2CO_3	$\bar{2}$·72428
	=0·06701 gm. $Na_2C_2O_4$	$\bar{2}$·82614
	=0·040005 gm. NaOH	$\bar{2}$·60211
	=0·014008 gm. N	$\bar{2}$·14638
	=0·01703 gm. NH_3	$\bar{2}$·23121
	=0·06607 gm. $(NH_4)_2SO_4$	$\bar{2}$·82000
	=0·05350 gm. NH_4Cl	$\bar{2}$·72835
	=0·08501 gm. $NaNO_3$	$\bar{2}$·92947
	=0·08205 gm. $Ca(NO_3)_2$	$\bar{2}$·91408
	=0·03003 gm. $CO(NH_2)_2$	$\bar{2}$·47756
1 ml. N NaOH	=0·036465 gm. HCl	$\bar{2}$·56188
	=0·049038 gm. H_2SO_4	$\bar{2}$·69053
	=0·12212 gm. C_6H_5·COOH	$\bar{1}$·08679
	=0·20422 gm. COOH·C_6H_4·COOK	$\bar{1}$·31010
	=0·18818 gm. $KHC_4H_4O_6$	$\bar{1}$·27457
1 ml. N/10 $KMnO_4$	=0·0031605 gm. $KMnO_4$	$\bar{3}$·49976
	=0·006701 gm. $Na_2C_2O_4$	$\bar{3}$·82614
	=0·006303 gm. $H_2C_2O_4,2H_2O$	$\bar{3}$·79955
	=0·039213 gm. $FeSO_4,(NH_4)_2SO_4,6H_2O$	$\bar{2}$·59343
	=0·005584 gm. Fe	$\bar{3}$·74695
	=0·007984 gm. Fe_2O_3	$\bar{3}$·90222
	=0·002004 gm. Ca	$\bar{3}$·30190
	=0·002804 gm. CaO	$\bar{3}$·44778
	=0·000711 gm. K	$\bar{4}$·85187
	=0·000856 gm. K_2O	$\bar{4}$·93247
1 ml. N/10 $K_2Cr_2O_7$	=0·0049035 gm. $K_2Cr_2O_7$	$\bar{3}$·69051
	=0·005584 gm. Fe	$\bar{3}$·74695
	=0·007984 gm. Fe_2O_3	$\bar{3}$·90222

1 ml. N/10 $Na_2S_2O_3$	=0·012692 gm. I	$\bar{2}$·10353
	=0·0031605 gm. $KMnO_4$	$\bar{3}$·49976
	=0·0049035 gm. $K_2Cr_2O_7$	$\bar{3}$·69051
	=0·003567 gm. KIO_3	$\bar{3}$·55230
	=0·006357 gm. Cu	$\bar{3}$·80325
	=0·02497 gm. $CuSO_4,5H_2O$	$\bar{2}$·39742
1 ml. N/10 I	=0·012692 gm. I	$\bar{2}$·10353
	=0·02482 gm. $Na_2S_2O_3,5H_2O$	$\bar{2}$·39480
	=0·004946 gm. As_2O_3	$\bar{3}$·69425
	=0·005746 gm. As_2O_5	$\bar{3}$·75937
	=0·001351 gm. HCN	$\bar{3}$·13066
1 ml. N/10 $AgNO_3$	=0·016989 gm. $AgNO_3$	$\bar{2}$·23017
	=0·005845 gm. NaCl	$\bar{3}$·76678
	=0·003546 gm. Cl	$\bar{3}$·54974
	=0·001900 gm. F	$\bar{3}$·27875
	=0·005204 gm. CN	$\bar{3}$·71634
	=0·005405 gm. HCN	$\bar{3}$·73280
	=0·009803 gm. NaCN	$\bar{3}$·99136
	=0·013023 gm. KCN	$\bar{2}$·11471
	=0·009212 gm. $Ca(CN)_2$	$\bar{3}$·96435

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