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THE ANALYST

PROCEEDINGS OF THE SOCIETY OF PUBLIC ANALYSTS AND OTHER ANALYTICAL CHEMISTS

THE Annual General Meeting of the Society was held in the Chemical Society's Rooms, Burlington House, W.1, on Wednesday, March 6th, at 2.50 p.m., the President, Professor W. H. Roberts, being in the chair. The Honorary Treasurer presented the accounts for the year, and the Honorary Secretary presented the Annual Report of the Council. The President delivered his Presidential Address.

The following were elected as Officers and Council for the year 1940:

President.—E. B. Hughes, D.Sc., F.I.C.

Past Presidents serving on the Council.—F. W. F. Arnaud, Bernard Dyer, John Evans, Edward Hinks, G. Roche Lynch, W. H. Roberts, G. Rudd Thompson.

Vice-Presidents.—E. B. Anderson, S. E. Melling, F. G. H. Tate, J. R. Stubbs (*Chairman, North of England Section; ex officio*), T. Cockburn (*Chairman, Scottish Section; ex officio*).

Honorary Treasurer.—G. Taylor.

Honorary Secretary.—Lewis Eynon.

Other Members of Council.—C. A. Adams, F. C. Bullock, H. E. Cox, D. C. Garratt, L. H. Lampitt, G. W. Monier-Williams, C. J. Regan, T. Rendle, A. Scholes, W. M. Seaber, W. H. Simmons, W. W. Taylor, J. B. McKean (*Honorary Secretary, Scottish Section; ex officio*), A. Lees (*Honorary Secretary, North of England Section; ex officio*).

The following candidates have been elected members of the Society:

Aage Jorgen Christian Andersen, B.A., M.Sc. (Copenhagen).
Isaac Berg, B.Sc. (Lond.), A.I.C.
William Edwin Joseph Richard Calcutt, B.A., D.I.C., A.I.C.
Richard Arthur Dalley, A.I.C. (*Through North of England Section.*)
William Thomas Elwell, A.I.C. (*Through North of England Section.*)
Joseph Markland, B.Sc. (Lond.), A.I.C. (*Through North of England Section.*)
Cecil Hancorn Robins, B.Sc. (Lond.), A.I.C.
Robert Orr Scott, B.Sc., A.R.T.C., A.I.C. (*Through Scottish Section.*)
Joseph Henry Singer, A.I.C.
George Edgar Turfitt, B.Sc., Ph.D. (Lond.), A.I.C. (*Through North of England Section.*)

SCOTTISH SECTION

THE Fifth Annual General Meeting of the Section was held in the Bath Hotel, 154, Bath Street, Glasgow, on February 22nd, 1940.

The Chairman, Mr. T. Cockburn, gave an appreciation of the late Dr. T. W. Drinkwater, who had been Vice-Chairman of the Section during 1935 and 1936.

The Secretary read the Report and Financial Statement for 1939, which were adopted.

The following Office-bearers were elected:

Chairman.—T. Cockburn; *Vice-Chairman.*—J. W. Hawley; *Committee.*—J. Brown, A. Dargie, A. R. Jamieson, R. G. Thin, R. T. Thomson and J. F. Tocher; *Honorary Secretary and Treasurer.*—J. B. McKean; *Honorary Auditors.*—M. Herd and R. S. Watson.

The following papers were read and discussed:—"The Pollution of Water by Fish," by R. T. Thomson, F.I.C., and "Notes on some War Gases: The Detection of Mustard Gas in Food," by J. B. McKean, F.I.C., and J. A. MacNair, F.I.C.

Death

WITH great regret we record the death, on January 9th, of George Arthur Pingstone, Public Analyst for the Municipality of Bulawayo. He had been a member of the Society for 36 years.

Annual Report of Council

MARCH, 1940

THE Roll of the Society numbers 886, an increase of 29 over the membership of last year.

The Council regrets to have to record the death of the following members:

W. T. Burgess
R. A. Cripps
T. W. Drinkwater
F. G. Edmed
D. L. Howard
H. F. E. Hulton
H. T. Lea
Sir G. T. Morgan
G. A. Pingstone
Sir W. J. Pope
F. T. Shutt
K. E. N. Williams

and of G. Stubbs, a former member.

Burgess, who died at the age of 78, had been a member of the Society for 40 years and served on the Council as an Ordinary Member and as a Vice-President. During almost the whole of his career he was engaged in work connected with water supply, first as an assistant to Sir Edward Frankland, then as Water Analyst to the Local Government Board, and finally as a private practitioner. (Obituary, ANALYST, 1940, **65**, 1.)

Cripps, who died at the age of 76, had been a member of the Society for 43 years, and served as a member of Council in 1910–11. He was for many years Public Analyst for Bournemouth. His work was chiefly in connection with pharmaceutical chemistry, and he published numerous papers on pharmaceutical subjects. (Obituary, ANALYST, 1939, **64**, 394.)

Drinkwater, who died in his 90th year, had been a member of the Society for 16 years. He was for over 60 years a lecturer in the School of Medicine of the Royal College of Physicians and Surgeons, Edinburgh, and held a number of appointments as Public Analyst.

Edmed, who died at the age of 62, had been a member of the Society since 1930, and served as a Member of the Council in 1934–35. He was for many years Admiralty Chemist at H.M. Dockyard, Portsmouth.

Howard, who died at the age of 73, had been a member of the Society since 1900, and served on the Council in 1906–07 and 1918–19. He was a Director of Messrs. Howards & Sons, and was for many years a Justice of the Peace for Essex.

Hulton, who died at the age of 64, was elected a member of the Society in 1913, and served as a Member of Council in 1923–24. Practically the whole of his professional life was devoted to the brewing industry, and in collaboration with J. L. Baker he published a number of papers on brewing subjects. (Obituary, ANALYST, 1939, **64**, 639.)

Lea, who died in his 50th year, had been a member of the Society since 1919, and served on the Council 1926–27. During the war of 1914–18 he served in the Special Brigade, Royal Engineers. He subsequently became a Consulting Chemist and held appointments as Public Analyst and Official Agricultural Analyst. (Obituary, ANALYST, 1939, **64**, 778.)

Sir G. T. Morgan, who died in his 70th year, was an Honorary Member of the Society. After a long and distinguished academic career, during which he made many important contributions to pure and applied chemistry, he became, in 1925, Director of the Chemical Research Laboratory at Teddington, and held that position until 1937, after which he became the Research Director of the Institute of Brewing. He was awarded the honour of Knighthood in 1936.

Sir W. J. Pope, who died in his 70th year, had been a member of the Society since 1915. He was for over 30 years Professor of Chemistry at Cambridge. His discovery of the optical activity of compounds of nitrogen, sulphur, selenium and tin gave him an international reputation as a research worker in the field of pure chemistry. During the war of 1914–18 he developed a process for the synthesis of mustard gas far in advance of that used in Germany. (Obituary, ANALYST, May, 1940.)

Shutt, who died in his 81st year, became a member of the Society in 1916. He had a long and distinguished career in the field of agricultural chemistry in Canada, and held the post of Dominion Chemist in the Department of Agriculture, Ottawa. He was awarded the honour of C.B.E. in 1935.

Stubbs, who died at the age of 75, was elected a member of the Society in 1926, and served on the Council as an Ordinary Member and as a Vice-President. He was for many years in the Government Laboratory, finally as Deputy Government Chemist.

BIENNIAL LECTURE.—Following the Annual General Meeting on March 3rd, 1939, the Biennial Lecture was given by Sir Henry Dale, C.B.E., M.D., F.R.S., the subject being "Biological Standardisation" (ANALYST, 1939, **64**, 554).

ORDINARY MEETINGS.—Owing to the outbreak of war it was found necessary to cancel the October and November meetings. During the year, five meetings were held, and the following papers were communicated:

"Food Inspection and Analysis in Holland." By J. Straub, Chem. Ing., Director of the Food Inspection Laboratory for Amsterdam.

"The Determination of Traces of Zinc in Biological Material and Natural Waters." By Noel L. Allport, F.I.C., and C. D. B. Moon, A.I.C.

"The Evaluation of Hydrogen Peroxide." By Mrs. S. M. L. Tritton, M.P.S., F.I.C.

"Extract of Malt with Cod-liver Oil: Determination of Oil and Vitamin A." By D. C. Garratt, B.Sc., Ph.D., F.I.C.

"The Presence of Leuco-anthocyanins in Criollo Cacao." By the late A. W. Knapp, M.Sc., M.I.Chem.E., F.I.C., and J. F. Hearne, A.I.C.

"Notes on the Examination of Textiles in Cases of Suspected Dermatitis." By H. E. Cox, Ph.D., D.Sc., F.I.C.

"The Examination of Lard." By R. W. Sutton, B.Sc., F.I.C., A. Barraclough, B.Sc., F.I.C., R. Mallinder, B.Sc., F.I.C., and O. Hitchen, B.Sc., F.I.C.

"The Estimation and Examination of 2-Methyl-1:4-naphthoquinone." By J. L. Pinder, B.Sc., F.I.C., and J. H. Singer, A.I.C. With Introductory Remarks on the Relationship of 2-Methyl-1:4-naphthoquinone to Vitamin K, by A. L. Bacharach, M.A., F.I.C.

The February meeting was a joint meeting with the Food Group of the Society of Chemical Industry, at which the following papers on Carotene and Allied Pigments were presented:

"The Constitution and Physiological Significance of Carotene and Allied Pigments." By R. A. Morton, D.Sc., F.I.C.

"The Commercial Determination of Carotene and Allied Pigments, with especial reference to Dried Grass and other Leafy Materials." By W. M. Seaber, B.Sc., F.I.C.

THE ANALYST.—Our journal continues to reflect the satisfactory position of the Society. It was feared at first that the outbreak of war would have a serious effect on the number of papers submitted for publication. Happily this expectation has not been realised, and the Editor is still receiving numerous contributions. By a coincidence the number of pages (924) in THE ANALYST was exactly the same as in the preceding year. The total number of papers published was 48, and these may be roughly classified into 15 on food and drugs, 15 inorganic, 6 organic, 5 biological, 2 on water, 3 on gas analysis, 1 agricultural, and 1 forensic. It will be noted with satisfaction that our journal is now recognised as a suitable medium for the publication of papers on inorganic analysis and metallurgy. In addition to the papers there were also 38 notes on subjects of analytical interest, and the usual abstracts from the reports of Government Analysts and Public Analysts. Publishers, as well as readers of the journal, still value the critical reviews published in THE ANALYST, for no fewer than 72 books were reviewed in the course of the year.

HON. TREASURER'S REPORT.—The Hon. Treasurer reports that the financial position of the Society continues to be satisfactory; the year's income has more than balanced the year's expenditure. It is hoped that no need will arise for increasing the subscription beyond one guinea, the traditional subscription to the Society.

EMERGENCY COMMITTEE.—At the time of the international crisis in September, 1938, an Emergency Committee was formed to act for and on behalf of the Council in a state of national emergency. On the outbreak of war in September, 1939, the Committee met and decided that, in view of the impossibility of holding

meetings of the Society in the near future and the uncertainty as to the holding of meetings subsequently, the procedure for the election of new members should be temporarily as follows:

(*a*) All applications for membership shall first be considered by the Committee.

(*b*) Those approved shall be circulated for comment to all members of the Council.

(*c*) The Committee shall reconsider them with the comments received, if any, and decide finally which applicants shall be admitted to membership.

FOOD AND DRUGS ACT, 1938.—Draft Regulations for bread and flour and applications for regulations for cheese, cream, ice-cream, honey, vinegar, coffee and coffee mixtures, have been subjects of correspondence between the Ministry of Health and the Society. Shortly after the outbreak of war, however, the Ministry informed the Society that the consideration of these draft regulations and applications must be deferred for the time being.

CENTRAL REGISTER FOR NATIONAL SERVICE.—The Society has co-operated with other scientific and professional bodies in supplying the Ministry of Labour with the names and qualifications of persons willing to be enrolled in the Central Register for National Service.

SOCIETY'S ARCHIVES.—Minutes of meetings of Council and Committees and other important records and a set of early numbers of THE ANALYST have been sent to Messrs. W. Heffer & Sons, at Cambridge, for safe storage against war risks, and the Council desires to express thanks to Messrs. Heffer for this accommodation.

METROPOLITAN WATER BOARD BILL.—The Society presented a Petition against the Metropolitan Water Board Bill, and in particular against Clause 51 of the Bill whereby the Metropolitan Water Board would be empowered to establish a general practice in water and sewage and effluent analysis and examination extending over the whole of the country and to receive remuneration for such practice.

The Petition was successful, Clause 51 being deleted from the Bill. The Council desires to record its thanks to the Institute of Chemistry for supporting the Petition and for defraying part of the cost of the Petition.

ANALYTICAL METHODS COMMITTEE.—Two reports from the Committee have been published during the past year, *viz.*:

"Determination of Copper in Food Colouring Materials." ANALYST, 1939, **64**, 339.

"Assay of Lobelia." ANALYST, 1939, **64**, 581.

A new Sub-Committee has been appointed, under the chairmanship of Dr. E. B. Hughes, to consider methods for determination of dry solid matter and of copper in tomato purée and other tomato products.

The work of the Sub-Committees has been seriously interfered with by war conditions, and in some cases has had to be suspended for the time being.

The Council appointed Mr. J. R. Nicholls to the Committee in place of the late Mr. E. R. Bolton, and has also appointed Mr. N. Evers an additional member.

ANALYTICAL INVESTIGATION SCHEME.—One research ("Manganese and Caffeine Contents of some Teas and Coffees") by Dr. R. K. Coleman and F. G. Gilbert has been completed and published in THE ANALYST (1939, **64**, 706), and three problems are still under investigation. Discussions have been in progress with a view to extending the usefulness of the Scheme by increased co-operation with the chemical departments of different universities and colleges; it is hoped that as conditions become more settled these discussions will be resumed.

NORTH OF ENGLAND SECTION.—Owing to the war the usual number of five meetings has been reduced to three. Six papers have been read:

"Silicosis and the Analyst." By Dr. F. S. Fowweather, M.Sc., Ch.B., F.I.C., D.P.H.
"Some Notes on the Examination of Paraldehyde." By A. R. Tankard, F.I.C., D. J. T. Bagnall, A.C.G.F.C., F.I.C., and A. Smith, B.Sc., F.I.C.
"An Improved Table for use with the British Standard Specification Hydrometers for Milks, B.S.S., No. 734—1937." By J. G. Lunt, B.Sc., F.I.C.
"Lead in Food." By G. W. Monier-Williams, O.B.E., M.C., M.A., Ph.D., F.I.C.
"Some Aspects of the Purification of Polluted Waters for Industrial Use." By J. G. Sherratt, B.Sc., F.I.C.
"The Estimation of Lead in Drinking Waters." By C. H. Manley, M.A., F.I.C.
The Annual Address by the Chairman.

There has been a good attendance at the meetings, and the President has attended.

The tenth Summer Meeting, held at Scarborough, was as successful as former ones. Dr. G. W. Monier-Williams gave the Paper, which was followed by a very interesting discussion. The number attending the meeting was above the average for previous years.

Eight candidates have applied for membership of the Parent Society through the Section.

The Section now numbers 130 members, an increase of seven on the previous year.

The Honorary Secretary wishes to acknowledge with thanks the loyal support and help of the Chairman, Officers and members of the Committee in carrying out the work of the Section.

SCOTTISH SECTION.—Two meetings were held in the year, the usual November meeting being cancelled owing to war-time difficulties of travelling and lighting.

The following papers were read and discussed.

"Sampling." By W. M. Cameron.
"Seasoning Materials." By R. H. McKinlay, F.I.C.
"Counterfeit Coins." By A. Scott-Dodd, B.Sc., Ph.D., F.I.C.
"The Assay of Mercury." By James Sandilands, F.I.C.
"Description of a Closed Respiration Apparatus for Plant Tissue." By A. M. Smith, Ph.D., D.Sc., A.I.C.

Two new members joined the Parent Society through the Section and one member resigned.

With the approval of the Council, a Memorandum was sent to the Local Government and Public Health Consolidation (Scotland) Committee requesting the introduction into Scotland of a Food and Drugs Act on similar lines to the Food and Drugs Act, 1938. A formal acknowledgment of the Memorandum was received.

CONGRESSES.

The Society was represented by the President and Mr. A. L. Bacharach at a meeting on the subject of "Milk in its Nutritional Aspects" convened by the British Association.

Royal Sanitary Institute Congress, Scarborough, 1939.—Mr. A. R. Tankard represented the Society at the Congress of the Royal Sanitary Institute at Scarborough.

Congress of Industrial Chemistry, Warsaw, 1939.—Dr. L. H. Lampitt was appointed to represent the Society at the 19th Congress of Industrial Chemistry, Warsaw. Owing to the outbreak of war, however, the Congress was cancelled.

British Standards Institution.—The President was appointed to represent the Society on the Chemical Divisional Council of the British Standards Institution in place of the late Mr. E. R. Bolton.

The Council desires to record its thanks to the members of the Society who have served on the Committees and Sub-Committees and to those who have represented the Society on other bodies, and to various organisations and members of the Society who have afforded accommodation and hospitality to the Committees.

W. H. ROBERTS, *President*
LEWIS EYNON, *Hon. Secretary*

The Determination of small amounts of Formaldehyde in Air

By R. W. KERSEY, B.Sc., J. R. MADDOCKS, A.I.C., and T. E. JOHNSON, A.I.C.

The health hazards arising from formaldehyde are known to be serious. Information published by the Association of British Chemical Manufacturers[1] indicates that the limit of toleration of formaldehyde in air is of the order of 0·025 mg. per litre. Accordingly the authors were led to devise a method for the determination of formaldehyde when present in air at such a low concentration. The quantities involved were so small as to preclude the use of methods normally available for concentrated solutions. The following methods were examined:—

A. *Absorption in iodine solution* (*Romijn's Method*[2]).—Solutions were prepared by mixing equivalent amounts of standard ($N/10$) iodine solution and sodium hydroxide solution and diluting to a known volume with water. Known volumes of air under test were aspirated through portions of the solution and the change in iodine-content was determined. Very low figures were obtained, corresponding to 0·0003 mg. of formaldehyde per litre of air, even in an atmosphere artificially charged by contact with 40 per cent. formaldehyde solution.

B. *Absorption in water followed by colorimetric determination.*—The usual type of cylindrical bubbler, with the inlet tube drawn out to a fine orifice and the cylinder packed with glass beads to give small bubbles and provide an increased surface for absorption, gave unsatisfactory results. The concentration of formaldehyde in the water failed to "build up" with increasing volumes of air.

A Jena glass distribution tube, with fused-in fritted glass disc (Type 33 c. G.1; diameter of pores 100–120 microns), which divided the air into a fine gas spray, was next tried. An atmosphere was artificially produced, having such a high concentration of formaldehyde that it caused acute discomfort; its formaldehyde-content must therefore have approached 0·025 mg. per litre (*cf.* Ref. 1). When this atmosphere was passed through water *via* the glass distributor tube, figures obtained were of the order of only 0·003 to 0·004 mg. per litre. With two absorbers in series, the first containing 600 ml. of water and the second 150 ml., the

concentration in the first absorber rose to 10 parts per 1,000,000 without any being detectable in the second vessel, the test used (Schryver's method, see later) being sensitive to 0·5 part per 1,000,000. Mechanical loss by non-absorption was therefore unlikely, and a possible explanation of the low results was the formation of oxidation products due to passing a continuous air stream through the solution containing absorbed formaldehyde.

C. *Application of Schryver's Method.*[3]—This method depends on the formation of an intense magenta colour, when dilute solutions of formaldehyde phenylhydrazone are treated with potassium ferricyanide in presence of an excess of hydrochloric acid, the depth of colour, between certain limits, being proportional to the concentration of formaldehyde present. Examination of the method, using solutions of formaldehyde of known concentration, prepared by triple dilution of the 40 per cent. aqueous solution, indicated that the working range of the test was approximately 1 to 5 parts of formaldehyde per million of solution, though 0·5 p.p.m. was detectable. Above 5 p.p.m. the colour was too intense for accurate evaluation.

The possibility of absorbing the formaldehyde from the atmosphere in a dilute aqueous solution of phenylhydrazine hydrochloride seemed to offer a method free from the disadvantages associated with absorption in water alone.

TABLE I

Ref. No.	Volume of air, litres	Duration of test, hours	Formaldehyde in test solution p.p.m.	Concentration of formaldehyde in atmosphere, mg. per litre	Remarks
1	22	3	6	0·014	Atmosphere too strong for continuous breathing.
2	10	1	3	0·015	Ditto.
3	14½	2	5	0·017	Formaldehyde distributed on shed floor.
4	29	4	1	0·0016	Slight smell of formaldehyde.
5	34	3½	2 to 3	0·0037	Stronger than 4.
6	41	3½	1	0·0012	Test done after running fan for ½ hr. after test 3.
7	35	2½	0·5 to 1	0·001	Test after running fan for 2 hrs. after test 5.

A solution of phenylhydrazine hydrochloride was prepared by adding 2 ml. of conc. hydrochloric acid to a mixture of 1 g. of phenylhydrazine with water, making up to 100 ml. with water and filtering from traces of suspended matter.

Ten ml. of this solution were diluted with 50 ml. of water, and air free from formaldehyde was drawn through a Jena glass distribution tube (0·75 inch diameter) set in an absorption bottle of slightly larger diameter; the rate of flow was approximately 10 litres per hour. Slight coloration of the solution began to take place after the passage of 40 litres air. In subsequent tests, therefore, the volume of air was kept below this figure whenever feasible.

When air containing formaldehyde was passed through this solution, according to the method described later, figures for formaldehyde-content were in

direct proportion to the volume of air used, *i.e.* the formaldehyde "built up" in solution, in contradistinction to the experiments in which water only was used.

When two absorption bottles in series were used, each having a Jena glass distribution tube, no formaldehyde was detected in the solution from the second absorption bottle, showing that complete absorption was taking place in the first bottle.

The results obtained are summarised in Table I. Although the accuracy of the method has not been verified formally by analysis of mixtures of air and formaldehyde of known composition, there is every reason to suppose that the results obtained are correct within the usual limits of error of a colorimetric method.

Method.

Special Reagents Required.—(1) *Phenylhydrazine hydrochloride solution*, 1 per cent. Suspend 1 g. of phenylhydrazine in about 5 ml. water, add 2 ml. of concentrated hydrochloric acid (sp.gr. 1·16) and dilute to about 80 ml. Filter off any insoluble matter and dilute the filtrate to 100 ml. with water.

(2) *Standard formaldehyde solution.—Solution A.*—Dilute 25 ml. of 40 per cent. w/v formaldehyde to a litre with water (1 ml. ≡ 0·01 g. of formaldehyde). *Solution B.*—Dilute 10 ml. of Solution A to a litre with water (1 ml. ≡ 0·0001 g. of formaldehyde). *Solution C.*—Dilute 10 ml. of Solution B to a litre with water (1000 ml. ≡ 1 mg. of formaldehyde).

If desired, a stronger Solution C may be used by diluting a larger volume of Solution B to a litre with water, *e.g.* 50 ml. of Solution B diluted to a litre will give a solution containing 5 mg. of formaldehyde per litre. Solutions A, B, and C must be made freshly when required.

Procedure.—Into a cylindrical wash-bottle fitted with a Jena glass distribution tube (diameter, 0·75 in.) with fused-in fritted glass filter disc (Type 33 c. G.1—pore diameter 100 to 120 microns) place 10 ml. of the phenylhydrazine hydrochloride solution and dilute to 50 ml. with water. Aspirate through this solution, at a rate of about 10 litres per hour, such a volume of air to be tested that the final solution will contain between 1 and 5 mg. of formaldehyde per litre. (The volume of air used should not exceed 40 litres.)

Dilute the solution to 50 ml. (Test Solution). Transfer 10 ml. of the Test Solution to a 20-ml. Nessler cylinder, add 1·0 ml. of potassium ferricyanide solution (5 per cent., freshly prepared) and 4·0 ml. of conc. hydrochloric acid (sp.gr. 1·16), dilute to 20·0 ml. with water, mix well and allow to stand for ten minutes.

Prepare simultaneously a series of standard comparison solutions in similar Nessler cylinders, using 2 ml. of the phenylhydrazine hydrochloride solution (reagent 1), 7 ml. of water, measured amounts of Solution C (say, 1 ml., 2 ml., 5 ml.), 1·0 ml. of potassium ferricyanide solution (5 per cent.) and 4·0 ml. of conc. hydrochloric acid (sp.gr. 1·16); dilute to 20·0 ml. with water, mix each well and allow to stand for ten minutes. Compare the colour of the Test Solution against those of the standard comparison solutions.

We wish to thank Imperial Chemical Industries, Ltd., for permission to publish this paper.

REFERENCES

1. Association of British Chemical Manufacturers Quarterly Summary, October–December, 1934. No. 2 (Vol. 5), p. 58. List of harmful concentrations of various gases.
2. *Z. anal. Chem.*, 1897, **36**, 9. Abst., ANALYST, 1897, **22**, 221.
3. S. B. Schryver, "On the Application of Formaldehyde to Meat." Food Report No. 9, 1909. Reports of the Local Government Board on Public Health and Medical Subjects. New Series, No. 12.
   See also E. H. Callow, ANALYST, 1927, **52**, 391. "The Presence of Formaldehyde in Wood Smoke and in Smoked Foodstuffs."

December 17th, 1939

The Determination of Members of the Sulphanilamide Group of Drugs

BY G. V. JAMES, M.Sc., F.I.C.*

BIOCHEMICAL control during and after use of drugs of the sulphonamide group is essential to secure a concentration in the blood that is bacteriologically effective and yet sufficiently low for no toxic effects to be produced; such effects, however, are difficult to predict purely from the known drug concentration in the blood, since individuals vary in their reaction to the drug.

Probably the two most widely used members of the class are sulphanilamide itself and sulphanilamido-pyridine, but there are many other substances which owe their activity to the liberation of sulphanilamide in the body; some of these are included here, since they are still sold and may be used should a temporary shortage of the more active members of the class arise.

The sulphanilamide class may be divided broadly into those in which a substituent is introduced into the amino group, such as prontosil, supron, etc., and those in which the substituent is introduced into the sulphonamide group. Moreover, the body helps to rid itself of the drugs by acetylation of the amino group and by oxidation; oxidation applies more particularly to sulphanilamide than to sulphanilamido-pyridine, which seems only to be acetylated. The determination of the acetyl derivatives and oxidation products, however, is important, as it enables an estimate to be formed of the quantity of drug absorbed, and hence potentially active.

Table I shows the fate of many of these drugs, so far as is at present known, in the human subject, and of some of them in mice.

The results in Table I are averages, the number of experiments being shown in brackets; the individual figures often differ widely, as is to be expected in biological work. The experiments on mice were made with at least 8 animals in a metabolism cage, from which the excreta could be collected.

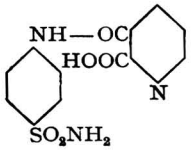
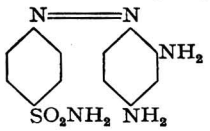
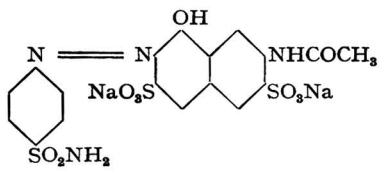
SULPHANILAMIDE (Streptocide, Ambeside, Prontylin, Prontosil Album) is a white crystalline powder (m.p. 165–166·5° C.), slightly soluble in cold water (to

* Working under a full-time grant from the Medical Research Council.

form a neutral solution), soluble in hot water, hot alcohol and cold acetone, slightly soluble in ether, benzene or chloroform, and soluble in acid or alkaline solution. In acetic acid solution it condenses with an alcoholic solution of xanthydrol to

TABLE I

THE FATE OF VARIOUS DRUGS OF THE SULPHANILAMIDE GROUP

Drug	Formula	Animal	Urine		Faeces Per Cent.	Total Per Cent.
			Total Per Cent.	Oxidin Per Cent.		
Sulphanilamide	$NH_2C_6H_4SO_2NH_2$	Mouse (4)	44·2	2·9	0·0	47·1
		Man (5)	48·8	17·2	0·0	66·0
Sulphanilamido-pyridine	$NH_2C_6H_4SO_2NHC_5H_4N$	Mouse (2)	80·9	0·0	8·4	89·3
		Man (3)	78·6	0·0	15·6	94·2
Supron		Man (2)	50·7	—	0·0	50·7
Uleron	$NH_2C_6H_4SO_2NHC_6H_4SO_2N(CH_3)_2$	Mouse (1)	75·0	0·0	16·7	91·7
Ilvin	$NH_2C_6H_4SO_2NHC_6H_4COCH_3$	Mouse (2)	12·3	—	55·0	67·3
Benzyl sulphanilamide or prosepta-sine	$C_6H_5CH_2NHC_6H_4SO_2NH_2$	Mouse (3)	1·9	—	87·0	88·9
		Man (3)	17·8	—	—	17·8
Soluseptasine	$C_6H_5CH(SO_3Na)CH_2CH(SO_3Na)NHC_6H_4SO_2NH_2$	Mouse (1)	98·5	0·0	0·0	98·5
Prontosil rubrum*		Mouse (1)	9·3	—	—	9·3
Prontosil soluble*		Mouse (1)	55·0	—	—	55·0

* The results for the two prontosils are taken from Fuller's paper.[1]

form a crystalline compound (m.p. 209° C.); on this reaction has been based a gravimetric method of determination.[2] Other methods depend on diazotisation and coupling in either acid or alkaline solution. The mono-acetyl derivative melts at 219° C.

Fuller's method[1] of determining sulphanilamide has remained in use (with

modifications) in these laboratories. The urine is diluted according to the supposed sulphanilamide-content so that 100 ml. will contain about 5 mg., and to 2 ml. of the diluted liquid are added 2 ml. of $N/2$ hydrochloric acid, followed by 0·2 ml. of a freshly prepared 0·5 per cent. solution of sodium nitrite, and the mixture is allowed to stand for 3 to 5 minutes at a temperature below 20° C., and then tested with potassium iodide and starch to ensure excess of nitrous acid. If there is not an excess, more nitrite is added; otherwise 0·5 ml. of 20 per cent. urea solution is added to destroy the excess, and the mixture is left for 5 minutes. It is then treated with 0·5 ml. of a 1 per cent. solution of thymol or β-naphthol in 5 per cent. sodium hydroxide solution followed by 1 ml. of 40 per cent. sodium hydroxide solution. The resulting colour is compared with a standard or the concentration is estimated by a photo-electric colorimeter using appropriate colour filters.

Marshall, in a series of investigations,[3] made use of dimethyl α-naphthylamine and acid coupling, and used ammonium sulphamate to destroy the excess of nitrous acid. Proom[4] used the same reagent, and MacLaglan[5] introduced the photo-electric-colorimeter and the necessary colour-filters.

TABLE II

Fuller's method	Werner's method	Remarks
26·0	47·0	Oxidation products present.
20·1	46·5	Oxidation products present.
107·0	102·0	Oxidation products absent.
125·0	200·0	Oxidation products present.
112·0	100·0	Oxidation products absent.
70·4	106·0	Oxidation products present.
32·0	42·2	Oxidation products present.
179·0	250·0	Oxidation products present.

The removal of nitrous acid is necessary, since, in presence of urine, colours are produced with the reagents, which tend to vitiate the results, although for approximate results this refinement is not essential. The purity of the dimethyl α-naphthylamine is also important, as certain oxidation products entirely alter the shade of colour; the pH also appears to have some effect, and in his final method Marshall buffered the solution at the same time as he removed excess of nitrous acid. His final method is as follows:—An aliquot portion of the diluted urine is treated with N hydrochloric acid followed by 1 ml. of a freshly-made 0·1 per cent. solution of sodium nitrite, and then, after 3 minutes, by 1 ml. of M sodium dihydrogen phosphate solution containing 0·5 per cent. of ammonium sulphamate. After 5 minutes 5 ml. of a 0·4 per cent. alcoholic solution of dimethyl α-naphthylamine is added and the colour is compared with a standard.

Werner[6] condensed the amino group with p-dimethylamino-benzaldehyde, adding 1 ml. of a 3 per cent. solution of this reagent in 7 per cent. v/v sulphuric acid to 9 ml. of the urine previously diluted one or two hundredfold, and comparing the colour with those of similarly treated standards. Here, again, care is necessary, since the reagent itself has a pronounced colour which readily becomes

darker, and this colour may mask that produced by small amounts of sulphanilamide in the highly diluted urine.

The reagent also determines all amino groupings and so includes *p*-aminophenol, an oxidation product of sulphanilamide. Nitrous acid oxidises the phenol to quinone which does not couple. Table II compares results obtained by Fuller's diazotisation method and by Werner's method with urines in which oxidation products were present and absent. The results are expressed as mg. of sulphanilamide per 100 ml.

It has already been mentioned that the human organism acetylates the amino group before excretion, and estimations of the total sulphanilamide are necessary to compute the actual excretion of the drug, the term total being used to designate the amount of drug excreted with a free amino group plus that part in which the amino group is conjugated with acetyl radical. The acetyl radical is readily split off by heat treatment with hydrochloric acid; when, however, urine, or even diluted urine, is thus directly treated, low recovery results are obtained, but the use of nascent zinc hydroxide as a decolorising agent effects an improvement, as is shown in Table III.

TABLE III

	Free sulphanilamide, mg. per 100 ml.			Acetylsulphanilamide, mg. per 100 ml.		
	Present Per Cent.	Found Per Cent.	Recovery Per Cent.	Present Per Cent.	Found Per Cent.	Recovery Per Cent.
Unheated urine decolorised	2	2·04	102			
	4	3·92	98			
	8	8·3	104			
Heated urine diluted 25-fold	5	2·6	52	5	3·6	72
	10	8·8	88	10	9·2	92
Decolorised	5	4·2	84	5	4·8	96
	10	10·1	101	10	10·6	106

In the method adopted for determining acetyl sulphanilamide the urine is heated with an equal volume of 2 *N* hydrochloric acid in a boiling water-bath for 30 minutes and then cooled, and the water lost by evaporation is replaced. Five ml. of this heated urine are treated with 2 ml. of 10 per cent. zinc sulphate solution, the *p*H is adjusted to 7–8 by addition of 10 per cent. ammonia, the whole is diluted to 10 ml., allowed to stand and filtered, and aliquot parts of the filtrate are taken. The number of dilutions must not be forgotten in making the final calculations.

Marshall's procedure differs slightly in that he heats 1 ml. of urine with 2 ml. of *N* hydrochloric acid for 30 minutes and neutralises with *N* sodium hydroxide solution before diazotisation and coupling.

For determinations in blood it is necessary first to remove proteins. In Fuller's[1] method 1 ml. of blood is laked with 2 ml. of *N*/2 hydrochloric acid, 1 ml. of 20 per cent. trichloroacetic acid is added, and the mixture is allowed to stand for a short time, and then filtered with the aid of the pump. Aliquot portions of the filtrate are diazotised, and, after removal of excess of nitrous acid, are coupled with thymol (β-naphthol must not be used, since it develops a colour with some

normal constituent of the blood filtrate). Acetyl sulphanilamide is estimated in the filtrate by heating for 30 minutes in a boiling water-bath with an equal volume of 2 *N* hydrochloric acid, cooling and diazotising.

Marshall's method[3] differs somewhat, and he considers that, unless nitrous acid is destroyed, trichloroacetic acid should only be used if free sulphanilamide is to be estimated. He uses *p*-toluene sulphonic acid as a protein precipitant and his final method for blood is as follows:—One ml. of blood is laked with 7 ml. of 0·05 per cent. saponin solution, 2 ml. of 20 per cent. *p*-toluene sulphonic acid are added, and the mixture is filtered. Ten ml. of the filtrate are treated with 1 ml. of 0·1 per cent. sodium nitrite solution, followed, after 3 minutes, by 1 ml. of *M* sodium dihydrogen phosphate solution containing 0·5 per cent. of ammonium sulphamate; the liquid is allowed to stand, and then treated with 5 ml. of 0·4 per cent. alcoholic dimethyl α-naphthylamine solution, and the colour is matched. The total sulphanilamide may be estimated by heating 10 ml. of the *p*-toluene sulphonic acid filtrate in a boiling water-bath for 90 minutes, replacing the water lost by evaporation, cooling and diazotising.

The diazotisation method is suitable for the determination of those drugs in which there is a free amino group, such as Ilvin, Uleron and sulphanilamido-pyridine, or in which the amino group is readily set free as in Supron, but with the substituted sulphonamides they must be developed from the particular drug under examination, since the colours developed are often different from that produced by treatment of the correct equivalent of sulphanilamide. This is especially noticeable with sulphanilamido-pyridine, with which the colour developed is more intense than with sulphanilamide.

SULPHANILAMIDO-PYRIDINE (M. & B. 693, Dagenan).—This is a white crystalline powder, much less soluble in water than sulphanilamide and sparingly soluble in organic solvents. It melts at 191° C., and is soluble in acids and alkaline solutions, being precipitated over the range *p*H 4 to 9. The mono-acetyl derivative melts at 224° C.

Owing to its free amino group it can be estimated by direct diazotisation, but great dilution is necessary because of the intense colour produced with the usual coupling agents.

So far as I have at present been able to ascertain, neither this drug nor any other of the group in which the sulphonamide radical is substituted appears to split up to give free sulphanilamide, and there is thus little likelihood of the necessity of a "mixed estimation" arising.

Baines and Wein[7] estimate the drug by diazotisation and coupling with dimethyl α-naphthylamine. The free amino body is estimated in blood and urine by diluting 1 ml. of the sample with 2 ml. of water and then adding 1 ml. of 20 per cent. trichloroacetic acid solution. The mixture is shaken and filtered with the aid of the pump, 1 ml. of filtrate is treated with 0·05 ml. of 0·5 per cent. sodium nitrite solution, and left for 3 minutes, after which 0·5 ml. of 1 per cent. alcoholic dimethyl α-naphthylamine solution is added and the whole is mixed and allowed to stand for 15 minutes before matching the colour with standards.

The conjugated drug is determined in urine by heating under reflux 5 ml. of the sample with 5 ml. of 20 per cent. sodium hydroxide solution for one hour,

cooling, neutralising to litmus with hydrochloric acid, and measuring the volume. To 1 ml. of this liquid, 2 ml. of water and 1 ml. of 20 per cent. trichloroacetic acid are added, and 1 ml. of this mixture is then diazotised and coupled as before; the total drug is thus determined and the amount conjugated is found by difference.

For blood the process is similar. Three ml. are treated with 1·5 ml. of water and 1·5 ml. of 20 per cent. trichloroacetic acid and the mixture is filtered. Three ml. of the filtrate are then heated under reflux for one hour with 3 ml. of 20 per cent. sodium hydroxide solution, the mixture is cooled and neutralised with hydrochloric acid to litmus, and the volume is measured. One ml. of the neutralised solution is diluted with 0·5 ml. of water and treated with 0·5 ml. of 20 per cent. trichloroacetic acid, and 1 ml. of this solution is diazotised and coupled as before.

The method I have used is similar, but involves substitution of thymol for dimethyl α-naphthylamine; the mixture is made alkaline, but decolorising with zinc hydroxide is necessary after heating under reflux with sodium hydroxide solution; for this purpose the alkaline liquid is brought to *p*H 8·0, 2 ml. of 10 per cent. zinc sulphate solution are added, and the whole is allowed to stand and filtered; an aliquot part of the cool filtrate is acidified with hydrochloric acid, diazotised and coupled in the usual way.

The following recoveries (Table IV) are illustrative of the results obtained.

TABLE IV

RECOVERY OF ACETYL-SULPHANILAMIDO-PYRIDINE FROM URINE

Actual, mg. per 100 ml.		Found, mg. per 100 ml.		Recovery	
Free	Acetyl	Free	Acetyl	Free Per Cent.	Acetyl Per Cent.
20·0	4·8	21·0	3·82	105	80
	3·5		3·45		99
	7·5		7·5		100
	10·0		9·7		97
	6·0		6·3		105
	9·5		9·2		97

The drug also occurs, both free and conjugated, in vomit, etc., and the same methods of determination as for blood may be applied, but in faeces, on account of its insolubility, it becomes necessary to extract the sample by warming for 15 minutes with 10 per cent. sodium hydroxide solution; this, of course, renders it impossible to differentiate between the free and conjugated drug, although, since the conjugated form is excreted into the alimentary canal, it probably occurs in the faeces also.

PRONTOSIL AND PRONTOSIL SOLUBLE.—These two compounds have been determined by comparing the colour of the urine with that of a solution of the corresponding prontosil of known strength and of the same *p*H.[1] Prontosil Soluble can be estimated in presence of Prontosil by making the sample strongly alkaline, the colour due to Prontosil almost disappearing under this condition.

Both these drugs owe their activity to the liberation of sulphanilamide, and the total sulphanilamide may be estimated by boiling the strongly acidified urine

with activated charcoal (Norit), filtering, cooling, diazotising and coupling an aliquot part of the filtrate.

ILVIN.—This drug is a yellow crystalline powder (m.p. 194·5° C.), slightly soluble in water. It shows a reactive ketonic group and will form an insoluble bisulphite compound.

No examination was made for conjugated compound, although it probably occurs. The free substance may be estimated in a manner similar to that used for sulphanilamide.

It may be estimated in faeces by grinding with sand and heating under reflux with methyl alcohol. An aliquot part of the extract is mixed with dilute hydrochloric acid, diazotised and coupled.

ULERON.—This drug, in the free and conjugated form, is determined in the same manner as sulphanilamide. For the faeces, repeated extraction with 2 *N* hydrochloric acid is necessary, and the extract is best decolorised by treatment with zinc sulphate.

SOLUSEPTASINE.—This is the disodium salt of *p*-(γ-phenyl propylamino) benzene sulphonamide $\alpha\gamma$-disulphonic acid and is a white substance, readily soluble in water. It is administered by injection, and in the body some undergoes hydrolysis, liberating sulphanilamide. So far, it has not been found possible to differentiate between acetyl sulphanilamide and soluseptasine, as the heat treatment with 2 *N* hydrochloric acid completely hydrolyses both compounds, but the free sulphanilamide may be estimated in the usual manner.

BENZYL SULPHANILAMIDE (Proseptasine).—This is a white, micro-crystalline powder melting at 169–174° C.; it is very slightly soluble in water, and more so in acid and alkali. The compound is very stable, and is hydrolysed to sulphanilamide with great difficulty. Oxidation to benzoic acid is possible, but the yield is not quantitative; nitrous acid gives an insoluble yellow product which has the characteristics of a nitroso compound, but this reaction is valueless for its determination.

TABLE V

RECOVERY OF BENZYL SULPHANILAMIDE FROM URINE

Actual mg. per 100 ml.	Found, mg. per 100 ml.	Recovery Per Cent.
6·0	6·3	105
5·9	6·2	105
1·9	1·9	100
2·2	2·1	95·5
7·5	7·2	96·0
1·5	2·0	133·5

On account of its stability it is still possible to estimate free and conjugated sulphanilamide by the usual methods, and the following method was devised for urine: The urine is saturated with sodium sulphate, adjusted to *p*H 7·0 and extracted with petroleum spirit in a continuous extractor for eight hours. The solvent is then distilled off, and the residual benzyl compound is hydrolysed by heating with 70 per cent. sulphuric acid for one hour in a boiling water-bath.

The ammonia thus produced from the benzyl compound is estimated after dilution and neutralisation.

By this procedure the results in Table V were obtained with urines in presence of free and acetyl sulphanilamide.

Continuous extraction with petroleum spirit may also be applied to dried faeces after grinding with sand.

Walti[8] determines the compound by hydrogenation, which decomposes it into sulphanilamide and toluene; the former is then estimated by the usual method. As hydrogenation may not be convenient on account of the apparatus required, hydrolysis offers advantages.

SUPRON.—This is a compound of sulphanilamide and quinolinic acid; the sodium salt is very soluble and is given intramuscularly. In the body a small quantity of sulphanilamide is liberated and the remainder of the supron appears to be excreted unchanged.

The compound is easily hydrolysed; heating for 30 minutes in a boiling water-bath with an equal volume of 4 per cent. acetic acid effects complete hydrolysis while not affecting acetyl sulphanilamide. Both compounds are entirely decomposed by treatment with 2 *N* hydrochloric acid for 30 minutes in a boiling water-bath.

Decolorising with zinc hydroxide is necessary. By differential means it is possible to determine free sulphanilamide in addition to the other two derivatives, but the error in the amount of conjugated sulphanilamide is subject to the effect of all errors, as this substance is estimated by subtraction of the sum of the other two from total sulphanilamide.

TABLE VI

RECOVERY OF SUPRON, SULPHANILAMIDE AND ACETYL-SULPHANILAMIDE FROM URINE

	mg. per 100 ml.					
	Present	Found	Recovery Per Cent.	Present	Found	Recovery Per Cent.
Sulphanilamide	21·5	25·5	118·5	10·0	12·4	124·0
Supron	25·0	22·0	87·0	40·0	33·3	83·0
Total sulphonamide	43·6	47·7	92·0	38·0	40·2	95·0
Sulphanilamide	16·0	14·6	91·5	16·2	16·8	104·0
Supron	24·0	25·0	104·0	16·2	18·2	112·5
Total sulphonamide	52·8	40·0	76·0	27·2	27·0	99·0
Sulphanilamide	28·0	31·0	90·0	33·5	23·0	68·0
Supron	20·0	23·5	85·0	24·0	25·0	104·0
Total sulphonamide	40·0	40·0	100	63·0	56·0	89·0
Sulphanilamide	16·0	16·6	104	20·0	19·2	96·0
Supron	16·0	16·6	104	20·0	20·8	104·0
Total sulphonamide	27·0	27·9	103	47·5	48·0	101·0

Applying the method to blood necessitated modification of the usual protein precipitant, since trichloroacetic acid on heating causes hydrolysis of the acetyl sulphanilamide, and when this acid was neutralised some hydrolysis still occurred,

as the sodium trichloroacetate becomes alkaline on heating. The use of sulphosalicylic acid as a protein precipitant solved the difficulty, and the following process was evolved:

Three ml. of blood were mixed with 6 ml. of $N/2$ hydrochloric acid and 3 ml. of 25 per cent. sulphosalicylic acid solution, shaken and filtered with the aid of the pump; aliquot parts of the filtrate were taken for the determinations of free and total sulphanilamide by the usual methods and of supron by neutralising with N sodium hydroxide solution to pH 5·0 (for 2 ml. of the filtrate approximately 0·5 ml. are required), adding 2 ml. of 4 per cent. acetic acid, heating the whole for 30 minutes on the boiling water-bath, cooling, treating with 1 ml. of $N/2$ hydrochloric acid, diazotising and coupling.

By this method the results in Table VII were obtained when different amounts of the various substances were added to normal blood.

TABLE VII

RECOVERY OF SUPRON, SULPHANILAMIDE AND ACETYL-SULPHANILAMIDE ADDED TO BLOOD

	mg. per 100 ml.					
	Present	Found	Recovery Per Cent.	Present	Found	Recovery Per Cent.
Sulphanilamide ..	4·5	5·2	116	3·6	3·6	100
Supron	18·0	20·0	111·5	7·2	7·2	100
Total sulphonamide	17·1	18·4	107	10·2	10·2	100

OXIDATION PRODUCTS OF SULPHANILAMIDE.—Many urines from patients receiving sulphanilamide darken on exposure to the air; examination of the inorganic and ethereal sulphates shows a much lower ratio than the normal 10:1. From urines of this type acetyl hydroxylamino benzene sulphonamide (1:4) and p-amino-phenol have been isolated, the latter especially from cyanosed patients; a purple pigment of the indophenol class may also occur, but its exact composition is not yet known.

Since hydrolysis is necessary to set these substances free, the following process has been worked out, in which the compounds are separated from the urine and also from other interfering substances.

Ten ml. of urine are heated for 30 minutes in a boiling water-bath with 0·5 ml. of conc. hydrochloric acid, and then cooled and neutralised. The neutral solution is extracted several times with ether, and the combined ethereal solutions are evaporated, water being added before the last of the ether has disappeared, since this aids the solution of the extracted compounds. The aqueous solution is made up to 20 ml. and divided into two portions for the following estimations:

p-*Amino-phenol.*—Ten ml. of the aqueous solution are heated in a boiling water-bath for ten minutes with 2 ml. of 5 per cent. solution of phenol, then cooled, treated with 2 ml. of 2 per cent. calcium hypochlorite solution, left for ten minutes for the blue colour to develop to its maximum and compared with a standard solution of p-amino-phenol similarly treated. It is essential that the reagents should be freshly prepared.

Hydroxylamino benzene sulphonamide.—When pure, this is a white crystalline

powder (m.p. 161–163° C.), slightly soluble in water, its aqueous solution on exposure to air depositing fine needle crystals. For this reason standard solutions must be prepared every two days.

Ten ml. of the aqueous extract, neutralised to phenolphthalein, are treated with 10 ml. of absolute alcohol, well cooled, and treated with 2 drops of redistilled benzoyl chloride followed by 2 ml. of 2 per cent. sodium acetate solution. After thorough mixing the liquid is treated with 2 ml. of a 0·5 per cent. solution of ferric chloride hexahydrate in 2 per cent. v/v hydrochloric acid, then diluted to a known volume, and compared with a standard solution similarly treated.

It is essential that the solution after addition of the alcohol should be kept cool and that freshly prepared reagents should be used. This method, which is due to Pucher and Day,[9] depends on the interaction of the benzoyl chloride and hydroxylamine compound to form a benzyl hydroxamic acid which produces a reddish-violet colour with ferric chloride.

Use of this method on urines, to which the compounds had been added, gave the following results (Table VIII) :

TABLE VIII

RECOVERY OF *p*-AMINO-PHENOL AND *p*-HYDROXYLAMINO BENZENE SULPHONAMIDE

	Present	Found	Re-covery Per Cent.	Present	Found	Re-covery Per Cent.	Present	Found	Re-covery Per Cent.
p-Amino-phenol	2·5	2·4	96	2·0	2·0	100	5·0	4·5	90
Hydroxylamine compound ..	4·5	6·0	133	5·0	3·2	62	7·3	7·0	96

A successful alternative method for the hydroxylamine compound is as follows: Add to the aqueous extract 2 ml. of *N*/2 hydrochloric acid and 1 ml. of 0·5 per cent. sodium nitrite solution, stand, add 1 ml. of 20 per cent. urea solution, again stand, then add 0·5 ml. of thymol solution and 1 ml. of 40 per cent. sodium hydroxide solution. The nitrous acid reduces the hydroxylamine compound to sulphonamide, which is determined by diazotising and coupling. Comparison should be made with hydroxylamine-compound standards which have been similarly treated.

I wish to express my thanks to Dr. L. Colebrook for performing the animal experiments and to Dr. A. T. Fuller for helpful advice.

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The Determination of Tin in Foodstuffs by means of Dithiol

By R. DE GIACOMI

The determination of small amounts of tin in foodstuffs by gravimetric methods necessitates taking a large sample, with the attendant difficulty of the destruction of much organic matter. An attempt has therefore been made to find a suitable method which could be carried out on 10 g. of material. The colorimetric method of Schryver[1] was not found satisfactory for this purpose. On the other hand, the use of Clark's 4-methyl-1.2-dimercaptobenzene (dithiol) reagent,[2] with the procedure here outlined, proved successful. Hamence[3] suggested the use of dithiol in a scheme designed for determining several metals in one solution. I have found, however, that the presence of ammonium sulphate, ammonium citrate and hydrogen sulphide water in the test and standard solutions renders the method unreliable.

In Clark's[4] method agar is added to the test solution to keep the precipitate in suspension, and the colour is evaluated by means of a Lovibond tintometer. In the following method the use of agar is avoided by keeping the concentration of tin in the test solution below 30 p.p.m. The colour is matched by comparison with standard tin solutions, and this is of use when a Lovibond tintometer is not available.

Reagents.—(*a*) *Strong solution of tin.*—Tin (0·1 g.) is dissolved in 10 ml. of conc. hydrochloric acid, 0·5 ml. of thioglycollic acid is added, and the solution is diluted to 100 ml. with water.

(*b*) *Dilute solution of tin.*—One ml. of the strong solution of tin and 2 drops of thioglycollic acid are diluted to 100 ml. This solution should not be kept for more than one day.

(*c*) *Reagent.*—Dithiol (0·1g) and 0·25 ml. of thioglycollic acid are dissolved in 50 ml. of a 1 per cent. aqueous solution of sodium hydroxide.

Procedure.—Careful control of the conditions under which the tin sulphide is precipitated is essential. Ten g. of the sample are treated in a 300-ml. Kjeldahl flask with 10 g. of potassium sulphate and 30 ml. of conc. sulphuric acid, and the flask is heated over a flame until the organic matter is destroyed; further additions of acid may be made if necessary. When cool, the solution is washed into a 250-ml. conical flask, so that the final volume does not exceed 100 ml. It is then neutralised with ammonia (sp.gr. 0·880), a small piece of litmus paper being used as indicator (the use of indicator solutions introduces difficulties at a later stage). Conc. hydrochloric acid is added, drop by drop, until the solution is just acid and then 2 ml. excess. The litmus paper is rinsed and removed, the solution is cooled, and hydrogen sulphide passed through it first for 10 minutes in the ordinary way and then for 1 hour under pressure, obtained by passing the delivery tube through a rubber stopper inserted firmly in the neck of the flask. The solution is next boiled gently for 5 minutes and filtered through a Buchner funnel containing a No. 5 Whatman filter-paper, 4·25 cm. in diameter. The filtrate is re-filtered two or three times until it is clear and colourless, after which it is again saturated with hydrogen

sulphide, allowed to stand under pressure for half-an-hour, again boiled, and filtered through the original filter. If the filtrate is not crystal clear the filtration is repeated, but this is not usually necessary. The flask and filter are washed two or three times with water. The filter-paper is placed in a squat 100-ml. beaker, 10 ml. of 10 per cent. sodium hydroxide solution are added, and the beaker is heated on a water-bath for at least 10 minutes. The pulp and solution are poured on to the funnel previously used, a No. 5 paper again being used, and the beaker and paper are washed several times by decantation with water (10 to 20 ml. in all). The filtrate is washed into a 100-ml. flask and made just acid by adding conc. hydrochloric acid, drop by drop, a small piece of litmus paper being used as indicator. Two drops of thioglycollic acid are added, and the solution is made up to 100 ml. with water.

Five ml. of the solution are treated in a boiling-tube with 5 ml. of water, 0·5 ml. of conc. hydrochloric acid and 0·5 ml. of reagent. The tube is immersed in a boiling water-bath for 30 seconds and then allowed to stand for 1 minute, after which the colour is compared with standards prepared in the same way from the dilute tin solution and made up to the same volume. The comparison is made by viewing the tubes from the side by light reflected at right angles. When an approximate match has been obtained the test is repeated, the standard and test solutions being prepared simultaneously. To obtain the best results the amount of test solution taken should not give a greater depth of colour than is given by 4 ml. of the dilute tin solution. The test solution is diluted accordingly, or smaller aliquot portions are taken, to bring it within this range. If tin is present in a very small quantity, 10 ml. of the test solution are used.

TABLE I

Expt. ..	1	2	3	4	5	6	7	8	9	10	11	12
Added, mg.	0·30	0·70	0·75	1·00	1·25	1·40	1·50	1·75	2·10	2·80	4·00	6·00
,, p.p.m.	30	70	75	100	125	140	150	175	210	280	400	600
Found, mg.	0·25	0·65	0·75	0·90	1·20	1·30	1·40	1·60	2·00	2·60	3·75	5·20
,, p.p.m.	25	65	75	90	120	130	140	160	200	260	375	520
Diff., p.p.m.	5	5	—	10	5	10	10	15	10	20	25	80

The experimental results given in Table I were obtained by adding known amounts of standard tin solution to 10 g. of minced beef or veal known to be free from tin.

It will be observed that above 150 p.p.m. the error increases. This is necessarily a limiting factor in all colorimetric work. The error was found to be chiefly due to small differences in the tint between the standard and test solutions; by adopting the following modification greater accuracy may be achieved with the higher concentrations of tin.

The dilute solution of tin is prepared by adding to 1 ml. of the strong solution of tin 95 ml. of water and 5 ml. of conc. sulphuric acid. The solution is then treated in exactly the same way as the acid solution obtained after the destruction of the organic matter. The dissolved sulphide is treated with hydrochloric acid and thioglycollic acid as before, and made up to 100 ml., and this solution is used as the standard. Some results thus obtained are given in Table II.

TABLE II

Expt.		Mgms.	p.p.m.	Diff. p.p.m.
(*a*)	Added	1·50	150	nil
	Found	1·50	150	
(*b*)	Added	2·00	200	nil
	Found	2·00	200	
(*c*)	Added	3·00	300	nil
	Found	3·00	300	
(*d*)	Added	4·00	400	15
	Found	3·85	385	

OBSERVATIONS.—(*a*) In the destruction of organic matter the use of nitric acid is avoided, since this may give rise to a precipitate of insoluble metastannic acid, which has to be filtered off and fused with caustic soda.

(*b*) The reagent is best kept in an atmosphere of hydrogen and should be rejected on the appearance of a white precipitate of the disulphide. I have been unable to preserve the solution satisfactorily for more than two weeks.

(*c*) If the filter is washed by decantation, not by means of a jet, there is no risk of colloidal tin sulphide being carried through it. By using No. 5 Whatman paper, in conjunction with a Buchner funnel and a suction pump, a fine filter, which retains the precipitate, is obtained.

(*d*) Specimen tubes (6 in. × 1 in.) have been found more suitable than boiling-tubes for comparing the solutions. They should be selected so as to be of the same diameter and of the same tint of glass.

(*e*) The colour matching should be carried out by daylight, as I have found that with artificial light accurate work is impossible. Also, the matching should be carried out on the same day as that on which the final test solution is prepared.

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2. R. E. D. Clark, ANALYST, 1936, **61**, 242.
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November 9th, 1939

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.

AN ALKALINE AGEING TEST FOR TEXTILE OILS

OILS for lubricating textile fibres should be examined for liability to undesirable oxidation, from two aspects:—(*a*) The rate of oxidation (which depends upon the catalyst/antioxidant balance and is measured by such methods as the Mackey test and the peroxide accumulation rate); (*b*) the type of end-product (which depends upon the chemical constitution and is indicated by the yield of petrol-insoluble bromides, iodine value of the liquid fatty acids, etc.).

The present test gives visual evidence of the formation of undesirable end-products, by the production, under controlled laboratory conditions, of effects similar to those that may result from the use, in bulk, of unsuitable oils. White worsted serge, previously extracted with ether to remove soap and oil, is cut into 3-inch squares; 0·1 ml. of the oil is dropped into the centre of one of these squares, which is then suspended in an oven at 80° C. six inches above a dish of dilute ammonia (100 ml.). After 4 hours the pattern is removed from the stove and cut into two halves. One-half is retained for reference; the other is scoured for 10 minutes in 300 ml. of a solution containing per litre 0·2 per cent. of soap and 0·1 per cent. of ammonia at 40° C., drained, scoured again in the same solution, washed off, dyed in a dye bath (5 g. of serge) containing 0·04 g. of Coomassie Blue R.L.S. (I.C.I.), 10 g. of Glauber's salt and 3 ml. of acetic acid (added in portions of 1 ml. at 20-minute intervals), beginning cold, heating to boiling during 20 minutes, and boiling for 1 hour.

The following table gives some typical results:

Oil	Discoloration after alkaline ageing	Resist after scouring and dyeing	Mackey test (mins. to 400° F.)
Nilox ester oil	Brownish	nil	No rise
Nilox arachis oil	Cream	nil	No rise
Olive oil	Pale yellow	Very slight	215
Olive oil containing iron	Pale fawn	Slight	125
Arachis oil (edible)	Fawn	Slight to mod.	310
Cottonseed oil (edible)	Brownish-fawn	Moderate	245
Linseed oil (refined)	Brownish-yellow	Great	95
Linseed oil containing antioxidant	Pale brownish-yellow	Great	340

The discoloration produced by the alkaline ageing appears to be due partly to oxy-acids (which form brownish alkali salts) and partly to yellowish dimers and polymers resulting from the oxidation and condensation of constituents which yield petrol-insoluble bromides.

The depth of colour increases with the unsaturation (*e.g.* linseed oil becomes darker than olive oil), the amount of oxy-acids present (*e.g.* blown olive oil becomes darker than olive oil), and the amount of effective catalyst (*e.g.* olive oil with a bad Mackey test and high peroxide accumulation rate becomes darker than an olive oil with a slow rate of oxidation).

The coloured bodies due to oxy-acids are scoured out easily and do not cause a resist in dyeing; those due to the condensation products are not scoured out, and the residue after scouring acts as a resist in dyeing. It is the resist effect after dyeing which is therefore to be taken as the significant indication of unsuitability.

It will be noticed that the test is sensitive to the presence of effective catalysts (which are not shown by such tests as the yield of petrol-insoluble bromide) and of di- or poly-ethenoids (which are not shown by the Mackey test, etc.); the effect of polyethenoids is only slightly masked by the presence of antioxidants.

An oil which produces a resist greater than that given by an olive oil that is free from antioxidant and has a good Mackey test and low peroxide accumulation rate should be considered unsuitable for textile use.

An oil which gives a resist less pronounced than this standard is not necessarily suitable, but if, in addition, it answers to the following requirements it can be considered reasonably safe:—iron-content less than 1 p.p.m., Mackey test and peroxide accumulation rate satisfactory, and yield of petrol-insoluble bromides less than 10 per cent. (octo- and hexa-bromides being absent). W. GARNER

March, 1940

UNIVERSAL BUFFER MIXTURE

IN THE ANALYST, 1939, **64**, 490, Johnson and Lindsey described what they regard as an "Improved Universal Buffer Mixture," this being presumably an improvement on the mixture introduced by Robinson and myself in 1931 (*J. Chem. Soc.*, 1931, 1456). Apparently they have overlooked the fact that Welford and I described the same modification in the *J. Chem. Soc.*, 1937, 1848, and, moreover, calibrated it for the temperatures: 12·5°, 25°, 34° 53°, 63°, 75°, and 91° C., instead of at one temperature, *viz.* 18° C., as Johnson and Lindsey have done. This Universal Buffer mixture has the advantage over other similar mixtures, that none of its acids is volatile, so that it can therefore be used for *p*H work at elevated temperatures. H. T. S. BRITTON

March, 1940

COPPER IN TOMATO PULP

IN the notice of the Annual Report of the Chief Medical Officer of the Ministry of Health (ANALYST, 1940, **65**, 103) reference is made to an agreement reached at a conference of Port Medical Officers of Health in October, 1938, as to the tolerance of copper in imported tomato pulp.

Information has now been received that Port Medical Officers of Health, at a later conference, agreed to postpone until further notice the intended reduction of the tolerance from 100 to 50 parts per million in the dried total solids.

EDITOR

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 special interest to the Society. Notes made from such Reports are submitted to the Publication Committee.

COUNTY OF KENT

REPORT OF THE COUNTY ANALYST FOR THE FOURTH QUARTER, 1939

OF the 694 samples received from the County sampling officers, 43 were purchased informally.

TOWN REFUSE AS MANURE.—Two samples of treated household refuse from different sources gave the following percentage results:

Water	Nitrogen	Phosphoric acid	Potash	Organic matter
7·7	0·67	0·59	0·24	33·1
6·6	0·80	0·51	0·22	30·8

In both samples the organic matter consisted essentially of paper and carbon in the form of partly burned coal. Household refuse is often advocated as an organic manure, but the proportion of organic matter, even in the dried refuse, is invariably comparatively low. In these two samples only about one-third of the material was organic in nature and the preponderating substance was coal ash, ash material amounting to more than 60 per cent. The value of treated house refuse can only be ascertained by actual experiment, and at the moment these experiments would best be carried out on the soils to which the manure might be applied in the future. The results of a few experiments carried out at both Rothamsted and Woburn have been published (*cf.* ANALYST, 1940, 104), and they would tend to show that a proportion, at all events, of the nitrogen in house waste is in a form that renders it available as a plant food. It may well be that these treated household refuses would have a manurial effect extending over two years and, further, they may advantageously affect the soil condition. It seems to me that whether these wastes will ever attain popularity must depend on the price at which they can be brought to the farm. Rather large quantities will be necessary to supply the available nitrogen essential for crop production, and still larger quantities if dressings are required to improve soil tilth. F. W. F. ARNAUD

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.

IS CARBOLIC ACID A DRUG?

ON February 3rd a druggist was summoned at Bishop Auckland under the Food and Drugs Act for selling carbolic acid not of the quality demanded.

Mr. H. C. Underwood, prosecuting for Durham County Council, said that two bottles of carbolic acid had been purchased from the defendant by an inspector under the Food and Drugs Act. The substance was labelled "Carbolic Acid (Crude). Contains Phenols 90 per cent. weight in volume. For disinfecting purposes." Whilst according to the label it contained 90 per cent. of phenols, analysis showed it to contain only 14 per cent. Although there was no fixed

standard for crude carbolic acid, the B.P. stated that carbolic acid contained not less than 98 per cent. of phenols. The fact that the article was sold for use as a disinfectant brought it within the meaning of a drug.

Mr. C. J. H. Stock, F.I.C., County Analyst, said that the sample examined by him contained only 14 per cent. of phenols. In his opinion, such a substance was practically valueless as a disinfectant. In cross-examination he said that he thought that there was some doubt whether the substance came within the Food and Drugs Act.

Mr. Brown-Hughes, for the defence, contended that the product could not be described as a medicine, and, if that were so, it could not be defined as a drug coming within the ambit of the Food and Drugs Act. He submitted that he had no case to answer.

Mr. Underwood maintained that the term drug meant anything that could be used in the prevention or alleviation of disease, and, as a disinfectant came within this category, it should therefore come within the scope of the Food and Drugs Act.

The Magistrates decided that there was no case to answer and dismissed the charge.

CARBOLIC OINTMENT

ON February 26th a druggist was summoned at Stalybridge for selling carbolic ointment deficient in carbolic acid to the extent of 92 per cent.

Mr. Gregory, prosecuting for the Cheshire County Council, said that, as there was no fixed standard for carbolic ointment, the magistrates could fix their own standard on the evidence.

Mr. S. E. Melling, F.I.C., Public Analyst for Cheshire, said that the sample of ointment examined by him contained 0·24 per cent. of carbolic acid. The British Pharmacopoeia did not fix a standard for the ointment, but prescribed that, in compounding it, 3 per cent. by weight of phenol should be added; in his opinion, as the sample contained less than one-tenth of 3 per cent., it was of very little use as an antiseptic.

In cross-examination the witness said that phenol might evaporate to the extent of 28 per cent. unless kept in an air-tight container. The British Pharmacopoeia did not give directions as to keeping carbolic acid, and witness agreed that ointment kept in the jar produced in court might lose 25 per cent. of its phenol by evaporation in 2 years.

Mr. Henriques, for the defence, said that before the magistrates could fix a standard there must be evidence before the Court from which they could assume a standard recognised in the trade.

Dr. Mumford, F.R.C.P., consulting dermatologist at the Manchester Royal Infirmary, said that the medical profession was not in the habit of prescribing carbolic ointment. For medicinal purposes 0·24 per cent. of phenol in carbolic ointment would be as effective as 3 per cent.; in other words, it would be "completely ineffective." Three per cent. of phenol might be dangerous if used on an open cut or abrasion. The only form in which he prescribed carbolic acid therapeutically was as a 1 per cent. lotion.

The defendant said that he had obtained the ointment from a firm of manufacturing chemists and had kept the jar on a shelf in his shop. There was very little demand for it, and he had not sold more than 2 oz. a year.

Mr. Thomas Tickle, F.I.C., said that the sample he had analysed for the defendant contained 0·36 per cent. of phenol. There was no acknowledged standard; knowing what he did of the sample, he would regard it as genuine.

The Magistrates decided that the case for the prosecution had not been proved, but would not allow costs.

Department of Scientific and Industrial Research

METHODS FOR THE DETECTION OF TOXIC GASES IN INDUSTRY

CARBON MONOXIDE*

OCCURRENCE.—Among the industrial situations in which this gas may be encountered in dangerous concentrations are blast furnaces, brick kilns, chemical works, foundries, gas works, coke ovens, lime kilns, and gas and water gas producers.

POISONOUS EFFECTS.—The first symptoms of poisoning are shortness of breath and palpitation on exertion, accompanied by headache which increases in severity. With increasing intoxication the judgment becomes disturbed, and the affected person may not realise his danger. An atmosphere in which a concentration as low as 1 part in 2000 is present may prove fatal in about one hour to a person engaged in an active occupation. On the other hand, concentrations below 1 in 10,000 may be regarded as relatively harmless for all practical purposes.

METHODS OF DETECTION.—Methods based on the reaction of carbon monoxide with haemoglobin, though accurate and sensitive, are unsuitable for industrial purposes. The iodine pentoxide method has the drawback that the apparatus and the procedure are too complicated for routine tests.

The standard method developed consists in drawing samples of the atmosphere under examination through a known area of test-paper impregnated with palladium chloride, at a slow and constant rate, by means of a 5-litre aspirator.

The palladium chloride solution is prepared by dissolving 0·1 g. of the pure salt in about 20 ml. of boiling water, filtering and cooling the solution, and making up the filtrate to 20 ml. This solution is then mixed with 20 ml. of pure acetone. The test-papers (3 in. × 2 in.) are impregnated with this solution.

To remove interfering gases the sample of air is made to pass through a tube of activated charcoal before passing through the test-paper. Sampling is continued until a stain is obtained on the test-paper which comes within the range of the standard colour chart; the concentration is then found by comparing the time required to reach the necessary colour with the times given on the chart. In this way concentrations of 1 part in 500 can be detected in less than two minutes, and of 1 part in 10,000 in half-an-hour. Full instructions for carrying out the test are contained in the leaflet.

In the colour chart, which is included in the Report, two standard stains are given, and it is directed that the test-paper should be examined at intervals of 5 minutes until a stain is obtained which is darker than Standard No. 1 and lighter than Standard No. 2.

FOOD INVESTIGATION BOARD

REPORT FOR THE YEAR 1938†

THE most important development during the year under review has been the expansion of the work of the Board to cover research on the processing of food. A committee has been set up to consider the organisation of research in this field in the interests alike of the consumer, producer and manufacturer. Although some work has already been carried out on the manufacture of bacon, the smoking and salting of fish and the canning of fruit, the work of the Board has previously

* Leaflet No. 7. H.M. Stationery Office, York House, Kingsway, London, W.C.2. 1939. Price 1s. 6d. net.

† H.M. Stationery Office, York House, Kingsway, January, 1940. Price 4s. 6d. net.

been mainly concentrated on the problems involved in preserving the "fresh" properties of unprocessed food during transport and storage.

TRANSPORT OF MEAT.—Attention is directed to the progress in the transport of chilled beef from Australia and New Zealand by gas storage in an atmosphere enriched, to a controlled extent, with carbon dioxide. During the five years since the method was introduced our imports of chilled beef from Australia and New Zealand have increased nearly tenfold to a combined annual figure of 850,000 cwts. Experiments have indicated that the "bloom" of the meat might be improved by increasing the rate of evaporation from the meat in the course of the voyage.

WASTAGE OF ORANGES IN TRANSPORT.—During the year arrangements were made to carry out on behalf of the Government of Palestine a survey of the wastage of oranges during transport. The temperature in the holds is an important feature in the wastage of the fruit, and the main object was to ascertain how far temperature variations were responsible for the wastage and to what extent the temperature conditions might be improved. Experiments were also carried out on the storage conditions of Palestinian grapefruit after its arrival in England.

GAS STORAGE OF FRUIT AND VEGETABLES.—Further successful experiments have been carried out on the gas storage of home-grown pears, apples and broccoli. No form of storage for apples can be reckoned successful unless it conserves their flavour, and this is especially important in the case of Cox's Orange Pippin. A full-scale demonstration was arranged to remove any doubt in the trade as to whether this variety developed its full flavour after gas storage. Twenty-six tons of Cox's Orange Pippins were put into a gas store at the Ditton Laboratory at the end of September, 1937. The composition of the atmosphere of the store was 2·5 per cent. of oxygen, 5 per cent. of carbon dioxide and 92·5 per cent. of nitrogen, and was obtained by the removal of the excess of carbon dioxide by a scrubber of commercial design, together with controlled ventilation. The temperature of storage was 39° F. The store was opened on February 22nd, 1938, in the presence of about 150 fruit-growers and other experts. The demonstration was completely successful, the fruit being in excellent condition; in fact, 80 per cent. of it was graded as "Fancy" or "Extra Fancy." On the other hand, a subsequent survey of fruit of this variety from 10 representative areas has shown that the extent to which the flavour is developed depends, in the main, on pre-storage conditions, such as soil, manurial treatment and maturity at the time of gathering.

GLYCOGEN IN MEAT.—No adequate explanation of the variations in the glycogen-content of pig's muscle is yet forthcoming. The amount of glycogen in the livers of oxen is usually about 1 per cent., as compared with about 0·1 per cent. in pig's liver. Animals whose muscle contains little glycogen also have little in the liver, so that the deficiency is general and not confined to the muscular tissue.

RANCIDITY OF HERRING OIL.—The potency of the enzyme that causes oxidative rancidity in herring oil is decreased by storing the fish at temperatures ranging from 0° to −10° C., the decrease being most rapid at the higher temperatures.

ESTIMATION OF THE FRESHNESS OF FISH.—Rapid chemical methods for determining the concentration of di- and tri-methylamines have been devised. The determination of either compound can be used as a means of detecting incipient staleness, but to follow the course of deterioration prior to this stage it is necessary to make a series of colorimetric estimations of dimethylamine.

"PINK" SPOILAGE OF SALTED FISH.—The micro-organisms responsible for this type of spoilage have been found to belong to two groups, *Serratia* and *Micrococci*, the latter being responsible for the final stage of deterioration. The best remedy, so far discovered, is to chill the fish below 5° C.

METABOLISM OF POTATO CARBOHYDRATES.—The potato tuber has been found to contain a zymohexase, and by the action of this enzyme on hexosediphosphate

(the presence of which has also been established) triosephosphate is formed; this transformation is regarded as the initial stage of the breakdown of sugar in yeast and in muscle.

STIMULATION OF RIPENING OF PLUMS BY ACETYLENE.—The recent finding in South Africa (*Nature*, 1938, **141**, 876) has been confirmed. Immature *Monarch* plums, when treated at 18·3° C. with an atmosphere containing acetylene, ripened more rapidly and developed a better flavour than untreated plums.

ENGINEERING.—Among the other investigations described is the effect of turbulence in increasing the rate of evaporation from a wet surface. Further studies have also been made of the properties of refrigerants.

All-India Institute of Hygiene and Public Health

ANNUAL REPORT FOR THE YEAR 1938

IN discussing the work of the Institute (*cf.* ANALYST, 1939, **64**, 196) the Director (Dr. John B. Grant) directs attention to the paradox that, whilst it is fully recognised that the country is badly in need of a vigorous and forward policy with regard to public health organisation, even the small number of trained workers are unable to find employment; this is due to the magnitude of the problem on the one hand and to the shortness of funds on the other.

In addition to the teaching work various investigations have been continued. These include the following:

SALMONELLA ENQUIRY.—Fever and diarrhoea of unknown origin are widely prevalent in Indian towns. Preliminary investigations have shown that a high percentage of rats, caught at random in Calcutta, carry salmonella organisms. The facts so far revealed should serve to stimulate public interest in the destruction of this pest, even in the absence of plague.

STUDIES IN CALCIUM METABOLISM.—Three types of Indian diets: (1) good North Indian, (2) well-to-do lacto-vegetarian, and (3) poor Indian, were fed to groups of rats. Each group was made to bear its litter to enable the study to be continued over three generations, and, for comparison, a control group was fed on a well-balanced diet. The bones and teeth were studied by four methods: (1) X-ray pictures, (2) chemical analysis of bone and teeth, (3) histological, and (4) micro-incineration of histological sections. X-ray pictures showed lighter shadows as calcium intake was decreased, and chemical analysis showed that the percentage of ash from the bones decreased with lower calcium intakes. In the teeth, however, the percentage of ash remained fairly constant, and the calcium: phosphate ratio remained practically constant in both the bones and teeth in all groups. Histological examination revealed no difference in the bones, but striking differences were noticed in the structure of the teeth.

Micro-incineration of histological sections and examination of the ash under dark-ground illumination is an excellent method for the study of inorganic constituents in cells.

One conclusion drawn from the investigation is that even the North Indian diet, which is considered to be ideal, is inferior to the stock diet if the histological picture of the teeth is studied.

VITAMIN A CONTENT OF FISH OILS.—Oils obtained from 15 varieties of local fresh-water fish were examined. The body and depot oils were very poor sources of vitamin A, but the liver oils contained more than was present in an ordinary brand of Norwegian cod-liver oil. The liver oils of two species, "Air" and "Boal,"

had a vitamin A content nearly half that of halibut liver oil, and the liver oils of "Dhain" and "Shole" were nearly equal to halibut oil. It would thus appear that the industrial preparation of Bengal fish-liver oils would be feasible. These oils showed the typical absorption bands of vitamin A_2.

DETECTION OF SULPHANILAMIDE POISONING.—Since a certain number of patients using sulphanilamide preparations have developed severe types of anaemia, the popularity of these drugs has greatly suffered. The anaemia is due to the formation of sulphaemoglobin in the blood, and this substance can easily be detected by the spectrograph, as it gives specific absorption at 6200 Å. Blood samples from a group of patients receiving diaminodiphenyl sulphone glucoside were examined daily, and it was noticed that sulphaemoglobin could be detected about six days after the beginning of the treatment and a week before any clinical signs of anaemia could be observed. It would thus appear that a spectrographic examination of blood will be a useful guide in chemotherapy with sulphanilamide derivatives. It is interesting to record that in meningitis patients large doses of the drug were administered without the appearance of sulphaemoglobin in the blood.

EPIDEMIC DROPSY AND *ARGEMONE MEXICANA* OIL.—In the previous Report (ANALYST, 1939, **64**, 196) it was shown that the use of certain kinds of mustard oil produced epidemic dropsy. It was found that the incriminated oils gave a brown colour in Hauchecorne's nitric acid test and a green colour when mixed with an equal volume of glacial acetic acid and treated with a 3 per cent. solution of cupric acetate. Moreover, the toxic oils had a characteristic bluish-violet fluorescence in ultra-violet light, and, when examined spectrophotometrically, showed a broad absorption band between 2900Å and 2600Å with a maximum about 2750Å. The possibility of the mustard seeds having been mixed with some poisonous seeds was investigated, and it was found that the oil expressed from a mixture of mustard seed with 6 per cent. of *Argemone mexicana* seeds answered to the same physical and chemical tests as the known toxic oil from Rangpur. A white crystalline substance, soluble in hot alcohol and in dilute hydrochloric acid, was isolated from *Argemone* oil, and this gave intense reactions in the differential tests. Further work on this substance is in progress. *Argemone mexicana* grows wild and is plentiful in different parts of India, and the seeds may get accidentally mixed with mustard seed at the time of harvesting or possibly there may be intentional adulteration before the seeds reach the millers.

Commonwealth of Massachusetts

ANNUAL REPORT OF THE DIVISION OF FOOD AND DRUGS DEPARTMENT OF PUBLIC HEALTH

THE Report of the Director of the Division (Dr. H. C. Lythgoe) for the year ending November 30th, 1938, gives an outline of the routine work relative to the enforcement of the laws pertaining to the sale of milk, sale of food and drugs, the bakery law, mattress law, etc. The total number of milks examined was 5154, of which 529 were below standard. Of the 2325 samples of other foods examined, 417 were adulterated, and 18 of the 182 samples of drugs were adulterated or misbranded. Among the points of interest discussed are the following:

GOATS' MILK.—A New York dairy is now selling pasteurised certified goats' milk in Massachusetts, and one goat breeder in the Commonwealth sells the milk pasteurised. Exaggerated claims are made as to the curative and medicinal properties of this milk, and there is some risk of its becoming a fad. Investigations have shown that practically all the animals are tested for Bang's disease as well

as for tuberculosis; recently one animal reacted to the tuberculin test. The following table summarises the results of the analysis of samples of milk of known purity obtained from 21 goats:

	Highest Per Cent.	Lowest Per Cent.	Average Per Cent.
Total solids	18·40	11·96	14·42
Fat	7·25	2·95	4·93
Solids-not-fat	11·17	8·39	9·49
Lactose	5·65	4·25	4·78
Total proteins	5·62	3·38	4·11
Albumin	1·37	0·77	1·06
Ash	1·04	0·75	0·89
Copper serum refraction at 20° C.	40·2	36·3	38·2
Acetic serum ash, g. per 100 ml...	1·105	0·845	0·970
Freezing-point, °C.	—0·550	—0·595	—0·573
Protein: fat ratio	0·67	1·30	0·83
Albumin in proteins, per cent...	—	—	25·79

The average total solids are somewhat higher than for cows' milk, as is also the fat; the total proteins are considerably higher, as is also the ash. Hence, the average freezing-point applied to cows' milk for the detection of added water is too high for goats' milk.

ADDED WATER IN SHELLFISH.—Of 145 samples of shelled clams examined, 85 were found to have been soaked, *i.e.* allowed to absorb a considerable amount of water. Prosecutions were instituted in many of these cases. Three samples of oysters containing added water were also obtained.

PORK IN HAMBURG STEAK.—A sample of Hamburg steak containing pork was taken in Western Massachusetts. The store manager admitted having used up pork ends in this way, and stearine crystals characteristic of pork fat were separated from the fat of the sample. It is unfortunate that pork is so often put into Hamburg steak, because the material is assumed by the public to be beef. It is frequently eaten semi-raw and is very rarely cooked at a temperature sufficiently high to kill trichinae. It is possible that the apparent increase in trichinosis in this part of the country may be due to the practice of adding pork to Hamburg steak without giving a notification to the customer.

COLON BACILLI IN CRAB AND LOBSTER MEAT.—The bacterial counts of crab and lobster meat indicated that the business is not conducted under any greater degree of sanitation than in the previous year, when so much sickness resulted. Of 18 samples of crab meat examined, 14 contained colon bacilli with counts ranging from 220 to 95,000. All the 10 samples of lobster meat contained colon bacilli with counts ranging from 260 to 70,000.

COLON BACILLI IN VEGETABLES.—During September, 1938, there was a disastrous flood in Western Massachusetts. Eleven samples of vegetables which had been in the flood were examined and 5 contained *B. coli*. Carrots that had been in the flood waters were found, after the usual washing in cold water, to be free from colon bacilli, but cabbages were found to have been contaminated.

Manchester Chamber of Commerce

TESTING HOUSE AND LABORATORY

ANNUAL REPORT FOR THE YEAR 1939

THE outbreak of war caused a decrease in the work of the Testing House, but the position is rapidly becoming normal and large orders placed for Empire defence purposes are being inspected. A special feature has been the inspection, for local A.R.P. authorities, of gas-proof oiled fabric suits. Investigations of general interest include the following:

STAINS AND DEFECTS IN FABRICS.—Bleaching of the blue "silk" trimming of a masonic apron was found to be due to its contact with the goatskin, which was strongly acid; the cotton weft in the ribbon had also suffered loss in strength.

Pink Staining of Bleached Cotton.—In one instance this was due to aniline vapour, but in another it set an interesting problem. Pink stains developed on stocks of white polo helmets, and these could only be attributed to the vapour from amino-compounds in the rubber solution used for securing the bleached drill to the cork foundation.

Red stains on coloured frocks were attributed to a perspiration corrective affecting the dyestuffs.

SURGICAL DRESSINGS.—The total number of dressings submitted under the National Health Insurance Acts was 1156, and 69 of these (6 per cent.) were deficient in quality and 20 (1·7 per cent.) in quantity. The corresponding figures for the previous year were 5·8 and 1·3 per cent. Dressings supplied in the original sealed packets showed less deficiencies than those not so packed. A number of samples of white and boric lint contained fewer weft threads than the standard. A more serious defect was deficient absorbency, some of the dressings taking several hours, instead of 10 seconds, to sink in water. Other materials, mainly bandages, yielded two or three times the permissible amount of foreign matter. The proportion of medicament in a number of dressings differed considerably from that specified. Thus a sample of boric lint contained 55 per cent. of boric acid instead of 35 to 45 per cent.; a double cyanide gauze had a total cyanide-content of 1 per cent., instead of the 2 to 4·5 per cent. required, and an iodoform gauze contained no perceptible amount of iodoform, whereas 4 to 6 per cent. was stipulated.

Adhesive-coated or rubber- or oil-proofed appliances were deficient in adhesive or proofing, some to the extent of 40 per cent., and the fabrics used were sometimes low in weight by as much as 20 per cent. and contained fewer threads than required.

COTTON WOOL.—Many samples were inferior to the standard in respect of freedom from "neps" and particles of leaf and shell. One sample contained pieces of coloured thread in addition to particles of cotton seed, and hard bast fibres were found in another sample. A third sample contained 25 per cent. of rayon fibre, presumably added to impart a soft "handle" to the material.

South Africa

ANNUAL REPORT OF THE DIVISION OF CHEMICAL SERVICES, 1939

THE Division of Chemical Services (Chief: Dr. J. P. van Zyl) undertakes work for all the State Departments and for provincial administrations. Its work consists of (*a*) regulatory and control work, and (*b*) investigational and research work. The chemical work required in the working of Acts administered by the different Departments comes under the first category, whilst the second category comprises work on soil classification, soil fertility and micro-biological problems, food and nutrition, toxicological research and miscellaneous agricultural and industrial investigations.

REGISTRATION OF FERTILISERS AND FEEDING STUFFS.—All applications are scrutinised by the Division before a list of fertilisers and farm foods registered with the Department of Agriculture and Forestry is published. This list gives the registered number, the name of the person registering, and the chemical composition of fertiliser or farm food, so that the public can see exactly what products are on the market and an official check can be kept to see that they conform to their declared composition.

ARSENIC IN PEARS AND APPLES.—The total number of samples examined was 4202, and 430 were found to contain arsenic in excess of the legal export limit of 1/100 grain of arsenious oxide per lb.

FOOD AND DRUGS.—Of the 5235 samples examined for the Department of Public Health, 545 were adulterated or below standard. The quality of milk was still unsatisfactory, for 321 of the 3435 samples examined were adulterated or below standard. Of 223 samples of ice-cream received, 40 contained less than the 10 per cent. of fat required by the regulations.

EFFECT OF FLUORINE INSECTICIDES ON CITRUS-TREES.—Experiments with fluorine insecticides, such as cryolite and sodium fluosilicate, have been continued, to ascertain if there is any danger involved in the use of these remedies in the form of spray or dust against American ball worm. The young oranges are treated when they are about the size of a pea, and when ripe both the fruit and peel are analysed for fluorine. This work has already been in progress for 3 years, in view of the possibility that the effect may be cumulative. The effect of superphosphates has also been tested, since most superphosphates contain about 1·5 per cent. of fluorine. The results so far obtained indicate that there is no danger of navel oranges taking up fluorine from the soil or of the quality of the oranges being affected by dusting or spraying with fluorine insecticides.

ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

Food and Drugs

Determination of Creatinine with *m*-Dinitrobenzoic Acid. E. Komm and H. Pinder. (*Z. Unters. Lebensmittel*, 1939, **78**, 113–123.)—The suitability of *m*-dinitrobenzoic acid for the quantitative determination of creatinine has been confirmed; it is more specific than picric acid. A disadvantage of picric acid is its own colour, whereas *m*-dinitrobenzoic acid is only pale yellow, and after purification is quite colourless. Purification is effected by crystallisation from glacial acetic acid and drying for 45 minutes at 80° C., and the reagent is kept in a vacuum desiccator in the dark. The determination of creatinine in a 5 per cent. solution of broth-cubes (defatted) or a 0·5 per cent. solution of meat-extract is made as

follows:—Evaporate 50 ml., after addition of 20 ml. of *N* hydrochloric acid, in a porcelain dish on the water-bath. Dissolve the residue in water, neutralise, and make the volume up to 50 ml. Decolorise the solution in a cylinder by means of aluminium hydroxide. Add to 20 ml. of the filtrate 5 ml. of *N* hydrochloric acid and make up to 50 ml. Add to 2 ml. of this solution 4 ml. of a 2 per cent. solution of dinitrobenzoic acid in absolute alcohol and 1 ml. of 5 per cent. sodium hydroxide solution. At the same time prepare a standard solution (0·01 per cent. of creatinine in *N*/10 hydrochloric acid) and treat it similarly. Measure the colour in a Duboscq colorimeter or with a photo-electric colorimeter, preferably the latter. Take the readings of the instruments for different dilutions of the standard creatinine solution, and obtain the corresponding percentages of creatinine from a table. In broth cubes 0·32 to 0·8 per cent. of creatinine was found, and in meat extracts 5·3 to 6·3 per cent. The amounts in urine ranged from 0·082 to 0·193 per cent. D. A.

Oil from the Seeds of *Canarium commune* L. A. Steger and J. van Loon. (*Rec. Trav. Chim. Pays-Bas*, 1940, **59**, 168–172.)—*Canarium commune* (*Burseraceae*) is a tree indigenous to the Moluccas, Java, the Celebes and Indo-China. The three-cornered oval fruits have three compartments, each containing an oily kernel resembling the sweet almond in form and taste and sometimes known as Java almonds. Samples of the oil were obtained by cold pressing in a small laboratory press and by extraction of the kernels with petroleum spirit. The kernels yielded 68·6 per cent. of oil (10·4 per cent. on the whole fruit). The press-cake contained 16 per cent. of water, 11·3 per cent. of mineral matter and 39 per cent. of crude protein; it is therefore a valuable fertiliser and cattle-food. The expressed oil had the following constants, and the corresponding figures for the extracted oil are given in brackets:—acid value, 0·22 (1·12); saponification value, 195·3 (194·4); iodine value (Wijs, 2 hours), 74·0 (68·8); iodine value (Wijs, 24 hours), 73·5 (70·2); Reichert–Meissl value, 0·64 (0·58); Polenske value, 0·43 (0·60); n_D^{70}, 1·4497 (1·4495); sp.gr. at 78°/4° C., 0·8740 (0·8728). The oil is solid at room temperature. It yielded 95·0 (93·8) per cent. of fatty acids soluble in petroleum spirit, 0·61 (0·24) per cent. of unsaponifiable matter, 4·4 (4·4) per cent. of glycerol residue C_3H_2 and 0·0 (1·54) per cent. of volatile and insoluble matter. The constants of the fatty acids were as follows:—iodine value (Wijs 2 hours), 76·6 (72·4); iodine value (Wijs, 24 hours), 77·1 (—); neutralisation value, 206·0 (205·1); mean molecular equiv., 272·3 (273·5); n_D^{70}, 1·4401 (1·4394); saturated acids per cent., (Bertram), 38·7 (40·7); mean molecular equiv. of the saturated acids, 263·3 (263·3). The fatty acids were separated into solid and liquid fractions by Twitchell's method, converted into ethyl esters, and fractionated by vacuum distillation. The results indicated the following composition (per cent.):—stearic acid, 9·7 (10·2); palmitic acid, 29·0 (30·5); 9-oleic acid, 38·3 (39·9); 9, 12-linolic acid, 21·8 (18·7); 9, 12, 15-linolenic acid, 1·2 (0·7). The statement of Grün and Halden (*Analyse der Fette und Wächse*, Vol. I, p. 378; Vol. II, p. 261) that canari oil polymerises when heated to 240° to 250° C. could not be confirmed, and it is suggested that these authors may have confused canari oil with Java-olive oil (Wedermeyer, *Z. Unters. Nahr. Genussm.*, 1906, **12**, 210; Abst., ANALYST, 1906, **31**, 361). A. O. J.

Liver Oils of Some Terrestrial Animals. M. Tsujimoto and H. Koyanagi. (*J. Soc. Chem. Ind. Japan*, 1939, **42**, 421–422B.)—The liver oils of (*a*) elephant, (*b*) dog, (*c*) rabbit and (*d*) hen, were obtained by extraction with alcohol, followed by ether, and subsequent treatment of the combined extracts with acetone. The oils had the following characteristics:

	(*a*)	(*b*)	(*c*)	(*d*)
Sp.gr. at 30°/4° C.	0·9137	0·9246	0·9222	0·9239
n_D^{40}	1·4695	1·4730	1·4660	1·4700
Saponification value	147·9	152·7	169·8	167·8
Iodine value (Wijs)	146·0	144·9	109·3	95·9
Unsaponifiable matter, per cent.	21·13	22·35	14·81	14·60
Acid value	7·0	4·5	8·1	2·3

Oils (*b*) and (*d*) contained appreciable amounts of vitamin A. Notable amounts of highly unsaturated acids were present in each oil. Palmitic, stearic and oleic acids were identified in the free acids from hen-liver oil. The unsaponifiable matter from each oil contained hydrocarbons. Hydrogen chloride addition products were formed by saturating the ethereal solutions with hydrogen chloride below 0° C. (*a*) The hydrochloride sintered above 120° C. and melted at about 130° C. with decomposition, and contained 29·7 per cent. of chlorine. (*b*) From 2·6 g. of unsaponifiable matter 0·7 g. of crude hydrocarbon was obtained (of iodine value 292·3) the highest yield yet observed; m.pt. over 115° C., with decomposition at 128–129° C., and chlorine content 30·15 per cent. (*c*) There was only a trace of precipitate of hydrochloride owing to the small amount of hydrocarbon present. (*d*) The m.p. of the hydrochloride was about 130° C. and its chlorine-content 29·83 per cent. All these hydrochlorides are similar to the analogous compounds obtained from pig, horse, sheep, ox and human liver, as also to the compounds from ishinagi, sperm and finback whales and dolphin liver oils. D. G. H.

Analysis of Chaulmoogra Oils. *Hydnocarpus anthelmintica* Oil and *Taraktogenos Kurzii* (Chaulmoogra) Oil. H. I. Cole and H. T. Cardoso. (*J. Amer. Chem. Soc.*, 1939, **61**, 3442–3445.)—The oil of *Hydnocarpus anthelmintica* ranks next to that of *Hydnocarpus Wightiana* for the treatment of leprosy. The analysis (*J. Amer. Chem. Soc.*, 1938, **60**, 614) was made by saponifying and liberating the fatty acids in the usual way, and separating the solid from the liquid acids by crystallisation from 80 per cent. ethyl alcohol. The two fractions, as ethyl esters, were fractionally distilled, and two final crystallisations for the separation of the liquid acids were made with 80 per cent. acetone to prevent formation of ethyl esters. The native name "Chaulmoogra" of *Taraktogenos Kurzii* oil has latterly been used to indicate any oil that contains chaulmoogric acid and so might be used for the treatment of leprosy. The collection of the seeds is difficult and dangerous, and usually a long time elapses before they can be expressed, so that the commercial oil is of poor quality, and is often adulterated, and has therefore fallen into some disrepute. Plantations would eliminate the difficulty, and in fact *Taraktogenos Kurzii* has been acclimatised in Brazil, whence came the oil used in this investigation. The high iodine values of certain fractions, which led previous investigators to suspect the presence of linolic or linolenic acids, has

now been found to be due to gorlic acid, which has two double bonds. If it is found that the value of the oil in the treatment of leprosy is due to the gorlic acid rather than to the other optically active fatty acids, then this, the true chaulmoogra oil, would be preferable to the other oils analysed. The keeping properties of the oil appear to be good, but, as with all the chaulmoogra oils, that from old seeds is liable to cause irritation.

	Oils of:				
	Hydnocarpus Wightiana	*Hydnocarpus anthelmintica*	*Taraktogenos Kurzii*	*Carpotroche brasiliensis*	*Oncoba echinata*
Sp.gr. at 25/25° C.	0·955	0·952	0·952	0·955	—
Free fatty acid (as oleic), per cent.	2·7	2·9	1·3	3·6	4·3
Saponification value ..	201·0	203·3	200·6	201·8	193·7
Iodine value (Hanus) ..	98·4	89·2	101·5	108·0	96·4
Spec. optical rotation $[\alpha]_D^{25}$	55·0	49·7	49·8	53·8	51·7
Refractive index, n_D^{25} ..	1·4799	1·4772	1·4790	1·4790	—
Unsaponifiable matter, per cent.	0·25	0·50	0·29	—	—
Percentage composition of acids—					
Hydnocarpic ..	48·7	67·8	34·9	45·0	none
Chaulmoogric ..	27·0	8·7	22·5	24·4	74·9
Gorlic	12·2	1·4	22·6	15·4	14·7
Lower homologues of hydnocarpic ..	3·4	0·1	0·4	?	?
Oleic	6·5	12·3	14·6	6·3	2·2
Palmitic	1·8	7·5	4·0	6·6	7·8
Loss	0·4	2·2	1·0	2·3	0·4

D. G. H.

Quantitative Estimation of Coloured Components in Paprika. L. Cholnoky. (*Z. Unters. Lebensm.*, 1939, **78**, 157–161.)—For the commercial evaluation of paprika a knowledge of the proportions of the different paprika pigments is important. Red, ripe paprika contains the following polyene colour components:—capsorubin ($C_{40}H_{60}O_4$), capsanthin ($C_{40}H_{58}O_3$), zeaxanthin ($C_{40}H_{56}O_2$), cryptoxanthine ($C_{40}H_{56}O$), β-carotene ($C_{40}H_{56}$) and a trace of α-carotene ($C_{40}H_{56}$). The polyene alcohols are present as esters of higher fatty acids. Capsicum red is a coloured wax. Capsanthin and capsorubin esters, especially the former, are responsible for the bright red colour of paprika. The percentages of the different colour components may vary widely, and hence an estimation of the total colour is not sufficient for the evaluation. The only accurate method is to estimate the separate colour components by applying the chromatographic adsorption process of Tswett (*cf.* L. Zechmeister and L. Cholnoky, *Die chromatographische Adsorptionsmethode*, Vienna, 1938). For the extraction, 0·5 to 1·5 g. of the ground sample are transferred to a funnel, provided with a cottonwool plug of 3 to 5 mm. The colour is extracted with boiling petroleum spirit (b.p. 60 to 70° C.) in successive portions of 10 ml., and the extraction is continued until the solvent remains colourless. About 100 to 150 ml. of petroleum spirit are required. The separation is made in an adsorption tube (23 × 3·5 cm.), the lower part of which is filled with calcium hydroxide and the upper half with calcium carbonate. The latter adsorbs

the capsorubin and capsanthin esters, the former the yellow components. The chromatogram shows layers due to the separate colours, which may be measured in a colorimeter. The average amounts of coloured components per kg. found in 19 samples of paprika from different crops were as follows: carotene, 0·52; cryptoxanthin, 0·16; zeaxanthin, 0·41; capsanthin, 2·19; capsorubin, 0·49; total, 3·77 g. The paprika fruit-shells are usually ground up with 40 to 70 per cent. of seeds. The above-mentioned components estimated in 18 samples as supplied by the factories, amounted on the average to 0·31, 0·10, 0·20, 1·28 and 0·27 g., respectively; total 2·16 g. per kg. The loss of colour on keeping was also determined; for pure paprika it amounted to 0·86 per cent. after 6 months and 8·56 per cent. after 12 months. For 100 kg. of paprika ground with 40 kg. of seeds the losses after 6 and 12 months were 5·45 and 12·73 per cent. of the initial colour, and when the same mixture was stirred daily, the losses after 1, 2 and 3 months were 24·54, 35·34 and 48·48 per cent., respectively. Under normal conditions the pigment-content is not affected much. Pure polyenes are very sensitive to oxygen, and as a rule are destroyed within 1 to 2 months, with decolorisation. That this is not so with paprika pigments may be explained by their enclosure in lipoids, which protect them from oxidation. Thus paprika is a good stable source of provitamin A. D. A.

Tests for Tannic Acid. D. B. Dott. (*Pharm. J.*, 1940, **144**, 137.)—The solubility of 0·5 g. of tannic acid in 10 ml. of acetone is recommended as a test for the purity of the sample, and the colour and turbidity of a solution of 0·5 g. of the acid in 10 ml. of collodion (meth.) as a test for the suitability of the specimen for hospital use. The following procedure is suggested for quantitative examination:— Cupric sulphate (1·5 g.) is dissolved in 40 ml. of water, and the solution is added to a solution of 1 g. of the tannic acid in 20 ml. of water. A solution of crystalline sodium acetate (1·7 g.) in 10 ml. of water is added, and the liquid is finally treated with 0·5 ml. of acetic acid and mixed. After 3 or 4 hours the precipitate is collected on a weighed filter, washed free from sulphate and dried at about 100° C. to constant weight. These tests were applied to 4 samples of tannic acid which had passed the B.P. tests but had proved to be unsatisfactory in hospital practice, with the following results:

Sample	Collodion solution	Solubility in acetone	Weight of copper ppt. g.
A	Very turbid	Considerable amount insoluble	1·143
B	Almost clear, slowly giving slight deposit	Nearly clear, slowly giving small quantity of brown sediment	1·179
C	Clear straw-colour, no deposit	Clear, almost colourless, faint straw colour but no deposit after 24 hours	1·163
D	Clear brownish colour, soon changing to objectionable green colour	——	—

E. M. P.

New Solanaceous Alkaloids from *Duboisia myoporoides*. W. Mitchell. (*Pharm. J.*, 1940, **144**, 137.)—"Duboisine," the total alkaloids extracted from *Duboisia myoporoides*, has previously been found to contain hyoscine and four new

alkaloids, tigloidine, valeroidine, poroidine, and isoporoidine (*J. Chem. Soc.*, 1937, p. 1820; 1938, p. 1685); there is no trace of hyoscyamine or other similar alkaloid. The four new alkaloids have now been identified. Tigloidine is tiglyl-ψ-tropëine; it has been synthesised. Valeroidine is the mono*iso*valeryl ester of a dihydroxytropeine previously isolated from Peruvian coca leaves as the dibenzoyl ester, and a structural formula has been suggested. Poroidine and *iso*poroidine have been shown to be *iso*valerylnortropëine and *d*-α-methylbutylnortropëine, respectively, and both have been synthesised. They have been isolated in the form of a mixture, known as base Z, which has been partly separated by an indirect method. *Iso*valerylnortropëine has been isolated from this mixture, and although *d*-α-methylbutylnortropëine has not been isolated, its presence is practically certain. A mixture of 10 parts of the former with 1 of the latter has been found to resemble base Z closely. E. M. P.

Rapid Method for the Determination of Nicotine. A. Verda and E. Herzfeld. (*Z. anal. Chem.*, 1939, **118**, 9–13.)—Existing methods, which are reviewed, are considered troublesome and lengthy. The following procedure, which depends on the nephelometric measurement of the turbidity produced when nicotine reacts with silicotungstic acid in presence of gum arabic, requires only 60 to 90 minutes. A mixture of the tobacco (5 to 20 g., according to the nicotine-content), 2 g. of magnesium oxide, 30 g. of sodium chloride and 300 ml. of water is steam-distilled, and 300 ml. of distillate are collected in a receiver containing 3 g. of gum arabic; this ultimately dissolves in the distillate, which is then filtered through glass wool. A standard solution is prepared by dissolving 1 g. of pure nicotine in 100 ml. of a 1 per cent. solution of gum arabic (which has been filtered through glass wool), and 10 ml. of this solution are diluted to 100 ml. with the same gum arabic solution. Volumes of this solution corresponding with 5, 2·5, 1·25, etc., down to 0·0391 mg. of nicotine are then pipetted into each of 8 nephelometer tubes, and the volume is made up to 5 ml. (where necessary) with the gum arabic solution. The reagent is prepared by dissolving 1 g. of gum arabic and 10 g. of silicotungstic acid in a mixture of 50 ml. of water and 50 ml. of glycerol; if the solution is allowed to stand overnight and is then filtered through glass wool, it keeps well. On the addition of 0·5 ml. of reagent to the solutions containing the nicotine, a milky turbidity is produced which remains unchanged after 1 day and cannot be separated in the centrifuge, and this is used to match the sample against the series of standard tubes, the sensitiveness of the method being 0·31 mg. of nicotine; it may therefore be used to recognise "nicotine-free" tobaccos having a limiting nicotine-content of 0·1 per cent. Up to 10 per cent. of pyridine or ammonia does not interfere, and the accuracy of matching is unaffected by the yellow colour of the mixture observed in transmitted light. The recorded errors for experiments in which 15 to 100 mg. of nicotine were used varied from + 5 to −7 mg.; in general, the higher the quantity of nicotine present, the greater the negative error. J. G.

Determination of Nicotinic Acid Diethylamide and Phthalic Acid Bis-diethylamide. K. A. Jackerott and F. Reimers. (*Z. anal. Chem.*, 1939, **117**, 415–420.)—The method is based upon the hydrolysis of the acid amide into its constituent acid and diethylamine, and determination of the diethylamine by

distillation into a standard acid solution. Preliminary experiments showed that nicotinic acid diethylamide (coramine) is completely hydrolysed when boiled for 30 minutes with 5 *N* sodium hydroxide solution. The method is as follows:—Nicotinic acid diethylamide (0·4 g. or a corresponding amount of its preparations) is mixed in a distillation flask with 100 ml. of water and 100 ml. of conc. sodium hydroxide solution, and after the addition of pumice the flask is connected with a Kjeldahl distillation apparatus. The receiver contains 25 ml. of 0·1 *N* hydrochloric acid, and the orifice of the condenser should dip well beneath the surface of the liquid. The flask is heated at such a rate that its contents are maintained at boiling-point for 20 minutes with scarcely any distillation. The rate of heating is then increased, and 100 ml. of liquid are distilled. Then 25 ml. of water are introduced by means of a separating funnel fitted into the stopper of the flask, and distillation is continued until the distillate measures 125 ml. The liquid in the receiver is titrated with 0·1 *N* sodium borate or sodium hydroxide solution, methyl red being used as indicator, and the result of a blank determination (obtained by distillation of a mixture of 100 ml. of water and 100 ml. of sodium hydroxide solution) is deducted. Each ml. of 0·1 *N* hydrochloric acid is equivalent to 0·01781 g. of nicotinic acid diethylamide. In recent years commercial preparations with constituents resembling nicotinic acid diethylamide in constitution and pharmacological properties have been sold. The application of the method to one of these compounds, *viz.* phthalic acid bis-diethylamide (Neospiran) was investigated. Preliminary experiments showed that hydrolysis of this compound is effected more rapidly by dilute hydrochloric acid than by alkali. The method recommended is as follows:—The sample (0·3 g.) is heated under a reflux condenser with 20 ml. of dilute hydrochloric acid for 30 minutes. When cold the product is transferred to the distillation flask with 80 ml. of water, and, after the addition of 100 ml. of conc. sodium hydroxide solution, the liberated diethylamine is distilled into 0·1 *N* hydrochloric acid in the manner previously described. The blank determination is made upon a mixture of 20 ml. of dilute hydrochloric acid, 80 ml. of water and 100 ml. of the sodium hydroxide solution. Each ml. of 0·1 *N* hydrochloric acid is equivalent to 0·01381 g. of phthalic acid bis-diethylamide. Unlike nicotinic acid diethylamide, phthalic acid bis-diethylamide is hydrolysed only slowly in alkaline solution. It is therefore necessary to ensure complete hydrolysis before the liquid is transferred to the distillation flask. The time of 30 minutes for hydrolysis may be exceeded if required. A. O. J.

Biochemical

Biochemical Behaviour of Lead. I. Influence of Calcium, Phosphorus and Vitamin D on Lead in Blood and Bone. A. E. Sobel, H. Yuska, D. D. Peters and B. Kramer. (*J. Biol. Chem.*, 1940, **132**, 239–265.)—Experiments were carried out to determine the effect of calcium, phosphorus and vitamin D on the amount of lead present in the blood of young rats and on the deposition of lead in the bones. Groups of animals were fed on a low calcium—low phosphorus diet, a low calcium—high phosphorus diet, and a high calcium—low phosphorus diet all containing the same amount of lead, while some of the rats on each of the diets

were given vitamin D in addition. A satisfactory explanation of the results observed is that lead deposition is directed by a system of its own, this being governed by the same laws as calcium deposition, but not necessarily in the same direction. Thus there is an ideal lead—phosphorus ratio in the diet at each lead level that is most favourable for lead deposition in the bones, and the addition or removal of phosphorus decreases the amount of lead deposited. The addition of any agent, such as calcium, which behaves as if it removed phosphorus has the same effect. Vitamin D increases the deposition of lead with all types of diet by compensating for a disproportionate dietary ratio. The low calcium—low phosphorus diet was found to bring about the greatest degree of lead deposition. The effect of the three dietary factors on blood lead values may be similarly explained by assuming a relationship between blood-lead and dietary lead—phosphorus ratios analogous to the relationship between blood-calcium and dietary calcium—phosphorus ratios. As the lead—phosphorus ratio is increased the blood lead value is increased, so that the addition of phosphorus depresses the blood lead, whilst the addition of calcium, which has the effect of removing phosphorus, increases it. The effect of vitamin D on blood lead is similar to its effect on blood calcium, that is, blood lead is increased on a low calcium—low phosphorus diet, but is not markedly affected on a high calcium—low phosphorus diet or on a low calcium—high phosphorus diet. F. A. R.

Molecular Weight of Egg Albumin. F. W. Bernhart. (*J. Biol. Chem.*, 1940, **132**, 189–193.)—Analyses were made of the phenylalanine, tyrosine and tryptophane contents of recrystallised, electro-dialysed egg albumin. From the results obtained the minimal molecular weight was estimated to be 18,400. When multiplied by 2 this gives a value of 36,800, which is in close agreement with the molecular weight found either by physical methods or by estimating the polysaccharide-content and assuming that one molecule is present in each molecule of the protein. On the assumption that no unknown basic amino acids or nitrogen-containing prosthetic group is present, the number of amino-acid groups in egg albumin was calculated to be 310 per molecule. This value approximates closely to the value of 288 calculated by Bergmann and Niemann. F. A. R.

Estimation of Arginine by means of Flavianic Acid. H. B. Vickery. (*J. Biol. Chem.*, 1940, **132**, 325–342.)—A sample of the protein, weighing 25 g., or more according to the anticipated arginine-content, is hydrolysed by boiling for 24 hours with 500 ml. of 20 per cent. hydrochloric acid. The hydrolysate is repeatedly evaporated to a syrup under reduced pressure and then made up to 250 ml.; 1-ml. aliquot portions are removed for nitrogen determination, from which the amount of protein present is calculated. The solution is diluted, boiled with 5 g. of Norit and filtered, and the Norit is extracted twice with boiling water. The combined filtrate and washings are concentrated to 250 ml., and 50-ml. aliquot portions are taken for each of 4 arginine estimations. For single estimations the amounts of materials taken can be correspondingly reduced. For each mole of arginine present, calculated from the best estimate available, 4 to 5 moles of flavianic acid are added to the solution (1 g. of arginine requires 1·805 g. of flavianic acid for 1 mole) at room temperature. The samples are placed in the refrigerator

for 4 days, being thoroughly stirred at least once a day. Pale yellow needles of arginine diflavianate separate, occasionally in admixture with orange-yellow monoflavianate. The precipitate from each sample is filtered off on a small sintered funnel and washed with water saturated at room temperature with arginine monoflavianate. During this operation much of the diflavianate is converted into the monoflavianate. The precipitate is then stirred with a little hot water, 5 *N* ammonium hydroxide is added drop by drop from a 1-ml. pipette, and the precipitate is stirred until all is in solution; the minimal amount of ammonia is used. The solution is drawn through the filter into a filter-flask, and the filter is washed with hot water and, if necessary, a drop of ammonia. The filtrate is transferred to a beaker, the total volume being about 40 ml., and is then heated to boiling, and a slight excess of *N* sulphuric acid is added. Arginine monoflavianate usually crystallises at once from the hot solution, and crystallisation is allowed to become complete at room temperature before the solution is stirred. It is allowed to stand overnight in the refrigerator and then filtered on to a sintered glass crucible, and the precipitate is washed several times with water saturated at room temperature with arginine monoflavianate, and finally with a little alcohol. It is dried for a few hours at 105° C., cooled in a desiccator and weighed with as little exposure to the air as possible (it is hygroscopic). The weight of arginine monoflavianate, multiplied by 0·3566, gives the weight of arginine. Very good agreement was obtained in estimations carried out in quadruplicate on pure proteins, and in only one instance was the average variation greater than ± 1 per cent. The following are the results obtained with various proteins (per cent.): edestin, 16·76; tobacco-seed globulin, 16·09; cottonseed globulin, 14·92; arachin, 13·94; amandin, 13·31; gliadin, 2·57; zein, 1·60; casein, 3·72; haemoglobin (horse), 3·59; γ-pseudoglobulin (horse), 2·66; fibrin (cattle), 7·70; gelatin, 8·68; egg albumin, 5·66; hair (human), 9·37; wool (sheep), 10·4; silk fibroin, 0·76.

F. A. R.

Naphthalene-β-Sulphonic Acid as a Reagent for Amino Acids. M. Bergmann and W. H. Stein. (*J. Biol. Chem.*, 1939, **129**, 609–618.)—Naphthalene-β-sulphonic acid forms sparingly soluble salts with leucine, phenylalanine, arginine, histidine, tryptophane and cysteine. These salts, for which the term nasylates (not to be confused with the naphthalenesulphonyl or nasyl derivatives) is proposed, promise to be of great value in the isolation, purification and determination of these amino-acids. The determination of *l*-phenylalanine is given as an example. Three 15-ml. samples of a solution containing 0·906 g. of *l*-phenylalanine in 0·5 *N* hydrochloric acid were added to 1·004 g., 1·254 g. and 1·509 g. samples of ammonium nasylate (the most stable form of the reagent), each dissolved in 60 ml. of 0·5 *N* hydrochloric acid. The mixtures were continuously agitated at exactly 0° C. for 3 days, and the precipitates were collected at 0° C. on sintered glass filters, which were weighed, then dried to constant weight in a desiccator and re-weighed. The loss in weight enabled corrections to be applied for the weight of solid in the mother liquors retained by the precipitates. The amount of *l*-phenylalanine in the original solution was calculated from the corrected dry weights of the precipitates, and the average was found to be 99·3 per cent. of the quantity actually

taken. *l*-Arginine and *l*-histidine form sparingly soluble dinasylates as well as mononasylates. Some polypeptides also form sparingly soluble nasylates. All these salts appear to be well-crystallised compounds with definite, characteristic melting-points as follows: *l*-leucine mononasylate 187·5 to 189° C., *l*-phenylalanine mononasylate 232 to 233° C., *l*-arginine dinasylate 209 to 211° C., *l*-arginine mononasylate 243° C., *l*-histidine dinasylate 265° C., *l*-histidine mononasylate 206 to 207° C., glycyl-*l*-leucine mononasylate 211 to 212° C., all with decomposition. The amino acids can be regenerated by dissolving the salts in absolute alcohol, adding pyridine, allowing the mixture to stand for 2 days at room temperature, and filtering off the precipitated amino acid. This is purified by a second treatment.

F. A. R.

Experiments on Amino-Acids. I. Partition of Acetamino-acids between Immiscible Solvents. II. Separation of Amino-acids by means of their N-Acetyl Derivatives. III. Isolation of Hydroxy-amino-acids from Protein Hydrolysates. IV. Methyl Ethers of some N-Acetylhydroxy-amino-acids. R. L. M. Synge. (*Biochem. J.*, 1939, **33**, 1913–1917, 1918–1923, 1924–1930, 1931–1934.)—I. The partition coefficients of a number of acetamino-acids between chloroform and water, ether and water, and ethyl acetate and water, were measured, and very wide variations were found, suggesting a possible basis for the separation of amino-acids. II. A mixture of 14 amino-acids, intended to simulate a gelatin hydrolysate, was acetylated at 0° C. with acetic anhydride and sodium hydroxide solution. The solution of acetylated acids was made slightly acid with sulphuric acid and extracted with chloroform in a Neuberger continuous extractor. The aqueous phase, which contained about 20 per cent. of unchanged amino-acids, was re-acetylated. After a second re-acetylation, the three chloroform extracts were examined for individual amino-acids. Amino-acids susceptible of separation by extractional fractionation were obtained in high yield, namely, leucine, phenylalanine, proline, methionine, valine, alanine and glycine. III. Serine and hydroxy-proline were acetylated separately by means of acetic anhydride and alkali, as already described. The resulting alkaline solutions were allowed to stand for some time to ensure hydrolysis of any O-acetyl groups, and were then neutralised and treated with benzoyl chloride at 0° C. in the absence of excess alkali. Good yields of N-acetyl-O-benzoyl-amino acids were obtained, and these were readily extractable from aqueous solution into chloroform; on treatment with dilute aqueous alkali at room temperature they were rapidly converted into N-acetyl derivatives. A protein hydrolysate, after being freed from bases by precipitation with phosphotungstic acid, was acetylated and, after being kept alkaline overnight, was benzoylated as described above. The mixture, containing acetamino-acids, N-acetyl-O-benzoyl-hydroxy-amino-acids and benzamino-acids, was extracted with chloroform in a Neuberger continuous extractor. The chloroform was removed from the extract, and the residue dissolved in dilute alkali solution at room temperature. This saponified the O-benzoyl group only, and on acidifying the solution and exhaustively extracting with chloroform, a solution containing N-acetyl-hydroxy-amino-acids was obtained. The free amino-acids were prepared by acid hydrolysis. A preliminary fractionation of hydrolysates of

fibrin, wool and gelatin was attempted by the method. IV. The individual components of the hydroxy-amino-acid fraction were not readily separable from one another by direct crystallisation. Accordingly, a number of N-acetyl-hydroxy-amino-acids were methylated by means of silver oxide and methyl iodide, and the methyl esters of the N-acetyl-O-methyl-hydroxy-amino acids were isolated by distillation and hydrolysed to the free acid. The partition coefficients between chloroform and water of the compounds derived from *dl*-serine, *dl*-*allo*threonine *l*-hydroxyproline and *l*-tyrosine were sufficiently different to suggest that this procedure provided a basis for fractionating a mixture of these amino-acids, but the separation has not yet been attempted. F. A. R.

Colorimetric Estimation of Quinine in Biological Fluids and Tissues. R. O. Prudhomme. (*J. Pharm. Chim.*, 1940, **1**, 8–17.)—When a few drops of a 2 per cent. solution of eosin are added to a 1 per cent. solution of a quinine salt a red precipitate is formed which, when shaken with chloroform, dissolves to form an intensely red solution. The colour is perceptible when the amount of quinine present is only 1/1000 mg. in 10 ml. of water. The reaction is given by other alkaloids, *e.g.* cinchonine, ephedrine, eserine, pilocarpine and atropine, but not by caffeine. The colour attains its maximum intensity at *p*H 6·5 to 7·5, and this condition may be secured by the use of a buffer solution made by mixing 13·617 g. of monopotassium phosphate dissolved in 300 ml. of water with 41·566 g. of disodium phosphate dissolved in 700 ml. of water. Standards for the colorimetric estimation of quinine in urine are prepared by adding amounts of quinine sulphate ranging from 0·01 to 2 mg. to 10-ml. portions of normal urine. Each portion is treated with 5 ml. of a 10 per cent. lead acetate solution and filtered. The filtrate (12 ml.) is treated with 5 drops of conc. sulphuric acid and filtered repeatedly until clear, and 9 ml. are neutralised to the lilac end-point of litmus solution with sodium hydroxide solution. The mixture is treated with 2 ml. of the phosphate buffer solution and 4 drops of a 2 per cent. eosin solution and is finally shaken thoroughly with 3 ml. of chloroform. After 4 hours the chloroform layer is transferred by means of a pipette to a tightly stoppered tube. Sealed tubes may be used, but care should be taken that the portion of the tube to be heated is not contaminated with chloroform, the decomposition products of which affect the colour of the solution. The urine to be investigated is treated in the same manner. Comparison of sample with standard should not be made until the chloroform layer has been separated and transferred to a clean tube since the inclusion of the supernatant liquid between the chloroform layer and the walls of the tube may lead to erroneous results. For the determination of quinine in blood and serum, the standards are prepared as follows:—The coagulating power of the blood is destroyed by addition of powdered potassium oxalate, and amounts of quinine sulphate ranging from 0·005 to 0·1 mg. are added to 10-ml. portions. Each portion is treated with 20 g. of crystalline sodium sulphate and 7 ml. of *N* sulphuric acid, and the mixture is heated in a water-bath at 45° to 50° C. for 30 minutes with occasional shaking. The brown solution is filtered, and the filtrate is cooled until the sodium sulphate crystallises. The supernatant liquid (10 ml.) is neutralised with sodium hydroxide solution, phenolsulphonephthalein being used as external

indicator, and is treated with 2 ml. of the buffer solution, 6 drops of eosin solution and 3 ml. of chloroform. After being vigorously shaken the mixture is allowed to stand for 6 hours, and the chloroform layer is transferred to a stoppered tube. The sample of blood to be tested is treated with potassium oxalate and subjected to the same process. For organs or tissue the procedure is as follows: —The material (10 g.) is finely ground in a mortar with 30 g. of crystalline sodium sulphate and, if necessary, a little sand. The paste so obtained is rinsed into a flask with 10 ml. of water, 10 ml. of *N* sulphuric acid are added, and the mixture is heated for 30 minutes at 45° to 50° C. The method is then as described for blood. To obtain standards different amounts of quinine sulphate are added to similar material in the mortar. It is important to notice that with normal fresh organs a blank estimation may give results corresponding with 0·01 mg. of quinine. It is not possible therefore to determine less than this amount, and when putrefaction has set in the colour given in a blank estimation may be intense. Although not specific, the reaction is useful for the study of the distribution of quinine and other alkaloids in the organs and their rate of excretion. The reaction is not given by aspirin, the sulphonamides, veronal and gardenal, nor by excretory substances such as urea and the amino acids. The red compound was isolated and was found to have no bitter taste and to be devoid of the therapeutic properties of quinine for malaria. A study of several of the eosin-alkaloid compounds showed that they can be distinguished by their ultra-violet absorption spectra—a method that may prove useful in toxicological practice. A. O. J.

Photometric Estimation of Silicic Acid in Biological Substances. J. Bodnár and T. Török. (*Z. physiol. Chem.*, 1939, **261**, 257–268.)—The chief difficulty in the colorimetric estimation of silica as silicomolybdic acid is the interference caused by the presence of iron and phosphate. In the method described this is overcome by converting the iron into a water-soluble complex and precipitating the phosphate with magnesia mixture. From 0·05 to 0·25 g. of the powdered tissue is weighed into a small platinum crucible and mixed with 0·25 g. of fusion mixture (potassium and sodium carbonates). The crucible is heated over a Teclu burner, gently at first, but with gradual increase of the temperature as the organic matter becomes charred, and is finally ignited for 40 minutes (not less) with the full heat of the burner. The fused mass is stirred at intervals with a platinum wire. After cooling, the melt is dissolved in water, 1 to 2 drops of methyl orange solution are added, and the solution is made slightly acid by neutralising with 3 per cent. hydrochloric acid and adding 0·5 ml. in excess. The solution is transferred to a 15-ml. graduated flask and treated in turn with 0·5 ml. of 6·5 per cent. potassium cyanide solution and 1·5 ml. of acid magnesia mixture (42 g. of ammonium chloride, 22 g. of magnesium chloride, 1 g. of citric acid and 1·3 g. of ammonium dihydrogen phosphate in 200 ml. of 3 per cent. hydrochloric acid). Three ml. of 8 per cent. ammonia solution (prepared from ammonium chloride, the aqueous vapours being condensed and collected in an apparatus protected by a film of paraffin) are added dropwise to the solution, which is constantly shaken. The solution is then diluted to the mark, transferred to a centrifuge tube, and centrifuged for 5 to 10 minutes. Ten ml. of the supernatant liquid are transferred

to a 25-ml. graduated flask with the aid of a pipette and treated with 1·5 ml. of ammonium molybdate solution (10 g. of the salt are dissolved in 200 ml. of 5 per cent. sulphuric acid, and the solution is filtered after standing for 24 hours) and 1·5 ml. of hydroquinone solution (4 g. of hydroquinone in 200 ml. of water containing 4 drops of conc. sulphuric acid) and thoroughly shaken. After standing for 5 minutes the blue solution is diluted to the mark with sodium sulphite solution (made by dissolving 30 g. of anhydrous sodium sulphite in 200 ml. of water and 160 g. of anhydrous sodium carbonate in 800 ml. of water, and mixing the two filtered solutions) and again shaken. The solution is transferred to the cell of the photometer and exposed to the light of the photometer for 10 minutes, and the reading is taken, a red filter being used. The extinction value of a solution prepared in the same way from the reagents only is measured and subtracted from that of the test solution. With pure silica solutions the error was never in excess of 2 per cent., whilst in presence of different amounts of iron and phosphorus the greatest error found was + 6·7 per cent. In estimations of the silica-content of organic materials, duplicate experiments agreed usually within ± 2 per cent., whilst the error in the recovery of added silica never exceeded ± 5 per cent.

F. A. R.

Fatty Acid Dehydrogenase and its Co-enzyme. O. St. A. K. Lang and H. Mayer. (*Z. physiol. Chem.*, 1939, **261**, 249–252.)—Extracts of liver and muscle-tissue brought about dehydrogenation of saturated fatty acids when incubated with the potassium salts of those acids in presence of methylene blue and a co-enzyme preparation. The product obtained from palmitic acid was a hexadecenoic acid, presumed to be the $\alpha\beta$-unsaturated acid, though the position of the double bond is not certain. The dehydrogenation of oleic acid proceeded more slowly, and of linolic acid more slowly still. The co-dehydrogenase was shown to be identical with muscle adenylic acid. F. A. R.

On the Fatty Acid Dehydrogenase IV. Product of the Dehydrogenation of Stearic Acid. O. St. A. K. Lang and F. Adickes. (*Z. physiol. Chem.*, 1939, **262**, 123–127.)—In an earlier paper it was stated that stearic acid is dehydrogenated by the enzyme to the corresponding $\alpha\beta$-unsaturated acid. This is not correct, the product being oleic acid, as was shown by the isolation (as the 2 : 4-dinitrophenylhydrazone) of azelaic aldehydic acid from the product of ozonolysis. F. A. R.

Estimation of Vitamin C by means of the Zeiss Step-Photometer. F. Bukatsch. (*Z. physiol. Chem.*, 1939, **262**, 20–28.)—The usual method of estimating ascorbic acid by titration with 2 : 6-dichlorophenolindophenol in strongly acid solution suffers from three disadvantages. First, the end-point may not be very sharp, especially with biological fluids such as tissue-extracts and press-juices. Secondly, unless the titration is carried out very rapidly, large errors are introduced because of fading of the indicator. Thirdly, the method is not applicable to certain extracts containing plant-pigments. All these disadvantages are overcome in the method now proposed, and good results are recorded. About 3 g. of the fresh material, *e.g.* leaves, are minced and ground in a mortar with sand

and 3 ml. of 20 per cent. metaphosphoric acid. The resulting paste is diluted to 30 ml. with air-free water and filtered. An aliquot portion of the filtrate is transferred to a test-tube and 5 ml. of *pure* nitrobenzene are added. A quantity of 2 : 6-dichlorophenol indophenol solution equivalent to 0·5 mg. of ascorbic acid is added from a pipette, and the tube is gently inverted. The colour of the dye slowly fades and may disappear, in which event a further quantity of indicator solution should be added. When no further fading occurs the tube is vigorously shaken to transfer the remainder of the indicator to the nitrobenzene phase. Xylene may be used instead of nitrobenzene and the indicator must then be introduced below the xylene layer by means of a long pipette. The colour of the dye in either solvent remains practically unchanged for at least ten minutes, and the extinction value of the solution is measured in the 0·5-cm. cell of a Zeiss step-photometer, filter S53 being used with nitrobenzene and S50 with xylene. By using solutions of pure ascorbic acid the relationship between the extinction value of the indicator solutions and the concentration of ascorbic acid was shown to be linear in both instances. From the resulting graph it is possible to calculate the ascorbic acid content of the solution under examination. Even bright red extracts, such as are obtained from red cabbage leaves or beetroot, give satisfactory results by this method, for the red pigments are not extracted by either nitrobenzene or xylene. F. A. R.

The Red Oxidation Products of the Tocopherols. W. John and W. Emte. (*Z. physiol. Chem.*, 1939, **261**, 24–34.)—Furter and Meyer (*cf.* ANALYST, 1939, **64**, 217) made use of the intense red colour produced by heating tocopherols with nitric acid in their method of assaying vitamin E preparations. It has now been found that α- (or β-) tocopheryl quinone, which is the first oxidation-product, is further oxidised to a red substance, which is itself converted into another compound by prolonged treatment. Thus, in Furter and Meyer's method, not only are the biologically active tocopherols converted into the red substance, but also the inactive tocopheryl quinones. This observation would account for the fact that Emmerie and Engel (*cf.* ANALYST, 1939, **64**, 446) obtained higher values by the chemical method of assay than by the biological method. F. A. R.

Fluorescence Analysis of Human Urine. W. Koschara, S. von der Seipen and P. A. Aldred. (*Z. physiol. Chem.*, 1939, **262**, 158–167.)—Urine itself does not fluoresce in daylight, but if it is stirred with active charcoal, pigments are adsorbed from urine; by eluting the charcoal with hot 0·02 *N* sodium hydroxide solution, an eluate is obtained that exhibits a blue fluorescence. Uropterin, the chief fluorescent pigment of urine, is quantitatively recovered from a solution by such treatment, but the fluorescence of the eluate from urine is not due entirely to uropterin, solutions of which have a blue-green fluorescence. Uropterin is selectively adsorbed from 0·1 *N* hydrochloric acid solution on to bleaching-earth, from which it is eluted by aqueous pyridine, and by applying this technique to the charcoal eluates from a large number of specimens of urine it was shown that, in general, one-third to one-half of the fluorescence is due to uropterin. The charcoal adsorption and elution method was applied to a large number of

urines voided by patients suffering from many different diseases, and an attempt was made to assess the clinical value of the uropterin estimation. The results are regarded as confirmation of the authors' hypothesis, that the uropterin content of urine is a measure of oxidative nitrogen metabolism. F. A. R.

Bacteriological

Silicic Acid Nutrient Media. O. Hettche. (*Zent. f. Bakt.* I. Abt. Orig., **1939, 5**, 144; *Bull. Hyg.*, **1940, 15**, 48.)—The author has previously reported on the value of silica jelly for bacterial cultivation. He here describes a simplification of this medium, water-glass being used as a substitute for agar or gelatin. By using 0·05 per cent. sodium sulphite and Cenovis (a yeast and vegetable extract), peptone can be dispensed with, and it is claimed that the size of the colonies is half as large again as on ordinary nutrient agar. The latest development is the use of 0·5 per cent. of peptone and 0·1 to 0·2 per cent. of Cenovis with 7 per cent. of water glass. Growth on this medium compares favourably with that on lactose agar. When sodium sulphite is used and the *p*H is adjusted to 7·2 the medium sets in about 6 minutes at 20° C. D. R. W.

Toxicological

Extraction of Alkaloids with Acetone. P. Cheramy and M. Papavassilou. (*J. Pharm. Chim.*, **1940, 30**, 316–321.)—A method based on that of Cheramy and Lobo for the extraction of barbiturates (*J. Pharm. Chim.*, 1934, **20**, 400–403; Abst., ANALYST, 1935, **60**, 50) is satisfactory for the extraction of alkaloids from viscera. The pulped organs are heated for two hours on the water-bath with 3 volumes of acetone in presence of tartaric acid, the solvent is removed (without heat), and the extraction is repeated. The residue is washed with acetone, and the combined acetone extracts are cooled to 0° C., filtered and distilled, the distillation being begun at normal pressure and finished under reduced pressure. The syrupy residue is dissolved in 300 ml. of warm anhydrous acetone, re-cooled, filtered through a filter moistened with acetone, and washed with the solvent. After distillation of the acetone, the soft residue is dissolved in 100 to 200 ml. of 20 per cent. ammonium sulphate solution, filtered cold and exhausted with various volatile solvents in acid and then in alkaline solution in the usual way. No appreciable loss of alkaloid occurs through the precipitation of impurities with ammonium sulphate solution. For some alkaloids (*e.g.* quinine and strychnine) which give too low results by this and by the Stas-Otto method, results of test determinations *in vitro* and *in vivo* have been greatly improved through the replacement of the first extraction on the water-bath by a Soxhlet extraction with acetone acidified with acetic acid; *e.g.* strychnine in egg, from 57 to 80 per cent. of theory, quinine in beef muscle, from 83 to 92 per cent. of theory. Other results of test analyses are given. E. B. D.

Gas Analysis

Separation of *Para*-Hydrogen from Oxygen and Carbon Monoxide. E. Bergmann, L. Farkas and L. Sandler. (*J. Amer. Chem. Soc.*, 1940, **62**, 445.)—Since *para*-hydrogen is often used as an aid to the elucidation of reaction mechanisms, it is necessary to determine the *para-ortho* ratio of the hydrogen, and therefore, to separate the hydrogen from all other gases concerned in the reaction in such a way that the *para-ortho* ratio remains unchanged. Most of the common gases can be separated by liquefaction with the aid of liquid air, but liquid hydrogen is necessary for carbon monoxide or oxygen, and, as it is not always available and is expensive to prepare, the following alternative methods are proposed:—Oxygen may be absorbed quantitatively in a saturated solution of triphenylmethyl sodium in dibutyl ether containing an excess of the solute; dibutyl ether is preferable to the diethyl ether recommended by Schlenk and Marcus (*Ber.*, 1914, **47**, 1664), as it has a lower vapour-pressure and will not dissolve the grease from stopcocks. The solution, prepared in the usual way (*loc. cit.*) and filtered, is then evaporated in a stream of nitrogen, dibutyl ether being added gradually during the process. The resulting solution is stable for many months if stored *in vacuo*, and if it is cooled its rate of absorption is very high. In one experiment a closed system of 5-ml. capacity was connected through a narrow capillary tube with a wash-bottle containing 20 ml. of the solution; when 130 cb. mm. of oxygen (at S.T.P.) had passed through the capillary tube, the residual oxygen was no longer measurable (*i.e.* it was less than 0·005 per cent.). The alteration in the *para-ortho* ratio of the hydrogen during the determinations was negligible, and was probably due to the presence of small quantities of free (paramagnetic) triphenylmethyl. The usual ammoniacal cuprous oxide solution is recommended for the absorption of carbon monoxide, and when under the conditions described above 500 cb. mm. (S.T.P.) of the gas (actual pressure, 70 mm.) had passed through the tube, less than 0·005 per cent. remained unabsorbed. Here, again, a negligible change in the *para-ortho* ratio was observed, and this is attributed mainly to divalent (*para*-magnetic) copper compounds. These methods may also be useful for other hydrogen modifications, *e.g.* mixtures of *ortho*-hydrogen and deuterium (*cf. J. Amer. Chem. Soc.*, 1939, **61**, 3393). J. G.

Agricultural

New Derivatives of Constituents of Derris Root. Th. M. Meyer and D. R. Koolhaas. (*Rev. Trav. Chim. Pays-Bas*, 1939, **58**, 1119–1123.)—(A) By the action of alcoholic potash on derride, 4-hydroxycumarone-5-carboxylic acid was formed (*cf.* Manjunath, Seetharamiah and Siddappa, *Ber.*, 1939, **72**, 93). (B) Oxidation of an alkaline solution of derridenone with hydrogen peroxide, by the Späth and Pesta method (*Ber.*, 1934, **67**, 853), yielded furan-2-3-dicarboxylic acid. (C) Rissic acid and (D) 2-hydroxy-4,5-dimethoxybenzoic acid were among the products of oxidation of dehydroderride with potassium permanganate in acetone. Full experimental details are given. E. B. D.

Water

Determination of Iodide in Mineral Waters containing Bromide and large quantities of Chloride. E. Müller and W. Stumpf. (*Z. anal. Chem.*, 1939, **118**, 90–93.)—Determination of iodine in mineral waters containing much chloride (1:100,000) by the usual method (oxidation with sodium nitrite and hydrochloric acid) gives inconsistent results. After the oxidation the carbon disulphide is not always violet, but, according to the conditions, more or less brown, so that the results are too low. It was found that oxidation with very small quantities of nitrite and of hydrochloric acid yielded comparative figures. Although all the iodine is not extracted, a factor can be used for calculating the true iodine-content. It is essential to keep the carbon disulphide constantly covered with water during the washings. The procedure is as follows:—(1) Approximate determination of the iodine-content without using a factor. (2) Preparation of a type-solution, containing the same proportion of salts as the water to be analysed and with the same iodine-content as found; from the results obtained in the iodine determination the factor to be applied can be calculated. (3) Accurate determination of the iodine-content, applying the factor found. Iron, manganese or bromine do not interfere; hydrogen peroxide oxidises a small amount of bromide very slowly after long shaking. D. A.

Organic

Preparation of Grignard Reagents from Magnesium Amalgams. E. G. Rochow. (*J. Amer. Chem. Soc.*, 1939, **61**, 3591.)—The phase diagram for the system Mg-Hg shows two compounds, $MgHg_2$ and MgHg. Below 168° C. the equilibrium condition at the low magnesium end is a mixture of $MgHg_2$ crystals and liquid, so that the reported solubility of magnesium in mercury (3 per cent. at 250° C., 1 per cent. at 100° C. and probably 0·1 per cent. at room temperature) must refer to the solubility of $MgHg_2$. Amalgams of 5·71 per cent. or greater magnesium concentration will be solids; those with 5 to 0·1 per cent. of magnesium will normally be mixtures of $MgHg_2$ with increasing proportions of liquid. Amalgams containing from 0·1 to 1·0 per cent. of magnesium were prepared in an all-glass apparatus under an atmosphere of purified nitrogen, and after cooling in nitrogen, 50 ml. of 0·1 *N* solution of methyl magnesium chloride were added through the condenser to eliminate difficulties in starting, and methyl bromide was admitted. After refluxing for some hours samples were withdrawn for determination of total CH_3MgX by evolution of methane with water, and the increase of CH_3MgX over that added at first was calculated as percentage yield based on the magnesium. Typical examples were:—Percentage of magnesium in amalgams, 0·1, 0·5 and 1·0 gave respectively 0, 4·1 and 25·3 yield of $RMgX$ per cent. The yield of $RMgX$ from magnesium amalgams increases with concentration of magnesium in the amalgam with increasing possibility of free magnesium in the amalgam mixture, and it is concluded that $MgHg_2$ does not participitate in the Grignard reaction as readily as magnesium, and that mercury has therefore an inhibiting effect.

D. G. H.

Gradual Decomposition by Oxidation of Fatty Acids into their next lower Homologues. H. Mendel and J. Coops. (*Rec. Trav. Chim. Pays-Bas.*, 1940, **58**, 1133–1143.)—A new method for the gradual decomposition of fatty acids into their next lower homologues has been tested on palmitic and stearic acids. The steps of the decomposition are:—Bromination of the original acid in the α-position, conversion into α-hydroxy fatty acid, oxidation of the acid to aldehyde with lead tetra-acetate, and subsequent immediate oxidation of the aldehyde to the required lower fatty acid with air and excess of lead tetra-acetate. Nearly quantitative yields (*i.e.* up to 96 per cent. of the theoretical) of the hydroxy fatty acids were obtained. The yields of lower fatty acids were 88 to 89 per cent. of the theoretical calculated on the hydroxy fatty acid, or approximately 84 per cent. on the original fatty acid. The original describes the decomposition in detail and discusses results obtained. E. B. D.

Reactions of Nessler's Reagent with Aqueous Solutions of Mustard Gas and Lewisite. J. Delga. (*J. Pharm. Chim.*, 1940, **1**, 5–8.)—It has been observed that Nessler's reagent when added to water contaminated with dichlorodiethyl sulphide or with the mixture of chlorovinyl chloroarsines and trichlorovinyl arsine gives reactions likely to cause error in the normal use of the reagent or, conversely, reactions available for the detection of these toxic substances in water. With mustard gas the reagent gives a white precipitate that becomes yellowish if the concentration of mustard gas is sufficiently high. A study of the composition of this compound is in progress. The reaction is given with as little as 0·078 g. of mustard gas per litre. Thiodiglycol (formed by hydrolysis of mustard gas) gives no precipitate with Nessler's reagent and only a faint yellow colour when its concentration exceeds 6 g. per litre; it is therefore not responsible for the reaction. With Lewisite the reaction varies with the concentration and the time of reaction. The relation between the reaction and the concentration (expressed as g. of arsenic per litre) may be summarised as follows:—2·93 to 1·47, a white precipitate becoming grey in 2 minutes; 0·293, a chestnut-coloured precipitate becoming grey; 0·147 to 0·0293, an orange-yellow to yellow colour becoming chestnut and followed by a grey precipitate; 0·0097, a clear pale yellow colour changing to rose in 3 minutes; 0·0029, a faint greenish-yellow colour becoming more intense. The reaction is still perceptible at a concentration of 0·001 g. of arsenic per litre. The arsenic was determined by the method of Fleury (*J. Pharm. Chim.*, 1920, **21**, 385; Abst., ANALYST, 1920, **45**, 389) after ignition. The solutions were prepared from the commercial liquid and were filtered and diluted until free from undissolved globules. The best results are obtained by adding 2 ml. of Nessler's reagent, drop by drop, with constant shaking, to 10 ml. of the solution. A. O. J.

Precipitation of Natural Tannins with Calcium Hydroxide. V. Nemec. (*J. Inst. Leather Trades Chem.*, 1940, **24**, 5–8.)—Calcium hydroxide in the form of a fine dry powder is added in excess (2 g. per 50 ml.) to the analytical tannin solution, and the mixture is boiled for 5 minutes beneath a reflux condenser, then cooled and filtered, and 25 ml. of the filtrate are evaporated to dryness in a quartz or platinum basin. The residue is weighed and then ignited in an electric oven at a

moderate temperature until a white ash is obtained. Errors due to partial conversion of calcium oxide into carbonate by atmospheric carbon dioxide are eliminated by adding a few drops of dilute ammonium carbonate solution to the cooled ash, which is then dried for one hour at 170° C. before weighing. In examining the portion not precipitated by calcium hydroxide the colours of the filtrates and dry residues are noted (*e.g.* valonea, colourless; sumach, yellow; quebracho, brown). The following amounts of tannin were thus determined:—*Hydrolysable tannins.*—Valonea, 68·9; myrobalans, 65·2; sumach, 25·4; oak wood (mean of 5 determinations), 66·8; chestnut wood (mean of 5), 70·3. *Condensed tannins.*—Ordinary quebracho, 69·5; sulphited quebracho, 73·3; mangrove, 65·6; mimosa, 69·3; pine bark, 65·0. With the hydrolysable tannins the portion not precipitated by calcium hydroxide is always smaller than the quantity of non-tans, as estimated by the hide-powder method; probably part of the sugar is precipitated. With the condensed tannins, however, sugar is not precipitated with the tannin.

D. G. H.

Absorption of Substantive Dyes by Oxy-Celluloses of the Acidic Type. S. M. Neale and W. A. Stringfellow. (*J. Soc. Dyers and Col.*, 1940, **56**, 17–18.)—Cotton that has been oxidised by alkaline oxidising agents exhibits weakly acidic properties, presumably due to carboxyl groups. It shows an enhanced affinity for basic dyes, *e.g.* methylene blue, and a much reduced power of absorbing substantive dyes. In the experiments described a good grade of bleached Egyptian cotton cloth was used, the oxidising agent being a 0·1 *N* solution of potassium hypobromite in free alkali; dyeing was carried out at 90° C. and *p*H 6·20, with solutions containing 0·05 g. of dye and 5·0 g. of sodium chloride per litre. It was found that the amount of Sky Blue-FF absorbed fell off rapidly as the carboxyl value (determined by the authors' method, *Trans. Faraday Soc.*, 1937, **33**, 881) increased, until a constant value was reached. The theory underlying these findings is discussed in detail.

J. G.

Precipitation Reactions of Organic Arsenic Compounds. M. Péronnet and R. H. Rémy. (*J. Pharm. Chim.*, 1939, **30**, 353–364.)—About 25 organic arsenic compounds were tested. *Reaction with hydrogen sulphide.*—To the solution of the compound in aqueous alcohol (10 per cent. alcohol) was added 1 ml. of hydrogen sulphide water. A yellow precipitate or turbidity formed immediately with the majority of the compounds. The precipitate obtained with phenyldichloroarsine and methyl phenylarsonate was identified as phenylarsine sulphide and that with β-chlorovinyldichloroarsine as chlorovinylarsine sulphide. It is noteworthy that arsinic and arsonic acids gave no precipitate. The reaction obtained in aqueous alcoholic solution did not always occur when absolute alcohol was used as solvent. Hydrogen sulphide in acetone solution gave similar results to hydrogen sulphide water. *Reaction with mercury reagent.*—The reagent contained 2 ml. of nitric acid (sp.gr. 1·42) and 10 g. of mercuric nitrate in 100 ml. of water. A white or yellowish precipitate of unknown composition was obtained with most organic arsenic compounds in absolute alcoholic solution. Exceptions were compounds in which several aliphatic or aromatic groups were linked to arsenic in open chain.

S. G. C.

Inorganic

Reaction of Copper with Benzidine. G. Spacu and C. G. Macarovici. (Abstr., *J. Pharm. Belgique*, 1939, **21**, 1005; *cf. Chem. Abstr.*, 1939, **33**, 6194[4].) —The effect of anions on the reaction is dealt with. In presence of iodide, a complex cupric benzidine iodide is formed yielding a blue colour at a dilution of 1 in 2,000,000. In absence of copper, benzidine and iodide give an evanescent blue colour at a dilution of 1 in 1,000,000. In presence of halogens the sensitiveness of the copper-benzidine reaction decreases in the order iodide, bromide, chloride. Fluoride and cyanide prevent the reaction. S. G. C.

Tests for Cadmium and Magnesium. E. Eegriwe. (*Z. anal. Chem.*, 1939, **118**, 98–100.)—*Cadmium.*—When a cadmium salt solution is treated with sodium carbonate solution and a certain quantity of a solution of diaminoazobenzene in acetone, an orange-red turbidity or precipitate is formed, which, when shaken with chloroform, is extracted and forms an orange-red to red solution. Most of the other cations give only a yellow turbidity or precipitate, colouring the chloroform slightly yellow. This test, which is very sensitive, is carried out as follows:—Add to one drop of the neutral solution to be tested (free from copper and ammonium) 0·5 ml. of sodium carbonate solution (15 g. of $Na_2CO_3.2H_2O$ in water made up to 100 ml.), 0·25 ml. of the reagent solution (0·5 g. of diaminoazobenzene in 100 ml. of acetone), and a few drops of chloroform and shake. In presence of 0·2γ of cadmium the chloroform becomes orange-red. With copper the reagent gives a greenish colour, extracted by chloroform; silver causes a brownish-yellow turbidity in the chloroform layer. Both cations interfere with the reaction, as do also coloured precipitates formed by sodium carbonate or large proportions of cations giving colourless precipitates; also ammonium salts. Reactions similar to that with cadmium are given by cobaltous salts and by nickel. Whereas, however, the cobalt colour disappears on shaking, whilst the red colour of the chloroform changes to brown, the nickel colour remains unchanged. Thus cadmium can be detected in presence of cobalt; also, nickel in presence of cobalt. For the detection of cadmium in presence of small amounts of nickel, solid dimethylglyoxime is added to remove the nickel from the chloroform layer, and after addition of the reagent an orange-red colour then indicates the presence of cadmium. By this method 3γ of cadmium can be detected in presence of ten times the amount of nickel. The limit for the identification of nickel is 0·05γ of nickel in 1 drop of the solution; 3γ of cadmium can be detected in presence of 500 times the amount of lead, manganese(ous) and zinc, but not of mercury(ous), bismuth, aluminium, calcium or magnesium; in presence of only 100 times the amount of these cations, however, the detection of cadmium is possible.

Magnesium.—On adding to 1 drop of pure water in a test-tube a few drops of conc. ammonia, then a small amount of solid *p*-aminophenol hydrochloride, shaking a few times and allowing the tube to stand, the liquid is gradually coloured by oxidation from faint yellow, through yellow-brown, to brownish. In presence of magnesium the oxidation product is adsorbed by the magnesium hydroxide, and instead of the yellow and brown colour a light to deep blue colour appears.

This reaction has not been observed with any other cation. In applying the test 1 drop of the neutral solution of the fifth group, free from ammonium salts, is used. Cations forming precipitates with ammonia, or anions giving less soluble magnesium components (phosphate or arsenate ions), interfere with the test; also sulphites, which prevent the necessary oxidation of the reagent. The blue colour, or the formation of blue flakes, is still visible with 5γ of magnesium in 1 drop (0·05 ml.) of solution. With smaller amounts of magnesium, or in presence of cations that interfere with the reaction (*e.g.* cadmium and zinc), no blue colour appears, but the solution shows an initial violet colour when the normal amount of reagent is used. Excess of the reagent gives a violet colour also with magnesium; the violet colour may be obtained with 1γ of magnesium. The characteristic blue colour may also be used as a macro-reaction. D. A.

New Method for the Volumetric Determination of Bismuth. L. Malaprade. (*Ann. Chim. anal.*, 1940, **22**, 5–8.)—The method involves (*a*) neutralisation of free acid in presence of an excess of sodium thiosulphate, yielding a complex sodium bismuthi-thiosulphate which is stable at *p*H 4; (*b*) titration of the complex bismuth salt with alkali according to the reaction

$$2Bi(S_2O_3)_3Na_3+5KOH+KNO_3\rightarrow BiONO_3.BiO_2H+3K_2S_2O_3+3Na_2S_2O_3+2H_2O.$$

(*a*) To the solution in dilute nitric acid (100 ml.) containing 0·17 to 0·5 g. of bismuth, 4 drops of methyl red indicator solution (2 per cent. in alcohol) are added, and potassium hydroxide solution is introduced until the indicator changes to yellow and a precipitate of basic bismuth salt is produced; 10 g. of sodium thiosulphate are dissolved in the solution. Dilute nitric acid is added, drop by drop, until the precipitate re-dissolves, and the solution is then neutralised with a few drops of potassium hydroxide solution, as necessary, yielding a pale orange colour (faint red colour of the methyl red superposed on the yellow colour of the bismuth complex). (*b*) Ten drops of phenolphthalein indicator (2 per cent. in alcohol) are added, and the solution is titrated with 0·2 *N* potassium hydroxide solution. The colour change at the end-point is from yellow to a neutral tint produced by the superposition of the yellow colour of the liquid on the faint pink of the phenolphthalein. It was established that, at the end-point, 1 ml. of 0·2 *N* potassium hydroxide solution is equivalent to 16·72 mg. of bismuth. The solution titrated should be free from chloride and sulphate, which interfere with the reaction in (*b*). The alkali solutions used should be as free as possible from carbonate. Bismuth may be determined after separation as sulphide. The sulphide precipitate is dissolved in conc. nitric acid, the liquid is diluted, the sulphur is filtered off, and a slight excess of barium nitrate is added to precipitate sulphate formed by oxidation of the sulphide. The barium nitrate and sulphate present are without effect on the subsequent determination of bismuth, which is carried out as described above. S. G. C.

Colorimetric Determination of Ferric Iron by means of Gallic Acid. Y. Volmar and A. Wagner. (*J. Pharm. Chim.*, 1939, **30**, 364–369.)—The method is applicable to 1 to 6 mg. of ferric iron. The solution in dilute hydrochloric acid is evaporated to remove excess acid. The residue is dissolved in 5 ml. of water,

3 ml. of gallic acid solution (2 per cent.) are added, and the solution is diluted to 15 ml. with saturated sodium acetate solution. The bluish-violet colour produced is compared colorimetrically in a Duboscq colorimeter with a similarly prepared standard containing 2 mg. of ferric iron. The method may be applied to the determination of iron in blood: 3 to 5 g. of blood are rendered alkaline with ammonia and evaporated to dryness. The residue is mixed with 5 to 15 g. of ammonium nitrate, and the mixture is heated to destroy organic matter and finally ashed. The ash is dissolved in 2 to 3 ml. of conc. hydrochloric acid with the addition of a little hydrogen peroxide, the liquid is evaporated to remove excess acid, and the iron is determined as described above. S. G. C.

Determination of Chlorides, Thiocyanates and Cyanides in Presence of each other, and a Qualitative Reaction for the Detection of Chlorides in Potassium Thiocyanate. A. Slooff and D. Van Duyn. (*Chem. Weekblad*, 1940, **37**, 69–72.)—In Treadwell's method (*Lehrb. anal. Chem.*, 1927, II, 621) the chlorides, thiocyanates and cyanides are titrated together by Volhard's method and the cyanides only by Liebig's method, the thiocyanates plus cyanides being determined by boiling a mixture of the three silver salts with nitric acid, and titrating the soluble portion with a standard ammonium thiocyanate solution. This assumes, although unjustifiably, that no silver chloride dissolves; even if this error is minimised by the use of less concentrated nitric acid, the decomposition of the silver cyanide and thiocyanate is slow and incomplete. In the present method the Volhard and Liebig titrations are used as in Treadwell's method, and the chlorides are then isolated by destruction of the cyanides and thiocyanates with hot nitric acid. Thus, a mixture of 100 ml. of the solution and 10 ml. of nitric acid (sp.gr. 1·3) is heated slowly to boiling so that the rapid oxidation of the cyanides and thiocyanates does not cause it to boil over; if thiocyanates are present the solution is dark red, but subsequently becomes colourless. The mixture is boiled gently for 30 minutes, precautions being taken to avoid bumping, an excess of 0·1 *N* silver nitrate solution is added, and, after further boiling for a short time (to coagulate the silver chloride), the solution is cooled, and the excess of silver nitrate is back-titrated with 0·1 *N* ammonium thiocyanate solution, with ferric ammonium alum as indicator. Sulphates produced by oxidation of the thiocyanates do not interfere. With 24·8 to 124·0 mg. of chloride, in presence of 58·4 to 292·0 mg. of thiocyanate and 51·3 to 256·5 mg. of cyanide, the differences between the amounts of chloride taken and found ranged from +0·2 to −0·4 mg. In the Liebig titration high values were found for the cyanide-content when the thiocyanate and chloride contents were high (*e.g.* above about 60 mg. per litre), and it is therefore desirable first to dilute such solutions. It was found that in the determination of small quantities of chlorides in potassium thiocyanate the authors' method gave results agreeing well with those obtained by difference from a determination of the thiocyanate content by Volhard's method or by Rupp's method (*i.e.* oxidation with iodine in presence of alkali, and back-titration of the excess of iodine). Rivot's method (precipitation of the thiocyanate with copper sulphate), or oxidation of the thiocyanate by means of bromine water to sulphate, which is determined as barium sulphate (*cf.* Treadwell, *loc. cit.*, pp. 290, 291) gave

low results for the thiocyanate-content. In the qualitative test for chlorides in potassium thiocyanate a solution of 1 g. of the sample in 100 ml. of water is boiled for 30 minutes with 10 ml. of nitric acid (sp.gr. 1·3), silver nitrate solution being added to the warm mixture so as to avoid the precipitation of silver sulphate; an opalescence indicates the presence of chlorides. The authors collected in sodium hydroxide solution the gases evolved from a mixture of 1 g. of potassium thiocyanate and 10 mg. of potassium chloride during the boiling process; they demonstrated by the above method that under these conditions no chlorides are lost by volatilisation. J. G.

Microchemical

Detection of Barium and Sulphate by means of Spot Reactions. F. Feigl and W. Aufricht. (*Rec. Trav. Chim. Pays-Bas*, 1939, **58**, 1127–1132.)—Potassium permanganate which has been adsorbed from solution by barium sulphate adheres to it permanently and the resulting violet colour of the sulphate is almost unaffected by the usual permanganate-reducing agents (*cf.* Wohlers, *Z. anorg. Chem.*, 1908, **59**, 203). A spot method based on this effect has been devised for the detection of small amounts of barium and sulphate. To obtain maximum visibility, it is essential to precipitate the barium sulphate in concentrated permanganate solution and to reduce the excess of permanganate immediately without filtering. For the detection of barium, sulphurous acid is the best reducing agent (as the sulphate formed decreases the solubility of barium sulphate). The test can be made with one drop of the test solution on the spot plate, in Emich's small pointed tubes, or on filter-paper—the limiting sensitivities for barium by these methods being 12, 2·5 and 5γ, respectively, and the corresponding limiting concentrations, 1 to 4200, 20,000 and to 10,000.

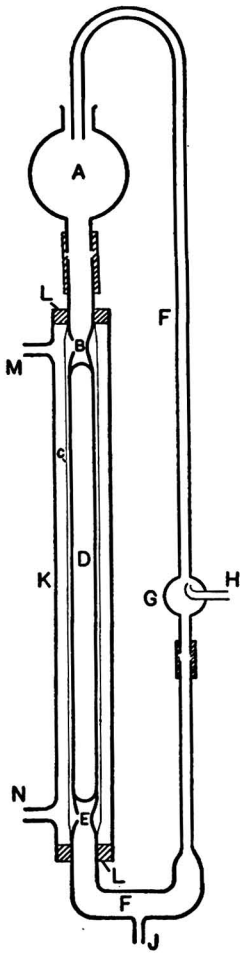
Detection of Barium.—(*a*) *Spot plate.*—One drop (0·05 ml.) of the test solution and 3 drops of cold saturated potassium permanganate solution are mixed on the plate, the barium is precipitated with a few drops of dilute sulphuric acid, and sulphurous acid is added immediately while the liquid is stirred with a sealed glass capillary tube until the solution is decolorised. A pink turbidity indicates barium. (*b*) *Emich tube.*—The reaction is carried out as before. After decolorisation of the solution the barium sulphate is centrifuged into the tip of the tube, where it can readily be recognised against a white background, particularly by means of a lens. (*c*) *Filter-paper.*—A sulphate paper which can be stored after drying is prepared by soaking strips of filter-paper in $N/2$ sodium sulphate solution. One drop of a mixture of 3 drops of the test solution with one drop of the permanganate solution is placed on the dry sodium sulphate paper, which is then kept for 7 to 10 minutes in an oven at 70° C. to 80° C. The original violet colour changes to brown, owing to reduction of the permanganate by the filter-paper; longer drying weakens the sensitivity of the reaction. The dried paper is soaked in sulphurous acid for 1 or 2 minutes, until the last traces of manganese dioxide have been removed. The presence of barium sulphate on the paper, which is now white, is shown by a violet fleck or ring. Under the same experimental conditions strontium sulphate is very slightly coloured by potassium permanganate solution,

whilst calcium sulphate does not adsorb permanganate. It is possible to detect 5γ of barium in presence of 2500γ of strontium if a blank test with pure strontium nitrate is made. While decolorisation of the coloured barium sulphate by conc. sulphurous acid is very slight, even in 24 hours, that of the coloured strontium sulphate is rapid and complete. *Detection of Sulphate.*—(i) *Spot method.*—To a mixture of one drop of the test solution and three drops of cold saturated permanganate solution on the spot plate is added 1 drop of $M/2$ barium chloride solution and, after mixing, 3 to 4 drops of 3 per cent. hydrogen peroxide to decolorise the excess of permanganate. The barium sulphate is coloured violet. For the better recognition of the colour in the spot-plate tests for sulphate and for barium it is advisable to fill an adjacent depression in the plate with water. Limiting sensitivity: 3γ of sulphuric acid; limiting concentration: 1 to 9000. (ii) *Emich tube.*—The test is made as in (i), hydroxylamine hydrochloride being used as reducing agent. The centrifuging and examination of the precipitate are as in (*b*). Limiting sensitivity: 2·5γ of sulphuric acid; limiting concentration: 1 to 20,000. (iii) *Filter-paper.*—One drop of a mixture (3:1) of the test solution and of saturated permanganate solution is placed on filter-paper which has been soaked in $M/2$ barium chloride solution and dried. After drying again at 70 to 80° C. in an oven for 7 to 10 minutes the excess of barium chloride is removed by washing out with water in a dish for one minute, then placing for an instant under the water-tap. On placing the paper in oxalic acid solution a pink ring indicates the presence of sulphate. Limiting sensitivity: 2·5γ of sulphuric acid; limiting concentration: 1 to 20,000. Lead sulphate gives a similar reaction under these conditions. The method for the detection of lead will be given later. E. B. D.

Microscopy of the Amino Acids and their Compounds. IV. Picrolonates. R. Dunn, K. Inouye and P. L. Kirk. (*Mikrochem.*, 1939, **27**, 154–160.)—Picrolonates were obtained from all naturally occurring amino acids and from some synthetic amino acids not found naturally. Proline yields a salt only from concentrated solution. A minute amount of the amino acid added to the solution of about 0·01 mg. of the reagent produces satisfactory crystals with alanine, arginine, cysteine, dibromotyrosine, dichlorotyrosine, glycine, histidine, norleucine, norvaline, tyrosine and valine. The remaining amino acids require a more concentrated solution to produce satisfactory crystals. The predominant crystal habit is acicular, the needles being arranged in rosettes; the appearance is insufficiently characteristic, but in nearly every instance the refractive indices serve for identification. These are measured in the two extinction positions 90° apart, designated N_1 and N_2. The values obtained are as follows: alanine, $N_1 = 1{\cdot}575$, $N_2 = 1{\cdot}580$; arginine, (*a*) $N_1 = 1{\cdot}716$, $N_2 = 1{\cdot}580$, (*b*) $N = 1{\cdot}578$; aspartic acid, (*a*) $N_1 < 1{\cdot}527$, $1{\cdot}527 > N_2 > 1{\cdot}512$, (*b*) N_1 and $N_2 > 1{\cdot}740$; cysteine, N_1 and $N_2 > 1{\cdot}740$; cystine, $N_1 = 1{\cdot}600$, $N_2 = 1{\cdot}548$; dibromo-tyrosine, (*a*) $N_1 > 1{\cdot}740$, $1{\cdot}616 > N_2 > 1{\cdot}549$, (*b*) $N_1 < 1{\cdot}549$, $N_2 > 1{\cdot}580$; dichlorotyrosine, $1{\cdot}740 > N_1 > 1{\cdot}698$, $1{\cdot}633 > N_2 > 1{\cdot}618$; diiodo-tyrosine, anisotropic; N_1 and N_2 could not be measured; glutamic acid, $N_1 = 1{\cdot}574$, $N_2 = 1{\cdot}596$; glycine, $N_1 = 1{\cdot}616$, $N_2 = 1{\cdot}531$; histidine, $N_1 = 1{\cdot}616$, $N_2 = 1{\cdot}557$; hydroxyproline,

$N_1 = 1{\cdot}658$, $N_2 = 1{\cdot}493$; hydroxy-valine, $N_1 = 1{\cdot}56$ (approx.), $N_2 = 1{\cdot}54$ (approx.); isoleucine, $N_1 = 1{\cdot}610$, $N_2 = 1{\cdot}520$; isoserine, (*a*) $N_1 > 1{\cdot}740$, $N_2 > 1{\cdot}70$ (approx.), (*b*) $N_1 = 1{\cdot}608$, $N_2 = 1{\cdot}520$, (*c*) $N_1 = 1660$, $N_2 = 1{\cdot}529$; leucine, $N_1 = 1{\cdot}617$, $N_2 = 1{\cdot}527$; lysine, $N_1 = 1{\cdot}645$, $N_2 = 1{\cdot}520$; methionine, $N_1 = 1{\cdot}62$ (approx.), $N_2 = 1{\cdot}494$; norleucine, $1{\cdot}740 > N_1 > 1{\cdot}658$, $1{\cdot}740 > N_2 > 1{\cdot}658$; norvaline, (*b*) $N_1 = 1{\cdot}684$, $N_2 > 1{\cdot}74$; phenylalanine, (*b*) N_1 and $N_2 > 1{\cdot}74$; proline, $N_1 = 1{\cdot}530$, $N_2 = 1{\cdot}605$; serine, (*a*) $N_1 = 1{\cdot}567$, $N_2 = 1{\cdot}530$, (*b*) refractive indices not measured owing to rapid solution in immersion media; tryptophane, $N = 1{\cdot}712$, tyrosine, $N_1 = 1{\cdot}596$, $N_2 = 1{\cdot}529$; valine, $N_1 = 1{\cdot}549$, $N_2 > 1{\cdot}740$; *dl*-α-amino-*n*-valeric acid, $N_1 > 1{\cdot}685$, $N_2 > 1{\cdot}740$. Twelve photomicrographs are given. The letters (*a*) and (*b*) represent two types of crystals. J. W. M.

Physical Methods, Apparatus, etc.



A Rapid Circulating Dialyser. A. R. Taylor, A. K. Parpart and R. Ballentine. (*Ind. Eng. Chem. Anal. Ed.*, 1939, **11**, 659.)—In this apparatus a current of water flows from M to N through the outer glass jacket K. The dialysing membrane C consists of a thin sheet of cellophane, and a glass cylinder D, which is enclosed in the membrane and kept in position by fusion with the inlet and outlet tubes B and E, ensures that the liquid under treatment is in a thin layer. The outlet J is closed with a rubber tube and clip, and the liquid to be dialysed is poured into the bulb A, whence it descends, filling the lower part of the apparatus and part of the tube F. A current of air or inert gas, passed through the jet H, causes the solution to circulate, and samples can be drawn off at J for examination. As an example of the efficiency of the apparatus it is stated that 25 to 100 ml. of protein solution semi-saturated with ammonium sulphate were completely freed from the salt in nine hours. Another advantage claimed for the apparatus is that there is no risk of rupturing the cellophane membrane, which can be used repeatedly.

Reviews

MICRO-DIFFUSION ANALYSIS AND VOLUMETRIC ERROR. By EDWARD J. CONWAY, M.B., D.Sc. Pp. xiii + 306. London: Crosby Lockwood & Son, Ltd. 1939. Price 25s. net.

The micro-diffusion method of analysis is so simple and the apparatus so cheap that it deserves a much wider application than as at present mainly in the problems of medical and biochemical research. It is to be hoped that Professor Conway's excellent book, which includes an account not only of the method but also of micro-volumetric procedure and the errors of volumetric determination, may achieve this result by interesting more analytical chemists in the method.

The standard micro-diffusion unit resembles two small concentric Petri dishes, the walls of the internal portion being half the height of the outer. When sealed with an airtight cover this simple apparatus may be used for the determination of volatile gases liberated by a reagent in the outer chamber, absorbed by a reagent in the centre chamber, and subsequently measured by titration or colorimetrically.

Three chapters are devoted to the use of the unit itself, the standard procedure and generalised conditions for the determination of amounts of test substance (ammonia, urea, chloride, bromide and carbon dioxide) ranging from 0·5 to 500γ per ml. (designated "gammis," by the author) in 1 ml. volume, with an accuracy of 0·5 per cent. for amounts of 20γ and more and 5 per cent. for 0·4 "gammil."

The chapters on volumetric procedure are extremely valuable. The greater accuracy, well known to micro-chemists, of using simple laboratory-made pipettes, is clearly explained. The simple tube-shape pipette is shown to be quite as accurate as the original Ostwald form, if not more so.

In the second part of the book the different methods are described in detail, in thirteen chapters, the final chapter being devoted to Karon and Webb's qualitative tests for acetone and alcohol which were described in this journal three years ago.

The chapters on error are readily understandable without any very great knowledge of mathematics, and are of great value to all those to whom accuracy is important, that is, to all analysts.

The work can be highly recommended not only to those interested in diffusion analysis, but to all who wish to have a clear exposition of micro-volumetric procedure, including "drop" technique, with especial reference to the accuracy of the results. This is the best book on micro-volumetric work that the reviewer has had the pleasure of reading. JANET W. MATTHEWS

A LABORATORY MANUAL OF QUALITATIVE ANALYSIS. By JOHN H. YOE, Ph.D. Pp. ix + 219. London: Chapman & Hall, Ltd. 1938. Price 12s. 6d. net.

In this text-book of elementary inorganic qualitative analysis, intended to cover a thirty-six weeks' course, only such reactions of cations and anions are included as are used for their identification in the schemes for separation. The space saved by the exclusion of the general reactions of the elements has been

devoted to a very complete description of the procedures used for their separation and detection and for detailed explanatory notes following each section.

Intended to be used solely as a bench book, it contains no physico-chemical theory; for this the student is referred to the author's or to other works on the subject. This should please those who consider that the art and the science of analytical chemistry are best treated separately; such a thought was probably in the mind of the author when he wrote, page 42, "Qualitative analysis was a well organised art long before Wilhelm Ostwald placed it upon a firm scientific basis when he published his '*Wissenschaftliche Grundlagen der analytischen Chemie*,' 1894."

The work covers the usual twenty-four common cations of the elementary course, and the same number of anions; it follows American practice in precipitating the hydroxide and alkaline sulphide groups together. An unusual feature is the scheme for the separation of arsenic, antimony and tin by successive precipitation, under controlled conditions of acidity, as sulphides from hydrochloric acid solution. The reviewer, having in mind the well-known adsorptive capacity of antimony sulphide for tin, was agreeably surprised to learn that one milligram of tin in a one per cent. mixture with antimony gave, under the specified conditions and after filtration, a characteristic and easily visible precipitate of stannic sulphide.

As a text-book it may be considered defective by many in this country in that it contains no provision for elimination of phosphates or other interfering elements in the iron group, and it is somewhat diluted by the inclusion of standing orders to the students of a particular school of chemistry.

A special feature is a section on delicate reactions by means of organic reagents, to which reference is made at appropriate points in the text. In this, possibly the most valuable part of the work, the manipulation is fully described, with warnings as to interfering elements and references to original sources; the reactions, wherever possible, are illustrated by structural formulae.

One misprint was noted on page 167, where "Janus Green" is termed "James Green."

Although there are elementary text-books of wider scope at more moderate price than this, there are but few in which the subject-matter is presented with more detailed precision. F. L. OKELL

HANDBOOK OF FOOD MANUFACTURE. By F. FIENE and S. BLUMENTHAL, B.Sc. Pp. vi + 603. London: Chapman & Hall. 1939. Price 25s. net.

This ambitious attempt to provide information of service to the various classes of workers engaged in the manufacture of foods and beverages deals mainly with the practical aspects of the industry, from the sources, grading and testing of the raw materials to the preparation of the finished products. The profusion of formulae and methods described are based upon large-scale industrial experience, and thus may be relied upon to produce successful results.

Most of the extensive range of materials dealt with would be included under the headings of milk, meat, fish, fruit and nut preparations, pâtisserie, confectionery, preserves, sauces, liqueurs, cocktails, and fruit drinks, while mixtures sold to the public for the home preparation of pastry, ices and so forth are also described.

The first part of the chapter entitled "An Outline of Elementary Chemistry" is too condensed to be of value either to the layman or the chemist and might well have been omitted; in contrast with this, the rest of the chapter contains admirable summaries of vitamin data and analytical values for the composition of many naturally occurring foods together with the physiological effects of the various metals, the halogens, phosphorus and sulphur in dietary. Over sixty pages are devoted to the numerous raw materials used in the food industry, including *inter alia* synthetic compounds, essential and fixed oils, drugs and cereals, the physical properties and taste effects of many of these being given in detail. This chapter is excellent, but it is a little disconcerting to find in a book on foods that human, horse, dog and wool fats are included in a table giving the solidifying points of fats (p. 68), and the value of 9 per cent. given for the yield of sucrose from the sugar-beet is much below the amount obtained at the present time.

The following twenty-three chapters contain a miscellaneous collection of formulae and procedures for the manufacture of hundreds of food products, the variety of which would overwhelm the famous Mrs. Beeton. This section is undoubtedly the best in the volume. Chapter XXV provides an admirable account of hydrogen ion control by means of indicators and its applications, *e.g.* in bread-making and canning. Among the "Miscellaneous" items is a useful section on the use of alkalis for bottle-washing together with the requirements of many of the American States in this respect. The striking diversity in the concentrations of alkali (2·5 to 5 per cent.), temperatures (100 to 160° F.), and time of soaking (3 to 20 minutes) causes one to wonder if such regulations have any value, especially as some States make no requirements whatever. One table giving the common adulterants of about sixty food materials will prove of considerable value to the analyst.

In the chapter dealing with "Analyses and Tests" a number of qualitative and quantitative tests for a wide range of food materials are given. Many of these are satisfactory, but the precautions necessary for the correct interpretation of results obtained in the colour-tests for oils are omitted, and no reliance can be placed upon the results of some of the tests as described. It is evident that the proofs were not read by a qualified chemist, otherwise numerous erroneous statements in this section would have been eliminated.

The final pages of the volume contain the definitions and standards for food products adopted in the United States under the Food and Drugs Act, and an extensive and accurate index which, whilst not quite complete, yet contains nearly 2500 references. There is much sound and valuable matter in the text, but its merits are greatly discounted by the number of errors present, and it may be hoped that these will be eliminated by more thorough proof-reading in the next edition.

T. J. WARD