THE ANALYST

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

NORTH OF ENGLAND SECTION.

THE Second Summer Meeting was held at the Queen's Hotel, Scarborough, from July 3rd to 6th, when forty-four were present, including several ladies. The President (Dr. J. T. Dunn) was accompanied by Mrs. Dunn, and the Hon. Secretary of the Society (Mr. F. W. F. Arnaud) by Mrs. Arnaud, and other members from the south included Mr. E. M. Hawkins, Mr. E. Hinks and Mr. A. Lucas.

On Saturday afternoon Dr. C. Ainsworth Mitchell (Editor of THE ANALYST) read a paper, illustrated by lantern slides, on "Documentary Evidence in Criminal Trials," and Mr. A. Lucas contributed to the discussion.

The Chairman (Mr. C. J. H. Stock) thanked the members of the Section and the members of the Society in general for their support, which had helped to make the Summer Meeting such an unqualified success. He recalled the fact that in former years such meetings were regularly held by the Society, and laid stress on their social value in enabling members to become better acquainted.

He then proposed the following resolution, which was carried unanimously:

> "That the members of this Summer Meeting of the North of England Section of the Society of Public Analysts and Other Analytical Chemists desire to convey an expression of loyalty and goodwill to the Council and of devotion to its aims and interests. They wish also to express their sense of the honour paid to the Section by the presence at the meeting of officers and members of the parent body."

A vote of thanks to the Hon. Secretary of the Section (Mr. J. R. Stubbs) was proposed by the President and unanimously passed.

Death.

WITH deep regret we record the death of Mr. S. G. Agar (Official Analyst for the Island of Guernsey), on March 2nd, 1931.

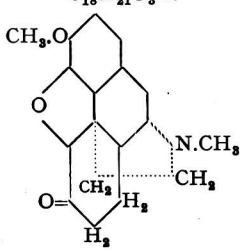
The Identification and Determination of Dicodide, Eucodal and Dilaudide.

By JOHN KING, F.I.C.

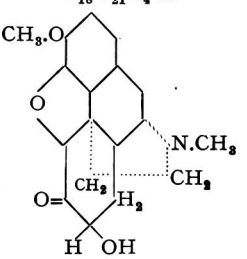
The identification and determination of dicodide, eucodal and dilaudide have become important, owing to their recent inclusion in the list of drugs falling within the provisions of the Dangerous Drugs Act, 1920, Part III. Eucodal and dicodide are included under Statutory Rules and Orders No. 644 of Aug. 14, 1928, and dilaudide under No. 530 of June 13, 1930.

Their constitutions may be given as follows:

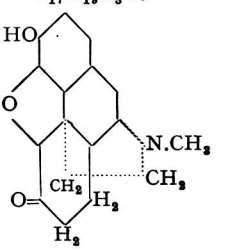
Dicodide
(dihydro-codeinone)
$C_{18}H_{21}O_3N$.



Eucodal,
"dihydro-oxycodeinone"
(dihydro-hydroxycodeinone)
$C_{18}H_{21}O_4N$.



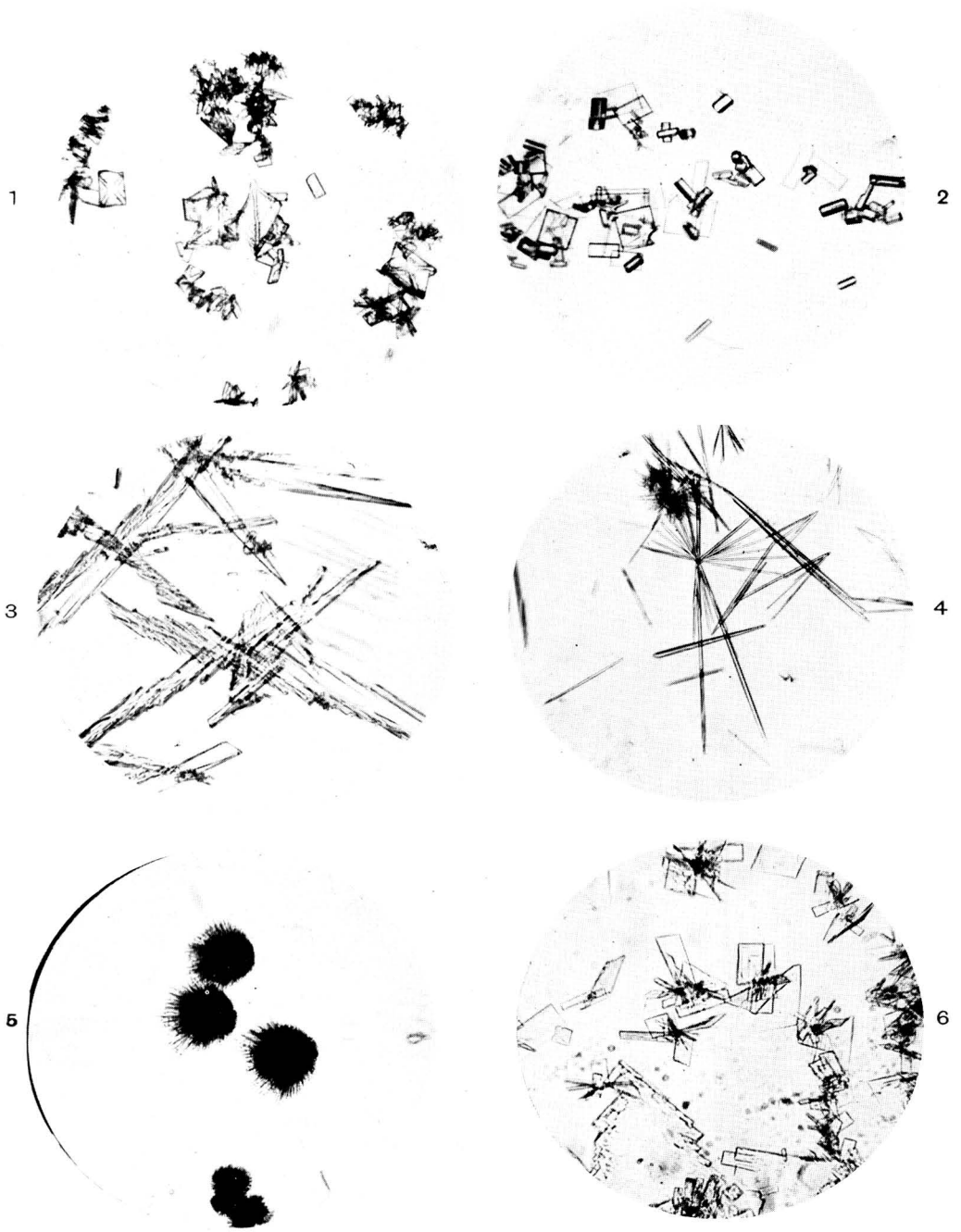
Dilaudide
(dihydro-morphinone)
$C_{17}H_{19}O_3N$.



The structural formulae are based on Robinson's structure for codeinone (Gulland and Robinson, *J. Chem. Soc.*, 1923, **123**, 980; *Mem. Proc. Manch. Lit. Phil. Soc.*, 1925, **69**, 79), which Schopf's recent work (*Annalen*, 1927, **452**, 211) has shown to be more probable than that of Wieland (*Ber.*, 1925, **58**B, 2009), or of Freund and Speyer (*J. prakt. Chem.*, 1916, **94**, 135; *Ber.*, 1906, **39**, 844; 1924, **57**, 1404).

These substances have been prepared from morphine, codeine or thebaine by suitable oxidation and reduction methods, and, as would be expected from their structures and derivation, they exhibit many similarities in properties both amongst themselves and with morphine and its derivatives. They are prescribed medicinally as hydrochlorides, bitartrates and phosphates. The solubility of the free bases in water at ordinary temperatures is very small, but increases rapidly with rise of temperature in a manner similar to that of morphine. The hydrochlorides are readily soluble in water, giving neutral solutions, the bitartrates being less soluble and giving acid solutions.

Dicodide and eucodal are precipitated by alkalis from solutions of their salts and are not soluble in excess of the reagents. The precipitates are crystalline, so that the bases may be identified microscopically. The crystalline forms of the bases and of some of their derivatives are shown on the accompanying photomicrographs. The bases may remain in super-saturated solution for a considerable



(1) Dicodide (5 per cent.) and 7 per cent. ammonia. × 100.
(2) Dilaudide (5 per cent.) and 7 ,, ,, ,, ,,
(3) Eucodal (1 per cent.) and 7 ,, ,, ,, ,,
(4) Morphine (1 per cent.) and picrolonic acid. ,,
(5) Dilaudide (0·5 per cent.) and picrolonic acid (1 per cent.) ,,
(6) Dilaudide (10 per cent.) and palladous chloride. ,,

time, and it is generally necessary to scratch the microscope slide in order to induce crystallisation when employing a micro-crystalline test.

Dilaudide is precipitated from its salts by alkalis, and, as might be expected from the presence of a phenolic hydroxyl group, is soluble in excess of the reagents, its solubility in ammonia distinguishing it from morphine.

Eucodal is moderately soluble in petroleum spirit, dicodide sparingly soluble, and dilaudide insoluble. All three bases are moderately soluble in ether and readily soluble in chloroform, differing from morphine in these respects.

MICRO-CRYSTALLINE TESTS.—The bases are precipitated from solutions of their salts at concentrations of 1 per cent., or even less, by the following alkaloid reagents: —Mayer's reagent, Scheibler's reagent, Wagner's reagent, phosphomolybdic acid, silicotungstic acid, picrolonic acid and bromine water, and, at concentrations somewhat higher, by chromic acid, mercuric chloride and palladous chloride. These precipitates, with the following exceptions, are amorphous: Wagner's reagent gives a crystalline precipitate only with eucodal, which somewhat resembles that given by morphine, but is sufficiently characteristic to make a micro-crystalline test of value in identification. Picrolonic acid (1:4-dinitro-1-phenyl-3-methyl-5-pyrazolone) gives a crystalline precipitate only with dilaudide, its form resembling phenyl-lactosazone, and being of value in a micro-crystalline test. It is necessary to scratch the slide to induce crystallisation. The crystals given by morphine with picrolonic acid are readily distinguishable from those given by dilaudide. All three alkaloids give crystalline precipitates with a 1 per cent. solution of palladous chloride in dilute hydrochloric acid when the concentration of the alkaloid is 10 per cent. or over. They form circular clusters of plates or needles and resemble each other and the compound given by morphine. This micro-crystalline test should be carried out alongside known specimens under identical conditions, and should be used only as a confirmatory test.

The above, together with the crystalline form of the free bases, are the only micro-crystalline tests, as all other reagents tried have given amorphous precipitates.

COLORIMETRIC TESTS.—Marquis' reagent gives with each a bluish-violet colour, becoming red on standing. The colours given with the three alkaloids are not identical, nor are they quite the same as those given by morphine and heroin. In order to distinguish them, actual trials of known specimens must be made under conditions identical with those used with the substance under examination.

Iodic acid, in presence of dilute sulphuric acid, gives a yellowish-brown colour with dilaudide, as might be expected from its structural resemblance to morphine, but the colour is closer to yellow than that given by morphine. On adding ammonia a mahogany colour is given, and, on standing, this becomes distinctly more red, in contrast with morphine, which gradually assumes a dull brown colour. This test is quite characteristic for dilaudide.

When to 1 ml. of a dilute aqueous solution of a salt of these alkaloids 1 ml. each of 3 per cent. hydrogen peroxide and 7 per cent. ammonia solution are added,

followed by 1 drop of 10 per cent. copper sulphate solution, the following colours are given:—Dilaudide, brown to yellow; and morphine, brown to red. Eucodal and dicodide have no appreciable effect on the blue colour of the copper ammine. On adding a few drops of 10 per cent. potassium cyanide (which removes the colour of the copper ammine), the colours given are:—Morphine, red; dilaudide, yellow; eucodal and dicodide, very pale red. The test may be made extremely delicate for dilaudide by suitably reducing the amount of reagents added. As little as 1/20 mgrm. can be detected with certainty, and the test distinguishes dilaudide from all other alkaloids.

Radulescu's test (*Chem. Centrbl.*, 1906, **1**, 1378) for morphine is also given by dilaudide, but not by dicodide or eucodal. The test is carried out as follows:—To a dilute aqueous solution of the alkaloid are added a few drops of dilute hydrochloric acid and sodium nitrite solution. After five minutes a few drops of strong ammonia solution are added, a brown colour indicating morphine or dilaudide. The test is extremely sensitive and will detect 1/50 mgrm.

Like many ketones, the three bases may be condensed with aldehydes in presence of alkalis (Lapworth, *J. Chem. Soc.*, 1911, **99**, 1884). They condense readily with piperonal in alcoholic solution in presence of sodium hydroxide, giving yellow piperonylidenes. These cannot be obtained in crystalline form. The piperonylidenes give a brilliant violet colour with concentrated sulphuric acid, and this may be used as a confirmatory test for the alkaloids. They give precipitates with the usual alkaloid precipitants.

Fröhde's reagent gives with dilaudide a deep violet colour, changing on standing to intense blue. This resembles the colour change given by morphine and distinguishes these two alkaloids from eucodal and dicodide, which give no colour with the reagent.

Like morphine, dilaudide reduces potassium ferricyanide and gives Prussian blue with a mixed solution of ferric chloride and potassium ferricyanide. Eucodal and dicodide do not give this reaction.

All three alkaloids resemble morphine in giving a red colour when coupled with diazotised sulphanilic acid, and this test distinguishes the group from most other alkaloids.

Dicodide and dilaudide form oximes, unlike morphine, which has no ketonic group. The oxime of dicodide may be obtained by warming a solution of the hydrochloride in water with hydroxylamine hydrochloride, cooling and adding sodium hydroxide. It may be recrystallised from aqueous pyridine. The oxime of dilaudide may be prepared by heating as above and adding ammonia to the cooled solution. It should be recrystallised from alcohol. I have not been able to prepare the oxime of eucodal by the above methods, or by that of Lapworth (*loc. cit.*), and no reference to the oxime appears in the literature.

Separation and Determination of the Bases.—Where the bases occur in the pure state, they may be titrated, using as indicator methyl red, which gives

a well-defined end-point. Excess of $N/10$ acid is added and the excess back-titrated with $N/10$ alkali. Where they occur singly in the form of salts, the bases may be extracted from an ammoniacal solution by chloroform, and then titrated after removal of the solvent. The determination of mixtures is a matter of difficulty, and no one scheme is satisfactory for all cases.

The general scheme of H. C. Fuller described in his book, *The Qualitative Analysis of Medicinal Preparations*, 1920, has been in use in the Government Laboratory for some years, and is convenient in many cases. This scheme consists in extracting acid and neutral substances successively with petroleum spirit, ether and chloroform, and then basic substances (after addition of ammonia) with petroleum spirit, ether, chloroform, and, finally, a mixture of chloroform and alcohol. In this scheme, the three bases will appear in the separations after addition of ammonia in the following order:

(1) *Petroleum Spirit.* Eucodal will be extracted. If present in large quantity, the three extractions given by Fuller may not be sufficient to remove the whole of the base, and some may appear in the ether fraction. A small amount of dicodide will also appear here.

(2) *Ether.* Dicodide and dilaudide will be extracted; and if dilaudide is present in large quantity, it may not be completely extracted by three extractions, and will appear in the chloroform fraction.

Owing to the ease with which these three ketonic bases condense with aldehydes, ether free from aldehyde should be used. The bases extracted with impure ether are often yellow and non-crystalline.

(3) *Chloroform.* Any of the three alkaloids remaining will be removed in this fraction.

If heroin, benzoyl morphine, or other esters of morphine are present, traces will appear in the ether fraction, the bulk being removed in the chloroform fraction.

Morphine, if present, will appear only in traces in these extractions. It is completely removed by extraction with a mixture of alcohol and chloroform (Nicholls, ANALYST, 1922, **47**, 506). It may be determined either by titration or colorimetrically. Heroin or other esters of morphine, if present with dilaudide, may be hydrolysed to morphine, which may then be removed and determined in any convenient way.

The separation of dilaudide presents no difficulty in mixtures of dangerous drugs. The mixed bases should be extracted from an ammoniacal solution by a mixture of chloroform and alcohol, and the extract evaporated to dryness. The extract should be dissolved in dilute hydrochloric acid and the solution made alkaline with sodium hydroxide. A chloroform extraction will now remove only eucodal and dicodide, morphine and dilaudide remaining in the aqueous layer. This is saturated with ammonium sulphate and again extracted with chloroform, which removes dilaudide. Morphine remains in the aqueous layer and may be removed by a mixture of alcohol and chloroform.

Dilaudide may be determined colorimetrically by the iodate method or by the methods given later. In the presence of some coloured preparations, particularly those containing opium extracts, it is very difficult to obtain the alkaloid in a condition suitable for colorimetric determination. Extraction of the free base by lime water in the presence of freshly prepared free lime is often successful in reducing the amount of colour. The bases may be recovered from this solution by adding excess of ammonium sulphate and extracting with a mixture of alcohol and chloroform. In the case of opium preparations it may be impossible to eliminate the whole of the brown colour; this may often be matched, however, by that produced by a known amount of morphine or dilaudide acting on iodic acid. In this case the colours should be matched when the solutions are acid, as the addition of ammonia so alters the tint that matching is impossible. The amount of morphine or dilaudide equivalent to the initial colour of the preparation before the addition of iodate should be subtracted from that equivalent to the final colour, after adding the reagents to the test solution. The colour given in Radulescu's method is sometimes more convenient for matching than that given in the iodate method.

The directions given by Nicholls for the colorimetric determination of morphine (ANALYST, 1922, **47**, 506) apply equally to the determination of dilaudide. Iodic acid needs approximately eight times as much dilaudide to produce a given intensity of colour as morphine in acid solution, and approximately five times as much in ammoniacal solution. This should be borne in mind when adjusting quantities for comparison of colours with standards, which should contain about 2 mgrms. of dilaudide per ml.

A more delicate method for the determination of dilaudide than the above is the copper and hydrogen peroxide test, which should be employed when only very small quantities of material are available. It has the added advantage of being specific for dilaudide. The solution for the test should contain approximately 0·1 mgrm. of dilaudide per ml. and should be neutral or slightly acid. To approximately 20 ml. of the solution are added 6 drops of 3 per cent. hydrogen peroxide, followed by 3 drops of 1 per cent. copper sulphate solution and 3 drops of 7 per cent. ammonia solution. After standing 5 minutes, 2 drops of 10 per cent. potassium cyanide solution are added, and comparisons made with standards similarly prepared. It is often convenient to employ small flat-bottomed specimen tubes which are carefully matched for size and colour of glass, instead of the usual Nessler tubes, which may be too large for the quantity of material available. As little as 5 ml. of a 0·01 per cent. solution may be used in this way.

Dilaudide may also be determined, when present in minute quantities, by Radulescu's method previously mentioned. The concentration of the alkaloid should be about 1/20 mgrm. per ml. for convenient colour matching.

Eucodal and dicodide may be partly separated from each other by extraction from ammoniacal solution with petroleum spirit, in which dicodide is only sparingly soluble. A satisfactory method for their quantitative separation has not yet been found.

IDENTIFICATION.—The specific rotations of the alkaloids may be used as a check both in identifying an alkaloid and in determining its quantity. If a narrow-bore polarimeter tube is used, an accurate determination may be carried out with as little as 0·1 grm. The tube which I have used is a 2 dcm. tube of approximately 4·5 mm. bore, holding about 3 ml. The readings are practically as exact as those obtained when using the usual pattern of tube, and are often a reliable guide to the identity of an alkaloid. It is found that a slight excess of hydrochloric acid may be added to the free bases in order to dissolve them, without the excess altering the specific rotation of the hydrochloride. When only 0·1 grm., or less, of a base is extracted, the procedure is to dry and weigh in the anhydrous form, dissolve in the minimum volume of $N/2$ hydrochloric acid, transfer to a 5 ml. graduated flask, make up to volume, and filter into the narrow-bore polarimeter tube.

The physical constants of the alkaloids are as follows:

Dicodide	M. pt. of base	193–194° C.
	,, ,, oxime	265–266° C.
	$[\alpha]_D$ of hydrochloride ..	−143°
	$[\alpha]_D$,, bitartrate	− 79°
Eucodal	M. pt. of base	220° C.
	$[\alpha]_D$,, hydrochloride ..	−136°
Dilaudide ...	M. pt. of base	259–260° C.
	,, ,, oxime	231–233° C.
	$[\alpha]_D$ of hydrochloride ..	−132°.

The specific rotations are calculated for the anhydrous salts in 2 per cent. aqueous solutions at 20° C.

SEPARATION FROM COCAINE.—The separation of cocaine from eucodal and dicodide presents some difficulty. It is readily extracted by petroleum spirit, and the three alkaloids are extracted in the same fraction in Fuller's scheme. Cocaine in this case should be tested for qualitatively, and, if present, hydrolysed to ecgonine by gently warming with dilute sodium hydroxide solution. Eucodal and dicodide can be extracted from this product, but ecgonine is not removed by any of the immiscible solvents and remains in the aqueous layer. It may then be removed and determined by adding sulphuric acid and phosphomolybdic acid, which precipitates the phosphomolybdate. This is washed with dilute sulphuric acid, filtered off, and boiled with barium carbonate. The mixture is filtered, the filtrate (containing free ecgonine) evaporated, the residue (the free base) dried and weighed. The equivalent weight of cocaine can be calculated.

I wish to thank the Government Chemist for permission to publish this paper, and Mr. J. F. Hirst and Mr. R. Stewart for the preparation of the photomicrographs.

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A New Method for the Detection of the Nitro-Group in some Organic Compounds.

By P. K. BOSE, D.Sc.

(*Read at the Meeting, December* **3**, 1930.)

So far as I am aware, there is no characteristic colour reaction for aliphatic nitro-compounds. Although Victor Meyer's method (*Annalen*, 1875, **175**, 93) may be utilised for the characterisation of primary and secondary nitro-paraffins containing less than seven carbon atoms in the molecule, it cannot indicate a tertiary nitro-group. Konowalow's reaction (*Ber.*, 1895, **28**, 1851) suffers from a similar disadvantage, although, unlike Victor Meyer's reaction, it is given by higher nitro-paraffins (primary and secondary). Moreover, nitrous acid (as in V. Meyer's method) gives colour reactions with many other compounds of a different chemical nature (*e.g.* secondary amines, aromatic tertiary amines, phenols, etc.), and it can hardly be employed with advantage for the identification of nitro-paraffins.

In the aromatic series, the well-known diazo test for amines is usually applied after reduction of the nitro-bodies in acid solution. But a similar behaviour is shown by hydrazo-, azo-, azoxy- and nitroso-compounds, which are also convertible into primary amines under identical conditions. Moreover, the diazo test is hardly suitable for showing the presence of a nitro-group in a primary nitro-amine, since the parent compound, as well as its reduction product, will obviously respond to the diazo test with almost equal facility. Mulliken and Barker's method (*Amer. Chem. J.*, 1899, **21**, 271), which consists in the reduction of nitro-bodies to hydroxylamine derivatives, and subsequent identification of the latter by their reducing action upon silver nitrate, suffers from many of the aforesaid drawbacks, inasmuch as nitroso-, azo- and azoxy-compounds behave like nitro-compounds. The adaptation of Coupier's process for the production of magenta, as a test for an aromatic nitro-group, is equally unsuitable, since a positive reaction is given by many organic compounds containing oxygen directly linked to nitrogen, as also by iodo- and iodoso-benzene (Mulliken and Barker, *loc. cit.*).

References in this connection should be made to the colour reactions given by certain nitro-compounds, both aliphatic and aromatic, with unsaturated compounds, aromatic hydrocarbons, phenols and phenol ethers, bases and metallic salts (Werner, *Ber.*, 1909, **42**, 4324; Graham and Macbeth, *J. Chem. Soc.*, **1921**, **119**, 1362; Will, *Ber.*, 1914, **47**, 964; Harper and Macbeth, *J. Chem. Soc.*, **1915**, **107**, 87; Giua and Giua, *Gazzetta*, 1921, **51**, 313; Sudborough and collaborators,

J. Chem. Soc., 1911, **99**, 209, and subsequent papers; Ciusa and Vecchiotti, *Atti. R. Accad. Lincei*, 1911, v, **20**, I, 803; **20**, II, 377; Goddard, *J. Chem. Soc.*, 1921, **119**, 1161). These, however, are not general, and are not applicable to nitro-bodies as a class. Dimethylaniline (Walter, *Z. Farbenind.*, 1911, **10**, 49) and aluminium bromide (Olivier, *Rec. trav. chim.*, 1918, **37**, 241) give colour reactions with many aromatic nitro-compounds, and apparently these two reagents may be used with advantage for the characterisation of aromatic nitro-compounds.

Janovsky (*Ber.*, 1891, **24**, 971) discovered that aromatic dinitro-compounds in acetone solution give characteristic colorations with aqueous potassium hydroxide, and this sensitive reaction has subsequently been extended by Bitto (*Annalen*, 1892, **269**, 377), Reitzenstein and Stamm (*J. prakt. Chem.*, 1910, **81**, 167) and by Rudolph (*Z. anal. Chem.*, 1921, **60**, 239). These chemists found that many other aldehydes and ketones (and ethyl alcohol) serve the same purpose as acetone, but they recorded at the same time some exceptions so far as the nitro-bodies were concerned. Thus Bitto found that trinitroresorcinol does not give any coloration with acetone and alkali, whilst trinitroxylene gives a green colour with alkali alone. Reitzenstein and Stamm noticed that *p*-dinitrobenzene and dinitro-resorcinol do not give Janovsky's reaction, whilst some mononitro-compounds give colorations which are difficult to distinguish from those given by polynitro-compounds (*e.g.* *m*-nitrophenol and trinitro-phenol give identical colorations). Further instances of the failure of Janovsky's reaction which I have observed include 2:4-dinitromesitylene, trinitromesitylene, 3:5-dinitro-*o*-cresol, 2:4:6-trinitro-*m*-cresol and 3:5-dinitro-1:2:4-xylenol. It is thus evident that Janovsky's reaction is hardly suitable for even the diagnosis of aromatic polynitro-compounds.

It was thought that if the nitro-group could be dislocated as nitrous acid a method of wider applicability than those mentioned would be available, since nitrous acid can be detected easily by Griess-Ilosvay's reagent (*Bull. Soc. Chim.*, 1899, [iii], **2**, 388), which is said to have a sensitiveness of 1 in 1,000,000,000. Accordingly a large number of nitro-compounds were treated with various hydrolytic agents with a view to realise a decomposition of the following type:

$$R.NO_2+H.OH=R.OH+HNO_2.$$

After numerous experiments the following procedure was found to be the most satisfactory: A small quantity (0·01–0·05 grm.) of the substance is boiled in a clean Pyrex test tube with 1 c.c. of a concentrated solution of pure potassium hydroxide (10 grms. in 6 c.c. of water) over a Bunsen flame for a period not exceeding two minutes. The colour of the solution deepens during the boiling, and finally a yellow to dark brown-red product is usually obtained, profound decomposition occurring in some cases. The test tube is then cooled under the tap and, 1 c.c. of water added. A few drops of this alkaline solution are poured into a test tube and acidified with about 1 c.c. of 50 per cent. acetic acid. On acidification, the solution usually assumes a straw-yellow colour. Should this not be the case, the solution may be diluted with water until it becomes pale in colour, and it is then treated with about 0·5 c.c. of Griess-Ilosvay's reagent, prepared according to

the directions given by Lunge (*Z. angew. Chem.*, 1899, 666).* The development of a rose-red colour indicates the presence of free nitrous acid, and, indirectly, that of a nitro-group in the substance taken. Control experiments may be performed, omitting the nitro-compound, whenever any doubt arises as to the purity of the reagents.

I have tested fifty-two nitro-compounds with the following results:

REACTION POSITIVE.

1. Nitromethane.
2. Nitroethane.
3. 1:2-Dinitrobenzene.
4. 1:3-Dinitrobenzene.
5. 1:4-Dinitrobenzene.
6. 1:2:4-Dinitrotoluene.
7. 1:2:6-Dinitrotoluene.
8. 1:2:4-Chlorodinitrobenzene.
9. 1:2:4-Bromodinitrobenzene.
10. 1:2:4-Dinitrophenol.
11. 1:2:4-Dinitroaniline.
12. 1:2:4-Dinitrobenzoic acid.
13. 1:3:5-Dinitrobenzoic acid.
14. 1:2:4:6-Dinitroxylene.
15. 1:2:3:4-Dinitroxylene.
16. 2:4-Dinitromesitylene.
17. 3:5-Dinitro-1:2:4-xylenol.
18. 3:5-Dinitro-*o*-cresol.
19. 3:4-Dinitro-*o*-cresol.
20. 2:4-Dinitrostilbene.
21. 3:5-Dinitro-*p*-toluic acid.
22. Dinitrophthalic acid.
23. 2:4-Dinitro-6-bromophenol.
24. 1:5-Dinitronaphthalene.
25. 1:8-Dinitronaphthalene
26. 1:5-Dinitro-2-methylanthraquinone.
27. Dinitro-2-methoxy-6-methylanthraquinone.
28. 1:3:5-Trinitrobenzene.
29. 2:4:6-Trinitrophenol.
30. Trinitromesitylene.
31. 2:4:6-Trinitrotoluene.
32. Trinitroresorcinol.
33. 2:4:6-Trinitro-*m*-cresol.
34. α-Trinitronaphthalene.

REACTION NEGATIVE.

1. Nitrobenzene.
2. *o*-Nitrotoluene.
3. *m*-Nitrotoluene.
4. *p*-Nitrotoluene.
5. *o*-Nitrophenol.
6. *m*-Nitrophenol.
7. *p*-Nitrophenol.
8. *p*-Nitroanisole.
9. *p*-Nitroaniline.
10. *o*-Nitroaniline.
11. *p*-Chloronitrobenzene.
12. *o*-Chloronitrobenzene.
13. *p*-Nitroacetanilide.
14. *m*-Nitrobenzaldehyde.
15. *m*-Nitrobenzoic acid.
16. *p*-Nitrobenzoic acid.
17. α-Nitronaphthalene.
18. 2:2-Dinitrostilbene.†

It will be seen from the above tables that, with the exception of aromatic mononitro-compounds, all nitro-compounds decompose under the experimental conditions, yielding nitrous acid. It was further observed that aliphatic nitro-compounds are hydrolysed to nitrous acid, even when shaken with cold dilute alkali for a few seconds. Amyl nitrite, being an ester of nitrous acid, is also readily hydrolysed by cold dilute alkali. Consequently, it is not possible to distinguish

* The reagent is prepared by dissolving 0·5 grm. of sulphanilic acid in 150 c.c. of 2 *N* acetic acid and mixing the solution with a colourless solution of 0·1 grm. of pure α-naphthylamine in 20 c.c. boiling water and 150 c.c. 2 *N* acetic acid.

† Although its name does not imply it, this substance is really a mononitro-compound of benzene, in the sense that each of the two benzene rings carries a nitro-group and they are separated by an ethylenic linkage.

between a nitrite and an aliphatic nitro-compound by their action upon alkali.* These may, however, be readily distinguished from aromatic polynitro-compounds, which are not so easily affected by cold alkali. It is thus possible to distinguish between the following types of nitro-bodies:

I. *Aliphatic nitro-compounds and nitrites:* decomposed by cold dilute alkali.

II. *Aromatic mononitro-compounds:* not affected by hot alkali.

III. *Aromatic polynitro-compounds:* decomposed by hot alkali.

Instances of the decomposition of di- and poly-nitro-compounds into nitrous acid under the influence of alkali have been known for a long time, prominent workers in this field being Lobry de Bruyn, Holleman, Blanksma, Kenner and Jackson (for a complete bibliography dealing with nitro-derivatives of benzene, see Clark and Carter, *Trans. Roy. Soc. Canada*, 1927, [iii], **21**, 322). Clark and Carter (*loc. cit.*) made an extensive quantitative study of the replaceability of nitro-groups in the benzene series by OH-groups by means of dilute alkali at 100° C. Their results, together with those of previous investigators, show that a nitro-group in the ortho- or para-position to another nitro-group is always displaced, as demanded by Fry's polarity hypothesis. They also find that, as a rule, the nitro-group in *m*-dinitro-compounds is not displaced. I have found, however, that under the experimental conditions mentioned before a nitro-group may be displaced (partly at least) from *m*-dinitro-compounds. Consequently, this method is applicable to *all* polynitro-derivatives, irrespective of the orientation of the nitro-groups in the molecule.

I have submitted *p*-nitrosophenol and *p*-nitrosodiphenylamine to the above-described process of hydrolysis, and in neither instance did I find any evidence of the formation of nitrous acid. Hence, nitroso derivatives of phenols and amines do not give the reaction.

I wish to express my sincere thanks to Sir P. C. Rây for his interest in this investigation.

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* Alkyl nitrites may be differentiated from aliphatic nitro-compounds by Liebermann's reaction, the former giving a positive result (see Houben, *Methoden der Organischen Chemie*, 2nd Ed., Vol. IV, p. 100), or by Konowalow's test, which is negative in the case of a nitrite.

Determination of the Carbonyl and Aldehyde Content of Organic Compounds: Estimation of Phenylhydrazine.

By L. MARKS, M.Sc., A.I.C., and R. S. MORRELL, M.A., Ph.D., F.I.C.

(*Read at the Meeting, December 3rd*, 1930).

The object of this investigation was to discover a reliable method for the accurate determination of the carbonyl, >CO, content of certain oily and gummy substances of unknown constitution which had been isolated during the oxidation of β-elaeostearin from tung oil (Morrell and Marks, *J. Oil and Colour Chem. Assoc.*, **1927**, **10**, 186; **1929**, **12**, 183). Parallel investigations into the determination of the peroxide-oxygen (Analyst, **1929**, **54**, 503) and of the hydroxyl contents (Analyst, **1931**, 429) of substances of this class have already been published.

Most of the methods for the determination of aldehydes and ketones can be applied only to certain substances, but the three processes described below, all of which depend on the use of phenylhydrazine, appeared to be capable of general application, and were, therefore, carefully examined. (The iodimetric method of Ardagh and Williams (*J. Amer. Chem. Soc.*, 1925, **47**, 2983) is obviously unsuitable for unsaturated compounds of this type.)

1. MacLean's Method.—I. Smedley MacLean (*J. Biol. Chem.*, **1913**, **7**, 611) obtained satisfactory results for pyruvic acid by treating a weighed quantity with excess of phenylhydrazine. The amount of this left over is then determined by adding excess of Fehling's solution; the precipitated cuprous oxide is dissolved in an acid solution of ferric sulphate, and the ferrous sulphate produced is titrated with potassium permanganate solution. A blank phenylhydrazine test, without the ketonic substance, is made at the same time.

A series of experiments with each of the following substances has been carried out: (*a*) Pyruvic acid, (*b*) salicylaldehyde, (*c*) benzaldehyde, (*d*) aceto-acetic ether. In each series the relative quantity of phenylhydrazine employed was successively reduced so as to determine the effect of the mass action of this reagent. The practical details given by MacLean were followed, with the following exceptions:

(i) Since an acid solution of ferric sulphate oxidises cuprous oxide in the cold, the mixture was not heated.

(ii) Phenylhydrazine hydrochloride, mixed with its own weight of sodium acetate, was used instead of the free base. The phenylhydrazine hydrochloride was that supplied by the B.D.H. as "specially pure for osazone tests."

(iii) The end-point in the permanganate titration was obtained with potassium ferricyanide as external indicator. This was necessary because of the difficulty, after having filtered off the cuprous oxide, of removing all traces of tartrate, the presence of which delays the end-point considerably. (See also Jones and Carpenter, *J. Chem. Soc.*, 1903, **83**, 1399.)

MacLean showed that the reaction between phenylhydrazine and Fehling's solution reaches an equilibrium value in approximately half-an-hour at ordinary temperatures, after which no further oxidation takes place. We have confirmed this observation. The reaction involved approximates to the following equation:

$$\text{(i)}\quad 2C_6H_5NHNH_2+3O=C_6H_6+C_6H_5OH+2N_2+2H_2O,$$

but it varies to some extent with the conditions, the most important of which is the temperature. The following reactions may also take place:

$$\text{(ii)}\quad C_6H_5NHNH_2+O=C_6H_6+N_2+H_2O.$$

$$\text{(iii)}\quad 4C_6H_5NHNH_2+3O=2C_6H_5NH_2+2C_6H_6+3N_2+3H_2O.$$

The weight of cuprous oxide precipitated is also influenced by oxidation of the phenylhydrazine through contact with air; hence the permanganate equivalent of the phenylhydrazine must be independently determined. MacLean found that 1 c.c. of 0·1 *N* potassium permanganate solution was equivalent to 0·00295 grm. of phenylhydrazine. In our experiments the equivalent varied between 0·00330 and 0·00357 grm. The theoretical figure required by the equation (i) above is 0·00360 (*cf.* Foster, *Proc. Camb. Phil. Soc.*, 1907, **14**, 90).

We have carried out two other series of preliminary experiments, which showed that the weight of cuprous oxide precipitated (i) does not vary with the amount of Fehling's solution added, provided excess of that reagent is maintained; (ii) has a slight tendency to decrease as the dilution of the reacting mixture is increased.

The following results were obtained with the substances mentioned:

(*a*) *Pyruvic Acid* (*freshly distilled*).

Expt. No.	Wt. of acid. Grm.	Phenylhydrazine solution (approx. 3 per cent.). c.c.	Molecular proportions pyruvic acid: phenylhydrazine hydrochloride.	Carbonyl >CO found. Per Cent.	Percentage of theoretical figure.
1	0·055	10 (0·3358 grm.)	1:5·0	22·4	70·2
2	0·056	,,	1:4·9	24·0	75·2
3	0·123	,,	1:2·2	26·8	84·0
4	0·183	,,	1:1·5	28·2	88·4

Theoretical figure = 31·9 per cent. The purity of the sample used was not otherwise determined.

(*b*) *Salicylaldehyde* (*freshly distilled*).

Expt. No.	Wt. of salicyl-aldehyde. Grm.	Phenyl-hydrazine solution (approx. 3 per cent.). c.c.	Molecular proportions salicylaldehyde: phenylhydrazine hydrochloride.	Carbonyl >CO found. Per Cent.	Percentage of theoretical figure.
1	0·050	10 (0·3358 grm.)	1:7·6	6·2	27·1
2	0·106	,,	1:3·6	17·5	76·4
3	0·208	,,	1:1·8	22·7	99·1
4	0·279	,,	1:1·4	23·1	100

Theoretical figure = 22·9 per cent. The purity of the sample was not otherwise determined.

(*c*) *Benzaldehyde.*

Expt. No.	Wt. of benzal-dehyde. Grm.	Phenyl-hydrazine solution (approx. 3 per cent.). c.c.	Molecular proportions benzaldehyde: phenylhydrazine hydrochloride.	Carbonyl >CO found. Per Cent.
1	0·057	10 (0·3358 grm.)	1:5·8	24·5
2	0·118	,,	1:2·8	24·9
3	0·196	,,	1:1·7	26·6

Theoretical figure (for sample 93·5 per cent. pure) = 24·7 per cent. The sample was found by the sodium sulphite method to have a purity of 93·5 per cent.

(*d*) *Ethyl acetoacetate.*

Expt. No.	Wt. of acetoacetic ether. Grm.	Phenyl-hydrazine solution (approx. 3 per cent.). c.c.	Molecular proportions ethyl acetoacetate phenylhydrazine hydrochloride.	Carbonyl >CO found. Per Cent.
1	0·055	10 (0·3358 grm.)	1:7·3	17·3
2	0·097	,,	1:4·2	18·7
3	0·216	,,	1:2·4	17·6

Theoretical figure for one CO group = 21·5 per cent. The purity of the sample was not otherwise determined.

The data given permit of the following conclusions:

(1) The method is reliable only for those cases in which the hydrazone formed is not hydrolysed by water, *e.g.* for pyruvic acid and benzaldehyde. Even in these cases, however, slight hydrolysis of the hydrazone takes place, which accounts for the low results. Where the hydrazone left in solution undergoes appreciable hydrolysis (*e.g.* acetone and ethyl acetoacetate) the method fails (see also Bodforss, *Z. physik. Chem.*, 1924, **109**, 223).

(2) Considerable excess of phenylhydrazine should be avoided; the best ratio is approximately 1½ molecular proportions of phenylhydrazine to 1 molecular proportion of the carbonyl compound.

Additional series of experiments were carried out with the following ketonic compounds: (*e*) Acetone, (*f*) laevulinic acid, (*g*) methyl ester of oxidised β-elaeostearic acid, $C_{18}H_{29}O_6(CH_3)$ (Morrell and Marks, *loc. cit.*). With each of these substances the first conclusion (*supra*) was confirmed. The effect of variation of the molecular proportions and of the concentration was particularly pronounced with the last-mentioned compound; this is shown by the following figures:

Expt. No.	Number of molecular proportions of phenylhydrazine to one of the compound $C_{18}H_{29}O_6(CH_3)$.	Number of molecular proportions of phenylhydrazine which reacted.	Solution.
1	30	nil	dilute
2	53	7·2	,,
3	166	12·8	,,
4	159	62·2	concentrated

It is possible, however, that in this case slight oxidation of the phenylhydrazine, as well as hydrazone formation, may have been taking place.

An example of an estimation of salicylaldehyde showing the calculation follows:

Example:

Salicylaldehyde: 1 molecular weight = 122.

Phenylhydrazine hydrochloride: 1½ molecular weight = 217 (approx.).

A weighed quantity (0·208 grm.) of salicylaldehyde was dissolved in about 80 c.c. of distilled water; 10 c.c. of a solution (in 50 per cent. acetic acid) of phenylhydrazine hydrochloride, containing 4·477 grms. (together with an equal weight of sodium acetate) in 100 c.c., were added; the mixture was made up to 100 c.c. and filtered, leaving, approximately, a third of the hydrazine (*i.e.* ½ molecular proportion) in the filtrate, 25 c.c. of which were withdrawn for treatment with Fehling's solution

Titration with *N*/10 potassium permanganate solution = 11·0 c.c.

Therefore phenylhydrazine excess ≡ 44·0 c.c. of *N*/10 permanganate solution.

For the blank experiment 3·50 c.c. of the phenylhydrazine solution (*i.e.* about one-third of the quantity used in the previous experiment) was made up to 100 c.c., and 25 c.c. again withdrawn for the determination of the permanganate equivalent. *N*/10 potassium permanganate titration = 8·40 c.c.

Therefore, *N*/10 permanganate titration for 10 c.c. of the phenylhydrazine solution

$$= \frac{100}{25} \times \frac{10}{3{\cdot}5} \times 8{\cdot}40 = 96{\cdot}0 \text{ c.c.}$$

Hence, 1 c.c. of *N*/10 permanganate $= \frac{0{\cdot}4477}{96}$ grms. of phenylhydrazine hydrochloride.

The phenylhydrazine hydrochloride used after treatment of 10 c.c. with 0·208 grm. of salicylaldehyde ≡ 96·0 − 44·0 ≡ 52·0 c.c. of *N*/10 permanganate

$$\frac{C_6H_5NH.NH_2.HCl}{144{\cdot}5} = \frac{>CO}{28}$$

Therefore, >CO content of salicylaldehyde $= \frac{52{\cdot}0}{1} \times \frac{0{\cdot}4477}{96} \times \frac{28}{144{\cdot}5} \times \frac{100}{0{\cdot}208} = 22{\cdot}6$ per cent.

It was thought that the above-described method might be rendered more reliable by the substitution of *p*-nitrophenylhydrazine for the phenylhydrazine, since the nitro-derivatives of the hydrazones are generally less soluble in, and more

stable towards, water. Trials with this substance, however, showed that the subsequent reduction of the Fehling's solution with excess of the nitro-derivative cannot be depended upon to act quantitatively, because metallic copper is precipitated together with cuprous oxide. The introduction of an acid nitro-group in the *para* position obviously renders the compound more sensitive to oxidation, at any rate in an alkaline medium. The final conclusion with regard to the MacLean process is that, in the case of a substance of unknown composition, and in the absence of information concerning the behaviour of the hydrazone formed from it, the method is not safe.

2. WATSON SMITH'S METHOD.—This method is a modification of Strache's method, and is fully described in the reference cited (Smith, *Chem. News*, 1906, **93**, 83), and in most reference works on organic analysis. [Thorpe and Whiteley, *Organic Chemical Analysis* (London), 1925, p. 182; Kingscott and Knight, *Quantitative Organic Analysis* (London), 1914, p. 121; Barnett and Thorne, *Organic Analysis* (London), 1921, p. 125.] It resembles the method just described in that the excess of phenylhydrazine left after formation of the hydrazone is treated with Fehling's solution, but instead of the cuprous oxide thereby precipitated being collected, the nitrogen evolved is collected and measured. The errors involved in the reaction between the phenylhydrazine and the Fehling's solution, with formation of cuprous oxide, are thereby avoided, but this advantage is outweighed by the very elaborate apparatus which is now necessary, and the consequent considerable errors involved. The apparatus for this process was set up independently by each of us, working in separate laboratories. A large number of determinations were carried out on substances of known carbonyl, >CO, content, but the results, independently obtained, were unsatisfactory. Figures for the same compound often showed variations of 10 per cent., or even more (in some cases too low and in others too high), and it became obvious that the data obtained for a substance of unknown composition would not permit of a definite answer as to the number of >CO groups present. This experience of the Watson Smith method is confirmed by Ardagh and Williams (*loc. cit.*), who describe it as being "tedious and cumbersome," and whose best results for acetone showed an error of 5 per cent., as well as by Ellis (*J. Chem. Soc.*, 1927, **130**, 848), according to whom the process is "unnecessarily tedious and often untrustworthy." It would appear desirable, therefore, that, in future works on organic analysis, attention should be drawn to the lack of accuracy associated with this method.

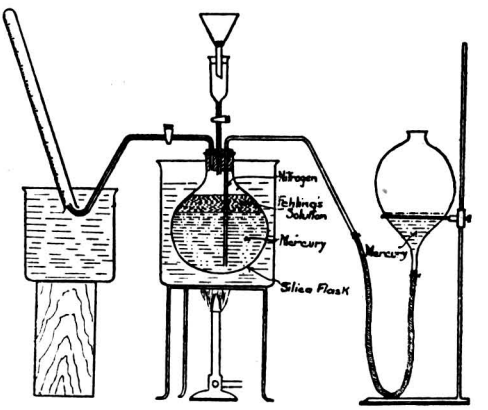
Some of the figures obtained are as follows:

Substance.	>CO found (Per Cent.)	>CO calculated. Per Cent.
Pyruvic acid	29·3; 28·4; 35·4	31·9
Salicylaldehyde	26·6; 21·0; 27·2	22·9
p-Nitro-benzaldehyde	23·2; 22·5	18·5
Methyl ester of oxidised β-elaeostearic acid, $C_{18}H_{29}O_6(CH_3)$	11·2; 15·3	7·8 or 15·6*

* Either one or two CO groups may have been present in this substance.

3. ELLIS'S METHOD.—Some of the possible causes of the discordant results obtained by the last-described method are as follows:—(*a*) Incomplete formation of the hydrazone, (*b*) hydrolysis of the hydrazone, (*c*) the hydrazone is itself attacked by the boiling Fehling's solution, (*d*) some of the evolved nitrogen remains dissolved in the reacting mixture or in the benzene-absorbent solution, or in the potash solution below the eudiometer, (*e*) atmospheric oxidation of the phenylhydrazine.

Of these, (*a*), (*b*) and (*c*) produce low results; (*e*) leads to high results; the effect of (*d*) will be to produce a low or a high result according to whether more or less nitrogen is retained in the actual estimation than in the blank experiment. Ellis (*loc. cit.*) has modified the details for the collection of the nitrogen so as to ensure that no loss takes place. The nitrogen is completely evolved from the



boiling reaction mixture by reducing the pressure in the containing flask, which for this purpose is connected with a mercury reservoir (see diagram). In addition, the benzene vapour, which is simultaneously evolved, is not absorbed, but its presence is allowed for in the subsequent calculation. Although the apparatus is thus modified considerably, it must be specially made for the purpose, and involves (for safety) also a silica flask. Some of the results obtained by us with it are set out below. In each case the substance was treated with approximately 1½ molecular proportions of phenylhydrazine.

Compound.	>CO found. Per Cent.	>CO calculated. Per Cent.
Phenylhydrazine hydrochloride A.R. ..	98·2;* 101*	100*
Salicylaldehyde redistilled	22·0; 23·1	22·9
Acetone A.R.	48·6; 47·3	48·2
Pyruvic acid	29·5	31·9
Oxidised β-elaeostearin $(C_{18}H_{29}O_5)_3C_3H_5$	8·3; 9·4	8·3

* Percentage purity.

It should be mentioned that in our experiments, (*a*) any precipitated hydrazone was removed by filtering through glass wool or asbestos fibre in a small

porcelain funnel fitted into the cup of the apparatus; (*b*) the special pipette described by Ellis was not used; the reacting solutions were mixed in a very small beaker, and the freshly boiled distilled water, alcohol or glacial acetic acid that was used for washing the hydrazone was run out of a burette, the same volume being used in the blank; (*c*) it was found that the apparatus as sketched has the advantage over that of Ellis in that the phenylhydrazine solution can now be decomposed and the nitrogen collected *in portions*, if necessary; the use of a two-way stopcock necessitates, for accurate results, that *all* the phenylhydrazine and the washings are run into the Fehling's solution before any nitrogen is driven over into the eudiometer, because of the small volume of liquid which is retained in the tube between the two-way stopcock and the flask; (*d*) the nitrogen collected was saturated with benzene vapour by introducing a drop into the eudiometer, and the partial pressure of the benzene was then allowed for by reading the graph constructed from the data given by Woringer (*Z. physik. Chem.*, 1900, **34**, 257); (*e*) glacial acetic acid, or 50 per cent. aqueous acetic acid solution, was employed as a solvent, when the ketonic compound was insoluble in both water and alcohol; care was taken in these cases to maintain the Fehling's solution in the silica flask alkaline by addition of more potassium hydroxide.

It is advisable to filter off the hydrazone, because it is liable to undergo hydrolysis, with the result that the volume of nitrogen evolved is high and the figure for ketonic content is low. This is liable to take place, for instance, in the case of acetone, the hydrazone of which, being soluble, cannot be removed. At the outset there is a vigorous evolution of nitrogen (from the excess of phenylhydrazine), followed by a further slow evolution; the initial rapid evolution is always greater when the reaction mixture is allowed to stand for a few hours before being introduced into the Fehling's solution.

Ellis's process was finally adopted as the most rapid and satisfactory for the determination of the ketonic content of a large number of oily complex substances of unknown constitution (Morrell and Marks, *J. Soc. Chem. Ind.*, 1931, **50**, 27T).

It will be observed that the blank experiment in the above estimations is, in effect, the determination of the amount of phenylhydrazine solution used. It is obvious that the procedure can be applied to the estimation of the purity of a sample of phenylhydrazine. The results vary by ± 2 per cent.

SUMMARY.—The use of phenylhydrazine and Fehling's solution in three methods (due to MacLean, Watson Smith, and Ellis) for the determination of the carbonyl, $>CO$, content in aldehydes and ketones has been investigated. The MacLean method is not safe in the case of a substance of unknown composition, and in the absence of information concerning the behaviour of the hydrazone formed from it. The Watson Smith method is unreliable. Ellis's modification of the Watson Smith method gives satisfactory results, and it can also be used for the estimation of phenylhydrazine.

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The Reichert, Polenske and Kirschner Values of Rancid Butters and Margarines.

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(*Read at the Meeting of the North of England Section, December* 6, 1930.)

At one of the meetings of the North of England Section of the Society, during the discussion following a paper by two of us, an enquiry was made regarding the effect, if any, of keeping (and, therefore, presumably, the action of rancidity) on the Reichert, Polenske and Kirschner value of fats. Since then we have had an opportunity of investigating these points rather more fully than had been the case up to that time, and we now present the results obtained.

It is, of course, well known that when oils become rancid there is a very considerable increase in the volatile acids present. (Elsdon and Hawley, *Year Book of Pharmacy*, 1913, p. 575.) Some years ago one of us extracted from a rancid sample of cotton-seed oil a considerable quantity of fatty acid which had a valeric odour, but this was not further examined. Apart from such experiences as this, no work appears to have been published showing what increases may be expected in the case of ordinary fats. Certain old samples of butter have been examined (Radcliffe and Maddocks, *J. Soc. Chem. Ind.*, 1907, **26**, 3), and D. Crispo (Analyst, 1911, **36**, 64) found that some samples of butter increased in their Reichert value with age, and some lost, but that, on the whole, the losses were greater than the gains.

For our present purpose, certain mixtures of coconut oil, palm-kernel oil, margarine and butter fats, which were prepared for special reasons in 1925 and 1926, were used, as these had been placed on one side in open beakers of about 250 c.c. capacity in a dark cupboard at a medium temperature, and with free access to air. They had been standing for some fourteen months. When the Reichert value was being determined, it was observed that the fat turned dark brown when saponified with sodium hydroxide and that the fatty acids, on liberation, were more liquid than they were in the original samples. The figures obtained with these samples, both when freshly prepared and after standing for fourteen months under the conditions mentioned above, are given in the following tables:

Table I.

Palm-kernel Oil and Oleo-margarine.

Composition.	Reichert value.		Polenske value.		Kirschner value.	
	Dec. 1925.	Feb. 1927.	Dec. 1925.	Feb. 1927.	Dec. 1925.	Feb. 1927.
20 per cent. Palm-kernel oil	1·8	2·1	1·7	1·8	0·5	0·6
40 ,, ,, ,,	2·6	4·0	3·2	3·7	0·6	1·2
80 ,, ,, ,,	4·3	5·5	7·4	7·4	0·9	1·5
*20 ,, ,, ,,	1·8	3·7	1·5	2·1	0·5	1·4

* Prepared from different ingredients, February, 1925, and determinations made then and after two years.

TABLE II.

COCONUT OIL, PALM-KERNEL OIL AND OLEO-MARGARINE.

Composition.	Reichert value.		Polenske value.		Kirschner value.	
	Dec. 1925.	Feb. 1927.	Dec. 1925.	Feb. 1927.	Dec. 1925.	Feb. 1927.
20 per cent. Palm-kernel oil 20 ,, Coconut oil	4·2	5·7	4·4	4·4	0·6	1·8
30 ,, Palm-kernel oil 30 ,, Coconut oil	5·2	6·7	7·2	7·2	0·9	2·0
40 ,, Palm-kernel oil 40 ,, Coconut oil	6·2	7·0	9·5	10·3	1·2	2·1
50 ,, Palm-kernel oil 50 ,, Coconut oil	6·7	7·4	13·0	13·5	1·4	2·2

TABLE III.

PALM-KERNEL OIL, BUTTER-FAT AND OLEO-MARGARINE.

Composition.	Reichert value.		Polenske value.		Kirschner value.	
	Feb. 1925.	Feb. 1927.	Feb. 1925.	Feb. 1927.	Feb. 1925.	Feb. 1927.
60 per cent. Palm-kernel oil 10 ,, Butter-fat	6·0	8·0	6·0	6·3	3·3	4·3
40 ,, Palm-kernel oil 10 ,, Butter-fat	5·2	6·7	3·5	4·1	3·0	3·6
50 ,, Palm-kernel oil 10 ,, Butter-fat	5·6	8·0	4·7	5·0	3·2	4·1
20 ,, Palm-kernel oil 10 ,, Butter-fat	4·4	6·6	1·8	2·4	3·0	4·5
*20 ,, Palm-kernel oil 10 ,, Butter-fat	4·5	7·5	1·8	2·5	2·8	5·2
20 ,, Palm-kernel oil 2 ,, Butter-fat	2·2	4·2	1·5	2·1	0·9	2·2

* Mixture prepared from different ingredients, January, 1925.

From an examination of these tables, it will be observed that the Reichert value has increased in every case, and that in some cases the increase is very marked, corresponding with a considerable increase in the apparent percentage of butter-fat present. The Polenske value has increased in some cases, but usually by a much smaller proportion than the Reichert. The Kirschner value has also increased in every case, but there is no obvious relationship between the increase in the Kirschner value and the increase in the Reichert value.

All these results suggest that when the amount of butter-fat present in the margarine mixture is determined by means of the Reichert, Polenske and Kirschner process, if this is not carried out while the margarine is quite fresh, special care should be taken in the interpretation of the results obtained.

In view of these results, samples of butter-fat were examined after various lengths of time. Five samples of butter were melted, the fat filtered, and about 35 grms. placed in glass crystallising basins. A portion of each was allowed to stand in a dark cupboard covered with paper, and another portion of each was allowed to stand uncovered on the bench. The samples were examined after nineteen weeks, with results as given in the following table:—

TABLE IV.

No. of sample.	Reichert value.			Polenske value.			Percentage of free fatty acids.*		
	Original.	in dark.	in light.	Original.	in dark.	in light.	Original.	in dark.	in light.
1	30·5	31·0	31·2	2·7	3·6	3·7	0·28	0·79	1·29
2	30·1	30·6	30·9	3·2	3·8	4·2	0·33	1·07	1·12
3	30·4	30·3	31·0	3·3	3·4	4·0	0·28	0·39	1·01
4	29·9	29·7	30·6	2·6	2·8	3·4	0·50	0·50	1·01
5	29·9	29·9	30·2	2·7	3·1	3·4	0·45	0·62	1·1
Sample of lard	0·2	—	1·6	0·3	—	0·6	0·62	—	1·58

* Expressed as oleic acid.

From these results it would appear that the Reichert value of butter is not changed very much when exposed to the activities of light and air over a period of a considerable number of weeks, under conditions when the free fatty acids have risen to as much as 1·3 per cent. It is apparent that, as would be expected, the increase in acidity is very much more in the case of those samples which are exposed to light than in the case of those samples which are kept in the dark. The increase in the Polenske value is quite marked, particularly in those samples which are exposed to the light.

A number of samples of canned butter were very kindly placed at our disposal by Messrs. Nestlé, to whom our thanks are due. These samples had been canned for approximately twelve months at the time the determinations were made. The results obtained are set out in the following table:

TABLE V.

Mark.	Reichert value.	Polenske value.	Kirschner value.	Free fatty acids. Per Cent.
SP. 1095	29·1	2·6	24·45	1·01
SP. 1098	29·0	2·5	24·75	0·95
SP. 1097	29·1	2·5	24·7	0·62
SP. 1102	27·35	2·9	23·75	0·95
SP. 1096	27·6	2·6	23·65	1·41
SP. 1099	27·6	2·5	23·35	2·54
SP. 1100	25·85	2·2	22·3	1·01
SP. 1101	26·8	2·3	22·75	0·90
SP. 1103	30·35	3·1	25·9	1·80
SP. 1104	24·6	1·6	21·35	6·20

Beyond the considerable increase in the percentage of free fatty acids there is little to distinguish these results from those obtained with fresh samples.

A quantity of butter-fat obtained by mixing eleven samples of butter, received under the Food and Drugs Act, melting and clarifying, was allowed to stand for nineteen months uncovered in a dark cupboard at laboratory temperature. During this time, the Reichert value increased from 28·0 to 28·9, whilst the Polenske value remained unchanged at 2·1.

There is, as a general rule, therefore, little increase in the Reichert values of butter fats as these become rancid, or at least not sufficient to invalidate the results obtained under any likely commercial conditions.

P. Arup (ANALYST, 1929, **54**, 736) has shown that during a period of nearly six years the Reichert value of a sample of butter kept in a bottle fitted with a screw cap and cork disc fell from 20·7 to 8·0, but other samples, although behaving in the same way, did not do so to the same extent.

The available evidence (*cf.* J. S. Hepburn, *7th International Congress of Applied Chemistry*, 1909, Sec. VIII C., p. 268) would suggest that during comparatively short periods of time there is a tendency for volatile acids to be produced as oils become rancid, but that under certain conditions these may be consumed or decomposed by biological agencies. The somewhat contradictory results which have been published are probably due to different types of organisms causing varying types of decomposition.

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The Determination of Small Quantities of Phosphate by Pouget and Chouchak's Method: the Determination of Phosphorus in Small Samples of Steel.*

BY S. G. CLARKE, PH.D., A.I.C.

THE necessity has arisen for methods for the analysis of steel which are suitable for dealing with small samples. Such a method for the determination of sulphur in steel has already been published (Clarke, ANALYST, 1931, 436), and the present paper contains the results of an investigation of a method for the determination of phosphorus in small samples of steel weighing 0·1 grm. As in the case of sulphur, a photometric method of determination was called for, owing to the minute quantities of phosphorus in question.

There are in existence two main methods for the determination of traces of phosphate, depending, respectively, upon the turbidity produced by the formation of phosphomolybdates of certain bases, in particular strychnine, and upon Denigès'

* Communication from the Research Department, Woolwich.

discovery that, under certain conditions of acidity of the solution, etc., blue reduction products of molybdenum can be formed only in the presence of phosphate or arsenate. While this latter type of method is attractive on account of its great sensitiveness, it would appear from recent work (Truog and Meyer, *Ind. Eng. Chem., Anal. Ed.*, 1929, **1**, 136) that there is a serious interference even by small amounts of iron. There is, therefore, little prospect of this method being applied to the determination of phosphorus in steel, owing to the difficulty in separating traces of phosphorus from iron in a simple manner.

The method to be described is based upon the work of Pouget and Chouchak (*Bull. Soc. chim.*, 1909, **5**, [iv], 104; **1911, 9, 649**) who, in their papers on the determination of minute amounts of phosphoric acid by means of the turbidity produced by the formation of strychnine phosphomolybdate, suggest a method for determining phosphorus in steel. While the investigation of these authors is pioneer work of the highest value, a re-investigation of the subject was found to be justified.

THE REAGENT.—The strychnine-molybdic acid reagent, as used in this work, is prepared as follows: Neutral sodium molybdate* (33 grms.) is dissolved in water (150 c.c. approx.), and nitric acid (50 c.c. of 1·42 sp. gr.) added slowly, the solution being well shaken during the addition; the resulting clear solution is cooled and diluted to 250 c.c. To a portion of this stock solution (which keeps well) is added, also with vigorous shaking, one-tenth of its volume of a hot solution of strychnine sulphate, prepared by dissolving strychnine (2 grms.) in hot water to which a few c.c. of dilute (1:3) sulphuric acid have been added, and diluting to 100 c.c.† A slight precipitate appears on adding the strychnine solution; the reagent is filtered through a No. 42 Whatman paper before use. This strychnine-molybdic acid reagent is not very stable and, on being kept for a few hours, becomes opalescent and develops a distinct yellow colour; it should, therefore, be freshly prepared for each series of determinations.

Kober and Egerer (*J. Amer. Chem. Soc.*, 1915, **37**, 2373) have suggested a strychnine-molybdic acid reagent for the nephelometric determination of phosphate, in which nitric acid is replaced by hydrochloric acid, claiming that it is colourless and stable. This claim was not completely verified. A specific objection exists to the use of this hydrochloric acid reagent for the photometric determination of phosphorus in presence of iron, since, whereas one decigram of iron (as ferric nitrate) in 50 c.c. of solution containing nitric acid is very nearly colourless, the addition of hydrochloric acid causes the development of a decided yellow colour which would interfere with the estimation of turbidity in the liquid. This hydrochloric acid reagent has been used by Kober (*J. Ind. Eng. Chem.*, 1918, **10**, 560; Yoe, *Nephelometry*, p. 144) for the nephelometric determination of phosphorus in *iron* (presumably of high phosphorus content); this method could not be used for steel, since the larger weight of sample necessary would introduce sufficient iron into the test solution to vitiate the process.

* See note at end of paper.

† This solution deposits strychnine sulphate on cooling, and it therefore requires to be heated on a steam bath before use.

Steps have been taken to ascertain the most suitable conditions for the formation of a satisfactory turbidity of strychnine phosphomolybdate upon which quantitative measurements could be made. In a solution of very low acidity the addition of the reagent causes the formation of a turbidity in the absence of phosphate, and large amounts of acid seriously retard the formation of visible strychnine phosphomolybdate. The presence of 10 c.c. of nitric acid (sp. gr. 1·2) in a final volume of 50 c.c. appears to be the optimum concentration, and, on the addition of 5 c.c. of the reagent, which tests had shown to be a suitable quantity, a turbidity quickly appears in the solution (containing a few hundredths of a milligram of phosphorus as orthophosphate) and reaches a steady amount in about ten minutes, thereafter remaining practically constant during the next half-hour.

The density of the turbidity has been found, contrary to the statements of Pouget and Chouchak, not to be directly proportional to the amount of phosphorus added. In order to determine phosphorus by comparison of the turbidity produced with that produced under the same conditions by a known amount of phosphate, it is necessary to apply a correction.

The correction to be applied has been determined, both in the presence and the absence of iron, by preparing series of turbidities with known amounts of phosphorus (added as a standard solution of potassium dihydrogen phosphate) and obtaining the ratio of the opacity of each member of the series to that of a given one of the members. From the results obtained, curves were drawn from which the correction to be applied, within the range of amounts of phosphorus examined, can be read.

The photometric comparison of the turbidities of strychnine phosphomolybdate was carried out, in the present work, with the use of *transmitted* light, and not by what is known as nephelometry, which employs the reflected and diffracted light produced by a turbidity under illumination normal to the length of column. The method employed was adopted because the turbidity in question is not strictly colourless. A Klett colorimeter was used, which permits of the accurate measurement of the ratio of the lengths of the columns of two unequal turbidities when the amount of light transmitted by each is the same. The light transmitted, as viewed in the divided field of the instrument, is clear yellow. The cups for holding the turbid liquid were those supplied for ordinary colorimetric work, *i.e.* made of black glass with clear fused-on bottoms; the black glass cups are essential to screen the turbid liquid from incidental light.

Method for Pure Phosphate Solution.—The method, which can be used for the determination of phosphorus in a pure orthophosphate solution containing from 0·01 to 0·06 mgrms. of phosphorus, is as follows:

Into two glass cylinders graduated at 50 c.c. (Nessler glasses are suitable) are run 10 c.c. of nitric acid (sp. gr. 1·2) from a pipette. The neutral phosphate solution is placed in one of the cylinders, and 3 c.c. of a standard phosphate solution (0·0439 grm. KH_2PO_4 per litre; 1 c.c.=0·01 mgrm. P.) is run into the other. Water

is added to both up to approximately 40 to 45 c.c., followed by exactly 5 c.c. of the strychnine and molybdic acid reagent. The solutions are diluted to 50 c.c. and stirred by a glass plunger (say six strokes up and down). After being kept for 10 minutes, sufficient of the solutions (about 10 c.c.) are poured into the cups of the colorimeter, and when the divided field of view in the instrument has been made uniform by raising or lowering either of the cups (the standard being preferably set at a definite depth) the ratio of the depths of the solutions is measured. From this ratio the apparent amount of phosphorus is obtained. The true amount is ascertained by reference to the curve (Figure 1).

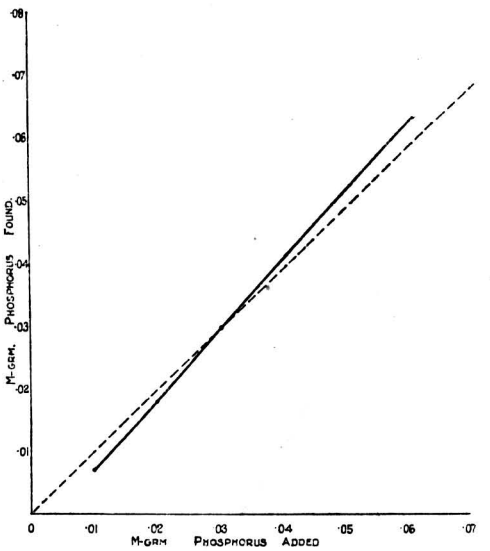

Fig. 1.*

Table I shows the apparent amounts found by comparing turbidities from known amounts of phosphorus with a 0·030 mgrm. standard.

TABLE I.

Phosphorus added. Mgrm.	Depth ratio. (0·030 mgrm. standard.)	Apparent phosphorus found. Mgrm.
0·010	10/40·4	0·007
0·020	25/41·3, 20/33·4	0·018
0·030	Used as standard	0·030
0·040	30/21·6, 20/14·7	0·0415
0·050	20/11·3	0·053
0·060	30/14·0, 20/9·4	0·064
0·070	30/11·2, 20/7·8	0·080

* For use in this process, this curve must be drawn on a large scale on squared paper, using the data in Table I.

EFFECT OF SILICON AND ARSENIC.—The observation of Pouget and Chouchak (*loc. cit.*), that silicon does not interfere, has been confirmed, a correct result for phosphorus having been obtained in presence of several mgrms. of this element (added as sodium silicate). Their statement that arsenic interferes has been verified. Whilst, on the one hand, it has been found that comparatively large amounts of arsenic (added as a neutralised solution of sodium arsenate) give no turbidity when treated by the method described for phosphorus, *e.g.* with 0·5 mgrm. of arsenic alone, no turbidity was produced within one hour; on the other hand, a solution containing 0·03 mgrm. of phosphorus and 0·1 mgrm. of arsenic gave a turbidity in ten minutes, corresponding with 0·056 mgrm. of phosphorus. In Kober's method for determining phosphorus in iron, the effect of arsenic is ignored.

PHOSPHORUS IN STEEL.—Iron in the ferric condition seems to exercise no very marked effect on the development of the strychnine phosphomolybdate turbidity; but it has been found that, in order to obtain a quantitative measure of this turbidity in the presence of iron, it is necessary that the standard turbidity should be produced in the presence of the same amount of iron. The principal requirements of a method for the determination of phosphorus in the solution of a steel in acid are:—(i) That the whole of the phosphorus should be converted into the form (orthophosphate) in which it will react with the reagent; (ii) that arsenic must be eliminated; (iii) that the final solution in which the turbidity is to be produced must be of the correct degree of acidity and should contain the iron in a form which shows the least possible neutral colour. Halogen acids must be absent.

The following method provides for these requirements:

METHOD.—The sample of steel and the standard (see note below) are treated in exactly the same way. The directions given are to be taken as applying to both.

One-tenth grm. is dissolved in 5 c.c. of dilute nitric acid (sp. gr. 1·2) by heating in a 400 c.c. squat beaker. Nitrous fumes having been dispelled, three drops of a saturated solution of potassium permanganate are added, the liquid is evaporated to dryness, and the residue is heated for 5 to 10 minutes. The residue is dissolved by warming with 5 c.c. of concentrated hydrochloric acid. One c.c. of hydrazine hydrochloride solution (20 per cent.) is added, the liquid is evaporated to dryness, and the brown residue is heated until it changes to yellowish-white and a slight evolution of white fumes occurs. To the residue are added 5 c.c. of concentrated hydrochloric acid, and the liquid is again evaporated to dryness. (This second evaporation is suggested as being necessary to remove all traces of arsenic.)

The residue is covered with 5 c.c. of concentrated hydrochloric acid and the beaker heated; a similar volume of concentrated nitric acid is added, and the heating continued until the vigorous effervescence has subsided.

About 30 c.c. of hot water are now added, and the liquid rendered slightly alkaline with ammonia. The liquid is boiled for a few moments and filtered through a 12·5 cm. Whatman paper (No. 41). The beaker is washed out twice with dilute potassium nitrate solution (5 per cent.), and the liquid poured on the filter, which is finally washed with two successive quantities of the same solution.

The filter paper is opened out in the funnel, and the ferric hydroxide washed back into the original beaker, *using not more than* 25 *c.c. of water* (a brown stain generally remains on the paper, but this is neglected). Ten c.c. of dilute nitric acid (sp. gr. 1·2) are added from a pipette to the contents of the beaker, a very small granule of potassium *nitrite* is also added, the liquid is heated (not boiled) until a clear solution is obtained, and is finally cooled in a bath of running water.

The liquid is filtered, to remove shreds of filter paper, through a small wad of damped cotton wool contained in a small funnel, a small volume of water being used to rinse out the beaker and to wash the filter. The filtrate is collected in a 50 c.c. Nessler cylinder, and its volume should be *not more than* 45 *c.c.*

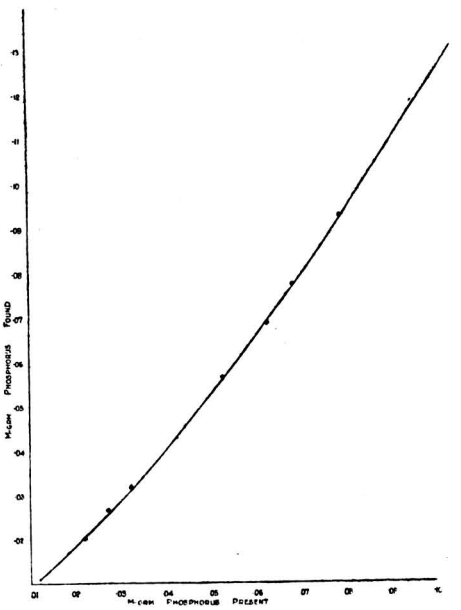

Fig. 2.*

To the solution from the steel to be tested, and also to the standard which has been treated in the same way, are added exactly 5 c.c. of the reagent (the preparation of which is described in the earlier part of the paper), the volumes are adjusted to 50 c.c., and each solution mixed by stirring six times with a glass plunger.

After the lapse of 10 minutes, the two colorimeter cups are filled with the respective liquids, and the ratio of the opacities of the liquids determined by finding the depth of liquid which transmits the same amount of light as a definite depth of the standard liquid, *i.e.* when the divided field seen in the eye-piece of the instrument is uniform.

* For use in the process, this curve must be drawn on a large scale on squared paper, using the data in Table II.

The apparent phosphorus content of the sample is simply

$$\frac{x}{y} \times s$$

where x represents the depth (in mm.) of the standard liquid; y the depth (in mm.) of the sample; and s the phosphorus content of the standard. The true phosphorus content of the sample is obtained by reference to the curve (Fig. 2).

THE STANDARD.—Two alternative standards can be used. Probably the most convenient in ordinary work would be a standard steel. Since the essential correction curve connecting the apparent phosphorus found with the true phosphorus content was worked out by using a standard containing 0·032 mgrm. of phosphorus with 0·1 grm. of iron (0·032 per cent.), a standard steel giving very nearly this value must be chosen. British Chemical Standard Steel "0·1" (0·031 per cent. P) is suitable.

In the present work 0·1 grm. of electrolytic iron was used, to which 0·030 mgrm. of phosphorus was added (as a standard solution of phosphate added to the iron in a beaker before dissolving). Analyses by independent methods showed that the electrolytic iron used contained 0·002 per cent. of phosphorus.

THE CALIBRATION CURVE.—The data for this curve were obtained by adding known amounts of phosphorus (as a standard solution of potassium dihydrogen phosphate) to 0·1 grm. samples of electrolytic iron, which were then submitted to the process just described. Each member of the series (except that containing 0·032 mgrm. of phosphorus) was compared in turn, the 0·032 mgrm. member being used as the standard. The results shown in Table II were obtained.

TABLE II.

Added.			Depth ratio.	Found.
Electrolytic iron. Grm.	Phosphorus (as std. solution). Mgrm.	Total phosphorus. Mgrm.	0·032 mgrm. standard.	Apparent phosphorus. Mgrm.
0·1	0·010	0·012	10/29·3, 15/44·4	0·011
0·1	0·020	0·022	15/24·2, 20/31·6	0·020
0·1	0·025	0·027	20/24·2, 30/35·2	0·027
0·1	0·030	0·032	Used as the standard	
0·1	0·035	0·037	20/18·1, 30/27·4	0·035
0·1	0·040	0·042	20/15·0	0·043
0·1	0·050	0·052	20/11·3, 30/16·7	0·057
0·1	0·060	0·062	20/9·6, 30/13·5	0·069
0·1	0·065	0·067	30/12·3	0·078
0·1	0·075	0·077	20/6·9, 30/10·0	0·094
0·1	0·100	0·102	30/7·2	0·133

These results are plotted as a curve in Fig. 2.

Some results which have been obtained by this method of the determination of phosphorus in various standard steels are recorded in Table III.

TABLE III.

Standard used: 0·1 grm. of electrolytic iron (containing 0·002 per cent. of phosphorus + 0·030 mgrm. of phosphorus added as KH_2PO_4).

	Taken.		Found.				
	Steel.	Wt. Grm.	Depth ratio.	Apparent phosphorus. Mgrm.	Corrected phosphorus (from Fig. 2). Mgrm.	Per Cent.	Mean certificate result. Per Cent.
B.C.S.	A_2	0·1	10/49·0, 5/24·4	0·007	(0·007	0·007)	0·008
B.C.S.	H_1	0·1	20/30·6, 30/46·3	0·021	0·023	0·023	(0·024)[1]
B.C.S.	O_1	0·1	20/20·8, 30/31·1	0·031	0·031	0·031	0·031
B.C.S.	N_1	0·1	20/19·0, 30/28·7	0·033	0·033	0·033	0·036
B.C.S.	S_1	0·1	20/11·4, 30/17·1	0·056	0·052	0·052	0·051
B.C.S.	V[2]	0·1	20/25·6, 30/39·2	0·025	0·026	0·026	0·024
B.C.S.	P	0·1	20/5·0, 30/7·2	0·131	0·099	0·099	0·105
Bureau of Stds.	32[3] B.	0·1	15/40·3	0·012	0·013	0·013	0·016

[1] This is the value obtained by the author with the ammonium phosphomolybdate process. The certificate result (0·027 per cent. of phosphorus) was not confirmed.

[2] Chromium-vanadium steel: Cr, 0·86 per cent.; V, 0·27 per cent.

[3] Nickel-chromium steel: Cr. 0·64 per cent.; Ni, 1·21 per cent.

NOTE ON THE PREPARATION OF SODIUM MOLYBDATE.—According to Pouget and Chouchak (*loc. cit.*) at least two substances go under the name of sodium molybdate: neutral sodium molybdate $Na_2MoO_4.2H_2O$, and acid sodium molybdate $Na_6Mo_7O_{24}.22H_2O$, and it is not a matter of indifference which substance is used in the preparation of the reagent which they specify. In order to be free from uncertainty, Pouget and Chouchak gave directions for preparing their reagent from molybdic acid by dissolving this in a certain quantity of sodium carbonate solution and adding a specified amount of nitric acid. The two specimens of molybdic acid which were available to me contained combined ammonia, so that the preparation of a reliable reagent from molybdic acid appeared to be no more certain than its preparation from commercial sodium molybdate. Neutral sodium molybdate for the preparation of the reagent used in the present work was made from molybdic acid as follows:

Molybdic acid (72 grms.) is dissolved in sodium hydroxide solution (150 c.c. containing 45 grms. of NaOH) contained in a Pyrex vessel. The solution is boiled for 30 minutes to expel ammonia (if present). The solution is cooled, filtered if necessary, and the sodium molybdate precipitated by the addition of about two volumes of alcohol. It is filtered on a Buchner funnel, washed several times with alcohol and dried in an oven at 100° C.

Notes.

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

VOLUMETRIC DETERMINATION OF SULPHATE.

MODERN methods of boiler-feed water treatment require the maintenance of a definite ratio between sulphate and carbonate, the sulphate being calculated as sodium sulphate, and the alkalinity, made up of hydroxide and carbonate, as sodium carbonate.

This ratio is determined by the boiler pressure, and should be capable of being measured quickly and easily by a boiler attendant or a shift engineer.

Now, the total alkalinity is easily carried out, the two methods for such a determination suitable for boiler water being (1) the Winkler method; (2) the excess acid method. The details of these are well known and need not be described.

For the sulphate determination, the only method recommended for boiler room purposes is the turbidity method, consisting in adding barium chloride to an aliquot portion of water and measuring the turbidity of the solution against a standard.

Differences in size of the barium sulphate particles upset the calculations, and the following method appears to offer definite advantages and can be carried out with the same apparatus as that used for the alkalinity test.

The method consists in shaking a measured quantity of water with a suspension of barium carbonate, and titrating the sodium carbonate formed, with phenolphthalein as indicator.

The barium carbonate suspension can be prepared either by shaking barium carbonate in water or by mixing equivalent amounts of barium chloride and sodium carbonate dissolved in water. The supernatant liquor is tested with a drop of sodium carbonate solution to make sure that there is no soluble barium salt present, and the precipitate is filtered off on a Buchner funnel and washed. The washing need not be complete.

The precipitate is re-mixed with fresh distilled water to make a convenient suspension of the carbonate and water, and a few drops of phenolphthalein solution are added. If a purple colour develops, $N/10$ hydrochloric acid is added, drop by drop, until only the faintest colour is left.

The determination is carried out as follows:—One hundred c.c. of water are placed in a conical flask of about 300 c.c. capacity. Ten drops of phenolphthalein solution are added, and $N/10$ acid run in, drop by drop, until the liquid is colourless. The burette reading is then taken, and about 10 to 20 c.c. of barium carbonate suspension are added to the flask. A pink colour immediately develops, due to the interaction of the barium carbonate and soluble sulphate, with formation of barium sulphate and soluble carbonate. The acid is now run in slowly until the pink colour is discharged.

Care should be taken not to run the acid in too quickly, lest it should act upon the suspension of barium carbonate, but with care there is no apparent action until the soluble carbonate has been converted into bicarbonate. The number of c.c. of acid used: $\times 96$=parts per million SO_4, or $\times 142$=parts per million of sodium sulphate.

Whilst the method is not suggested as one to replace the gravimetric method as a final check, yet it can be carried out quickly and is sufficiently accurate to determine whether the sulphate to carbonate ratio is being maintained in the boiler. For example, a water was tested by the gravimetric and the volumetric methods and the results obtained were as follows: Gravimetric method, 1572 parts per million SO_4; volumetric method, 1585 parts per million SO_4.

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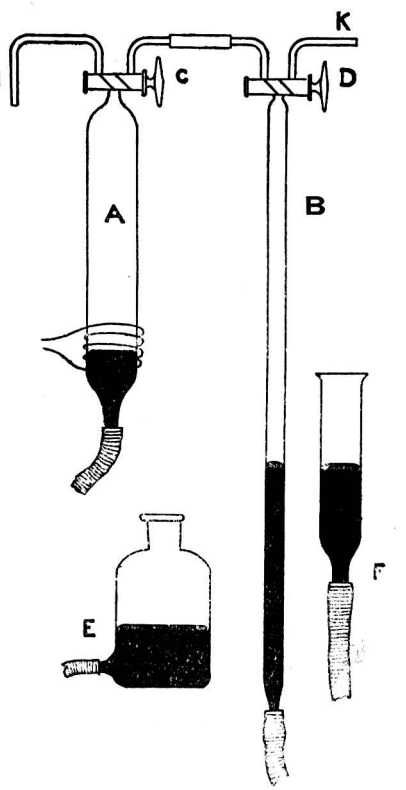
AN APPARATUS FOR THE DETERMINATION OF SMALL QUANTITIES OF AMINO NITROGEN.

THE determination of amino nitrogen present in solution at low concentration appears to be a matter of some difficulty; but the apparatus shown in the diagram enables one part of amino nitrogen per million parts of solution to be determined.

At the outset it should be pointed out that the low pressures and small amounts of gases involved in the process render efficient lubrication of the stopcocks necessary. The paraffin and rubber lubricant supplied by British Drug Houses, Ltd., is eminently satisfactory for the purpose.



Referring to the diagram, *A* is the reaction vessel, of 100 c.c. capacity. The lower end is wrapped with several turns of resistance wire, which heats the tube sufficiently with a current of 1–2 amperes. *B* is a 10 c.c. measuring tube, graduated in hundredths of c.c.

PROCEDURE.—Stopcocks *C* and *D* are turned, so that on raising *F*, the mercury completely fills *B* and runs into *A*. *D* is then turned off, and *C* turned so that, on raising *E*, mercury fills *A* and the side tube *H*. By lowering *E*, the sample solution (as much as 50 c.c.) may be drawn through *H*, into *A*, followed by 1 c.c. of water to wash the tube. If any air has entered, it must be forced out by raising *E* so that the liquid just reaches the stopcock *C*, which is then closed. *E* is then lowered, so that a Torricellian vacuum exists above the surface of the solution. *A* is now heated (electrically), and the dissolved gases expelled. These are driven out of *A* after a few minutes, and their complete removal is shown when only a very small bubble exists between the surface of the solution and the stopcock upon levelling the mercury. After this, *C* is turned and the solution allowed just to fill *H*; *C* is then turned off and *E* lowered. Two to 4 c.c. of glacial acetic acid, contained in a small tube, are drawn in at *H* by opening *C*, care being taken that no air enters, after which 2 c.c. of 5 per cent. sodium nitrite solution, contained in another tube, are made to follow the acid, and both are drawn into *A*. *C* is then

closed so that no air enters *A*, and by raising and lowering *E* a few times the solutions are made to mix. After a period of 10 to 30 minutes (depending on the nature of the amino-substance), the evolved gases are sent into *B* by manipulation of *C*, *D*, and *E*. The dissolved gases are boiled out as before, and passed over into *B*, until rather more than 10 c.c. are collected. *C* is then turned, *E* raised, and the spent solution forced out of *H*. *A* is washed by drawing in and ejecting, successively, three small quantities of water. Eight c.c. of "alkaline permanganate" solution (50 grms. of potassium permanganate and 25 c.c. of potassium hydroxide per litre) are drawn in, care being taken to avoid entrance of air. The gases are then brought back into *A*, and the nitric oxide absorbed, after which the nitrogen is returned to *B* and measured. A blank determination must be made with the nitrite solution. After removal of the permanganate solution, the mercury in *A* may be washed with acid and water.

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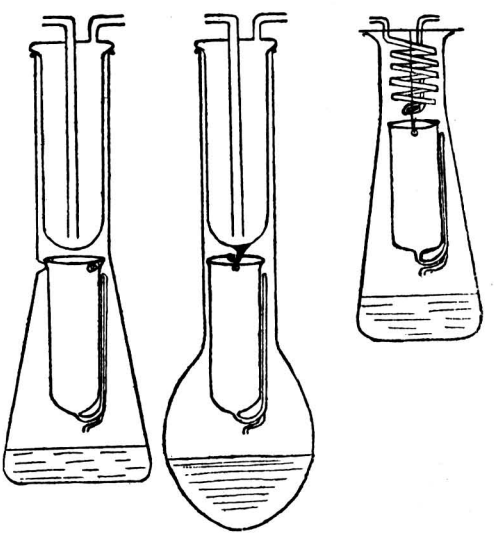
EXTRACTION APPARATUS FOR RUBBER, CELLULOSE, FATS, ETC.

A "SIMPLE all-glass extraction apparatus" was described in 1913 by C. Beadle and H. P. Stevens (ANALYST, 1913, **38**, 143). This apparatus was based on a modification of the Soxhlet or Knoefler principle, and was designed to avoid the use of ground-glass joints, corks or mercury seals. In its original form, or in a slightly modified form, in which a round-bottomed flask replaced the conical flask, it has



I II III

been in use in these laboratories since that time. In recent years a modification, in accordance with suggestions then made (*loc. cit.*), has been introduced in order to overcome the only disadvantage attaching to the apparatus, namely, the necessity for the transference of the extract for the purpose of evaporation, drying

and weighing. The apparatus in its final form would appear to be generally used on the Continent and in America (G. S. Whitby, *Trans. Inst. Rubber Ind.*, 1925, **1**, 18; *Rubber Age* (*London*), Oct., 1924; *cf. Elektr. Z.*, 1922, **43**, 295, 483), but is seldom seen in this country.

For this reason the following description and drawings, which are self-explanatory, are appended:

Form I is that originally employed, the drawings being approximately a quarter of the natural size. Form II, which is that at present employed, is especially adapted for use on a water-bath. Form III, the improved type, is very similar to Form I except that, as originally suggested by Beadle and Stevens (*loc. cit.*), a more effective condenser (lead or tinned copper coil) has been introduced, thus enabling the total length of the flask to be reduced, so that this may be accommodated on an ordinary balance pan. When removing the solvent after extraction it is, therefore, unnecessary to transfer it to a separate weighed flask, as is the case with Forms I and II.

The advantages of Form III may thus be summarised:—(1) Absence of corks, ground-glass joints and mercury seals; (2) efficient extraction due to adherence to Knoefler principle; (3) general simplicity and compactness of the apparatus, thus giving: (*a*) Comparative cheapness. (*b*) Interchangeability of parts should breakages occur; and ready replacements of any part to recomplete the apparatus. (*c*) No transference of extract necessary. (*d*) Type of extraction thimble at choice, *i.e.* straight through or with Soxhlet siphon tube. (*e*) Adaptability to water-bath or hot plate. In connecting up the condensers of several of these pieces of apparatus it is advisable to use thin-walled rubber tubing, so that the weight of the connection has no tendency to upset the flasks.

The apparatus has two limitations: first, the size of the sample that may be extracted is restricted to the capacity of the balance used for weighing the flask; and, secondly, solvents must not be used which might attack the metal of the condenser.

W. H. Stevens.

15, Borough High Street,
S.E.1.

CALCULATION OF ALCOHOL FROM THE SPECIFIC GRAVITY.

Some years ago, when calculating the relations of the various ways of stating alcoholic strength (Analyst, 1919, **44**, 167), I assumed, without trial, that the method of calculation of Thorpe's "*Alcoholometric Tables*" was the same as that used by Hehner (Analyst, 1880, **5**, 42), and gave values with five or more significant figures.

Hehner, after accepting relationships between specific gravities and percentages of alcohol by weight, used constants to calculate from them the corresponding percentages of proof spirit and alcohol by volume.

I am indebted to Mr. F. C. H. Tate for pointing out that the percentages of proof spirit in Thorpe's tables are obtained by multiplying the percentage of alcohol (*v*/*v*) at 50° F. by 1·7535. Also, that the ratio of the percentage of alcohol (*v*/*v*) at 50° F., to that of alcohol (*v*/*v*) at 60° F. is not constant, and, therefore, the ratio of alcohol (*v*/*v*) at 60° F. to proof spirit is not constant. The differences are not serious; for example, division of the percentage of alcohol (*v*/*v*) at 60° F. into the corresponding percentage of proof spirit gives the factor 1·7535 with absolute alcohol, and 1·7482 with 25 per cent. alcohol.

Owing to this fact, calculations assuming constants will have small errors except at one particular strength of alcohol. In the following revised formulae, proof spirit is taken as a convenient mean strength:

$$\text{Per cent.} = \frac{0{\cdot}7940\, v/v}{S} = \frac{w/v}{S} = \frac{0{\cdot}4533P}{S}.$$

v/v = per cent. $\times$ $1{\cdot}259S = 1{\cdot}259\, w/v = 0{\cdot}5711P$.

w/v = per cent. $\times$ $S = 0{\cdot}7940\, v/v = 0{\cdot}4533P$.

P = per cent. $\times$ $2{\cdot}206S = 1{\cdot}751\, v/v = 2{\cdot}206\, w/v$.

S = specific gravity at 60°/60° F.

Per cent. = grms. of absolute alcohol per 100 grms.

v/v = c.c. of absolute alcohol per 100 c.c.

w/v = grms. of absolute alcohol per 100 c.c.

P = c.c. of proof spirit per 100 c.c.

J. F. LIVERSEEGE.

161, ROTTON PARK ROAD,
BIRMINGHAM.

Official Appointment.

THE Minister of Health has confirmed the following appointment:

JOHN SINLEY WILSON, as Additional Public Analyst for the County Borough of Burnley (June 16th, 1931).

Legal Notes.

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

"HOUSEHOLD TURPS."

ON July 10th a retail trading firm was charged at the Salford City Police Court, under Sec. 2 (2) of the Merchandise Marks Act, 1887, with selling a mixture of paraffin and turpentine, to which a false trade description ("Finest Household Turps") had been applied. The manufacturers of the article were also charged, under Sec. 2 (1) of the Act, and with aiding, abetting, counselling, or procuring the trading firm to commit the offence.

The City Analyst (Mr. H. E. Monk, B.Sc., F.I.C.) said that the sample consisted of 30 per cent. of turpentine and 70 per cent. of paraffin of the nature of "white spirit." The fact that the sample was not genuine would be apparent to anyone who was accustomed to deal in such products, from both the smell and the appearance of the bubbles formed on shaking.

In cross-examination the witness said that he had heard of an article called "Household Ammonia," and knew that it contained ingredients other than ammonia, such as soap. He did not consider the description "Household Turpentine" to be on the same footing.

Mr. H. Stout, a pharmacist, gave trade evidence for the prosecution to the effect that he understood "Household Turps" or "Household Turpentine," to mean genuine turpentine and nothing else. The price of turpentine was about four times that of "white spirit."

The secretary of the manufacturing firm said that they had been packing the article for eight years. He considered it equal to, if not better than, turpentine for all household purposes.

The solicitor for the defendants argued that household turps had been supplied, and that there was no standard whereby this article should consist wholly of turpentine. If the magistrate found against him on this point, he must fall back upon the defence that his clients had acted innocently.

The Stipendiary Magistrate (Mr. Percy Macbeth) found the charges against both defendants proved. The retail firm would be fined £15 with £5 5s. costs, and the manufacturers £15 (£7 10s. on each charge) with £5 5s. costs.

Department of Scientific and Industrial Research.

REPORT OF THE FOOD INVESTIGATION BOARD FOR THE YEAR 1930.*

SECTION A. MEAT.—An expedition visited New Zealand in September, 1929, to make a comprehensive scientific survey of the trade in frozen mutton and lamb from the abbatoir in New Zealand to the wholesale market at Smithfield. A brief account of the ground covered is given. Interesting results have been obtained in the further researches on the analyses of the function of muscle and nerve, and it is shown that the mechanism for oxidising lactic acid is not the same as for synthesising glycogen, since when muscles are frozen so intensely that they do not recover their function on thawing, the capacity for oxidising lactic acids still persists, whereas that for forming glycogen is lost. Heart and nerve tissues are also being studied in order to obtain further light on the mechanism of the injury which freezing occasions. Further work on the water relations in colloidal systems shows that there is very little water bound to the gelatin in dead mammalian muscle, most probably not more than 6 per cent. A paper by J. Brooks (*Biochem. J.*, 1930, **24**, 1379) summarises some of the work on changes in muscle pigment.

Rancidity in Fats.—The Kreis test is now applied by dissolving 1 grm. of fat in 2 c.c. of benzene; 1 c.c. of concentrated hydrochloric acid is added, followed, after shaking for 1 minute, by 1 c.c. of a 0·1 per cent. solution of phloroglucinol in ether; shaking is continued for 1 minute and, after centrifuging, the pink aqueous layer is compared with standards. At low temperature the rate of increase of the Kreis reaction is much less than that of the active oxygen content (but in sunlight the opposite occurs), so that rancidity, as measured by any chemical test, may not necessarily bear the same relationship to rancidity measured by taste and odour. Pre-cooling of carcases for 24 hours at an average temperature of 18·5° C. had no appreciable effect on the keeping qualities of the fat when stored at −5° C., or less, for 30 weeks; a temperature of −10° C. is satisfactory for over 30 weeks, but at −5° C. the growth of moulds and yeasts is not completely inhibited, and sweating caused by exposure to ordinary temperatures for short times

* Obtainable at H.M. Stationery Office, Adastral House, Kingsway, W.C.2, pp. 175. Price 3s. 0d. net.

during storage has a bad effect on the fat. The chemical changes occurring in the fat of beef stored at 0° C. have been studied. After 25 days, followed by 4 days at 10° C., the fat was in good condition, but in 42 days a definite taint had appeared in places. The movement of air had a striking effect in reducing the growth of moulds and prolonging the storage life of the fat. A considerable amount of work has been done on the *Actinomyces* of cold stores, which appear to be the only micro-organisms giving rise to a musty odour.

SECTION B. FRUIT AND VEGETABLES.—The biochemical study of senescence in apples is not yet complete. The study of the effect of acetaldehyde on the germination of fungal spores (ANALYST, 1930, **55**, 509) has been extended to the growth of moulds, and it is found that, although their rate of growth is generally at first inhibited or much retarded, it usually increases with the time of exposure. The age of the colony, temperature, and concentration of the acetaldehyde influence the result. As a whole, volatile substances fall into two classes: those which retard growth to a constant value, *e.g.* chloroform, and those with which "adaptation" occurs, *e.g.* acetaldehyde. The study of the loss of water from apples confirms the view that the mechanism by which water passes from the cell vacuoles to the outer air is complicated, and the rate of loss seems to be governed by the resultant of a number of still not understood internal factors. The work on the action of moderate strengths of cyanide on potatoes at 15° C. shows that changes in respiration are mainly related to the action of cyanide on the sugar content, probably affecting the starch-hydrolysing system.

Investigation of the vitamin content of apples still leaves Bramley's Seedling richer than other varieties, Lane's Prince Albert coming next. Storage in the frozen state does not affect the vitamin content. Vitamin *C* is at least six times as concentrated in the peel as in the core region. Peas may be preserved without objectionable flavour if they are first cooked for about 8 minutes before freezing in water at 0° F.

SECTION C. PIG PRODUCTS.—The freezing and storage of pork and mild-cured bacon, and the scientific basis of canning, including the swelling of gelatin in solutions of sodium chloride, and the effect of sodium chloride on pork muscle are dealt with.

SECTION D. BIOLOGICAL ENGINEERING.—The long-felt need of an experimental chamber intermediate in scale between the small constant-temperature room and the immense ship's hold has now been supplied by the erection of a large experimental store at Ditton Laboratory capable of holding some 120 tons of fruit, and an empirical study of the distribution of temperature inside a mass of fruit consisting of 6,000 boxes of apples is being made. The determination of various biological constants for engineering use, particularly in connection with the rate of evaporation from eggs as affected by changes of temperature, humidity and air movement, was continued.

SECTION E. CANNING.—See ANALYST, 1931, **56**, 315.

SECTION F. FISH.—The work on the low-temperature preservation of haddock has been carried further, and, so far as commercial practice goes, the fish may be successfully stored at 29° to 25° F, or −9° to −13° F. or lower. The first temperature has the disadvantage of allowing bacterial growth to proceed, and the second involves greater expense in refrigeration. At intermediate temperatures the "drip" (*i.e.* easily expressible fluid) is most obvious.

Work on post-mortem changes in the liver fat of various fish shows that there are different rates of development of free fatty acids for different species, and that after storage the percentage of free fatty acids developed in the oil of the outer

richer layers of liver is less than in the inner. Lipase is probably evenly distributed in the liver, and the existence of a fat-splitting enzyme other than lipase is suspected.

The effect of bacteria on the spoilage of fish has been investigated, and of the numerous bacteria occurring in the slime on the fish, the group *Achromobacter* is most prevalent, and there was almost complete absence of spore-bearing organisms. The fish muscle was found to be sterile, and of two batches of fish, one untreated, and the other treated with germicidal solution, the muscle of the first group was contaminated several days before the second, which took 7 to 10 days. Invasion from the intestine seems to be an unimportant factor in spoilage. The smoke curing of fish has also received attention, and control of humidity seems to be an important factor.

SECTION G deals with researches conducted at the National Physical laboratory under the direction of the Engineering Committee.

SECTION H. RESEARCHES ON FRUIT.—The chemical work on fruit, at the Imperial College of Science and Technology, has been mainly concerned with the effect of maturity at gathering on subsequent behaviour of apples in cold store. Chemical changes in apples in store at 1° C., gathered at different dates, involved many determinations of fructose and glucose, and it is emphasised that, as stated in the report for 1929, clearing with basic lead acetate and subsequent de-leading, to remove oxidisable substances other than sugar, is an essential preliminary to iodimetric determination of the sugars. If potassium oxalate is used as the de-leading agent, the copper-reducing values are too low, and the iodine values too high (owing to slight oxidation of the oxalate), but if sodium phosphate is used as the de-leading agent, no corrections are necessary. Alcoholic solutions of apple pulp cleared with sodium phosphate, however, develop a yellow colour on standing, and, to obtain concordant results, such solutions should be decolorised by boiling with charcoal before the sugar is determined. Both these reagents are satisfactory with extracts from mature apples, but sodium phosphate only is suitable for very immature apples, owing to the coloured solution obtained with the oxalate. Since analyses with immature apples showed that the alcohol-insoluble residue increased considerably before iodine showed the presence of starch, a direct method for the determination of starch by enzyme hydrolysis was used. In order to obtain the tissue sufficiently disintegrated, some of the pectin was first removed by shaking the material in the cold with potassium oxalate and washing free from oxalate; the hydrolysis was then effected with taka diastase, and the resulting solution of maltose and glucose used for the determination of each sugar, by a combination of the iodimetric method and of Hane's modification of the Hagedorn and Jensen method of oxidation by potassium ferricyanide.

D. G. H.

THE RELATIVE VALUES OF COD-LIVER OILS FROM VARIOUS SOURCES.

J. C. DRUMMOND AND T. P. HILDITCH.

(*Empire Marketing Board, No.* 15, *December*, 1930.)*

PRIMARY SOURCE OF VITAMIN *A*.—Confirmatory evidence was obtained that the primary source of vitamin *A* is the minute green plant life of the sea, but there was no evidence as to the formation of vitamin *D* in these microscopic

* Obtainable at H.M. Stationery Office, Adastral House, Kingsway, W.C.2. Price 1s. 0d. net.

plants. The small crustacea which feed on these plants, and themselves form a main supply of the cod's food, are curiously deficient in both vitamins, but the balance of evidence favours the supposition that the vitamins are retained by the liver of the cod, and the mature liver is many times richer in them than the foods of the fish. Variations in the vitamin activity of cod-liver oils appear to be wider for vitamin *A* than for vitamin *D*, but, generally, an oil rich in vitamin *A* will be a good source of vitamin *D*, and the nature of the food supply and the quantity of oil stored in the liver appear to be the main causes of variation. The higher the yield of oil from the livers, the lower, relatively speaking, will be the vitamin content, and the richest oils will be obtained from areas where abundant food is available at seasons when the oil content of the liver tends to be low. The Newfoundland area yields the richest oil, followed by Iceland, and then by Scotland, and the Norwegian oils are much poorer.

ASSAY OF VITAMINS.—The assay of vitamins in cod-liver oil is unfortunately complicated by the use of three methods: the Norwegian (Poulsson method) giving a value of 250 units, where the British (Pharmaceutical Society assay) gives 100 units, and the American method giving 15 units. In the opinion of the authors, an unfailing indication of the medicinal value of an oil is the intensity of the blue colour produced with arsenic or antimony trichloride, and evidence is accumulating that it is vitamin *A* itself which is responsible for the blue colour. The natural colour of the cod-liver oil tends to run parallel with the vitamin content, and the pale oils from the Lofoten fisheries are, generally speaking, of low vitamin (certainly vitamin *A*) activity. Analyses of a large number of samples of cod-liver oil of known origin are given to elucidate the composition, and include detailed investigations of the fatty acids present in typical oils.

PREPARATION OF MEDICINAL COD-LIVER OIL.—In the preparation of medicinal cod-liver oil it is of the greatest possible importance to use absolutely fresh livers, and the oils should on no account whatever have free acidities above 0·5 per cent. The fat-splitting enzymes in the liver must be destroyed, and for this reason a temperature above 70° C. must be reached, and steam at a pressure of 60–100 lbs. is recommended. Insufficient cooking produces oils tending to rancidity on storage. At all stages, contact with air should be reduced to a minimum, but the use of inert gases appears to have little to recommend it. The air-spaces left in the containers should be as small as possible, and, if glass is used, it should be dark amber in colour.

SUGGESTED STANDARDS FOR COD-LIVER OIL.—It is suggested that cod-liver oil for human use should have a colour (in 1 inch cell.) of not more than 10 yellow and 0·5 red Lovibond units; (for farm stock, 15 yellow; 1 red); that the free acidity (as oleic acid) should not exceed 0·5 per cent. (1 per cent. for farm stock); that the unsaponifiable matter should not be greater than 1·5 per cent. (the same for farm stock), and that the vitamin *A* reaction should show more than 7 blue units when measured by technique B or C (see below), that is in 10 per cent. dilution (same for farm stock). It is further suggested that containers should bear on the label a statement of the free acidity and response to the colour reaction and the date of the tests.

VITAMIN *A* REACTION.—*Technique B.*—One grm. of oil is accurately weighed and dissolved in 10 c.c. of redistilled chloroform; 0·2 c.c. of this solution is placed in a 1 cm. cell and 2 c.c. of a 30 per cent. solution of antimony trichloride in chloroform are quickly added. The cell is shaken during mixing, and exactly 30 seconds after addition of the antimony solution was begun the colour is matched in a Rosenheim-Schuster tintometer. At least four separate estimations are made, and then

a series of estimations on greater or lesser concentrations, and from the plotted results (which are nearly always linear) the value for a 10 per cent. solution is obtained.

Technique C.—The same weight of oil is weighed into a 10 c.c. flask and dissolved in 10 c.c. of the chloroform. The temperature of the solution and that of the antimony trichloride reagent must be between 15° and 17° C. The oil solution is pipetted into the cell, the reagent added as above, and the blue colour matched in the Rosenheim-Schuster tintometer, as before. This is conveniently expressed in the Lovibond scale, and three estimations are made, and a further set at two lower concentrations if the test shows more than 10 blue Lovibond units, or on higher concentrations if it shows less. If the relation is not linear, it may be advisable to separate the non-saponifiable matter, with precautions against oxidation.

D. G. H.

Connecticut Agricultural Experiment Station.

REPORT ON FOOD PRODUCTS AND DRUG PRODUCTS FOR THE YEAR 1929.

THIS Report summarises the work done in connection with the inspection of food and drugs, most of the samples being submitted by the Dairy and Food Commissioner. The Department of Analytical Chemistry also tests and issues certificates as to glassware used in carrying out the Babcock test on milk and cream, and of thermometers used in the control of the pasteurisation of milk. The total number of foods examined was 1462, of which 131 were adulterated or below standard, and 17 of 57 drugs were condemned. Investigations of special foods, and of tobacco, have also been made.

CARBONATED BEVERAGES.—Only 3 of the 149 samples of the soda water type failed to comply with the requirements. Saccharin was present in 1, artificial colour was not declared in 1, and one sample bore misleading statements as to equivalent food value. The minimum limit for sugar was always exceeded.

"NEAR BEER."—Twenty-three samples of malt beverages of the "near beer" type were examined. Two were found to contain caffeine in the proportion of 0·8 and 0·9 grain per 12 oz. bottle. Objection was taken to these, on the ground that caffeine is an added ingredient foreign to the article generally known as beer, which these articles purported to be.

CEREAL PRODUCTS, ETC.—Analyses have been given of various cereal breakfast preparations in previous reports (*cf.* ANALYST, 1929, **54**, 161; 1930, **55**, 129). Eighty-six new analyses have been added, from which the following are quoted:

	Water. Per Cent.	Ash. Per Cent.	Protein. Per Cent.	Fibre. Per Cent.	Carbohydrate other than fibre. Per Cent.	Fat. Per Cent.	Calories per 100 Grms.
Barley Preparations.							
Cream of barley ..	9·2	1·4	11·1	0·6	76·1	1·6	363
Quaker Scotch brand pearled barley ..	12·1	1·0	9·5	0·3	76·2	0·9	351

	Water. Per Cent.	Ash. Per Cent.	Protein. Per Cent.	Fibre. Per Cent.	Carbohydrate other than fibre. Per Cent.	Fat. Per Cent.	Calories per 100 Grms.
Corn (Maize) Preparations.							
Cerealine	11·2	1·5	6·9	0·1	79·9	0·4	351
Jersey cornflakes	7·7	0·9	8·5	0·3	82·3	0·3	366
Post toasties	11·7	1·8	6·6	0·2	79·4	0·3	347
Quaker corn puffs	12·0	0·4	8·7	0·1	78·5	0·3	352
Quaker homing grits	13·2	0·5	7·9	0·2	77·7	0·5	347
Oat Preparations.							
Keen & Robinson's granulated Scotch oatmeal	10·4	1·9	13·7	0·8	64·1	9·1	393
Quaker oats	10·8	1·9	15·9	0·9	64·5	6·0	376
Robinson's patent groats	8·4	1·8	12·8	0·7	67·7	8·6	399
Scotch porage oats	10·1	1·7	13·3	0·4	64·9	9·6	399
Rice Preparations.							
Cook's flaked rice	12·6	0·4	7·8	0·2	78·9	0·1	348
Milk rice	12·3	3·2	6·9	0·2	77·2	0·2	338
Quaker puffed rice	12·2	0·4	7·6	0·1	79·5	0·2	350
Rye Preparations.							
Cream of rye	11·5	1·7	12·0	1·4	71·8	1·6	350
Ry-krisp	5·8	2·8	14·0	1·3	74·4	1·7	369
Wheat Preparations.							
Granose biscuit	11·3	3·9	10·3	1·8	71·1	1·6	340
Force	10·7	2·8	10·6	1·1	73·7	1·1	347
Quaker puffed wheat	11·5	1·8	13·1	1·6	70·2	1·8	349
Triscuit	10·3	1·7	11·0	1·7	73·9	1·4	352
Shredded wheat biscuit	8·5	1·5	11·0	2·6	75·0	1·4	357
Wheat Bran.							
Jireh wheat bran	11·1	4·3	16·8	6·3	56·7	4·8	337
Kellogg's sterilised wheat bran	9·6	6·0	16·3	8·5	54·4	5·2	330
Wheat Bran Biscuits, etc.							
Bran bisque	8·5	3·1	12·1	2·2	61·0	13·1	410
Dietetic bran biscuit	9·3	5·0	9·9	1·7	69·1	5·0	361
Fruit nut cereal	7·3	3·2	13·5	2·4	72·4	1·2	354
Laxative biscuit (Kellogg)	9·4	3·0	16·7	2·4	57·7	10·8	395
Miscellaneous.							
Grape nuts	10·3	1·9	11·5	1·5	74·2	0·6	348
Sea Moss Farina	15·6	13·6	9·1	1·5	59·9	0·3	279
Trix	6·2	1·5	14·5	0·3	77·3	0·2	369

"CAFFEINELESS" COFFEE.—A product, sold as "Al-Mo-Co," consisted of a mixture of cereal, coffee, molasses and chicory. It contained 0·18 per cent. of caffeine, and claimed to be 99·74 per cent. caffeineless. It may not be clear to every purchaser that ordinary coffee is about 98·8 per cent. caffeine-free, so that this product contained about one-seventh of the amount of caffeine in ordinary coffee.

SPECIAL FOODS.—Analyses were made of 54 special and miscellaneous foods, including some for diabetic patients. Of the articles included in the following table, Nouron is made from soya beans, whole wheat flour and egg yolk, and "Fiddle heads" are a species of native ferns said to have been used by the Indians as food.

					Carbohydrate.		
	Water. Per Cent.	Ash. Per Cent.	Protein (N × 6·25). Per Cent.	Fibre. Per Cent.	Starch + Water-soluble (as dextrose). Per Cent.	Undetermined. Per Cent.	Fat. Per Cent.
Flour of cooked chestnuts	6·30	2·00	6·63	2·05	65·28	14·76	2·98
Loeb's gluten breakfast cereal	6·18	3·12	37·28	0·98	27·44	8·07	16·93
Mellin's food	2·20	3·90	10·63	none	81·54		1·75
Nouron	8·30	2·48	24·38	3·05	52·46		9·33
"Flour of Algae" ..	7·48	35·62*	5·38	7·43	43·46		0·63
Soy cheese	77·20	0·55	14·44	—	trace	4·31	3·50
Fiddle heads (*Osmunda cinnamomea*) ..	87·03	1·24	4·72	1·04	5·56		0·41

* Total P_2O_5, 0·60; Fe_2O_3, 0·24; CaO, 1·75; iodine, 0·15 per cent.

ICE CREAM.—Of the 301 samples submitted, 4 were unsatisfactory. The State standard for fat content in plain ice cream is 8 per cent., and for fruit and nut ice cream 6 per cent. A Federal standard, fixing the fat limit for plain ice cream at 12 per cent., has never become official.

CIDER VINEGAR.—The law requires that vinegar (cider vinegar) shall contain not less than 1·6 per cent. of total solids. The Federal standard has been revised, so that the only numerical standard is that for acid strength (4 per cent.). Since it is now known that genuine vinegar may sometimes contain less than 1·6 per cent. of total solids, it may be unfair to adhere strictly to that limit.

TOBACCO SEED.—In addition to 167 partial analyses of tobacco, proximate analyses of tobacco seed and of fresh and cured leaves were made. The tobacco analyses gave the following results:—Water, 3·34; ash, 3·71; protein (N × 5·34), 20·76; fibre, 14·44; carbohydrate other than fibre starch, none; water-soluble after hydrolysis (as dextrose), 3·08; water-insoluble after hydrolysis (as dextrose), 0·55; undetermined carbohydrate, 11·89; fat (ethereal extract), 42·23 per cent.

The fresh and cured leaves contained 1·88 and 1·51 per cent. of starch, calculated on the air-dry substance. The starch was determined by hydrolysis of the material with malt, after successive extractions with ether, alcohol, and water.

International Atomic Weights.

FIRST REPORT OF THE COMMITTEE ON ATOMIC WEIGHTS OF THE INTERNATIONAL UNION OF CHEMISTRY.*

THE report comprises investigations published since January 1st, 1930. The atomic weight of nitrogen was calculated from specific gravity determinations of ammonia gas as 14·009; the specific gravity of phosphine also was determined, giving P = 30·977. Synthesis of silver sulphide furnished the value S = 32·066. The atomic weight of calcium was checked by chlorine determinations on anhydrous calcium chloride fused in hydrogen chloride and cooled in nitrogen; average, 40·085. The value 50·948 for vanadium almost agrees with the present figure. Determination of chlorine in chromyl chloride gave the figure Cr = 52·02; arsenic trichloride, also analysed for chlorine, led to As = 74·938. Halogen determination in tantalum chloride and bromide resulted in a lower figure, namely, Ta = 181·36. The atomic weight of rhenium, 186·31, as determined by analysis of silver perrhenate prepared by three different methods, is 2·4 units lower than Noddack's provisional figure. Thallous bromide was analysed, the result (Tl = 204·390), confirming the earlier one.

A revised complete table of atomic weights is appended; such alterations as occur affect only the second decimal place. The following figures are of interest: Re = 186·31; Ta = 181·4; Ti = 47·90; Zr = 91·22.

W. R. S.

* By G. P. Baxter, Mme. M. Curie, O. Hönigschmid, P. Le Beau, and R. J. Meyer. (*J. Amer. Chem. Soc.*, 1931, **53**, 1627–1639).

ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS.

Food and Drugs Analysis.

Chemical Changes in the Fat of Frozen and Chilled Meat. Part I. Frozen Mutton and Lamb. C. H. Lea. (*J. Soc. Chem. Ind.*, 1931, **50**, 207–212T.)—To follow the progress of oxidation a very thin layer of surface fat, 3 to 4 sq. in. in area was removed from the breast of the carcase, frozen at −20° C., trimmed at 0° C. until only a very thin layer (about 0·5 mm.) of fat-impregnated connective tissue remained. This material was crushed and cut up finely, 1 grm. weighed into a test tube, and the active oxygen content determined, using 19 c.c. of glacial acetic acid and chloroform mixture (*Proc. Roy Soc.*, 1931, 3, **108**, 175, and Reports of the Food Investigation Board, ANALYST, 1930, **55**, 570; 1931, **56**, 531). Free acidity was determined by the direct titration method of Pennington and Hepburn for chicken fat (*J. Amer. Chem. Soc.*, 1910, **32**, 568). The fat (10 grms.) was cut up, ground at 0° C. with silver sand (1 grm.), boiled with 95 per cent. alcohol (100 c.c.) for 5 minutes for kidney fat and 10 minutes for breast fat, and titrated to phenolphthalein with 0·05 *N* potassium hydroxide. The changes

in the free acidity and superficial oxidation in the fat of carcases of lamb were followed during pre-cooling, cold storage at various freezing temperatures, and at ordinary temperatures subsequent to storage. The maximum values reached for free acidity were about 1·2 per cent. (as oleic acid) for external fat, and rather less for kidney fat; 24 hours' pre-cooling at the ordinary temperature gave rise to no signs of superficial oxidation, and no definite increase in susceptibility to oxidation during subsequent storage. The fat of carcases held at −5° C., or below, for periods up to 7 months, showed no oxidation sufficient to affect the flavour after 3 days' exposure to ordinary temperatures in not too strong a light. Sweating only exerts a markedly deleterious effect on the keeping properties of the fat when held subsequently at ordinary temperature if it has occurred in the presence of light; but even for short periods it produces a bad effect on the appearance of the carcase, although the lower the temperature of storage the less the bad effect of exposure for short periods to temperatures above 0° C. No visible growth of moulds or yeasts occurred at storage temperatures of −10° C. or −20° C., but growth was not inhibited at −5° C. In no case did micro-organisms appear to be present in the fat when the meat was held for 3 days after storage at 12° C.

D. G. H.

Two New Sugars of Human Milk, Gynolactose and Allolactose. M. Polonovski and A. Lespagnol. (*Compt. rend.*, 1931, **192**, 1319–1320.)—The divergent results obtained on determining the sugar in the serum of human milk by the ordinary methods of reduction and polarimetry are due to the presence of sugars other than lactose. Two such sugars have now been isolated by means of a long series of fractional crystallisations from water and from methyl and ethyl alcohols. (1) *Gynolactose*, readily soluble in water, but almost insoluble in ethyl alcohol, has m.pt. about 205° C. and, in 3 per cent. aqueous solution, $[\alpha]_D$ −27°. It shows no apparent mutarotation, and its reducing power, as given by Bertrand's method, is about 40 per cent. of that of dextrose. It yields no phenylosazone, and gives galactose (50 per cent.) and dextrose on hydrolysis by strong acids. (2) *Allolactose*, also highly soluble in water, has m.pt. 165° C. and $[\alpha]_D$ +20° in aqueous solution. It exhibits slight and extremely transitory mutarotation. Towards iodine it behaves like an aldobiose, and its reducing power is about two-thirds of that of dextrose. The phenylosazone, which dissolves in boiling water, crystallises in mammillary masses of radiating structure and has the instantaneous m.pt. 172°C. This sugar also furnishes dextrose and galactose on hydrolysis. T. H. P.

Evaluation of Honey on the Basis of the Diastase Content. K. Braunsdorf. (*Z. Unters. Lebensm.*, 1931, **61**, 411–420.)—Of the 62 German honeys examined, one had a diastatic value of 62·5; 18 of 23·8 to 50; 18 of 11 to 18; and 8 less than 10; where the value was medium to low the phloroglucinol test indicated strong heating (*cf.* Fiehe and Kordatzki, ANALYST, 1928, **53**, 388). The value 23·8 is, therefore, suggested as a lower limit for first-class diastatic honeys, with 17·9 as the lowest permissible limit for unheated honeys and 10 for honeys assessed on the basis of the (1930) German Regulations (*cf.* following abstract, and

Braunsdorf, *Z. Unters. Lebensm.*, 1930, **60**, 575). On the whole, there was correspondence between the Fiehe and phloroglucinol tests (*cf. loc. cit.*), but the latter does not readily distinguish between unheated genuine honeys having a naturally low diastatic power and those the diastase of which has been crippled by heating. This is important, since the effect of heat is greatest for diastatic values of about 11 to 14. Absence of colour change, or a change to pale yellow on addition of the reagent in the phloroglucinol test, is an indication of unheated or slightly heated honey (*i.e.* natural diastase is present), whilst a yellow-gold colour indicates weakening of the diastase due to strong heating. It is stated that, as a rough guide for this test, a honey of fairly high diastatic value heated for 1 hour at 60 to 65° C. gives a colour (fading after 5 minutes) similar in shade and intensity to that of a 0·1 *N* solution of potassium dichromate. J. G.

Honey Diastase. J. Fiehe. (*Z. Unters. Lebensm.*, 1931, **61**, 420–427.)—The diastatic powers of 190 German and foreign honeys were determined by Fiehe and Kordatzki's modification of Gothe's test (ANALYST, 1928, **53**, 388) before and after melting at 60° C. in a steam-heated vessel provided with a stirring apparatus. Under these conditions the lowering of diastatic value is small and, in many cases, nil; the value was reduced to 8·3 in only 12 cases, and to less than 8·3 (to 5 and 6·5) in 2 cases, and 8·3 is, therefore, considered a suitable lower limit of diastatic power, and is becoming recognised as such in the trade. Attention is directed to certain Californian honeys which are naturally poor in diastase, and which cannot, therefore, be said to contravene the German Regulations, March, 1930, since these stipulate that the low diastatic value must not be the result of heating. Analyses of two such honeys ("orange-blossom" honey having a crystalline consistence, pale yellow colour and orange-blossom odour, and "belvedere" honey, crystalline, bright yellow, with the odour of alfalfa or lucerne honey) gave, respectively:—Water, 16·75 and 11·50; invert sugar, 72·84 and 76·03; sucrose, 1·07 and 3·90; glucose, 35·65 and 38·08; fructose, 37·19 and 37·95; ash, 0·04 and 0·052 (percentages); acidity, 1·2 and 0·84 c.c. of 0·1 *N* sodium hydroxide solution per 100 grms.; diastase value, 2·5 and 1·0. The end-point in the above diastase test may be sensitised by addition to the tubes concerned of a little aluminium hydroxide, precipitated from alum by ammonia and washed. This adsorbs any blue colour, which may thus be distinguished from the purple of the iododextrins remaining in solution (*cf.* preceding abstract). J. G.

Determination of the Acidity of Red Wines by means of Fluorescent Indicators. Y. Volmar and S. M. Clavera. (*J. Pharm. Chim.*, 1931, **13**, 561–568.)—Titration of red wines in presence of litmus or phenolphthalein gives high values for the acidity. By the use of the fluorescent indicator, umbelliferone, results agreeing closely with the theoretical acidity, as determined by potentiometric titration, are, however, obtainable. Five c.c. of the wine, freed from carbon dioxide by agitation in a vacuum, are diluted to 100 c.c., and the solution transferred to a thin glass or, preferably, Pyrex Erlenmeyer flask, and treated with from 2 to 5 drops of alcoholic umbelliferone solution of 1:100,000 concentration.

The flask is placed in a dark room, in the path of the rays from a silica mercury-vapour lamp of Westinghouse type, working at 110 volts. This lamp is enclosed hermetically in a box furnished with a window of Wood's nickel oxide glass, which allows of the passage of only ultra-violet light of wave-length above 0·35μ. While exposed to this light, the liquid is titrated with 0·1 or 0·05 N sodium hydroxide until it just exhibits blue fluorescence. If acridine is used in place of umbelliferone, the initial green fluorescence changes suddenly to indigo-blue on titration, at pH 4·9, so that this indicator gives correct results only if a suitably-adjusted solution is used for comparison purposes. These indicators are useless in the determination of the acidity of beer, owing to the excessive intensity of the fluorescence of this beverage under Wood's light. T. H. P.

Systematic Examination and Evaluation of the Kreis Reaction. K. Täufel. (*Chem. Ztg.*, 1931, **55**, 434.)—For normal fats the Kreis test provides a very sensitive indication of the beginning of the series of reactions producing rancidity, phloroglucinol being preferable as a reagent to resorcinol or naphthoresorcinol. The diethyl and glycol acetals of the unstable epihydrinaldehyde gave reactions in solutions corresponding with 0·5γ of aldehyde per c.c. This aldehyde, which has been suggested as an intermediate product of the rancidity reaction, has a slight stability in acid or neutral solution, but decomposes rapidly in an alkaline medium. The Kreis reaction may be used as a test for epihydrin aldehyde, but the interference of fatty substances should be eliminated by directing a jet of steam containing the vapour of the aldehyde against a wad of wool or lignin-free paper which has been soaked in a mixture of an ethereal solution of phloroglucinol and concentrated sulphuric acid. J. G.

Glyceride Structure of Butter-Fats. T. P. Hilditch and J. J. Sleightholme. (*Biochem. J.*, 1931, **25**, 507–522.)—Four English butter-fats from members of the same herd of cows fed on various diets and a New Zealand pasture-fed (December) butter-fat have been oxidised in order to determine their content of fully-saturated glycerides, and the fatty acids of the latter have been analysed in detail. The results obtained, with similar data for two other New Zealand butter-fats and an Indian cow ghee, are discussed with reference to the general glyceride structure of butter-fats (*cf.* ANALYST, 1931, 161). The six butters from cows on "normal" (usually pasture-fed) diets show many features in common, but in two cases in which specific fatty oils had been given to the cows the results stand apart in certain respects; all eight fats conform to the usual rule (in animal fats; *i.e.* beef and mutton tallows, lards, rabbit-fat) that the content of fully-saturated glycerides is a function of the proportion of the total saturated acids present in the mixed acids of the whole fat, irrespective of the nature of the saturated acids (the unsaturated acids are throughout almost constant in composition, *viz.* about 90 per cent. oleic and about 10 per cent. linoleic). In the "normal" butters, the component acids of the fully-saturated parts are present in approximately the same proportions, the composition of the fully-saturated

glycerides tending to be the same whatever the original unsaturation of the butter as a whole. Similar concordances are shown in the combined butyric-lauric acid contents, and in the combined myristic-palmitic acid contents of the whole fats and of their two divisions, fully- and non-fully-saturated glycerides, but the content of unsaturated acids in the non-fully-saturated glycerides increases steadily and slowly with increase of unsaturation in the fats as a whole. When coconut-fat (a relatively saturated fat) formed part of the diet, the fully-saturated part of the butter showed a marked increase in butyric-lauric acid content, but the composition of the non-fully-saturated glycerides was normal. With a soya bean (predominantly unsaturated) oil diet the structure of the butter glycerides was different; the fully-saturated components were almost normal in composition, but the lower acids of the non-fully-saturated portion were present in excess of the usual proportion. Study of the glyceride structure of these eight butters confirms the general rules which have been observed to connect the amount of fully-saturated glycerides with the degree of unsaturation of animal fats, but it also reveals influences due to diet more clearly than the consideration of the component fatty acids of the whole fats, and affords some additional evidence as to the mode of utilisation of ingested fat in milk-fat metabolism. P. H. P.

Detection of Riegel's "New Preservative for Pumpernickel." P. Weinstein, J. Muesmann and W. Bodschwinna. (*Z. Unters. Lebensm.*, 1931, **61**, 436–442.)—"Antibacterin" and "Mikrobin-P," which have been suggested as preservatives for bread, were identified as sodium *p*-toluene sulphochloramine (also known as chlorazene, miamine and chloramine-T), and benzoic acid containing 10 per cent. of chlorobenzoic acid, respectively. The former is detected in 400 grms. of crustless bread by reducing it to fine crumbs and heating it under a reflux condenser for 15 minutes with 500 c.c. of ether containing 2 c.c. of fuming hydrochloric acid. After filtration the residue is washed with 200 c.c. of ether, the total filtrates evaporated to 50 c.c., the extract transferred to a large dish, and the last traces of ether removed. Fat is then eliminated by extraction with hot 50 per cent. alcohol, and, after filtration on kieselguhr, the residue is washed with two 10 c.c. portions of hot alcohol, and the combined extracts evaporated. (Benzoic acid may separate in feathery masses at this stage.) The residue is extracted with 25 c.c. of warm ether, the mixture filtered into a 50 c.c. cylinder, and the residue washed with warm ether until the 50 c.c. mark is reached. The filtrate is then divided into 25 c.c. portions, which are evaporated, and the respective residues are used for the (Kjeldahl) nitrogen and sulphate determinations. In the latter case the residue is gently fused with a powdered mixture of 2 grms. of dry sodium carbonate and 1 grm. of sodium nitrate, the melt is extracted with water, 10 c.c. of fuming hydrochloric acid added, and the sulphate precipitated with barium chloride. The Mikrobin-P is detected in the ethereal extract by the usual method. Blank tests on preservative-free bread gave no barium sulphate and 7 mgrms. of nitrogen by the method described. Experiments in which known amounts of the preservatives were added to the unbaked meal and the final loaf

analysed led to the conclusion that more than 15 mgrms. of barium sulphate and 10 mgrms. of nitrogen per kilo. in the test indicate the addition of antibacterin.
J. G.

Trigonelline Content of Coffee. F. E. Nottbohm and F. Mayer. (*Z. Unters. Lebensm.*, 1931, **61**, 429–435.)—Determinations of trigonelline by the authors' method (ANALYST, 1931, **56**, 405) gave values between 0·228 and 0·245 per cent. for all ordinary trade coffees, whilst in certain "wild" African coffees only 0·1 to 0·2 per cent. was found, such values being associated with a relatively high caffeine content (1·2 to 2·2 per cent.). Surinam-Liberian coffee gave the lowest value obtained (0·11 per cent.). In such cases the lead precipitate of the alcoholic extract (*loc. cit.*) was pure yellow, whilst for normal coffees it is orange-red. There is no evidence of loss or decomposition of trigonelline after roasting at 250° C., but the precipitate with iodine and potassium iodide (*loc. cit.*) is oily in character, probably on account of the presence of basic nitrogenous decomposition products produced by heat. The "alkaloid value," given by the ratio caffeine : trigonelline content is constant (4·3 to 5·5) for all ordinary coffees, but is high (9·7 to 13·7) for wild (*e.g.* Liberian) coffees; treated coffees contain the normal quantities of trigonelline. Aqueous infusions of coffee contain only about 82 per cent. of the total caffeine and 70 per cent. of the trigonelline, so that in such cases it is necessary, if possible, to determine these alkaloids both in the extract and in the residual grounds. The double platinum salt of trigonelline $(C_7H_7NO_2, HCl)PtCl_4$ exists in only one form (*cf.* the gold salt, *loc. cit.*), *viz.* in anhydrous dense orange-red prisms, m.pt. 215° C.
J. G.

Colloidal Iodine Preparations. S. J. Hopkins. (*Pharm. J.*, 1931, **126**, 538.)—Some commercial colloidal iodine preparations, paler in colour than usual, were found to be acid to litmus and to give no colour with starch mucilage, and no colour could be removed by chloroform or carbon tetrachloride, but addition of chlorine water was followed by liberation of iodine. The average composition was: Iodine (in combination), 0·21; sodium chloride, 0·74; gelatin, 0·35; and water, 98·70 per cent.; and the acidity (as hydriodic acid) agreed with the amount of iodine present. It was found that when finely divided iodine was heated with a solution of 0·74 per cent. of sodium chloride and 0·35 per cent. of gelatin under pressure in an autoclave, combination occurred, with complete loss of colour and production of acid, so that in these preparations the iodine was not in the colloidal state, nor was the gelatin a protective colloid.
D. G. H.

Biochemical.

Effect of Desiccation upon Nutritive Properties of Egg-White. II. M. A. Boas Fixsen. (*Biochem. J.*, 1931, **25**, 596–605.)—It was previously shown by Boas (*Biochem. J.*, 1927, **21**, 712) that dried egg-white, when used as the sole source of protein in a diet for young rats, produces a train of characteristic symptoms—notably dermatitis, baldness and spastic gait terminating in death in from 4 to 6 weeks, and that this syndrome could be completely prevented if certain substances

such as yeast, potato, fresh egg-white and others were added to the diet, and the presence of an unknown protective dietary factor X in these substances was postulated. It was suggested (*a*) that dried egg-white is lacking in some essential dietary factor which is supplied by protective factor X, or (*b*) that a toxic substance is formed in dried egg-white as a result of desiccation, and that this is neutralised in some way by protective factor X. As there is no prospect at present of continuing this work, which has been carried a stage further but is still incomplete, the results so far obtained are now published. Some of the different constituents of egg-white, fresh or dried, were investigated as the source of protein both by curative and by preventive tests. It is shown that pure ovalbumin, prepared by crystallisation from crude egg-white, forms a satisfactory protein for young rats. The same is true of ovoglobulin and of the total albumin and ovomucoid fraction. Desiccation does not alter the nutritive properties of these materials as is the case with crude egg-white. Therefore, the alteration in nutritive value observed when egg-white is dried is probably due, not to the loss of some essential factor, but to the formation of a toxic substance. The evidence suggests that the toxic substance is formed from some non-protein constituent of the egg-white. A protein fraction uncontaminated by this toxic substance can be prepared from dried egg-white. The protective factor X, capable of neutralising the toxic substance in dried egg-white, is present in raw liver, and also in a dried preparation of the total albumin and ovomucoid fraction of egg-white, but not in raw beefsteak. It is only partly extracted from yeast by boiling dilute acetic acid. It is not removed from caseinogen by washing with dilute acetic acid and extraction with alcohol. Rats suffering from the effects of the dried egg-white can become spontaneously refected in a similar manner to that observed by Fridericia and his colleagues (*J. Hyg.*, 1927, **27**, 70), and by Roscoe (*J. Hyg.*, 1927, **27**, 103) in the case of rats fed upon diets devoid of water-soluble *B* vitamins. This condition can be transmitted from one rat to another by ingestion of the faeces (bulky white faeces containing undigested starch). Refection can be induced in rats receiving the dried egg-white diet, by feeding them with the bulky white faeces of refected rats on diets deprived of *B* vitamins. This suggests that the agency is the same in both cases. The facts afford little support to the idea of Findlay and Stern (*Arch. Dis. Childhood*, 1929, **4**, 104) that the egg-white syndrome is identical with "pink disease" (Swift's disease) in children. P. H. P.

Effect of High-Pressure Hardening of Oils for the Margarine Industry. J. A. Van Dijk, R. T. A. Mees and H. I. Waterman. (*Chem. Weekblad.*, 1931, **28**, 319–320.)—It has been shown that it is possible to harden fatty oils by using a nickel catalyst at temperatures of 30 to 60° C. if hydrogen at very high pressure is used, and that with such low temperatures there is much less loss of important constituents. The carotene in palm oil hardened in this way is not destroyed, and cod-liver oil will still show the blue coloration in Carr and Price's reaction. It seems probable that the activity of vitamins may thus be retained in hardened oils, and biological tests are to be made with oils hardened at these low temperatures.

D. G. H.

Characterisation of Vitamin *A*. Part I, Spectroscopic Evidence. I. M. Heilbron and R. A. Morton. Part II, Biological Experiments. B. Ahmad and J. C. Drummond. (*J. Soc. Chem. Ind.*, 1931, **50**, 183T–186T.)—It has already been shown with liver oils and concentrates that quantitative determination of the intensity of absorption at 328$m\mu$ (maximum) provides a criterion of vitamin *A* potency, and that the blue colour obtained with the antimony trichloride test for vitamin *A* is complex in nature, being the resultant of two absorption bands with maxima at 572$m\mu$ and 606$m\mu$ in fish-liver oils (displaced to 583$m\mu$ and 620$m\mu$ in concentrates). With the idea of establishing definitely if the chromogenic property of liver oils is in the same molecule as the entity giving rise to the 328$m\mu$ maximum, the physical measurements have been extended to embrace as wide a range of liver oils and concentrates as possible. Various discrepancies have been disclosed. The most striking anomaly, from the point of view of the colour test, was the occasional appearance (in relatively fresh oils) of the 572$m\mu$ maximum at an intensity great enough to mask the 606$m\mu$ band almost completely. Re-examination of these oils after several months disclosed a remarkable spontaneous increase in the intensity of the 606$m\mu$ band, so that the oils appeared normal. In spite of this striking change, which could be induced artificially, neither the ultra-violet maximum nor the 572$m\mu$ colour band had undergone significant alteration. The authors had already concluded that a closer correlation, both in oils and concentrates, existed between the 328$m\mu$ maximum and the absorption at 572$m\mu$ (583$m\mu$ in concentrates) than between the former and the 606$m\mu$ (620$m\mu$ in concentrates) band, in spite of the customary predominance of this longer wave-length maximum. The experiments on induced or spontaneous oxidation, concerned as they are with very extreme deviations, have led definitely to the view that, whilst the 328$m\mu$ band and the 572$m\mu$ chromogen cannot be differentiated, the 606$m\mu$ chromogen must be a distinct and separate entity, which, although not vitamin *A*, cannot be rejected as unrelated, and may well provide an indication of vitamin potency which is approximately valid in practice though not in principle. There is probably a fairly close connection between the two chromogens. It is suggested that the most promising criteria for vitamin *A* will be a colourless, or almost colourless, substance, exhibiting (*a*) an absorption band without fine structure and with a maximum at 320–330$m\mu$, and (*b*) giving with antimony trichloride a sharp absorption band at about 583$m\mu$, the intensity of absorption for both bands being approximately the same. The reduction product of carotene prepared by Smith (*J. Biol. Chem.*, 1931, **90**, 597), and claimed to be "dihydrocarotene," has been prepared and tested. It approaches in its characteristics these criteria for vitamin *A*, but fails to satisfy them completely, and is not a pure single substance. When it was tested biologically on rats negative results were generally obtained, although one or two cases of irregular growth occurred. These results are not in agreement with those of Karrer, Euler, Hellström and Rydbom (*Svensk. Kem. Tidskr.*, **1931**, **43**, 105). Possibly only a small proportion of the "dihydrocarotene" preparation is physiologically active, and the irregularities are due to differences

in the amount absorbed. An investigation of the absorption of carotene from the mammalian intestinal tract and of the mechanism of the conversion of the pigment into vitamin *A* in the tissues is in progress. The results so far obtained are given. It is noted that the addition of fat to the diet improves absorption considerably. The experiments suggest that a large proportion of the carotene absorbed into the organism may be rapidly transformed into other substances, but that the formation of vitamin *A* is relatively a slow process, possibly concerning quite a small proportion of the assimilated pigment. It is highly probable that the breakdown of the complex, unsaturated molecule (carotene) in the organism would follow several paths; reduction might be one of the changes involved, but it must be remembered that there are grounds for the belief that vitamin *A* contains hydroxyl groups.

P. H. P.

Critical Study of the Antimony Trichloride Colour Test for Vitamin *A*. W. R. Brode and M. A. Magill. (*J. Biol. Chem.*, 1931, **92**, 87–98.)—There is much difference of opinion with regard to the specificity of the antimony trichloride colour reaction for vitamin *A*; a systematic study has now been made of the absorption spectra of the solutions with the use of a Bausch and Lomb spectrophotometer to which a modified Duboscq colorimeter was attached. This spectrophotometer permitted either a qualitative examination over a wide portion of the spectrum to determine the position of the band, or a quantitative determination, within a narrow portion of the spectrum, of its intensity. It has been shown that the antimony tricyloride colour solutions in the test for vitamin *A* may have two different absorption bands, one at 578$m\mu$ and the other at 608$m\mu$. Both of these bands fade and the respective solutions develop new bands at 472 and 532$m\mu$. Conditions of concentration have been determined for a few commercial samples of cod-liver oil by which only one of the two bands is produced (608$m\mu$). A saturated solution of antimony trichloride in anhydrous chloroform gave the best results. Under the conditions described cod-liver oils yield extinction coefficient values of the 608$m\mu$ band which are proportional to the concentration of the oil. The procedure adopted for the analysis of oils is as follows:—Five c.c. of saturated antimony trichloride solution are placed in a spectrophotometric cell with 1 drop of acetic anhydride (to react with hydrochloric acid, water, etc., present). Then 0·5 c.c. of a chloroform solution of the oil to be tested is added to form a layer on top of the reagent, the cell is shaken, and the intensity of the 608$m\mu$ band is observed exactly 20 seconds after mixing. A reading is made at 578$m\mu$, to note if there is a band there; if so, the oil is too concentrated, and a more dilute solution should be used. As a check, another solution of oil of a different concentration is made and observed. The readings of the 608$m\mu$ band should be proportional to the amount of oil used. A 10 per cent. solution of oil should be tried first. If the extinction coefficient of the 608$m\mu$ band for 2 cm. cell thickness is less than 1·50 to 1·75, the check solution may be 20 per cent.; if greater than 1·75, but less than 2·00 to 2·50, 15 per cent.; if greater than 2·00 to 2·50 the check solution should be 7 or 8 per cent. There appears to be a definite relation between the extinction coefficient values of the blue solution (608 and 578$m\mu$ bands), and the faded

or red solutions (532 and 472$m\mu$ bands). Results will be published in a subsequent paper on the comparisons of results obtained by this method with those obtained by biological assay. P. H. P.

Photographic Effects of Vitamins *A* and *B*. S. Botcharsky and A. Foehringer. (*Nature*, **1931**, **127**, 856.)—These two vitamins are capable of affecting photographic plates, the effects produced being similar in character, in spite of the different origins of the vitamins. An ethereal extract of dried ox-liver, subsequently freed from solvent in an atmosphere of nitrogen, was used as the source of vitamin *A*, and an aqueous extract of purified brewery yeast as that of vitamin *B*. The photographic plates were covered with aluminium foil and letters were cut out of the foil covering the glass side. Letters were then painted on the same side with the vitamin extracts, which had been previously tested biologically. After being left for 3 days wrapped in black paper, the plates were developed, clear images of the letters being obtained. Similar images are formed by the vitamins sealed in separate glass tubes. If the vitamins are destroyed, but not carbonised, no effect is produced on the plates. T. H. P.

Isolation of the Antineuritic Vitamin. A. Seidell and V. Birckner. (*J. Amer. Chem. Soc.*, **1931**, **53**, 2288–2295.)—The preparation of the "activated solid" is the first step in the process of production of the vitamin itself, and for this purpose 160 kilos. of pressed brewer's yeast (equivalent to about 45 kilos. of dried yeast) are quickly added to about 300 litres of rapidly stirred water at 80° C. After slight cooling, the mixture is filtered and 15 kilos. of fuller's earth are added, after which the whole is stirred for 1 hour and left for 2 hours for subsidence of the activated fuller's earth. The supernatant liquid is decanted, and the "activated solid" washed with slightly acidified water (1 c.c. of concentrated hydrochloric acid per litre). The extraction and concentration are effected by vigorously agitating the activated solid in 0·4 to 0·5 *N* sodium hydroxide solution for 5 minutes, and separating the alkaline liquid as quickly as possible by means of a Sharples super-centrifuge. The extract is quickly acidified with sulphuric acid and adjusted to a *p*H of 3·0, this being indicated by the separation of a light fluffy precipitate. The slightly acid extract is distilled under reduced pressure to about a tenth of its volume, and the brownish material which separates is removed by deposition in a large cup centrifuge. The nearly clear supernatant solution is seeded with sodium sulphate decahydrate and kept cool for crystallisation of the large excess of the salt. The brown material is very rich in the thermo-stable growth factor (B_2 or G) required as a supplement to the anti-neuritic vitamin, and, when dried, is about five times as active as yeast. The solution from which the excess of sodium sulphate crystallises is treated with about an equal volume of methyl or ethyl alcohol to precipitate more of the inorganic salts and organic impurities present. By sacrificing some of the vitamin, much material interfering with subsequent purification may be eliminated at this stage. The clear, approximately 50 per cent. solution, usually contains about 30 grms. of dissolved solids per litre, of which about 30 per cent. are inorganic salts. The alcoholic solution is distilled

down in separate portions and benzoylated, and the vitamin salts are extracted by rotating for 24 hours with a mixture of 3 volumes of normal propyl alcohol and 1 volume of concentrated hydrochloric acid, using about 3 c.c. of this mixture for 1·0 grm. of salts. This extract is separated by centrifuging and the residue extracted again. The two extracts are then distilled under reduced pressure to about 50 c.c., the liquid centrifuged, and the clear solution added, drop by drop, to about 1600 c.c. of acetone. The resulting precipitate is separated by centrifuging, dissolved in about 15 c.c. of methyl alcohol, and the solution added, drop by drop, to about 800 c.c. of acetone. The white flaky precipitate is centrifuged, washed with acetone, and dried in a vacuum. The activities of 16 precipitates obtained in this way were tested by M. I. Smith's rat method (*Public Health Reports*, **1930, 15, 116**), and the most active was curative in 0·03 mgrm. doses, *i.e.* an activity about one-fourth greater than that of the Jansen and Donath crystals. Apparently, small variations of conditions at certain stages, such as the proportion of sodium carbonate used in the benzoylation and differences of temperature, greatly affect the quantity and quality of the final product. D. G. H.

Vitamin Studies. III, Vitamin Content of Fruits. F. V. v. Hahn. (*Z. Unters. Lebensm.*, 1931, **61**, 369–411.)—Growth experiments with guinea pigs (full details of which, including weight-curves, are given in the original) indicate that the 40 types of fruit examined may be classified according to their vitamin-*C* contents as follows:—*Very rich :* strawberry, orange, lemon; *rich :* gooseberry, raspberry, hips (dried and cooked); *vitamin-containing :* red currant, mandarin; *poor in vitamin :* freshly picked apple, morella cherry, greengage, plum (various kinds), peach, banana, pineapple, cooked elderberry, white-hearted cherry; *almost free from vitamin :* whortleberry, cranberry (cooked), stored apple, pear, quince (raw and cooked), grape, dried fruits (*e.g.* date, currant, fig, etc.). The minimum weight of fruit to prevent symptoms of scurvy in man is given as 50 times that required by guinea-pigs. J. G.

Effects of Overdosage of Vitamin *D*. II. R. F. Light, G. E. Miller and C. N. Frey. (*J. Biol. Chem.*, 1931, **92**, 47–52.)—In a study of the effect of low and high vitamin *D* overdosage on the reproduction of white rats the authors had available a number of animals of the third and fourth generation, some of the progenitors of which had received 40 units and others 2500 units of vitamin *D* as supplements to the basal diets. It was decided to use some of these animals in a study of the ash content of bones and of organs which had been found to be particularly susceptible to calcification, namely, heart and kidneys. The animals were examined in four groups. The results show that moderate overdosage (40 units) of vitamin *D* daily has no effect on the mineral metabolism of white rats when continued through the third and fourth generations. No pathological calcification occurs, and the ash of the bones is normal. Animals receiving a moderate overdosage of vitamin *D* for a long period of time are more susceptible to excessive overdosage than normal animals. A large overdosage (2500 units) of vitamin *D* just insufficient to produce toxic symptoms in the first and second generations, given for a long

period of time, does produce striking pathological changes in the third and fourth generations. These changes are (*a*) decalcification of the bones, (*b*) severe calcification of the kidneys, and (*c*) certain pellagra-like symptoms, namely, scabby conditions of the feet, nose and forequarters. P. H. P.

Organic Analysis.

Detection and Separation of Hydrocarbons with Branched Chains from Natural or Artificial Mixtures of Hydrocarbons. A. Schaarschmidt. (*Chem. Ztg.*, 1931, **55**, 424.)—The olefines and aromatic compounds present in hydrocarbon mixtures are easily determined and separated, and in the residual mixture of saturated aliphatic and alicyclic hydrocarbons, the latter may be determined with moderate accuracy, although not isolated, by means of the aniline point, in accordance with Tizard and Marshall's method (ANALYST, 1921, **46**, 155). It is now found that, with the more simple mixtures obtained from natural or synthetic hydrocarbons, treatment with a metallic halide with a mobile halogen atom, such as antimony pentachloride, renders it possible to detect and separate saturated aliphatic and alicyclic hydrocarbons containing trebly-linked carbon atoms; the complex compounds formed are insoluble in excess of the hydrocarbon mixture, and form characteristic greenish-blue or violet products with water or alcohol. Normal hydrocarbons and those containing quaternary-linked carbon atoms remain behind. This method has been applied to different fractions obtained from synthetic and natural hydrocarbons, it being found, for example, that benzine fractions with b.pt. about 100° C. consist mostly of aliphatic and alicyclic hydrocarbons with branched chains. T. H. P.

New Acid in the Oil of *Conepia grandiflora*, Benth. F. Wilborn. (*Chem. Ztg.*, 1931, **55**, 434.)—An oil, with n_D^{21} 1·5094, has been extracted from the seeds of *Conepia grandiflora* (*Rosaceae*); it has similar properties to Chinese wood oil, and contains a highly unsaturated acid, probably isomeric with elaeostearic acid, having neutralisation value, 200·3; m.pt., 94° C.; n_D^{96}, 1·4865; and molecular weight (Rast), 267. J. G.

Identification of Mesaconic Acid. H. H. Mottern and G. L. Keenan. (*J. Amer. Chem. Soc.*, 1931, **53**, 2347–2349.)—Mesaconic acid (a natural constituent of plants), its hydrazide, and *p*-nitro-benzyl mesaconate are crystalline bodies readily studied by the optical immersion method. The pure acid prepared by the method of Fittig (*Annalen*, 1877, **188**, 73) had a melting point of 204·5° C. (corr.). When sublimed it yields colourless rod-like forms in laminated fibrous masses. With crossed nicols the rods show parallel extinction, and the elongation is + or −; the double refraction is very strong, and the index of refraction (immersion method) is less than $n = 1{\cdot}445$. The acid is soluble in oily mixtures; the maximum refractive index was 1·740 (methylene iodide), but this was infrequent; the most significant refractive index is shown when the elongated crystals are oriented with their long axis parallel to the vibration plane of the lower nicol, when the rods

frequently match a liquid with $n = 1{\cdot}690$ (conveniently monochloronaphthalene and methylene iodide). Diethyl mesaconate was prepared by heating the acid under reflux for 18 hours with 300 c.c. of absolute alcohol, dissolving the ester in ether, and washing with dilute sodium hydroxide solution, drying with anhydrous sodium sulphate and distilling off the ether under reduced pressure. The diethyl mesaconate boiled at 93–95° C., under 10 mm. pressure. Mesaconic hydrazide was made from the ester by adding 1 c.c. of 42 per cent. hydrazine hydrate in water to a solution of 0·7 grm. of the ester in 5 c.c. of absolute alcohol and, after standing, crystallising the hydrazide; the purified crystals melted at 217–218° C. with decomposition. The *p*-nitrobenzyl mesaconate prepared by the method of Reid (*J. Amer. Chem. Soc.*, 1917, **39**, 124) had a melting point of 134° C. Optical crystallographic data are given for both these derivatives. D. G. H.

Iodine Mercerisation Test. W. F. A. Ermen. (*J. Soc. Dyers and Col.*, 1931, **47**, 161.)—Mercerised and unmercerised samples of cotton fabrics are immersed in a solution of six grms. of iodine in 100 c.c. of potassium iodide solution. They are washed until the unmercerised sample is colourless and are then dropped into a boiling solution of Indigosol Black 1B. The mercerised sample is deeply dyed, the unmercerised emerging colourless. The samples are now washed, first in cold water, and finally in boiling soap solution. The Indigosol requires to be freshly prepared every time. In the case of an unknown sample, starch should be tested for, and, if present, removed either by boiling with dilute acid or by treatment with malt extract. Comparative tests must be made with cloths of similar substance. R. F. I.

Inorganic Analysis.

Volumetric Determination of Carbon Monoxide by means of a Suspension of Iodine Pentoxide in Fuming Sulphuric Acid. H. A. J. Pieters. (*Chem. Weekblad*, 1931, **28**, 335–337.)—The gas is measured in a water-jacketed (35 c.c.) burette, and carbon dioxide then removed in an absorption vessel containing 40 per cent. potassium hydroxide solution. The residual gas is passed into a 10 per cent. suspension of iodine pentoxide in fuming sulphuric acid (10 per cent. SO_3), contained in a double absorption pipette, the outlet from which leads through a wash-bottle containing sulphuric acid to the air. The gas is then returned to the absorption vessel, when the carbon dioxide formed by oxidation of the carbon monoxide is absorbed in the alkali, the absorption being measured manometrically. The reaction period varies with the carbon monoxide content (10, 15 and 20 minutes for less than 0·06, 2·8 and 5·2 per cent. by volume, respectively), but the end of the reaction is indicated when no further absorption of carbon dioxide occurs. An accuracy of 0·02 per cent. was obtained with mixtures of known composition and as compared with results obtained by the ammoniacal cuprous chloride reagent, and the method is unaffected by the presence of hydrogen or methane, both of which, however, are slowly oxidised by a reagent containing 25 per cent. SO_3. J. G.

Volumetric Determination of Copper based on Spacu's Reaction. L. Cuny. (*J. Pharm. Chim.*, 1931, **13**, 513–518.) J. Golse (ANALYST, 1931, 272) published criticisms of Cuny's original method (*J. Pharm. Chim.*, 1924, **30**, 240), and proposed a modified process. Cuny now agrees with practically all Golse's findings, and states that the grossest fault of his original method lay in the use of the empirical and inaccurate permanganate titration method for determining the excess of thiocyanate remaining in the solution after the precipitation of the copper as copper pyridine thiocyanate. S. G. C.

Determination of Lead and Copper in Bordeaux and Lead Arsenate Mixtures. J. C. Bubb. (*J. Assoc. Off. Agric. Chem.*, 1931, **24**, 260–263.)—As an equally accurate alternative to the more lengthy official method of the A.O.A.C. for the determination of lead oxide and copper, the following method is proposed: A mixture of 1 grm. of the sample with 50 c.c. of "acetic acid solution (1 + 2)," contained in a 250 c.c. beaker, is heated on a steam-bath for 5 to 10 minutes; 0·5 grm. of calcium arsenate is added to convert any tri-lead arsenate, $Pb_3(AsO_4)_2$, or other lead compound, into the acid arsenate, $PbHAsO_4$, followed by nitric acid, drop by drop, with stirring, until any blue coloration in the insoluble residue clears up and the residue of lead arsenate is white (up to 1·5 c.c. of nitric acid is required, and the amount used is noted). The mixture is cooled in a water-bath, ammonia is added in amount exactly equivalent to the nitric acid added; the mixture is kept at room temperature for 20 minutes, and then filtered (No. 44 Whatman paper), the insoluble residue being washed with small portions of hot water. The residue is dissolved by heating in dilute nitric acid (20 per cent.) the lead determined in the solution by the official chromate method of the A.O.A.C. (*Methods of Analysis*, 1925, 58), and the result calculated to PbO. The copper in the filtrate is determined by adding potassium iodide and titrating with thiosulphate in the usual manner. S. G. C.

Study of Travers' Method for the Determination of Fluorine with Reference to Insecticides. C. M. Smith, E. H. Hamilton, and J. J. C. Graham. (*J. Assoc. Off. Agric. Chem.*, 1931, **24**, 253–260.)—The following modified form of Travers' method is proposed for the analysis of insecticides containing fluorides: To 0·5 grm. of the sample, contained in a small beaker, from 20 to 25 c.c. of water, 0·3 grm. of finely divided *precipitated* silica and a few drops of methyl orange indicator are added. Concentrated hydrochloric acid is added, drop by drop, to the mixture until the indicator changes to pink, followed by 2 c.c. of the acid in excess; the liquid is boiled for 1 minute and then cooled. Four grms. of solid potassium chloride are dissolved in the liquid, 25 c.c. of ethyl alcohol (96 per cent.) are added, and the whole kept for 1 hour with frequent stirring. The solution is filtered through a Gooch crucible with an asbestos filtering-pad (fritted glass will not do), and the precipitate washed with an alcoholic solution of potassium chloride [60 grms. of potassium chloride dissolved in 400 c.c. of water with 400 c.c. of ethyl alcohol (96 per cent.) added; this solution should be made neutral to phenolphthalein] until the washings are practically neutral to

phenolphthalein (three or four washings are usually sufficient). The crucible and contents are placed in a 400 c.c. beaker together with 100 c.c. of freshly boiled water and 1 or 2 c.c. of phenolphthalein solution (1 per cent.); the liquid is heated and titrated with standard sodium hydroxide solution (0·2 *N*; freedom from carbonate specified; 1 c.c.=0·0057 grm. of fluorine), the titration being finished with the liquid actively boiling. The method is stated to be satisfactory for, *e.g.* chicken-lice and cockroach powders, which consist of commercial sodium fluoride containing as impurities sodium carbonate, sodium sulphate, sodium bifluoride, and sodium silicofluoride. Most fluoride insecticides are mixtures of fluorine compounds either with other insecticidal materials or with diluents. The effect on the method, of a number of different substances was tried. The following (about equal in amount to the fluoride taken) gave no interference: lime, sulphur, starch, flour, tobacco, talc, *p*-dichlorobenzene, naphthalene, cresols, coal-tar neutral oils, Paris green and calcium arsenate. Pyrethrum and lead arsenate caused a tendency to high results. Slightly low results were obtained with added kaolin, diatomaceous earth and ferric chloride. The process fails in presence of soluble aluminium or boron compounds, very low results being obtained. S. G. C.

Electrometric Titration of Uranium by means of Ceric Sulphate. D. T. Ewing and M. Wilson. (*J. Amer. Chem. Soc.*, 1931, **53**, 2105–2110.)—The hot sulphate solutions in 2 per cent. sulphuric acid were passed through a Jones reductor, collected in an atmosphere of nitrogen, and titrated electrometrically with ceric sulphate. Two distinct end-points were obtained: (1) Trivalent to quadrivalent, and (2) quadrivalent to hexavalent uranium. If ferrous salt is also present, its oxidation to ferric salt follows the complete oxidation of the uranium, and a third end-point is observed. W. R. S.

Colorimetric Determination of Sodium. R. A. McCance and H. L. Shipp. (*Biochem. J.*, 1931, **25**, 449–456.)—A method is described by which 0·02–0·8 mgrm. of sodium may be directly determined; in the absence of phosphates the range is 0·01–0·4 mgrm. of sodium. The free acids and phosphates are removed with zinc acetate and hydroxide in 50 per cent. alcohol, then the sodium is precipitated as sodium uranyl zinc acetate, and the uranium in the precipitate is determined colorimetrically with potassium ferrocyanide. The method is applicable to neutral or acid solutions. Calcium, magnesium and iron do not interfere; phosphates interfere, but are removed. Sodium may be determined directly in the presence of 30 times its weight of potassium. The following reagents are necessary:—(1) *Alcoholic zinc acetate with zinc hydroxide.*—A slight excess of ammonia (sp. gr. 0·880) is added to a hot strong solution of A.R. zinc sulphate; this is filtered on a Buchner funnel, washed with hot water, and sucked dry. The zinc hydroxide paste formed is added in small amounts at a time, until in slight excess, to 12·5 c.c. of glacial acetic acid, filtered, washed, the combined filtrate and washings made up to 100 c.c., then 3 c.c. of ammonia and 300 c.c. of 95 per cent. alcohol are added. (2) *Alcoholic uranyl zinc acetate reagent.*—(*a*) In 50 c.c. of

boiling water containing 2·0 c.c. of glacial acetic acid 10 grms. of uranyl acetate are dissolved. (*b*) In 50 c.c. of boiling water containing 1 c.c. of glacial acetic acid 30 grms. of zinc acetate are dissolved. Both solutions are mixed while boiling, brought to boiling point, left overnight, then filtered. The filtrate is mixed with an equal volume of absolute alcohol, left for 48 hours at 0° C., and filtered at 0° C. The reagent is stable at room temperature. (3) *Ninety-five per cent. alcohol saturated with the triple acetate.*—Uranyl zinc acetate reagent is added to sodium chloride dissolved in 50 per cent. alcohol, filtered or centrifuged, and the precipitate of sodium uranyl zinc acetate washed with 95 per cent. alcohol. The precipitate is suspended in 95 per cent. alcohol and left to settle in the ice-chest. The fluid is used for washing the precipitate. It must be filtered before use if not absolutely clear. (4) *Twenty per cent. potassium ferrocyanide.* (5) *Standard sodium chloride.*—The stock solution contains 1 grm. of pure dry sodium chloride in 100 c.c. of water. For use, 2 c.c. are diluted to 100 c.c.; thus 1 c.c. contains 0·2 mgrm. of sodium chloride (0·0786 mgrm. of sodium). (6) *Standard triple acetate.*—To 10 c.c. of 1 per cent. sodium chloride solution, 80 c.c. of water, 100 c.c. of alcohol and 100–120 c.c. of reagent (2) are added, and left for 1 hour; the precipitate is collected, washed with ice-cold 95 per cent. alcohol, dissolved in water, and made up to 1000 c.c. to form the stock solution. A portion is diluted accurately 1 : 5. To 5 c.c. of this solution, diluted with water in a 25 c.c. flask, 1 drop of glacial acetic acid and 0·5 c.c. of reagent (4) are added, and the whole made up to 25 c.c. The resulting colour is close to that obtained from 0·2 mgrm. of sodium chloride (0·0786 mgrm. of sodium) in 2 c.c. of water submitted to all stages of the method and made up to 25 c.c. This triple acetate solution must be accurately standardised against sodium solutions. Four samples of exactly 0·2 mgrm. of sodium chloride in 2 c.c. of water are subjected to all stages of the determination, and the precipitates transferred to 25 c.c. flasks. In 2 other flasks 5 c.c. of the dilute standard are placed, and water is added to 18–20 c.c. The uranium colour is developed in all six flasks. Both standards are matched against each of the quadruplicate flasks, setting the latter at 20 mm.; the mean is taken. If this should be 23 mm., it is best always to set the standard at 23 mm. in the colorimeter. (This is equivalent to the colour obtained by determination from 0·0786 mgrm. of sodium, the precipitate being made up to 25 c.c., and the colorimeter set at 20 mm.); or, if preferred, the stock solution may be diluted so that 5 or 10 c.c. of the weak standard diluted to 25 c.c. gives exactly the same colour intensity as that obtained from 0·0786 mgrms. of sodium. *Procedure.*—An amount of the unknown solution containing 0·04 to 0·16 mgrm. of sodium is placed in a centrifuge tube, diluted to 2 c.c. with water, 4 c.c. of reagent (1) added, stirred, and covered with a rubber cap (10 c.c. vaccine cap), then left 2–3 hours at room temperature, and at 0° C. overnight. It is centrifuged while cold, and 3 c.c. of the supernatant liquid are placed in another centrifuge tube, 4 c.c. of reagent (2) are added, and the whole is stirred with a glass rod until the precipitate begins to appear. It is covered with a rubber cap and left for 1 hour at 0° C., centrifuged, the liquid poured off, and the tube drained by inverting on filter-paper. The mouth of the

tube is wiped, and the inside and contents are washed once with 5 c.c. of the ice-cold alcohol saturated with the precipitate. The precipitate should be stirred up, centrifuged, and again drained. It is dissolved in water, and transferred to a 25 c.c. volumetric flask (unless too bulky, when a larger flask is necessary). For the standard either 1 c.c. of the dilute sodium chloride solution is treated in the same way as the unknown and transferred to a 25 c.c. flask, or 5 c.c. of the dilute standard triple acetate solution are taken in a 25 c.c. flask. To both standard and unknown, 1 drop of glacial acetic acid and 0·5 c.c. of reagent (4) are added, the solutions are made up to the mark with water, left for 3 minutes, and matched. *Calculation.* (*a*) *With standard sodium chloride solution.*—Sodium, mgrm. per 100 c.c.

$$= \frac{20\ (\text{standard colorimeter reading})}{\text{Reading of unknown}} \times 0{\cdot}0786 \times \frac{100}{\text{Volume of unknown taken}}.$$

(*b*) *With standardised triple acetate solution.*—Suppose the colorimeter set at 23 mm. is equivalent to 0·2 mgrm. of sodium chloride submitted to all stages of the determination made up to 25 c.c. and set in the colorimeter at 20 mm. Then, sodium, mgrm. per 100 c.c.

$$= \frac{20}{\text{Unknown}} \times 0{\cdot}0786 \times \frac{100}{\text{Volume of unknown taken}}.$$

A communication will shortly be made on the application of the method to blood and serum without incineration. P. H. P.

Determination of Traces of Chloride in Bromides. I. E. Orlow. (*Z. anal. Chem.*, 1931, **84**, 185–189.)—A solution of 5 grms. of bromide is treated with 50 c.c. of 20 per cent. sulphuric acid and 3 grms. of freshly-precipitated dried manganese peroxide. The mixture is diluted to 150 c.c. and boiled for 30 minutes; if the volume is thereby reduced to less than 50 c.c., hot distilled water is to be added. The liquid should no longer smell of bromine, otherwise it is boiled for another 10 to 15 minutes with a small addition of the dilute sulphuric acid. The cold liquid is made up to 200 c.c., and 100 c.c. titrated with silver solution. The process, which requires half an hour, can also be used for the determination of chloride in potassium iodide. W. R. S.

Sensitive Test for Iodine. C. V. King and M. B. Jacobs. (*J. Amer. Chem. Soc.*, 1931, **53**, 1704–1714.)—In an investigation into the kinetics of the reaction between iodide and persulphate in highly dilute solution, which forms the subject of this paper, use was made of a balanced photo-cell connected with a sensitive galvanometer to detect the time of first appearance of iodine in the solution. The test is stated to be much more sensitive than the reaction with starch or with basic lanthanum or praseodymium acetate. A diagram and particulars of the photo-cell circuit are given. W. R. S.

Internal Indicators for Bromate Titrations. G. F. Smith and H. H. Bliss. (*J. Amer. Chem. Soc.*, 1931, **53**, 2091–2096.)—A number of dyes were studied with reference to their suitability as indicators for the bromate titration

of, *e.g.* arsenic and antimony, in which methyl orange or indigosulphonic acid are usually employed. Several monoazo- and triphenylmethane dyes proved satisfactory; fuchsine was found to be preferable to methyl orange, as the titration could be made at room temperature and at lower acidities. Whilst the action of bromate on methyl orange requires 3 *N* acid at a temperature not below 60° C., the indicators tested were bleached at *N* acidity, the required temperature varying between 25° and 85° C. W. R. S.

Volumetric Determination of Fluorine by means of Cerous Nitrate. G. Batchelder and V. W. Meloche. (*J. Amer. Chem. Soc.*, 1931, **53**, 2131–2136.)—Sodium fluoride solutions were treated with a known excess of cerous nitrate, and the non-precipitated cerium salt titrated with permanganate. Removal of the mixed precipitate by filtration in the neighbourhood of the end-point was found to be necessary, making the method rather cumbersone. The direct titration of neutral fluoride solutions with cerous nitrate in presence of an adsorption indicator was found to be convenient, ampho-magenta (0·02 grm. per litre) changing from deep blue to a less intense purple at the equivalence point (the dye is diazotised *p*-aminoethylacetanilide coupled with 1,8-dihydroxynaphthalene-3,6-disulphonic acid). Kurtenacker and Jurenka's procedure (*Z. anal. Chem.*, 1930, **82**, 210), which consists in carrying out the same titration in presence of methyl red, was also tried, and found to give satisfactory results. No end-points could be obtained in presence of sulphate ion. W. R. S.

Iodimetric Thiocyanate Titration. H. A. Pagel and H. J. Koch. (*J. Amer. Chem. Soc.*, 1931, **53**, 1774–1777.)—The neutralised thiocyanate solution is treated with 1 grm. of ammonium sulphate, nitrate, or chloride dissolved in 20 c.c. of *N* ammonia, and an excess of 5 c.c. of standard iodine solution, and left for 5 minutes. It is then acidified with 6 *N* hydrochloric acid (5 c.c. excess) and immediately titrated with standard thiosulphate solution, which gives the excess of iodine over $HCNS+3I_2+4H_2O=H_2SO_4+HCN+6HI$. Nickel, cobalt, and manganese cause erratic results; precipitated aluminium hydroxide, zinc, magnesium, and borax do not interfere. W. R. S.

Microchemical.

Contributions to the Micro-Dumas Method. O. R. Trautz. (*Mikrochem.*, 1931, **9**, 300–312.)—Sources of error in the micro-Dumas determination of nitrogen are investigated. (1) *Dissociation in the heated combustion tube.*—Dissociation of carbon dioxide into carbon monoxide and oxygen is shown by enlargement of the micro-bubbles, since these gases are not absorbed in 50 per cent. potash solution. In the conditions of the experiment no enlargement of micro-bubbles is observed, even on prolonged heating of the tube, with a rate of flow of carbon dioxide of 22 c.c. per minute (7 bubbles per second). Copper oxide wire

of 0·5 mm. diameter is used rather than coarser wire, as with smaller spaces between the wires there is a longer time of contact between the gases and the copper oxide. The copper oxide of the temporary filling must be oxidised after use by igniting for half-an-hour and cooling in air for a day or more. (2) *Purity of the carbon dioxide.*—The micro-bubbles consist chiefly of air which comes from that dissolved in the hydrochloric acid of the Kipp generator. It is found that 1000 bubbles of diameter 0·2 mm., or 40 to 50 c.c. of carbon dioxide, which is the volume used to sweep out the apparatus, cause an air error of 4 c.mm., which has not previously been taken into account. Purer carbon dioxide, with a constant air content and giving micro bubbles of 0·1 mm. diameter, is obtained by using two Kipp generators in series (Niederl, Trautz and Saschek, ANALYST, 1930, **55**, 771), or by evacuating the upper bulb of the Kipp generator, using a Hein pump (*Z. angew. Chem.*, 1927, 864). The air content should be determined, and the correction of 1–6 c.mm. per 100 c.c. of carbon dioxide applied. When the air content is greater than 4 c.mm. per 100 c.c. it is best to insert a gasometer between the generator and combustion tube so that a standard amount of carbon dioxide can be used for each analysis and the correction applied more accurately. (3) *Adsorption error in the combustion tube.*—The permanent filling is always left under pressure of carbon dioxide, and any air which enters it during the replacement of the temporary filling is removed by a stream of carbon dioxide in the cold, but the adsorbed air is not completely removed from the temporary filling in the cold. Pregl's method (*Quantitative Organic Micro-analysis*, 2nd Ed., 1930) is to heat the tube, but there is danger of loss in the analysis of volatile substances. Therefore, the tube is swept out in the cold for 5 minutes with carbon dioxide (about 150 c.c.) and a correction is made for the adsorption error of the temporary filling. This has been found to be 6 c.mm. (4) *Nitrometer errors.*—The adherence of bubbles to the mercury-potash surface is avoided by adding a few drops of mercury which have been shaken with ether, and, after removing the ether, washed with water and potash solution; both the mercury and the slight sludge formed are added to the mercury in the nitrometer. The wall error, due to the moistening of the sides of the nitrometer with potash has been re-determined and found to be 0·5 per cent., as compared with the value 1·7 per cent. used by Pregl. The correction for the vapour pressure is 0·3 per cent. of the volume of nitrogen. The amount of nitrogen absorbed in the potash solution has been found to be negligible, showing the possibility of determining even smaller amounts of nitrogen. (5) *Rubber connections.*—It is found that a piece of best quality rubber, 7 mm. long, 2 mm. inside diameter, and 1 mm. wall thickness in the pressure conditions of the experiment, allows 1 c.mm. of air per hour to diffuse through. Therefore the error is negligible when the rubber connections are good, but they must be tested frequently. It is better to use ground-glass joints. When a known amount of carbon dioxide is used for the sweeping out, and the corrections described are applied, the analysis can be carried out at a rate of 4 bubbles per second, so that the determination can be completed in 15 minutes, and results are obtained which show no difference from those at the slower rate of analysis of 1 bubble per second. J. W. B.

Micro-Determination of Copper by means of Salicyl-aldoxime. W. Reif. (*Mikrochem.*, 1931, **9**, 424–429.)—Salicyl-aldoxime, a reagent first used by Ephraim (*Ber.*, 1930, **63**, 1928) is used for the micro-determination of copper. *Method.*—From 2 to 5 c.c. of the test solution are treated, drop by drop, with dilute ammonia, until the blue colour is clearly visible, when ammonium acetate is added until the blue colour disappears again. The copper is then precipitated with a freshly prepared alcoholic solution of salicyl-aldoxime. The mixture is shaken until the green-white precipitate agglomerates, and further salycyl-aldoxime is then added until excess is present. After standing for 10 to 20 minutes the precipitate is filtered by means of a Pregl micro-filter tube and washed alternately with cold water and alcohol, and finally with alcohol, and dried in a Pregl drying block at 105° C., cooled and weighed. The factor is 0·1895, and the log. factor is $\bar{1}\cdot27761$. On amounts of copper from 0·7 to 1·3 mgrm. the error is about 0·5 per cent. The determination can be carried out with a similar accuracy in the presence of ammonium salts; the error is only 1 per cent. in the presence of 10,000 times the weight of ammonium chloride. In the presence of iron about 50 to 100 mgrms. of tartaric acid are added to the test solution before the first treatment with ammonia, to give a stable tartrate complex with the iron, which then does not affect the accuracy of the determination. For the determination of nickel and copper in the same solution, the test solution is treated with about 50 mgrms. of tartaric acid, and then with ammonia until the blue colour appears. The nickel is then precipitated with a 1 per cent. alcoholic solution of dimethyl-glyoxime, and the tube is heated over a water bath at 70° to 80° C. for ½ hour until the red precipitate coagulates. The solution is tested to see if the precipitation is complete; and after standing, the mixture is filtered through a Pregl filter tube, and the precipitate dried at 110° C. and weighed. The filtrate is treated with ammonium acetate until the blue colour disappears, when the copper is precipitated as before. J. W. B.

Physical Methods, Apparatus, etc.

Transference of Small Quantities of Liquids. G. Owen. (*J. Soc. Chem. Ind.*, 1931, **50**, 189T–190T.)—Small quantities of liquids (½–1 c.c.) which have been sealed up in ampoules may be manipulated with ease if a siphon of finely drawn-out and bent glass tubing of about 0·2 mm. bore is inserted to the bottom of the ampoule. The liquid automatically rises in the tube by capillarity and flows out, drop by drop. P. H. P.

Apparatus for Recording the Ultra-Violet Light of the Sky. J. R. Ashworth. (*Nature*, 1931, **127**, 893.)—Hill's method of measuring ultra-violet light by recording the fading of an acetone solution of methylene blue is simple and satisfactory, except that on short winter days the fading produced is too slight to be observable by this method. An alternative procedure, which has been under test during the last year or two in the neighbourhood of Rochdale, involves the use of

photographic printing paper and has given monthly average readings agreeing well with those furnished by the methylene blue method. A strip (3 × 1 inch) of ultra-violet glass which is opaque to visible light but transparent to a band of rays with the wave-length range 3400–3700 A.U., is fitted into a slot (3 × 1 inch) in the lid of a shallow metal box. A strip of photographic paper, on the bottom of the inside of the box, is covered with a stepped "wedge," constructed of layers of thin tissue paper of fine quality arranged to provide ten different thicknesses through which the light may pass.

The closed box is exposed to the light of the sky, and at the end of the day the photographic paper is examined to ascertain the greatest number of layers of the tissue paper which the light has penetrated. According to theory this number is the logarithm of the intensity of the light, and, the transmission factor of the paper being known, an arbitrary scale of light values may be constructed. This method gives readings on all winter days except when fog obscures light of all kinds. As summer approaches and the light becomes stronger, the wedge scale may need extension; a further suitable number of layers of the tissue paper may be superimposed on the wedge without detriment to the accuracy of the readings. If the photographic paper is wrapped on a rotating drum, a continuous record is obtainable. T. H. P.

Filters made of Porous Hard Rubber. E. Vossen. (*Chem. Ztg.*, 1931, **55**, 454.)—A filtering medium of porous hard rubber containing 32·08 per cent. of sulphur is completely resistant to the action of hydrofluoric acid, sulphurous acid, phosphorous acid, phosphoric acid, alkalis and solutions of salts, and also to sulphuric acid (less than 70 per cent. and below 70° C.) and hydrochloric acid (less than 20 per cent. and below 20° C.). The range of temperature within which it can be used is, in general, from −10° C. to 110° C. The manufacture of these filters is, in outline, as follows: Rubber, sulphur, and vulcanising fluxes are kneaded to a homogeneous mass which is then pulled out into sheets and vulcanised just sufficiently to allow of the product being ground into a powder. The pulverised material is separated, by sieving, into grades of different fineness which yield products of different degrees of porosity according to the fineness. The grades are separately vulcanised under pressure in iron moulds of the required shape; the porous product is finally treated with silica-gel in order to remove carbon disulphide which is present in the pores. These porous rubber filters find their main application as substitutes for filter cloth, etc., in the form of plates, rings and tubes for filter presses and suction filters. It is stated that it is not known at present whether it will be possible to reduce the pore-size sufficiently to render the porous rubber suitable for colloid or bacteria filters. S. G. C.

References to Scientific Articles not Abstracted.

PRESERVATION OF LEATHER BOOKBINDINGS. By R. W. FREY and F. P. VEITCH. *Leaflet No.* 69. *U.S.A. Dept. of Agriculture.*

Rotting of Leather Bookbindings—Dressings for Bindings—Application of Dressings—Treating Vellum Bindings—Lacquering Powdery Bindings.

NEW ALKALOIDS DISCOVERED, 1920–1929 INCLUSIVE. By J. F. COUCH. *Amer. J. Pharm.*, **1931**, **103**, 242–251 (May).

An Alphabetical Summary of New Alkaloids, with formulae and melting points. Bibliography containing 89 references.

THE PATHOLOGY OF SOME INDUSTRIAL POISONS. By A. J. AMOR. *Chem. and Ind.*, **1931**, **50**, 475–476 (June 5).

Carbon Monoxide: Action on Haemoglobin and on Brain Cells—Arsine: Haemolysis of Red Blood Corpuscles—Effect on Stomach and Kidneys—Lead: Anaemia of Lead Poisoning—Lead Paralysis—Lead Colic—Lead Encephalopathy—Effect on the Arteries—Effect on the Kidneys.

Reviews.

HANDBOOK OF CHEMICAL MICROSCOPY. Vol. II. By E. M. CHAMOT, Ph.D., and C. W. MASON, Ph.D. 2nd Edition. Pp. ix+411, with 181 Figures. London: Chapman and Hall. 1931. Price 22s. 6d.

The opinion has frequently been expressed that the attraction of chemical microscopy to those who employ it lies in the manipulations involved much more than in the results attained. That this view is erroneous is well illustrated by the present work in conjunction with Volume I previously reviewed (ANALYST, 1930, **55**, 470), and in the ever-increasing applications of microscopical analysis in many branches of science. Those chemists who have adopted these methods soon realise that for economy of materials, reagents and time, as well as certainty of the results obtained, the usual macroscopic methods are left far behind, and the brief practice necessary to acquire the special technique used is very soon justified.

This volume comprises chapters dealing with the technique of manipulating small amounts of material, methods of using reagents, reactions of the elements arranged according to the eight groups of the periodic system, detection of inorganic anions together with acetic, oxalic and tartaric acids, special reagents, qualitative micro-analysis of mixtures including alloys, and an accurate and well-arranged index. The general plan adopted in the descriptions given is roughly as follows: (*a*) discussion of the reactions of each member of a group,

(*b*) detection of one or more group metals present in the unknown substance, (*c*) behaviour of a group reagent with elements of other groups, (*d*) separation of the metals present when necessary, and (*e*) identification of the separated metals by appropriate reactions. The description of the methods and of the results obtained is particularly lucid, although in certain cases necessarily involved, but all redundant matter has been carefully excluded, and it will be found that the practical application of many of the reactions occupies much less time than their perusal.

The reactions described have been well selected, and are dependable, with, perhaps, the exception of those involving the use of potassium xanthate, but it appears to the reviewer that insufficient reference is made to the use of the flame test in conjunction with a small hand spectroscope and to the value of carrying out simultaneously on the same microscope slide three or four tests with the same reagent, using different concentrations of the solution under examination.

The numerous illustrations, of which a large proportion are photomicrographs depicting crystalline precipitates obtained in various reactions, are excellent, and add considerably to the value of the work, while the freedom from typographical and other errors, the legibility of the text, and the general style of the book make it a worthy successor to the previous volume. This work is a valuable addition to the literature of the student and the analytical chemist, and it is to be hoped that the authors will provide a third volume on similar lines dealing with the chemical microscopy of the organic acids and radicals.

T. J. WARD.

Erratum.—*Bell's Sale of Food and Drugs Acts.* The case cited in the review in the June issue (p. 421), should be *Lémy* v. *Watson* (not "*Lamy*").