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THE ANALYST

The Journal of
The Society of Public Analysts
and other Analytical Chemists

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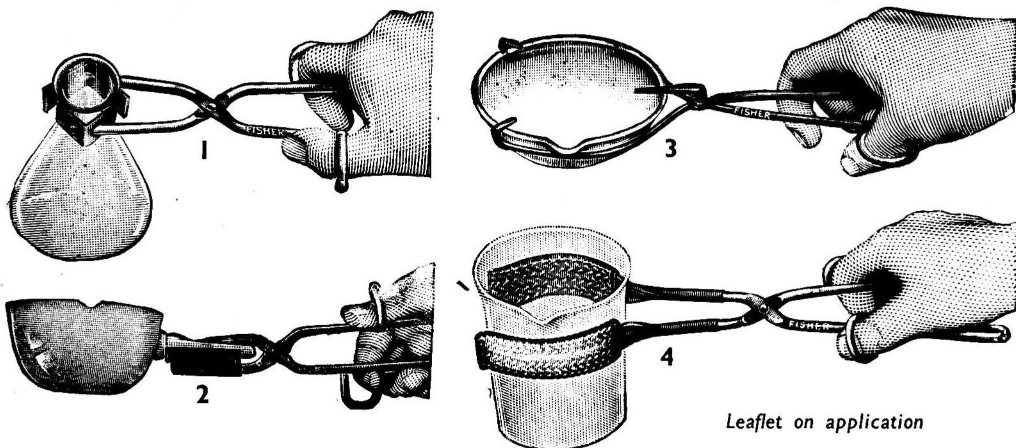

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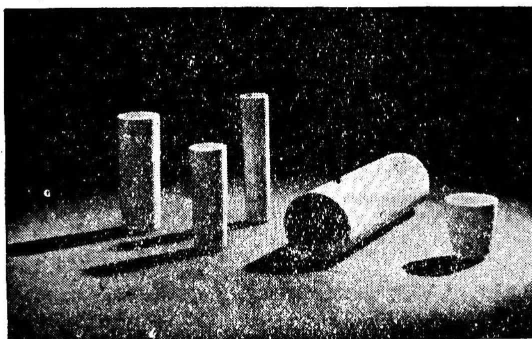

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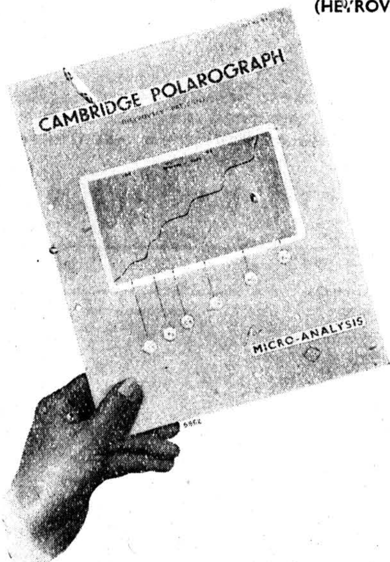

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THE ANALYST

PROCEEDINGS OF THE SOCIETY OF PUBLIC ANALYSTS
AND OTHER ANALYTICAL CHEMISTS

THE Annual General Meeting of the Society was held at 3.15 p.m. on Wednesday, March 1st, 1944, at The Chemical Society's Rooms, Burlington House, London, W.1. The chair was occupied by the President, Mr. S. Ernest Melling, F.I.C. The Financial Statement for 1943 was presented by the Hon. Treasurer and approved and the Auditors for 1944 were appointed. The Report of the Council for the year ending March, 1944, was presented by the Hon. Secretary and adopted. The following were elected Officers and Council for the coming year:

President—S. Ernest Melling, F.I.C.

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

Vice-Presidents—G. Hogan, B. G. McLellan, J. R. Nicholls and, *ex officio*, W. Gordon Carey (Chairman, North of England Section) and A. R. Jamieson (Chairman, Scottish Section).

Hon. Treasurer—George Taylor.

Hon. Secretary—Lewis Eynon.

Other Members of Council—C. A. Bassett, R. C. Chirnside, S. Dixon, R. H. Ellis, J. H. Hamence, H. M. Mason, (Mrs.) J. W. Matthews, (Miss) M. Pearson, W. H. Simmons, E. Voelcker, G. H. Walker, K. A. Williams and, *ex officio*, Arnold Lees (Hon. Secretary, North of England Section) and R. S. Watson (Hon. Secretary, Scottish Section).

The Annual General Meeting was followed by the postponed Presidential Address of Dr. E. B. Hughes, who was unable to deliver it when he retired from the presidential office last year. The subject of the Address was "The Technology of Tea."

Indicating first the world distribution of tea-growing areas, the speaker pointed out that these were not now confined to Asia; the necessary tropical or sub-tropical conditions existed elsewhere and tea was now grown extensively in Africa, even as far south as Natal, and also in Russia. In the production of black tea, as distinct from green tea, the main processes are: withering, rolling, fermentation and final drying. Withering is a partial natural drying process at as cool a temperature as possible. It dries the leaves to a condition in which they can be rolled and twisted by mechanical action simulating rotatory rubbing between the hands; this damages the cells, whereupon, possibly as a result of "disorganised respiration," oxidase of the leaf brings about "fermentation." This so-called fermentation is mainly oxidation of polyphenols of the leaf to quinone compounds, which readily produce reddish, copper-coloured condensation products. The polyphenols of the leaf are the so-called tea tannins, but they are not tannins in the ordinary sense, as they are not able to convert hide into leather. The speaker emphasised the importance of the rate of "fermentation" on the quality of the tea produced; if too rapid it gives inferior products, and the greater rate of fermentation, combined with lower quality of leaf grown in hot humid conditions, produces a commoner quality of tea. Indeed, the differences in quality and character between teas from different areas are due mainly to differences in geographical and climatic conditions, rather than to varietal differences in the plants grown. Changes in climatic conditions in the same area may produce much choicer tea at one time than a month earlier or later. It is the practice of blending, dependent on the remarkable skill of the tea taster, that enables the consumer to be supplied with brands of unchanging character. Most good teas, as supplied to the consumer, are blends of more than a dozen lots.

Green tea is not subjected to "fermentation"; the enzyme activity is destroyed by heating the leaf (steaming) as soon as possible after plucking, and the leaf is afterwards rolled and "fired".

NEW MEMBERS

Wilfred Norman Aldridge; Benjamin Bagshawe, A.Met. (Sheff.)*; Kenneth Charles Barraclough, B.Sc. (Lond.), F.I.C., A.Met. (Sheff.)*; Kenneth Booth Belcher, Major, A.I.C.; John Henry Bignell, B.Sc. (Lond.); George Broomhead, B.Sc. (Lond.)*; William Brown, A.I.C.; John Edmund Chivers; Gordon Shaw Doubleday; Edwin Edwards*; Gordon Westland Edwards, A.I.C.; William Thomas Field, B.Sc. (Lond.), F.I.C.; Eric Arthur Foster; Charles Henry Hands; Neil Heron, A.I.C.*; John Doogood Hill, A.Met. (Sheff.)*; John Douglas Hobson, A.Met. (Sheff.)*; William Horner*; Thomas William Jones; John Gwilliam Maltby, B.Sc. (Lond.), A.I.C.; Paul Michael Mooney, B.Sc. (Lond.), F.I.C.; George Parkin, A.I.C., A.Met. (Sheff.)*; Ronald Francis Porter, B.Sc. (Lond.), A.I.C.; Roger Tully Postlethwaite*; William Bennett Price, B.Sc., Ph.D. (Lond.), F.I.C.*; Alexander Robertson, M.A., B.Sc., Ph.D., M.R.C.V.S.†; Marcus Rosebery, B.Sc. (Lond.), A.R.C.S., A.I.C.*; James Edwin Sands, A.I.C.*; Thomas Arthur Frederick Sexton, B.Sc. (Lond.), F.I.C.; Frank Shaw, M.Sc. (Lond.), D.I.C., A.I.C.; Cyril Edward Spooner, M.Sc.Tech. (Sheff.), A.I.C.*; George Albert Storey, M.P.S.; Norman Strafford, M.Sc. (Lond.), F.I.C.*; Joseph Knight Thompson, M.Sc., B.Sc.Tech., Ph.D. (Sheff.)*; Harold George Tribley, A.C.G.F.C., F.I.C.; Jack Trask Unwin; Charles Walker, B.Sc. (Lond.), F.I.C.*; Lionel William Warner, A.I.C.; Harold Lawson Webster, A.M.C.T., A.I.C.*; Clifford Whalley, B.Sc. (Sheff.)*; Cecil Leeburn Wilson, M.Sc. (Belfast), Ph.D. (Glasg.)*; Kenneth Mackenzie Wilson, A.I.C.; Ronald Wood, M.P.S.*; Leslie Woods, M.Sc. (Liv.), A.I.C.*; Alfred Wright.*

STANDARDS FOR GELATIN

THE Public Analysts and Official Agricultural Analysts Committee of the Society has had under consideration the question of Standards for Impurities in Edible Gelatin on sale by retail. Consideration has been given to what limits of impurity are obtainable at the present time, and the Council has authorised the publication of the following provisional standards pending any official regulations.

It is recommended that no action should be taken under the Food and Drugs Act, 1938, where the following quantities are not exceeded:

Arsenic, as arsenious oxide ..	2	parts per million
Copper	30	,, ,, ,,
Lead	10	,, ,, ,,
Zinc	100	,, ,, ,,

Annual Report of Council: March, 1944

THE roll of the Society numbers 1080, an increase of 77 over the membership a year ago.

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

R. M. Clark	J. Golding	J. A. MacNair	H. L. Smith
L. Cooksey	F. W. Jackson	W. G. Messenger	K. Wallis
M. B. Elliott	A. Jaffé	H. S. Redgrove	S. A. Woodhead

Clark, who died in his 66th year, had been a member of the Society for 36 years and served as a member of the Council in 1912–13. After completing his course as a student at Glasgow University, he became assistant to his father, Dr. John Clark, and subsequently succeeded to the practice. He held appointments as Public Analyst to a number of Scottish Counties and Burghs.

Cooksey, who died at the age of 49, studied at East London College, graduating B.Sc. with first class honours in chemistry in 1915. From then until his death he was assistant to Messrs. Rideal and Sciver.

Miss Elliott, who died in her 59th year, became associated with the Society as indexer and business manager of THE ANALYST in 1921, and unofficial Assistant Secretary in 1922. In 1937 pressure of other work compelled Miss Elliott to give up most of her work for the Society and, in recognition of her eminent service, she was elected an Honorary Member of the Society. (Obituary, ANALYST, 1944, 69, 33.)

Golding, who was 72 at the time of his death, had been a member of the Society for 41 years and served for two periods as a member of the Council. Practically the whole

* Through the North of England Section. † Through the Scottish Section.

of his professional career was spent in the service of agricultural chemistry. From 1912 to 1937 he was head of the Chemistry Department of the National Institute for Research in Dairying, Shinfield. During the war of 1914–18 he served with great distinction in the Army in France. (Obituary, ANALYST, 1944, 69, 69.)

Jackson, who died in his 53rd year, received his scientific training at the Central Technical College, and obtained the B.Sc. degree with honours in chemistry in 1911. From 1912 to 1915 he had control of the Vinegar Brewery of Holbrooks, Ltd., and more recently, was chemist to Messrs. Marsh & Baxter, Ltd.

Jaffé, who died at the age of 68, had been a member of the Society for 40 years. He was for over 50 years engaged as a consulting and analytical chemist in partnership with F. W. Richardson. (Obituary, ANALYST, 1943, 68, 199.)

MacNair died at the early age of 36. He was for many years on the staff of R. R. Tatlock & Thomson, Public Analysts, and was lecturer in chemistry at Stow College, School of Engineering, Glasgow. He was prominently associated with the Scottish Section of the Society.

Messenger, who died in his 50th year, had been a member of the Society for 15 years and served as a member of the Council in 1926–27. He obtained the B.Sc. (Birm.) degree with first class honours in chemistry in 1914. During the war of 1914–18 he served, first in the infantry and then in the Special Brigade, R.E. In 1919 he became a chemist with J. Lyons & Co., Ltd., and subsequently an assistant chief chemist.

Redgrove, who died at the age of 56, was a lecturer on mathematics and chemistry at the Regent Street Polytechnic and subsequently at other Institutions. He was an authority on perfumery and cosmetics and on alchemy. (Obituary, ANALYST, 1943, 68, 199.)

Smith, who died in his 68th year, had been a member of the Society for 36 years and served on the Council in 1916–17. He was for some years a lecturer at King's College, London, and afterwards Professor of Chemistry in the School of the Pharmaceutical Society. After the war of 1914–18 he was chemist to the Scientific Instrument Research Association.

Woodhead, who died in his 71st year, had been a member of the Society for 44 years and served as a member of the Council in 1911–12. He began his professional career as lecturer in chemistry at the Uckfield Agricultural College and subsequently became Principal. He afterwards set up in practice as a consulting chemist and bacteriologist and held a number of appointments as Public Analyst. (Obituary, ANALYST, 1943, 68, 297.)

ORDINARY MEETINGS—In the course of the year five meetings were held and the following papers were communicated:

"The Determination of Fluorine in Wool treated with Fluorides." By F. F. Elsworth, B.Sc., Ph.D., and J. Barritt, B.Sc., A.R.C.S., A.I.C.

"Polarographic Studies. III. The Determination of Vanadium." By J. E. Page, B.Sc., Ph.D., F.I.C., and F. A. Robinson, M.Sc., A.I.C.

"The Determination of Cantharidin in Beetles and Native Medicines." By J. C. Bodenstein, M.Sc.

"The Separation of Vitamin A from Xanthophylls in the Presence of Egg Yolk Sterols." By T. Barton Mann.

"Two New Colour Tests for Stilboestrol." By T. Tusting Cocking, F.I.C.

"The Determination of Copper Volumetrically by the Iodine-Thiocyanate Method." By C. C. Oglethorpe, B.Sc., and C. G. Smith, B.Sc., Ph.D., F.I.C.

"The Determination of Carotene in Grass and Silage Mixtures." By T. Barton Mann.

"The Rapid Determination of Arsenic in Glass." By H. N. Wilson, F.I.C.

"Three Pieces of Apparatus." By M. A. Fill and J. T. Stock, B.Sc., A.I.C.

"(1) Reflux Apparatus for Automatic Dispersion of Froth in the Determination of Fibre."

"(2) An Electrical Indicator for Collecting a Constant Volume of Distillate."

"(3) A Wash Bottle for Delivering Predetermined Volumes of Liquid."

"The Determination of Indolylacetic Acid." By P. F. Holt, Ph.D., D.I.C., F.I.C., and H. J. Callow, B.Sc., A.I.C.

"A Review of Micro-Volumetric Apparatus." By G. H. Wyatt, B.Sc., Ph.D., F.I.C.

"The Analysis of Commercial Lecithin." By H. H. Hutt, A.I.C., and H. Weatherall, F.I.C.

"A Micro-Method for the Quantitative Determination of Tannin." By M. Nierenstein, D.Sc., Ph.D.

"An Amperometric Cell." By J. H. Stock, B.Sc., A.I.C., and M. A. Fill.

The December meeting was a Joint Meeting with the Food Group of the Society of Chemical Industry. Ths subject was "Nutrition of the Public and Food Legislation," and the following papers were read and discussed:

"The Essential Natural Nutrients of Fresh and Manufactured Foods." By J. C. Drummond, D.Sc., F.I.C.

"The Rôle of Food Legislation in securing Adequate Nutrition." By H. E. Cox, Ph.D., D.Sc., F.I.C.

"The Part Played by the Food Supplier in Safeguarding the Nutritive Values of Food." By E. B. Hughes, D.Sc., F.I.C.

At the Annual General Meeting Dr. C. A. Mitchell, M.A., F.I.C., gave an address on "Ink in Relation to Crime."

GROUPS WITHIN THE SOCIETY—Proposals for the formation of Groups within the Society, to deal with special branches of analysis, were considered by the Council and arrangements for the incorporation of the Microchemical Club and for the formation of other Groups were submitted to the Society at the November meeting and approved.

A Group will only be formed on the application of members if, in the opinion of the Council, there is sufficient justification for its formation. Each Group will have its own Chairman, Honorary Secretary and Committee of Management. This Committee will arrange periodical meetings and, if desired, joint meetings with other Groups and/or other Societies. Persons wishing to join any Group must first join the Society and members of the Society are at liberty to join any Group or Groups. All members of the Society will be entitled to attend meetings of Groups and take part in the deliberations, but only members of a Group will be entitled to vote at meetings of the Group.

THE ANALYST—To comply with the regulations of the Paper Controller each number of THE ANALYST must now contain not more than 32 pages on the average, and it will be noted that the total number of pages for the year (388) is in accordance with this computation. Notwithstanding this restriction the number of papers published in 1943 (42) exceeds the number in 1942 (31), and there is a similar increase in the number of notes (38 as compared with 30 in the preceding year). This successful condensation of matter into space is largely due to the gradual permeation of the "Advice to Authors" issued by the Publication Committee. Once more the subject matter of the papers has been fairly evenly distributed between Food and Drugs (15) and Inorganic Chemistry (16), and the same trend is to be observed in the notes (14 relating to Food and Drugs and 16 to inorganic subjects). The remaining papers and notes cover Biochemistry, Bacteriology, Water Analysis, Agricultural Analysis, Organic Compounds, Physical Methods and Apparatus. To assist Public Analysts and Agricultural Analysts we have continued to draw attention to salient points in the various Food Orders issued by the Ministries of Food and Health. We have also been able once more to publish extracts from the Reports of Government Analysts in the Dominions and India, although the issue of some of these was suspended until after the war.

HON. TREASURER'S REPORT—The Hon. Treasurer anticipates that the Financial Statement for the year will show a very satisfactory position, probably better even than that of the previous year.

EXAMINATION OF GAS-CONTAMINATED FOOD—It has been agreed with the Ministry of Food that it would not be practicable to fix fees but that these should depend on the nature and circumstances of each case and left until the work is carried out.

FLUORINE IN FOODSTUFFS—The Public Analysts and Official Agricultural Analysts Committee has recommended maximum limits for fluorine in certain foodstuffs (ANALYST, 1943, **69**, 233.)

STATUS OF PUBLIC ANALYSTS—A Sub-Committee of the Public Analysts and Official Agricultural Analysts Committee has been appointed to consider the future status of the Public Analyst.

ANALYTICAL METHODS COMMITTEE—War conditions still restrict the activities of the Sub-Committees, and from these no reports have been forthcoming during the year. One new Sub-Committee has been appointed to investigate methods for determining small amounts of fluorine in foods and is making good progress. The Tomato Products Sub-Committee has been authorised to extend its work to the determination of moisture in other fruit products. A report on work carried out at the request of the Vitamin B_1

Sub-Committee of the Lister Institute and Medical Research Council, on the Determination of Crude Fibre in National Flour, was published in THE ANALYST, 1943, **68**, 276.

NORTH OF ENGLAND SECTION—Four meetings have been held during the year and the following papers have been read:

"The Resazurin Test for Milk." By G. Sykes, M.Sc., A.I.C.
"The Determination of Arsenic in Glass." By H. N. Wilson, F.I.C.
"Quadruplicate Tasting Tests." By H. M. Mason, M.Sc., F.I.C.
"Comments on the Colouring of Food." By D. J. T. Bagnall, A.C.G.F.C., F.I.C.
"Mustard." By Dr. J. W. Corran, B.Sc., F.I.C.
"Analysis and Research." By R. C. Chirnside, F.I.C.

There have been good attendances at the meetings. The Section now numbers 160, an increase of 13 on the previous year.

The Hon. Secretary wishes to express his appreciation of the loyal support and assistance accorded to him by the Chairman and members of the Committee during the year.

SCOTTISH SECTION—Three meetings were held in the year, at which the following papers by members of the Section were read and discussed:

"Effluents and Fish Life." By M. J. Robb, F.I.C.
"The Total and Reducing Sugars in Different Varieties of Potato Tubers, as determined by the Shaffer and Hartmann Iodimetric Method." By A. M. Smith, Ph.D., D.Sc.
"Some Aspects of the Application of Formulae to the Calculation of Milk Solids." By S. M. Boden, B.Sc., A.I.C. (Visitor to the Section.)
"Effect of Electrolytes on the Freezing Point of Milk." By A. R. Jamieson, B.Sc., F.I.C.
"Contaminated Beverages." By J. B. McKean, F.I.C.

The Committee record with regret the death of one of our members, Mr. J. A. McNair, F.I.C.

Forty-nine subscriptions were received, being the same number as in the previous year. Four new members joined the Parent Society through the Section and two members resigned on taking up residence outside the Scottish Area.

INTER-DEPARTMENTAL COMMITTEE ON FOOD STANDARDS—The Council accepted with much regret the resignation of Professor W. H. Roberts, one of the Society's nominees on the Inter-departmental Committee for Food Standards and nominated Mr. R. W. Sutton in his place.

BRITISH STANDARDS INSTITUTION—Mr. S. Dixon was appointed as representative of the Society on the Technical Committee of the British Standards Institution to prepare standards for filling materials for bedding and upholstery. Dr. R. Lessing was appointed as representative of the Society on a Committee to establish standard methods for the sampling and analysis of boiler waters. Mr. R. Belcher and Dr. G. H. Wyatt were appointed as representatives of the Society on a Sub-Committee for the standardisation of microchemical apparatus.

Attendance at meetings of the Council and Committees has been well maintained, although travelling conditions have been still more difficult than in the earlier years of the War. The Council has again to record its thanks to organisations and members of the Society for accommodation and hospitality to the Committees.

S. E. MELLING, *President*
LEWIS EYNON, *Hon. Secretary*

Toluene-3:4-dithiol as a Selective Reagent for Tungsten: The Detection of Tungsten, especially in Molybdenum and Rhenium Compounds, and in Ferrous Alloys

BY CHRISTINA C. MILLER, D.SC., PH.D.

THE term "dithiol" was first applied by Clark[1] to toluene-3:4-dithiol, 4-methyl-1:2-dimercaptobenzene, which he used as a reagent for tin, and Hamence[2] used the compound as a reagent for tungsten and molybdenum, with which it forms bluish-green and green complexes, respectively. At the same time its reactions were under investigation by Miller and Lowe[3] in the analysis of the tantalum and the tungsten group of Noyes and Bray's qualitative scheme. Miller[4] found that rhenium also forms a green complex with

dithiol. Later,[5] she stressed the importance of the reagent for detecting tungsten in association with large amounts of aluminium, beryllium, chromium, uranium, vanadium, zinc and phosphate, singly or in admixture. The selectivity of the reagent was associated with the addition of its soln. in *n*-butyl acetate to test solns. in which the concn. of hydrochloric acid was high.

The main objects of this investigation were to extend the expts. on the detection of tungsten to solns. containing elements other than those cited, and to devise a means for eliminating interference, particularly that of the similarly reacting elements, molybdenum and rhenium.

It has been found that, in absence of molybdenum and rhenium, the above test is of wide applicability for the detection of a few μg of tungsten, but that there are some interfering radicals. When molybdenum and rhenium are present, a preliminary reduction with stannous chloride suppresses these reactions and removes some other interfering effects. A modified procedure has been evolved for detecting tungsten in presence of molybdenum and rhenium, and in ferrous alloys. As the test requires no preliminary separation of tungstic acid, many other applications are foreseen.

EXPERIMENTAL—*Simple test* (*molybdenum and rhenium absent*)—To about 1 ml of the test soln. (in a test-tube, 3 × 3/8 in.), 10–11 *N* with respect to hydrochloric acid, and containing a drop of orthophosphoric acid to ensure the complete solution of tungstic acid, add 0·25 ml of *iso*amyl acetate and *ca.* 5 mg of dithiol. Heat in a water-bath at *ca.* 70° C. for 5–10 min., shake the tube vigorously at intervals, then add a little more dithiol, and heat further for 5 min. The colour in the ester layer ranges from pale blue to deep emerald-green for 0·5–25μg of tungsten. If the two layers merge into one, or a minor amount of tungsten is present, remove the tube from the bath, add 0·5 ml of carbon tetrachloride, shake, centrifuge, and remove and reject the upper layer. Wash the residual layer with conc. hydrochloric acid.

Notes on the test—Dithiol does not react appreciably in absence of the ester, which cannot be replaced by carbon tetrachloride. Heating at 70° C. ensures the quantitative conversion of tungsten without much loss of hydrochloric acid. The addition of more dithiol is a safeguard when the ester layer indicates a negative or feeble reaction, and when other substances that react with the reagent or that retard the reaction are present. Carbon tetrachloride is added in preference to water for the restoration of two layers, as it does not induce interference by metals that react with dithiol at lower acidity. The solution of the complex seems to be very stable, and it is permissible to boil off the carbon tetrachloride in order to concentrate the complex in the ester. The blue colour obtained with a small amount of tungsten is highly characteristic. Minor quantities of molybdenum and rhenium give green colours. Impurities that give a pale yellow colour occasionally modify the colour given by tungsten.

Effect of various substances on the detection of 2μg of tungsten by the above method—The detection of tungsten was considered satisfactory if more than 50% of the amount added initially was indicated and the colour had a blue tinge. If less than 50% was found the sensitivity of the test was considered to be reduced.

Addition of 0·25 ml of any of the following acids had no important influence: glacial acetic, formic (90%), hydrobromic (48%, bromine-free), hydrofluoric (40%), perchloric (sp.gr. 1·68), orthophosphoric (sp.gr. 1·75), conc. sulphuric (test particularly good). Nitric acid decomposed the reagent, but 5–10 mg could be tolerated. Bromine-free hydrobromic acid could replace hydrochloric acid entirely. The presence of 250 mg of citric, oxalic, or tartaric acid did not prevent the detection of tungsten, but the first two lowered the sensitivity. Other acids, tested in 10-mg amounts, were added as their sodium or potassium salts. Carbonate, cyanide, ferricyanide (more dithiol needed), ferrocyanide, phosphite, sulphide, sulphite, and thiosulphate permitted a satisfactory test. Borate lowered the sensitivity a little. Anions, *e.g.*, hypochlorite, bromate, chlorate, iodate, iodide, periodate, and nitrite, which decompose the reagent, and thiocyanate are easily destroyed before applying the test in a hydrochloric or a hydrochloric and sulphuric acid soln. Quantities up to 1 mg (the greatest amount tested) of silicate did not influence the detection of tungsten.

In addition to the metals already mentioned, 10 mg (the greatest amount tested) of the following could be present: lithium, sodium, potassium, rubidium, caesium, silver, magnesium, calcium, strontium, barium, cadmium, mercury^{II}, scandium, yttrium, lanthanum,

cerium, praseodymium, neodymium, samarium, gadolinium, gallium, indium, thallium^{I}, titanium, zirconium, thorium, germanium, tin, lead, niobium^{V}, tantalum^{V}, arsenic^{V}, antimony^{III}, bismuth (colour greener), manganese^{II}, iron^{II} cobalt, and nickel. Ammonium ions (10 mg) were without influence.

Niobium and tantalum were present as fluorides in solns. containing 0·25 ml of hydrofluoric acid. The pptn. of zirconium and titanium phosphates, and of silver, sodium, potassium, and ammonium chlorides did not prevent the detection of tungsten. Manganese^{VII} and thallium^{III} should be pre-reduced with a little sodium bisulphite, which is used with advantage also when chromium, cerium, and vanadium are in their highest valence states.

Interference in various degrees was associated with 10 mg of the following: iron^{III}, copper$^{II \text{ and } I}$, arsenic^{III}, selenium$^{IV \text{ and } VI}$, tellurium$^{IV \text{ and } VI}$, gold^{III}, ruthenium^{III}, rhodium^{III}, palladium^{II}, osmium^{VIII}, iridium^{IV}, and platinum^{IV}. The yellow colour of the ester-soluble ferric chloride interfered, so that 1 mg of iron with 2μg of tungsten gave an olive-green colour (*cf.* Mo). Copper^{II} reacted with dithiol: 1 mg permitted the detection of tungsten but quartered the sensitivity. Arsenic^{III} suppressed the reaction with tungsten, but 1 mg could be present if an excess of dithiol were employed. Selenium and tellurium reacted with dithiol but 25-fold excesses were permissible (colours greener). Gold, ruthenium, rhodium, osmium, and platinum interfered by undergoing slow reduction to the elements. Two μg of tungsten could be detected with *ca.* 250μg of gold, if the separated metal coagulated well, and with *ca.* 1 mg of platinum, 2·5 mg of rhodium, 5 mg of ruthenium, and 50μg of osmium, provided that the period of heating was brief. One mg of iridium^{IV}, which was reduced to iridium^{III}, showed negligible interference, but with 10 mg the sensitivity of the test, which had to be completed within 5 min., was lowered. The brown dithiol complex of palladium[6] was formed under the prevailing conditions and seriously interfered. The remaining metals of the periodic table were not tried.

Modified test (molybdenum and rhenium present)—The formation of the dithiol complexes of moderate amounts of molybdenum and rhenium was suppressed by heating the customary test solns. with excess of stannous chloride before adding the ester and dithiol. The ester-insol. molybdenum^{III}[7] and rhenium^{IV}[8] produced did not react with dithiol, but continued heating led to the production in the ester layer, presumably by re-oxidation in air, first, of brown complexes, and later, of the green compounds. With tungsten the blue colour, which usually appeared, deteriorated to grey-green, but addition of carbon tetrachloride, brisk shaking, and replacement of the stannous chloride layer by conc. hydrochloric acid, restored it.

For the detection of 2μg of tungsten with 1 mg of molybdenum^{VI} or 0·5 mg of rhenium^{VII} the only modification required in the test already prescribed was addition of 100 mg of stannous chloride, and 5 min. heating at 70° C. before addition of the ester and dithiol. Traces of molybdenum and rhenium made the colours slightly greener, but the presence of tungsten was not in doubt. With 10 mg of molybdenum or rhenium *ca.* 10% of each accompanied the tungsten, and it was necessary to re-apply the test as follows: Separate and wash the soln. with conc. hydrochloric acid, boil off the organic solvents, add a drop of phosphoric acid to the residue, and destroy organic matter by heating with a few drops of conc. sulphuric acid to which a drop of conc. nitric acid is added at intervals. Expel the latter, add 1 ml of conc. hydrochloric acid, and repeat the modified test.

Traces of molybdenum and rhenium that remained after 2 and 3 treatments, respectively, could be eliminated by an additional treatment which involved no loss of tungsten. The presence of 0·25 ml of sulphuric or phosphoric acid initially did not influence the reduction process, but perchloric acid counteracted it and had to be absent. It was difficult to find tungsten-free molybdenum compounds. Samples of AnalaR ammonium molybdate contained 0·005%.

Effect of stannous chloride on the detection of tungsten in the presence of other metals—The treatment outlined above effectively eliminated interference with the detection of 2μg of tungsten by 10 mg of iron^{III}, copper^{II}, arsenic^{III}, tellurium$^{IV \text{ and } VI}$, and gold^{III}. It was unnecessary to remove the elements pptd. from the last three. In the presence of stannous chloride and copper^{I} the blue colour of the tungsten complex deteriorated to pale grey, but was immediately restored when the carbon tetrachloride layer was shaken briskly with fresh acid. This aeration should never be omitted.

No tungsten was found after the deposition by stannous chloride of 10 mg of elementary selenium from selenium$^{IV \text{ and } VI}$, only 50μg of which could be tolerated as such in the test.

Deposition in conc. hydrochloric acid by means of sulphur dioxide was satisfactory, but, if the stannous chloride was afterwards required, it was necessary to expel most of the sulphur dioxide and restore the high concn. of hydrochloric acid. The interference of the precious metals was increased, and only 25-fold excesses of ruthenium, osmium, and iridium could be tolerated. Rhodium, palladium and platinum had to be absent. Interference was not anticipated from the majority of the metallic radicals previously tested. The modified test was applied successfully in presence of mercury, titanium, vanadium, tantalum, niobium, uranium, praseodymium, and samarium.

Detection of small amounts of tungsten in ferrous alloys—Any combination of the following acids, hydrochloric, nitric, phosphoric, hydrofluoric, perchloric and sulphuric, is permissible for effecting solution. A little phosphoric acid should be present, and all but traces of nitric and perchloric acids ultimately removed. The following method is applicable to alloy steels and cast irons.[9] Dissolve 10 mg of steel by heating in a test-tube with 0·15 ml of phosphoric acid (sp.gr. 1·75) and 0·07 ml of perchloric acid (sp.gr. 1·68). Add further 0·1 ml of the latter and 0·2 ml of sulphuric acid (80%) and expel, first, the excess water, and then the perchloric acid, which oxidises the steel components and converts the tungsten into silicotungstic and phosphotungstic acids. When vigorous ebullition ceases raise the temp. until the sulphuric acid fumes. Allow the tube to cool, add 1 ml of conc. hydrochloric acid, and proceed with the modified method for the detection of tungsten, repeating the treatment if necessary.

RESULTS FOR FERROUS ALLOYS—Samples to which it was intended to add a little tungsten were first analysed alone for tungsten, with the following results: British Chemical Standard—(i) Stainless Steel, No. 209 (Mo = 0·093%; no mention of W). W found (2 expts., one with 25 mg), *ca.* 0·007%. (ii) Carbon Steel "R," No. 161 (no W reported by one analyst in qualitative tests). W found (2 expts.), 0·02–0·03%. National Bureau of Standards, U.S.A.—(i) Chromium-Molybdenum Steel, No. 72A (Mo = 0·202%; W not detected by two analysts). W found (2 expts. with 20 and 50 mg), *ca.* 0·001%. (ii) Nickel-Chromium-Molybdenum Cast Iron, No. 107 (Mo = 0·687%; W = 0·002% by 1 analyst). W found, *ca.* 0·005%.

Expts. with 10-mg samples of Nos. 209 and 72A were made with addition to their solns. of 2μg of tungsten in the form of a small portion of a similarly prepared soln. of a tungsten steel. *Ca.* 3μg and 2μg were found, respectively. With all the alloys containing molybdenum a trace entered the organic solvents, necessitating a repetition of the treatment with stannous chloride and dithiol in order to ensure the production of the characteristic *blue* colour.

CONCLUSION—The modified test prescribed for tungsten is so sensitive and distinctive, and the conditions of application so favourable, that it would appear to be suitable for its detection in a variety of materials. The stability of the complex and its ready extraction by means of organic solvents suggest that it may be suitable for the colorimetric determination of tungsten. Quantitative expts. are under consideration.

SUMMARY—Toluene-3 : 4-dithiol in *iso*amyl acetate soln. can be used for the detection of minor amounts of tungsten^{VI} in conc. hydrochloric acid solns. containing large excesses of many foreign cations and anions. The similar reactions of molybdenum^{VI} and rhenium^{VII} can be considerably suppressed, and some important interfering effects (*e.g.*, of iron, copper, gold, arsenic and tellurium) eliminated, by reducing the test soln. with stannous chloride before adding the reagent. Methods are prescribed for the detection of tungsten in molybdenum and rhenium compounds, and in ferrous alloys. As a preliminary separation of tungstic acid is not required, the test would appear to be of wide applicability qualitatively, and perhaps quantitatively.

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CHEMISTRY DEPARTMENT
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Determination of Residual Carbon Dioxide in Aerating Powder

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(*Read at the Meeting, April 5, 1944*)

INTRODUCTION—Aerating powders are evaluated from the available carbon dioxide, which may be determined either directly or by the difference between the total and residual carbon dioxide. Determination of total carbon dioxide presents no difficulty, but results for available and residual carbon dioxide are empirical and cannot be precisely stated without reference to the method of determination. Direct determination of available carbon dioxide is often preferred, since it represents the real value of the product to the consumer. Determination of residual carbon dioxide has an advantage from the analytical point of view, since this figure does not materially alter if the product loses strength on storage through interaction of the components, although there are complications in certain powders which have suffered serious loss in strength.

The Food Standards (Baking Powder and Golden Raising Powder) Order, 1944, S.R.O. No. 46, prescribes standards for available carbon dioxide in baking powder and golden raising powder and requires that they shall yield not more than 1·5% of residual carbon dioxide, determined in the following manner: "A sample of 2 g of baking powder or golden raising powder, as the case may be, shall be treated with 25 ml of water and evaporated to dryness on a boiling water-bath and subsequently treated with a further 25 ml of water and evaporated in like manner. The residual carbon dioxide is the weight thereof evolved when the sample so treated is further treated with excess of dil. sulphuric acid at room temperature, the evolution being completed either by boiling or by means of reduced pressure."

The purpose of this paper is to show the relationship between the results obtained by the specified "double evaporation" method and the calculated excess of sodium bicarbonate for a series of baking powders. An alternative "salt-reflux" procedure having certain advantages in speed and convenience for routine work is also given.

EXPERIMENTAL—*Methods*—The determination of residual carbon dioxide is conveniently considered in two stages: (1) preliminary treatment for removal of "available" gas, and (2) determination of the "residual" gas. We are here primarily concerned with the first of these. The second stage is in line with the determination of total carbon dioxide and a number of satisfactory methods are available. Results quoted in this paper have been obtained using the methods of (i) Bagnall, Potter and Fleming,[1] (ii) Chittick[2] and (iii) Harley and Green.[3] The three methods gave results in excellent agreement.

The method quoted above expresses clearly the preliminary treatment for removal of available gas by the double evaporation method. An alternative salt-reflux procedure is in use in our respective laboratories: Two g of the aerating powder are boiled with 50 ml of saturated sodium chloride soln. in a 250-ml carbon dioxide flask under reflux for 30 min. Preliminary expts. with water as reaction medium had shown that the ratio of water to sample, the total water plus sample, and the time and rate of boiling, could all lead to variations in the result, particularly with powders containing acid sodium pyrophosphate. In the salt-reflux method it is important that the rate of boiling should be brisk and the Bunsen flame should be adjusted so that the contents of the flask reach b.p. within 4 to 5 min. The flame is then maintained at this height throughout the 30 min. boiling. The salt greatly reduces the tendency towards frothing, which can be completely controlled by adding a few mg of cetyl alcohol or 1 to 2 ml of amyl alcohol. The size and type of the water condenser has no effect on the result. The salt soln. should have a sp.gr. of *ca.* 1·20 (Twaddell 40°) and its neutrality should be confirmed with bromothymol blue indicator.

The salt-reflux procedure has been used for direct determination of available CO_2 with the apparatus of Bagnall, Potter and Fleming,[1] a water condenser being introduced after the reaction flask and the time of boiling extended to 30 min. Subsequently, residual CO_2 can be estimated on the same portion of the sample, by successive acid treatment and absorption in fresh portions of barium hydroxide soln. Bagnall *et al.* report that they were unable to repeat their results with sufficient accuracy with the gasometric apparatus of Chittick,[2] used in the official A.O.A.C. method. We find that this apparatus

can give reproducible and precise results for total CO_2 and for residual CO_2, provided that the preliminary treatment in the latter determination is standardised. We find the apparatus of Hartley and Green[3] convenient for determining both total and residual carbon dioxide. It is based on the principle of absorption in barium hydroxide soln. at reduced pressure, as in the Hepburn[4] method, which was adapted for routine work by Bagnall *et al.*[1] Available CO_2 cannot be determined directly in the Chittick[2] apparatus and has not been attempted by the Hartley and Green[3] method.

Baking Powders—Two baking powders with 12% of total carbon dioxide were prepared, one containing tartaric acid and the other acid sodium pyrophosphate, as representing the two main classes of aerating powders. The tartaric acid had an acidity of 100% and 100 parts of the acid sodium pyrophosphate were equiv. to 73·5 parts of sodium bicarbonate, both being comparable with commercial materials. The baking powders were formulated to have a calculated excess balance of 5% of sodium bicarbonate and so to give values for residual carbon dioxide approaching the prescribed limit of 1·5%. This figure of 5% excess is appreciably greater than would be considered good trade practice, although a small excess of alkali is usual.

Determinations of residual CO_2 were carried out on the powders as made and also with increments of the respective acids calculated so that results were available with sodium bicarbonate in excess balance at 5%, 3%, 1% and minus 1%. Further determinations on these powders were made with added increments of sodium bicarbonate to give excess balances of 10% and 15%.

Sodium Bicarbonate—To obtain evidence of its decomposition under the conditions of the present tests, expts. were carried out with sodium bicarbonate alone. Results by the reflux method are shown in Table III as the proportion of the original CO_2 found as "residual," *i.e.*, as % retention. Thus 50% retention represents complete conversion of bicarbonate into carbonate.

Expts. with bicarbonate by the double evaporation method (not included in the table) showed 50% retention, as would be expected by consideration of the details of the procedure. It cannot be assumed that the same degree of decomposition takes place in presence of the baking powder residues.

RESULTS—Table I gives the results of the individual determinations. Average figures from this table are entered in Table II and are expressed as the retention % of the CO_2 present in the excess sodium bicarbonate which was found as "residual." The results at the high excesses of 10% and 15% show that there is about 50% retention of the CO_2 in the salt-reflux method compared with about 60% in the double evaporation method. At the lower excess levels of 5% and 3% this difference is masked to some extent because of the ratio of the experimental error to the residual CO_2 found. As a consequence, for normal aerating powders there is good agreement between the two methods, but products exceeding the limit of 1·5% residual CO_2 will give significantly higher results by the double evaporation method than by salt-reflux.

Table II shows that the retention of CO_2 is less than the 75% reported by Macara[5] after 30 minutes' boiling under somewhat different conditions.

Reverting to Table I, it is shown that there is good agreement between the results with the two types of powder at the same calculated excess balance.

Table III gives the results of expts. with sodium bicarbonate only, when boiled under reflux in salt soln. and water respectively. Conditions are different from those when baking powder residues are present; a point of interest is that the retention of CO_2 is much the same in salt soln. as in water after normal boiling times, but that retention is greater in the salt soln. on prolonged boiling. This effect is attributed to suppression of hydrolysis of carbonate and bicarbonate ions by the high concn. of sodium ions. A point of more general interest is the formation of some degree of caustic alkalinity on boiling a 1% soln. of sodium bicarbonate under reflux for 3 hr.

DISCUSSION—A general principle in devising an empirical method of this type is that an attempt should be made to simulate the conditions under which the material is to be used. The double evaporation procedure fits in with this principle, since the water treatment may be considered comparable with conditions in a moist dough or batter and the evaporation as corresponding to the baking process. In the reflux method, it may be argued that the use of saturated salt soln. is different from the conditions in a cake batter; but in cake the medium is certainly not distilled water but a strong soln. of sugar and other extractives,

TABLE I

Sodium bicarb. balance	Percentage residual carbon dioxide: By double evaporation — Individual			Aver.	By salt-reflux — Individual			Aver.
Tartrate baking powder								
Minus 1%	0·07	0·05			nil	nil		nil
	0·03	0·09		0·06	nil	nil		
Plus 1%	0·31	0·46			0·29	0·22		
	0·18	0·19		0·28	0·17	0·13		0·20
„ 3%	0·98	0·97			0·91	0·91	0·84	
	0·76	0·78		0·87	0·70	0·74		0·82
„ 5%	1·40	1·40	1·50		1·34	1·43	1·37	
	1·38	1·38		1·41	1·38	1·22		1·38
„ 10%	3·15	3·15			2·50	2·55		
	3·24			3·18	2·69			2·58
„ 15%	4·50			4·50	3·94			3·94
Phosphate baking powder								
Minus 1%	0·07	0·07			0·06	0·09		
	0·18	0·14		0·11	nil	nil		0·03
Plus 1%	0·28	0·33			0·22	0·30		
	0·31	0·30		0·30	0·18	0·27		0·28
„ 3%	0·98	0·97			0·91	0·88		
	0·87	0·85		0·92	0·80	0·76		0·82
„ 5%	1·40	1·42	1·46		1·37	1·47	1·45	
	1·47	1·46	1·35	1·42	1·47	1·43		1·44
„ 10%	2·91	3·10			2·50	2·53		
	3·11			3·04	2·69			2·57
„ 15%	4·65			4·65	3·85			3·85

TABLE II

Sodium bicarbonate balance: As $NaHCO_3$ %	CO_2 equiv. %	Residual carbon dioxide: Double evaporation — Found %	Retention %	Salt-reflux — Found %	Retention %
Tartrate baking powders					
Plus 3	1·57	0·87	55	0·82	52
„ 5	2·62	1·41	54	1·38	53
„ 10	5·24	3·18	61	2·58	49
„ 15	7·85	4·50	57	3·94	50
Phosphate baking powders					
Plus 3	1·57	0·92	59	0·82	52
„ 5	2·62	1·42	54	1·44	55
„ 10	5·24	3·04	58	2·57	49
„ 15	7·85	4·65	59	3·85	49

TABLE III

Bicarbonate Concentration	Time of reflux	Residual CO_2 as % of original: In salt soln.		In water	
0·25 g in 50 ml	30 min.	61	62	65	69
0·5 g in 50 ml	30 „	60	58	59	62
1·0 g in 50 ml	30 „	60	61	60	62
0·5 g in 50 ml	1 hour	54	—	53	—
0·5 g in 50 ml	3 hours	47	49	43	44

at a *p*H largely influenced by that of the flour. It is generally accepted that powders based on acid sodium pyrophosphate give more difficulty than tartrate powders when water is used as reaction medium. The sodium chloride in the salt-reflux method reduces the hydrolysis of $Na_4P_2O_7$ and sharpens the end-point of the reaction, which is complete at a lower *p*H than in absence of the sodium chloride. Gerber and Miles[6] illustrate this effect by titration curves for pyrophosphoric acid. At *p*H 8·4, 97% of the acid is neutralised in presence of 20 g of sodium nitrate per 100 ml as compared with 75% without the added salt. At *p*H 9·1, the degrees of neutralisation are 100% and 93% respectively. A similar effect occurs in the tartrate powder, as shown by the lower *p*H found with both the experimental baking powders after reflux with salt soln. as compared with water. This problem of hydrolysis is eliminated in the double evaporation method by the actual process of evaporation.

It should be noted that *p*H results on solns. of aerating powders in water or salt soln. bear no relation to *p*H values for the crumb of goods baked with these powders. Any attempt to correlate the conditions in any such chemical determination with actual baking conditions will be unsuccessful unless it can take into account the acidity and buffer action of the flour and other ingredients of the dough or batter.

The conditions of rate and time of boiling must be adhered to in the reflux method, since further loss of CO_2 by hydrolysis takes place slowly on continued boiling. This method shows a definite advantage in time and attention required over double evaporation, but appears to be less suitable for general work where analysis of aerating powders is not a routine matter.

Aerating powders normally contain a proportion of cereal matter as filler and this material may have a slight acidity. Maize starch was the filler in the baking powders used in our expts. and the acidity was found to be small and not to have any significant effect on the results.

SUMMARY—Official standards have been prescribed for baking powder and golden raising powder. The official "double-evaporation" treatment for removal of available carbon dioxide is compared with an alternative "salt-reflux" procedure having advantages in speed and convenience for routine work. Both methods are applied to baking powders having known excess of sodium bicarbonate.

Retention of the carbon dioxide present in the excess of sodium bicarbonate is of the order of 60% by the double-evaporation method and 50% by the salt-reflux method.

Results with sodium bicarbonate only, under similar conditions of test, are given.

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DISCUSSION

Dr. J. R. NICHOLLS confirmed that the authors' salt reflux method gave results in good agreement with the Official method. He was particularly interested in the tables showing the relationship between residual carbon dioxide and the excess sodium bicarbonate.

Mrs. G. DIMMICK asked whether release of carbon dioxide was complete from the hard gelatinous film formed in the double evaporation method. How was the difficulty of frothing overcome?

Mr. G. TAYLOR agreed that both methods gave concordant and reproducible results. He actually used double evaporation and considered some degree of standardisation of procedure was essential; this point was covered by both methods.

Dr. J. H. HAMENCE also confirmed the agreement and emphasised the ease of manipulation in the salt reflux method. He noted 50% decomposition of the excess sodium bicarbonate compared with 25% reported by Macara.

Dr. D. C. GARRATT asked whether the authors had any information on the allied subject of determination of residual carbon dioxide in self-raising flour.

Mr. R. W. MORRIS, in reply to Mrs. Dimmick, stated that no difficulty had been experienced in releasing the carbon dioxide after double evaporation. In the Potter, Bagnall and Fleming method, boiling with dilute acid caused rapid dissolution of the mass. The salt reduced frothing and this feature was readily controlled by cetyl or amyl alcohol as mentioned in the published text. In reply to Dr. Hamence, Mr. Morris pointed out that the conditions under which Macara found 25% decomposition of the excess sodium bicarbonate were different from those in the present experiments. In particular, the rate of boiling was considerably less rapid. On the question of self-raising flour raised by Dr. Garratt, the authors had carried out some preliminary experiments and it appeared that the salt reflux procedure was applicable, but it would probably be necessary to reduce the time of boiling to 5 minutes to obtain results in agreement with the S.R. & O. method. These tests had been carried out with 4 g of sample in 150 ml of salt solution but, owing to the small amount of carbon dioxide involved, the authors proposed to use 10 g in 300 ml in any further experiments.

A Rapid Method of Micro Gas Analysis*

BY W. B. PRICE, B.SC., PH.D., F.I.C., AND L. WOODS, M.SC., A.I.C.

SEVERAL procedures have been proposed in recent years for the quantitative determination of the components of gas mixtures of small volume. These methods differ in the complexity of the apparatus needed and in the volumes of gas with which they will deal. The present account refers to one such method, details of which were published about 10 years ago, and which has now been somewhat amplified. It is particularly suited to the examination of the gases which occur in bubbles found in glass.

The method originated with a biochemist, Krogh,[1] who wished to examine the gases dissolved in blood. It was then developed and applied to the examination of the gases in bubbles in glass by Enss.[2] It consists in isolating the bubble of gas, confining it in glycerin, and measuring the diameter by means of a microscope. The bubble is then surrounded with a series of liquid reagents in turn of the type used in the macro-analysis of inorganic gases. After each treatment it is returned to glycerin, and its diam. is re-measured. A decrease after treatment with any reagent is taken to indicate the presence of the corresponding gas, and the amount of such decrease to represent the quantity present. Thus, assuming the bubble to be spherical, the original volume

$$v_1 = \frac{\pi D_1^3}{6} \text{ and the final vol. } v_2 = \frac{\pi D_2^3}{6};$$

the percentage decrease then

$$= \frac{v_1 - v_2}{v_1} \times 100 = \frac{D_1^3 - D_2^3}{D_1^3} \times 100 \text{ or } 100\left(1 - \left[\frac{D_2}{D_1}\right]^3\right)$$

This procedure has a number of advantages and also some disadvantages. The advantages are, briefly, comparative simplicity of apparatus, speed, and ability to deal with quite small vols. of gas. Of the apparatus needed, a microscope fitted with a mechanical stage, and having an eye-piece scale, is the most expensive. The remainder consists largely of glass pipettes of special shape, which are easily made, glass capillary tubes provided with taps, and metal boxes to hold the glycerin. Dissecting needles, tweezers, and a good hand magnifying glass are also necessary. There have also been developed two pieces of apparatus specially for controlling the piercing of bubbles in glass, one being for use in conjunction with the microscope. They are used when it is desired to allow a liquid reagent to flow into a bubble in a piece of glass and to observe the effect, either at the moment of piercing or at leisure afterwards.

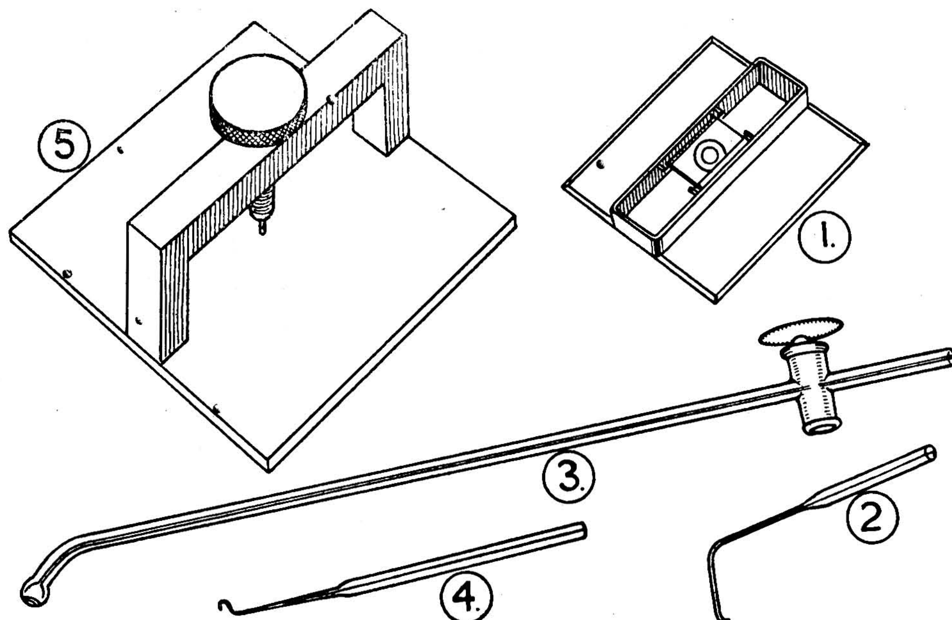
It has been found that about 1 hr. is sufficient for a full analysis which includes the determination of hydrogen sulphide, carbon dioxide, oxygen, carbon monoxide and hydrogen. If the greater part of the mixture consists of one of the first two or three gases to be tested for, it is often possible to stop at that point, with a consequent shortening of the time. This obviously depends on the amount of information required about the minor constituents of the mixture.

The min. volume of gas which can be comfortably dealt with is that contained in a bubble whose diameter is about 0·2 mm, *i.e.*, about 0·004 cb.mm. Much, of course, depends on the actual composition of the gaseous mixture, since if there is, *e.g.*, *ca.* 90% of hydrogen

* Paper read at the symposium held by the Microchemical Club, the South Yorkshire Section of the Royal Institute of Chemistry and the Sheffield Metallurgical Association, at Sheffield on October 9th, 1943.

sulphide in a gas bubble of 0·2 mm diam., the bubble will shrink after treatment with the first reagent to so small a size as to render further manipulation impossible. With such mixtures, however, the composition of the residue is often of little importance.

Method—Grind the surface of the glass nearest to the bubble by means of an abrasive, such as carborundum, until about 1/1000″ separates it from the interior of the bubble. Then immerse the glass in the glycerin contained in one of the metal boxes or troughs mentioned above (Fig. 1). It is convenient to have a number of these boxes at hand;



suitable internal dimensions are 3″ long, 3/4″ wide, and 1/2″ deep, and the material may be brass or copper sheet. Each box is provided with a central circular window in the bottom, which may be 1/2″ in diameter, and two narrow brackets in the centre of each long side near the top to hold a shortened microscope slide, which must be directly over the window. The bubbles are more easily followed by eye if the interior of the box is painted black. Pour glycerin into the box until it makes contact with the underside of the microscope slide, taking care to avoid the introduction of air bubbles. A cover should be provided to exclude atmospheric moisture when the box is not actually being used, since the highly hygroscopic nature of the glycerin causes rapid absorption of any moisture that is available and subsequent measurements of bubble diameter are made difficult or impossible by the resulting striae.

During the introduction of the glass into the glycerin take care once more to avoid the carrying in of air bubbles. Push the glass under the piece of microscope slide, pierce the bubble wall with a sharp point, and remove the gas bubble from the cavity by means of a bent dissecting needle, allowing it to rise through the glycerin until it comes to rest under the slide. Next measure the diameter; it is sufficient to know it in terms of scale divisions, since only the relative decreases are needed. Remove the bubble by sucking it into a bent capillary pipette (Fig. 2) which has previously been partly filled with glycerin. The internal diameter of the pipette should not be appreciably smaller than that of the bubble; about 0·5 mm has been found to be generally suitable. Transfer the bubble to a soln. of cadmium acetate in glycerin, contained in a small basin. Immersed in the solution is a piece of glass tube, *ca.* 1 cm in diam. and 1½–2 cm. long, lying on its side; introduce the bubble into this through one of the open ends. Roll the tube over so that the bubble is carried down, and then allow it to rise, passing through fresh reagent as it does so; repeat the operation as often as desired. After an immersion of 5 min. remove the bubble by means of the pipette (the outside of which has first been wiped) and transfer it first to a glycerin wash trough and allow it to rise through clean glycerin several times to remove cadmium acetate solution, and then to the original trough, and again measure its diameter.

The decrease is taken to represent hydrogen sulphide. Repeat the whole procedure, using a soln. of caustic potash in glycerin, for the determination of carbon dioxide.

The remaining reagents are aqueous solns., and, as the low viscosity of water compared with that of glycerin causes difficulty in the control of the bubble, a different form of apparatus has been devised (Fig. 3). This, consisting essentially of a piece of capillary tubing, about 30 cm long and 1·5 mm in internal diam., has the advantages, first that the bubble can be caused to move along the length, and so comes continuously into contact with fresh reagent; secondly, that the exposure of the reagent itself to the atmosphere is reduced to a minimum, thereby practically preventing the absorption of atmospheric oxygen by two of the reagents (sodium hydrosulphite and cuprous chloride); thirdly, that the reagent does not become contaminated with glycerin.

The capillary tube is provided with a tap 5 cm from one end. At the other end a small bulb, about 5 mm in internal diam., is blown, with an opening, about 3 mm across, opposite the capillary. Through this opening the bubble is introduced and removed. About 3 cm from this bulb the capillary tube is bent through an angle of about 15 degrees. The tube is supported on a small stand; its normal position is horizontal, but provision is made to turn it almost vertically in either direction. These tubes, of which several are needed, must be kept perfectly clean to prevent the bubbles from adhering, and it is desirable to keep them in chromic acid cleaning soln. when not in use.

For use, the tube is filled with reagent by means of a rubber teat, great care being taken to avoid the entry of air bubbles. The meniscus at the bulb end should lie across the opening in the bulb; when the tap is closed and the tube held vertically so that the open end hangs downwards, no liquid runs out. Before the bubble is introduced the tube is arranged horizontally, with the bend at the bulb end pointing downwards. The bubble is introduced from the pipette, and the tube is then tilted so that it travels up the tube almost up to the tap. It is then brought back and removed by means of a pipette of a slightly different shape (Fig. 4). Usually one passage through the reagent suffices. Some practice is needed at first in introducing and removing the bubble, but the necessary skill is easily acquired, and failures thereafter are rare. Separate tubes are used for each of the three aqueous reagents, and the washing and measuring already described are repeated after treatment in each of them. These reagents, for oxygen, carbon monoxide and hydrogen, are respectively alkaline sodium hydrosulphite soln. (which does not discolour), ammoniacal cuprous chloride soln., and colloidal palladium soln. containing sodium picrate. The last of these is rather more difficult to use than the others, since it is black and opaque, and considerable care is needed in watching the bubble during treatment. The residue after the final operation is assumed to be nitrogen.

TESTS FOR SULPHUR DIOXIDE, HYDROGEN SULPHIDE AND WATER VAPOUR IN BUBBLES IN GLASS—Qualitative tests have been developed for these substances in bubbles in glass. For the first two of them a simple pricker is sufficient (Fig. 5). This consists of a base plate, about 6″ square, carrying two pillars and a cross bar. Through the centre of the latter there acts a vertical screw carrying a sharp steel point at its lower end. It is only necessary to place the glass containing the bubble, prepared as already described, under the point and to screw the latter down, to effect a controlled piercing of the bubble. In use, for the test for sulphur dioxide, after the glass is in position, with the steel point touching the glass over the bubble, a drop of Steigmann's reagent[3] is placed round the point. Since the gas in almost all bubbles in glass is under reduced pressure, the reagent is sucked in as soon as the wall has been pierced. In presence of sulphur dioxide a reddish-purple colour is developed on standing.

For hydrogen sulphide tests the conditions are slightly modified. The sharp point is replaced by a blunt rounded one, and a small piece of filter-paper, wet with mercuric chloride soln., is interposed between it and the surface of the glass. The screw is turned only so far as to crack the glass, without piercing the paper. In this way the filter paper is exposed to the action of the gases in the cavity. It is removed and washed, and is then treated with a drop of sodium azide-iodine soln,[4] and observed under the microscope. An evolution of bubbles from that part of the paper previously in contact with the gases indicates the presence among the latter of hydrogen sulphide.

The test for water vapour is less certain, but can be useful on occasion. Use is made of Karl Fischer's reagent,[5] and a more elaborate form of pricker is used. This is similar in construction to a microscope mechanical stage and is used mounted on a microscope, the tube of which is set horizontally. Its purpose is to allow of microscopical inspection

of the bubble at the moment when the reagent enters the cavity and immediately afterwards. The reagent is so sensitive to atmospheric moisture that it is essential to observe its behaviour in this way. A uniform and immediate change of colour from brown to yellow indicates the presence of water or water vapour.

DISADVANTAGES OF THE METHOD—The chief of these is that some of the gases encountered are appreciably soluble in glycerin alone. Thus sulphur dioxide is very readily soluble, hydrogen sulphide appreciably so, and carbon dioxide fairly so. The presence of any of these, but especially of the first, therefore reduces the accuracy of the results. The solubilities of the remaining gases are lower and have little effect in the time normally taken for an analysis.

Other disadvantages are that a tendency towards decomposition has been observed on the part of some of the reagents, that the bubbles are not quite spherical, as is assumed in making the calculations, and that there may be a small error in making the measurements. A way, although rather a laborious one, of allowing for these errors (except the decomposition of the reagents, which is thought to be affected chiefly by temperature changes) is to make analyses of a systematic series of mixtures the compositions of which are already known. The accumulation of a series of such results would in time greatly increase the usefulness of the method and the analyst's confidence in the accuracy of his results.

APPLICABILITY OF THE METHOD—The method is capable of providing valuable information where great accuracy is not needed, but it deals only with inorganic gases, and in the present instance has been applied exclusively to the examination of glass, for which it is particularly adapted. There is no reason, however, why it should not be useful under other conditions in which gaseous mixtures are confined in a small space from which they can easily be released.

REAGENTS—(1) *Cadmium acetate soln.*: 10 g of cadmium acetate in 100 ml of anhydrous glycerin. (2) *Potassium hydroxide soln.*: 10 g of potassium hydroxide in 100 ml of anhydrous glycerin. The dishes containing this and the previous soln. should be kept in a desiccator when not in use. (3) *Sodium hydrosulphite soln.*: 0·5 g of sodium hydrosulphite ($Na_2S_2O_4$) is dissolved in 3 ml of a soln. containing 40 g of potassium hydroxide in 277 ml of water. (4) *Cuprous chloride soln.*: 11·25 g of ammonium chloride are dissolved in a mixture of 14 ml of ammonia (sp.gr. 0·880) and 33 ml of water, and 9 g of freshly purified cuprous chloride are added. Both this and the previous soln. may conveniently be stored in an atmosphere of nitrogen. (5) *Colloidal palladium soln.*: 0·06 g of solid palladium preparation is dissolved in 4·35 ml of a sat. soln. of sodium picrate.*

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PILKINGTON BROTHERS, LTD.
RESEARCH LABORATORIES, ST. HELENS

December, 1943

* The colloidal palladium preparation is made as follows: Dissolve 0·5 g of palladium chloride by boiling in a mixture of 8 ml of water and 2·8 ml of 2 *N* hydrochloric acid. Also, dissolve 0·65 g of sodium protalbinate (see below) in 16 ml of water and add 10 ml of approx. 2 *N* sodium hydroxide, followed by the soln. of palladium chloride (slow addition). Add slowly an excess (about 5 ml) of a 50% aq. soln. of hydrazine hydrate. This causes the soln. to turn black, with evolution of nitrogen. Dialyse the resulting liquid in diffusion shells for 3 days. During the first day use running tap water, followed on the second and third days by twice-daily changes of distilled water. At the end of the period remove the soln. from the shells and evaporate it to dryness in a vacuum desiccator over fused calcium chloride. It forms black shining plates, soluble in water.

The sodium protalbinate is made as follows (Paal)[6]: Add 100 g of egg albumin in small portions to a soln. of 15 g of sodium hydroxide in 500 ml of water in a flask. Distribute the albumin uniformly by shaking, and heat the mixture on the water-bath until everything has dissolved, except a small flocculent residue; this takes about 1 hr. During the heating there is a continuous evolution of ammonia. Next filter the soln. and treat the alkaline filtrate in a large dish with dil. acetic acid as long as a ppt. forms. During the addition of the acid the strongly foaming mass gives off considerable quantities of hydrogen sulphide. After about 12 hr. collect the protalbinic acid (which will have settled, partly in fine flocks and partly in larger white lumps) on a filter and wash with a little water. Dissolve the protalbinic acid in the min. amount of sodium hydroxide soln., and dialyse the resulting soln. for 3 days. Then concentrate the soln. and carry out the final evaporation in a vacuum desiccator. The residue of solid sodium protalbinate is a white amorphous powder or a yellow brittle vesicular mass.

Notes

IDENTIFICATION OF DEMOLITION DUST

SHORTLY before the war we were asked to examine several new costumes which, it was alleged, had been spoiled by the dust produced in the demolition of an adjoining building penetrating into the room in which the costumes were hung. Samples of the demolition dust were submitted for comparison.

To extract the dust from the garments, a piece of finely woven white silk was stretched across the penultimate joint of the tube of an ordinary vacuum cleaner and over it was fitted the nozzle joint. By passing the nozzle over the surface of the different dresses a few mg of dust were extracted from each and could afterwards be brushed from the silk diaphragm without detaching any significant amount of silk fibres.

Microscopical examination of the demolition dust showed that it contained numerous light particles which could be separated by flotation on water. These were identified as wood by their cellular structure and analogous particles were separated from the dust extracted from the dresses. It was concluded that other yellow particles floating on the water consisted of paint, and similar particles were present in the dust from the dresses (see Figs. 1, 2 and 3). The wood was separated from the paint by treatment with

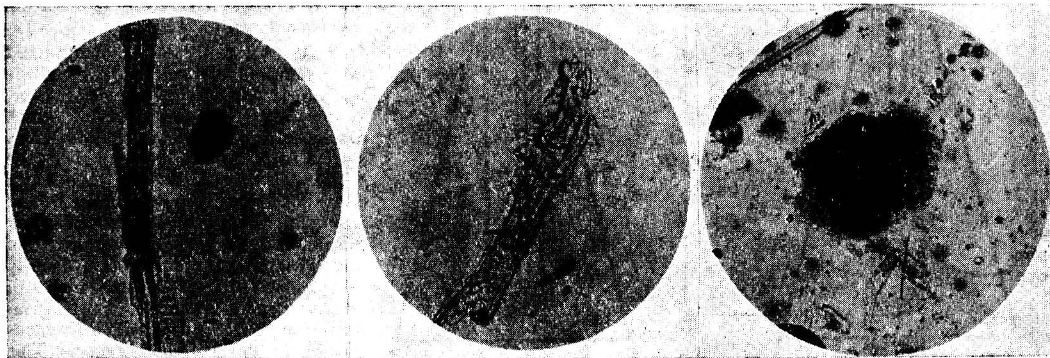

Fig. 1	Fig. 2	Fig. 3
Wood and paint in demolition dust × 125	Wood in dust from dress × 125	Paint and wood in dust from dress × 125

50% alcohol, in which the paint sank. The heavy sediment which settled in the treatment with water had sp.gr. 2·28. It contained calcium sulphate and an aluminium salt and was probably Keene's cement (sp.gr. *ca* 2·3). The following table shows the comparative results obtained in typical tests:

	Demolition dust	Violet dress	Black coat
Micro-crystalline sediment from water:	$CaSO_4$.Al salt	$CaSO_4$.Al salt	$CaSO_4$.Al salt
Paint:	Lead, iron, trace of zinc	Lead, iron, no zinc found	Lead, iron, trace of zinc

Samples of ordinary street dust did not contain wood or paint and consisted mainly of siliceous material and organic débris.

February, 1944

T. J. WARD
C. AINSWORTH MITCHELL

REFLUX APPARATUS FOR AUTOMATIC DISPERSION OF FROTH IN THE DETERMINATION OF FIBRE

THE estimation of fibre in cocoa and feeding stuffs by successive heatings under reflux with acid and alkali is invariably accompanied by some frothing and necessitates constant attendance. Frothing can be checked by applying a damp cloth to, or by blowing on the walls of the flask. The use of anti-froth agents does not comply with the official method of the Fertilisers and Feeding Stuffs Regulations (S.R. & O., 1932, No. 658).

Even with moderate frothing, particles of material tend to adhere to the walls of the flask, and are very difficult to swill down by rotation. Such particles as remain attached are removed from the hydrolysing action of the reagent, and hence are more or less incompletely digested, depending upon the stage of the process at which they are deposited.

Recently a series of fibre determinations was carried out on a cattle food which, when digested under reflux, frothed excessively in every test, and this could be suppressed only by frequent removal of the Rose burner. The resulting fibre figures were not concordant, ranging in 4 tests from 10·31 to 10·64; mean 10·47%. Under such conditions it is essential to suppress frothing.

We have found that froth can be dispersed automatically by maintaining the cooling of the condensate after it has passed from the condenser to the mixture under reflux. This is effected by allowing the condensate to flow down a secondary water-cooled condenser supported inside the reflux flask, thus effecting a cooling at the surface of the liquid.

The anti-froth device (Fig. 1) can be used in the form of an adaptor by permanently connecting the secondary condenser in series with the reflux condenser. When required for fibre determinations the delivery end of the latter condenser is merely inserted into the socket at B, and lowered until contact is made with the feed-bridge C. The dimensions assigned to the secondary condenser are suitable for a litre Pyrex flask. Although the secondary condenser can be considerably elaborated to give a more efficient cooling of the condensate, it is shown here in a simple form.

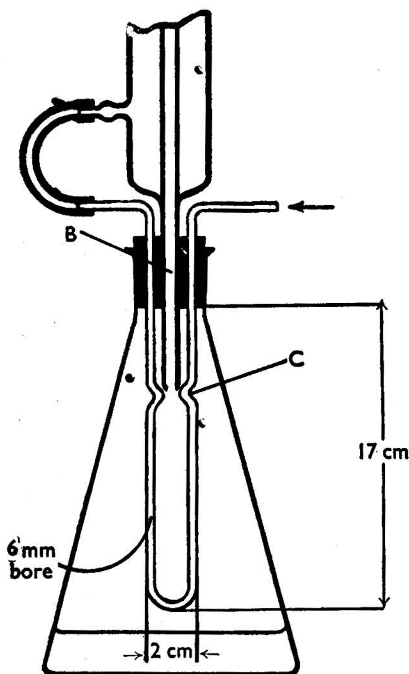

Fig. 1

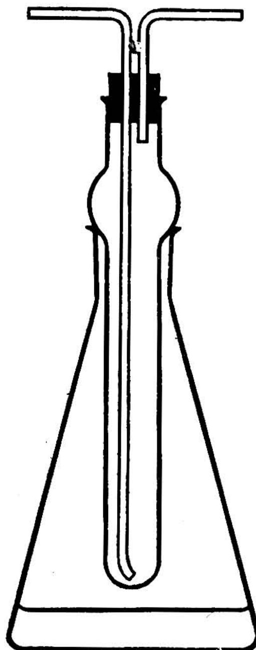
Fig. 2

A parallel set of fibre determinations on the same sample was made with the aid of this device, and the results were much more consistent than before, ranging in 4 tests from 10·24 to 10·28; mean 10·27%. The mean figure obtained was a little lower than in the previous test, suggesting more complete digestion. Moreover, the subsequent filtration was easier.

Fig. 2 is an alternative modified form particularly suitable for routine work. It consists of a boiling tube, 24 cm long × 2·5 cm in diam., which fits loosely into the neck of a litre Pyrex flask, and is supported by the 4-cm diam. bulb. The condenser end should be approximately 1·5 cm from the surface of the mixture under reflux. Units can be ganged in series without the congestion usually associated with reflux condensers of normal type. The rate of condensation for both forms of apparatus should be *ca.* 1 drop per sec. This corresponds to gentle boiling. In most instances frothing is completely eliminated.

It is important when transferring the material to the reflux flask to reserve an adequate portion of the reagent for rinsing down adhering particles from the walls of the flask. Since particles can be deposited by external agitation as well as by frothing, the normal procedure of rotating the flask is not only unnecessary but undesirable.

We intend to apply a similar technique to other problems involving frothing.

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August, 1943

THE DETECTION OF INDOLE BY MEANS OF XANTHYDROL

During a study of the effect of interfering substances on the pptn. of urea with xanthydrol it was observed that indole forms a violet pigment with xanthydrol in acid solns. As this colour reaction was not given by any other familiar biological compound examined, it should be of value in the detection and estimation of indole.

Method—Mix 0·5–1 ml of the test soln. with 2–5 ml of glacial acetic acid. Add a small quantity (5 mg) of xanthydrol and boil. In presence of indole in concns. as low as 1 : 50,000 in the final mixture a bright violet colour develops in *ca.* 1 min. and deepens as the mixture is shaken with air. The reaction also proceeds, but much more slowly, at room temp. The lower limit of the test is *ca.* 1 : 10^6, and hence it approximates in delicacy to the indole-aromatic aldehyde reactions studied by Denigès.[1] The pigment is stable in acid solns. and survives at least 6 months' exposure to light and air, in absence of direct sunlight. As the unchanged xanthydrol is pptd. by addition of water, it is necessary to use acetic acid or alcohol as diluents for colorimetric work.

Selectivity—The test appears to require the presence of an unsubstituted H in the β-, or 3-, position in the indole nucleus. The reaction is not given by scatole, β-indole propionic acid, tryptophan or indoxyl, or by any of the common amino acids, proteins, purines, amides, amines, ureides, guanidines or water-sol. vitamins. The test is also negative with normal urine, blood plasma, milk, saliva, gastric juice, bile, pancreatic juice, and cerebro-spinal fluid, although it will reveal traces of indole that have been added to these liquids. The presence of urea in urine does not seriously interfere with the test, and the characteristic colour can be seen even before the ppt. of dixanthyl urea has separated out. Among non-biological compounds, free pyrrole gives a similar reaction, while barbituric acid, but not the substituted barbiturates, yields a deep purple colour (Kidd[2]).

Reagents—The xanthydrol may be applied as the solid or as a 5–10% soln. in aldehyde-free methyl or ethyl alcohol. Such solns. are active for at least a year, although xanthydrol is unstable, and even in

solid form slowly reverts to a mixture of xanthene and xanthone (Kny-Jones and Ward)[3], neither of which gives a colour with indole. Acids stronger than acetic, if used as condensing agents, have the disadvantage that they form a yellow oxonium salt with the xanthydrol. Even the soln. in acetic acid acquires a faint yellow on boiling, but this disappears when the soln. cools. With conc. hydrochloric acid colour formation occurs rapidly in the cold and, if the mixture be boiled, the test loses some of its selectivity, and both free tryptophan and tryptophan-containing proteins eventually react, presumably owing to indole formation by decomposition. Acetic acid contaminated with glyoxylic acid will give a pink colour when boiled with indole; this effect, however, is suppressed in presence of excess of xanthydrol.

Mechanism—The properties of the pigment and the circumstances of its preparation suggest that it is a compound of the indolidene-methane type investigated by Burr and Gortner,[4] formed by oxidation of the 3-xanthyl-indole precursor (Illari)[5]. Fosse[6] has obtained a colourless, crystalline dixanthyl-indole, but does not refer to the formation of the violet pigment, although he observes that the dixanthyl derivative decomposes at 205°–214° C., "pour donner un liquide rouge foncé." Fosse does not appear to have published the exact details for the preparation of dixanthyl-indole. The compound is readily obtained when a conc. soln. of indole in glacial acetic acid is boiled with excess of xanthydrol. Under these conditions the acid is buffered to such an extent that the violet pigment does not form, which may be the reason why Fosse has not referred to it. The pigment can be isolated as an acetate (m.p. 170–172° C.) from the interaction between equimolecular proportions of indole (117 mg) and xanthydrol (198 mg) in 90% acetic acid (100 ml). The mixture is gently boiled for 10 min., left overnight, and treated with 30–40 ml of aldehyde-free ether, which ppts. any dixanthyl-indole that may have formed. Excess of ether bleaches the pigment, and the pigment-base is pptd. After filtration from any xanthyl-indole present, the mixture is concentrated *in vacuo* until the pigment separates as a crust on the side of the container. It is extracted with 50–70% acetic acid, and recrystallised.

SUMMARY—Dil. solns. of indole yield a stable purple pigment on being boiled with xanthydrol and excess of acetic acid. Among biological compounds, the reaction appears to be selective for indole.

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DEPARTMENT OF BIOCHEMISTRY
TRINITY COLLEGE, DUBLIN

W. R. FEARON
February, 1944

Ministry of Food

STATUTORY RULES AND ORDERS*

1944 No. 135. The Canned Fruit and Vegetables Order, 1944. Dated February 8, 1944. Price 3d.

This Order revokes and replaces (with Amendments) S.R. & O., 1943, Nos. 144 and 901; S.R. & O., 1943, No. 236; S.R. & O., 1943, No. 1087. *The sale of canned fruit and vegetables other than those for which maximum prices are prescribed (viz., apples, cherries, plums [including damsons and greengages], blackberries, gooseberries, loganberries, rhubarb ; beans, beetroot, carrots, celery, macedoine, parsnips and turnips, potatoes [other than mashed], peas, spinach, beans [baked or steamed in sauce], vegetable salad [with mayonnaise]) is prohibited, except for ships' stores or if sold before April* 1, 1944, *at prices regulated by the revoked Orders.*

The preservation of fruit hitherto regulated under the Jam, Marmalade and Preserved Fruit (Licensing and Control) Order, 1941, is now controlled under this Order.

— No. 136. The Flour Confectionery (Control and Maximum Prices) Order, 1944. Dated February 9, 1944. Price 2d.

This Order revokes S.R. & O., 1943, No. 688, but re-enacts most of its provisions. *Its principal alterations include the following :*

(i) Trifles, ready-made puddings and uncooked pastry have been brought within the scope of the Order.

(ii) A minimum fat and sugar content of 14% has been introduced for goods retailed at more than 8d. per lb. and a minimum fat content of 25% is prescribed for uncooked pastry.

In Sec. 9 (*e*) *ether or other appropriate solvent may now be used (instead of ether only) for extracting the sample for the determination of oils and fats.*

— No. 167. The Retail Prices (Notices) Order, 1944. Dated February 17, 1944. Price 1d.

This Order revokes and re-enacts S.R. & O., 1942, *No.* 494, *and its amending Orders. The Schedule gives a complete list of the groceries and provisions in respect of which a price list must be available together with the respective Orders controlling them. Soft drinks and soya flour are now included.*

"Sale by retail" is defined as any sale to a person buying otherwise than for the purpose of re-sale, but does not include any sale to a person for the purposes of an establishment carried on by him or to a manufacturer for the purposes of a manufacturing business carried on by him.

* Obtainable from H.M. Stationery Office. Italics signify changed wording.

NATIONAL FLOUR AND BREAD. FOURTH REPORT FROM THE SCIENTIFIC ADVISER'S DIVISION, MINISTRY OF FOOD*

THE present report deals with the 440 samples of bread-making flour and 1408 loaves examined from May to December, 1943, inclusive (*cf.* ANALYST, 1943, 68, 306). *Flour*—During most of the year 10% of diluents (mainly barley, but also oats and rye) were added, and the addition of *ca.* 0·16% of calcium carbonate has now become general. The "national average" figures for fibre (corrected for added fortified Canadian flour) and vitamin B_1 were 0·55% and 1·0 I.U./g, respectively. The protein contents depend on the proportions of Manitoba and English wheats (mean protein contents 14·0 and 9·4%, respectively); 5 average monthly values (Aug.-Dec.) were 12·5, 12·1, 11·1, 11·1 and 11·0%, respectively. The size of the bran particles (420 samples, corrected for added white flour) were:—1·5% total over 5 silk (aperture, 0·27 mm), and 5·4% total over 8 silk (aperture 0·19 mm). Calcium carbonate contents (1310 samples) determined by the method of Greer *et al.* (ANALYST, 1942, **67**, 352; 1944, **69**, 14) were:—4·5 oz. per sack and less, 4·4%; 5·6–6·4 oz., 20·9%; 6·5–7·5 oz., 47·0%; 7·6–8·5 oz., 13·5%; 9·6 oz. per sack and over, 1·0%. It is very difficult to feed Creta Praeparata uniformly, and this is illustrated by the variations, recorded above, although the values for each sack as a whole were almost exactly 7 oz. Classification for (laboratory) baking quality was:—good, 44; fair-good, 26; fair, 23; poor, 7%. *Loaves*—These were classified for quality as:—good, 21; fair to good, 30; fair, 34; poor, 15%. These data represent a lower general level, which is due mainly to the high % of English wheat and the presence of diluent grains with grist. The difference between the quality figures for the flour and the loaves is a rough measure of the lower efficiency of commercial, as compared with laboratory, baking methods. Against this, however, must be set the fact that the commercial loaves had been sent by post from different parts of the country; the laboratory loaves were baked under controlled conditions of water absorption, fermentation and temperature; certain loaves are made to suit local taste (*e.g.*, with thick crusts, which may rank as a fault in a quality evaluation). The principal faults in the commercial loaves were incorrect fermentation of the dough, faulty water absorption, poor manipulation and inadequate baking. J. G.

Legal Notes

The Editor would be glad to receive particulars of cases with points of special legal or chemical interest

MEANING OF "SHOP" IN THE PHARMACY AND MEDICINE ACT†

SUMMERS *v.* ROBERTS

A JUDGMENT with an important bearing on the taking of samples under the Pharmacy and Medicines Act, 1941, was delivered in the King's Bench Divisional Court on November 23, 1943, in an appeal from a decision of the Leeds Stipendiary Magistrate.

The appellant (Summers) had been convicted of the sale of a certain liniment "not effected in a shop," contrary to the provisions of Sub.-sec. 2, Sec. 12 of the Act, and appealed against the conviction.

The appellant's premises consisted of a stall formed by two trestles and a board in the uncovered portion of Leeds City Market on a site allotted to him and for which he paid the Corporation.

Having heard the arguments of counsel, the Lord Chief Justice (Lord Caldecote) confined himself in his judgment to the significance of the word "shop" in the Act. By the interpretation of Sec. 17 of the Act "shop" had the same meaning as in Sub.-sec. 1, Sec. 19 of the Shops Act, 1912, and included any premises where any retail trade or business is carried on. But the definition must be considered and interpreted with reference to the objects and scope of the Pharmacy Act. Provisions in many places of that Act appeared to suggest that the legislature contemplated that the premises or shop dealt with in the Act should be a place defined by precise limits upon which there was some sort of structure. There was a provision in the Pharmacy Act requiring a place to be registered and he thought that this provision was also in the 1941 Act. He considered that the Act as a whole indicated at various points that it contemplated permanent places where the work or business was carried on in a place which had limits and which could be identified precisely.

In the Irish case of *Wallace* v. *Dixon* (1917) the definition of "shop" in the Shops Act, 1912, was under consideration and in the course of their judgments the three members of the Court used expressions which supported the argument that the definition in Sec. 19 included a retail shop where orders were taken. But this was not the question under consideration in the present case, and there was little, if anything, in the decisions in the Irish case which ought to affect the decision in this case, except that which seemed quite obvious, that the legislature meant to give an extended and unnatural meaning to the word "shop," which went far beyond the recognised meaning of the word. Hence his Lordship did not find that the words used by Mr. Justice Pim in the Irish case—premises where a retail business is carried on—were so wide as to require a finding in the present case that a place in no way limited or bounded by any ascertainable marks or fence, with no structure upon it except two trestles and a board, and with no continuity or regularity except that it was there twice a week, was a "shop" for the purposes of the Pharmacy and Medicines Act. He held that the place or space was not a shop within the meaning of the Act, and the appeal was accordingly dismissed.

Mr. Justice Macnaghten and Mr. Justice Tucker concurred.

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* *Nature*, 1944, **153**, 154–155.

† By courtesy of the Pharmaceutical Society.—EDITOR.

Notes from the Reports of Public Analysts

The Editor would be glad to receive reports containing matter of special interest

BIRMINGHAM: REPORT OF THE CITY ANALYST FOR THE FOURTH QUARTER, 1943

OF the 1280 samples submitted under the Food and Drugs Act, 32 were taken formally.

THE SOFT DRINKS ORDER, 1943—During the 3rd quarter of 1943 seven samples of mineral water were examined; not one complied with the standards of the Order. During the 4th quarter 6 samples of mineral water (5 from retail shops previously visited) and 3 samples of cordials and squashes were taken, and again none was satisfactory. In some, the quantities of the various ingredients were too great, and in others too small. In general, there was too little sugar and (in the mineral waters) too much saccharin. The citric acid contents were very irregular but on the whole nearer to the standard than the sugar and saccharin. The regional secretary of the Soft Drinks (War Time) Association was asked for an explanation of these irregularities. He attributed them largely to defective bottling machinery, to the withdrawal of experienced workers, and to careless handling of bottles during filling.

FRIAR'S BALSAM—A sample contained only 12·9% of solid matter, the normal amount being *ca.* 18% The makers suggested that the deficiency might be due to the poor quality of the drugs used—benzoin, storax, tolu balsam and aloes. In reply they were informed that even under present conditions most manufacturers were able to maintain the total solids between 17 and 18% (aver. 17·9%), which compared well with the pre-war average of 19·6%. Owing to the difficulties of supply, which were fully recognised, 4 samples containing only 14·8–15·9% had been passed during the last 3 years, but after every allowance had been made for poor drugs, it was impossible to pass 12·9%.

HEALTH SALTS—A sample was labelled as containing the equivalent of 20% of sodium bicarbonate, 38% of Rochelle salt, 10·5% of tartaric acid, and 15% of exsiccated magnesium sulphate, together with flavouring and sweetening ingredients. A powder of this composition would be strongly alkaline (8·2% excess of sodium bicarbonate), but the alkalinity of the sample was equivalent to only 1·2% of sodium bicarbonate. In addition, the amount of exsiccated magnesium sulphate ranged from 17·4 to 19·6% according to the amount of residual water. The packers disclosed the actual ingredients, and from a consideration of these it appeared that the alkalinity should have been 1·15% and the exsiccated magnesium sulphate 14·75%. One of the ingredients (citric acid) had been omitted from the label as it had been considered to be merely a bulk and effervescence producing ingredient. They agreed to alter their printed labels to correspond with the actual amounts present.

N. H. BAGNALL

KINGSTON UPON HULL: CITY ANALYST'S REPORT FOR THE FOURTH QUARTER, 1943

OF the 642 samples examined under the Food and Drugs Act, 222 were taken formally.

DRIED HERBS—Two samples contained 5·2 and 7·2% of earthy matter instead of not more than 3%, and were yellowish-brown and devoid of parsley flavour. Two other samples, which had only a slight flavour of parsley, contained 4·7 and 18·2% of earthy matter respectively, and another sample contained 6·6%. Cautions were issued.

FLUID BEEF—A sample, labelled in large letters "Fluid Beef," had a secondary label, in small letters on the back of the jar, "Prepared from beef, vegetables and seasoning." A caution was issued, and the manufacturers agreed to amend the wording of the main label.

PEPPERMINT FLAVOURED DRINK—This contained 143 p.p.m. of sulphur dioxide, and its sale was considered illegal on the ground that a drink of this type is not in the list of foods in the Public Health (Preservatives, etc. in Food) Regulations (1927) that may contain preservative, nor was the preservative derived from an article in that list. Moreover, the description "Peppermint Flavoured Drink" is not specified in Part I of Schedule I of the Soft Drinks Order, 1943. Caution issued.

BISMUTH TABLETS—A sample contained only 1·0% of chloroform instead of the 7·23% stated on the label. The manufacturers were cautioned and amended the label to "2·0% (approx.)"

D. J. T. BAGNALL

The Iron and Steel Institute

THE DETERMINATION OF SULPHUR AND PHOSPHORUS IN PIG IRON*

SAMPLING—Alternative methods are recommended, pigs being prepared and drilled nearly to the bottom skin, and molten samples taken by the pot sampling method.

DETERMINATION OF SULPHUR—The recommended method consists in burning the sample (0·3–1 g) in a rapid stream of oxygen (2 to 3 litres per min.) at 1250°–1300° C., collecting the resulting sulphur dioxide in neutral $N/50$ silver nitrate, and titrating the liberated nitric acid with approx. $N/200$ sodium hydroxide standardised against iron or steel of known sulphur content, using methyl red as indicator. To prevent entrainment of alkaline particles from a soda-lime drying tube, it is recommended that the oxygen should be dried by passage through silica gel only. The gas stream is freed from iron oxide by passage through a filter of cotton wool (previously dried at 105°–110° C.) before entering the absorbent; glass wool may adsorb sulphur dioxide. If the combustion tube projects 10 in. from the furnace there is no risk of the cotton igniting. Addition of methylene blue to the indicator provides a colour change easily detected in artificial light. The titration is done during the combustion, the whole process requiring 3½ to 4 min.

DETERMINATION OF PHOSPHORUS—Results on standard samples (*ca.* 0·02% of P) by the method in the British Cast Iron Research Assoc. handbook, "*The Sampling and Chemical Analysis of Cast Iron Ferrous Metals*" (E. Taylor-Austin), showed a range of *ca.* 0·005% on 0·02% of phosphorus. It was found that the

* By the Blast Furnace Materials Analysis Sub-Committee of the Blast Furnace Committee. Pp. 16, November, 1943.

temp. after addition of the molybdate reagent should not exceed 75° C., otherwise the formation of the yellow ppt. was affected. A modified B.C.I.R.A. method is described.

Interference by Arsenic—In an alternative method, prior to pptn. of the phosphorus, arsenic is removed by the method of E. Gregory (*J. Iron and Steel Inst.*, 1942, I, 279P). The mean errors in results of this method on 2 samples (*ca.* 0·02% P) were 0·0011 and 0·0017%, as compared with 0·0022 and 0·0027 by the B.C.I.R.A. method. Further work suggested that the superiority of the Gregory method does not primarily depend on the removal of arsenic. It was also found that the presence of as much as 0·17% of titanium introduces an error of only 0·001% in the phosphorus determination, and the Sub-Committee are therefore of opinion that interference from titanium may be ignored except when the highest accuracy is required. The following adaptation of the colorimetric method of N. T. Wilkinson (*J. Soc. Chem. Ind.*, 1938, **57**, 292) for determining phosphate in boiler water should then be used to determine the loss of phosphorus in the silica-graphite residue.

Colorimetric Method—Ignite the residue in a platinum crucible, treat with hydrofluoric acid to remove silica, and fuse with 1 g of sodium carbonate. Extract in 50 ml of 10% sulphuric acid, adjust the vol. of the soln. to 100 ml, pipette off 50 ml, add 5 ml of 1% gum acacia soln., and make faintly ammoniacal. Boil to expel all traces of ammonia, cool, and add 5 ml of molybdate reagent (100 ml of 1% aq. ammonium molybdate soln. mixed with a cooled soln. of 4·5 ml of conc. sulphuric acid in 100 ml of water; the whole diluted to 1 litre). Dilute to 100 ml with water, mix thoroughly, and add 0·1 ml of stannous chloride reagent (1 g of pure tin and 0·025 g of copper sulphate [$5H_2O$] heated with 20 ml of conc. hydrochloric acid, cooled, treated with *ca.* 20 ml of water, filtered and diluted to 100 ml with water). Stir well and match the resulting blue colour against a series of standards (0·000002–0·00001 g of phosphorus) prepared by dissolving 1 g of sodium carbonate in 50 ml of 10% sulphuric acid, adding the required vol. of standard dil. sodium phosphate soln. (2·29 g of $Na_2H.PO_4$ per litre; 1 ml = 0·0005 g of P; for dil. solns., 10 ml diluted to 1 litre), and treat exactly as described. The reagents must be added in accurately measured amounts; a slight excess of molybdate increases the depth of the blue colour and excess of acid or alkali reduces it. If much iron is present in the residue, remove it after the extraction in sulphuric acid by pptn. with cupferron, evaporate the filtrate with nitric acid to a small vol. to destroy the reagent, dilute to 100 ml, pipette off 50 ml and treat it as described above.

British Standards Institution

British Standard 997: Part 2: 1943—Filtered Sperm Oil*

This Standard, which is one in the series for Marine Animal and Fish Oils, provides the following specification for filtered sperm oil:

The oil shall be yellow, clear, free from sediment or insol. matter and uncontaminated with other oils and fats.

Sp.gr. at 15·5°/15·5° C.: min. 0·875; max. 0·886. Weight of 1 ml at 15·5° C.: min. 0·873 g; max. 0·884.

Iodine val. (Wijs): min. 80; max. 90.

Viscosity (by I.P.T. method, serial L.O.8 at 70° F.): min. 140 sec.; max. 170 sec. Viscosity in kinematic units (serial G.8 and B.S. 188): min. 0·343 stokes; max. 0·418 stokes at 70° F.

Cold test: *Cloud point* of "winter-prepared" oil (determined as described) must not exceed 38° F., unless otherwise agreed with the purchaser.

Sap. val. by specified method: min. 120; max. 145.

Acidity (by specified method) in benzene and alcohol, must not exceed 2·0% (as oleic acid) unless otherwise agreed. There must be no mineral acid or added organic acid.

Unsap. matter (by S.P.A. method) min. 32; max. 42%. Extracted unsap. matter must remain clear and homogeneous in warm soln. after being boiled with 2 to 3 times its weight of acetic anhydride.

Sampling. Recommended methods are given in B.S. 627.

ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

Food and Drugs

Carbohydrate Characterisation. Identification of *d*-Ribose, *l*-Fucose and *d*-Digitoxose as Benzimidazole Derivatives. R. J. Dimler and K. P. Link (*J. Biol. Chem.*, 1943, **150**, 345–349)—The benzimidazole procedure previously described has been extended to *d*-ribose, *l*-fucose and *d*-digitoxose. *d*-Ribose and *l*-fucose were converted into the aldo-benzimidazoles by oxidation of the aldose to aldonic acid with potassium hypoiodite in methyl alcohol, liberation of the acid from the pptd. potassium or barium salt and condensation with *o*-phenylenediamine in presence of hydrochloric and phosphoric acids at 135° C. (*cf.* Analyst, 1940, **65**, 419; 1942, **67**, 307). With *d*-digitoxose, however, it is necessary to use a different method, and the benzimidazole is prepared in good yields by oxidative condensation with *o*-phenylenediamine in presence of cupric acetate and acetic acid (*cf. J. Org. Chem.*, 1940, **5**, 637). The following are the constants of the aldo-benzimidazoles:

	m.p. °C.	*$[\alpha]_D^{25}$	Hydrochloride m.p. °C.	Picrate m.p. °C.
d-Ribo-benzimidazole	190	+22·5°	196–198	185–186
l-Fuco-benzimidazole	248–249	−41·2	224–225	189–191†
d-Digitoxo-benzimidazole	207–209	−45·7	Oil	124–127

* Rotations in 1 *N* HCl with *c* = 2 (approx.). † Sinters at 150° C.

* Obtainable from British Standards Institution, 28, Victoria Street, London, S.W.1. Price 2s post free.

During the oxidation of *d*-ribose some epimerisation occurs, but the resulting *d*-arabo-benzimidazole, being very insoluble, is easily separated from the *d*-ribo-benzimidazole during isolation. F. A. R.

Azoyl Derivatives of Sugars and Separation by Chromatographic Adsorption. G. H. Coleman and C. M. McCloskey (*J. Amer. Chem. Soc.*, 1943, **65**, 1588–1594)—Previous work (*J. Amer. Chem. Soc.*, 1942, **64**, 1501) on the separation by chromatographic adsorption of azoyl derivatives of sugars has been extended.* α-*d*-Galactose pentaazoate has been separated from β-*d*-galactose pentaazoate, and β-*d*-glucose pentaazoate has been separated from both α-*d*-xylose tetraazoate and β-*l*-arabinose tetraazoate. No separation was obtained with the azoates of the enantiomorphs β-*d*-arabinose and β-*l*-arabinose. The method has been applied also to azoyl derivatives of methyl glycosides and of partially acetylated sugars. The separation of methyl heptaazoyl-β-*d*-cellobioside and methyl tetraazoyl-α-*d*-glucoside was accomplished, and an unusually sharp separation made with 1-azoyltetraacetyl-β-*d*-glucose and 1-azoylheptaacetyl-β-*d*-cellobiose. The separation of the azoates of the methyl glycosides was not as clean cut under the conditions used as was that of the 1-azoylacetyl sugars. The technique has been applied also to the separation of mixtures of more than two sugar derivatives. A mixture of β-*l*-arabinose, β-*d*-glucose, α.α-trehalose and β-cellobiose azoates has been separated. The purity of each band is difficult to estimate from the optical rotation, but is thought to be at least 90% for each compound that has been obtained.

The reported method for the preparation of sugar azoates from the sugar and azoyl chloride has been modified in that the preliminary reaction is allowed to take place at 0° C. and is then completed at 90° C. After the hydroxyl group on carbon atom one had been azoylated at 0° C. no change was observed in the configuration on prolonged heating at 90° C. The preliminary low temp. minimises the mutarotation but does not completely prevent it, so that a small amount of the other member of the anomeric pair is always obtained. To prevent formation of azoic anhydride, a little water is added to the pyridine soln. to decompose the azoyl-pyridine complex before pptng. the azoate by addition of the soln. to a larger vol. of water. Azoic anhydride was prepared in 98% yields by adding a pyridine soln. of the acid chloride to cold water. The azoates were separated from the azoic acid by dissolving them in chloroform and pptng. by adding alcohol. Most of the azoates had sharp m.p., but only derivatives containing 4 or less azoyl groups have been obtained with easily discernible crystalline form. The m.p. of diacetoneglucose azoate agreed with that found by Freudenberg and Plankenhorn (*Ber.*, 1940, **73**, 621). E. M. P.

Occurrence of Citric and *Iso*citric Acid in Blackberries and Dewberry Hybrids. A. L. Curl and E. K. Nelson (*J. Agric. Res.*, 1943, **67**, 301–303)—Previous work on the non-volatile acids of blackberries, etc. (Nelson, *J. Amer. Chem. Soc.*, 1925, **47**, 568; 1927, **49**, 1300; 1930, **52**, 2928) has been extended to include three named varieties of blackberry, the Brainerd, Crandall (Macatawa) and Texas Wonder and the dewberry hybrids Young (Youngberry) and Boysen (Boysenberry). The ester distillation method (Franzen *et al.*, *Z. physiol. Chem.*, 1922, **115**, 9; 1922, **122**, 46; 1923, **124**, 65; 1923, **127**, 14; 1923, **129**, 80; Nelson, *J. Amer. Chem. Soc.*, 1925, **47**, 568; 1930, **52**, 2928) was employed, whereby the non-volatile acids are converted into their ethyl esters, which are fractionated and the hydrazides then formed from the various fractions. The individual acids are identified by the physical properties of the esters and of the hydrazides. The proportions of the acids found in the fruit examined are as follows.

Variety	*iso*Citric acid %	Citric acid %	*l*-Malic acid %
Brainerd ..	65	0	35
Crandall ..	85	0	15
Texas Wonder	75	0	25
Boysen ..	4	85	11
Young ..	6	86	8

The last two, in which citric acid predominates, are trailing varieties, as is also the logan (loganberry), which contains citric acid exclusively, and it is suggested that there may be some correlation between the principal non-volatile acid and the type of berry. J. A.

Quantitative Extraction of Laboratory Samples of Oils from Fish Tissues. W. S. Rapson, H. M. Schwartz and N. J. van Rensburg (*J. Soc. Chem. Ind.*, 1943, **62**, 221–223)—Three methods of extracting oil from fish tissues for subsequent analysis have been compared: (*a*) Mince the tissue, mix with anhydrous sodium sulphate, set aside for 3 min., grind and extract with peroxide-free ether. (*b*) Add to 100 g of tissue 0·5–1 g of pepsin in 100 ml of water containing 2 ml of hydrochloric acid (sp.gr. 1·2). Replace the air in the vessel by carbon dioxide and hold at 45–50° C. with occasional stirring for 12–36 hr. When almost all the tissue protein has been peptised add 10 ml of saturated sodium carbonate soln., heat in a boiling water-bath for 10–15 min. to destroy lipolytic enzymes and complete the digestion, and extract with peroxide-free ether. Break persistent emulsions by addition of alcohol or by whirling in a large bucket centrifuge. (*c*) Mix the minced tissue with twice its weight of 1% sodium hydroxide soln. and steam the mixture or hold it at 70–90° C. until the tissue is completely disintegrated (20–30 min.). Break the cold emulsion, if necessary, by adding 1 vol. of alcohol to 4 vols. of digestion mixture, and extract with peroxide-free ether. The second and third methods are better than the sodium sulphate desiccation method for the extraction of vitamin A- and D-bearing oils from visceral tissues. The recovery of vitamin A and of unsap. matter generally is quantitative, although that of free fatty acids is variable. Alkali digestion is applicable also to the rapid and approx. estimation of the oil content of fish heads and fish flesh. E. M. P.

Thermal Decomposition of Lard. C. D. Larsen and H. P. Morris (*J. Amer. Chem. Soc.*, 1943, **65**, 2301–2303)—Changes in lard heated at 200–350° C. for 30–150 min. are recorded. Uncatalysed pyrolysis is slow below *ca.* 300 C., but much more rapid at *ca.* 350° C. Total chromogenic sterols were assayed colorimetrically by the Bloor modification (*J. Biol. Chem.*, 1928, **77**, 53) of the Liebermann–Burchard reaction, and sterol in the unsap. matter by a modification of the digitonin

* Azoic acid is benzene-azo-*p*-benzoic acid, $C_{13}H_{10}O_2N_2$.—Ed.

method of Schoenheimer and Sperry (*J. Biol. Chem.*, 1934, **106**, 745). The intact cholesterol was pptd. with digitonin and the washed and dried digitonide was used for the colour test, the absorption values being translated into cholesterol values with the aid of a standard absorption curve. The latter method appeared to give a true picture of the fate of cholesterol *per se* in drastically heated lard. The iodine vals. of the free fatty acids decreased from 80·1 to an average of 42·2 in the most vigorously heated samples, whilst the iodine vals. of the unsapon. fraction increased from 73·0 to 87·0. Of the original sterol, approx. 40% was not precipitable with digitonin after the lard had been heated at 300° C. for 30 min., and 75% after ½ hr. at 300° C. Heating at 350° C. for 30 min. reduced the precipitable sterol to zero. The sterol content (determined colorimetrically), however, increased with increased severity of heating; this is thought to be due, partly at least, to formation of the isomeric cholestadienes, as it has previously been found that under the conditions of analysis 3·5-cholestadiene develops about 70% more colour than cholesterol. About 50% of the unsap. matter from lard heated at 350° C. solidified at room temp.; it consisted of a mixture of aliphatic ketones derived chiefly from 16- and 18-carbon acids. With hydroxylamine hydrochloride the ketone mixture formed a colourless oil. Acidification of a dil. alcoholic soln. of the oximes regenerated the ketones. Attempts to prepare a semicarbazone or phenylhydrazone were unsuccessful. E. M. P.

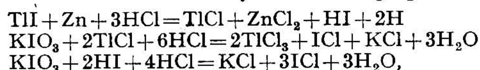
Palisade Ratio of the Official Leaf Drugs of the Solanaceae, *Solanum carolinense* and *Phytolacca americana*. P. L. Bogarosh (*Amer. J. Pharm.*, 1943, **115**, 373–385)—The diagnostic significance of the palisade ratios of the leaves of *Atropa Belladonna, Datura Tatula, Hyoscyamus niger, Solanum carolinense* and *Phytolacca americana* has been studied. *Method*—Cut pieces, 2–3 mm square, from the base, apex, margin and centre of each leaf, clear the segments in a soln. of 50 g of chloral hydrate in 20 ml of water for 5–15 min, mount in Berlese mountant (distilled water, 10 ml; dextrose, 5 g; powdered acacia, 8 g; chloral hydrate, 74 g; glacial acetic acid, 3 ml; in that order) and examine at ×430. With the help of a camera lucida, count the number of palisade parenchyma cells lying beneath any 4 contiguous upper epidermal cells, including all those whose half-area or more comes within the boundaries of the chosen cells, and divide this number by 4 to obtain the palisade ratio. For each species except *Phytolacca americana* 5 leaves were taken and five counts were made of each of the 4 sections from each leaf, whence the average palisade ratio was obtained as the mean of 100 determinations. In examining *Phytolacca americana*, one of the chief adulterants of Belladonna, 8 leaves were selected, giving the average palisade ratio as the mean of 160 determinations. The following results are set out in 5 tables together with the max. and min. values: *Atropa Belladonna*, 5·6 (4·0–9·5); *Datura Tatula*, 5·4 (4·0–7·0); *Hyoscyamus niger*, 9·5 (7·3–11·5); *Solanum carolinense*, 3·3 (2·3–4·3); *Phytolacca americana*, 2·9 (2·0–3·8). It is concluded that from the aver. palisade ratio the leaves of *Hyoscyamus niger, Solanum carolinense*, and *Phytolacca americana* can be distinguished from one another and from the leaves of *Atropa Belladonna* and *Datura Tatula*, but that the leaves of the last two cannot thus be distinguished. Further, there is no marked variation in the average palisade ratio of a given leaf in going from base to apex, except with *Hyoscyamus niger*, in which the ratio decreases progressively in passing from base to apex. J. A.

Quantitative Determination of Sulphanilamide and Sulphathiazole in Mixtures. D. T. Englis and D. A. Skoog (*Ind. Eng. Chem., Anal. Ed.*, 1943, **15**, 748–749)—Barnard and MacMichael (*Ind. Eng. Chem., Anal. Ed.*, 1930, **2**, 363) showed that a colour system of two components may be quantitatively analysed, even if both components show absorption at the selected wave length, provided that the degree of absorption is sufficiently different and the sample represents a definite total amount of the two constituents only. It is necessary to determine the extinction coefficients of each pure component at selected wave lengths that give the widest difference of absorption between one and the other component. The extinction value of the mixture is then determined at the selected wave length, and the proportions of the constituents are calculated by means of a series of simultaneous equations. A Bausch and Lomb medium quartz spectrograph was used with the slit adjusted to 0·06 mm. The source of illumination, a Wood's type hydrogen discharge tube, was placed with the exit window 10 cm from the slit, and a 1-cm cell with quartz windows was used to hold the liquids. Separate exposures of the solvent and the solns. were taken for a period of 2 min. each, and the spectra were recorded on Eastman polychromatic plates. Each plate was calibrated by making a series of separate successive exposures in which the time interval was varied regularly, *e.g.*, 4, 8, 16, 32, 64 and 128 sec. The plates were developed for 6 min. in Eastman X-ray developer, fixed, washed and dried, and the optical densities of the spectrograms at selected wave length intervals were determined with a Leeds and Northrup recording microphotometer. A family of plate calibration curves for the selected wave lengths was constructed; then, by reference to the appropriate curve, the relative intensity values for the pure solvent and the soln. were found, and from these the extinction value for the soln. was calculated. Sulphanilamide in 95% ethanol soln. shows an absorption peak at 261·0$m\mu$, and sulphathiazole in the same solvent has absorption peaks at 258·$m\mu$ and 87·5$m\mu$. Sulphanilamide has a slight absorption peak at 287·5$m\mu$ also. The absorption values at different concns. of each compound were determined. The solns. of both compounds obey the Lambert–Beer law at 260$m\mu$, and a quantitative estimation of each component is thus possible in the mixture. The data necessary are—extinction for 1 g of sulphathiazole per litre at 260$m\mu$ (E'_a), the corresponding figure for sulphanilamide (E'_b), the extinction for 1 g per litre of sulphathiazole at 287·5$m\mu$ (E''_a), the corresponding figure for sulphanilamide (E''_b), the extinction for the mixture at 260$m\mu$ (E'_m) and the extinction for the mixture at 287·5$m\mu$ (E''_m). If $x =$ g of sulphathiazole per litre and $y =$ g of sulphanilamide per litre, then $xE'_a + yE'_b = E'_m$; $xE''_a + yE''_b = E''_m$. From these equations the amount of each constituent of the mixture is determined. A. O. J.

Biochemical

Chemical Method for the Determination of Minute Traces of Thallium in Tissues. K. Samaan and M. N. Mikhail (*Quart. J. Pharm.*, 1943, **16**, 342–3)—The method of Kluge (*Z. Unters. Lebensm.*, 1938, **76**, 152; Abst., ANALYST, 1938,

63, 748) has been found unreliable, as there were variations of as much as 15%. The recommended method is stated to give concordant results, with recoveries of 94–96% in control expts. on rabbit's liver with added thallium. Add a sufficient quantity of a mixture of 1 part of conc. sulphuric acid and 5 parts of fuming nitric acid to a known amount of the tissue in a deep porcelain dish (*ca*. 40 ml required for 30 g of rabbit's liver) and, when the first vigorous reaction has ceased, heat gently to expel all fumes. Transfer to a Kjeldahl flask with 5-ml quantities of conc. sulphuric acid, add 5 g of potassium sulphate and heat until colourless or pale yellow. Cool, add an equal vol. of water, cool, chlorinate with conc. hydrochloric acid and potassium chlorate and extract 4 times with ether; re-chlorinate the aqueous layer with freshly prepared chlorine water and extract twice more with ether. Remove the solvent from the combined ethereal extracts, treat the residue with 5 ml of water and evaporate to dryness. Dissolve the residue in the min. quantity of water, transfer to a centrifuge tube, add excess of 20% potassium iodide soln., discharge any liberated iodine with thiosulphate soln., centrifuge for 15 min., decant, and wash the thallous iodide free from soluble iodide with successive portions of 90% alcohol by centrifuging and decanting. Dry the ppt. *in vacuo*, suspend it in 2 ml of water and treat it with sufficient zinc and hydrochloric acid to convert the thallous iodide into chloride, as indicated by production of a clear soln. Transfer the soln., free from undissolved zinc, to a 100-ml glass-stoppered flask by means of conc. hydrochloric acid, add more of the acid and 2 ml of chloroform and titrate the hydriodic acid with 0·0005 *M* potassium iodate. The reactions are shown by the following equations:



$$TlI + Zn + 3HCl = TlCl + ZnCl_2 + HI + 2H$$
$$KIO_3 + 2TlCl + 6HCl = 2TlCl_3 + ICl + KCl + 3H_2O$$
$$KIO_3 + 2HI + 4HCl = KCl + 3ICl + 3H_2O,$$

whence 1 ml of 0·0005 *M* potassium iodate ≡ 0·131707 mg of thallous acetate. J. A.

Rapid Determination of Iron in Ferric Phytate. J. R. Foy and J. B. Thompson (*Ind. Eng. Chem., Anal. Ed.*, 1943, **15**, 747–748)—Gently shake 0·7–1·0 g with 10 ml of hydrochloric acid in a 250-ml glass-stoppered flask until dissolved. Add 25 ml of 16% potassium iodide soln., stopper and rotate several times to ensure thorough mixing, set aside for 5 min., add 100 ml of water, and titrate with 0·1 *N* thiosulphate soln. (starch indicator). Subtract the blank result obtained under identical conditions (1 ml ≡ 0·05585 g of Fe). It is recommended to pipette the volumes of acid and iodide soln. accurately and to take the time of standing by a stop watch to secure uniform results. There is no afterblueing for 2–3 hrs., which is ascribed to the formation of non-dissociated ferrous potassium phytate. W. R. S.

Application of the Millon Reaction to the Determination of Chlorophenols in Body Fluids and Tissues. B. Zondek, B. Shapiro and S. Hestrin (*Biochem. J.*, 1943, **37**, 589–591)—*Aqueous solns. Estimation of* p-*chloro*-m-*xylenol* (*CX*)—To 15 ml of test soln. add 2 ml of reagent (1) (50 ml of 20% mercuric acetate soln. mixed with 30 ml of 25% *v/v* nitric acid) and then, if no ppt. forms, add 1 ml of reagent (2) (a soln. of 100 g of mercury in 200 g of nitric acid diluted four-fold). If a ppt. forms, filter and add reagent (2) to a measured portion of the filtrate. Leave at room temp. for 30 min., cool in ice-water, and add 5 ml of cold ether. Shake and, within the next hour, transfer the ethereal extract to a 0·5-cm. cuvette covered with a glass slide. Measure the extinction in a Pulfrich photometer with filter S 43. Prepare a calibration curve from standard solns. of *CX* similarly treated. A fresh curve should be prepared for each new batch of reagent. *Estimation of* p-*chloro*-m-*cresol* (*CC*)—To 10 ml of test soln. add 1 ml of reagent (1) and 1 ml of reagent (2). Incubate for 2 hr. at 37° C., cool in ice-water for 5 min., and shake with 5 ml of cold ether. Measure the colour within 1 hr., using filter S 50 and a 0·5-cm cuvette. *CX* forms a dirty-red colour in the aqueous phase and a clear yellow in ether, whilst *CC* gives a red soln. in water and ether. Concentrations of either substance greater than 0·5 mg per 100 ml were estimated with an error of 5%. Lower concns. could be estimated by concentrating the phenol by extraction with one-third vol. of toluene and re-extraction with a suitable vol. of 0·1 *N* sodium hydroxide. *Biological materials. Estimation of CX in urine*—Dilute 2 ml of urine with 4 ml of water and add 2 ml of reagent (1). After 3 min. centrifuge, and to 4 ml of the clear supernatant liquid add 0·3 ml of reagent (2). Leave for 30 min. at room temp., cool and extract with 4 ml of cold ether. Measure the colour as before. *Estimation of CC in urine*—Steam-distil 5 ml of urine, made just acid to litmus, until 15 ml of distillate have been collected; foaming is prevented by adding a few drops of liquid paraffin. Treat the distillate as described for aqueous solns. *Estimation of CX and CC in blood*—De-proteinise 5 ml of oxalated blood by mixing with 20 ml of acetone. Filter and wash the ppt. with 10 ml of acetone and 5 ml of ether. Transfer the filtrates to a distillation flask, add 0·3 ml of 4% sodium hydroxide soln. and a few drops of liquid paraffin, and remove the acetone and ether by distillation. Acidify the residual liquid by adding 1 ml of 25% sulphuric acid and steam-distil. Collect 15 ml of distillate and treat as described for aqueous solns. *Estimation of CX and CC in minced tissue*—Extract the minced tissue by boiling in acetone for 1 hr. under a reflux condenser and then follow the procedure described for blood. The recoveries of both phenols added to urine and blood were practically quantitative. The method was highly specific for halogenated phenols, and blood and urine from normal human subjects gave consistently negative results. F. A. R.

Estimation of Haemoglobin by Photometric Absorptiometers. J. M. Peterson and D. H. Strangeways (*Brit. Med. J.*, 1944, i, 43–44)—To avoid errors due to changes in light intensity and in the electric properties of the photo-cell in the estimation of haemoglobin with a photo-electric absorptiometer (*cf.* M.R.C. Report, *Brit. Med. J.*, 1943, i, 209) a procedure is described in which a null point technique with an apparatus incorporating two opposed cells is used. Set the instrument by allowing light to pass through a standard filter on to one cell with the corresponding slit set to the haemoglobin value of the filter; adjust the light to the other cell so that the galvanometer gives a null reading. Replace the filter by the blood soln. to be measured and adjust the slit to give a null reading. Check the instrument by replacing the filter and adjusting the slit to the filter value; the galvanometer should then again read zero. A reliable result will only be obtained if this last condition is satisfied; significant changes in either

or both of the cells or in the light source will be indicated by a galvanometer reading other than zero, and the light to the balancing cell will have to be re-adjusted before repeating the determination. For accurate results the current output of the photo-cells must be the same for all effective wave-lengths, or the wave-length range employed must be sufficiently narrow for any difference between the wavelength of the light transmitted by the filter and that transmitted by the blood soln. to be without appreciable effect. With an absorptiometer of the opposed cell type, using barrier-layer selenium photo-cells and the alkaline haematin method, the accuracy of the procedure is unaffected by the intensity of the illumination or by the marked alteration in the condition of the cells on continued use. The region of the spectrum most suitable for accurate determinations of alkaline haematin has been investigated and it is concluded that the use of a Wratten 45A filter gives the most satisfactory results. Although the absorption range of this filter (430–540$m\mu$) is not so specific for alkaline haematin as is the 600 $m\mu$ band, its use is justified by the fact that the change in absorption with change in concn. of alkaline haematin is greater than in the longer wavelength region with the filters available, while the absorption of blood plasma in the region transmitted by the Wratten 45 A filter is negligible. The fact of specificity could be significant only with abnormal bloods.

The use of 0·1 *N* sodium hydroxide is recommended for the production of alkaline haematin; addition of 0·02 or 0·04 ml of human blood to 5 ml of 0·1 *N* sodium hydroxide gives a value greater than 95% of the max. in 5 min. For very accurate work it is advisable for the mixture to stand overnight, the supernatant fluid then giving a constant max. absorption for *ca.* 5 days. This rapid rate of denaturation is not necessarily true of all bloods, that of the ox taking some hours for the establishment of a satisfactory equilibrium. With this method replicates from the same sample of blood with an oxygen capacity of about 20 ml per 100 ml can be determined with a standard deviation of 0·1 and a standard error of 0·04 for 12 determinations.

J. A.

Estimation of Creatine and of Diacetyl. P. Eggleton, S. R. Elsden and N. Gough (*Biochem. J.*, 1943, **37**, 526–529)—*Creatine*—To a neutral soln. of creatine containing not more than 60μg, add 2 ml of 1% α-naphthol soln. in alkali (30 g of sodium hydroxide and 80 g of sodium carbonate in 500 ml of water), followed by 1 ml of a 20-fold diluted 1% stock son. of diacetyl (prepared by heating 1·6 g of pure dimethyl glyoxime with 200 ml of 5 *N* sulphuric acid in an all-glass distillation apparatus, collecting the first 50 ml of distillate and diluting to 100 ml with water; the yield is practically constant at 85% of theory). Mix, dilute to 10 ml and leave for 30 min. Measure the colour with a Zeiss–Pulfrich photometer with an S 53 filter or compare directly with standard creatine solns. prepared in the same way. The colour is stable for at least 2 hr. Other guanido compounds react under these conditions; of these, arginine, guanidine and glycocyamine produce about one-ninth of the colour given by an equiv. amount of creatine. Creatinine and freshly prepared creatine phosphate do not react. *Diacetyl*—To a soln. of diacetyl (containing not more than 100μg), add 4 ml of a soln. made by mixing 1 part of saturated aqueous creatine and 3 parts of 1% α-naphthol soln. in the stock alkali. Shake, dilute to 15 ml and leave for 30 min. Estimate the colour in a photometer or by direct colorimetry, the latter method being preferred. The same method can be used for estimating acetyl methyl carbinol by first converting this into diacetyl by heating under reflux with ferric chloride soln. and recovering the resulting diacetyl by distillation. The estimation of creatine in urine and liver extracts is not satisfactory, owing to the presence of interfering substances. Glycine, β-alanine and carnosine, *e.g.*, were found to suppress the colour, but in most animal tissues, except liver, only insignificant amounts of these are present. The possibility of interference should, however, always be borne in mind.

F. A. R.

Photometric Micro Method for the Estimation of Inulin in Serum and Urine. H. Ranney and D. J. McCune (*J. Biol. Chem.*, 1943, **150**, 311–313)—The method of Reinecke (*J. Biol. Chem.*, 1942, **142**, 487; ANALYST, 1942, **67**, 234) for the estimation of fructose was adapted to the estimation of inulin in serum and urine. Add 0·2 ml of serum to 5·0 ml of dil. tungstic acid, centrifuge and transfer 2·0 ml of the supernatant liquid to each of two Bailey–Myers sugar tubes calibrated at 10 ml. Add 4 ml of saturated ethyl alcoholic hydrogen chloride soln. to each, stir and close the mouth of each tube with a glass marble. Immerse the tubes for 30 min. in a water-bath at 60° C. and then cool for 2–4 min. in an ice-bath. Add to each 0·1 ml of a 1% alcoholic skatole soln. and, after 5–7 min., stir the mixture, dilute with ethanol at 60° C., and transfer to an Evelyn colorimeter tube. Take the readings 10–15 min. later, using a filter transmitting at 520$m\mu$. Dilute urine to contain 5–10μg of inulin per ml and then treat it in the same way as serum. Calculate the results from a calibration curve prepared with pure inulin. To minimise the interfering colour produced by the interaction of skatole and ethyl alcoholic hydrogen chloride soln., it is necessary to dilute with ethyl alcohol within 5–7 min. after adding the skatole soln.; under these conditions the optical density of the solns. is directly proportional to the concn. of inulin. The standard deviation in 27 recoveries of inulin added to serum in concns. of 4–10μg/100 ml was ± 3·4%.

F. A. R.

Glucose Dehydrogenase from Germinating Seeds of Green and Black Grams (*Phaseolus Radiatus* and *Phaseolus Mungo* Linn). K. P. Basu and J. N. Karkun (*J. Indian Chem. Soc.*, 1943, **20**, 229–238)—An oxidising enzyme from the germinating seedlings of green and black gram has been identified as a glucose dehydrogenase, this being the first observation of this enzyme in the higher plants. The max. activity is when the seedlings are 3 days old; after 5 days the activity is low. The enzyme oxidises glucose both aerobically and anaerobically, galactose and mannose also reacting. Fructose, xylose and arabinose are little affected; hence the dehydrogenase is group-specific. Methylene blue cannot be used as a hydrogen acceptor, activity being reduced by about 25% in its presence, but 2 : 6-dichlorophenol-indophenol can also act. Flavin, adrenaline and ascorbic acid are incapable of acting as intermediate carriers, but in presence of 1 in 2000 of glutathione, an acceleration of *ca.* 8% was observed. Inhibition of the enzyme is caused by various narcotics, 0·1% of octyl alcohol producing a loss of 99% of the activity and 5% of vanillin, 95%. Potassium cyanide of 0·001 *M* concn. inhibits the

enzyme by *ca.* 66%; inhibition increases with increasing concn., 91% inhibition being produced by 0·01 *M* potassium cyanide. Other inhibitors are: sodium pyrophosphate (0·02 *M*), 8% inhibition; sodium sulphide (0·004 *M*), 66·7% inhibition; sodium fluoride (0·1 *M*), 17·5% inhibition; copper (0·2 mg in 3 ml), 55·0% inhibition; iron^{II} or ^{III}, in a concn. of *ca.* 0·2 mg in 6 ml, is without effect. The inhibiting power of xanthine oxidase and of sodium bisulphite in an acid medium is evidence that the enzyme contains an aldehyde group. It is probable that the product of oxidation is gluconic acid. The physiological significance of this enzyme in plants is discussed. J. A.

Succinic Acid Dehydrogenase from Cucumber Seeds. K. P. Basu and J. N. Karkun (*J. Indian Chem. Soc.*, 1943, **20**, 277–281)—The preparation and properties of succinic acid dehydrogenase from cucumber seeds are described. For active enzyme preparations, the fruits must be fresh; extracts from fruits kept for 2–3 days after picking are completely inactive. The enzyme cannot use methylene blue as hydrogen acceptor, but in presence of succinate 2 : 6-dichlorophenolindophenol is completely reduced. Narcotics cause some inhibition: vanillin (0·1%), 12·2%; urethane (1%), 21·7%; barbitone (1%), 17·2%; octyl alcohol (0·001%), 25·0%. Up to 40° C. extracts retain their activity unimpaired, but above it declines, and at 60° C. it is zero. It is further shown that sodium pyrophosphate and sodium fluoride affect both aerobic and anaerobic activity, but that sodium arsenite and potassium fluoride affect only the aerobic (oxidase factor). It is concluded that the enzyme contains the –SH group because copper sulphate produces no initial inactivation, whereas after incubation of the enzyme preparation with copper sulphate for 1 hr. 70–100% inhibition occurs. J. A.

Colorimetric Estimation of Cholesterol. W. M. Sperry and F. C. Brand (*J. Biol. Chem.*, 1943, **150**, 315–324)—The usual methods of estimating cholesterol based on the Liebermann–Burchard colour reaction are unsatisfactory, and the following modification is therefore recommended. Pipette 0·4 ml of blood serum into approx. 5 ml of alcohol-acetone (1 : 1), and heat the mixture to boiling. Cool, dilute to 10 ml with alcohol-acetone and, after mixing and filtering, pipette 5 ml of the filtrate into a 25-ml flask containing 0·15 ml of potassium hydroxide soln. (10 g in 20 ml of water). Mix gently and put the flask in an incubator at 37–40° C. for 40 min., add 1 drop of phenolphthalein soln., titrate with 10% acetic acid in abs. alcohol, add 1 drop in excess and evaporate just to dryness on the steam-bath with the aid of a current of air. Cool immediately, add 0·1 ml of 50% alcohol and wash the walls of the flask with about 3 ml of light petroleum. If the salt does not dissolve within 10 min., add a further 0·05 ml of 50% alcohol. Decant the light petroleum into a dry bottle and extract the aq. alcohol soln. with 5 additional quantities of light petroleum. Remove the solvent from the combined extracts completely in a current of air and pipette 5 ml of acid-free chloroform into the bottle. Add 5 ml of chloroform likewise to 3 bottles containing 0·24, 0·4 and 0·6μg of pure cholesterol. Stopper the bottles and immerse them in a water-bath at 24° C. placed in a dark cabinet. Add 1 ml of conc. sulphuric acid to 20 ml of pure acetic anhydride cooled in ice, and 9 min. after putting the bottles into the water-bath add 2 ml of this reagent to one of the standard solns., shake for 10 sec. and return it to the bath. The operation should be so timed that this occurs 10 min. (± 5 sec.) after immersion of the bottle in the water-bath. Treat all the other bottles similarly, and then measure the colours in a colorimeter 17–18 min. after each bottle has been returned to the bath. Standardise a soln. containing 14 mg of naphthol green B per 100 ml, and then match the colours of the unknown solns. against the standard settings of the dye. The results of replicate estimations agreed within 3%. F. A. R.

Vitamin Content of Beverages. J. C. Drummond and T. Moran (*Nature*, 1944, **153**, 99–100)—Preliminary experimental values for the vitamin contents of some common beverages are reported. The riboflavin content of beer seems to be related to the strength of the brew; samples of malt gave values of 5·6μg/g (compared with 2·5μg/g for barley), indicating a limiting riboflavin content of *ca.* 1·3μg/ml for present-day beer. Values have been reported ranging from 0·5 to 1·7μg/ml (*cf.* ANALYST, 1943, **68**, 339). A sample of pre-war strong ale gave a value of 3·9μg/ml, but a beer of 1798 gave the disappointing figure of 0·8μg/ml, due, no doubt, to destruction of the vitamin during prolonged storage. The results show that beer is a good source of nicotinic acid (*ca.* 15μg/ml) and probably of biotin, pantothenic acid and inositol. There is appreciable destruction of phytic acid during germination, with a corresponding increase in inositol content; thus, a sample of malted English wheat contained 190 mg of phytate phosphorus per 100 g, compared with *ca.* 270 mg/100 g before germination. Brewed malt vinegars contain *ca.* 0·7μg/ml of riboflavin; artificial vinegars are devoid of nutrient substances.

A few samples of tea, coffee and cocoa have been examined for riboflavin content, with the following results: tea, *ca.* 9·0μg/g, coffee, *ca.* 1·7μg/g and cocoa, *ca.* 2·7μg/g. The daily intake of riboflavin by an habitual tea drinker would not be negligible; this fact, together with the riboflavin content of beer, might explain the relative rarity of clear-cut symptoms of deficiency conditions in Great Britain.

Five proprietary brands of meat extract contained 15·4–25·8μg of riboflavin per g and 410–1025μg of nicotinic acid per g, whence a breakfast cup from these extracts would provide *ca.* 0·2 mg of riboflavin and *ca.* 0·7 mg of nicotinic acid. Any such concentration of vitamin in the extract, however, means a corresponding poverty in corned beef. The importance of these results is stressed and some possibly fruitful lines of research are indicated. J. A.

Micro-method for the Estimation of Vitamin B_1. J. J. C. Hinton (*Biochem. J.*, 1943, **37**, 585–589)—A fluorimetric method for estimating as little as 0·005μg of vitamin B_1 makes use of a modified Spekker Photoelectric Fluorimeter, in which the glass cell of the standard instrument is replaced by a suitable holder so arranged that a capillary tube containing the test soln. receives the full irradiation transmitted. First estimate the concn. of ferricyanide soln. required to produce max. fluorescence with the test material; with cereal extracts, this was found by testing a range of the concns. from 0·05–0·5% in steps of 0·05%. Next measure 0·1 ml of 30% sodium hydroxide soln. into a 2 × 3/8″ tube, run 0·2 ml of the appropriate ferricyanide soln. on top and leave undisturbed. Measure out 0·1 ml of the test soln., then mix the sodium hydroxide and ferricyanide

solns., and immediately add the test soln., and again mix. At the same time treat three standard aneurine solns. of different strengths in the same way. Add 0·2 ml of water-saturated *iso*butanol to each tube, stir for 30 sec. and then leave for 10–15 min. or centrifuge for 3–4 min. Draw up the *iso*butanol layer from the first tube into the capillary tube used for measuring the fluorescence. This is a glass tube of oval section, 8 cm long with a 1 × 3 mm bore. It is filled and emptied by means of a rubber "policeman" slipped over one end. Before placing it in position clean the tube with ethyl alcohol and polish with a cloth, and between measurements rinse out the tube with two lots of the soln. to be measured. Transfer the capillary to the special holder, so constructed that the angle formed by the oval section of the capillary with the light beam can be adjusted to the optimum, and the tube adjusted squarely in the beam. Always take care to place the tube in the holder the same way up. Measure the fluorescence, using a No. 39 Wratten filter in front of the right-hand photo-cell. Repeat the measurements with the other solns. in turn, and finally prepare a calibration curve in the same way, using solns. containing 0·001 to 0·2μg of aneurine per 0·1 ml. With solns. of pure aneurine the extreme variations between replicates was ±20% with 0·001μg and ±3% with 0·05μg. F. A. R.

New Modification of the *p*-Amino-acetophenone Method for Estimating Nicotinic Acid in Urine. Y. L. Wang and E. Kodicek (*Biochem. J.*, 1943, **37**, 530–538)—If the total amount of urine excreted daily is less than 1500 ml, dilute to this vol. To 20 ml add 2 ml of 40% sodium hydroxide soln. and heat on a boiling water-bath for 45 min. Cool, adjust to *p*H 2–3 with conc. hydrochloric acid (thymol blue), dilute to 25 ml and shake for 2 min. with a vol. of *iso*butanol equal to one-half that of the vol. of the digested urine before dilution. Separate and remove the dissolved *iso*butanol from 20 ml of the aqueous layer by heating on a boiling water-bath for about 15 min. Add 15 ml of water and cool to 40–50° C. Add 6 drops of conc. hydrochloric acid and then 4% potassium permanganate soln., drop by drop, with stirring, until the urine is completely decolorised and the pink colour persists for at least 30 sec. Cool, leave for at least 15 min. and neutralise to *p*H 6·5–6·8 with 40% sodium hydroxide soln. (bromothymol blue); the *p*H should not exceed 6·8. Dilute to 50 ml and remove any ppt. by centrifuging. Label 4 graduated 15-ml flasks *A*, *B*, *C* and *D*. To flasks *C* and *D* add 0·5 ml of standard nicotinic acid soln. (20μg per ml in 25% ethyl alcohol) and to flask *A* (the blank) 0·6 ml of 10% hydrochloric acid. Put 10 ml of urine into each of the 4 flasks, mix, and then add 2 ml of cyanogen bromide soln. (a saturated soln. of ice-cold bromine water titrated with 10% ice-cold sodium cyanide soln. until colourless) to each. Immerse the flasks for 4 min. in a water-bath at 56–58° C. and then cool for 5 min. in tap-water in the dark. To each flask add 1 ml of alcoholic amine reagent (10 g of recryst. *p*-amino acetophenone in 96% ethyl alcohol diluted to 100 ml) and shake. To flasks *B*, *C* and *D* add 0·6 ml of 10% hydrochloric acid and make up the vols. to 15 ml. After mixing, leave for 5 min. in the dark and immediately afterwards measure the colours in a Pulfrich photometer, using 3-cm cells and filter S 47. The nicotinic acid content in μg per ml of urine is equal to

$$\frac{B-A}{(C-A)-(B-A)} \times S \times \frac{F}{10} \times 1{\cdot}1 \times \frac{25}{20} \times \frac{1}{V_{\circ}}$$

where *A* and *B* are the extinctions of the solns. in flasks *A* and *B*; *C* is the mean of the extinctions of the solns. in flasks *C* and *D*; S = amount of nicotinic acid (μg) added to flasks *C* and *D*; F = final vol. of urine after washing with *iso*butanol, oxidation and neutralisation; $V_{\circ}$ = original vol. of urine taken for analysis; 1·1 = factor allowing for the increase in vol. of the washed urine due to *iso*butanol. The standard deviation of a single determination was ±2·4%, and the recoveries of added nicotinic acid ranged from 92 to 108% of the theoretical, with an average value of 98·5% in 13 expts. F. A. R.

Comparison of Thiamine Values by Chemical and Bioassay Methods. E. B. Brown, J. C. Hamm and H. E. Harrison (*J. Biol. Chem.*, 1943, **151**, 153–161)—In a collaborative assay of the aneurine contents of various samples carried out in different laboratories it was observed that the chemical results were, on the average, 20% lower than the bioassay results. The discrepancy was reduced to about 10% by adopting the following modification of the chemical method. Activate the Decalso used for adsorption by washing four times for 15 min. each with 4 vols. of hot 5% acetic acid, then four times with 4 vols. of hot 25% potassium chloride soln., and finally twice with hot water. Put the activated Decalso into the adsorption tube in small portions at a time, and allow the water to drip through by gravity. Wash the column with 5 ml of acidified potassium chloride soln. (250 g of potassium chloride and 10 ml of conc. hydrochloric acid per litre) and then with 50 ml of hot water. Just before use run 5 ml of 2% acetic acid through the column, followed by 5 ml of water and then by the sample. To obtain the best results it is necessary that the final solns. of both sample and standard should contain approx. the same amount (0·2μg per ml) of aneurine. Make up 5 ml of standard soln., containing 100μg per ml, to 200 ml, dilute 10 ml of this soln. ten-fold and run 20 ml of this diluted soln. through another column of activated Decalso. Wash both columns with 45 ml of hot water and then elute with 25 ml of acidified potassium chloride soln. Pipette 5 ml of standard eluate into each of two centrifuge tubes, and to one add 3 ml of 15% sodium hydroxide soln. and 25 ml of re-distilled *iso*butyl alcohol. To the other tube add 3 ml of 15% sodium hydroxide soln., 0·2 ml of 1% potassium ferricyanide soln. and then, after 30 sec., 25 ml of re-distilled *iso*butyl alcohol. Shake each tube for 90 sec. and centrifuge. Treat 5 ml of eluate from the sample in the same way. Finally, transfer 20 ml of the alcoholic layer in each tube to a dry centrifuge tube containing 1–1·5 g of anhydrous sodium sulphate, shake and centrifuge. Transfer the clear solns. to the cuvette of a fluorophotometer and take readings in the ordinary way. The preparation of the sample has also been found to be of considerable importance, and the following technique is recommended. Suspend the sample in 50 ml of 0·1 *N* sulphuric acid and heat under reflux for 30 min. After the b.p. has been reached, cool to 55° C., add 20 ml of takadiastase soln. (1·25 g in 100 ml of buffer soln., prepared by dissolving 136 g of sodium acetate and 60 ml of glacial acetic acid in water and diluting to 1 litre) and incubate at 50–55° C. for 2 hr. Add 5 ml of *N* sulphuric acid to stop the hydrolysis, dilute to 100 ml and filter. (The filtrate is used for adsorption). Clarase and polydase were not as good as takadiastase for enzymic hydrolysis. F. A. R.

Specific Reaction for Ascorbic Acid. L. F. Levy (*Nature*, 1943, **152**, 693)—The reversible oxidation of ascorbic acid with 2 : 6-dichlorophenolindophenol and with iodine becomes progressively slower with increasing hydrochloric acid concn., being completely inhibited by 20% of acid with the former reagent and by 29% with the latter; on dilution of the mixture with water, the reducing capacity of the ascorbic acid is fully restored. The reaction is specific and it enables account to be taken of substances normally interfering with the determination, being particularly useful for glucoreductone from glucose and hot alkali which, in presence of 20% of hydrochloric acid, can be titrated with indophenol, the pink colour persisting for 20 sec. at the end-point. With iodine in presence of 29% hydrochloric acid, however, the result is unreliable. By this method the high apparent ascorbic acid figure obtained with metaphosphoric acid extracts of biscuits to which has been added conc. orange juice, has been proved to be due to a reductone-like substance only; orange juice was found to contain a small proportion of reducing substance, the effect of which was not inhibited by mineral acid, whilst in fruits and vegetables of high vitamin content (guava, lucerne and parsley) no interfering substance was found. It is suggested that the effect might be due to the conversion of the ascorbic acid into the non-reducing keto form by the abundance of hydrogen ion, dilution with water regenerating the enol form.

J. A.

Determination of Ascorbic Acid in Presence of Sulphur Dioxide. L. F. Levy (*Nature*, 1943, **152**, 599)—Addition of an equal vol. of 3% hydrogen peroxide to the slightly acidified extracts of sulphited fruits and vegetables enables the ascorbic acid content to be accurately determined, the peroxide having no appreciable effect on the indophenol in absence of salts of iron and copper. The end-point is a permanent pink colour deepening very slowly on standing; smaller quantities of hydrogen peroxide cause low titration figures and more rapid deepening of the pink colour. The method has been used successfully for the last 3 years.

J. A.

Vitamins from Rose Hips. F. Wokes, E. H. Johnson, J. Duncan, J. G. Organ and F. C. Jacoby (*Quart. J. Pharm.*, 1943, **16**, 269–274)—Further evidence is given of the instability of vitamin C in rose hip syrup (see *Quart. J. Pharm.*, 1942, **15**, 314; ANALYST, 1943, **68**, 24), and it is shown that, although storage in a refrigerator diminishes the rate of loss, destruction of the vitamin still takes place, even out of contact with air. Dried rose hip extract, made by rapidly evaporating carefully prepared aqueous extracts to dryness *in vacuo*, is described, the average vitamin C content of 5 such extracts being 1250 mg per 100 g (1000 to 1400 mg per 100 g). Stability tests indicate that dried extracts are more stable than syrups, only 15% loss taking place after 248 days at 37° C., while no significant change occurs after 8 months in a refrigerator or at room temp. Dried rose hip extracts are shown by biological and clinical tests to be a good source of vitamin P; one sample, prepared with especial care to avoid loss, contained 520 G.L. units per g, as compared with 3 units per g for National rose hip syrup and 95 units per g for recrystallised hesperidin. The following figures for various fruit products are reported:

	Vitamin C mg per 100 g	Vitamin P G.L. units per g	Ratio C/P
Dried rose hip extract (mean)	1250	520	2·4
Rose hip syrup	140 to 150	3	49·5
Black currant purée ..	70	2·5	28
Flesh of rose hips (*Rosa arvensis*)	80	6·8	12
Flesh of rose hips (*R. coriifolia*)	1080	5·6	193

There is reason to believe that the higher ratios observed with rose hip syrup and black currant purée are due to loss of vitamin P in manufacture. The presence of carotene in some species of British rose hips is also reported and biological tests are in progress.

J. A.

Water

Sensitive Indicator for Volumetric Determination of Boiler Feedwater Alkalinity. H. Fleisher (*Ind. Eng. Chem., Anal. Ed.*, 1943, **15**, 742–743)—The ideal indicator for titration of boiler feedwaters would show a sharp colour change at the equilibrium point for the completion of the neutralisation reaction. This point was computed for boiler feedwaters at *p*H *ca.* 4·89 by interpolating data reported by Cooper (*Ind. Eng. Chem., Anal. Ed.*, 1941, **13**, 466; ANALYST, 1941, **66**, 429) to a millimolarity of 0·333, corresponding with the average sodium carbonate content of such waters. Methyl orange has been shown by Cooper (*loc. cit.*) to have a *p*H colour response in disagreement with the point of complete neutralisation. A modification of the indicator described by Johnson and Green (*Ind. Eng. Chem., Anal. Ed.*, 1930, **2**, 2), *viz.*, an alcoholic soln. of methyl red and alphazurine showed great promise but was unstable. This disadvantage was removed by substituting the sodium salt of methyl red (dimethylaminobenzene sodium carbonate) for methyl red, and water for alcohol; the indicator consisted of 0·45 g of methyl red sodium salt and 0·55 g of alphazurine in 1 litre of water; *p*H of the soln., *ca.* 7·3. The accuracy of the indicator was checked by titrating 100 ml of 0·00033 *M* sodium carbonate with 0·033 *N* hydrochloric acid, 3 drops of phenolphthalein being used for the first end-point and 3 drops of the prepared indicator for the second. Potential changes were measured with a glass electrode valve *p*H meter, and colour changes and max. potential deflection agreed well with the calc. equivalence point, and the vol. of acid required for the second end-point was double that required for the first. The *p*H values corresponding with the colour changes were determined in a series of adjusted solns. of acid potassium phthalate (0·02 *M*) with slightly lower results—4·8 for the grey and 4·6 for the purple-grey end-point. This discrepancy should not affect the accuracy in dil. unbuffered feedwaters. The sensitivity of the indicator is shown by a colour change from green-grey to grey with 1 drop (0·05 ml) of acid and grey to purple-grey with a second drop. The grey intermediate colour gives ample warning of the approaching purple-grey, which is used as the true end-point. To determine the effect of neutral salts, 100-ml portions of 0·00033 *M* sodium carbonate were prepared containing 400 p.p.m. of sodium chloride, 400 p.p.m.

of sodium sulphate, and 400 p.p.m. each of both salts respectively, and the solns. were titrated with 0·033 *N* hydrochloric acid. Each soln. required 2·0 (±0·025 ml) of the acid and the *p*H ranged from 4·6+ to 4·9+. These results show that, although neutral salts increase the apparent *p*H (as indicated by the glass electrode) of the equivalence point by 0·3 unit, the stoichiometric end-point, as indicated by the colour change, is not affected. Hence the indicator is suitable for titrating any feedwater containing salts within the above limits, which are those for most samples. A. O. J.

Determination of Oxygen in De-aerated Water. D. P. Evans and N. T. Simmons (*J. Soc. Chem. Ind.*, 1944, **63**, 29–30)—Dissolved oxygen in de-aerated water can be determined with an error of ±0·0005 ml per litre by means of the modified Winkler test described by the authors, in which the liberated iodine is titrated with 0·002 *N* thiosulphate in a soln. containing at least 0·2% of potassium iodide, the end-point being determined amperometrically by the "dead-stop" end-point method of Foulk and Bawden (*J. Amer. Chem. Soc.*, 1926, **48**, 2045). For full details the original paper should be consulted. W. R. S.

Organic

Substitute for Benzene in Determination of Acid Values. P. L. Gordon, M. A. Gildon and F. L. Rubin (*Ind. Eng. Chem., Anal. Ed.*, 1943, **15**, 765)—In the determination of the acid value of oils, resins and varnishes (Amer. Soc. Testing Materials, *Standards on Paint, Varnish, Lacquer and Related Products*, Jan., 1941, 137, 181) the customary solvent is a mixture of benzene and 95% ethyl alcohol, and the procedure involves titration of free acid with standard alcoholic potassium hydroxide, with phenolphthalein as indicator. A substitute for benzene being required owing to war conditions, the aromatic petroleum product Solvesso No. 1 (Colonial Beacon Oil Co.) was investigated. This solvent had the following properties, the figures in brackets being the corresponding figures for benzene for comparison—sp.gr. 0·815 (0·884); b.p. range, 93·3°–135° C. (80°–80·5° C.); Kauri-butanol value, 72·5 (120); mixed aniline point, 26·7° C. (7·3° C.). A mixture of 2 parts of Solvesso No. 1 to 1 part of denatured anhydrous ethyl alcohol proved to be an effective solvent for a representative selection of oils, resins and varnishes, the acid values obtained agreeing with those obtained with the benzene-alcohol solvent. With heavy bodied linseed oil the new solvent mixture had the advantage of maintaining a clear soln. throughout the titration. A. O. J.

Neatsfoot Oil [from Shin Bones]. C. C. Kritzinger (*Leather Ind. Res. Inst.* [*S.A.*], 1943, **2**, Circ. 24; *J. Int. Soc. Leather Trades Chem.*, 1944, **28**, 49)—The "neatsfoot" oil prepared by boiling the shin bones of cattle and sheep is of the same type as ordinary neatsfoot oil.

	Pure neats-foot oil, chilled and pressed	Commercial neats-foot oil	Neats-foot and shin oils	Shin oil	Sheep's foot oil
Acid. val.	0·17	0·21	0·24	0·26	0·27
Iodine val.	71·0	72·4	73·0	73·3	73·9
Sap. val.	195·4	195·9	194·8	192·7	192·2
Unsap., %	0·30	0·35	0·40	0·50	0·20

Resolution of Enantiomorphs by Chromatographic Adsorption. H. B. Hass, T. de Vries and H. H. Jaffé (*J. Amer. Chem. Soc.*, 1943, **65**, 1486–1488)—Chromatographic adsorption was applied to the resolution of *dl*-α-phenylethylammonium bi-*d*-tartrate and of brucine *dl*-mandelate. *dl-α-Phenylethylamine*—The following expt. illustrates the method. Dissolve 20 g of *dl*-α-phenylethylammonium bi-*d*-tartrate in 100 ml of water and filter through a 10 × 100-mm column packed with magnesium oxide mixed with 2 parts of Hyflo-supercel. Develop the chromatogram with 600 ml of water. Collect 4 successive liquid chromatograms (40, 100, 200, 200 ml). Recover the amine in ethereal soln., and measure the rotation to ±0·01° in a 1-dm tube. The results ranged from $[\alpha]_D$ −0·4 to +4. The $[\alpha]_D$ of pure α-phenylethylamine is +39·9; hence, practically no resolution was effected. *dl-Mandelic acid*—Filter a soln. of 105 g of brucine *dl*-mandelate in 1000 ml of benzene through a 5·2 × 120-cm column packed with dextrose; develop the chromatogram with benzene. Recover the mandelic acid from 5 liquid chromatograms and measure its rotation in aqueous soln. The results ranged from $[\alpha]_D$ −2·8 to +4·4. The $[\alpha]_D$ of pure *d*-mandelic acid in aqueous soln. is +155·5; the max. resolution was therefore about 3%. E. M. P.

Polysaccharide Hydroxylation by means of *p*-Toluenesulphonyl Chloride and Triphenylchloromethane. W. Low and E. V. White. (*J. Amer. Chem. Soc.*, 1943, **65**, 2430–2432)—Two methods have been used to confirm the presence of primary hydroxyl groups in arabo-galactan (White, *J. Amer. Chem. Soc.*, 1942, **64**, 2838). The first method uses *p*-toluenesulphonyl chloride, which reacts rapidly with primary hydroxyl groups and more slowly with secondary groups; treatment of the product with sodium iodide in acetone causes substitution only in the primary position. The second method is based on reaction with triphenylchloromethane in pyridine soln.; the primary groups react rapidly, and the remaining hydroxyl groups may be determined by esterification with acetic anhydride. The arabo-galactan was extracted from larch sawdust, *Larix occidentalis*, with the min. quantity of water, and the extract was filtered through Norit and Super-Cel; the arabo-galactan was purified by fractional pptn. with ethyl alcohol (White, *J. Amer. Chem. Soc.*, 1941, **63**, 2871). The white amorphous powder was dried under reduced pressure at 50° C. before reaction. Experimental procedures are as follows. *Reaction with p-toluenesulphonyl chloride*—Add 20 ml of anhydrous pyridine containing 5 g of the reagent to 1 g of polysaccharide in a glass reaction tube. Seal the tube, shake and warm in a rocking heater at 55° C. for 28 hr. Cool and pour the mixture slowly into rapidly stirred ice-water. Filter, wash free from chloride ion and dry. The yield was 2·5 g.; found, S 13·18%; calcd. tosyl substitution, 12·5 moles. *Iodination of the* p-*toluenesulphonate*—Dissolve 5 g of ester in 50 ml of acetone and treat with 10 g of sodium iodide. Heat in a sealed tube for 6 hr. at 100° C., cool and filter from the crystallised sodium *p*-toluenesulphonate. Drop the soln. slowly into rapidly stirred ice-water, filter, wash free from iodide ion and dry. Yield was 4·8 g; found, I 13·51%; S 10·23%; calcd. iodine substitution 3·08 moles, tosyl substitution 3·21 moles. *Reaction with triphenylchloromethane*—Dissolve 3 g of arabo-galactan in 20 ml of anhydrous pyridine, add 12 g of triphenylchloromethane dissolved in 40 ml of

pyridine, seal the tube, shake and warm at 50° C. for 12 hr. Cool, drop the soln. slowly into an excess of dry acetone, centrifuge, wash the ppt. free from chloride and carbinol with fresh acetone, and dissolve in pyridine. Pour the pyridine soln. into rapidly stirred ice-water, wash to remove chloride ion and dry. Yield, 5 g; found, C 62·76%, H 6·59; calcd. substitution, 2·80 moles. The slightly low carbon-content was constant and cannot be explained. *Acetylation of the tritylated derivative*—Dissolve 2 g of the ether in 5 ml of pyridine and warm on a steam-bath for 4 hr. with 5 ml of acetic anhydride. Cool and add the soln. dropwise to rapidly stirred ice-water. Filter, wash and dry. Dissolve in pyridine, precipitate as above, wash and dry. Yield 2·5 g; found, acetyl-content 27·2%; calcd. acetyl substitution 15·6 moles. Re-acetylation under the same conditions and similar experiments with boiling 1 : 1 soln. of pyridine and acetic anhydride gave products of the same acetyl content. E. M. P.

Determination of the Acetic Acid Yield of Acetylated Celluloses. F. Howlett and E. Martin (*J. Textile Inst.*, 1944, **35**, 1–6T)—The methods employ hydrolysis with sodium hydroxide in presence of sodium chloride for the evaluation of acetate rayons, and hydrolysis with potassium hydroxide in presence of alcohol for cellulose triacetates or samples difficult to wet or to hydrolyse. Procedures are as follows. *Undyed acetate rayon*—Weigh accurately 0·2–0·4 g of the acetate rayon and determine its dry weight either by drying or by making a moisture-content detmn. on a separate sample (drying for at least 3 hr. at 110° C. has no effect on the final result). Transfer to a stoppered conical flask, add 5 ml of saturated sodium chloride soln. and run in 10 ml of *N* sodium hydroxide. Leave overnight or for at least 2 hr. Add 10 ml of *N* sulphuric acid and, after 30 min. titrate with *N*/10 sodium hydroxide, using phenolphthalein as indicator. Make duplicate detmns. and blank tests. The *N* solns. do not require to be accurately standardised, but, to ensure a positive "blank," the acid should be slightly more concentrated than the alkali. Standardise the *N*/10 sodium hydroxide against potassium hydrogen phthalate (AnalaR). The acetic acid yield is defined as the weight of acetic acid produced by the complete hydrolysis of 100 g of dry cellulose acetate and is calculated according to the formula: acetic acid yield

$$= \frac{0{\cdot}6 \times f \times (T-t).}{W}$$

where f = factor of the $N/10$ sodium hydroxide, T = actual titre in ml, t = blank titre in ml, and W = dry weight of cellulose acetate in g. This method can be used also with commercial cottons acetylated to about 30% acetic acid yield (Cotopa 30); such materials do not swell greatly in alkali, and sodium chloride need not be added.

Dyed Acetate Rayon—Proceed as above to the titration stage. Add 15 ml of carbon tetrachloride, and also to the blank, stopper the flasks, shake vigorously for about 30 sec., and titrate as before. The carbon tetrachloride generally absorbs all the dye from the aqueous soln.; further dilution with water may be necessary if absorption is not complete. During the titration, support behind the flask a piece of mirror so tilted that the flask can be seen from the side; the coloured carbon tetrachloride layer does not then interfere with the perception of the colour change in the aqueous layer. Phenolphthalein may be used as indicator except where the aqueous layer is red or pink after carbon tetrachloride extraction; bromthymol blue (0·5% soln. in 20% ethyl alcohol) should then be used. Purify the carbon tetrachloride by distillation, or by shaking with aluminium oxide, activated charcoal or silica gel, preferably aluminium oxide, and filtering. *Materials difficult to hydrolyse*—The technique is the same as in the first method, but potassium hydroxide dissolved in a mixture of equal vols. of water and methyl or ethyl alcohol is used as the hydrolysing agent and no salt is added. Dilution with water before titrating is advisable, as the alcohol may cause fading of the indicator. E. M. P.

Determining Mixtures of Shirlan and *p*-Nitrophenol in Rot-proofed Cotton. F. Fancutt and M. S. J. Twiselton (*J. Soc. Chem. Ind.*, 1943, **62**, 205–206)—The following method has been devised for the determination of Shirlan NA and *p*-nitrophenol together in rot-proofed cotton railway wagon sheets, in which the limits have been fixed as 0·05–0·1% of each rot-proofing agent, expressed on the weight of the cloth. *Extraction of rot-proofing agents from cotton*—Extract 10 g of fabric in a Soxhlet apparatus with industrial alcohol or methyl alcohol for at least 4 hr., or until colourless. Remove the sample and recover the alcohol. Take up the residue of Shirlan NA and *p*-nitrophenol in water containing 1 ml of 2 *N* sodium hydroxide, transfer to a graduated flask, and make up to 200 ml with water. The extraction is accurate within 10% for Shirlan NA and 5% for *p*-nitrophenol. The dried cloth is used for determining fluidity (Clibbens and Geake, *J. Text. Inst.*, 1928, **19**, 77; Clibbens and Little, *id.*, 1936, **27**, 285), being compared with a sample of grey cloth before treatment. *Estimation of Shirlan NA*—This is based on the intensity of the colour of the indophenol formed by reaction between Shirlan NA and dimethyl-*p*-phenylenediamine (Steigmann, *J. Soc. Chem. Ind.*, 1942, **61**, 180). Put into eight 50-ml Nessler tubes 2·5, 5, 7·5, and 10 ml, respectively, of the extract and of the standard mixed soln. (containing 0·00375% of Shirlan NA and 0·00375% of *p*-nitrophenol and 5 ml of 2 *N* sodium hydroxide per litre). To each tube add 20 ml of 2·5% boric acid soln. and 1 ml of 0·2% aqueous dimethyl-*p*-phenylenediamine soln. (Prepare the latter by shaking a 0·2% aqueous *p*-nitrosodimethylaniline soln. with zinc dust and 1 ml of 10% copper sulphate; filter, and use within 2 hr. This reagent may turn pink or violet.) Add drops of 3% aqueous chloramine-T soln. until the red colour first developed disappears, leaving either a bluish-green soln. or one showing fluorescence; preferably, add 3 drops to all the tubes and repeat this until sufficient has been added (usually 10–14 drops). Add 10 ml of pure carbon tetrachloride to each tube and shake vigorously. Match the blue colour in the carbon tetrachloride layer against the standards and estimate the % of Shirlan NA present. The addition of the boric acid is the critical point which controls the development of the bluish-green soln. and fluorescence, which in turn controls the final colour. *Estimation of* p-*nitrophenol*—This is based on the intensity of the colour of the indophenol formed by interaction of *p*-aminophenol and excess of *o*-cresol. Acidify 100 ml of the above extract with 2 *N* acetic acid (5–10 ml in excess), using the *p*-nitrophenol present as indicator. Add 3–5 g of zinc dust, stir with a glass rod and set aside for 1 hr. to reduce the nitro-compound. At the same time reduce an equal

vol. of the standard soln. (as above) under the same conditions. Into eight 100-ml Nessler tubes introduce 2·5, 5, 7·5, and 10 ml respectively of the filtered reduced sample and standard. Add to each tube 5 ml of 1% aqueous *o*-cresol soln. made slightly alkaline with sodium hydroxide and make the vol. up to about 80 ml. Add 10 ml of 2 *N* sodium hydroxide and make up to 100 ml. Match the intensity of the blue colour, which usually reaches a max. in about 2 hr., against the standards and estimate the % of *p*-nitrophenol present.

E. M. P.

Reactions to Establish the Origin of Gelatins. A. Steigmann (*J. Soc. Chem. Ind.*, 1943, **62**, 206–208)—Application of the following tests to gelatin gives valuable indications as to the nature and treatment of the particular gelatin raw material. (1) *Determination by the Briefer–Cohen reaction* (*Ind. Eng. Chem.*, 1920, **20**, 408) *of the* pH *at which max. turbidity occurs*—Adjust aliquot parts of a 1–2% gelatin soln. to *p*H between 4·5 and 10·5 and judge the turbidity after some hours, when the gelatin has cooled and set. Gelatins derived from acid-conditioned raw material show the max. turbidity at *p*H 7–8 and those from alkali-conditioned raw material between 4·8 and 5·5. Gelatins from insufficiently limed but fully acid-plumped raw materal usually show a wide turbidity-*p*H range and a turbidity max. according to the predominant treatment given to the raw material. (2) *Reaction for free aldehyde, with benzidine,* o-*dianisidine or* 2·7-*diaminofluorene*—To prepare the benzidine reagent, dissolve 2 g of benzidine in 20 ml of glacial acetic acid, make up to 200 ml with water and dilute 1 part of the soln. with 9 parts of 5 *N* acetic acid. If the gelatins contain relatively high amounts of sulphites or sulphurous acid, add 1 ml of 30% hydrogen peroxide to 200 ml of the diluted reagent. Add 2–3 ml of the reagent to 2–3 ml of 10–20% gelatin soln.; a yellow to orange colour indicates free aromatic aldehyde; few aliphatic aldehydes react. The colour develops fully in 3 min.; darker colours developing later with the hydrogen peroxide reagent may be ignored. A marked difference in colour in presence or absence of peroxide indicates sulphites or sulphurous acid. A more sensitive reagent is made by dissolving 1 g of *o*-dianisidine in 100 ml of glacial acetic acid, adding 50 ml of water, decolorising with 5–10 g of active carbon and filtering through pulp in a Buchner funnel (Feigl, "*Qualitative Analysis with the Aid of Spot Tests*," 3rd Ed., p. 390, Leipzig, 1938). Add 1 ml of reagent, in absence of hydrogen peroxide, to 3 ml of 10–20% gelatin soln.; a yellow-red to orange-brown colour indicates free aldehyde, especially when no aldehyde can be detected before incubation; carry out this test also after incubation. (3) *The slow resorcinol and hydrochloric acid reaction for hexoses and free aldehyde* (more rapid for aldehyde than for hexoses)—To 3 ml of 15–20% gelatin soln. add 0·2 g of resorcinol and then 5 ml of conc. hydrochloric acid. Immerse the test-tubes for 1 min. in boiling water; an almost immediate yellow colour, changing after 3–12 hr. to red-violet, is characteristic of free aldehyde. These two last tests distinguish between (*a*) alkali-conditioned raw material, in which the original hyaluronic acid and chondroitinsulphuric acid have been split into proteins, amino-sugars and glycuronic acid (unadjusted *p*H of the final gelatin, 6–7·3); (*b*) both alkali- and acid-plumped raw material, showing no reaction for free aldehydes or for amino-sugars and glycuronic acid (unadjusted *p*H of the final gelatin, 4·5–5·5); (*c*) acid- or predominantly acid-plumped raw material, free from aldehydes, but containing hyaluronic acid and chondriotinsulphuric acid that can be hydrolysed and in which the amino-sugars can be converted into aldehydes by strong mineral acids or by incubation of the gelatin soln. (*p*H 7·0–7·3) at 37° C. (unadjusted *p*H of the final gelatin, 4·5–5·5 or lower). The tests should be carried out in comparison with 3 or 4 standard gelatins: (1) one giving no colour reaction with benzidine, *o*-dianisidine or resorcinol (raw material both alkali- and acid-plumped to give the gelatin *p*H 4·5–5·5); (2) one giving no colour reaction with benzidine or *o*-dianisidine, but a strong reaction with resorcinol (raw material acid- or predominantly acid-treated to give the gelatin *p*H 4·5–5·3); (3) one giving a strong benzidine or *o*-dianisidine reaction (raw material alkali-conditioned and faintly acidulated to give the gelatin *p*H 6·3–7·3); (4) bone gelatin, which contains neither hyaluronic nor chondroitinsulphuric acid and therefore gives no aldehyde reaction, whatever the treatment of the gelatin raw material. Liberation of aldehyde by incubation for 1–6 days at 37° C. is of interest for the investigation of mucopolysaccharides and sugars, which, if necessary, are added to a 5–10% soln. (*p*H 7·0–7·3) of a gelatin free from aldehyde or aldehyde-producing substances and then incubated; incubation, followed by the *o*-dianisidine test, confirms the conclusions drawn from the resorcinol test.

Formation of aldehyde on incubation at *p*H 4·5–7·0 is the slower the more acid the gelatin soln.; the formation of aldehyde passes through a maximum and the aldehyde disappears the more rapidly on prolonged incubation (in absence of preservatives) the sooner putrefaction begins in the gelatin soln. The suggestion that this is due to a bacterial or enzyme action is supported by the fact that incubation in the presence of 1–2 ml of 1% sodium dichlorophenoxide plus 1 ml of hydrogen peroxide in 100 ml of 10% soln. did not cause the formation of aldehyde; phenols alone did not inhibit the production of aldehyde.

E. M. P.

Inorganic

Electrolytic Determination of Copper in Cast Iron. W. M. MacNevin and R. A. Bournique (*Ind. Eng. Chem., Anal. Ed.*, 1943, **15**, 759–762)—A direct micro-electrolytic method was worked out. Take sufficient of the sample to yield 0·3 to 2 mg of copper in 5 ml, and 0·3–0·5 ml of 70% nitric acid (nitrate, sulphate, or perchlorate soln.). If molybdenum is present, add 0·15 ml of 85% phosphoric acid to prevent deposition of black sesquioxide on the cathode. Filter the acid soln. through a sintered-glass micro funnel into the electrolytic cell, adjust the vol. to 9–10 ml, add 3 drops of alcohol—to eliminate spray, stir by means of a current of air (2–3 bubbles per sec.) and electrolyse for 20 min. at 2·5–2·8 volt. Add 0·02–0·03 g of urea, rinse down the cell walls, and electrolyse for a further 5 min. Drain the cell while adding water until the current drops to zero. Rinse the cathode with alcohol, dry at 110° C. and weigh. The iron in the nitrate soln. does not interfere with the deposition of the copper, owing to elimination of oxides of nitrogen by the current of air.

W. R. S.

Factors affecting the Precipitation of some Insoluble Quinaldates [Quinaldinates]. J. F. Flagg and F. T. McClure (*J. Amer. Chem. Soc.*,

1943, **65**, 2346–2348)—The pptn. of metallic ions by quinaldinic acid is considered in relation to the solubility product of the pptd. complex. The values of the *p*H at which a number of metallic quinaldinates begin to ppt. have been determined. Further determinations of the fractions of metal ions pptd. at various stages of the *p*H range between the points of incipient and complete pptn., have been made for copper, cadmium and zinc. The formula derived is—

$$[H^+] = \frac{K_a\sqrt{[M_o^{++}](1-\alpha)}}{\sqrt{S}}\left([HQ_o] - 2\alpha[M_o^{++}]\right)$$

where $[H^+]$ is the hydrogen ion concn.; K_a, the ionisation constant of quinaldinic acid (*i.e.*, $1 \cdot 2 \times 10^{-5}$); $[M_o^{++}]$, the initial molar concn. of metal ions; $[HQ_o]$, the initial molar concn. of quinaldinic acid; α, the fraction of the metal ions pptd.; S, the solubility product. The results are tabulated as follows:

Ion	$[M_o^{++}]$	$[HQ_o]$	α	*p*H	pS
Ag^+	$1 \cdot 00 \times 10^{-3}$	$3 \cdot 00 \times 10^{-3}$	0	~0	17·9
Cd^{++}	$2 \cdot 33 \times 10^{-3}$	$8 \cdot 40 \times 10^{-3}$	0·976	3·25	12·44
	$2 \cdot 33 \times 10^{-3}$	$8 \cdot 40 \times 10^{-3}$	0·389	2·45	12·14
	$2 \cdot 43 \times 10^{-3}$	$6 \cdot 14 \times 10^{-3}$	0·860	3·19	12·24
				Av.	12·3
Co^{++}	$1 \cdot 0 \times 10^{-3}$	$3 \cdot 00 \times 10^{-3}$	0	3·50	10·9
	$5 \cdot 0 \times 10^{-4}$	$1 \cdot 50 \times 10^{-3}$	0	4·10	10·6
				Av.	10·8
Cu^{++}	$2 \cdot 43 \times 10^{-3}$	$7 \cdot 71 \times 10^{-3}$	0	0·50	15·68*a*
	$1 \cdot 65 \times 10^{-3}$	$5 \cdot 23 \times 10^{-3}$	0·817	0·81	16·94
	$1 \cdot 65 \times 10^{-3}$	$5 \cdot 23 \times 10^{-3}$	0·560	0·68	16·56
	$4 \cdot 13 \times 10^{-4}$	$13 \cdot 1 \times 10^{-4}$	0·441	1·50	16·52
	$4 \cdot 13 \times 10^{-4}$	$13 \cdot 1 \times 10^{-4}$	0·890	1·74	17·20
	$4 \cdot 13 \times 10^{-4}$	$13 \cdot 1 \times 10^{-4}$	0·954	2·00	17·12
				Av.	16·8
Fe^{+++}	$1 \cdot 0 \times 10^{-3}$	$3 \cdot 00 \times 10^{-3}$	0	0·5	16·9
Hg_2^{++}	$1 \cdot 0 \times 10^{-3}$	$3 \cdot 00 \times 10^{-3}$	0	~0	17·9
Hg^{++}	$2 \cdot 0 \times 10^{-3}$	$4 \cdot 8 \times 10^{-3}$	0	0·16	16·8
MoO_4^-	1×10^{-3}	$3 \cdot 00 \times 10^{-3}$	>0	<0	
Ni^{++}	$1 \cdot 00 \times 10^{-3}$	$3 \cdot 00 \times 10^{-3}$	0	3·90	10·1
Pb^{++}	$1 \cdot 00 \times 10^{-3}$	$3 \cdot 00 \times 10^{-3}$	0	3·60	10·6
Pd^{++}	$1 \cdot 0 \times 10^{-3}$	$2 \cdot 4 \times 10^{-3}$	0	2·50	13·0
	$5 \cdot 0 \times 10^{-4}$	$1 \cdot 5 \times 10^{-3}$	0	2·99	12·8
				Av.	12·9
WO_4^-	1×10^{-3}	$3 \cdot 00 \times 10^{-3}$	>0	<0*b*	
Zn^{++}	$1 \cdot 30 \times 10^{-3}$	$4 \cdot 76 \times 10^{-3}$	0	1·85	12·78*a*
	$1 \cdot 30 \times 10^{-3}$	$4 \cdot 76 \times 10^{-3}$	0·483	2·30	13·32
	$1 \cdot 30 \times 10^{-3}$	$4 \cdot 76 \times 10^{-3}$	0·900	2·50	14·04
	$1 \cdot 30 \times 10^{-3}$	$8 \cdot 90 \times 10^{-3}$	0·916	2·18	13·84
	$1 \cdot 30 \times 10^{-3}$	$8 \cdot 90 \times 10^{-3}$	0·961	2·35	13·82
			..	Av.	13·8

a Not included in average. *b* Quantitative between *p*H = 0 and *p*H = 1. Solubility of molybdate and tungstate complexes increases with *p*H; both dissolve completely at *p*H 4.

The equation can be used to calculate: (*a*) the min. *p*H necessary for complete pptn. by making $\alpha = 0 \cdot 999$; (*b*) the possibility of a separation, by calculating the min. *p*H required for the complete pptn. of one metallic ion and the *p*H for incipient pptn. of the other (for this second calculation $\alpha = 0$ and allowance must be made for the quinaldinic acid removed by the first ion). If the second *p*H is greater than the first, a separation should be possible and the best *p*H for it will be mid-way between these two values. The separations of copper from zinc and from cadmium are discussed in detail and the authors find Majumdar's results for the latter separation in accordance with their theory (ANALYST, 1939, **64**, 874).* Silver, mercury, tungsten and molybdenum quinaldinates are insoluble at such low *p*H values that interference with copper, zinc or cadmium determinations would be certain. The equation can also be used to calculate the fraction of metallic ion (α) which will be pptd. under given conditions of *p*H and quinaldinic acid concentration. C. F. P.

Specific Test for Thallium. P. Wenger and Y. Rusconi (*Helv. Chim. Acta*, 1943, **26**, 2263–2264)—Treat a drop of cold acid thallous salt soln. on a spot plate with a drop of 0·4% bismuth nitrate soln. in 20% nitric acid and a drop of 10% sodium iodide soln. Thallium gives a red ppt. at $1 : 5 \times 10^4$. Lead, selenium and tellurium interfere. Platinum, iron and cerium do not react after addition of a drop of strong sodium thiosulphate soln. Rhodium and palladous salts gradually yield a brown ppt. which may mask the thallium reaction. W. R. S.

Method for the Continuous Measurement of Atmospheric Ozone. E. Gluckauf, H. G. Heal, G. R. Martin and F. A. Paneth (*J. Chem. Soc.*, 1944, 1–4)—The method is based on the reaction of ozone with buffered potassium iodide soln. (KI 100, $NaH_2PO_4.H_2O$ 1·38, NaOH 0·20 g in 500 ml of water). The liberated iodine is determined by its depolarising effect on polarised platinum electrodes; the current generated is amplified and works a

* See also A. K. Majumdar, *Id.*, 1943, **68**, 242; A. J. Lindsay and R. J. Shennan, *Id.*, 1940, **65**, 636; R. J. Shennan, *Id.*, 1939, **64**, 14; C. F. Pritchard and R. C. Chirnside, *Id.*, 1943, **68**, 224.—ABSTRACTOR.

relay which releases a small fixed volume of 0·0005 *N* thiosulphate from an automatic burette. The elaborate semiportable apparatus, fully described and illustrated with diagrams, is suitable for the continuous automatic determination of atmospheric ozone over periods up to 24 hr.

W. R. S.

Physical Methods, Apparatus, etc.

Portable Aspirator for Gas Sampling. H. C. Stephenson (*Chem. and Ind.*, 1944, 3–4)—A double aspirator previously designed for continuous aspirating consists of two aspirator bottles in a frame. Water runs from the upper aspirator to the lower and, after inversion of apparatus, *vice-versa.* Use of a modification of this, illustrated, is as follows: As a preliminary, fill the upper aspirator with water to above the zero mark and close with a stopper having a tube dipping to the bottom of the bottle. Run the water down to zero level of the top aspirator into the lower aspirator; close the pinch cock; invert the apparatus and add water to the aspirator now uppermost, until the level is at the lowest calibration mark. The apparatus is in a case which, in another compartment, holds a water-seal unit for maintaining unidirectional water- and air-flow through a detector tube; the action of this unit is automatic. For use, remove the seal from its case and place it where the atmospheric test is to be made; place the case on end nearby, with full aspirator uppermost and connect seal and aspirators through a hole in the back of case. Invert the case as often as required. Capacity of aspirator, 175 ml; rate of air flow, 25 ml per min. The apparatus is useful in factories where the air is to be tested for contamination with toxic gas.

E. B. D.

Corrigendum.—In the March issue the abstract beginning on p. 97 and ending on p. 98 was inserted in error; please ignore.

Reviews

THE VITAMINS. A General Survey for the Practising Pharmacist. 2nd (Revised) Edition. Pp. 64. London: The Pharmaceutical Press, 17, Bloomsbury Square, W.C.1. Price 2s. 6d. post free.

The substance of this booklet consists of a revision of a series of articles which appeared in *The Pharmaceutical Journal.* The second edition here reviewed has again been revised and all the information on this important and topical subject is practically up to date.

There are nine chapters dealing with such aspects as:—terminology, units of measurement, chemical and physical properties and structural formulae, vitamin values of common foods, requirements of human subjects, effects of overdosage, physiological action and signs of deficiency, pharmaceutical preparations, and the potency of commercial preparations. Under all these headings the information is comprehensive and accurate. Questions concerned with the analytical characterisation (apart from melting points) and determination of the vitamins are not discussed to any extent.

Although designed primarily for the use of pharmacists, this well planned and well written booklet should be of considerable value to food analysts and all whose work is concerned with problems of nutrition.

S. K. CREWS

THE SPECTROCHEMICAL AND POLAROGRAPHIC ANALYSIS OF ALUMINIUM AND ITS ALLOYS. Pp. 84. London: The British Aluminium Co., Ltd. Publication No. 401, 1943. Gratis.

A recent publication of the British Aluminium Company (No. 399, 1941; reviewed in THE ANALYST, 1942, **67**, 181) contained about fourteen pages of notes on the application of spectrographic and polarographic methods to the analysis of aluminium and its alloys. It is some measure of the growing importance of these two techniques that it has now been found necessary to issue an eighty-four page book to deal adequately with them.

The spectrochemical section opens with a brief description of the necessary equipment, including the devices for preparing the samples in the recommended form. All the methods proposed employ a medium quartz spectrograph and spark excitation of the spectra. The theory of plate calibration is then discussed in some detail and descriptions are given of two types of mechanical calculator which greatly facilitate the calibration process. The section concludes with notes on the various analytical problems for which the spectrographic method is considered suitable; these include the recommended line pairs for the various impurities, the concentration ranges for which the method is suitable and the probable accuracy of each determination. This information is given for a number of alloys as well as for pure aluminium.

The polarographic methods described in the second section of the book have all been carefully tested and some are in daily routine use in the laboratories. An all-glass dropping electrode of simple and satisfactory design is described, and new methods are given for the

following determinations: copper in pure aluminium; copper, antimony and tin in alloys; zinc in an aluminium-zinc-magnesium alloy; nickel and zinc simultaneously in secondary aluminium and in alloys.

In this book The British Aluminium Company have continued their policy of publishing the full details of their analytical experience; once again they have earned the thanks of a wide circle of analysts.

B. S. COOPER

INORGANIC CHEMISTRY. FRITZ EPHRAIM. Fourth English Ed. By P. C. L. THORNE, M.A., Ph.D., F.I.C., and E. R. ROBERTS, A.R.C.S., Ph.D., D.I.C. Pp. xii + 921. London: Gurney and Jackson. 1943. Price 28s.

That a fourth English edition of this book should have been called for only four years after the publication of the third, and in times so difficult as these, is perhaps the best testimony that can be paid to its outstanding quality. In this edition Dr. Thorne has had the collaboration of Dr. E. R. Roberts, and although there is an increase of only 11 pages over the 1939 volume, "substitution rather than addition has been the rule."

There is little to add to what was said about the third edition (ANALYST, 1940, **65**, 584); if the arrangement is still somewhat unconventional it has the merit that through it inorganic chemistry appears to have some sense of order and unity rather than to be a collection of unco-ordinated facts. Indeed, so pronounced is this quality of continuity that having begun, one is as reluctant to stop reading as with the most absorbing novel. From the first chapter concerning the structure of the atom to the last on intermetallic compounds it is a romantic tale and, to quote an earlier reviewer, "the only adequate method of reviewing it is simply to issue an order that it be read by all chemists." The analyst cannot fail to find inspiration within its covers. From time to time facts of direct interest to him are given, *e.g.*, p. 447, the necessity to ignite alumina at 1100°–1200° C., before weighing.

One notices a few statements that need correction or amendment; on p.448, synthetic sapphire does not form "hexagonal prismatic twins" nor is the addition of 0·2–0·3% of Cr_2O_3 likely to give a very good ruby. The existence of some of the oxides having the formulae given on pp. 449 and 451 is doubtful, *e.g.*, $BeO.H_2O$ and Ni_2O_3. Further X-ray evidence will resolve these doubts. On pp. 455 and 457 böhmite is obviously intended, while on p. 455 it is not clear what is meant by the statement that "AlO.OH has a completely unorientated structure." There may be other isolated inaccuracies, but nothing can detract from the all round excellence of this book.

The publishers are to be congratulated on the quality of the paper and of the binding.

R. C. CHIRNSIDE

FORMATION OF A MICROCHEMICAL GROUP

MEMBERS of the Society who wish to become members of the Microchemical Group now in course of formation should send in their names to the Hon. Secretary. Members of the Society who are also members of the Microchemical Club need not do so, as they will automatically become members of the Microchemical Group.

MICROCHEMICAL APPARATUS

WITH reference to standardisation of microchemical apparatus, all users of the Pregl micro combustion methods are invited to write to the Chairman, Panel C/8/9/1, British Standards Institution, 28, Victoria Street, Westminster, London, S.W.1, so that their experience and preferences can be consulted.

ADVICE TO AUTHORS

THE Council has approved the following notice by the Publication Committee, which is here given in condensed form.

The Society publishes papers concerned with all aspects of analytical chemistry, inorganic and organic, as, for example, food and drugs analysis, analysis of water (including its bacteriological examination), gas analysis, metallurgical assays, biological standardisation and micro-analysis. Papers on these and allied subjects may be submitted for presentation and publication; they may:

(1) Record the results of original investigations into known methods or improvements therein;
(2) Record proposals for new methods and the investigations on which the proposals are based;
(3) Record analytical results obtained by known methods where these results, by virtue of special circumstances, such as their range or the novelty of the materials examined, make a useful addition to analytical information;
(4) Record the application of new apparatus and new devices in analytical technique and the interpretation of results.

Communications.—Papers (which should be sent to the Editor) will normally be submitted to at least one referee, on whose advice the Publication Committee will be guided as to the acceptance or rejection of any communication. Papers or Notes accepted by the Publication Committee may not be published elsewhere except by permission of the Committee.

Abstracts.—The MS. should be accompanied by a brief abstract of about 100 to 150 words indicating the scope and results of the investigation.

Notes on the writing of papers for THE ANALYST

Manuscript.—Papers and Notes should preferably be typewritten.

The title should be descriptive and should set out clearly the scope of the paper.

Conciseness of expression should be aimed at; clarity is facilitated by the adoption of a logical order of presentation, with the insertion of suitable paragraph or sectional headings.

Generally, the best order of presentation is as indicated below:

(*a*) General, including historical, introduction.
(*b*) Statement of object of investigation.
(*c*) Description of methods used. Working details of methods are usually most concisely and clearly given in the imperative mood, and should be given in this form, at least while economy of paper is pressing, *e.g.*, "Dissolve 1 g in 10 ml of water and add . . .". Well-known procedures must not be described in detail.
(*d*) Presentation of results.
(*e*) Discussion of results.
(*f*) Conclusions.

To be followed by a short summary (100 to 250 words) of the whole paper: items (*e*) and (*f*) can often be combined.

Illustrations, diagrams, etc.—The cost of setting up tabular matter is high and columns should therefore be as few as possible. Column headings should be brief or replaced by a number or letter to be used in combination with an explanatory footnote to the table.

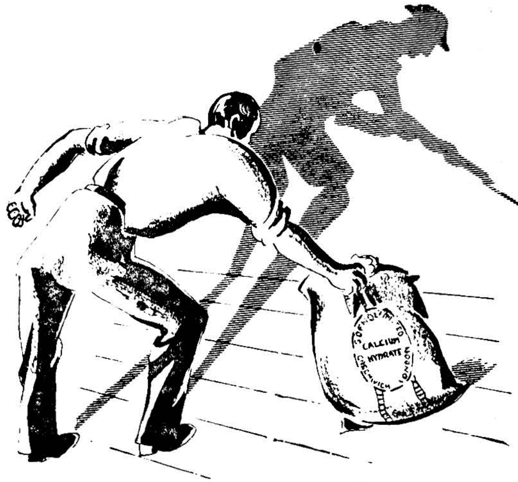
Sketches or diagrams should be on white Bristol board, not larger than foolscap size, in Indian ink. Lettering should be in light pencil.

Tables or graphs may be used, but normally not both for the same set of results. Graphs should have the co-ordinate lines clearly drawn in ink.

References.—References should be numbered serially in the text and collected in that order under "REFERENCES" at the end of the paper. They should be given in the following form:

1. Dunn, J. T., and Bloxam, H. C. L., *J. Soc. Chem. Ind.*, 1933, **52**, 189T.
2. Allen, A. H., "*Commercial Organic Analysis*," Churchill, London, 1882.

Notes on the Presentation of Papers before Meetings of the Society are appended to the "ADVICE," copies of which may be obtained on application to the Secretary, 7/8, Idol Lane, London, E.C.3.



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No. 817, Pages 105—140

Contents

PROCEEDINGS OF THE SOCIETY OF PUBLIC ANALYSTS AND OTHER ANALYTICAL CHEMISTS

PAGE

Annual General Meeting of the Society, March 1 .. 105
New Members .. 106
Standards for Gelatin .. 106
Annual Report of Council; March, 1944 .. 106
Toluene-3:4-dithiol as a Selective Reagent for Tungsten: The Detection of Tungsten, especially in Molybdenum and Rhenium Compounds, and in Ferrous Alloys—Christina C. Miller, D.Sc., Ph.D. 109
Determination of Residual Carbon Dioxide in Aerating Powder—C. K. Boundy, A.I.C., and R. W. Morris, B.Sc., F.I.C., A.C.G.F.C., D.I.C. .. 113
A Rapid Method of Micro Gas Analysis—W. B. Price, B.Sc., Ph.D., F.I.C., and L. Woods, M.Sc., A.I.C. 117
Notes—Identification of Demolition Dust; Reflux Apparatus for Automatic Dispersion of Froth in the Determination of Fibre; The Detection of Indole by means of Xanthydrol .. 121
Ministry of Food—Statutory Rules and Orders .. 123
Legal Notes .. 124
Notes from the Reports of Public Analysts .. 125
The Iron and Steel Institute—The Determination of Sulphur and Phosphorus in Pig Iron .. 125
British Standards Institution—B.S. for Filtered Sperm Oil .. 126

ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

Food and Drugs—

Carbohydrate Characterisation. Identification of *d*-Ribose, *l*-Fucose and *d*-Digitoxose as Benzimidiazole Derivatives—R. J. Dimler and K. P. Link .. 126
Azoyl Derivatives of Sugars and Separation by Chromatographic Adsorption—G. H. Coleman and C. M. McCloskey .. 127
Occurrence of Citric and *Iso*citric Acid in Blackberries and Dewberry Hybrids—A. L. Curl and E. K. Nelson .. 127
Quantitative Extraction of Laboratory Samples of Oils from Fish Tissues—W. S. Rapson, H. M. Schwartz and N. J. van Rensburg .. 127
Thermal Decomposition of Lard—C. D. Larsen and H. P. Morris .. 127
Palisade Ratio of the Official Leaf Drugs of the Solanaceae, *Solanum carolinense* and *Phytolacca americana*—P. L. Bogarosh .. 128
Quantitative Determination of Sulphanilamide and Sulphathiazole in Mixtures—D. T. Englis and D. A. Skoog .. 128

Biochemical—

Chemical Method for the Determination of Minute Traces of Thallium in Tissues—K. Samaan and M. N. Mikhail .. 128
Rapid Determination of Iron in Ferric Phytate—J. R. Foy and J. B. Thompson .. 129
Application of the Millon Reaction to the Determination of Chlorophenols in Body Fluids and Tissues—B. Zondek, B. Shapiro and S. Hestrin .. 129
Estimation of Haemoglobin by Photometric Absorptiometers—J. M. Peterson and D. H. Strangeways 129
Estimation of Creatine and of Diacetyl—P. Eggleton, S. R. Elsden and N. Gough .. 130
Photometric Micro Method for the Estimation of Inulin in Serum and Urine—H. Ranney and D. J. McCune .. 130
Glucose Dehydrogenase from Germinating Seeds of Green and Black Grams (*Phaseolus Radiatus* and *Phaseolus Mungo* Linn.—K. P. Basu and J. N. Karkun .. 130
Succinic Acid Dehydrogenase from Cucumber Seeds—K. P. Basu and J. N. Karkun .. 131
Colorimetric Estimation of Cholesterol—W. M. Sperry and F. C. Brand .. 131
Vitamin Content of Beverages—J. C. Drummond and T. Moran .. 131
Micro-method for the Estimation of Vitamin B_1—J. J. C. Hinton .. 131
New Modification of the *p*-Amino-acetophenone Method for Estimating Nicotinic Acid in Urine—Y. L. Wang and E. Kodicek .. 132
Comparison of Thiamine Values by Chemical and Bioassay Methods—E. B. Brown, J. C. Hamm and H. E. Harrison .. 132
Specific Reaction for Ascorbic Acid—L. F. Levy .. 133
Determination of Ascorbic Acid in Presence of Sulphur Dioxide—L. F. Levy .. 133
Vitamins from Rose Hips—F. Wokes, E. H. Johnson, J. Duncan, J. G. Organ and F. C. Jacoby .. 133

Water—

Sensitive Indicator for Volumetric Determination of Boiler Feedwater Alkalinity—H. Fleisher 133
Determination of Oxygen in De-aerated Water—D. P. Evans and N. T. Simmons .. 134

Organic—

Substitute for Benzene in Determination of Acid Values—P. L. Gordon, M. A. Gildon and F. L. Rubin 134
Neatsfoot Oil [from Shin Bones]—C. C. Kritzinger .. 134
Resolution of Enantiomorphs by Chromatographic Adsorption—H. B. Hass, T. de Vries and H. H. Jaffe .. 134
Polysaccharide Hydroxylation by means of *p*-Toluenesulphonyl Chloride and Triphenylchloromethane—W. Low and E. V. White .. 134
Determination of the Acetic Acid Yield of Acetylated Celluloses—F. Howlett and E. Martin .. 135
Determining Mixtures of Shirlan and *p*-Nitrophenol in Rot-proofed Cotton—F. Fancutt and M. S. J. Twiselton .. 135
Reactions to Establish the Origin of Gelatins—A. Steigmann .. 136

Inorganic—

Electrolytic Determination of Copper in Cast Iron—W. M. MacNevin and R. A. Bournique .. 136
Factors affecting the Precipitation of some Insoluble Quinaldates [Quinaldinates]—J. F. Flagg and F. T. McClure .. 136
Specific Test for Thallium—P. Wenger and Y. Rusconi .. 137
Method for the Continuous Measurement of Atmospheric Ozone—E. Glueckauf, H. G. Heal, G. R. Martin and F. A. Paneth .. 137

Physical Methods, Apparatus, etc.—

Portable Aspirator for Gas Sampling—H. C. Stephenson .. 138

Reviews .. 138

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