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THE ANALYST

PROCEEDINGS OF THE SOCIETY OF PUBLIC ANALYSTS AND OTHER ANALYTICAL CHEMISTS.

AN Ordinary Meeting of the Society was held in the Chemical Society's Rooms, Burlington House, on Wednesday, November 5, 1924. The President, Mr. G. Rudd Thompson, F.I.C., was in the chair.

Certificates were read for the first time in favour of:—Messrs. A. C. Barnes, B.Sc., F.I.C., J. J. Fox, D.Sc. (Lond.), F.I.C., I. C. Hay, and H. R. Jonson, M.Sc. (Liv.), A.I.C.

Certificates were read for the second time in favour of Messrs. A. C. Brooks, A.R.C.Sc.I., A.I.C., W. Donovan, M.Sc. (New Zealand), A. G. Flower, B.Sc. (Lond.), G. Hollingsworth, F.I.C., G. M. Norman, B.Sc. (Lond.), A.R.C.S., F.I.C., C. E. Sage, F.I.C., P. F. Spendlove, B.Sc., A.R.C.S., A.I.C., C. W. Spiers, M.Sc. (Bristol), A.I.C., L. H. Trace, B.Sc. (Lond.), A.I.C., and J. R. Walmsley, A.M.S.T., F.I.C.

Messrs. J. E. Nyrop and C. P. Thorpe were elected members of the Society.

The following papers were read:—"On certain new Methods for the Estimation of Arsenic and its Occurrence in Fish and Urine," by H. E. Cox, M.Sc., Ph.D., F.I.C.; "The Estimation of Cadmium in Brass," by A. T. Etheridge, M.B.E., B.Sc., F.I.C.; "The Hoyberg Method of Milk and Cream Testing," by N. C. Wright, B.A., and J. Golding, D.S.O., F.I.C.; and "An Apparatus for the Catalytic Dehydrogenation of Alcohols," by S. G. Willimott, Ph.D., B.Sc.

Deaths.

We deeply regret to have to record the deaths of the following Members of our Society:—

Sir Charles Cameron.
Dr. Robert Hellon.

Application of "Formol Titration" to the Kjeldahl Method of Estimating Nitrogen.

By W. S. SHAW, M.Sc., A.I.C.

(*Read at the Meeting, October* 1, 1924.)

If, in the Kjeldahl process, some means could be devised of estimating the ammonia formed which would render distillation unnecessary, considerable time and attention would be saved. It has been shown by Nanji and Shaw (Analyst, 1923, **48**, 571) that the hypobromite method of estimating ammonia is unsuitable, since more than one reaction takes place when ammonia and hypobromite are brought together. The so-called "formol titration" method then suggested itself, and experiments were made to determine its accuracy and to devise a suitable modification of the method.

The "formol titration" method was devised by Sörensen (*Comptes rend.*, Carlsberg, 1907, **7**, I.; *Biochem. Zeitsch.*, 1907, **7**, 45), and was applied by him to the measurement of the velocity of the hydrolysis of proteins by different enzymes. The reaction in the case of ammonium salts proceeds in accordance with the equation:

$$2(NH_4)_2SO_4 + 6HCHO = (CH_2)_6N_4 + 6H_2O + 2H_2SO_4.$$

Thus with ammonium salts, neutral hexamethylene tetramine is formed, with the liberation of acid equivalent to the nitrogen present as ammonium salts.

Malfatti (*Zeitsch. anal. Chem.*, 1908, **47**, 273) and Ronchese (*Bull. Soc. Chim.*, 1908, **3**, 840) obtained satisfactory results in the estimation of ammoniacal nitrogen by the "formol titration" method; but Van Bers (*Chem. Weekblad*, 1917, **14**, 968) found that the direct titration of the liberated acid gave unsatisfactory results. Kolthoff (*Pharm. Weekblad*, 1921, **58**, 1463) claimed, however, that accurate results could be obtained by the direct titration of the acid, provided that a sufficient excess of formaldehyde were used and the mixture allowed to stand for a minute before titration.

In order to test the accuracy of the method, and especially its application to the estimation of nitrogen in ammonium sulphate (the form in which it is present after the Kjeldahl digestion) experiments were made with a standard ammonium sulphate solution.

I. The Indicator.—It is apparent that the indicator most convenient for use in the titration should be one whose colour changes should be definite and well marked, even at a low concentration of hydroxyl or hydrogen ions. The most sensitive indicator for acid and alkali in ordinary use is methyl red, as it is not affected by carbon dioxide, but it was found difficult to recognise the change to a distinct and definite orange coloration, and its use was discarded. Similarly with methyl orange.

The indicator finally adopted was phenolphthalein, and, in order to obtain stability at the end-point, the solutions were left slightly acid before the "exact" neutralisation and were boiled vigorously to expel carbon dioxide; they were then cooled before continuing the estimation. When this was done, no trouble was experienced in obtaining the required permanent tint.

II. NEUTRALISATION OF THE FORMALDEHYDE SOLUTION.—All previous workers on the "formol titration" followed the original method of Sörensen, and neutralised the formaldehyde with sodium hydroxide before use, phenolphthalein being used as indicator. The difficulty in the neutralisation of the formaldehyde lies in the fact that the "exact" neutral point cannot be obtained with a sufficient degree of certainty. The only method possible is to add alkali in the presence of phenolphthalein until a definite, slightly alkaline reaction is obtained. This seemed a possible source of error. Further, it was found that the degree of alkalinity obtained was very unstable owing to the high tendency of an alkaline solution of formaldehyde to become acid.

In order to overcome this difficulty of the neutralisation of the formaldehyde and escape the possible errors introduced by it, another method was adopted.

A solution of commercial formaldehyde (formalin) has usually a comparatively large free acidity, which may be determined by diluting 5 c.c. with about two or three times its bulk of water (previously boiled to expel carbon dioxide), and titrating the acidity with 0·1 *N* sodium hydroxide solution in the presence of about 3 to 6 drops of a 1 per cent. solution of phenolphthalein. Correction for the acidity of the formaldehyde solution used in the estimation can then be made, and by subtraction of this amount from the total titre obtained after the addition of the formaldehyde to the ammoniacal solution, a measure of the nitrogen is obtained. An advantage of this method is that the acidity of the formaldehyde solution remains constant during a considerable period, thus making only one estimation of the acidity necessary.

III. NEUTRALISATION OF AMMONIUM SULPHATE SOLUTION.—In dealing with the application of the "formol titration" to ammonium salts in general the type of the acid radicle of the salt must be borne in mind. It cannot be assumed that the salt of ammonia and a weak acid, such as acetic acid, would have a neutral reaction to indicators, for, by the ordinary laws of ionisation in solution, this is not so. Thus it is necessary in applying the method to ammonium salts, especially in the case of weak organic acid salts, that the solution be neutralised in the presence of phenolphthalein.

The actual neutralisation-point with phenolphthalein cannot theoretically be obtained, as the solution being titrated is always either colourless (hence acidic) or pink (hence alkaline). The actual tint of pink obtained in the estimation of the liberated acid by neutralisation is immaterial, provided it is not too remote from the neutral point, as a correction can be made for this. The main object is to obtain an end-point with a coloration which can be reproduced with ease and certainty. The best means of doing this is as follows:—Neutralise very carefully

until the faint change from colourless to pink has taken place; add *one* extra drop of alkali, when a definite and clear pink colour will be obtained. After the addition of the formaldehyde solution the same procedure should be carried out in the neutralisation of the liberated acid, and the same tint will then be obtained. Thus any error which may be introduced by the varying behaviour of ammonium salts in solution may be overcome.

As the author was particularly concerned with the application of the "formol titration" to the Kjeldahl method of estimating nitrogen, experiments were carried out with an ammonium sulphate solution, under the conditions already given.

Five or ten c.c. of an ammonium sulphate solution containing 1 grm. per 200 c.c. are pipetted into a flask, and 5 drops of a 1 per cent. phenolphthalein solution added as indicator. No preliminary neutralisation of the ammonium solution is necessary, as it is already slightly acid. The contents of the flask are boiled vigorously to expel carbon dioxide, and the flask then cooled rapidly under cold running water. To this solution 0·1 *N* sodium hydroxide solution is added until the faint change to pink is noted; one extra drop of 0·1 *N* sodium hydroxide solution is added to produce a definite pink colour. The acidity of a formaldehyde solution is then determined as already described, 5 c.c. of this formaldehyde solution are added, and the mixture allowed to stand a few minutes. The liberated acid is titrated with 0·1 *N* sodium hydroxide solution, and the titre remaining after the deduction of the acidity due to the 5 c.c. of formaldehyde solution then affords a measure of the nitrogen present in the ammonium sulphate solution.

Following this procedure, a number of estimations were carried out on varying quantities of the ammonium sulphate solution, and the results only varied very slightly from the theoretical, usually within the magnitude of about $\pm 0{\cdot}03$ to $\pm 0{\cdot}1$ c.c. of 0·1 *N* sodium hydroxide solution (a deviation equal to approximately 0·07 mgrm. of nitrogen).

For comparison between the value obtained by the "formol titration" and by the distillation of the ammonia, 25 c.c. of the ammonium sulphate solution were distilled with an excess of sodium hydroxide. The result obtained, calculated as a percentage of nitrogen, showed a deviation of 0·19 per cent. from the value obtained by the "formol titration." The agreement is still closer if 0·05 *N* alkali is used.*

Application to the Kjeldahl Method.—The consistency and accuracy of the "formol titration" method of estimating nitrogen having been established, attempts were made to apply the method to the estimation of nitrogen in substances in which it can be estimated by the Kjeldahl method. It was recognised at the outset that two new factors would present themselves, namely (1) the presence of a comparatively large amount of electrolyte due to the neutralisation of the sulphuric acid and also of the potassium bisulphate added to raise the boiling point of the sulphuric acid with 40 per cent. sodium hydroxide solution; and (2) the presence of copper sulphate used as a catalyst in the digestion.

*Prof. A. R. Ling attributes the discrepancy to the presence of carbon dioxide in the sodium hydroxide.—Editor.

In order to test the influence of sodium sulphate 10 c.c. of the standard ammonium sulphate solution were saturated with sodium sulphate and used for the estimation in the usual manner, but no deviation from the original results was obtained.

The copper sulphate present has a slight, though not material influence on the results of the titration. In alkaline solution a blue colour results, due to the formation of compounds with copper and alkali hydroxide and carbonates; the copper sulphate added, however, is only a very small quantity, and the alkalinity being also very slight, in the place of a blue coloration a slight turbidity results as the titration approaches the neutral point. The turbidity does not interfere with the estimation, since it is the same in the first neutralisation, *i.e.* before the addition of formaldehyde, as in the second neutralisation, so that, provided the tint, due to the phenolphthalein and the turbidity, is matched after each neutralisation, no error in the results is involved. Any difficulty in matching the tints may be avoided by adding two extra drops of alkali instead of one, so as to produce a still more definite pink coloration.

All the conditions present in the ordinary Kjeldahl process now being satisfied, two forms of procedure were devised according to whether there was a large or small amount of the material available for analysis.

A. Large amount of Material Available.—From 0·5 to 1 grm. (depending on the percentage content of nitrogen) of the material is weighed out and transferred to a Kjeldahl flask, and 15 c.c. of concentrated sulphuric acid and a crystal of copper sulphate added. The mixture is heated gently over a low flame until charring begins; 5 grms. of fused potassium sulphate are added, and the contents are then heated strongly until complete decarbonisation has taken place. This usually takes between 30 and 40 minutes. To ensure complete digestion the heating is continued for at least one hour after the liquid has become transparent.

The liquid is allowed to cool, 50 c.c. of distilled water added, and the mixture is boiled to expel any sulphur dioxide. After cooling, a piece of red litmus paper is added. Then 40 per cent. sodium hydroxide solution is run in cautiously, care being taken that the solution is left just acid, and that it is kept cool by immersing the bulb of the Kjeldahl flask under running water. The 40 per cent. sodium hydroxide solution added always contains appreciable quantities of carbonate, with the result that there is much carbon dioxide in the liquid; this fact makes it absolutely essential to boil before proceeding with the "formol titration" (*vide supra*). The slightly acid solution is transferred to a 250 c.c. graduated flask and made up to the mark, and is now ready for the titration of the nitrogen.

The procedure of the titration is divided into three definite parts, *viz.* (i) Preliminary neutralisation; (ii) "Accurate" neutralisation; (iii) Neutralisation of the acid liberated on addition of the formaldehyde. Twenty-five c.c. of the ammoniacal solution are pipetted into a flask, and 5 drops of a 1 per cent. solution of phenolphthalein added. The preliminary neutralisation, consisting in the neutralisation of the acid remaining after the approximate removal of the sulphuric

acid used in the digestion by 40 per cent. sodium hydroxide solution is now effected by adding approximately 0·1 *N* sodium hydroxide solution, drop by drop, until a definite alkalinity is obtained, the alkali being well washed from the sides of the containing vessel. The solution is now made just acid with 0·1 *N* sulphuric acid, and boiled vigorously to expel carbon dioxide. It is then cooled rapidly and treated with 0·1 *N* sodium hydroxide solution, drop by drop, until the change from colourless to pink is just apparent, after which one drop more is added, to produce a decided pink coloration. This is the "accurate" neutralisation and, if any other dilution of sodium hydroxide is used for the titration, this same dilution must be used in the "accurate" neutralisation. Five c.c. of a formaldehyde solution, the acidity of which has previously been determined, are added, and the mixture allowed to stand for a few minutes. The acid liberated is now titrated with 0·1 *N* sodium hydroxide solution to the first appearance of a colour, and on the addition of one drop further (or, if necessary, 2 drops), the original tint should be obtained. Correction is then made for the acidity of the formaldehyde solution, and the amount of nitrogen in mgrms. present in the 25 c.c. of the solution is calculated.

It is probable in some cases that the formaldehyde solution may not have been added in sufficient quantity to react quantitatively with the nitrogen present; under such conditions 5 c.c. more of the formaldehyde solution are added and if, after the deduction of the extra acidity due to the extra 5 c.c. of formaldehyde solution, there is no change in the titre representing the nitrogen, no further addition is necessary. Five c.c. of an undiluted 40 per cent. formaldehyde solution were found to be more than sufficient to react with 15 mgrms. of nitrogen.

It is advisable to make two or three estimations with 25 c.c. of the solution, and to take the mean of the readings.

The results obtained with a number of nitrogenous substances are shown in the following table:

TABLE I.

Substance.	By Formol Method Per Cent	By Distillation Method Per Cent	Difference
Ammonium sulphate	20·58	20·77	−0·19
Acetanilide	9·91	9·71	+0·21
Asparagine	18·16	18·45	−0·29
Amygdalin	2·46	2·51	−0·05
Alanine	15·40	15·56	−0·16
Peptone	13·93	14·19	−0·26
Quinine sulphate	5·22	5·07	+0·15
Succinamide	24·06	24·20	−0·14

B. SMALL QUANTITIES OF MATERIAL AVAILABLE.—In cases where quantities of only 0·05 to 0·1 grm. of material are available the digestion is carried out in a boiling tube, as employed in micro-Kjeldahl digestions. The material (0·05 grm.) is weighed out and transferred to a boiling tube, 7·5 c.c. of concentrated sulphuric acid and a small crystal of copper sulphate are added, and the tube heated over a

micro-Bunsen burner. As soon as charring begins 0·5 grm. of fused potassium bisulphate is added, and the liquid heated until colourless. It is then diluted and boiled, cooled and approximately neutralised, transferred to an Erlenmeyer flask, and titrated by the "formol" method.

Estimations of nitrogen in small quantities of the substances mentioned in Table I. gave results which did not vary from those obtained previously. Great care must be taken in the neutralisations that no alkali adheres to the side of the vessel, as considerable error can be introduced in this manner.

APPLICATION TO THE MICRO-KJELDAHL METHOD.—O. Folin and C. J. Farmer (*J. Biol. Chem.*, 1912, **11**, 493) were the first to estimate nitrogen (in urine) by a micro-Kjeldahl method. In the digestion of the urine they used a mixture of 83 per cent. syrupy phosphoric acid (5 volumes) and concentrated sulphuric acid (1 volume). This mixture was advantageous from the standpoint of digestion, but had the disadvantage of attacking and devitrifying the glass of the boiling tube. The estimation of the nitrogen in the digested liquid was made by direct nesslerisation.

The most recent contribution to the literature on micro-Kjeldahl work is that of Ling and Price (*J. Soc. Chem. Ind.*, 1923, **41**, 149T). These authors discarded the digestion method of Folin and Farmer and adopted a new method on similar lines to the digestion in the ordinary Kjeldahl method, the ammonia in the distillate being determined colorimetrically with Nessler's reagent.

Experiments were carried out in which the strength of the alkali used in the "formol titration" was lowered considerably, the strengths varying from 0·1 *N* to 0·02 *N*. Nitrogen was estimated in equal portions of the digested acetanilide solution remaining from the former analysis in Table I., 0·1 *N*, 0·05 *N*, 0·04 *N*, and 0·02 *N* sodium hydroxide solution being used successively. The procedure of the titration is identical with that already given, with the exception that the lower the concentration of alkali used, the greater the number of drops of phenolphthalein that must be used. The results obtained were reduced to terms of 0·1 *N* alkali for comparison, and only a very slight divergence, amounting to a fraction of a mgrm. of nitrogen, was noted.

TABLE II.

Acetanilide solution taken	Strength of alkali used	Equivalent of nitrogen in 0·1*N* NaOH	Theoretical equivalent 0·1 *N* NaOH	Variation
c c	*N*	c c	c c	c c
25	0·1	6·55	6·55	—
25	0·05	6·51	6·55	0·04
10	0·04	2·63	2·62	0·01
10	0·02	2·64	2·62	0·02

From these figures it is clear that 0·02 *N* alkali may be used with accuracy in the "formol" titration. It is also evident that the error made in the titration is equivalent to 0·02 c.c. of sodium hydroxide solution, indicating an error of 0·028 mgrm. of nitrogen.

Two series of experiments were made with a standard ammonium sulphate solution. In Series I., 5 c.c. of a formaldehyde solution, containing 25 c.c. of 40 per cent. formaldehyde diluted to 100 c.c. were used; and in Series II., 5 c.c. of a formaldehyde solution containing 20 c.c. of 40 per cent. formaldehyde. The results obtained are given in Table III.

TABLE III.

Series.	Nitrogen by "Formol" Micro-Kjeldahl method. Mgrm.	Nitrogen calculated from "formol" method. Mgrm.	Error. Mgrm.
1.	0·802	0·824	−0·022
1.	0·423	0·412	+0·011
1.	0·211	0·206	+0·005
11.	0·825	0·824	+0·001
11.	0·409	0·412	−0·003

The degree of accuracy obtainable with the solution of digested quinine sulphate (*supra*) is shown in Table IV.

TABLE IV.

Amount of nitrogen solution taken. c.c.	Nitrogen found. Mgrm.	Nitrogen calculated. Mgrm.	Error. Mgrm.
2	0·409	0·418	−0·009
1	0·204	0·209	−0·005

The digestion is carried out under the conditions of Ling and Price (*loc. cit.*). An accurately weighed amount of the substance, containing approximately 1 mgrm. of nitrogen, is introduced into a boiling tube of hard glass, together with 1 grm. of fused potassium sulphate and 0·02 grm. of anhydrous copper sulphate. Five c.c. (*not* 8 c.c) of concentrated sulphuric acid are added, together with two drops of a 2·5 per cent. platinum tetrachloride solution. A small funnel is placed in the mouth of the tube, and the contents are boiled gently until the liquid is colourless. The liquid is cooled, 10 c.c. of distilled water are added, and the solution boiled to expel sulphur dioxide.

The solution is again cooled and approximately neutralised with 40 per cent. sodium hydroxide solution, the amount added not exceeding 15 c.c., thus leaving 20 c.c. for the subsequent washing. The solution is now transferred to a 50 c.c. graduated flask, the boiling tube being well washed out. The nitrogen in an aliquot portion of the resulting liquid, say 10 c.c., is now estimated under the conditions necessary for the "formol titration" (*supra*).

When the nitrogenous material to be analysed is in the liquid form, as, for example, in the case of vaccines, a slightly modified digestion is necessary. If the 5 c.c. of concentrated sulphuric acid are added to the vaccine and heated, there is

an inclination to excessive bumping, which must be avoided. To overcome this, 5 c.c. of the vaccine are pipetted into the boiling tube, 1 c.c. of concentrated sulphuric acid is added, and the tube immersed in a boiling water bath until a slight amount of charring occurs; the remainder of the 5 c.c. of concentrated acid is now added, and the digestion continued in the usual manner.

The author wishes to thank Professor A. R. Ling and Mr. D. R. Nanji for their advice and encouragement in the course of this work.

UNIVERSITY OF BIRMINGHAM,
DEPARTMENT OF THE BIOCHEMISTRY OF FERMENTATION.

The Pemberton-Neumann Method for the Estimation of Phosphorus.

BY MARION B. RICHARDS AND WILLIAM GODDEN.

(*Read at the Meeting, October* 1, 1924.)

THE authors have had occasion to use this method for the estimation of phosphorus in a large number of samples of urine and fæces, and at the outset, during a trial of the method, found certain discrepancies in the results if the conditions of experiment were varied to any marked extent. An examination of the literature showed that this fact was recorded by numerous workers, who had also investigated the cause. There was, however, no definite agreement as to the cause of the errors or as to the steps to be taken to avoid them. Accordingly it was decided to enquire further into the method, with a view to establishing a definite procedure which would give consistent results with varying amounts of material.

ORIGINAL METHOD.—The method, as originally outlined by Pemberton[1] for inorganic phosphates, is briefly as follows:—The phosphate is dissolved in nitric acid, the solution made just alkaline with ammonia, and 5 c.c. of nitric acid (sp. gr. 1·4) and 10 c.c. of a saturated solution of ammonium nitrate are added. Into this solution, when boiling, a slight excess of a cold aqueous solution of ammonium molybdate is run, and, as soon as the precipitate has settled, it is filtered off, washed with cold water and dissolved in a known volume of 0·5 *N* potassium hydroxide solution. The excess of alkali is then titrated back in the cold with 0·5 *N* sulphuric acid, without boiling off the ammonia, phenolphthalein being used as indicator. Pemberton gives the molecular ratio of P_2O_5 to KOH as 1:46, *i.e.* 1 c.c. of 0·5 *N* KOH is equivalent to 0·001543 grm. of P_2O_5.

Neumann[2] modifies this method, in that he precipitates in the presence of sulphuric and nitric acids, using 50 c.c. of a 50 per cent. solution of ammonium nitrate instead of 10 c.c. of a saturated solution, and he precipitates at 70°–80° C. Finally he boils his precipitate with an excess of 0·5 *N* sodium hydroxide solution to remove the ammonia and then titrates back the excess alkali, in the cold, with

0·5 *N* acid. He gives the molecular ratio P_2O_5 to NaOH as 1:56, *i.e.* 1 c.c. of 0·5 *N* NaOH is equivalent to 0·001268 grm. of P_2O_5.

COMPOSITION OF PRECIPITATE AND FACTORS.—It should be noted that both these authors cite Hundeshagen[3] as to the composition of the phosphomolybdate precipitate, but each takes a different formula without, apparently, having paid particular attention to the details given by Hundeshagen. This author found that the composition of the ammonium phosphomolybdate precipitate, when obtained under the most varied conditions, after washing with cold dilute nitric acid, and drying at 130°–150° C., in all cases corresponded in composition with the formula $(NH_4)_3PO_4$, $12MoO_3$. This was the case whether the precipitation took place in strongly or weakly acid solution, whether in the presence of nitric, hydrochloric or sulphuric acid, whether concentrated or dilute, in hot or cold solution, with phosphate or molybdic acid in excess, and whether other salts (*e.g.* of potassium) were present or not.

If precipitated in the presence of excess of nitric acid, washed with cold dilute nitric acid and dried in a desiccator over calcium chloride and potassium hydroxide to constant weight, the precipitate had the composition $(NH_4)_3PO_4$, $12MoO_3$, $2HNO_3$, H_2O. The acid and water were probably in loose combination and were quickly and completely removed by heating at 150° C.

Pemberton, in calculating his factor, took the formula without the two molecules of nitric acid and did not allow for the alkali necessary to replace the ammonia, as this was not removed by boiling. Neumann, on the other hand, took the formula with the two molecules of acid and allowed for the alkali necessary to replace the ammonia removed by boiling. Both of these workers washed their precipitate with water, Pemberton stating, contrary to Hundeshagen's findings, that the precipitate is not dissolved by the water even if one litre is used for the washing. Their two equations are respectively:—

$$2(NH_4)_3PO_4,\ 24MoO_3 + 46NaOH = (NH_4)_4H_2P_2O_8 + (NH_4)_2MoO_4 + 23Na_2MoO_4 + 22H_2O$$

$$2(NH_4)_3PO_4,\ 24MoO_3,\ 4HNO_3 + 56NaOH = 24Na_2MoO_4 + 2Na_2HPO_4 + 4NaNO_3 + 6NH_3 + 32H_2O.$$

Gregersen[4] modified Neumann's method in so far as the treatment of the precipitate is concerned. After boiling with the 0·5 *N* acid he recommends the addition of a slight excess of the acid and second boiling to expel carbon dioxide, the solution being finally titrated back when cold with the 0·5 *N* sodium hydroxide solution. He apparently retains the factor, 1 c.c. of 0·5 *N* NaOH = 0·001268 grm. P_2O_5, as given by Neumann.

Heubner[5], and later Jodidi[6], examined Neumann's method and were in agreement that Neumann's factor is too low. They both recommend as the factor 1 c.c. of 0·5 *N* NaOH = 0·0057 grm. of P (or 0·001305 grm. of P_2O_5), basing their figure, not on any equation, but by checking it against pure phosphate solutions which had been analysed gravimetrically. Wardlaw[7] suggests that the factor needs to be varied with the amount of phosphorus present.

PHOSPHORUS IN BIOLOGICAL MATERIAL.—The method has in all cases been tested upon pure inorganic phosphates, but in the estimation of phosphorus in biological material the process for the conversion of the phosphorus into phosphoric acid and the subsequent preparation of the solution for the precipitation need to be considered. Neumann (*loc. cit.*) recommends that the material should be digested with 20 c.c. of a mixture of equal volumes of concentrated sulphuric and nitric acids, more of this mixture being added from time to time if necessary. This solution he simply dilutes and then proceeds as described. Gregersen (*loc. cit.*) uses 20 c.c. of the acid mixture at the commencement, as described by Neumann, but, if further acid is necessary, adds nitric acid alone, as had been previously recommended by Plimmer and Bayliss[8]. Further, he does not neutralise after dilution before the precipitation. The procedure we have adopted, as being more in accord with Hundeshagen's results, is to carry out the oxidation as described by Gregersen, thereby limiting the amount of sulphuric acid to 10 c.c. Subsequently, when oxidation is complete, the solution is diluted to 200 c.c., made just alkaline with ammonia and then just acid with nitric acid. Thirty c.c. of a 50 per cent. solution of ammonium nitrate are now added, thus bringing the concentration of the ammonium nitrate in the total bulk of solution to about 7 per cent. (*cf.* Hundeshagen). The precipitation is carried out at 70 to 75° C., a solution of ammonium molybdate, prepared, as described by Mathews[9], from ammonium molybdate and not from molybdic acid, being used. We do not wash the precipitate with water only, but twice with 10 per cent. nitric acid to remove any excess of ammonium molybdate, three to four times with 2 per cent. ammonium nitrate solution to remove the acid, and then twice with cold water to remove the ammonium nitrate. The reason for this will be discussed later. The filtration and washing are carried out with the aid of suction on a Hirsch funnel, a small disc of hardened filter paper being used. By this means washing is more rapid and thorough, and the amount of filter paper present during the boiling with the alkali is reduced to a minimum.

EXPERIMENTAL.—The possible sources of error in the method, which were examined, were:—(1) Variation in the volume of semi-normal alkali used to dissolve the precipitate by Neumann's method; (2) failure to boil with excess of acid before final titration; (3) variation in the amount of phosphate present keeping the volumes of precipitation reagent constant; and (4) variation in the volume of ammonium molybdate used for the precipitation. Finally the correct factor to be used in the calculation had to be considered in the light of these results.

(1) *Variation in the volume of standard alkali used to dissolve the precipitate.*—When boiling the precipitate with alkali by Neumann's method, taking no particular precautions as to the amount of the alkali in excess, it was found that duplicate results often showed wide variations. Accordingly a solution of pure sodium phosphate was prepared which, on analysis, both gravimetrically and by the uranium acetate method, was found to contain 0·02575 grm. of P_2O_5 in 25 c.c. Of this solution, 25 c.c. portions were pipetted out, and the precipitate obtained was filtered off and washed as described above. This precipitate was dissolved

in varying amounts of 0·5 *N* sodium hydroxide solution, and, after the liquid had been boiled for twenty minutes and cooled, the excess of alkali was titrated.

TABLE I.

Vol. of 0·5 *N* NaOH used to dissolve precipitate.	Vol. of 0·5 *N* H_2SO_4 required for back titration.	P_2O_5 in 25 c.c. of solution, calculated with Neumann's factor.
c.c.	c.c.	Grm.
20·00	0·15	0·02517
20·00	0·15	
21·00	0·95	0·02542
21·00	0·95	
22·00	1·70	0·02574
22·00	1·70	
24·00	3·20	0·02637
24·00	3·20	
26·00	5·00	0·02663
26·00	5·00	
28·00	6·85	0·02681
28·00	6·75	0·02694

Thus the amount of alkali used to dissolve the precipitate, under Neumann's conditions, played a considerable part in determining the result, which was by no means constant when the amount of alkali varied. The most accurate result was obtained when 1 to 2 c.c. in excess of alkali was used. (Neumann used 5 to 6 c.c. in excess.)

(2) and (3) *Failure to boil with excess of acid and variations in the amount of phosphate present.*—For this purpose a solution of pure potassium dihydrogen phosphate was prepared and analysed gravimetrically, (*a*) by weighing the phosphorus as $Mg_2P_2O_7$, and (*b*) as KPO_3.

50 c.c. of the solution contained by (*a*) 0·02589 P_2O_5.
50 c.c. of the solution contained by (*b*) 0·02596 P_2O_5.

The mean figure 0·02593 was taken.

Varying amounts of this solution were used, and the estimations carried out as usual, the end-point being obtained first under Neumann's conditions and then under Gregersen's conditions. The results were all calculated for 50 c.c. of the original solution and are given in Table II.

TABLE II.

	Neumann's conditions.		Gregersen's conditions.
Vol. of phosphate solution taken.	0·5 *N* alkali consumed.	Same calculated for 50 c.c. of solution.	0·5 *N* alkali used calculated for 50 c.c.
c.c.	c.c.	c.c.	c.c.
5	2·60	26·00	19·04
10	4·81	24·05	19·04
20	8·14	20·35	19·04
30	12·10	20·16	19·08
40	15·67	19·59	19·08
50	19·49	19·49	19·04

With Neumann's factor volume of 0·5 *N* alkali should have been 20·45 c.c.
,, proposed new ,, ,, ,, ,, ,, ,, ,, ,, 19·00 c.c.

It will at once be seen that concordant results are not obtained with varying amounts of phosphate, under Neumann's conditions, but that the agreement is excellent if Gregersen's modification of boiling with the excess of acid, prior to the final titration, is adopted. The question of the proposed new factor will be discussed later.

That this discrepancy between the results, under Neumann's conditions, is due to carbon dioxide absorbed by the alkali during the boiling and cooling, as stated by Gregersen, is readily shown by the following data. Ten c.c. portions of a solution of sodium phosphate were taken, and diluted to 200 c.c., and to each of them were added 5 c.c. or 10 c.c. of 0·5 *N* sodium hydroxide solution. The solutions were then boiled in a round-bottomed flask for twenty minutes and, after cooling, titrated back with 0·5 *N* sulphuric acid in the presence of phenolphthalein. An excess of acid was next added and, after further boiling for twenty minutes, the solutions were again cooled and titrated back with 0·5 *N* sodium hydroxide solution. The results are given in Table III.

TABLE III.

Before boiling with excess of acid.			After boiling with excess of acid.		
Vol. of 0·5 *N* NaOH added.	Vol. of 0·5 *N* H_2SO_4 required.	Difference.	Total Vol. of 0·5 *N* NaOH used.	Total Vol. of 0·5 *N* H_2SO_4 used.	Difference.
c.c.	c.c.	c.c.	c.c.	c.c.	c.c.
5	4·3	0·7	6·0	6·0	0·00
5	2·85	2·15	6·0	5·95	0·05
10	8·90	1·10	11·0	10·90	0·10
10	6·65	3·35	11·0	10·95	0·05
10	8·25	1·75	11·0	11·00	0·00

Had there been no absorption of carbon dioxide the figures in columns (1) and (2) should have been identical. They are different, however, and there is no regularity, owing probably to the different rate of boiling, time of cooling, and state of the atmosphere. After expelling the carbon dioxide, however, by boiling with excess of acid the agreement is, with one exception, excellent. Finally, when the experiment was repeated, with 10 c.c. of 0·5 *N* NaOH, with boiling and cooling under such conditions as to exclude carbon dioxide, the first titration required 10 c.c. of 0·5 *N* sulphuric acid, and boiling with excess of acid did not alter the agreement. (Jodidi recommends the use of a correction of 0·2 c.c., based on a series of blank analyses to compensate for the errors due to (*a*) impurities in the reagents, (*b*) carbon dioxide in the liquids to be titrated, and (*c*) acidity caused by the action of the boiling alkali on the filter paper (a 12½ cm. folded paper). In the light of the above figures such a correction is not permissible.)

(4) *Variation in the volume of molybdate solution used for the precipitation.*—A solution of disodium hydrogen phosphate, containing 0·01574 grm. of P_2O_5

in 25 c.c. was used. Separate 25 c.c. portions were treated with varying volumes of molybdate solution, as shown, but otherwise the procedure described above was followed, and the liquid was boiled with excess of acid before the final titration.

TABLE IV.

Vol. of molybdate used. c.c.	Vol. of 0·5 *N* NaOH consumed. c.c.	Vol. of 0·5 *N* NaOH calculated from.
10	11·48	Factor 0·001365
20	11·50	11·53 c.c.
30	11·52	Factor 0·001268
50	11·65	12·41 c.c.

NOTE.—Ten c.c. of this molybdate solution are about 1 c.c. in excess of that required theoretically to precipitate all the phosphate which was present.

Within reasonable limits, therefore, the volume of the molybdate solution used for the precipitation does not interfere with the results.

FACTOR.—It will be noticed that in Tables II. and IV. a new factor (1 c.c. of 0·5 *N* NaOH $\equiv$ 0·001365 grm. of P_2O_5) has been used, and this is the factor by means of which it is proposed all calculations should be made under the conditions laid down in this paper. It is based in the first place on the work of Hundeshagen (*loc. cit.*), and in the second place on our own experimental results. Hundeshagen has shown that, while the composition of the yellow 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 (*a*) by heating at 130° C., and (*b*) by washing with neutral aqueous ammonium nitrate solution. He suggests that it is possible that this nitric acid may be replaced in the molecule by ammonium nitrate under such conditions. As, however, we follow the washing with the ammonium nitrate by washing with cold water, we are of the opinion that the resulting precipitate left on the filter paper has the composition $(NH_4)_3$, PO_4, $12MoO_3$. This is confirmed by the figures given in Tables II. and IV. Further confirmation is given by the following data. Three separate 50 c.c. portions of the solutions used for the experiments recorded in Table II. were treated with molybdate, and the precipitates filtered off and washed in the usual manner. In two cases the precipitates were heated at 150° C. before solution in the standard alkali, whilst the third precipitate was dissolved at once. The following results were obtained:

Duration of heating at 150° C.	Vol. of 0·5 *N* NaOH used up. c.c.	Vol. of 0·5 *N* NaOH calculated with factor 0·001365. c.c.
2½ hours	19·00	—
4½ ,,	19·02	19·00
nil	19·02	—

The heating at 150° C. would certainly, according to Hundeshagen, remove any loosely-combined nitric acid and, as the heating in this manner has not affected the results, the precipitate left under the given conditions must be free from combined nitric acid. Thus the reaction with the alkali will be represented by— $2(NH_4)_3PO_4,24MoO_3 + 52NaOH = 24Na_2MoO_4 + 2Na_2HPO_4 + 28H_2O + 6NH_3$; the molecular ratio of P_2O_5 to NaOH is 1:52, and the factor 1 c.c. of 0·5 *N* NaOH = 0·001365 grm. of P_2O_5.

PROPOSED METHOD FOR BIOLOGICAL MATERIAL.—The following details are given for the estimation of total phosphorus in fresh fæces: From 1·5 to 2·0 grms. are weighed out and transferred to a 500 c.c. round-bottomed flask, 10 c.c. of concentrated sulphuric acid and 10 c.c. of concentrated nitric acid are added, and 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, a further 5 c.c. 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. Thirty c.c. of a 50 per cent. solution of ammonium nitrate are added, the liquid is heated at 70 to 75° C., and 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 one hour is sufficient, but the result is not altered if it is left to stand overnight). It is filtered off, by suction, on a disc of hardened filter paper in a Hirsch funnel, and is washed twice with 10 per cent. nitric acid, three to four times with 2 per cent. ammonium nitrate and twice with cold water. The precipitate and filter paper are washed back into the precipitation flask with cold water and dissolved in a known volume of 0·5 *N* sodium hydroxide solution (about 1 c.c. in excess being used). The solution is diluted to about 250 c.c. and boiled for twenty minutes. While still warm it is titrated back with 0·5 *N* sulphuric acid, phenolphthalein being used as indicator, and an excess of 1 to 2 c.c. of acid is run in. Boiling is repeated for 15 minutes and, after cooling, the excess acid is accurately titrated with 0·5 *N* sodium hydroxide solution. The end-point is taken as soon as the first definite pink tint is obtained, although the colour will be found to fade fairly rapidly. The total alkali used, less the total acid, gives the volume of alkali equivalent to the P_2O_5 in the original weight of fæces (1 c.c. of 0·5 *N* NaOH = 0·001365 grm. of P_2O_5). It is desirable that the actual amount of phosphoric anhydride present for precipitation should not exceed 22 mgrms., or there may be some difficulty in washing the precipitate completely free from nitric acid and ammonium nitrate.

Note.—The authors have found one Winchester of nitric acid out of a large consignment to contain very decided amounts of phosphoric anhydride. One lot of ammonium nitrate also contained 0·003 per cent. of P_2O_5. The impurity in the nitric acid was presumably due to faulty washing of the containing vessel prior to its being filled by the manufacturer.

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The Estimation of Cadmium in Brass.*

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(*Read at the Meeting, November* 5, 1924.)

CADMIUM is associated with zinc in zinc ores, and is therefore present in commercial zinc, and, consequently, in brass. The amount found in brass varies from a mere trace (less than 0·01 per cent.) up to 0·2 per cent., depending on the quality of the zinc used. The effect of cadmium on the properties of brass is not yet completely known. In particular cases specifications are laid down fixing a maximum amount permissible, usually 0·05 per cent.

Cadmium sulphide can be precipitated practically free from zinc from slightly acid solutions, but it is necessary to remove copper first. In the work described in this paper the electrolytic method, which has great advantages over chemical methods, was used. It is necessary to take at least 5 grms. of brass. If the copper is removed as thiocyanate, in addition to the difficulty of filtering and washing, it is almost certain that a large proportion of the cadmium will be occluded in the precipitate. If the copper is removed as metal by sodium hypophosphite it is impossible to adjust the solution afterwards for the precipitation of cadmium in presence of zinc, on account of the phosphoric acid formed. In the electrolytic method 5 grms. of brass can be easily handled and no disturbing compounds are left behind.

The method is as follows:—Five grms. of brass, preferably drillings or turnings (freed from iron particles by a magnet) are dissolved in a mixture of 80 c.c. of dilute sulphuric acid (1 vol. conc. acid to 3 vols. water) and 7 c.c. of nitric acid (sp. gr. 1·42) in a 400 c.c. beaker. When the brass is dissolved and the nitrous fumes have been expelled, 150 c.c. of water are added and the beaker is cooled in running water for about 1 hour. Any lead present is partly precipitated at this stage as lead sulphate. The precipitation is not quite complete owing to the

* Communication from the Research Dept., Woolwich.

slight solubility of lead sulphate in liquids containing small amounts of nitric acid. As it is important that there should be no suspended lead sulphate carried forward to the next stage, it is better in all cases to pass the liquid through a small close pulp filter, whether any lead is visible or not. This point is referred to again later. The filtrate is caught in a 400 c.c. squat beaker and with the washings its volume is about 200 c.c. The electrolysis is carried out in the manner described by the author in the paper on "Analysis of Copper-Tin Alloys" (ANALYST, 1924, 371). A rotating platinum gauze cathode and platinum foil anode are used. The initial current is 4 amps. After 30 minutes 0·5 grm. of urea is added to destroy nitrous acid. When the liquid becomes colourless the current is continued at 4 amps. for another 30 minutes, after which it is reduced by ½ amp. every 15 minutes. At each change 0·5 grm. of urea is added. At the end of the last 15 minutes at ½ amp. the beaker is lowered away from the cathode, which is washed from the wash-bottle jet at the same time. The anode is also washed and lifted out. Traces of lead will probably be observed, as peroxide, on the anode, due to the presence of nitric acid, in which lead sulphate is slightly soluble. The remainder of the lead thus held in solution is completely separated on the anode at this stage. It would not be safe, however, to assume that suspended lead sulphate could be quantitatively transferred to the anode in this manner; hence the necessity of filtering before electrolysis. There must be no lead in the liquid after electrolysis, as it would contaminate cadmium separated in subsequent stages.

The liquid left contains all the cadmium and zinc, and the following metals may also be present: Arsenic, antimony, tin, bismuth, iron, nickel, manganese. No cadmium is deposited on copper under the conditions given, but antimony and bismuth are partly precipitated, as shown by discoloration of the deposited copper. The next step is to remove all nitric acid by careful evaporation to incipient fuming. This operation requires several hours, and must be carefully watched to avoid spirting when zinc sulphate begins to separate out. The elimination of nitric acid is necessary for the subsequent adjustment of acidity for the next operation.

After cooling, 200 c.c. of cold water are added quickly, and the liquid is stirred to dissolve the sulphates. Over-heating must be avoided in case tin is present. (Admiralty brass contains 1 per cent. of tin.) The liquid is cooled and neutralised, as shown by litmus paper, with dilute ammonia (one vol. strong ammonia to one vol. water). The volume should be approximately judged (usually 300 c.c.); it is then adjusted to correct acidity for precipitation of cadmium by adding 7 c.c. of dilute sulphuric acid (1 vol. conc. acid to 3 vols. water) per 100 c.c.

FIRST ACID PRECIPITATION.—The liquid is cooled, hydrogen sulphide is bubbled through it in a rapid stream for 30 minutes, and the beaker is then set aside to stand over-night or for at least 12 hours. The cadmium sulphide is not usually visible at this stage unless it is large in amount (of course it may be obscured by tin). On standing, it gradually collects and falls to the bottom of the beaker. It is probably in a colloidal state at first, like arsenic sulphide. The

failure to recognise the importance of long standing has probably led to recommendation of the use of trichloracetic acid instead of sulphuric acid; also to the practice of purposely allowing some zinc sulphide to form, and increasing the acidity afterwards. These variations may cause cadmium sulphide to collect more rapidly, but they have disadvantages. In any case, in dealing with minute precipitates, it is generally a mistake to attempt to filter too soon after precipitation. Sulphuric acid is quite satisfactory in this case if long standing is allowed.

The precipitate may contain, as well as cadmium sulphide, traces of copper (if directions for electrolysis are not properly followed), tin, arsenic, antimony, bismuth and zinc, but it is nearly free from iron, nickel and manganese. The precipitate is filtered on a paper pulp filter and washed with 1 per cent. ammonium nitrate solution. Washing with water tends to carry some precipitate through the pulp. If tin is present as a constituent, as in Admiralty brass, the bulk of the precipitate is large. In that case some zinc sulphide is certain to be in it, apart from the impossibility of washing all the zinc sulphate out. In the absence of tin the amount of zinc entangled is small, but it is always present at this stage.

Arsenic is probably partly removed as hydrogen arsenide during electrolysis, but the remainder is precipitated at this stage. Antimony and bismuth are partly deposited on the cathode, as mentioned above, and the remainder is precipitated at this stage. Iron, etc., are washed out (except traces), the amount left depending on the bulk of the precipitate.

First Alkaline Precipitation.—The precipitate is dissolved in hot dilute (1:1) *aqua regia* (1 vol. nitric acid of sp. gr. 1·42, and 3 vols. hydrochloric acid of sp. gr. 1·2 and 4 vols. water) and the filtrate is caught in the original beaker. After boiling for a few minutes to decompose the sulphides and cooling to room temperature, it is made alkaline with a slight excess of dilute (1:1) ammonia solution, and a crystal of potassium cyanide is added in cases where traces of copper are suspected. Hydrogen sulphide is then passed through in a rapid stream for about 15 minutes. Tin is precipitated by ammonia but is re-dissolved after hydrogen sulphide has passed through for a short time. The precipitate is cadmium sulphide together with any zinc and bismuth in the first "acid precipitate," but is free from arsenic, antimony and tin, if these were only present as traces in the original alloy. If tin is a constituent of the alloy, traces of tin will still remain in the precipitate. Traces of iron are also usually to be found here. It is not necessary to allow this precipitate to stand for 12 hours; cadmium sulphide collects very quickly from an alkaline liquid and may be filtered after 2 hours' standing. It is washed, as before, with 1 per cent. ammonium nitrate solution.

Second Acid Precipitation.—The precipitate is dissolved as before, the liquid is caught in a 200 c.c. tall narrow beaker, and after the addition of 20 c.c. of dilute sulphuric acid, it is evaporated until fumes appear. After cooling and diluting to 80 c.c. it is neutralised with ammonia. The volume is judged (usually 100 c.c.) and the acidity adjusted, as before, by the addition of 7 c.c. of dilute sulphuric acid per 100 c.c. When cool, a rapid current of hydrogen sulphide is

passed through for 30 minutes, and the liquid is set aside for at least 12 hours, as in all "acid precipitations."

The precipitate contains all the cadmium, with traces of tin, if tin was present originally as a constituent, with part of the original bismuth, which also follows the cadmium throughout after the electrolysis stage, but it is now nearly free from zinc and quite free from iron, nickel, etc.

SECOND "ALKALINE PRECIPITATION."—(If tin, arsenic and antimony are present as impurities only in the original alloy, this stage should be omitted.) The precipitate is filtered off, dissolved, etc., exactly as in the first alkaline precipitation (with omission of potassium cyanide, which is unnecessary at this stage). This eliminates the remaining traces of tin in cases where tin is present as a constituent.

THIRD "ACID PRECIPITATION."—This is exactly the same as the second acid precipitation. This stage eliminates the remainder of the zinc, and the precipitate is now pure cadmium sulphide, possibly contaminated with bismuth sulphide.

Weighing as Cadmium Sulphate.—The cadmium sulphide is filtered off and dissolved as before. In this stage the liquid is caught in a small beaker and, after addition of 5 c.c. of dilute sulphuric acid, it is evaporated on the hot plate until fumes appear. After cooling and dilution, it is transferred to a weighed platinum basin of about 100 c.c. capacity, evaporated to dryness on the hot plate, and ignited in a low temperature muffle for a few minutes, to drive off all traces of sulphuric acid. The increase in weight of the dish gives cadmium sulphate and bismuth sulphate, together with residue from the acids used in this stage. It is therefore necessary to know the volume of acid used and to carry out a blank test, pouring the same volume of acid through a pulp filter made from the same pulp, evaporating and igniting as described. The blank may amount to 0·02 per cent. on 5 grms. taken, and therefore cannot be ignored. The net weight of cadmium sulphate includes the bismuth which has escaped deposition on the cathode in the electrolysis. Bismuth follows cadmium throughout after that stage. No brasses have been met with containing so much as 0·01 per cent. or even 0·005 per cent. of bismuth. It is, therefore, not necessary to consider the separation of bismuth and cadmium. Since some of the bismuth is deposited on the copper, it is hardly likely that the cadmium sulphate would ever be appreciably contaminated. Bismuth can easily be estimated in the residue, after weighing, by the colorimetric method with potassium iodide.

CONCLUSION.—The process is lengthy and the manipulation considerable, but the purity of the cadmium sulphide finally obtained is assured and the loss by manipulation is negligible.

DISCUSSION.

Mr. J. MYERS, who read the paper on behalf of Mr. Etheridge, said that the chief point about the author's method was that it was designed to separate very small quantities of cadmium when very large quantities of other metals were

present. Copper and lead having been removed, the problem resolved itself into separating cadmium sulphide from large quantities of zinc. The method meant a lengthy manipulation, several hydrogen sulphide separations being involved, and, in his opinion, it compared unfavourably with the method published by Barr in the *J. Soc. Chem. Ind.* (1924, **43**, 24), which had been evolved for the estimation of small quantities of cadmium in zinc ores. From the results of his experience that method was twice as quick as the author's method, and he suggested that Mr. Etheridge should try Barr's method.

Dr. B. S. EVANS said that, so far as he knew, Mr. Etheridge had not seen Barr's paper, and he would certainly draw his attention to it.

The length of the process was undoubtedly a serious drawback, but Mr. Etheridge had told him that, in his experience, the Admiralty bronzes did not contain cadmium, and this, in practice, cut down the number of hydrogen sulphide precipitations. He hoped to publish shortly a method for elimination of tin that would in any case render unnecessary the three alkaline sulphide separations, but all methods of cadmium estimation had the common drawback of the uncertainty of the last weighing; it was necessary to keep the temperature of ignition between that at which sulphur trioxide was volatilised and that at which cadmium sulphate was decomposed, and these limits seemed to be rather narrow. The high blank obtained with the acids was another source of trouble.

Dr. W. R. SCHOELLER (in a written communication) said that the process described was tedious, but that the estimation of cadmium in brass was bound to be troublesome, especially if tin were also present. The only criticism he had to offer was on the statement that, if copper were precipitated as thiocyanate, it was almost certain that a large proportion of the cadmium would be occluded in the precipitate. The precipitation of copper as thiocyanate was a very valuable reaction, extensively applied in metallurgical laboratories; the separation, by this means, of copper from many other metals had been investigated by Hampe and others, and found to be satisfactory. Therefore, a statement calculated to cast doubt on the accuracy of a well-known analytical method should not be made unless substantiated by experimental evidence.

Notes.

The Editor wishes to point out that the pages of the Journal are open for the inclusion of short notes dealing with analytical practice and kindred matters. Such notes are submitted to the Publication Committee in the usual manner.

INVERT SUGAR AS A REAGENT FOR BORIC ACID ESTIMATIONS.

THE writer was the first to show the effectiveness and cheapness of invert sugar as a reagent in the titration of boric acid (ANALYST, 1921, **46**, 3). Since then Böeseken and Couvert (*Rec. trav. chim.*, 1921, **40**, 354) and, more recently, Mellon and Morris (*J. Ind. Eng. Chem.*, 1924, **16**, 123) have arrived at similar conclusions. There should now be no hesitation in adopting invert sugar instead of the older and more expensive reagents—glycerin and mannitol.

It has been found more convenient to prepare the reagent by inverting with sulphuric acid rather than with hydrochloric acid, because in routine analysis, particularly in the case of butter and margarine, it is often necessary to estimate salt as well as boric acid in the same solution, the boric acid being estimated first; and, if the inversion has been done with hydrochloric acid, the invert sugar will contain a small amount of chloride, an allowance for which would have to be made.

The following method for making a laboratory stock solution will be found useful:—Dissolve 7lb. of commercial granulated sugar in 1 litre of distilled water and boil the solution for a few minutes until clear (it is best to do the operation in a large tin). Remove the source of heat and add quickly 25 c.c. of 3 *N* sulphuric acid from a beaker, stir for half a minute, and then add 1½ litres of distilled water in which has been previously mixed 25 c.c. of 3 *N* sodium hydroxide solution, again stir and cool. The solution should be neutral and almost colourless.

The resulting volume is about 4½ litres and the solution contains approximately 55 per cent. of invert sugar. Three c.c. of this solution are sufficient to enable 10 c.c. of 0·1 *N* boric acid solution to be titrated.

G. VAN B. GILMOUR.

THE COMPOSITION OF COCONUT OIL.

NOTWITHSTANDING the apparently good results which I have obtained by the fractional distillation of the methyl esters of coconut oil, I find myself more or less in agreement with the conclusions of Professor Drummond (ANALYST, 1924, **49**, 311) and of Mr. Elsdon (ANALYST, 1924, 423).

The assumption made by certain American investigators and others that the individual fractions obtained in the distillation consist solely of two esters, together with oleic ester, is without any scientific justification. In my work, duplicate distillations yielded results which agree very closely, but this I am inclined to attribute to the fact that the two operations were carried out under identical conditions (*i.e.* as regards the apparatus used, rate of distillation, etc.) rather than to the accuracy of the method. Hence duplicate fractionations made by the same observer will probably show a much closer agreement than results obtained in different laboratories. I am also of opinion, in agreement with Mr. Elsdon, that the differences in the composition of different samples of coconut oil, as judged by their analytical characteristics, are not sufficient to account for the variations in the quantitative results.

Mr. Elsdon quotes in his paper (*loc. cit.*) a quantitative analysis of coconut oil, referred to by Dr. E. F. Armstrong and Mr. John Allan in the recent Presidential address to the Society of Chemical Industry (*J. Soc. Chem. Ind.*, 1924, **43**, 207T), and he evidently believes that these figures have been obtained by the method of alcoholysis. This is not the case, for the figures are those published by Paulmeyer (*La Savonnerie Marseillaise*, 1907, 78), and were obtained by fractional distillation of the fatty acids in steam.

This may have caused Mr. Elsdon to be more severe in his condemnation of the method of alcoholysis than he would otherwise have been, for it cannot be gainsaid that many workers of repute seem to have obtained very fair results with the method; besides which, the results of my own work are not so widely different from those of Elsdon, though they differ more from those of Paulmeyer.

While admitting, therefore, the unsound theoretical aspect of the method, I am inclined to the opinion that the method gives approximate values when applied to coconut oil or palm-kernel oil, and that the degree of accuracy is within

± 10 per cent. of each fraction, *i.e.* the error on a figure of 50 per cent. would be of the order of ± 5 per cent., for a 10 per cent. component, ± 1 per cent., etc.

As an example of the figures obtained with samples of different origin, the following results given by deodorised coconut oil refined in Hull and in Holland, respectively, may be quoted:

The oils had the subjoined analytical characteristics:

	Sapon. value.	Iodine value.	Reichert-Meissl value.	Polenske value.	Kirschner value.	M.Pt. °C	Free fatty acid (as lauric) Per cent.
A. English	258·0	9·2	7·15	16·0	1·81	25·3	0·05
B. Dutch	258·9	8·0	7·54	16·6	1·59	26·4	0·08

The methyl esters, prepared by the method of Haller, were freed from unchanged alcohol and distilled from a 1 litre Claisen flask, and the final redistillation was carried out in a 250 c.c. Ladenburg flask. The results of the final distillation were as follows:

A.

Fraction.		Temperature. °C.	Pressure.	Weight. Grms.	Sapon. value.	Iodine value.
Added A+B	1	up to 172	atmos.	4·79	366·0	—
,, C	2	172–209	,,	34·08	335·1	—
,, D	3	130	12–14 mm.	34·58	287·0	·02
,, E+F	4	130–150	12 mm.	158·97	259·2	·40
,, G	5	150–180	,,	72·69	232·1	7·55
,, H	6	180–200	,,	34·41	202·1	40·25
original residue	7	200–206	,,	10·58	192·1	61·09
	8	—	,,	9·83	175·8	53·90
			Total	359·93		

B.

Fraction.		Temperature. °C.	Pressure.	Weight. Grms.	Sapon. value.	Iodine value.
Added A+B	1	up to 172	atmos.	1·80	381·5	—
,,	2	172–200	,,	23·10	346·3	—
,, C	3	99–130	14 mm.	39·44	315·0	·04
,, D	4	130–150	,,	205·00	264·6	·21
,, E+F	5	150–180	,,	103·40	237·2	4·57
,, G	6	180–200	,,	56·66	202·3	38·75
,, H+I	7	200–218	,,	9·97	192·4	54·30
Residue	8	—		7·30	211·6*	33·20
			Total	446·67		

By the use of the now discredited assumption as a basis for the calculation, conventional figures for comparison of these oils were obtained.

	Caproic acid. Per cent.	Caprylic acid. Per cent.	Capric acid. Per cent.	Lauric acid. Per cent.	Myristic acid. Per cent.	Palmitic acid. Per cent.	Stearic acid. Per cent.	Oleic acid. Per cent.	Loss Per cent.
A	0·2	7·4	9·5	49·1	17·6	4·3	1·2	10·3	0·5
B	0·2	7·2	10·7	48·7	17·5	5·4	0·8	9·0	0·8

* This evidently contained unchanged coconut oil.

Duplicate determinations gave results agreeing closely with these.

An attempt was made to fractionate the fatty acids themselves of sample B, but, owing to difficulties caused by solidification of the distillates, the distillation was only taken up to 137° C. under a pressure of 1 mm. The approximate composition of the three first redistilled fractions was as follows:—Caproic acid, 0·2; caprylic acid, 7·3; and capric acid, 10·1 per cent.

In the main, the results obtained with these two samples of coconut oil agree with those of Elsdon (ANALYST, 1913, **39**, 8).

W. N. STOKOE.

NOTE.

Claisen flask.—This vessel (employed for the preliminary distillation) is a 1-litre flask with a straight neck, into which is fused a second neck, in the form of a half-U tube, which carries the thermometer, and has the delivery outlet fused into it. The other neck of the flask is fitted with a cork holding a finely-drawn out tube, by means of which a current of air can be drawn in, while, at the same time, the pressure can be accurately regulated with a screw clip and rubber tube fitted to this inlet.

Ladenburg flask.—This vessel, of 250 c.c. capacity, consists of a bulb 8 cm. in diameter and a neck 24 cm. in length, blown out in the form of a fractionating column of 3 bulbs, each of 4 cm. diameter. The delivery tube is fused into the straight portion of the neck above the top bulb, and for this flask, thermometer and air inlet tube are fitted in the one cork.

Receiver.—This is of the type described by Lewkowitsch (*Chem. Tech. of Oils, Fats and Waxes*, 5th Ed., Vol. I., p. 664). In order to prevent loss of uncondensed distillate, a small condenser with double surface was included between the receiver outlet and the vacuum pump, and, to avoid variation of pressure when changing the distillate, it was necessary to use two vacuum pumps, one connected with the upper receiver through the condenser, and the other direct with the lower receiver.

In work of this nature extraordinary precautions must be taken to avoid loss from imperfect condensation of the lower fractions.

TOTAL SULPHUR IN RUBBER.

IN the "Standard Methods for Analysis of Rubber Articles," abstracted in the July ANALYST (p. 352) a method of ascertaining the amount of "total sulphur" is proposed. For this purpose 0·5 grm. of the rubber is treated for one hour with 15 c.c. of nitric acid saturated with bromine, then heated on the water-bath for a further hour, and evaporated to dryness. The residue is treated with 3 c.c. of nitric acid and 5 grms. of sodium carbonate, dried and fused, and the amount of sulphate is estimated by precipitation as barium sulphate. An alternative process is given which is also open to adverse criticism, but it is to the first one that most exception can be taken, for there is a risk of loss of sulphate as sulphuric acid during the earlier part of the process. For many years I have always added potassium nitrate during the treatment with nitric acid; this fixes the sulphuric acid formed, and the result is much more satisfactory. It is also desirable to eliminate the nitric radicle by evaporation once or twice with hydrochloric acid before proceeding to the final precipitation stage.

F. H. ALCOCK.

Notes from the Reports of Public Analysts.

The Editor would be glad to receive the Annual or other Reports of Public Analysts containing matter of interest to the Society. Notes made from such Reports would be submitted to the Publication Committee.

CITY OF BIRMINGHAM.

REPORT OF THE PUBLIC ANALYST FOR THE SECOND QUARTER, 1924.

DURING the quarter 1414 samples were examined, of which 1218 (1024 informal) were examined under the Food and Drugs Acts. Of the informal samples, 50, and of the formal, 22, were adulterated.

MILK.—Thirty-eight of the 495 informal samples, and 20 of the 188 formal samples, were found to be adulterated.

COFFEE, CHICORY.—One of 26 informal samples of coffee contained about 25 per cent. of chicory. Three of the 20 samples of chicory were adulterated, one yielding 8·5 per cent. of ash, including 2 per cent. of sandy matter. One informal and one formal sample were sold as chicory, but were coffee of inferior quality (a war-time residue) which had only been slightly roasted. The vendor was fined 10s.

FLOUR.—Four of 14 samples of flour contained about 3 parts of potassium persulphate per 100,000. The matter was communicated to the Ministry of Health, who referred it to the Preservatives Committee.

CUSTARD POWDER.—Seven informal samples were, as usual, coloured and flavoured maize or sago flours. One was labelled "Extra Creamy Custard Powder," but, as it only contained 0·2 per cent. of fat, the claim was not justified.

SENNA LEAVES.—One of 15 informal samples consisted of senna pods, and the vendor was cautioned. Thirteen other samples yielded from 8·4 to 11·6 per cent. of ash. The leaves of one sample yielded 11·9 per cent. of ash, and the packet also contained 3·1 per cent. of small stones, so that the total mineral matter was 3 per cent. above the B.P. limit. The vendor was cautioned.

SOLUTION OF FERRIC CHLORIDE.—Chloride of iron is required by the B.P. to contain 5 per cent. of iron. Five of the 10 samples contained from 4·5 to 5·4 per cent., and were passed as genuine. One contained 6 per cent. and four others contained 5·7 per cent., and the vendors were cautioned. The Pharmacopœia directions for making a solution which is four times stronger are unsatisfactory. The specific gravity given as "about 1·49" does not agree with the required strength, and it seems probable that manufacturers have been misled by the directions.

SULPHUR OINTMENT.—One informal and one formal sample contained 19 per cent. of sulphur and were prepared with a paraffin basis. The vendor was prosecuted, and the magistrates dismissed the case, on payment of costs, under the Probation of Offenders' Act.

J. F. LIVERSEEGE.

Legal Notes.

Under this heading will be published notes on cases in which points of special legal or chemical interest arise. The Editor would be glad to receive particulars of such cases.

SALE OF SPIRITS WITH NOTICE OF DILUTION.

PRESTON *v.* GRANT.

ON November 4 an appeal from a decision of Warwickshire justices was heard in the High Court before the Lord Chief Justice and Justices Shearman and Salter.

The appellant, an inspector under the Food and Drugs Acts, had bought half a pint of whiskey from the respondent, the licensee of a publichouse, and the sample, upon analysis, had been found to be 42·26 deg. under proof.

When the sale was made there was a notice in the bar, and a similar notice in a room at the back of the bar, which read: "All spirits sold on this establishment are diluted, and no alcoholic strength is guaranteed." The appellant had not seen this notice, nor had his attention been directed to it.

The Warwickshire justices considered that the printed notice was a sufficient notification to the appellant, and that, accordingly, even though he had not seen it, he had not been prejudiced by the sale.

The Lord Chief Justice, after hearing the arguments of counsel on each side, said that the question turned upon the opinion expressed by the justices that the notice was a sufficient notice where it was found, as a fact, that the purchaser did not see the notice and had not been told of it. In other words, was a constructive notice sufficient?

In the present case, it was obvious that the sale was to the prejudice of the purchaser, unless one took the view, disregarding the Sale of Food and Drugs Acts, that the weaker the mixture, the less the purchaser was prejudiced. The seller must show that the purchaser was not prejudiced, and an inspector must not be attributed with more knowledge than an ordinary purchaser. If the article was not of the nature, substance and quality demanded he was prejudiced.

A practice had grown up of exhibiting notices intended to serve as an answer to Sec. 6 of the Sale of Food and Drugs Act, 1875, and the use to which these notices were put was that the seller claimed that the purchaser was not prejudiced because he had had notice of the difference between that which he demanded and that which he received.

So far as he was aware, there was no case which said that it was a good answer for the seller to say that he exhibited a notice which the purchaser did not see, to which his attention was not called, and of the existence of which he did not know. The case of *Pearks, Gunston & Tee, Ltd.* v. *Houghton* (1902) was a very different case from this one. In that case the purchaser got what he demanded, whereas the present appellant got something very different.

There was no such thing as a constructive notice in a case of this kind, and, in his opinion, the respondent was not protected. He did not think that the presumption that the purchaser had been prejudiced was in the smallest degree rebutted by the existence of a notice which he had not seen and to which his attention had not been called. In his opinion the appeal must be allowed, and the case remitted to the Warwickshire justices to convict.

Judgments to the same effect were given by the other Justices.

RODBOURN *v.* HUDSON.

ON November 19th the decision was given in an appeal heard in the King's Bench Division (the Lord Chief Justice and Justices Avory and Salter) from a decision of the Hampstead justices convicting the appellant of having sold, to the prejudice of the purchaser, rum diluted to 41½ deg. U.P. (*cf.* ANALYST, 1924, **49**, 229). For the defence it had been urged that the vendor was protected by a notice displayed on his premises, which notice, it was admitted, had been seen and read by the purchaser and by his agent who actually made the purchase. This notice read:—"All spirits sold in this establishment are of the same superior quality as heretofore, but, to meet the requirements of the Food and Drugs Acts, they are now sold as diluted spirits; no alcoholic strength guaranteed."

The Lord Chief Justice, in a written judgment, said that the justices had given their reasons why the sale was to the prejudice of the purchaser, and unless the Court could say that there was no evidence upon which such a decision could be based, they could not disturb the justices' decision.

Reviewing the decisions in previous cases, he pointed out that the decision in *Sandys* v. *Small* (1878; 3 Q.B.D., 449) was based upon the fact that the purchaser was fully aware of the nature of the article supplied to him, and the question of what was "due and sufficient" notice was not raised. In *Gage* v. *Elsey* (1883; 10 Q.B.D., 518) there had been a notice substantially in the same terms as in this case, and the conviction was quashed. But in that case the only point raised was whether Sec. 6 of the Sale of Food and Drugs (Amendment) Act, 1879, deprived the seller of a defence, in view of the decision in *Sandys* v. *Small*. It was assumed that knowledge had been brought home to the purchaser that the spirits were diluted, and the sufficiency of the notice was not discussed. Hence the decision in that case could not be regarded as an authority on the sufficiency of the notice.

In the case of *Morris* v. *Johnson* (1890; 57 J.P., 612) the question as to the knowledge of the purchaser was regarded as one of fact for the justices; and in *Morris* v. *Askew* (1893; 57 J.P., 724) it was held that mere notice was not sufficient, and that it was for the justices to decide whether the necessary information had, in fact, been conveyed to the purchaser.

In *Palmer* v. *Tyler* (1897; 61 J.P., 389) the justices had found that the sale was not to the prejudice of the purchaser, and it was held that, as this finding of fact was conclusive, the conviction could not be upheld. In *Dawes* v. *Wilkinson* it was held that, to obtain protection, the seller must give such notice to the purchaser as will inform him that there has been such tampering with the spirit as is expressed by the word "dilution."

In the case of *Taylor* v. *Elder* (1923) he (the Lord Chief Justice) had expressed the opinion that the notice (which was in practically the same terms as in the present case) was a notice of grave and calculated ambiguity, and to that opinion he still held. In the present case the notice was both ambiguous and misleading. "Superior quality" was a misleading term to apply to spirits which had been reduced below the statutory minimum, and there was no provision in the Sale of Foods and Drugs Acts requiring any one to sell spirits, whether of superior or inferior quality, as diluted spirits. The words "no alcoholic strength guaranteed" might well be understood to mean no alcoholic strength above the statutory minimum. It rested with the seller to prove that such notice had been given as would ensure that the sale was not to the prejudice of the purchaser. The two questions to be decided, therefore, were (1) What was the substance of the information which must be given? (2) Were the steps taken sufficient, in all the circumstances, to convey this information to the average purchaser? (If the

purchaser did not see and was not made aware of the notice the question of its sufficiency did not arise (*cf. Preston* v. *Grant, supra*). With regard to (1) the purchaser must be told in substance that what he is getting is not what he asked for. As to (2) the actual sufficiency of the notice was a question for the justices. There was evidence here on which the justices could find that this notice would not convey the information.

The saying that "everyone is supposed to know the law" was too general; the maxim *ignorantia legis neminem excusat* could not be accepted as a defence against a breach of the law; but in considering this notice the justices would properly remember that the average customer knows nothing of the Food and Drugs Acts. For the Courts to "hall-mark" a notice, so that anyone by hanging it up could evade the Acts, would be unfortunate. The sufficiency of the notice was for the justices to decide in each case. The appeal must therfore be dismissed, and this judgment, if truly analysed, was, in his opinion, not in conflict with any of the authorities.

Mr. Justice Avory agreed with the judgment, subject to the reservation that he had grave doubts whether it could be reconciled with that in *Gage* v. *Elsey*, which had been acted on since 1883; also, he was not prepared to assent to what he understood to be the effect of the Scottish judgments (*Brander* v. *Kinnear*, *Kelso* v. *Soutar*, and *Williamson* v. *Soutar*; 1923, S.C.(J.), 42), *viz.* that the seller was not protected unless he specified in the notice the actual extent of dilution.

Mr. Justice Salter concurred with the judgment.

NEW NOTICES IN PUBLIC BARS.

According to *The Times* (Nov. 22, 1924) the licensed victuallers' associations have taken legal advice on the position created by the judgment of the High Court in the case of *Rodbourn* v. *Hudson*, and are of opinion that an amended form of notice will meet the requirements. They point out that, for many years past, it has been the custom to sell spirits for immediate consumption, diluted with water so as to be weaker than 35 degrees U.P., and a printed notice has been exhibited to the effect that the spirits sold are diluted and that no alcoholic strength is guaranteed.

In view of the decision in the case mentioned above, the printed notice affords no defence if the customer has not seen, or had his attention directed to the notice; and, even then, it is open to the magistrates to find that the notice would not convey adequate information to an average purchaser, and the High Court would not disturb a conviction based on this finding. It is not practicable, say the victuallers' associations, to sell all spirits not weaker than the statutory limit and to let the customer do his own dilution, since every customer cannot afford, and does not expect, to receive the full strength of spirit, the degree of dilution before sale being indicated more or less by the price charged. The situation will be met by changing the form of notice, and taking further steps to draw the attention of the customer to its existence, so that in no case can he plead ignorance of the fact that diluted spirits are being sold.

The notice in the case of *Rodbourn* v. *Hudson* is an obsolete one, and in later forms of notice there is no reference to the "superior quality of the spirits." Several summonses in different parts of London are pending, but all refer to the later form of notice, which reads:—"Notice to purchasers of spirits.—All spirits sold in this establishment are sold as diluted spirits, no alcoholic strength being guaranteed." It is proposed to fight these cases as being in a different category from that decided in the High Court.

PAREGORIC WITHOUT OPIUM.

On October 6, a herbalist was summoned at Blackburn for applying a false trade description to a certain mixture, contrary to Sec. 2 of the Merchandise Marks Act, 1887, and for selling a mixture to which a false trade description had been applied.

Evidence was given that the defendant had been asked for half an ounce of paregoric, and had supplied a mixture contained in a small bottle which was labelled

in writing "paregoric," without qualification of any sort. Prior to the analysis it was thought that the defendant was selling a poison, and was therefore offending against the Pharmacy Acts, but, when written to upon the subject, he replied that the mixture sold by him was "paregoric" which was "free from opium," and that he had put this upon the label.

Mr. F. Wokes, B.Sc., for the prosecution, said that he had analysed the sample, and had found that it did not comply with the accepted standard of the British Pharmacopoeia, according to which paregoric must contain a considerable proportion of tincture of opium. In cross-examination, he agreed that the other ingredients of paregoric were present, and had an antiseptic property, but said that these alone would not constitute paregoric. He did not agree that the mixture could be sold as "paregoric essence"; in his opinion the essence of paregoric was the opium tincture.

Mr. Linsey, for the defence, submitted that the offence, if any, was a highly technical one. He produced the catalogue of a wholesale firm to show that paregoric was sold both with and without the addition of opium. "Paregoric essence" had been used as a favourite remedy in Lancashire for generations, and it was usually procured from herbalists. Had the defendant sold a mixture containing opium, he would have been liable to a heavy penalty, but there was a notice in his shop to the effect that no dangerous or poisonous drugs were sold there. The Merchandise Marks Act was not intended to apply to a case like this, where there was no intention to deceive or to make a profit out of the deception. The bottle and the preparation had been sold for 3½d. with the dangerous element removed.

The Bench held that while the defendant was perfectly innocent of any intention to break the law, there had been an offence. Fines of 40s. for the first case, and 20s. for the second, were imposed, and defendant was required to pay the costs of the witnesses.

Federated Malay States.

ANNUAL REPORT OF THE CHEMICAL LABORATORIES FOR 1923.

THE total number of samples examined during the year was 6521, as compared with 2702 in 1922, the increase being mainly due to samples of chandu dross and counterfeit coins.

MEDICAL DEPARTMENT.—Of the 774 samples of milk examined, 107 failed to comply with the standards prescribed in the Sale of Foods and Drugs Enactment, 1913. Eighteen of these were deficient in milk fat, 86 were deficient in non-fatty solids, and 3 in both.

WATER.—Chemical analysis of 343 samples and bacteriological examinations of 40 samples were made. The water from the Ayer Kuning reservoir has been treated with chlorine since September, 1923. The addition of 0·5 part of chlorine per million reduced the number of bacteria by 71 per cent., and 0·75 part per million by 84 per cent.

TOXICOLOGICAL ANALYSES.—Three cases of human poisoning were investigated. In one of these spirits of salt had been accidentally swallowed, and free acid and chlorides were detected in the vomit and in the stomach.

In a case of arsenical poisoning $\frac{1}{80}$ grain of arsenic was recovered from the stomach and contents, kidney and a portion of the ileum.

In the third case $\frac{1}{80}$ grain of strychnine was found in the stomach and $\frac{1}{20}$ grain in the stomach contents.

Vitamin B Extract.—The preparation of this extract from rice polishings was continued throughout the year, and 5159 fluid ounces were issued to medical practitioners, dispensaries and hospitals for the treatment of beri-beri.

TRADE AND CUSTOMS DEPARTMENT.—The total number of samples examined was 3619, as compared with 719 in 1922. These included samples of liquors for alcoholic strength determinations, toddy, chandu, chandu dross, and deleterious drugs (2 of which contained morphine hydrochloride).

POLICE DEPARTMENT.—Of 993 samples of coins and coining materials examined, 967 were counterfeit coins.

Tests for blood stains were applied in 106 cases, of which 43 gave positive results. Of these, 36 were tested for human blood by the precipitin test, and 23 gave positive reactions.

Poisons were identified in only four cases, *viz.* potassium cyanide in two cases of suicide, and arsenic in two cases.

H. MARSDEN, *Acting Chemist.*

The Cellular Content of Milk.

INVESTIGATION MADE ON BEHALF OF THE MEDICAL RESEARCH COUNCIL.*

THERE exists the greatest divergence of opinion as to the significance of the cellular elements and micro-organisms that occur in milk. Few will deny that milk should not contain pus, but what constitutes pus in milk? All milk contains leucocytes, but do these leucocytes become converted into pus cells, and, if so, how can one be distinguished from the other? The presence of a certain number of leucocytes in milk is strictly physiological, though beyond a certain point it may, probably does, become a pathological feature. One school holds firmly to the idea that the cellular contents of milk are all derived from the blood; that they are true blood cells, and that under certain conditions they constitute pus. Another school asserts that the cells of milk are tissue cells of purely epithelial origin, which under no circumstances can be spoken of as pus cells. The truth appears to lie somewhere between these extremes.

METHODS OF EXAMINATION USED.—To distinguish the various cells that may be present in milk, a differential method of staining must be used, and the contradictory statements published must be attributed to failure to appreciate this condition. The following method of cell examination has been adopted in this enquiry, and a stain used which has been found to give constant and reliable results for a differential count:—

The animal is milked in the ordinary way and at the usual times. During the milking three samples of the milk from each quarter—12 samples in all—are milked direct into sterilised test-tubes, which are plugged immediately. Of the three samples, one is taken at the commencement of milking—called the "fore-milk"—another in the middle of the operation—the "middle-milk"—and a third at the end of milking (but without any undue straining)—the "strippings." Taking each of these samples in turn, the tube is well shaken and 10 c.c. poured

* By P. C. Varrier Jones, M.A., M.R.C.S. Abridged from *The Lancet*, 1924, **207** (Sept. 13), 537–542, by permission of the Editor of that journal and of Dr. Varrier Jones. The original report has 6 coloured diagrams of the different types of cells.

into a centrifuge tube of Houston's pattern the lower part of which is drawn out and accurately marked with a scale of 1/100 cm., and centrifuged at 3000 revolutions per minute for ten minutes. The cream and supernatant fluid are carefully removed, 0·5 c.c. of milk being allowed to remain above the deposit. These are thoroughly mixed with a capillary pipette on which is a bulb and into which the mixture is finally drawn up. The pipette is then sealed off and centrifuged until the deposit is carried to the bottom of the pipette, whence, with a still finer tube, it may be removed and spread on a glass slide. The film is then air-dried, stained at once by Jenner's method, examined with a Zeiss 1/12, and the cells noted, described, drawn, and counted.

This method is not put forward as giving correct numerical results, but rather as a differential method in which the well-stained cells of different types may be readily distinguished.

The question now arises: are all the cells thrown down as sediment in the centrifuge, or are some carried upwards and held in position by the cream which rises to the surface? In normal milk it has been found that the number of cells entangled in the cream is a negligible quantity, and it is necessary in any differential count to examine only the deposit found at the bottom of the pipette.

CHARACTERISTICS OF CELLS IN MILK.—Observations have led to the conclusion that the following cells are to be found in milk obtained from a normal animal:—

1. *The Finely Granular Eosinophile Cell.*—A polymorphonuclear cell (10 to 12 μ in diameter), more or less round or elliptical, or even irregular in outline, the shape depending on various factors, such as speed of centrifuging. Clear cytoplasm remaining unstained by Jenner's method. Nucleus, as a rule, stained deep blue (old cells stain badly); branched or lobulated in form. In cells of fresh milk pseudopodia stretch out in all directions; phagocytic—the bacteria being contained in vacuoles lying in the substance of the cell. In a normal milk the number is almost negligible; but in milk from an inflamed quarter the number may be very considerable.

2. *The Coarsely Granular Eosinophile Cell.*—The coarsely granular eosinophile cell is slightly larger than the finely granular polymorphonuclear cell; it is oval or round in shape, and measures 10 to 13 μ in diameter. The cytoplasm is perfectly clear and transparent, but in this case, again, is closely studded with highly refractile granules, measuring in some cases as much as 0·5 μ in diameter; by Jenner's method these granules stain a deep pink.

The nucleus, usually of a light blue tint, is placed either in the centre of the cell or, more commonly, towards the periphery. It is either kidney-shaped or lobed, the different lobes being connected by bands of nuclear substance. In many films the cells appear to have burst; the granules are then scattered in all directions.

3. *The Large Mononuclear Leucocyte.*—The large mononuclear leucocyte is one of the largest cells met with in the deposit from cow's milk. It is round or oval in shape, invariably staining a dark blue. The stain, however, is not evenly distributed, but appears as a tangled thread throughout the substance of the cell. This is the nucleus, which fills up nearly the whole of the cell. Usually, however, there is a small portion of cytoplasm on one side of the nucleus, but it rarely makes a complete circle round it. This portion of the cell is stained less darkly, but it is nevertheless of a distinct blue colour. These cells are invariably present in all samples of cow's and goat's milk, and in some are a very prominent feature.

4. *The Lymphocyte.*—The lymphocyte is a small blue cell which, as a rule, stains deeply. The cytoplasm is small in amount and forms a thin ring of light blue colour around the darkly-stained nucleus. These cells vary in size from 2 to 4 μ, the majority measuring about 3 μ. The nucleus is round or slightly oval in shape, stains darkly, and is not reticular. It occupies almost the whole of the cell. Such cells are fairly numerous in normal milk, but appear to be found in greater numbers in certain breeds of cattle than in others.

5. *The Basophile Cell.*—The basophile cell (8–10 μ), a rare cell, is almost circular in shape, but may be oval. The cytoplasm is clear and transparent, and has scattered unevenly throughout its substance dark blue granules, large and small. The large nucleus occupies two-thirds of the cell, it is oval or rounded, and is always single. By Jenner's method it stains a pale blue. As a rule, the basophile cell is absent from films of normal milk, but under certain conditions it appears, though never in great numbers.

6. *The Large Neutrophile Cell* (5–9 μ).—The large neutrophile cell is extremely rarely met with. It is oval in shape, rarely round. The cytoplasm is stained a faint light blue throughout, the whole being filled, except in the region of the nucleus, with very fine granules, which take on a pale mauve tinge. By Jenner's stain the nucleus is tinged light blue.

7. *Red Blood Corpuscles.*—Red blood corpuscles are invariably present in a sample of cow's milk. In normal milk they are exceedingly few in number; as a rule, only one or two are found in a film made from the deposit of 10 c.c. of milk. It must be remembered, however, that in milk films the individual red blood corpuscles are not exactly similar to those found in normal blood, but they are always easily recognisable.

8. *Large Epithelial Cells.*—Large epithelial cells are almost always present. They vary greatly in size, some measuring as much as 20 μ in diameter. The cytoplasm is somewhat granular, having a ground-glass appearance; it stains a more or less deep blue with Jenner's stain. The cells are usually rounded, but may be oval, and appear to have a well-developed envelope, which takes on a slightly deeper stain. The nucleus is large, oval or rounded, occupying a quarter of the cell, and stains a less deep blue than the rest of the cell. It usually has a mottled appearance as though composed of a chromatin network. In the cytoplasm of the cell are usually one, two, or even more clear spaces with well-defined outlines, usually round, more rarely oval in shape. These are fat globules surrounded by an envelope which prevents the fat from taking the ordinary fat stains.

9. *Mononuclear Eosinophile Cell.*—Other vacuolated cells appear from time to time in milk. Apart from the finely granulated, eosinophile cell which may become vacuolated through age, or otherwise, there are other cells which closely resemble them. The cytoplasm of these cells, instead of being clear and transparent, stains a light pink, the substance being more or less vacuolated. The nucleus stains a light blue and is usually pushed to the side of the cell by the large number of vacuoles in the cytoplasm. These cells are abundant, especially when the cow dries off; but they may be present at other times. They may, however, be unvacuolated, when they may measure 9–10 μ in diameter, but they are often less than this. The author regards this as an epithelial cell, probably derived from the secretory layer of the gland tissue.

Cellular Content of Normal Milk.—Should the cow happen to be a normal animal—*e.g.* a heifer some days after her first calf, without any complications or udder trouble after calving—the blue cells, both large and small, which occupy the field are numerous, and at once arrest attention; indeed, one is apt to make up one's mind that the milk is full of lymphocytes both of the large and small variety. Looking over the fields, one may observe here and there, not usually more than one per field, a finely granular eosinophile cell, with nucleus sharply stained, and with the granules distinctly visible; characters that enable one to pick it out without difficulty from the surrounding cells. It would be a great mistake, and very misleading, to class these cells together as pus cells; most of them are broken-down polymorphonuclear cells. Now and again a coarsely granular eosinophile cell which stains well, resembling very closely the coarsely granular eosinophile cell of ox's blood, may be seen. Again, a much larger cell, round or oval in shape, with large pale blue nucleus, the cytoplasm of the cell staining a mottled blue, may be present. Evidently these different cells cannot be lumped together as pus. Moreover, one finds in the field a number of small dots, stained deep blue, 1 μ or so in diameter, sometimes with only a tag of pink substance around the blue point, or near its edge. These are obviously nuclei extruded, possibly, from blood cells, but more probably from secretory epithelial cells of the acini. The pink portion is part of the cytoplasm of the cell which was torn away when the nucleus was extruded. From this picture, which is invariably presented in the sediment from normal milk, it is obvious that a simple count can give no information as to the condition of the secreted fluid or of the secretory tissue. The number of cells (lymphocytes), added to the stray polymorphs, coarsely granular eosinophiles and tissue cells, along with the extruded nuclei, may give a high "leucocyte" count; but under these circumstances we cannot justly claim that the cow is suffering from a diseased condition of the udder or that the fluid is "pus." A differential count of cellular deposit must always be made before a diagnosis is justified.

CELLULAR CONTENT OF ABNORMAL MILK.—A high cell count is not of necessity evidence of a diseased udder, nor, on the other hand, does a low count necessarily indicate an udder free from disease. The actual count may, of course, in certain doubtful cases help in the formation of a diagnosis, but it cannot by itself be depended upon. For the present attention is directed to the vital importance in all cytological examinations of milk, of applying this differential staining and counting method *quarter by quarter*; for it is not sufficient to make an examination of the mixed milk by this method.

INFLUENCE OF PHYSIOLOGICAL CONDITIONS.—*Breed of Cow.*—It appears that normal cows of different breeds have different proportions of cells in their milk. In the Hereford heifer the cells are of large mononuclear lymphocyte type. Large mononuclear leucocytes also appear to be present invariably in the milk from Hereford cows, but are not so noticeable a feature in the films prepared from the milk of the cows of the other breeds. The Welsh heifer's milk always contains many—indeed, they were almost the most noticeable feature—of the very small blue cells, which are almost entirely absent from the Hereford cow's milk. Owing to the large number of these small cells, the cellular content of the Welsh heifer's milk is always high. In the milk of the Shorthorn, the very small cells, so familiar by their presence in the milk of the Welsh heifer, are here seldom met with; their place is taken by cells with a pale-blue nucleus and a surrounding layer of granular protoplasm, the granules being very fine and staining blue. This divergence of cellular content still requires explanation.

Influence of Calving.—Besides the colostrum corpuscles—*i.e.* cells laden with fat globules probably derived from the epithelium of the acinus, the polymorphonuclear cells are very numerous, some staining sharply, others vacuolated and evidently in process of disintegration. These colostrum corpuscles appear in such great numbers that the stained film appears quite pink, in this respect differing markedly from the film of the ordinary milk, which has a blue tinge. Lymphocytes, both of the large and small variety, though present, are not numerous; the cell with the pale-blue nucleus and granular protoplasm (with fine blue granules) predominates. The polymorphs, even in colostrum, are actively phagocytic. As the milk becomes normal the colostrum corpuscles and the polymorphonuclear cells gradually disappear; and, if no inflammatory process supervenes, the milk cells soon return to their normal count. If, however, by any chance one quarter of the udder becomes inflamed, the polymorphonuclear variety of cell persists, and is present even when the milk from the other quarters affords no trace of its presence.

Effect of Stage of Milking.—As a rule, unless a method of milking such as the use of a milk tube is employed, the cells of all three samples are much the same as regards both type and proportion, although the absolute number may vary considerably.

Effect of Intervals in Milking.—When the interval between the morning and the evening milkings is unduly prolonged the cells increase in number, especially the polymorphonuclear cells and the red blood corpuscles.

Relationship between Fat Content and Cellular Content.—In no case during this investigation has there been found any increase in the fat content of milk where there has been a rise in the polymorphonuclear cells. The statement of Pennington and Roberts (*J. Infect. Dis.*, 1908, **5**, 72) that "in almost 75 per cent. of the herd, perfectly healthy, clean cows show *pus* at the close of the milking period, and *coincident chemical analysis*, that the percentage of butter fat is from 0·5 to 1·5 per cent. above the normal for that particular individual" has not been borne out by the present results.

Physiological "Drying-Off."—In the normal animal when drying-off occurs the increase in the number of cells comes on quite gradually; the milk yield slowly falls off, and with this there is a corresponding increase in the number of cells, especially of one definite type. The films all present common features, and with a little practice it is possible to make a diagnosis of drying-off with great confidence. Should no differentiation be made between the various cells the condition would be diagnosed as due to previous disease, probably chronic inflammation of long standing. These drying-off cells, however, are usually circular in outline; the cytoplasm, which is often "vacuolated," contains no granules, and takes on an irregular somewhat deep pink stain. The nucleus may be single, or may be bi- or even tri-lobed, with the lobes apparently not joined together. The one, two, or even more "vacuoles" present in a single cell are stained by Nile blue, and therefore contain fat.

Pathological "Drying-Off."—Any severe inflammatory trouble may result in drying-off of the quarter or quarters affected. The cells are then of several varieties, the polymorphonuclear form being found in equal or greater extent than those described above as usually present. The inflammatory process introduces an extra factor, and may alter the cellular content to such an extent that the deposit may now be described as pus:—

Phagocytosis.—An experiment is described in detail by which it was demonstrated that the polymorphonuclear cells described above were actively amœboid and phagocytic.

SOURCE OF THE CELLS.—It is held very generally that all the cells found in milk are purely blood cells, but Winkler (*Zeitsch. Landw. Vers., Oesterreich*, 1908, **9**, 562) and his school maintain that they are entirely of epithelial origin. On the one hand, we have such statements as the following: "The outstanding feature in the examination of some hundred films prepared both from normal cows at different periods of lactation and from cows presenting slight signs of mastitis and representing many thousands of cells, is that *no cell having any decided resemblance to a polymorphonuclear leucocyte has been detected*, and phagocytosis of bacteria present is conspicuous by its absence." (Hewlett, Villar and Reyis, *J. Hygiene*, 1909, **9**, 271; 1910, **10**, 56; 1911–12, **11**, 97; 1914, **13**, 87.) It is obviously difficult to concur in this view, the polymorphonuclear cells described in this paper being identical with those found in the blood.

These leucocytes must be regarded as pus cells when in the course of time they break down and disintegrate. The slightest inflammation of the udder is followed by the appearance of a considerable number of these cells the characters of which are always the same, and the phagocytic activity of which, when examined fresh, can be demonstrated. Many of the author's drawings show this very clearly, and he cannot agree with those observers who say that phagocytosis "is conspicuous by its absence." He is, however, in agreement with those who point out that the cell with a single oval or rounded nucleus, which stains well but not always uniformly, the cytoplasm of which is structureless and which stains well with eosine, is derived from the secretory layer of the gland tissue. This cell, which he regards as epithelial in type, is present in large numbers when the gland tissue ceases to perform its proper function as the cow "dries off." On account of its sometimes having a double nucleus it is apt to be mistaken for a polymorphonuclear cell, and by many authors it is regarded as a pus cell. The small blue cells described above as lymphocytes are really the lymphocytes of the blood-stream. Again, the coarsely granular eosinophile cells and the basophile cells which are so often present in milk undoubtedly correspond to similar cells found in the blood.

The other cells are epithelial in type, and a few are undoubtedly derived from lymphoid tissues.

Presence of the Red Blood Corpuscle in Milk.—It would not be accurate to say that *every* sample of milk contains red blood cells, but it is undoubtedly true that 99 per cent. of the milk samples which the author has examined have contained erythrocytes. Even in normal milk—using the term in its strictest sense—he has met with an occasional red blood corpuscle. In acute congestion of the udder red corpuscles make their appearance, and they are always present also in the first milk or colostrum after calving.

Causes of the Presence of Blood in Milk.—The commonest cause of the presence of blood in milk is said to be rupture of a blood-vessel. This may be true where large numbers of erythrocytes are met with, but it is certainly not the case where the blood is detected only on microscopic examination of the milk. Apart from pathological and mechanical sources of blood, it is not generally recognised with what ease the red blood corpuscle can get into the milk-stream, and the fact that this can occur is undoubtedly a factor to be taken into consideration when one considers the possibility of pathogenic organisms—*e.g* the tubercle bacilli getting into the milk-stream by the same route.

So far as these observations go, it is evident that the cells in milk are partly blood cells, partly lymphoid, and partly epithelial. While it is impossible to agree with the statement that "no cell having any decided resemblance to a polymorphonuclear leucocyte" is present in milk, it is obvious that to concede that all the cells in milk are pus cells is equally out of the question. Although it must be admitted that at certain times, and under certain conditions, pus cells are found in milk, it does not by any means follow that, whenever the cell count is high, we are dealing with a purulent condition.

In this report an endeavour has been made to point out that, although reliance cannot be placed upon a quantitative leucocyte standard, yet, by means of the differential count, it is possible to distinguish all the various conditions most accurately, and in many cases even to make a diagnosis, and to set up a qualitative standard which may be of great service. It is, indeed, necessary not only to modify the existing standards, but to revise our whole method of diagnosis. The method here described, together with the results of the investigation, indicates a method of procedure which certainly promises to give more satisfactory results as regards diagnosis than have yet been obtained.

United States Department of Agriculture.

FOOD INSPECTION DECISIONS 193, 194, 195.

THE following revised and amended definitions and standards for cider vinegar, marjoram and cumin seed were adopted by the Joint Committee on Definitions and Standards, composed of representatives of the United States Department of Agriculture, the Association of American Dairy, Food and Drug Officials, and the Association of Official Agricultural Chemists, August 10, 1923, and Feb. 27, 1924.

(193). Vinegar, cider vinegar, apple vinegar, is the product made by the alcoholic and subsequent acetous fermentations of the juice of apples, and contains in one hundred cubic centimetres (100 c.c.) (20° C.) not less than four (4) grms. of acetic acid.

(194). Marjoram, leaf marjoram, is the dried leaves, with or without a small proportion of the flowering tops, of the *Majorana hortensis* Moench. It contains

not more than sixteen per cent. (16%) of total ash, not more than four and five-tenths per cent. (4·5%) of ash insoluble in hydrochloric acid, nor more than ten per cent. (10%) of stems and harmless foreign material.

(195). Cumin seed is the dried fruit of *Cuminum cyminum* L. It contains not more than nine and five-tenths per cent. (9·5%) of total ash, not more than one and five-tenths per cent. (1·5%) of ash insoluble in hydrochloric acid, nor more than five per cent. (5%) of harmless foreign matter.

The foregoing definitions and standards are adopted as a guide for the officials of this Department in enforcing the Food and Drugs Act.

ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS.

Food and Drugs Analysis.

Changes in the Optical Activity of Sucrose produced by Heating. M. A. Rakusin and A. N. Nesmejanow. (*Zeitsch. Unters. Nahr. Genussm.*, 1924, **48**, 151–152.)—Caramelised sugar has become increasingly important as a constituent of diabetic foods. An optically active, Fehling-reducing product, of coffee-brown colour and sweet taste, is best prepared by heating cane-sugar to 150° to 160° C. for 2 to 4 hours. The effect of heat on a sugar having m.pt. 178–183° C. and $[\alpha]_D = +65{\cdot}33$ was as under:

Temperature.	Time.	$[\alpha]_D$.	Colour.
m.pt.	—	+48·33	pale yellow
m.pt.	—	+59·33	,, ,,
190°	½ hour	—	black
180°	,,	0	,,
160°	,,	+53·66	pale yellow
170°	,,	+22·66	dark brown
160°	1 hour	+15·00	pale brown
150°	2 hours	+13·20	,, ,,
140°	4 hours	+21·00	yellow

H. E. C.

Modifications of the Picric Acid Method for the Estimation of Sugars. J. J. Willaman and F. R. Davison. (*J. Agric. Res.*, 1924, **28**, 479–488.)—There is no reasonably permanent colour standard suitable for use in the picric acid method of sugar estimation. The best available is a 0·08 per cent. glucose or 0·076 per cent. sucrose solution in saturated picric acid; these will keep for about a week. Careful examination of the method brings out the fact that strict attention to empirical detail is essential for accuracy, and the following procedure is recommended. Solutions required are:—Saturated recrystallised picric acid, 20 per cent. (anhydrous) sodium carbonate and the above-mentioned standard sugar solutions. The solution to be examined is first clarified with lead acetate, de-leaded with sodium phosphate, and diluted so that the sugar content is between

0·02 and 0·24 per cent. Then, for the estimation of reducing sugars, 1 c.c. of the solution, 2 c.c. of saturated picric acid solution and 1 c.c. of the sodium carbonate solution are mixed in a test tube closed with a tin-foil covered cork and heated in boiling water for 20 to 30 minutes, cooled, and diluted to 10 c.c. For the total sugars, 1 c.c. of the solution and 2 c.c. of the picric acid are heated in the same way for 10 minutes, then 1 c.c. of sodium carbonate solution is added, and the heating continued for 20 to 30 minutes and, as before, diluted to 10 c.c. The colour so obtained is matched in a colorimeter with that of 2 c.c. of the standard sugar solution similarly treated. The amount of sugar is not quite proportional to the intensity of colour, though it is nearly so. The colour values, as compared with glucose = 1·000, of the various sugars, and the appropriate factors are:

Sugar.	Colour value.	Conversion factor.	Sugar.	Colour value.	Conversion factor.
Glucose	1·000	1·000	Rhamnose	1·298	0·774
Fructose	1·000	1·000	Lactose	0·750	1·333
Maltose	0·770	1·298	Arabinose	1·000	1·000
Mannose	1·000	1·000	Xylose	1·071	0·934
Galactose	0·883	1·132			

A special table is given showing the exact colorimeter readings corresponding to varying amounts of sugar. H. E. C.

Limits for the Detection of Traces of Alkali or Soap in Refined Coconut Oil. W. L. Brooke. (*Philippine J. Sci.*, 1924, **25**, 151–153.)—Alkali was introduced in varying proportions into refined coconut oil previously washed three times with distilled water. The oil was then ashed, the ash dissolved in distilled water, and a drop of phenolphthalein solution added. A pink coloration was taken to indicate the presence of alkali or soap in the oil. Less than 0·02 per cent. of sodium carbonate was thus detected, and 0·00065 per cent. of sodium hydroxide with certainty, and as little as 0·00033 per cent. of the latter gave a pale pink coloration. D. G. H.

Non-Volatile Acids of the Peach. E. K. Nelson. (*J. Amer. Chem. Soc.*, 1924, **46**, 2337–2338).—According to Kunz and Adam (*Zeitsch. Nahr. Genussm.*, 1906, **12**, 670) the peach contains citric acid, but no malic acid. Bigelow and Dunbar, however (*J. Ind. Eng. Chem.*, 1917, **9**, 762), concluded that probably only malic acid is present. In experiments to settle the question 18 kilos. of sliced peaches were boiled with water and the extract concentrated. The pectin was precipitated with alcohol, and the filtrate treated with lead acetate. The lead salts were decomposed with sulphuric acid, and the liberated acids were converted into their ethyl esters, which were then fractionally distilled at a pressure of 10 mm. The acids in the fractions were identified by means of their hydrazides, 0·7 grm. of the ester being dissolved in 3·5 c.c. of absolute alcohol and treated with 0·5 c.c. of hydrazine hydrate. A hydrazide melting at 175° to 178° C. was identified as *l*-malic dihydrazide, and one melting (not sharply) at 145° C. as the trihydrazide

of citric acid. The amounts of these compounds obtained indicated that the non-volatile acids of the peach consist principally of a mixture of *l*-malic acid and citric acid in almost equal proportions.

New Method of Estimating Allyl-isothiocyanate. Morvillez and Meesemaecker. (*J. Pharm. Chim.*, 1924, **30**, 236–240.)—One hundred c.c. of water are added to 5 grms. of powdered mustard previously mixed with 20 c.c. of 90 per cent. alcohol, and the whole left to stand at 35° C. for 1 hour in a corked flask. The mixture is then distilled into 20 c.c. of ammonium hydroxide solution until 100 c.c. of liquid are obtained. To 50 c.c. of the distillate, neutralised and subsequently acidified with 10 c.c. of sulphuric acid, 10 c.c. of 0·1 *N* iodine solution are added, and the whole left in the dark for 15 minutes and titrated with *q* c.c. 0·1 *N* sodium thiosulphate solution. Then the percentage of allyl-isothiocyanate present is $(10 - q) \times 0{\cdot}00495 \times 4 \times 10$. This method is more rapid than the various silver methods and as accurate. The presence of white mustard does not interfere with the reaction. D. G. H.

Estimation of Peroxide in Ether. A. W. Rowe and E. P. Phelps. (*J. Amer. Chem. Soc.*, 1924, **46**, 2078–2085.)—The method described depends on the reaction between a peroxide and cadmium and potassium iodides in acid solution. Ten c.c. of the ether sample are mixed with 15 c.c. of aqueous 5 per cent. cadmium iodide and potassium iodide solution, 15 c.c. of dilute sulphuric acid, and 25 c.c. of pure alcohol; after about one hour, the liberated iodine is titrated with 0·1 *N* thiosulphate solution. A control experiment is made at the same time, using the same quantities of reagents but without the ether. The alcohol used is purified by treating each litre of 95 per cent. alcohol with 4 grms. of *m*-phenylenediamine hydrochloride, allowing the mixture to stand for several days and distilling it slowly. W. P. S.

Biochemical, Bacteriological, etc.

Chemotherapeutic Experiments with Chaulmoogra and Allied Preparations. O. Schobl. (*Philippine J. Sci.*, 1924, **25**, 123–134; 135–150.)—Water-soluble compounds; sodium, copper and nickel salts of fatty acids; and double bond containing compounds (particularly with the double bond in the side chain of the ring in aromatic compounds) were all found to be antiseptic, but the most powerful class was that of the unsaturated alcohols containing phenyl groups. The antiseptic effect of phenols increased with the number of hydroxyl groups present, specially with those in the ortho position, and also with alkylation *on the ring*. The amino group had a reverse effect on the antiseptic powers of aromatic compounds, but when linked directly to the side chain was strongly antiseptic. Open chain terpenes were found to be more antiseptic than their cyclic isomers.

No relationship was found to exist between the degree of unsaturation of vegetable oils which stimulate or inhibit the growth of acid-fast bacteria, and

their growth-stimulating or inhibiting activity, but in those which stimulate the growth of acid-fast bacteria the condition of unsaturation is paramount, so that saturated chaulmoogra oil lacks the activity of the unsaturated oil, and, further, its growth-inhibiting activity is lost if the ring structure is altered by saturation with hydrogen. There are indications that acids from the chaulmoogric series with short side chains are more effective *in vitro* than those with long chains, but acid-fast bacteria will, after a time, withstand larger doses of the acids than at first. The growth-stimulating effect of certain vegetable oils was found to be due to the glyceryl and not to the acid part of the oil. D. G. H.

Colour Reactions of Antirachitic and Antiscorbutic Principles. Bezssonoff. (*Comptes rend.*, 1924, 572–574.)—Antirachitic principles may be identified by the orange colour produced on mixing a benzene solution of the fatty material with an aqueous solution of phosphomolybdotungstic acid, and the colour is intensified by prolonged heating and by oxidation by air. With antiscorbutic principles a blue coloration develops, but the reaction is not so well defined as that for antirachitic bodies. Cod-liver oils, egg yolk and butters were tested, the latter showing pale yellow colorations of the benzene layers, and blue aqueous layers. Olive and arachis oils and lard were found devoid of the fat-soluble factors, but a feeble blue coloration was apparent in the case of unheated olive oil (*cf.* ANALYST, 1921, **46**, 462). D. G. H.

Antiscorbutic Value of Fresh and Canned English Tomatoes. E. M. Delf. (*Biochem. J.*, 1924, **18**, 674–678.)—The minimal daily ration of raw fresh juice of English tomatoes protecting young guinea pigs from scurvy has been shown to lie between 1·5 and 2·5 c.c.; thus it is not quite as effective an antiscorbutic as fresh orange or lemon juice. The locally produced S. African tomato is decidedly inferior to the English fruit, the minimum protective dose under similar experimental conditions being about 4 c.c. daily. A sample of bottled tomatoes, which had been stored for six months, was found to have lost about two-thirds of its original value. By canning, by a method described, a loss of nearly three-quarters of the original value was sustained, and a further but smaller loss occurred after storage of the canned fruit for nearly 4 years. Canned tomato purée had lost about seven-eighths of the value of the original juice. The concentrated juice is, bulk for bulk, but little superior to the canned unconcentrated juice, when prepared as described in the paper. P. H. P.

Antiscorbutic Fraction of Lemon Juice. II. S. S. Zilva. (*Biochem. J.*, 1924, **18**, 632–637.)—In a previous communication Zilva (*Biochem. J.*, 1924, **18**, 182) showed that it is possible to remove the acids and sugar from lemon juice without perceptibly influencing its potency. A series of experiments is described in which some very active antiscorbutic fractions have been prepared. It is shown that still more extraneous matter may be removed without vitiating the activity of the antiscorbutic principle. The most potent preparations contain only 0·03–0·07 per cent. of solid matter in solution, while retaining approximately

the entire activity of the original juice. A table gives the scheme of fractionation. The fractions were tested on guinea pigs, and small daily doses were found to protect them from scurvy. One fraction was also tested curatively on monkeys which had been kept on a scorbutic diet and had developed scurvy. The fraction cured them. All the active fractions reduce ammoniacal silver nitrate and decolorise potassium permanganate. The purest preparations showed an extremely low nitrogen and phosphorus content. P. H. P.

Differential Dialysis of the Antiscorbutic Factor. II. S. J. B. Connell and S. S. Zilva. (*Biochem. J.*, 1924, **18**, 641–646.)—Adopting the technique of Brown (*Biochem. J.*, 1915, **9**, 591), Zilva and Miura (*Biochem. J.*, 1921, **15**, 422), showed that the antiscorbutic factor of lemon juice diffuses through collodion membranes which permit the passage of substances of such molecular dimensions as methylene blue, neutral red and safranine. The vitamin diffuses also through collodion membranes of higher permeability. The authors have extended this investigation in the hope of utilising this method of differential dialysis for the purpose of separating the active principle from extraneous matter, but this has not been accomplished. Their experiments are described and results given. They find that the diffusion of the antiscorbutic factor proceeds differently from that of the sugar and nitrogenous substances present in decitrated lemon juice, thus confirming the observations of Zilva (*Biochem. J.*, 1924, **18**, 182; **18**, 632) in this connection. The antiscorbutic vitamin in swede juice and in lemon juice diffuses through membranes of the same permeability. This suggests that the factor in both sources is of the same molecular magnitude. The vitamin will diffuse through collodion membranes of somewhat lower permeability than those which permit the passage of the above-mentioned dyes. Indications suggest that the size of the active molecule cannot be far removed from that of the hexoses. P. H. P.

Influence of Diet and Sunlight on Vitamins of Milk. E. M. Luce. (*Biochem. J.*, 1924, **18**, 716–739.)—The experiments which were carried out on a Jersey cow are described in detail, and the results are discussed. Milk from the same cow was shown to vary within wide limits as regards its antirachitic and growth-promoting power. The diet of the cow appeared to be the main factor in determining the growth-promoting value of the milk. When the cow received a diet of fresh green grass the milk possessed a higher growth-promoting value than when she received dry fodder deficient in fat-soluble vitamins. This result was obtained irrespective of whether the animal was kept out of doors in sunlight or in a dark stall. The evidence as to whether light does, or does not, contribute to the action, is not conclusive from these experiments. The antirachitic value depends on the diet of the cow, and possibly also on the degree of illumination to which she is exposed. Milk from a pasture-fed cow has a definite and high antirachitic value; the same animal, when stall fed in the dark, yielded a milk much inferior in antirachitic properties. It is conceivable that, in the absence of

sunlight, it would be impossible to raise the antirachitic value of the milk above a certain level. A possible contributory cause of the seasonal incidence of rickets in children, may be the use of milks the antirachitic value of which is higher in summer than in winter. P. H. P.

Yeast Growth-Promoting Vitamin Tested for its Effects on Animals. J. Deas. (*J. Biol. Chem.*, 1924, **61**, 5–8.)—The yeast growth-promoting vitamin, first described by Wildiers (*La Cellule*, 1901, **18**, 313) as a "bios," was long considered to be the same as vitamin *B*. Funk and Dubin (*J. Biol. Chem.*, 1921, **48**, 437) showed that the yeast growth-promoting vitamin, called by them vitamin *D*, can be separated from that which causes growth of rats. Lucas (*Science*, **1924**, **59**, 197) found malt rootlets to be a more abundant source of bios than malt. The purified bios has now been separated into two fractions—Bios I. and Bios II. The former is precipitated by baryta, is not adsorbed on charcoal, and is not precipitated by lead acetate except in the presence of excess of ammonia; the latter is adsorbed by charcoal, can be removed from its solution by shaking with yeast, and is soluble in acetone. Either of these two substances by itself, and especially Bios II., is detrimental to the growth of yeast. The feeding experiments with rats, described in detail, show that malt rootless contain little or no vitamin *B*. Neither Bios I. or II., nor both in a combination suitable to promote an abundant growth of yeast, will promote the growth of rats. Consequently, Wildiers' bios and Funk's vitamin *D* are not identical with the rat growth-promoting vitamin *B*. It is also shown that the "bios" is not identical with the antiscorbutic vitamin *C*. P. H. P.

Comparison of Certain Oxidising Enzymes of the Higher and Lower Plants. M. E. Robinson. (*Biochem. J.*, 1924, **18**, 543–548.)—A fundamental difference appears to exist between the oxidases of the higher plants and those of the basidiomycetes, in that those of the former which have been examined consist of three components, a catechol derivative and two enzymes, oxygenase and peroxidase, whilst the oxidase of the basidiomycetes has so far been separated into two constituents only—an enzyme-like peroxide and peroxidase. The experiments of Onslow (*Biochem. J*, 1920, **14**, 535) on certain phanerogam oxidases have been repeated and his results confirmed. In the case of the mangold, the oxygenase and peroxidase could be demonstrated, whilst the presence of the catechol derivative, though indubitable, was sometimes difficult to show. The mangold contains a substance which is probably of a pectic nature, and this may be in combination with the catechol derivative. Probably the small content of the catechol derivative in the mangold, and also in the potato, may be due to the fact that these plants have a number of cultivated varieties, the chemical compositions of which may vary from that of the original types. The work of Gallagher (*Biochem. J.*, 1923, **17**, 515) on extractable plant phosphatides has been repeated; its interpretation as regards the oxidase reaction is very fully discussed in the text. P. H. P.

Oxidising Enzymes. VII. The Oxygenase of the Higher Plants. M. W. Onslow. (*Biochem. J.*, 1924, **18**, 549.)—The author criticises a statement made by Gallagher (*Biochem. J.*, 1923, **17**, 515) that there is no definite evidence for the existence, in the higher plants, of an enzyme (oxygenase) which catalyses the formation of peroxide, and that the presence of an autoxidisable lecithin-like substance is the cause of the rapid blueing of guaiacum; such a compound has been extracted from the potato tuber and the mangold root. A thermo-labile catalyst can be demonstrated readily in the potato tuber, mangold root and many other plants, but not in those of which the tissues show no direct blueing. The crude autoxidisable lecithin-like substance can be obtained from the tissue of a plant, *e.g.* the turnip root, which shows no direct blueing of guaiacum, and it needs some time (5 to 19 days) to autoxidise before giving any blueing with guaiacum in presence of peroxidase, whereas in the plant tissues the reaction is usually almost instantaneous. Many substances have this power of autoxidation and after some time can act as peroxides. P .H. P.

Influence of Colloids on the Reductase Test. A. I. Virtanen. (*Zeitsch. Unters. Nahr. Genussm.*, 1924, **48**, 141–151.)—When methylene blue is added to milk, as in the reductase test, part is adsorbed by the colloids present and part is in true solution. The time of reduction might, therefore, be expected to be affected by the presence of electrolytes which would, of course, alter the physical state of the casein, or by the presence of added colloids which would absorb more of the dye, or by added water which would alter the ratio between the natural colloids and the water. Experiment shows, however, that there is no marked effect traceable to these causes; it appears that in the reduction of the colour the equilibrium is so readily adjusted between the two phases that the influence of these factors is negligible. Barthel's method of applying the test was used, and there was no effect produced by the addition of either salts, or colloids, or water. H. E. C.

Insulin from the Cod Fish. Direct Application of Picric Acid to the Islet Tissue. H. W. Dudley. (*Biochem. J.*, 1924, **18**, 665–668.)—A convenient method of extracting insulin from the islet tissue of fish is described. It consists in applying picric acid directly to the tissue, extracting the insulin-containing picrate fraction with watery acetone, and converting the picrate into a soluble hydrochloride. Even with material of less than perfect freshness, preservation in watery picric acid was successful. There is a large principal islet in the cod occurring as a cap over the top of the gall bladder, and it can be detached easily. A yield of 13·12 rabbit units per grm. of tissue was obtained from the islet tissue of the cod, although collected from fish which had been caught at varying intervals up to 24 hours previously. Probably more would be obtained from absolutely fresh islets. The islet tissue of the cod contains, weight for weight, apparently about ten times as much insulin as mammalian pancreas. P. H. P.

Note on the Estimation of Phosphorus in Blood. M. Martland and R. Robison. (*Biochem. J.*, 1924, **18**, 765–768.)—There are many pitfalls in the estimation of phosphorus compounds in blood, and although under normal conditions the consequent errors may not be very large, they may be considerably increased if the conditions are but slightly changed. Although not mentioned before, in the Bell-Doisy method (*J. Biol. Chem.*, 1920, **44**, 55) the intensity of the final colour varies considerably with changes in the degree of acidity of the solution in the first stage of the process. This may cause errors in the estimation of the total phosphorus after acid ignition. In estimating the total phosphorus by the modified method of Briggs (*J. Biol. Chem.*, 1922, **53**, 13), after acid ignition the total acidity comes near or above the upper limit of safety; hence the authors use ammonium molybdate solution without acid, but the usual Briggs molybdate solution for the standards. Losses traced to over-ignition were entirely avoided by using more moderate temperatures and stopping the ignition as soon as the white fumes of sulphur trioxide appeared. Trichloracetic acid produces a blue colour with the Briggs reagents in complete absence of inorganic phosphate, but it is produced slowly; hence, to avoid error, the colorimetric readings must be made not more than 30 minutes after adding the reagents. If, in estimating the inorganic phosphates in blood, even a few minutes are allowed to elapse between laking and precipitation of proteins, the results will be too high. The amount of inorganic phosphate in whole blood is increased after ether anæsthesia, and also as the result of shock consequent upon cardiac puncture. P. H. P.

Effect of Oxygen Supply on the Metabolism of *Bacillus Coli Communis*. M. Stephenson and M. D. Whetham. (*Biochem. J.*, 1924, **18**, 498–506.)—A technique previously applied to the study of the metabolism of a non-fermenting bacillus was extended to one of a fermenting type (one which breaks down carbohydrates and other compounds in such a way that intermediate products collect in the medium), *B. coli* being employed. Three strains of it gave similar results. Details of the work are given, and curves showing the results. The carbon dioxide excretion and oxygen consumption of *B. coli* on a glucose inorganic medium were followed. The organism was almost anaerobic during the early stages of glucose decomposition. In an atmosphere of oxygen there was an increase in carbon dioxide excretion and a larger increase in oxygen consumption. This did not correspond, in the early stages, to an increased utilisation of glucose. Metabolism continued longer in oxygen than in air, the limiting P_H not being reached so soon. The increase of gaseous exchange with increase of oxygen pressure was attributed to an effect on some acid decomposition product of glucose. Growth on lactic acid (in the form of the ammonium salt) was studied in air and oxygen. Increased oxygen tension greatly increased the disappearance of lactic acid and production of carbon dioxide. Growth did not occur on lactic acid in anaerobic conditions. Succinic and acetic acids and glycerol were also found to be suitable sources of carbon for *B. coli* in air, but not in anaerobic conditions. A possible explanation of these facts may be found in the energy relations of the reactions involved.

P. H. P.

Proteolytic Action of *Bacillus Granulobacter Pectinovorum* and its Effect on the Hydrogen Ion Concentration. W. H. Peterson, E. B. Fred and B. P. Domogalla. (*J. Amer. Chem. Soc.*, 1924, **46**, 2086–2090.)—*Bacillus granulobacter pectinovorum* causes a rapid hydrolysis of the proteins during the fermentation of maize mash; from 50 to 75 per cent. of the total protein is converted into soluble products in the course of the fermentation, which is usually complete in three to four days. One-half of the soluble products may be formed within twenty-four hours. The hydrolysis results chiefly in the formation of simple peptides and amino acids; due to these buffers and to acids of low dissociation, a high titratable acidity may be produced without causing much change in the hydrogen ion concentration. W. P. S.

Toxicological and Forensic.

Circulation of some Heavy Metals in the Organism (Mercury, Bismuth and Lead). S. Lomholt. (*Biochem. J.*, 1924, **18**, 693–711.)—These experiments were carried out on rabbits. The metal compounds to be investigated were injected at short intervals into different portions of the muscles of a rabbit's limbs, and, after 10–20 days, the animal was killed under a narcotic by bleeding. The principal organs were then removed, together with a sample of the blood, for examination. The material analysed comprised: (1) The daily eliminated amount of urine and of fæces; (2) parts of muscles containing the remaining deposits of the metal compounds injected; (3) the most important viscera; (4) in some cases the total remaining parts of the rabbit's body. The results are given, partly in numbered columns, partly—to simplify the survey—in a series of diagrams. Four mercury compounds were investigated, mercurium benzoicum, acidum mercurisalicylicum, calomel, and metallic mercury finely divided in oil, by means of the method of Lomholt and Christiansen (*Biochem. Z.*, 1917, **81**, 356), which comprised destruction of organic substances, precipitation with hydrogen sulphide, solution of the precipitate, and electrolysis on thin gold electrodes which were weighed on a Nernst micro-balance. A large proportion of mercury was eliminated in the urine. In percentage the kidneys are the organs by far the richest in the metal; the liver comes second. The percentage distribution of the mercury in the organism is almost uniform with these four compounds, but the actual amount of mercury found is proportionately greater with the quickly absorbed preparations (mercurium benzoicum and acidum mercurisalicylicum) than with calomel, and especially with metallic mercury.

Amounts of bismuth were estimated by the radio-chemical method of Hevesy and Paneth (Aston: The Isotopes, 1922, Cambridge, 1924). Small quantities of radium E were mixed with the inactive bismuth to be used. When the radio-activity of the mixture was known, the bismuth was estimated by determining the β-radiation of the radium E. [Christiansen, Hevesy and Lomholt (*Compt. rend.*, 1924, **178**, 1324)]. Bismuth quinine iodide (Viochine) and bismuth hydroxide were examined. With the latter a little lampblack was

added to the oil suspension, as it was difficult to find and excise the parts where the injections had taken place. The greatest amount was found in the kidneys, not only relatively, but positively, twice as much being in the kidneys as in the liver. Bismuth and mercury compounds resemble each other as regards elimination and circulation, and are alike in other points, as they cause the same symptoms of poisoning (nephritis, stomatitis and colitis) and possess the same peculiar effect on syphilis.

The analytical method for lead was analogous to that used for bismuth, except that radium D was used instead of radium E [Hevesy (*Biochem. J.*, 1923, **17**, 439)]. Lead hydroxide was used, and lampblack was added to the emulsion. About twice as much of the metal was found in the liver as in the kidneys. Therefore the three metals investigated showed much the same conditions in the organism, but whereas with bismuth, the kidneys (and the urine) play the most prominent rôle, with lead the liver (and the fæces) seem to be just as important. Mercury comes between the two, somewhat nearer to bismuth. P. H. P.

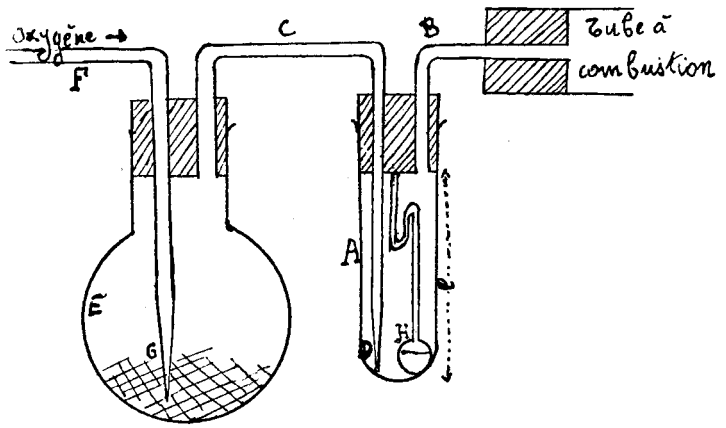
Agricultural Analysis.

Effects of the Method of Desiccation on the Nitrogenous Constituents of Plant Tissue. K. P. Link and E. R. Schule. (*J. Amer. Chem. Soc.*, 1924, **46**, 2044–2050.)—Experiments with beet leaves, maize leaves, maize ears, and common barberry leaves showed that drying at different temperatures had no effect on the total nitrogen content of the tissues. Drying at 80° and 90° C. caused coagulation of the soluble nitrogenous constituents in all cases. When the materials were dried at 32° and 54° C. proteolytic decomposition occurred, but in some instances this effect appeared to be counterbalanced by coagulation of soluble nitrogenous constituents. The general effect of all the methods of desiccation was a decrease in the amount of soluble nitrogen, due to coagulation; at 65° C. this was the only significant alteration. W. P. S.

Organic Analysis.

Organic Analysis by a Volumetric Method. J. Lindner. (*Chem. Zeit.*, 1924, **121**, 725.)—The usual method of combustion is employed, but connected with the combustion tube is an apparatus in which the products of combustion are brought into intimate contact with a halogen-phosphorus organic derivative, the compound $C_{10}H_7POCl_2$ being generally used. The water vapour produced during combustion is transformed by this compound into hydrochloric acid, which is absorbed by a small quantity of water and is readily estimated by titration. The carbon dioxide remaining in the products of combustion is absorbed by 0·1 *N* barium hydroxide solution and is estimated by back-titration. The analysis can be carried out on 20 mgrms. of the original substance and gives an accuracy of 0·05 per cent. in the case of the hydrogen, and of 0·1 per cent. in the case of the carbon. R. F. I.

Method of Analysis of Volatile Organic Substances by Combustion in an Open Tube. E. Carriere and C. Leenhardt. (*Bull. Soc. Chim.*, 1924, **35**, 1206–1207.)—By means of the device described a volatile liquid to be analysed by combustion is caused to evaporate regularly and is prevented from condensing in the front portion of the combustion tube. The substance is weighed in a stout bulb with a thin, sealed vertical neck having an S-bend. This is placed in a test-tube connected with the combustion tube by a tube flush with the bottom



of the stopper. Through the latter also a tube, drawn out at its lower end, passes to the bottom of the test-tube, its other end being flush with the bottom of the stopper of a small round-bottomed flask. Into this flask oxygen is passed through a tube reaching the bottom of the flask and with its drawn-out end below a quantity of broken glass. The oxygen is warmed in this flask and then passes into the test-tube and causes gradual evaporation of the liquid from the bulb, the end of the neck of which is broken by forcing in the stopper of the test-tube. The last traces of the liquid are eliminated by subjecting the test-tube to alternate heating and cooling. T. H. P.

New Reaction of Aldehydes. R. De Fazi. (*Gazz. Chim. Ital.*, 1924, **54**, 658–667.)—The unsaturated compounds formed by condensation of fluorene with piperonaldehyde, cuminaldehyde, *m*-tolualdehyde, cinnamaldehyde, *p*-dimethylaminobenzaldehyde, and furfuraldehyde give various colorations with concentrated sulphuric acid (*cf.* ANALYST, 1921, **46**, 376), but this is not the case with the compounds formed by reduction of these condensation products. Such products are not formed by acenaphthene under the conditions employed, but acenaphthenone reacts with cyclic aldehydes in presence of sodium hydroxide, giving compounds which are coloured violet-red by concentrated sulphuric acid. T. H. P.

Colour Reaction Common to Formaldehyde and Glyoxylic Acid. Fosse and A. Hieulle. (*Comptes rend.*, 1924, **179**, 636–638.)—Like formaldehyde, glyoxylic acid gives a deep magenta coloration with Schryver's reagent, composed of 2 c.c. of 1 per cent. phenylhydrazine hydrochloride solution, 1 c.c. of 5 per cent.

potassium ferricyanide solution, and 5 c.c. of concentrated hydrochloric acid. The reaction is detectable with 0·001 mgrm. of glyoxylic acid at the concentration 1:1,000,000. If the coloured liquid is shaken with ether, the ether extracts the base derived by the dissociation of the colouring matter and regenerates the latter if treated with a few drops of hydrochloric acid. T. H. P.

Exact Methods for the Analysis of Gaseous Fuels. E. Ott. (*Helv. Chim. Acta*, 1924, **7**, 886–898.)—The author, with H. Deringer, has investigated the different absorbents for gases occurring in illuminating or fuel gas. It is shown that fuming sulphuric acid is preferable to bromine water for the estimation of heavy hydrocarbons, as it is not only easier to handle but more accurate, especially for benzene or ethylene. Fixed pipettes loaded with glass rod are better than the ordinary moveable type. When only traces of ethylene or benzene are present and have to be estimated it is advisable first to remove any oxygen by means of phosphorus, and carbon monoxide by copper ammonium chloride. A summary of previous work on the same subject is given. H. E. C.

Examination of Commercial Pinene. A. Gawalowski. (*Zeitsch. anal. Chem.*, 1924, **64**, 471–473.)—The method of testing pinene by means of strong hydrochloric acid (*Zeitsch. anal. Chem.*, 1923, **63**, 121) may conveniently be carried out in a "pinenometer," a stoppered graduated cylinder of 15 c.c. capacity. The zero (top) mark corresponds to 15 c.c., the 50 mark to 10, and the 100 mark to 5 c.c. volume. The treatment of the sample of pinene is carried out as previously described; the volume of the top layer is read off after 24 hours; the figure multiplied by 0·7884 gives the percentage of pinene *plus* camphene. For the detection of chlorine, a flattened platinum wire is sprinkled with copper oxide, ignited, impregnated with the material, and again ignited. When the wire becomes incandescent after combustion of the organic matter, the flame is coloured green if chlorine is present. An approximate estimation of the chlorine content is possible if the persistence of the green coloration is measured in seconds and compared with that of an equal weight of a standard preparation of known chlorine content. W. R. S.

Estimation of Thymol and Carvacrol in Thyme Oils. C. E. Sage and W. G. Dalton. (*Perfumery and Essential Oil Record*, 1924, 345.)—The oil under examination is shaken with a 4 per cent. solution of sodium hydroxide in a boiling water bath and washed with water. The alkaline solution is acidified, and the liberated phenols extracted with petroleum spirit. This extract is dried with anhydrous sodium sulphate and allowed to evaporate in an open dish, complete removal of the petroleum spirit being effected by warming the residue to 35° C. for a few minutes. The solidification point of the mixed phenols is determined, and an allowance is made for the traces of the non-phenolic constituents of the oil also present. For this purpose a range of mixtures was prepared of pure thymol and pure carvacrol with non-phenolic constituents of the oil, the mixed phenols were prepared from these by the above method and their solidification points

determined and plotted on a curve. By comparing the freezing points of the mixed phenols of the oil under examination with the points on the curve the percentage of thymol can be estimated. The solidification point of thymol is given as 49° C. and that of carvacrol as 0° C. R. F. I.

Methylene Blue as an Indicator of Bleaching Damage. E. Ristenpart. (*Cellulosechem.*, 1924, **5**, 8; *J. Textile Inst.*, 1924, **15**, 254A.)—The fabric to be tested is dyed by immersing 5 sq. cm. of it in 500 c.c. of a cold 0·001 per cent. solution of methylene blue for 10 min. It is then rinsed, dried in contact with a smooth surface, and the "degree of white" is measured in terms of Ostwald's notation (*cf.* Abst., p. 607). In this, a matt surface of barium sulphate is taken as a standard, and between this and black are 100 degrees. Damage by bleaching is indicated by increased affinity for methylene blue, and a comparison may be obtained in the form of a ratio, Q, between the "degree of white" of the original sample and the "degree of white" of the sample dyed with methylene blue. If this ratio be Q in the bleached goods and Qu in the unbleached, then the "methylene blue value" is expressed as the difference produced by bleaching,

$$M = (Q - Qu)/Qu.$$

In hypochlorite bleaching the "methylene blue value" increases with the concentration of the chlorine, and also with the rise in temperature, but not with the alkalinity. A permanganate bleach produces far more oxycellulose than a hypochlorite bleach containing the same amount of active oxygen. R. F. I.

Inorganic Analysis.

Separation of Mercury from Arsenic. P. Wenger and M. Schilt. (*Helv. Chim. Acta*, 1924, **7**, 907–909.)—An accurate separation of mercury from arsenic may be effected by means of ammonia or sodium bicarbonate. The mixed sulphides are washed with cold ammonium hydroxide solution, which should also contain ammonium chloride in order to prevent the formation of any colloidal mercury sulphide, and then with ammonium chloride solution until the washings are neutral. Alternatively, sodium bicarbonate may be used, but in this case the solution must be warm to ensure complete solution of the arsenic sulphide. For the subsequent estimation of the mercury the sulphide is collected on a tared Gooch crucible, dried at 105° to 120° C., then washed with ammonium chloride solution and three times with alcohol, extracted with carbon disulphide to remove sulphur, washed again with alcohol and finally with ether, then dried and weighed. H. E. C.

Electrolytic Separation of Copper, Antimony and Bismuth from Lead. A. Lassieur. (*Comptes rend.*, 1924, **179**, 632–634.)—In the electrolytic separation of lead from copper, antimony and bismuth in presence of a reducing agent such as hydroxylamine, an acidity corresponding with at least 15 to 20 per cent. of

hydrochloric acid (1·19) is necessary. To obtain a quantitative lead deposit with the apparatus described by the author (*Comptes rend.*, 1923, **177**, 1114), the auxiliary potential should be 440 millivolts, the volume of the electrolyte being 200 c.c. and the temperature 50° to 60° C.; from 1 to 2 grms. of hydroxylamine hydrochloride are sufficient. Hydrofluoric acid, the preliminary use of which is sometimes necessary, may be present in the liquid in the proportion of 2·5 per cent. of the concentrated acid in place of some of the hydrochloric acid, the auxiliary potential being lowered in this case to 400 millivolts; glass vessels are still usable under such conditions.

In presence of lead, bismuth deposits well from a solution containing nitric acid, either alone or together with hydrochloric acid, and hydroxylamine hydrochloride, the auxiliary potential being from 200 to 240 millivolts; the lead forms a good cathodic deposit from a nitric acid solution containing hydroxylamine. The separation of copper and lead is easily effected in hydrochloric acid solution containing hydroxylamine hydrochloride, the copper being completely deposited at an auxiliary potential of 240 millivolts.

In separating antimony from lead, the antimony forms a black, loose coating if deposited from a solution of its trichloride in hydrochloric acid containing hydroxylamine, but a good, complete deposit is obtained from the antimonic salt with the auxiliary potential 240 millivolts. The lead is deposited on a cathode either of platinum or coated with antimony with the auxiliary potential 350 millivolts, but the concentration of the lead must not exceed 0·2 grm. per 100 c.c., since otherwise antimony becomes entangled with the lead. T. H. P.

Detection and Estimation of Traces of Calcium and Magnesium. F. Pavelka. (*Chem. Zeit.*, 1924, **121**, 728.)—Calcium and magnesium salts can be detected in alcoholic solution by means of ammonium ferrocyanide, which causes the formation of a finely crystalline precipitate. In dilute solution, *e.g* in water analysis, a permanent cloudiness is produced, and this effect may be made the basis of a nephelometric method of estimation. This test is claimed to be very much more sensitive than those with any of the usual reagents.

R. F. I.

Separation of Yttrium from the other Elements of Gadolinite. L. Rolla, V. Cuttica and L. Fernandes. (*Gazz. Chim. Ital.*, 1924, **54**, 617–622.)—Yttrium may be separated from the other elements occurring with it in gadolinite by fractional crystallisation of the mixed sulphates formed with thallous sulphate, the solubility being lowest for the yttrium double sulphate. These double sulphates are stable compounds and are more soluble at low than at high temperatures.

T. H. P.

Direct Nesslerisation Micro-Kjeldahl Method and a Modification of the Nessler-Folin Reagent for Ammonia. F. C. Koch and T. L. McMeekin. (*J. Amer. Chem. Soc.*, 1923, **46**, 2066–2069.)—The substance is heated with concentrated sulphuric acid until dissolved, the mixture is cooled, a small quantity

of 30 per cent. hydrogen peroxide is added, and the heating is continued. The addition of the peroxide causes very rapid oxidation of the carbonaceous matter present, and there is no loss of ammonia. The use of hydrogen peroxide in the ordinary Kjeldahl method is also advantageous in the case of substances containing a high proportion of carbohydrates and fats, since it prevents troublesome foaming. The modified Nessler-Folin reagent is prepared by dissolving 22·5 grms. of iodine in 20 c.c. of water containing 30 grms. of potassium iodide; 30 grms. of metallic mercury are then added, and the mixture is shaken and cooled until all yellow colour due to iodine has disappeared. The solution is then decanted and concentrated iodine solution is added, drop by drop, until a drop of the mixture gives a reaction with starch solution. The whole is then diluted to 200 c.c. and mixed with 975 c.c. of 10 per cent. sodium hydroxide solution. W. P. S.

Volumetric Estimation of Ammonium Salts. S. Lövgren. (*Zeitsch. anal. Chem.*, 1924, **64**, 457–470.)—The direct titration of ammonium salts at ordinary temperature by means of caustic alkali was investigated, thymolphthalein being used as indicator. The end-point (colourless to blue) was found to be sufficiently sharp in alcoholic solution, provided the final alcohol concentration was at least 50 per cent. The final volume should not much exceed 10 c.c., otherwise the sharpness of the end-point is impaired; one c.c. of a saturated solution of thymolphthalein in 50 per cent. alcohol is added. The maximum error is stated to be $\pm$ 1 per cent. The strength of the standard alkali solution should be chosen with a view to preventing undue dilution; with a 0·1 *N* solution it is unsafe to titrate more than 0·01 grm. of ammonium chloride. For larger quantities, *e.g.* 0·3 grm., the salt is dissolved in 2 c.c. of water, with addition of 7 c.c. of alcohol and 1 c.c. of indicator, and titrated with *N* sodium hydroxide to the appearance of a blue colour. The use of a standard ammonium salt solution is recommended. W. R. S.

Volumetric Estimation of Hydrazine by the Iodine, Bromate, Iodate, and Permanganate Methods. I. M. Kolthoff. (*J. Amer. Chem. Soc.*, 1924, **46**, 2009–2106.)—Under proper conditions, which are given below, all four methods yield accurate results. *Iodine Method.*—Twenty-five c.c. of approximately 0·1 *N* hydrazine sulphate solution are treated with 1 grm. of sodium hydrogen carbonate, and the solution is titrated with 0·1 *N* iodine solution until the yellow colour persists for two minutes; the last few drops of iodine solution required should be added slowly. *Bromate Method.*—The hydrazine sulphate solution is mixed with an equal volume of hydrochloric acid (sp. gr. 1·19), a few drops of indigo or methyl-red solution are added, and the mixture is titrated with 0·1 *N* potassium bromate solution until the colour disappears. When indigo is used, an excess of 0·14 c.c. of bromate solution is required; with methyl-red, the excess is 0·22 c.c. at the end-point. These amounts must be subtracted as a correction. *Iodate Method.*—Six c.c. of carbon tetrachloride and at least 25 c.c. of hydrochloric acid (sp. gr. 1·19) are added to 25 c.c. of hydrazine sulphate solution in a stoppered flask, and the mixture is titrated with standard iodate solution until the carbon

tetrachloride layer is decolorised after the mixture has been shaken vigorously: $N_2H_4+KIO+2HCl=KCl+ICl+N+3H_2O$. *Permanganate Method. A. In acid solution.*—Ten c.c. of 4 *N* hydrochloric acid are added to 25 c.c. of hydrazine sulphate solution, the mixture is heated to boiling and titrated with 0·1 *N* permanganate solution until a pink coloration is obtained; this colour disappears after a time *B. In alkaline solution.*—Twenty c.c. of hydrazine sulphate solution are mixed with 50 c.c. of 0·1 *N* permanganate solution and 10 c.c. of 4 *N* sodium hydroxide solution; after thirty minutes, 1·5 grm. of potassium iodide and 20 c.c. of 4 *N* sulphuric acid are added, and the liberated iodine is titrated with 0·1 N thiosulphate solution. In both cases the hydrazine is oxidised according to the equation:

$$N_2H_4+O_2=N_2+2H_2O.$$

W. P. S.

ERRATUM.

Separation of Rhodium from Platinum:—On page 545, line 33, read "*not* more than 1 grm."

Physical Methods, Apparatus, etc.

Colloidal Poles for observing the Emission Spectra of Solutions. J. Errara. (*Bull. Soc. Chim. Belg.*, 1924, **33**, 449–451.)—As has been shown by Gramont, the wave length of the ultimate rays of spark spectra of dilute solutions affords a ready means for the detection of metals. The spectra are obtained by sparking the solution between poles of gelose (an agar-agar product) at the secondary terminals of an induction coil. To prepare the poles the gelose should be washed for a week in slowly running distilled water, then dried by pressure to a water content of about 4 per cent., moulded into pencils and baked in an autoclave at 120° C. for 10 minutes. The pencils are cut into pieces about 7 by 50 mm., which are soaked for a few minutes in the solution to be tested, and used as poles for the sparking. The following rays are due to the gelose alone, the three rays of calcium and magnesium, one of sodium (3303), two of silver and copper, and 3064, 3067, 3089 due to water, and to air 3159, 3371, 3536, 3577.

Element.	Limit of concentration for μ_1.	Ultimate rays.
Silver	1/1000000	3280 $7\mu_1$; 3383, μ_2; 2437 μ_3
Cadmium	1/1000000	2288 μ_1;
Mercury	4·32/10000	2536 $5\mu_1$; 3650 μ_2; 4046 $6\mu_3$
Calcium	—	3933 $7\mu_1$; 3968 $5\mu_2$; 4226 $7\mu_3$
Magnesium	—	2852 $1\mu_1$; 2795 $5\mu_2$; 2882 $7\mu_3$
Copper	—	3247 $5\mu_1$; 3274 μ_2.

H. E. C.

Differentiation of Natural and Culture Pearls. A. Dauvillier. (*Comptes rend.*, **179**, 818–819.)—A method of distinguishing between natural and culture pearls has been based on the photographic examination of the figures of Laue or the diffraction rings produced when they are traversed axially by monochromatic X-rays. For this purpose the object is exposed for some hours to the K rays of rhodium and of silver produced by means of the quartz tube previously described (*Comptes rend.*, 1921, **172**, 1915), and the photographic plate is placed at a distance of 12 cm. while an intensifying screen of calcium tungstate is used. In the case of natural pearls the photograph shows a system of regular rings due to the doublets Kα and the rays Kβ. Pearl nacre, on the other hand, invariably gives more or less regular Laue figures composed of more diffused stains than crystals produce. These stains tend towards a hexagonal system when the irradiation is effected normally to the "plane of cleavage." In the perpendicular direction they are more numerous, and show quaternary symmetry. Thus culture pearls give at the same time both the system of rings characteristic of the true pearl and stains which reveal the internal nucleus of nacre.

Colour Measurement. O. Meissner. (*Zeitsch. Physik.*, 1924, **21** 68–72; *J. Textile Inst.*, 1924, **15**, 230A.)—In Ostwald's colour scheme (*cf. J. Soc. Chem. Ind.*, 1919, **38** 914A) each colour is defined by three characteristics, *viz.* the colour tone, c (in the scale of 100 divisions, 0 = yellow, 25 = red, 50 = blue, etc.), the proportion of white, w, and the proportion of black, s. From these the purity of the colour, r, is derived—$r = 100 - (w + s)$. The grey content is $w(w + s)$. All derivatives of a definite pure colour form a colour triangle, and by its rotation about the grey axis there arises a double cone, the properties of which are mathematically described; by means of it the characteristics of a mixed colour are determined from its components. It is shown that the purity of a composite colour is always lower than the arithmetical mean of the purities of the separate shades. An example is given of the formation of grey from three colours.

Reviews.

KONDUKTOMETRISCHE TITRATIONEN. By Dr. I. M. KOLTHOFF. Pp. viii. + 94. Dresden and Leipzig: Theodor Steinkopff. 1923. Price 2s. 9d. (paper cover).

This short monograph gives a clear and concise account of the various ways in which the measurement of conductivity can be applied to the solution of analytical problems. The first chapter deals generally with electrolytic dissociation, the hydrolysis of salts, the formation of complex molecules, etc., and in the second and third chapters the theoretical and practical considerations involved in conductivity measurements are discussed. In succeeding chapters the application

of the method to analytical problems is considered and diagrams are given of the various graphs obtained in titrating acids and bases of various strengths and in titrations involving precipitations. In the greater number of cases the conductivity graphs consist of straight lines which intersect at the points at which the reaction is complete. Accurate results may be obtained in the titration of weak acids with weak bases in cases where ordinary indicators give an uncertain colour change. The method may be used with highly coloured liquids and is of considerable practical value in the examination of wines, vinegar and possibly beer. It is by no means generally applicable, as in some cases the phenomena of dissociation and hydrolysis combine to produce a graph which is more or less uniformly curved, and in which no sharp indication of the completion of a reaction can be observed. In many natural products, such as beer and fruit juices, the significance of the data given by the conductivity method is not clear, and further research is needed. From a consideration of the many examples given the impression is gained that titration by conductivity methods is not likely to be of much value in the ordinary analytical laboratory. It is rather in the technical examination of a large number of samples of a particular product, such as wine, that it may often afford information which it would otherwise be difficult or even impossible to obtain. G. W. MONIER-WILLIAMS.

ANALYTICAL CHEMISTRY, Vol. II., QUANTITATIVE. By F. P. TREADWELL and W. T. HALL. Sixth Edition. Pp. xiii.+811. New York: Wiley & Sons; London: Chapman & Hall. 1924. Price 25s. net.

This well known work has been completely revised, but, as a comparison with the previous edition shows, the revision has been mainly textual. A few fresh methods have been included, and some of the old methods have been modified, apparently in accordance with the procedures adopted by the Bureau of Standards and by other American testing bodies. The standards of mass and volume now introduced into the book are those of the Bureau of Standards—so much so that the term millilitre (ml.) has been substituted for the more familiar cubic centimetre. The introductory section has been enlarged to include some treatment of topics such as the calculation of the precision of analytical data, adsorption by precipitates, the care of platinum, and sampling. Fuller consideration is given throughout the book to theoretical principles, *e.g.* the practical importance of a knowledge of the magnitude of the solubility products of certain sulphides, discussed on pp. 161–3. Fundamental analytical procedures also receive increased attention, in particular, the preparation of substances for analysis.

The treatment accorded to Indicators in the earlier editions was far from satisfactory, and an attempt has been made in the present issue to remedy this, by the inclusion of a brief study of Hydrogen Ion Concentrations, together with a description of Hildebrand's method of carrying out electrometric titrations with the use of the hydrogen electrode. The method is erroneously ascribed to Hillebrand. It is felt that the value of this knowledge from the standpoint of volumetric analysis has not been rendered sufficiently clear, and this is especially the

case in its application to the choice of indicators. Thus typical neutralisation curves of various acids and bases would have been invaluable to the student in his selection of suitable indicators, and so would have been the electrometric titration curves of Hildebrand to explain the use of hydroxy compounds, *e.g.* mannitol, glycerol, in the titration of boric acid in presence of phenolphthalein. Küster's method of preparing alkali free from carbonate is still retained, but no mention is made of the recent and more convenient electrolytic method (*J. Amer. Chem. Soc.*, 1920, **42**, 488). Greater attention is given to the calibration of apparatus. The general treatment of gas analysis remains unchanged; the estimation of carbonyl sulphide has been added.

The volume cannot be regarded as exhaustive, for many established methods have not yet been included and the estimation of many elements receives either a cursory note or no mention at all. The work is, however, and in its revised form will remain, a reliable and valuable aid to the analyst and will continue to rank high among the few standard works on analytical chemistry.

HUBERT T. S. BRITTON.

MODERN CEREAL CHEMISTRY. D. W. KENT-JONES, B.Sc., F.I.C. Pp. 324. The Northern Publishing Co., Ltd. Price 25/- net.

This book, the author states in his preface, is written for cereal chemists and progressive millers. It deals with the chemical and physical properties of wheat, wheaten flour, and bread, with their physical and chemical examination, and with the operations of milling and baking. In general, it may be said that the book deals only with roller milling and white flour and bread; other flours are hardly touched upon, and the bye-products of milling are almost unnoticed, so that "cereal chemistry" is perhaps rather too wide a title; there is no doubt, however, that the "modern" is justified. It is not unfair to say that the book is a plea, and a very interesting plea, for white flour and for the bleaching and "improving" of white flour.

The author considers milling, flour itself, and the baking of bread largely from the view points of colloid chemistry and H-ion concentration, whilst the vitamin point of view is not neglected. Results of researches of recent years are presented as a consecutive story, full references being given to original papers.

In order to prepare the reader for consideration of the subject from these modern points of view, a separate chapter is devoted to the above-mentioned three subjects. H-ion concentration is clearly expounded and the necessary apparatus described. The chapter on colloidal chemistry is less successful and would be more helpful if the principles were more carefully considered, and their bearing upon the matter at issue, namely flour, were kept more in mind. It is true that a further chapter is devoted to the colloidal chemistry of flour, but this deals also very largely with H-ion concentration and diastatic power, so that the colloidal aspect gets confused. The chapter on vitamins is a digest of the first edition of the well known report of the Medical Research Committee on this subject.

What may, for the want of a better expression, be termed the "ordinary"

analysis of flour occupies a subsidiary position in the book. The difficult question of the determination of moisture is fully and frankly treated. The fact that the figure for moisture content is almost meaningless unless the exact analytical procedure is given is fully brought out, and in this connection the progressive miller might well be pardoned if he doubted whether the cereal chemist knows what he is doing. A similar difficulty with regard to oil or fat determinations is referred to.

With regard to arsenic, the inclusion of a quantitative Reinsch process is surprising, and the use, in this test, of strips of copper 1 by $2\frac{1}{2}$ cm. in area is to be deprecated for delicate work. A modification of the Gutzeit process, in which the hydrogen and arsine are bubbled through lead acetate solution and dried by calcium chloride, takes the place of the usual method. The determination of gas-production power and diastatic activity are included. An omission is that of a process for the determination of total phosphorus. The determination of starch by washing out, drying, and weighing, is simple, but does not seem to be a very refined method.

A prominent place is given to the question of bleaching and flour improvers. Here the author is far from impartial. This part of the book is largely an account of the patent case of 1909 (*Flour Oxidising Co.* v. *Hutchinson*), and of the action taken by the Hull Corporation with regard to persulphate in 1913. It is hardly correct to say with regard to bleaching (even with oxides of nitrogen) that "in England the matter was settled" by the former case, nor can it be agreed that as a result of the latter case "persulphates are the one class of improvers that have definitely received legal sanction"; it is true that others have not, but it is going too far to say that persulphates have. There are many curious, and very disputable, statements in this connection. After being assured that the question of bleaching by oxides of nitrogen is settled (in their favour) we are comforted by the statement that "these lower oxides of nitrogen are, on the whole, quite harmless non-corrosive gases." It is not easy to see what is meant by "new flour improvers should only be used publicly after the fullest of careful investigations." "Should this matter" of persulphates "or a similar one be opened again the views expressed by Mr. Jago may be taken almost as a standard," seems to be pressing standardisation too far. Not many Public Analysts will agree with the statement, flattering though it is, that "with the just and necessary strict Food and Drugs Acts which are in existence, and with the exacting and widespread examination of food by expert and skilful analysts all over the country, it need hardly be pointed out that the presence of a harmful flour improver, or one which would be likely to be injurious to the public health in the slightest degree, would not long survive." The whole treatment of these subjects is obviously too partial to be accepted without much reserve. It must be admitted, all the same, that the author makes out a very good case for improvers of one sort or another. In a few months' time we may all be wiser on these matters.

The suggested limit for calcium sulphate in acid phosphate is erroneously referred to in more than one place as a Board of Trade limit.

This book deals with matters of great national importance and of particular interest at the present time; it is full of enthusiasm for research, is never dull, and contains many valuable data. Its very modernity carries with it the drawback that in a short time, no doubt, many of the views expressed and quoted will have to be revised: it is to be hoped that the demand for this edition will render possible another in the not distant future. E. HINKS.

THE MANUFACTURE OF SULPHURIC ACID. CHAMBER PROCESS. By WILFRID WYLD. Pp. xii+424. London: Gurney & Jackson. 1924. Price 31s. 6d,

In this, the second volume of the revised edition of Lunge's "Manufacture of Acids and Alkalis," only the chamber process is considered. This avoids the somewhat fortuitous arrangement of the last edition, and enables the author to include new matter and to discuss it adequately.

The first part gives a comprehensive description of the construction and erection of lead chambers in general, followed by details of the various types, such as the Mills Packard, Moritz, etc., and also the Opl and other systems, in which towers to replace or work in conjunction with chambers are used. The various methods of supplying water to, and draughting the chambers are given, together with figures of the various sprays and apparatus used for these purposes, although, in many cases, these are rather what are to be found in manufacturers' catalogues than in actual practice. The same applies to the description of acid-elevating plant.

The use of pumps is rapidly coming into general use now that they can be constructed without glands or packing, and it would have been useful had data been given as to the life of these and their behaviour after being in use for some time.

The design and working of the Glover and Gay Lussac towers are well described, and figures are given as to the cubic capacity and performance of several towers.

Probably the most useful part of the book is Chapter III., which discusses in detail the supply of water and nitre to the chambers and the various factors which cause, respectively, improper or correct working of the chamber. The causes and methods of rectifying various plant troubles are fully described, and in this connection stress is laid on the testing of the chamber exit gases. Methods are given for carrying this out and for estimating the content of nitrogen acids, sulphur acids, etc. The author recommends working with 9 per cent. of oxygen at the exit in the case of spent oxide, although Lunge (4th edition) and others use 6 to 8 per cent.

The older and more recent theories of the chamber process are surveyed, and the conclusion reached is that the principal reactions are:

1. $SO_2+NOOH+O=SO_2(OH)(ONO)$.
2. $SO_2(OH)(ONO)+H_2O=H_2SO_4+NOOH$.

Whether N_2O_3 exists for an appreciable time as undecomposed vapour or not, is not a decisive point in this case, as the hydrates NOOH may be introduced,

and as the components NO and NO_2 act in the same way as N_2O_3. The excess SO_2 in the first chambers also gives :

$$2SO_2(OH)(ONO)+SO_2+2H_2O=3H_2SO_4+2NO.$$

A chapter is given on the purification of sulphuric acid, and other methods of producing it are briefly mentioned, *e.g.* from sulphates, bisulphates, etc.

Costs are not dealt with at length, owing to the great variations in prices during the last few years. References to patent and other literature are given throughout the text; hence older theories and systems are not included, which makes the book more valuable for practical use, although, in many cases, it would have been better if some mention had been made of the actual working success of many of the types of plant and systems mentioned. A fuller discussion on the merits or otherwise of intensive working would also have been advantageous.

The author is to be commended on having produced what is, in fact, a new treatise, rather than a revision of chamber acid processes.

ERIC A. REAVELL.

LIME AND MAGNESIA. By N. V. S. KNIBBS, B.Sc. Pp. xii+306. London: Ernest Benn Ltd. 1924. Price 30s. net.

This very useful book, if only because it brings together matter relating to the subjects of which it treats, which otherwise would be widely separated and not easy to refer to. The book is divided into three parts: Chemistry, Manufacture, and Uses.

In Part I., which perhaps may be looked on as the really distinctive and new feature of the work, after a chapter on the origin and occurrence of lime and magnesia compounds, there is one on the physical and chemical properties of lime, in which are collected data on specific gravity, optical, thermal, and electrical properties, solubilities, etc., of calcium oxide, hydroxide, and carbonate; and whilst these in themselves are very full, the value of the chapter is enhanced by the collection of references to authoritative and relevant literature at the end. This admirable practice is continued throughout the book. The following chapter deals with the chemical reactions of the same three substances, and is followed by two corresponding chapters dealing with magnesium oxide, hydroxide, and carbonate in a similar way. A short chapter on dolomite and magnesium limestones follows. The next is on the chemistry and physics of lime and magnesia burning; here are theoretical data on the dissociation of the carbonate by heat, discussion of experimental work on it, of over burning, of the effect of steam in calcination, etc.; and this is followed by a similar chapter on the hydration of lime and magnesia. These two chapters are perhaps the most important in the book, as affording a basis for placing the manufacture of quicklime and hydrated lime on a scientific foundation. The last chapter in this part deals with the analysis and testing of the materials and products of the lime and magnesia industries. The account given of the methods in common use is fairly satisfactory, though it is not easy to understand why some of these methods, which quite properly belong here, have been described in an appendix.

The second part contains a description of the plant used in the preparation of limestone for calcination, of the various forms of kiln, the control of the operation of burning, the calculation of the thermal efficiency of the process, and the methods of production of hydrated lime from quicklime. All this is gone into very fully, and appears, to one who is not a technologist in the industry, to be very well done, though in places it suggests compilation rather than first hand knowledge. The corresponding portion of the work on magnesia is very short, and in a future edition might with advantage have a little more space devoted to it.

The third part, after a consideration of the effect of impurities and of physical condition on the uses of lime and magnesia, proceeds to an enumeration and description of those uses. The uses of lime in agriculture, whether as quicklime, hydrated lime, or limestone, are very fully discussed, as are also the characteristics of the varieties of lime when used in constructional work, the setting of mortars, etc. The section on magnesia refractories and still more that on magnesia cement, are far from having the same fulness, and an extension of these would greatly improve the work.

The writing is clear, and the style interesting, and the printing and general get up of the work are admirable. Misprints and errors are few, though on page 184, line 5, the words "per lb." have intruded themselves unnecessarily and entirely changed the meaning. Some novelties in spelling disfigure the volume: "dessicator" is hardly a novelty perhaps, but "cintering" and "insipient" are distinctly so. Some technical writers, American especially, have so inured us to "more data *is* needed," that only those with weak nerves will suffer seriously from the shock of meeting it here; but when the author goes on to tell us of the actions effected by "a bacteria," even the strongest shudders. The use of the word "clog" in the sense of "clot," and of "insular" for "insulator," is to be deprecated; so is the habitual use in the text of formulæ as substitutes for the names of substances. But these, after all, are small blemishes, and do little to soil the virtues of a book which, in a sense, breaks new ground, and will be found of great use by many, chemists as well as lime burners. J. T. DUNN.

THE SYNTHESIS OF NITROGEN RING COMPOUNDS CONTAINING A SINGLE HETEROATOM [NITROGEN]. By CECIL HOLLINS, B.Sc., A.I.C. Pp. xiv.+423. London: Ernest Benn, Ltd. 1924. Price 55s.

Even in our age of specialisation it is, to some extent, surprising to find an author as well as a publisher for such a highly specialised book, and both the author and publisher are to be congratulated on the production of this valuable addition to chemical literature. It can confidently be assumed that Mr. Hollins' compendium will be of some considerable value, especially if one considers that it covers a field dealing with the chemistry of the pyrroles, indoles, quinolines, etc.

The book under review seems to offer a complete summary of the different methods used for the synthesis of the many compounds mentioned in it. It gives a detailed summary of the methods and it frequently also refers to the yields which have been obtained for some of the substances. Of interest is also the fact that

Mr. Hollins refers to cases in which generally successful reactions have failed to give the expected results. Such negative observations are of value, and it would be most useful if other writers would follow his example. Negative results are too often omitted from the literature of a subject, and this is obviously to be regretted. To the research chemist negative results are as important as the positive ones, and chemistry would gain much if more prominence were given to the former.

The most valuable part of a book of this kind is obviously the bibliography. The reviewer has therefore deemed it desirable to compare some 30 to 40 references with the original literature and he has found them to be correct in every respect. This certainly adds greatly to the value of the book. M. NIERENSTEIN.

THE TECHNIQUE OF TISSUE CULTURE "IN VITRO." Pp. 80. Price 7s. 6d.

TISSUE CULTURE IN RELATION TO GROWTH AND DIFFERENTIATION. Pp. 50. Price 5s. By T. S. P. STRANGEWAYS. Cambridge: Heffer & Sons. 1924.

These two volumes appear to be the first attempts to give in book form the technique of, and results achieved by, tissue culture in the laboratory. Although perhaps outside the usual run of books reviewed in this journal, it is by no means improbable that in future the analyst, among his many other activities, will be called upon to carry out experiments such as those described in the first of these volumes, when dealing with preservatives, toxicological investigations and related subjects.

The larger volume is devoted to detailed descriptions of apparatus and manipulations in the removal, culture and staining of animal tissues which the author has evidently written with meticulous care and a wide knowledge of the subject. The descriptions are perhaps unnecessarily detailed, since the worker likely to take up tissue culture will have no use for the instruction in elementary glass working on page 42, nor for the process of coating apparatus with paraffin wax, to which two and a half pages are devoted. Some confusion is caused by the frequent references to different sections of the book instead of to pages which are much more readily found, and the letters on Fig. 18 are so small as to be almost illegible.

The second volume provides brief but fascinating reading dealing with the observation of growth "*in vitro*," its relation to the physiology of the cell, modification of the developing cells by external agencies, such as the addition of various substances to the medium, variation of temperature, exposure to Röntgen rays, etc., and the relation of tissue culture to inflammation and tissue repair. The four collotype plates provided are excellent reproductions illustrating the stages of cell growth and mitosis, but unfortunately the magnification is not given, although this appears to be in the neighbourhood of $\times 500$.

The author is to be congratulated upon the production of these two small volumes dealing with a subject which will undoubtedly become of increasing importance with the passage of time. T. J. WARD.