THE ANALYST

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

An Ordinary Meeting of the Society was held at the Chemical Society's Rooms, Burlington House, on Wednesday, October 7th, the President, Dr. J. T. Dunn, being in the chair.

Certificates were read for the first time in favour of:—Charles Hubert Francis Fuller, B.Sc., F.I.C., Ganesh Chandra Moitra, B.Sc., Eric Charles Wood, B.Sc., A.R.C.S., Robinson Pearson Wood, M.Sc., F.I.C.

Certificates were read for the second time in favour of:—Raymond Merefield Edwards, B.Sc., Llewelyn John Howells, B.Sc., Donald Neil McArthur, D.Sc., Ph.D., F.I.C., F.R.S.E., James Sword, M.A., B.Sc., Ph.D., A.I.C.

The following were elected Members of the Society:—George Brown, A.I.C., Charles Louden, B.Sc., A.I.C., Charles Percy Money, B.Sc., F.I.C., Martin Priest, F.I.C., Arthur Goodyear Simpson, M.A., Gerrish Smith.

The following papers were read and discussed:—"The Identification of Wood and Wood Charcoal Fragments," by J. Cecil Maby, B.Sc.; "The Examination of Dyed Leather in Cases of Alleged Dermatitis," by T. Callan, M.Sc., Ph.D., F.I.C., and N. Strafford, M.Sc., F.I.C.; and "The Determination of Chlorides in Dairy Products and Biological Material," by W. L. Davies, M.Sc., Ph.D., F.I.C.

NORTH OF ENGLAND SECTION.

A meeting of the Section was held in Manchester on October 10th, 1931. The Chairman (Mr. C. J. H. Stock) presided, and there was an attendance of thirty-five, including the President (Dr. Dunn).

An address was given by Prof. W. H. Roberts, M.Sc., F.I.C., on "The Analyst in the Witness-Box." A discussion followed in which many of the members took part.

In the morning, the members, by kind invitation, visited the premises of Messrs. Allied Dairies, Ltd., and inspected their plant. Lt.-Col. J. W. Brittlebank, C.M.G., on behalf of the firm, entertained the visitors to lunch.

Obituary.

HENRY DROOP RICHMOND.

HENRY DROOP RICHMOND was born at Hampstead on January 17th, 1867, and studied chemistry under Temple Orme at University College School.   He afterwards proceeded to Finsbury Technical College, and later became one of four or five assistants in the laboratory of Otto Hehner.   It was in this laboratory, about 40 years ago, that I first met Richmond.   He had a marked faculty for helping the younger members of the staff, and never failed to give good advice.   He was always recognised as a sound mathematician, and was the first to apply modern statistical methods to problems connected with milk analysis.

After leaving Hehner's laboratory, he joined the staff of the Khedival Laboratory at Cairo as second chemist, relinquishing this position in 1892.

On returning to England, he was appointed Chief Chemist to the Aylesbury Dairy Company, in succession to Vieth, whose admirable work he continued and developed.   He remained with this firm for some 23 years, and there enhanced his reputation as a chemist of outstanding ability.   While holding this appointment, he wrote his well-known standard book on Dairy Chemistry, invented the Milk Slide Rule, and contributed a large number of papers to THE ANALYST on many subjects connected with milk.   His extensive experience in the analysis of milk and the statistical treatment of milk records proved to be of great value to the Departmental Committee on Milk and Cream Regulations in 1901.

Droop Richmond was a valued member of several scientific societies.   He became a Fellow of the Institute of Chemistry in 1887, and served on its Council on two occasions.   He was also one of the older members of the Society of Public Analysts, having joined in 1890, acted as Treasurer in 1910–12, and as Vice-President in 1909 and 1914–15, and he was on the Council during 1893–96, 1900–1, 1907–8.   In addition to this, he served as Vice-Chairman of the Nottingham Section of the Society of Chemical Industry.

Richmond went to Messrs. Boots Pure Drug Company at Nottingham in 1915, primarily to work on the production of casein foods and of glycerophosphates. Shortly afterwards he was appointed Chief Analyst, and occupied that position until his death, although for the past two years his health had compelled him to abandon active work.

While with this company he published numerous papers in THE ANALYST and elsewhere, dealing with the analysis of drugs, some of the more important being his study of the selective hydrolysis of saccharin in the presence of its associated impurities (published in 1919), which led to a more exact method for the valuation of saccharin, and his paper on the action of hygroscopic drugs (potassium carbonate, etc.) on the glass containers in which they are stored, leading to the contamination of the drug with arsenic and lead (ANALYST, 1923, **48**, 260.)

His expressed belief in the value of physical methods of analysis (ANALYST, 1903, **28**, 141) was maintained in his series of papers, commenced before his period

of work at Nottingham and continued up to 1919, entitled "Studies in Steam Distillation," and in a number of other papers involving the use of physical methods, most of which were published in THE ANALYST.

As a further example of Richmond's many-sided activities, reference may be made to his communication to the Journal of the Chemical Society (1892, **56**, 491) on the supposed discovery of a new element in an Egyptian mineral. The reactions of the substance isolated were described, and its atomic weight was calculated to be 228.

His interest in the younger members of his laboratory staff at Nottingham was shown in many ways. He conducted classes for them in Latin and higher mathematics, took part in week-end walks with them, and, while his health allowed, joined them in both outdoor and indoor sports. His interest in education extended further than his own department, and for some time he conducted a "Laboratory Corner" in the firm's magazine, for the benefit of pharmaceutical students in the firm's retail branches.

After a severe operation in 1924, he never really recovered, and, although he attempted to struggle on, physical weakness compelled him to abandon active work early in 1930.

Those who worked with him during the last few years bear witness to his fortitude and patience, and his sheer determination to carry on his work in spite of growing pain and weakness, will always remain as a great trait of a kindly and lovable character.

I would like to bear testimony to the valuable help he so ungrudgingly gave me during a friendship which lasted over 40 years.

LEONARD BOSELEY.

Death.

WE greatly regret to have to record the death, on September 18th, of Dr. CHARLES A. KEANE, who was Vice-President of the Society in 1921–1922.

Standards for Jam.

PERCENTAGE OF SOLUBLE SOLIDS.

As the determination of total soluble solids set out in the Final Memorandum "by refractometer reading when cold" occasions small inaccuracies, the Council has decided that the refractometer reading shall be taken at a temperature of 20° C.

The appropriate Committee of the Food Manufacturers' Federation, Incorporated, has agreed to this alteration.

Evaluation of the Menthone Content of Peppermint Oil.

By J. REILLY, D.Sc., N. NOONAN, M.Sc., and P. J. DRUMM. Ph.D.

(Read at the Meeting, November 4, 1931.)

EXPERIMENTS on the possibility of the economic production of Irish oils of lavender, peppermint, and dill have engaged attention in this laboratory for a number of years (*Econ. Proc. Roy. Dub. Soc.*, 1926, **2**, Nos. 16, 17, 18, 19). The determination of the carvone and menthone content of dill and peppermint oils, respectively, presented some difficulty, inasmuch as the methods already in use did not always give concordant results.

Power and Kleber's method of determining menthone (*Pharm. Rundschau*, 1894, **12**, 162) consists in the determination of the menthol content of the oil before and after reduction. The menthol content is first determined by acetylation of the oil, followed by saponification. The oil is next reduced in alcoholic solution by means of sodium. The reduced oil is isolated, then acetylated, and the acetylated oil is afterwards saponified. From the increase in the menthol content the amount of menthone is determined. This method is tedious and leads to different values for the menthone content, according to the exact procedure.

A method in which carvone is determined as its semicarbazone was evolved by us, and has already been communicated to this Society (ANALYST, 1928, **53**, 209). In the present paper this method has been extended, with satisfactory results, to the determination of the menthone content of peppermint oil.

The pure menthone for preliminary experiments was prepared from the commercial product. This was converted into its semicarbazone by warming an aqueous alcoholic solution of the menthone semicarbazide hydrochloride (Kahlbaum) and sodium acetate. The precipitated *l*-menthone semicarbazone was collected and recrystallised from alcohol until it had a constant melting point (185° C.). The rotation of the menthone regenerated from its semicarbazone by hydrolysis with dilute acids was found to be considerably below that recorded in the literature for pure *l*-menthone. This is possibly due to the racemising action of the acid. In an attempt to avoid this, the method of Kon (*J. Chem. Soc.*, 1930, 1616) for the purification of sensitive ketones was employed. This consists in mechanically shaking the semicarbazone, suspended in petroleum spirit to remove the ketone from the sphere of reaction, with the calculated amount of $N/2$ sulphuric acid until hydrolysis is complete. This method was abandoned, as it was found that, under the conditions stated, very little hydrolysis of menthone semicarbazone took place.

Ultimately it was found that hydrolysis of the semicarbazone with the theoretical amount of $N/2$ sulphuric acid and removal of the menthone in a current of steam as it was formed, gave a menthone of fairly satisfactory rotation.

DETERMINATION OF PURE MENTHONE.—Two grms. of menthone were added to a solution of 3·4 grms. of semicarbazide hydrochloride and 3 grms. of fused sodium acetate in 45 c.c. of water. Forty-five c.c. of 93 per cent. alcohol were added to the mixture until a clear solution was obtained, and the whole was gently warmed on a water-bath for a few minutes. Separation of the semicarbazone occurred almost immediately. The whole was allowed to remain at room temperature over-night. The precipitated semicarbazone was collected, dried and weighed. The following results were obtained:—

Menthone taken. Grms.	Semicarbazone precipitated. Grms.	Semicarbazone in solution.	Total semicarbazone. Grms.	Menthone estimated. Grms.
2	2·49	45 c.c. water, 45 c.c. alcohol, 0·10 grm.	2·59	1·890
5	6·28	112·5 c.c. water, 112·5 c.c. alcohol, 0·25 grm.	6·53	4·765

In a series of determinations the amounts found were all approximately 95 per cent. of those taken, and a factor might be used to allow for the deficiency.

DETERMINATION OF MENTHONE IN PEPPERMINT OIL.—The method of procedure is the same as for pure menthone. Ten grms. of peppermint oil were added to a solution of 3·4 grms. of semicarbazide hydrochloride and 3 grms. of fused sodium acetate in 30 c.c. of water. Sixty c.c. of 93 per cent. alcohol were added to the mixture until a clear solution was obtained. The solution was then gently warmed on the water-bath, when precipitation of the semicarbazone commenced almost immediately. The whole was allowed to stand over-night, when most of the semicarbazone had separated in crystalline form. This was collected, dried and weighed. The crude semicarbazone in all the test determinations melted at 184–185° C. The solubility of menthone semicarbazone in a mixture of 30 c.c. of water and 60 c.c. of alcohol was 0·16 grm.

Several determinations with Irish peppermint oil gave closely agreeing results, the extreme figures being shown in the following table. No allowance is made for a factor.

Irish peppermint oil taken. Grms.	Semicarbazone precipitated. Grms.	Semicarbazone in solution (alcohol, 60 c.c.; water, 30 c.c.). Grm.	Total semicarbazone. Grms.	Percentage of menthone in Irish peppermint oil.
10	2·854	0·16	3·014	22·0
15	4·230	0·16	4·390	21·4

OXIME TITRATION METHOD.—In order to obtain comparative figures the menthone content of peppermint oil was also determined by means of hydroxylamine (for the particulars of which method we are greatly indebted to Messrs. Bush & Co., Hackney).

By this method hydroxylamine hydrochloride is allowed to react in the cold with the oil, and the liberated hydrochloric acid is titrated as described below. Each c.c. of $N/2$ potassium hydroxide solution is equivalent to 0·077 grm. of menthone.

About 10 grms. of peppermint oil are added to 25 c.c. of a solution of $N/2$ hydroxylamine hydrochloride in 80 per cent. alcohol (which has been previously neutralised, if necessary, with alkali). The solution is allowed to stand for half-an-hour and then back-titrated with $N/2$ alcoholic potassium hydroxide solution, brom-phenol blue being used as indicator. The figures by this method are about 2 per cent. lower than when the process is carried out with hot solutions.

The following table shows the results obtained with various oils by the above methods:—

	Percentage of menthone.	
Oil.	Semicarbazone method.	Oxime method.
Irish	22·00	21·40
American	17·25	17·00
English (Messrs. Bush & Co.)	18·25	17·45
English (Messrs. Stafford, Allen & Co.)	16·70	17·00

From the above table it is seen that the semicarbazone method, even when no allowance is made for the factor previously indicated, generally gives higher values than the oxime method. The semicarbazone isolated is a pure substance. This method, therefore, gives at least a minimum value for the menthone content of peppermint oil. It would appear, therefore, that the oxime method does not estimate all the menthone present in the oil.

THE CHEMICAL STATION,
UNIVERSITY COLLEGE, CORK.

DISCUSSION.

Mr. C. E. SAGE said that the authors' opening remarks on the possibility of the profitable production of essential oils of lavender, peppermint and dill in Ireland were not meant for criticism by a scientific meeting, but his commercial experience with the preparation of all three oils in Spain and America would suggest that further enquiries should be made there before embarking on the cultivation of the plants in Ireland; English growers could give some useful advice regarding the profits to be derived from peppermint growing in England. The climate of Ireland might have a very considerable effect on lavender, similar to that produced by different altitudes in Spain. The dill, to do any good at all, required sunshine and plenty of it, whilst the peppermint needed something more than chemical, botanical or agricultural experience to make its cultivation profitable.

The determination of menthone by the semicarbazone method might be theoretically possible and scientifically desirable, but in actual practice it was not reliable. His experience had been that the semicarbazone did not always separate immediately, that it did not all separate in 24 hours, and, even after standing three days and filtering, another small crop might be gathered. In comparison with the oxime method, the semicarbazone method was slow, cumbersome and un-certain, and yielded, in his hands, entirely erroneous results. Taking a com-mercial menthone from a well-known source, the oxime method gave consistent

results, indicating 88 to 89 per cent. purity, whilst by the carbazone method as described by the authors only 70 per cent. was obtained.   With an oil known to contain 25 per cent. of menthone, the hydroxylamine method yielded 25·2 and 25·4 per cent., whilst the semicarbazone method yielded only 14·3 per cent. after standing 24 hours, and a further crop of crystals after 48 hours.   Another comparative experiment with a mixture containing carvone yielded 55 per cent. by the oxime method, and only 40·5 per cent. by the semicarbazone method, as described by Reilly and Drumm in a paper read before the Society in 1928.   Such results indicated the need for further examination of the method before its acceptance as reliable.

The authors' statement that the hydroxylamine method gave lower results than that suggested by them might be accounted for by the fact that they made the test cold, whereas it was known that even at a temperature of 78° C. it took an hour to complete the reaction, and, in the cold, consistent results were not obtained by different workers.

Mr. T. T. COCKING sent the following communication, which was read to the meeting.

The authors' results for the menthone in peppermint oil strike me as being on the low side.   I have carried out a number of determinations by the hydroxylamine method, and, with one exception—that of a very old sample of English oil— all oils have contained from 22 to 35 per cent. of menthone.   The method I have used is that which has been recommended by C. T. Bennett and myself for the determination of ketones in essential oils, with special reference to carvone in the oils of caraway and dill.   The reagent is a normal solution of hydroxylamine hydrochloride in 90 per cent. alcohol adjusted to the full yellow colour of dimethyl yellow, and the reaction is carried out at a temperature of about 70° C., the free acid liberated being titrated intermittently with normal alcoholic potash until the reaction is complete.

The reaction proceeds rapidly at first, about 90 per cent. of the menthone being converted nito oxime in the first 20 minutes.   After this the reaction slows down, but is usually complete in from 40 to 60 minutes.   It was found that when the reaction was carried out in the cold with a half-normal reagent prepared with 80 per cent. alcohol, combination was very slow and did not proceed to completion even after many hours.   There is no doubt that the half-hour's contact in the cold allowed by the authors is insufficient, and is responsible for the low results given by this method.

I note that when using the semicarbazone method on peppermint oils, the authors make a correction for the solubility of the semicarbazone in the volume of dilute alcohol used, but do not state whether the solubility is affected by the presence of the non-menthone portion of the peppermint oil.

While the method advocated will, no doubt, be useful for the separation and identification of menthone, it is doubtful if the results will attain the accuracy of the hydroxylamine method when this is carried out under the best conditions.

Dr. REILLY sent the following reply: It has been shown by the staff of the Chemical Department, University College, Cork, in the *Economic Proceedings of the Royal Dublin Society (loc. cit.)* that the production of essential oils from Irish-grown plants is practicable, and that the oils compare favourably with the best Mitcham products.   The further development of the industry is mainly a question of finance.

The semicarbazone method is not intended to replace the oxime method, which gives figures of technical value.   The menthone semicarbazone, isolated as described, is a single organic compound with a definite melting point, and in

this respect it differs from the oxime reaction product, which may be more complex, especially if the reaction is carried out in a warm solution.   With experience, the method gives concordant results, and with synthetic mixtures the menthone content can be determined fairly accurately.   It is not claimed that the method can be used with all commercial oils, as it has only been studied to a limited extent.

In carrying out the hydroxylamine method the conditions as to temperature, given in the literature, are generally vague.   In the particular method followed, directions to carry out the reaction *in the cold* were definitely given.

Further work is in progress on the application of the semicarbazone method, especially for the separation of pure ketones.

A New Method for Detecting Decomposition Products in Anaesthetic Chloroform.

By NOEL L. ALLPORT, A.I.C.

(*Read at the Meeting, May* 6, 1931.)

IT is a well-established fact that perfectly pure chloroform is liable to oxidation and that one of the products formed is phosgene (carbonyl chloride).   This decomposition takes place so readily that all pharmacopoeias direct the addition of a small amount of alcohol which acts as a preservative.   According to Clover (*J. Amer. Chem. Soc.*, 1923, **45**, 3133), alcohol behaves as an anti-catalyst, and retards, without entirely preventing, the oxidation.   The formation of phosgene is, however, inhibited, since alcohol will at once react with any that is formed, producing mainly hydrochloric acid and ethyl carbonate.   Notwithstanding this, it has been customary to examine anaesthetic chloroform for traces of phosgene, which obviously could not be present until all the alcohol had been decomposed and a corresponding amount of hydrochloric acid formed.   It is obvious that, in the examination of chloroform for evidence of decomposition, attention should be directed to the detection of the minutest trace of hydrochloric acid, but the methods hitherto available are not as sensitive as could be desired.   A new test is here proposed which, it is hoped, may be found to be an advance on existing tests.   It is expressly applicable to B.P. chloroform.

Three of the tests at present commonly used are directed specifically to the detection of hydrochloric acid or phosgene.   These are the tests with silver nitrate, barium hydroxide, and benzidine.

The silver nitrate method involves shaking the chloroform with water, allowing separation to take place, and examining portions of the aqueous liquid for chlorides and for acidity.   This test is included in most of the pharmacopoeias, and is quoted as a limit test for hydrochloric acid.   As a rough preliminary examination it undoubtedly serves a very useful purpose, since any chloroform failing to pass the test could not possibly be considered as sufficiently pure for anaesthetic

use. It will be shown, however, that the method is unsuitable for detecting traces of hydrochloric acid.

The barium hydroxide test, originally suggested by Ramsay (*J. Soc. Chem. Ind.*, 1892, **11**, 772), was modified by Baskerville and Hamor (*J. Ind. Eng. Chem.*, 1912, **4**, 571). It is conducted by introducing 15 c.c. of the sample into a stoppered bottle of 25 c.c. capacity, adding sufficient aqueous barium hydroxide (1 in 19) to fill the bottle, and allowing the mixture to stand, without agitation, for three hours in a dark place. The formation of a film of barium carbonate at the junction of the two layers of liquid is said to indicate the presence of phosgene. Actually, this test responds to carbon dioxide; it is, therefore, unsatisfactory as a test for phosgene. It is also very difficult to operate, owing to the interference caused by the carbon dioxide in the air. Positive reactions are occasionally obtained by this test, owing to traces of carbon dioxide dissolved in the chloroform.

The benzidine method, which was devised by Utz (*Pharm. Zentr.*, 1917, **58**, 1), is a distinct improvement upon the foregoing. In its original form it has been included in the German Pharmacopoeia (D.A.B. VI). The benzidine (0·1 grm.) is added to 20 c.c. of chloroform, filling a completely dry, stoppered flask and allowed to stand in the dark for 24 hours. Utz states that 100 parts per million of phosgene will be indicated by a cloudiness, and that ten times this amount produces a yellowish-white precipitate. In the same paper it is pointed out that a turbidity or precipitate is also produced by hydrochloric acid. This test is more delicate when only one-fifth of the amount of benzidine originally prescribed is employed. In this way most of the colour due to the reagent itself is eliminated, but still the reaction is not sufficiently sensitive, and the necessity for standing over-night is a further disadvantage.

While investigating the foregoing process it occurred to me that advantage might be taken of the very delicate colour reactions produced by various phenols with aldehydes in the presence of condensing agents. The well-known test for detecting formaldehyde by the addition of resorcinol in the presence of sulphuric acid is an example of this type of reaction. The Kreis test for rancidity of oils and fats also depends upon the same principle, *viz.* the detection of traces of aldehyde formed prior to the development of free acid. Conversely, the reaction between phenol and aldehyde may be used to detect the presence of a condensing agent. This principle is embodied in the Gunsberg test for the detection of free hydrochloric acid in gastric juice. It consists in mixing an alcoholic solution of phloroglucinol and vanillin with the specimen and evaporating to dryness, a rose-red colour being produced if free hydrochloric acid is present. Panton (*Lancet*, 1918, [ii], 125) has suggested the substitution of either orcinol or resorcinol for phloroglucinol.

Preliminary experiments on these lines soon led to promising results, and it was found that by the adoption of the technique to be described one may detect extremely small amounts of hydrochloric acid in chloroform. The reagents employed are resorcinol and vanillin. The colour reaction is extremely delicate. In

this connection interest attaches to the work of La Wall (*Amer. J. Pharm.*, 1905, **77**, 392), who, when testing for the presence of formaldehyde in vanilla ice cream by the resorcinol test, obtained a positive reaction, although other tests gave negative results. The colour given by the resorcinol was found to be due to the vanillin present and not to formaldehyde, and further investigation showed the resorcinol test to be capable of detecting one part of vanillin in 200,000 of cream. Crocker (*Ind. Eng. Chem.*, 1925, **17**, 1158) has investigated these colour reactions between phenols and aldehydes, and has found that, by using the pure substances, resorcinol gives a colour with a 1 in 2,000,000 dilution of vanillin when mineral acid is present.

In applying the method for the purpose of detecting the formation of phosgene in chloroform it was necessary to prepare specimens of chloroform representing specific degrees of decomposition. This was done by the addition to B.P. chloroform of known quantities of phosgene. For convenience the concentration of decomposition products has been expressed in terms of phosgene.

EXPERIMENTAL.—An approximately 10 per cent. (w/v) solution of phosgene in B.P. chloroform was prepared by passing the gas into the solvent from a cylinder. The strength was determined by adding a known volume of the solution to a relatively large bulk of water contained in a stoppered flask and titrating the hydrochloric acid produced after agitating the mixture at frequent intervals during a period of about three hours. From this solution dilutions of the desired strength were made, the purest anaesthetic chloroform being used.

Preliminary experiments showed that if a solution of resorcinol and vanillin in B.P. chloroform was mixed with a moderately strong chloroformic solution of phosgene, a red precipitate formed more or less quickly according to the concentration of the reacting substances. This red substance probably belongs to the triphenylmethane group of dyes, and is only very slightly soluble in chloroform. A series of tests was made in which stoppered bottles, each holding 15 c.c. when completely filled, were used. Fifty mgrms. each of resorcinol and vanillin were added to each bottle, and the bottles were then completely filled with chloroformic solutions of phosgene, the dilutions ranging from one to twenty parts per million. After being securely stoppered the bottles were placed in a darkened cupboard. At the same time parallel experiments were made by the silver nitrate test and by the modified benzidine method. Blank tests with purest anaesthetic chloroform were made in each case. After 24 hours a slight turbidity was shown by the benzidine method in chloroform containing 5 parts per million. Silver nitrate revealed a slight opalescence when 10 parts per million or more were present. The resorcinol and vanillin test showed a distinct turbidity with 5 parts per million, and a faint but definite turbidity with 2 parts per million. Stronger solutions of phosgene showed pink precipitates in the latter test. When these reacting liquids are shaken with dilute aqueous alkali solutions the precipitate is dissolved in the alkaline layers, forming red solutions and thereby rendering the test much more delicate. By this method a rich pink aqueous layer is obtained with chloroform

containing 1 part per million of phosgene, whilst a blank test shows no pink coloration whatsoever, the aqueous layer remaining colourless for about two minutes, after which it begins to darken by reason of the resorcinol present. In subsequent experiments it was found that by reducing the quantity of reagent used to 20 mgrms. each of resorcinol and vanillin this tendency to darkening is decreased.

A series of tests was conducted in an endeavour to limit the time of standing. After remaining in a darkened cupboard for one hour the contents of the bottles were transferred to suitable tubes and immediately shaken with 5 c.c. of 1 per cent. ammonia solution. Although no coloration or turbidity was visible in the chloroform prior to the addition of ammonia, yet with it distinct pink aqueous layers were obtained, even with chloroform to which only 1 part per million of phosgene had been added, whilst pure chloroform yielded a colourless alkaline layer. The depth of colour was clearly proportional to the amount of phosgene present. With periods of shorter duration than one hour the results were not found to be so satisfactory.

Further experiments were made to ascertain the effect of light. With concentrations of phosgene of 5 parts per million and over, the pink colour developed in the chloroform itself after exposure to diffused daylight for one hour. Comparisons made with and without exposure to daylight showed that there was no apparent difference in the colour of the alkaline layer for equal concentrations of phosgene. It is recommended that the test mixtures should stand in darkness, since more uniform conditions are thus obtained. It was also shown that the exclusion of air by the use of completely filled bottles is immaterial to the success of the test. In consequence, transferring the chloroform to a larger vessel, prior to adding the ammonia, is unnecessary. The limit of sensitiveness for the resorcinol-vanillin test carried out as herein described is about one part of phosgene in two million parts of chloroform.

It is interesting to note that after the test mixtures have stood for about 15 minutes the aqueous layers have darkened in all cases, but, in the complete absence of free acid the chloroform is quite clear, and is turbid when a positive reaction has been obtained. Even when the equivalent of only 1 part per million of phosgene is present, the turbidity is quite obvious, and it becomes more marked with increasing proportions of impurity.

Besides resorcinol and vanillin many other phenols and aldehydes were tried in various combinations, but in no case was the result as satisfactory. Resorcinol and piperonal yielded a pink coloration or precipitate in the chloroform, varying with the concentration of phosgene employed, but the sensitiveness was much less than in the resorcinol and vanillin test. The substitution of phloroglucinol for resorcinol in the adopted test was ineffective.

The behaviour in this test of alcohol-free chloroform to which phosgene had been added was then examined, and the results showed the presence of alcohol to be necessary. Absolute chloroform was prepared by washing B.P. chloroform repeatedly with water, drying over anhydrous potassium carbonate and then

twice distilling, the first and last runnings being rejected. Dry phosgene was passed into a portion of this absolute chloroform and, after titration as before described, dilutions were made with the remainder of the same solvent.

Even when 0·1 per cent. of phosgene was present the resorcinol and vanillin test showed only a faint pink coloration in the chloroform. On adding about 1 per cent. of absolute alcohol, however, a red precipitate formed in a few minutes. On the other hand, the response to the benzidine test was better in the absence of alcohol. The turbidity was produced at once when only 5 parts per million of phosgene were present; whereas with B.P. chloroform and the same concentration of phosgene no reaction whatever was obtained until after several hours' standing.

The positive reaction for hydrochloric acid given by the benzidine test is doubtless due to the precipitation of benzidine hydrochloride. It was thought that the turbidity produced by phosgene might also be due to precipitation of the hydrochloride. To test this point a solution of benzidine in absolute chloroform was mixed with absolute chloroform containing about one per cent. of phosgene. The resulting precipitate was collected on a dry filter, well washed with absolute chloroform and then dried. It was found to be soluble in water and gave the ordinary reaction for chlorides. The yellowish white precipitate mentioned by Utz as being produced in the presence of much phosgene could not be obtained.

Summary.—A new and simple method for detecting decomposition products in chloroform, due to phosgene formation, has been described. It has been shown that the barium hydroxide test is untrustworthy and that the benzidine test is not sufficiently sensitive for the requirements of a pharmacopoeia. The following new test is recommended:

To 15 c.c. of medicinal chloroform contained in a dry stoppered bottle of about 25 c.c. capacity add 20 mgrms. each of resorcinol and vanillin. Stopper, and when the reagents have dissolved, set aside in a dark place for one hour. Add 5 c.c. of 1 per cent. aqueous ammonia solution, shake and allow the mixture to separate. When there has been prior formation of phosgene or in the presence of hydrochloric acid a pink or red colour develops in the aqueous layer, reaching its full value in about 30 seconds; its intensity varies with the quantity of impurity present.

In conclusion I wish to thank Mr. T. T. Cocking, F.I.C., for his helpful criticism and advice, and the Directors of The British Drug Houses, Ltd., for permission to publish this work.

Contamination in Morphine Deposited in the British Pharmacopoeia Process for the Analysis of Opium.

By JITENDRA NATH RAKSHIT, F.I.C.

(Read at the Meeting, May 6, 1931.)

IT was noted (ANALYST, 1919, **44**, 337; 1921, **46**, 482) that the British Pharmacopoeia method of determining morphine in opium is not very accurate. Hence it was thought advisable to find out what are the principal impurities in morphine deposited in this process, and how they can be eliminated.

A large sample of the mixed alkaloids was furnished by the crude morphine deposited in the B.P. (1914) process, being the residues from the analysis of nearly 10,000 samples of pure opium during the last five years. Two hundred grms. of this morphine were heated on a steam bath in a 4-litre flask with 200 c.c. of water, and about 150 c.c. of 30 per cent. (w/v) potassium hydroxide solution were added to dissolve the morphine. The alkaline solution was extracted 6 times with benzene, 500 c.c. being used each time. The last benzene extract did not leave any appreciable residue on evaporation. The united benzene extracts were filtered and distilled until about 100 c.c. were left in the distillation flask. This residual benzene, on slow evaporation, yielded crystals with the characteristics of codeine. The residue was dried for 6 hours in a steam oven, when it weighed 11·3 grms., *i.e.* 5·65 per cent. of the "B.P." morphine.

Five grms. of this dry benzene extract were dissolved in 200 c.c. of 1 per cent. (w/v) hydrochloric acid, heated on a steam bath for an hour and filtered. The filtrate was pink, indicating the presence of porphyroxine (Rakshit, *J. Chem. Soc.*, 1919, **59**, 455; *Ber.*, 1926, **115**, 2473). It was evaporated to dryness, powdered, and re-dissolved in 100 c.c. of the same dilute hydrochloric acid, and the solution was again evaporated to dryness. This process of solution in dilute hydrochloric acid and evaporation on a steam bath was repeated thrice more to complete the decomposition of the porphyroxine and some colouring matter. Finally, the dried salt was dissolved in 100 c.c. of hot water, and the solution was filtered and decolorised with 5 grms. of animal charcoal. The solution was heated again on a steam bath, filtered into a separator, made alkaline with 20 c.c. of 30 per cent. (w/v) potassium hydroxide solution, and thrice extracted with benzene, 100 c.c. being used each time. The united benzene extracts were evaporated until crystals formed. This crystalline residue was dried, powdered, and re-crystallised from 50 c.c. of 30 per cent. (w/v) alcohol, when 3·6 grms. (1st crop.= 3·1 grms. and 2nd crop,=0·5 grm.) of well-formed codeine crystals were obtained.

They gave $[\alpha]_D = -137 \cdot 4°$; m.pt., 155° C.   This sample, when mixed in equal quantities with codeine of known purity, did not alter in m.pt.

It is thus evident that morphine precipitated by the B.P. process is contaminated with about 3·6 per cent. of codeine.   Of course, besides codeine, there are other alkaloids to make up the total of 5·65 per cent. in the impure morphine. In experiments to eliminate such foreign matter by exhaustion with a solvent, batches of 100 grms. each of mixed alkaloids, containing 92 per cent. of pure morphine and 8 per cent. of codeine, prepared from their hydrochlorides by precipitation with dilute ammonia from 1 per cent. aqueous solution, were heated on a steam bath with various solvents, and the solutions were filtered.   All the filtrates were separately evaporated, and the residues were dried and weighed. The results of such repeated extractions with different solvents were as follows:

WEIGHTS IN GRMS. EXTRACTED BY DIFFERENT SOLVENTS FROM 100 GRMS. OF
MIXTURES OF 92 PER CENT. OF MORPHINE AND 8 PER CENT.
OF CODEINE.

No. of extractions.	Water = 500 c.c.	Water Ammonia (10 per cent.) = 50 c.c. 550 c.c.	Water 90 per cent. alcohol = 50 c.c. 550 c.c.	Benzene = 500 c.c.
1	3·40	2·90	1·85	3·04
2	1·87	2·37	1·61	1·46
3	1·51	2·45	1·50	0·52
4	1·05	2·05	1·60	0·60
5	0·86	1·50	1·11	0·57
6	0·75	1·91	0·96	0·15
7	0·51	1·96	0·95	0·07
8	0·51	1·10	0·90	0·07
9	0·65	1·78	0·81	0·06
Total extracts	11·10	18·02	11·29	6·54

Each of these total extracts was re-extracted separately with 50 c.c. of benzene, and the actual benzene extracts obtained were 1·61, 2·16, 2·40, and 5·06 grms. respectively.

The amounts of codeine in the main insoluble residues (chiefly morphine) left after repeated extractions were determined by extraction with benzene from potassium hydroxide solution, with the following results:—(1) 6·5 and 7·2 per cent.; (2) 6·7 and 7·2 per cent.; (3) 5·6 and 5·6 per cent.; and (4) 4·7 and 4·7 per cent.

It is thus evident that benzene removes some codeine from such mixtures, but that the results are far from satisfactory.

It seemed possible that extraction of a lime solution of opium, obtained, as in the B.P. process, with benzene prior to precipitation with ammonium chloride, might give a purer deposit of morphine, all other operations of the method remaining the same.   Accordingly 100 c.c. of a lime solution of opium were prepared

according to the B.P. process, and shaken well with 100 c.c. of benzene in a separator for 30 minutes; the separated aqueous lower layer was filtered and 51 c.c. precipitated with ammonium chloride in the presence of alcohol and ether in accordance with the B.P. (1914) process.   The same samples of opium were also analysed strictly according to the B.P. (1914) process.   The following comparative results were obtained by the two methods:—

	Morphine by	
Sample Nos.	B.P. (1914) method. Per Cent.	B.P. (1914) method, as modified. Per Cent.
1	9·8	10·6
2	9·5	10·5
3	10·4	11·3

When morphine deposited by this modified process was dissolved in potassium hydroxide solution and extracted with benzene, as described above, it gave only 0·3 per cent. of substances soluble in benzene; therefore such morphine is much purer than that given by the original B.P. process.

Annett and Singh (ANALYST, 1918, **43**, 208), when studying the effect of codeine in hindering the precipitation of morphine by ammonia from a solution of its lime compound, obtained deposits of morphine in the presence of codeine. It is not stated, however, whether such morphine was free from contamination by other bases.   By preliminary extractions of a lime solution of opium with benzene a deposit of morphine cleaner and in greater quantity than usual is obtained.   The higher yield need not interfere with the standard correction by 0·051 grm. (B.P. 1914) of the weight indicated by titration, since it has already been shown (Rakshit and D'Costa, ANALYST, 1919, **44**, 337) that this correction figure is too low, at all events for Indian opium.   If the B.P. process were modified in this way, the results would only approach the actual truth.

Dott (ANALYST, 1919, **44**, 50) stated that sufficient ether is present to hold all codeine in solution, but in the presence of two immiscible layers the codeine seems to remain distributed between them in a certain ratio.   Moreover, a good deal of ether [about 10 per cent. (v/v)] remains in the aqueous layer, which obviously increases the solubility of codeine in this layer.   Such codeine, however, appears to become contaminated with morphine during its precipitation in the B.P. process.

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The Use of Bromine as a Reagent in the Determination of Alkaloids.

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(Read at the Meeting, April 1st, 1931.)

THALLEIOQUIN REACTION.—The thalleioquin reaction for quinine has been dismissed as useless from a quantitative point of view (*cf. Allen's Commercial Organic Analysis*, 5th Ed., Vol. VII, p. 449; and W. B. Hart, *J. Soc. Chem. Ind.*, 1921, 72T), but in a critical examination of the test we have found that if it is carried out according to the procedure described below, it forms a simple and accurate method for the determination of this alkaloid. When it was found that the presence of many substances seriously interfered with the reaction it was elaborated and finally became an iodimetric determination.

In the course of the work new quantitative colour tests for other alkaloids were developed, bromine being used as a reagent in all cases. The most important of the tests recorded are those for codeine and emetine.

For convenience, the description of the investigation will be given in three groups:—(1) The cinchona alkaloids; (2) the opium alkaloids; (3) the ipecacuanha alkaloids.

With a view to elucidating the chemistry of the reactions obtained, some further work was undertaken with phenolic bodies. This is dealt with in Section IV of the paper.

PART I. THE CINCHONA ALKALOIDS.

THALLEIOQUIN REACTION.—In the initial experiments with quinine it was, noticed that an excess of halogen (bromine) impaired the delicacy of the thalleioquin reaction, the maximum colour being obtained when the halogen was just in excess. It was also found, in agreement with published procedure, that unless any large excess of bromine was removed the test was useless from a quantitative aspect. Whilst some success was obtained with other methods, it was quickly realised that aeration offered the best means of removing the excess.

QUININE.—The details of the method adopted for quinine are as follows:

The quinine salt (or quinine solution as nearly neutral as possible) is dissolved in 10 c.c. of water in a 25 c.c. graduated measuring cylinder. Four c.c. (or a suitable quantity) of $N/5$ freshly prepared bromine water are added, and the

excess of bromine, after standing 1 minute, is expelled by passing 20 litres of air through the liquid by means of a glass tube with a fine orifice reaching to the bottom of the cylinder, the rate of aeration being approximately 1 litre per minute. The contents of the cylinder are then diluted to 20 c.c. with distilled water and 10 drops of 32·5 per cent. ammonia solution are added. The bulk is made up to 25 c.c. and agitated to ensure uniformity. The solution is set aside for 5 minutes to permit of the maximum colour developing, and the colour obtained is compared with a series of standards made with a solution of a pure quinine salt and treated in exactly the manner described. In actual estimations it is advisable not to use more than 0·02 grm. of quinine, owing to the frothing caused during aeration by the precipitated quinine and bromine compound. After aeration the liquid is clear and of a yellowish colour, the depth of colour being proportional to the quinine content of the solution. In testing, using a series of standards, the ammonia is not added until all the solutions have had bromine added and have been aerated. The colour is thus developed in the standards and test solution simultaneously.

It was found necessary, in comparing the colour obtained, to have standards containing approximately the same amount of quinine as the solution being investigated, as weak solutions of quinine develop a blue colour, whilst strong ones are green. When the colour of the solution has been approximately matched by Nesslerising, the standard whose colour most closely resembles the solution under examination is selected, and the accurate proportion of colour is determined by means of a Duboscq colorimeter. The quantity of quinine is then calculated. A series of standards containing 0·002, 0·005, 0·007, 0·010, 0·015, and 0·020 grm. of quinine was found to be convenient.

The aspirator used in the determination was a 5-litre jar, with the inlet tube reaching to the bottom, of sufficient length to permit, after disconnection from the water tap, of its being used as a syphon to draw off the water without disturbing the sealed cork of the jar. The outlet tube had a fine orifice and was long enough to reach to the bottom of the liquid being aerated. Four jars were used, and after each 5 litres of air had passed, the cylinder was washed down by sluicing some of the liquid into the bubbling tube and letting it run down.

The concentration of the excess bromine was varied over a fairly wide range, and the time of aeration also up to one hour (60 litres of air), but neither variation had any effect on the green colour produced. The time of standing after the addition of bromine and before aeration, however, is important.

In comparative tests with several salts of quinine, viz. the sulphate, acid sulphate, hydrochloride, and acid hydrochloride, the colours obtained were found to be strictly proportional to the quinine content of the various salts used.

Chlorine water was substituted for bromine, and although a deeper colour was obtained while using the same amount of quinine, its use is not recommended, as it was found more difficult to free the solution from excess of halogen, and, also, because the chlorine reagent is usually more acid than bromine. This is a disadvantage, as will be seen later.

The delicacy of the test, if carried out as described, is approximately 1 part of alkaloid in 50,000 parts of water, using 50 c.c. of solution.   This is less sensitive than the maximum delicacy of the test where a bare excess of bromine is present, when a concentration of 1 part of alkaloid in 150,000 is just detectable.   Experiments showed that the maximum delicacy is reached when four atoms of bromine are attached to one molecule of alkaloid, one of them being removable by aeration. For testing purposes, however, aeration is recommended, as uniform conditions are easily obtainable and the test is, therefore, under control.   A slight excess of bromine above the 4 atoms mentioned is inimical to the test, and, in working with concentrations such as 0·0001 grm. of alkaloid in 10 c.c. of solution, ideal bromine conditions are hard to obtain other than by the aeration process described.

As, except in the testing of the various pharmacopoeial salts, a pure quinine solution is rarely met in practice, experiments were continued to investigate the influence of substances usually associated with this alkaloid.   It was found that neutral salts such as sodium sulphate, in moderate concentration, do not interfere, but that ethyl alcohol has a marked influence, as is shown by Table I.

(a) QUININE IN PRESENCE OF ETHYL ALCOHOL.—To vary the alcoholic strength, different proportions of (1) 0·1 per cent. solution of quinine (as sulphate) in water, and (2) 0·1 per cent. solution of quinine (as sulphate) in absolute alcohol were used, aqueous standards of quinine salt being employed for the comparison.

TABLE I.

Quinine taken. Grm.	Alcoholic strength of solution. Per Cent. (by vol.).	Quinine found. Grm.	Percentage found.
0·005	Nil	0·005	100
0·005	10	0·0044	88
0·005	20	0·0033	66
0·005	45	0·0008	16
0·005	75	Nil	—

The results show that the presence of even 10 per cent. (by vol.) of ethyl alcohol renders the method inaccurate, unless the standards are prepared with a quinine solution of an alcoholic strength exactly similar to that of the test.   The alcohol can, of course, be removed by distillation, the residue made up to a sufficient bulk, and a suitable quantity tested as before described.   The explanation of the failure of the test lies in the fact that dilute alcohol retains some of the excess bromine that cannot be expelled by aeration, and at a concentration of 70 per cent. of alcohol (by vol.), this excess is sufficient to mask even a qualitative reaction with 0·005 grm. of the alkaloid in 15 c.c. of solution.

(b) THE EFFECT OF FREE ACID.—Free acid (HCl) has a very marked effect on the strength of colour obtained.   With a solution of 0·005 grm. of quinine in 10 c.c. of $N/10$ acid, not more than 20 per cent. of the colour was obtained.   In 10 c.c. of $N/5$ acid, less than 10 per cent. of the colour was obtained.   If, however, the

free acid is almost neutralised with sodium hydroxide before adding the bromine, the reaction is satisfactory.

(c) IN PRESENCE OF CINCHONIDINE.—When 0·005 grm. quantities of each alkaloid in 15 c.c. of solution were taken, practically 100 per cent. yield was obtained, excepting that the colour was rather greener than when the quinine was used alone, and hence was difficult to match. In the presence of much cinchonine or cinchonidine, standards should be prepared containing approximately the same amounts of these substances.

(d) IN PRESENCE OF STRYCHNINE.—Two solutions were used throughout this series of experiments in order to obtain the various mixtures used:—(1) 0·1 per cent. solution of quinine sulphate; (2) 0·1 per cent. solution of strychnine.

TABLE II.

Quinine taken. Grm.	Strychnine taken. Grm.	Quinine found. Grm.	Percentage of quinine found.
0·005	Nil	0·005	100
0·005	0·0025	0·005	100
0·005	0·005	0·0045	90
0·005	0·010	0·0042	84
0·005	0·015	0·0036	72

After aeration, 10 c.c. of 90 per cent. alcohol were added before making up to 25 c.c. with distilled water. This is necessary, as the addition of ammonium hydroxide precipitates strychnine, which must be dissolved before the colour can be compared. The standards were treated in the same way. It will be seen that, although small quantities of strychnine do not interfere with the colour, as the proportion of strychnine increases the method becomes useless for purposes of determination.

In order to overcome the interference of large quantities of strychnine, the following method was used, 0·05 grm. of strychnine and 0·002 grm. of quinine being taken. These amounts were dissolved in chloroform and the solution was evaporated to dryness in a 7 cm. glass crystallising dish in order to get a mixture of alkaloids such as would be obtained after estimation in analytical practice. The dry alkaloids were treated with 10 c.c. of ethyl ether, and rubbed with a glass rod, and the liquid was filtered through a 5 cm. paper. The residue remaining in the dish, and the rod, were treated with 5 c.c. of ethy ether and filtered through the same paper. The total filtrate was evaporated in a 5 cm. glass crystallising dish. The residue after evaporation (quinine) was dissolved in 1 c.c. of $N/10$ hydrochloric acid, evaporated to dryness, and dissolved in 8 c.c. of hot water. The dish was washed with 2 c.c. of hot water and the contents were transferred to a 25 c.c. glass cylinder and cooled to 20° C. An excess of $N/5$ bromine was added, and the solution was aerated as described, treated with 10 drops of 32·5 per cent. ammonia solution, and made up to 25 c.c. with distilled water; 0·002 grm. of quinine in 15 c.c. solution was placed in a 25 c.c. cylinder, treated

with $N/5$ bromine, aerated, and the colours compared. The quinine found in the ethereal extract of the mixed alkaloids amounted to 0·0019 grm. It is thus seen that the colorimetric method for quinine is applicable, even in the presence of comparatively large quantities of strychnine, if the latter alkaloid is removed (*cf.* A. R. Bliss, *J. Amer. Pharm. Assoc.*, 1919, 804).

The method for the separation of quinine and strychnine described by Norman Evers (*Pharm. J.*, 1922, p. 90), was also investigated at this stage and found to be an excellent one. The quinine obtained by the use of this method was estimated colorimetrically and found to be pure. No reaction for quinine could be obtained with the strychnine separated.

(*e*) QUININE AND CAFFEINE.—Experiments with quinine in the presence of caffeine showed that when 0·002 grm. of quinine was mixed with 0·02 grm. of caffeine no thalleioquin reaction was obtained after aeration with bromine and addition of ammonium hydroxide.

As, from the foregoing experiments, the colour reaction for the determination of quinine was found to have a limited use, and as definite evidence had been obtained of a stable bromo-quinine compound, it was thought that by the addition of potassium iodide to the solution, after treatment with bromine and aeration, it would be possible to titrate the liberated iodine or iodo compound with thiosulphate solution and so make the determination volumetric. Experiments of this nature, as seen from Table III, gave satisfactory results, the tests being carried out at a temperature of 25° C. This is an important point.

The method used was identical with the one previously described for the colorimetric determination of quinine, excepting that a small amount of potassium iodide (approximately 0·5 grm.) was substituted for the 32·5 per cent. ammonia solution, and the quinine-iodo compound was titrated, without filtration, with $N/100$ thiosulphate solution, a 2 per cent. solution of clear soluble starch being used as indicator.

TABLE III.

Quinine used (alkaloid). Grm.	$N/100$ thiosulphate solution required. c.c.
0·003	1·75
0·006	3·60
0·010	5·95
0·015	8·9

From these results it was concluded that 1 c.c. of $N/100$ thiosulphate solution corresponds with 0·00166 grm. of anhydrous quinine (0·00162 grm., equivalent to 2 atoms of iodine, being liberated from 1 molecule alkaloid by reaction to potassium iodide and forming a di-iodo-quinine compound).

Various amounts of strychnine (from 0·001 to 0·010 grm.) were treated in the same way, but no iodine was liberated. When $N/5$ bromine was added to the strychnine solution a bromine compound of strychnine was precipitated in each

case, but where the strychnine was less than 0·006 grm. in 15 c.c. of solution it dissolved on aeration. Even when it remained undissolved no liberation of iodine was observed after aeration on testing with potassium iodide and starch solution.

TABLE IV.

EFFECT OF TEMPERATURE ON IODIMETRIC DETERMINATION OF QUININE.

Quinine taken. Grm.	Temperature of test solution °C.	$N/100$ thiosulphate solution required. c.c.	Quinine found. Grm.	Remarks.
0·006	0°	2·5	0·0041	Experiment carried out as described
0·006	0°	2·95	0·0049	Solution allowed to stand with excess of bromine for 30 mins. before aeration
0·006	15–16°	3·35	0·0056	Solution allowed to stand 15 mins. after addition of bromine before aeration
0·006	23–25°	3·55	0·0059	Solution allowed to stand 1 min. after addition of bromine before aeration
0·006	28°	3·55	0·0059	Do. do. do.
0·006	40°	3·4	0·0056	Do. do. do.
0·006	50°	3·4	0·0056	Do. do. do.

A temperature of 25 to 28° C. is most favourable for the maximum liberation of iodine, and this temperature was, therefore, used throughout the work detailed in this paper. At this temperature, on the addition of potassium iodide, 2 atoms of iodine are liberated and can be titrated with thiosulphate solution.

TABLE V.

QUININE AND STRYCHNINE: IODINE METHOD.

Quinine taken. Grm.	Strychnine taken. Grm.	$N/100$ thiosulphate solution required. c.c.	Quinine found. Grm.	Remarks.
0·005	Nil	3·05	0·00506	Cleared after aeration
0·010	0·001	6·05	0·01004	Do. do.
0·005	0·002	3·05	0·00506	Do. do.
0·005	0·005	3·1	0·00514	Did not clear completely on aeration. Titrated slowly, as precipitate seemed to dissolve slowly
0·005	0·010	3·1	0·00514	Do. do. do.

It is seen that the method yielded good results with a mixture of quinine and strychnine.

END-POINT OF TITRATION WITH MIXTURES OF QUININE AND STRYCHNINE.— The titration should be carried out slowly. The starch should be added approximately at the stage of 1 c.c. before the end of the titration with $N/100$ thiosulphate

solution. The solution at this stage has a slight precipitate of the quinine-strychnine-iodine compound and is very yellow. On the addition of starch, the solution becomes blue-green. The $N/100$ thiosulphate solution is added, 2 drops at a time, and the solution gradually loses its colour, becoming yellow, and also clears appreciably. The end-point is taken when no white rings are visible on the addition of 2 drops of $N/100$ thiosulphate solution. The end-point is very notice-able on looking at the solution against a white ground immediately after the addition of the thiosulphate. The quinine compound appears to give up its iodine véry slowly in the presence of strychnine. On this account the titration rate near the end-point is about 2 drops in 15 seconds. The starch must be clear and freshly made, and must not be added too early in the titration.

ALTERNATIVE END-POINT METHOD.—If, after the aeration and addition of potassium iodide, a slight excess of $N/100$ thiosulphate solution is added and the test is put aside for five minutes in order to permit the mixed iodine compounds to dissolve, and the slight excess of thiosulphate is then titrated with $N/100$ iodine solution; a sharp end-point is obtained. In carrying out this test for a mixture care must be taken to observe the following conditions:—(1) After the addition of bromine not more than about a minute should be allowed to elapse before aeration is begun, as otherwise the precipitated strychnine and bromine compound settles and is difficult to get into solution; and (2) a fair excess of bromine must be added before aeration. If this is not done, the strychnine may deprive the quinine of some of its bromine and the result will be low.

TABLE VI.

QUININE AND CAFFEINE TESTED IN 15 C.C. OF SOLUTION AT A
TEMPERATURE OF 25° C.

Quinine taken. Grm.	Caffeine taken. Grm.	$N/100$ thiosulphate solution required. c.c.	Quinine found. Grm.	Remarks.
0·003	Nil	1·75	0·0029	Experiment carried out as de-scribed
0·003	0·005	1·7	0·0028	Do. do. do.
0·003	0·015	1·3	0·0021	Do. do. do.
0·003	0·025	0·75	0·0012	Do. do. do.
0·003	0·050	0·45	0·0007	Do. do. do.
0·003	0·100	0·15	0·0002	Do. do. do.
0·003	0·025	1·75	0·0028	Allowed to stand 10 minutes after addition of bromine before aeration
0·006	0·025	3·55	0·0059	Do. do. do.

In the presence of much caffeine it is necessary to add a large excess of bromine and to allow the solution to stand for 10 minutes before aeration, as otherwise the caffeine absorbs the bromine at the expense of the quinine, and it is necessary, therefore, to saturate both. If these conditions are observed, the method is quite

good for comparatively small amounts of quinine in the presence of large amounts of caffeine. If desired, however, the caffeine can be separated from the quinine by extraction with chloroform from acid solution before making the test on the quinine.

Caffeine in the form of citrate was found to behave like the free alkaloid.

TABLE VII.

AMOUNT OF BROMINE REQUIRED FOR DETERMINATION OF QUININE IN MIXTURES OF QUININE AND CAFFEINE.

Quinine taken. Grm.	Caffeine taken. Grm.	$N/5$ bromine added. c.c.	$N/100$ thiosulphate solution required. c.c.	Remarks.
0·006	nil	0·6	2·75	Not sufficient bromine added
0·006	nil	1·0	3·6	No standing after addition of bromine before aeration
0·006	nil	1·0	3·6	Allowed to stand 10 minutes before aeration
0·006	nil	2·0	3·6	No standing before aeration
0·006	nil	2·0	3·55	Allowed to stand 10 minutes before aeration
0·006	0·025	2·0	2·6	Colour disappeared on standing 3 minutes; insufficient bromine
0·006	0·025	4·0	3·0	Allowed to stand 10 minutes before aeration
0·006	0·025	6·0	3·5	Do. do. do.
0·006	0·025	8·0	3·55	Do. do. do.

Table VII shows that in the presence of caffeine care must be taken to have an excess of bromine present. One c.c. of $N/5$ bromine should be present for every 0·006 grm. of quinine, and for every 0·003 grm. of caffeine. Although none of the absorbed bromine in the case of caffeine reacts on the addition of potassium iodide, it seems necessary to saturate this alkaloid, or it will deprive the quinine of its more loosely combined halogen, and a low result will be obtained.

TABLE VIII.

QUININE IN PRESENCE OF COCAINE.

Quinine taken. Grm.	Cocaine taken. Grm.	$N/100$ thiosulphate solution required. c.c.	Quinine found. Grm.
0·006	nil	3·55	0·0059
0·006	0·005	3·55	0·0059
0·006	0·02	3·6	0·0060

TABLE IX.

QUININE IN PRESENCE OF ATROPINE.

Quinine taken. Grm.	Atropine taken. Grm.	N/100 thiosulphate solution required. c.c.	Quinine found. Grm.
0·006	nil	3·6	0·0060
0·006	0·02	3·6	0·0060
0·006	0·05	6·3	0·0060

TABLE X.

QUININE IN PRESENCE OF CINCHONINE.

Quinine taken. Grm.	Cinchonine taken. Grm.	N/100 thiosulphate solution required. c.c.	Quinine found. Grm.
0·006	nil	3·55	0·0059
0·006	0·005	3·6	0·0060
0·006	0·010	3·6	0·0060

TABLE XI.

QUININE IN PRESENCE OF CINCHONIDINE.

Quinine taken. Grm.	Cinchonidine taken. Grm.	N/100 thiosulphate solution required. c.c.	Quinine found. Grm.
nil	0·010	nil	—
0·006	nil	3·5	0·0059
0·006	0·010	3·7	0·0062

From Tables VIII, IX, X, and XI it will be seen that the method can be used to determine quinine in the presence of cocaine, atropine, cinchonine and cinchonidine.

OTHER CINCHONA ALKALOIDS.—As the reaction permitted of the satisfactory determination of quinine in a mixture of other alkaloids, it was thought that the other cinchona alkaloids giving the thalleioquin reaction would also be capable of determination in a similar manner to quinine. The reaction was tested on cupreine, with satisfactory results, as shown in Table XII.

TABLE XII.

Cupreine Grm.	N/100 thiosulphate solution required. c.c.
0·006	3·75
0·010	6·3

Thus, 1 c.c. of $N/100$ thiosulphate solution equals 0·00158 grm. of anhydrous cupreine. Theoretically 1 c.c. should equal 0·00155 grm.

It seems most probable that the reaction would also be given by quinidine, hydroquinine, hydroquinidine and diquinicin, but these have not yet been tested.

SUMMARY OF WORK ON CINCHONA ALKALOIDS.—(1) The thalleioquin reaction has been made quantitative for the determination of quinine and its salts.

(2) The quantitative reaction is unreliable in the presence of such substances as free acid, alcohol, and various alkaloids, *e.g.* strychnine, and caffeine.

(3) An iodimetric development of the method is described which permits of the estimation of quinine in the presence of other alkaloids, *viz.* strychnine, caffeine, cocaine, atropine, etc.

(4) The method has been applied to cupreine, with satisfactory results, and it is thought that it would be applicable to those other members of the cinchona group which give the thalleioquin reaction, such as quinidine, hydroquinine, etc.

PART II. THE OPIUM ALKALOIDS.

During the preceding investigation it was observed that the presence of morphine seriously interfered with the thalleioquin reaction, and, on applying the iodine development of this test, it was found that, if suitable conditions were observed, morphine reacted in a somewhat similar manner to quinine, *i.e.* 2 atoms of iodine were liberated on addition of potassium iodide to the aerated bromine compound. The tests described in Table XIII were conducted at a temperature of 25° to 28° C., with a view to determining the details of the method to be adopted for the determination, the morphine being present as hydrochloride.

TABLE XIII.

Morphine (alkaloid). Grm.	Time of standing after aeration and addition of potassium iodide.	Quantity of acid added after aeration	$N/100$ thiosulphate solution required. c.c.	Remarks.
0·005	nil	nil	2·4	Indefinite end-point; comes back
0·005	5 mins.	nil	2·8	Do. do.
0·005	5 ,,	1 c.c. of $N/10$ HCl	3·1	Do. do.
0·005	15 ,,	1 c.c. do.	3·26	Do. do.
0·005	15 ,,	1 c.c. of $N/1$ HCl	3·4	Fairly good end-point
0·005	30 ,,	1 c.c. do.	3·45	Do. do.
0·005	60 ,,	2 c.c. do.	3·55	Satisfactory end-point
0·010	60 ,,	2 c.c. do.	7·15	Do. do.

From the results obtained satisfactory details of the application of the test were evolved as follows:

The alkaloid in the form of a salt in 15 c.c. of a solution, is made as nearly neutral as possible, placed in a 25 c.c. graduated measuring cylinder, and treated with an excess of $N/5$ bromine (freshly prepared). After 1 minute the excess of halogen is removed by aeration, as described for quinine (20 litres of air passed through at the rate of approximately 1 litre per minute). After aeration, 2 c.c. of N hydrochloric acid and excess of potassium iodide (approximately 0·5 grm.) are added, and the corked solution is set aside for 1 hour. A few drops of clear freshly prepared starch solution are then added, and the liberated iodine is titrated with $N/100$ thiosulphate solution. A good end-point is obtained.

Strict attention must be paid to the time of standing, the temperature, and the concentration of the acid used. The liberation of iodine corresponds with 2 atoms of iodine, being liberated by the morphine-bromine compound, after aeration, on the addition of potassium iodide. Therefore, 1 c.c. of thiosulphate solution equals 0·00142 grm. of alkaloid.

In an actual test, a 2·5 per cent. solution of morphine hydrochloride, as submitted to the laboratory for the determination of the alkaloidal strength, was taken. One c.c. of this solution was diluted to 15 c.c. with distilled water and tested as outlined above. It required 12·9 c.c. of $N/100$ thiosulphate solution, equivalent to 0·0244 grm. of morphine hydrochloride (containing 3 molecules of water of crystallisation). Morphine and atropine tablets were also tested by this method with excellent results, the atropine present apparently making no difference in the determination of the morphine.

DIFFERENCES OBSERVED IN QUININE AND MORPHINE REACTIONS.—Morphine in solution at the concentration tried, does not give a bromine alkaloid precipitate. After aeration the solution is of a very much more pronounced yellow colour than a quinine solution of the same strength.

DETERMINATION OF QUININE AND MORPHINE MIXTURE.—By comparison of the yellow colour obtained after aeration of the bromine alkaloid compound with standards prepared in the same manner, it was found that the morphine could be determined with an accuracy of 5 per cent. of the amount present. In a mixture of quinine and morphine the titration of the liberated iodine gives the total alkaloid. By varying the proportions of quinine and morphine within this amount and comparing the yellow colour produced after the addition of bromine and aeration, the quantity of each alkaloid could be determined.

A further development of this test is as follows:—On the addition of slight excess of $N/5$ bromine solution and then of excess of hydrogen peroxide (3 per cent. solution) to a dilute morphine solution, the excess of bromine is removed, but the solution remains yellow in proportion to the quantity of morphine present. This is determined by taking 0·5, 1, 1·5, 2 and 2·5 c.c. of a solution containing 0·001 grm. of morphine per c.c. in matched test tubes, and, after making up to 3 c.c. with distilled water, adding 2 to 3 drops in excess of $N/5$ bromine, than 1 c.c. of 3 per cent. hydrogen peroxide, setting aside for 2 minutes with occasional shaking, and then transferring with washing to a Nessler glass, making up to 50 c.c. with

distilled water, and comparing the colours produced. On adding, drop by drop, $N/10$ sodium hydroxide solution, or very dilute ammonium hydroxide (5 drops of 0·4 N), a red colour develops which is proportional to the amount of morphine present. Dilute alkali must be used, as the colour fades in the presence of strong alkali.

Codeine.—Codeine does not give a yellow colour with bromine and hydrogen peroxide, but develops a red colour after the addition of alkali. Table XIV shows the behaviour of common alkaloids in the test, 0·003 grm. of alkaloid to 3 c.c. of solution being taken and treated with bromine and peroxide as described above.

TABLE XIV.

Alkaloid 0·003 grm.	Yellow colour with $N/5$ bromine and hydrogen peroxide.	Red colour with alkali.
Morphine	Yes	Yes
Codeine	No	Yes
Strychnine	No	Very faint; rapidly disappears
Quinine	Faint; one-tenth that of morphine	No; becomes yellow
Veratrine	No	No
Atropine	No	No
Cinchonidine	No	No
Narcotine	Slight on filtration	No, but alkaloid precipitates
Narceine	No	Strong yellow, suggesting orange
Thebaine	No	Slight pink
Papaverine	Slight yellow. Heavy precipitate: does not clear with hydrogen peroxide until warmed.	No
Meconic acid	No colour	No
Emetine	Yes	No
Cephaeline	Yes	No

The presence of various alkaloids in admixture with morphine, did not seem to interfere with the depth of colour produced, *i.e.* 0·002 grm. of morphine, plus 0·002 grm. of strychnine, gave the same colour as 0·002 grm. of morphine alone. By means of the combined tests described it was often possible to determine the percentage of three alkaloids in a mixture.

Returning to the bromine aeration test, Table XV shows the behaviour of morphine in the presence of quinine, the test being carried out as described for morphine.

TABLE XV.

Morphine taken. Grm.	Quinine taken. Grm.	$N/100$ thiosulphate solution required. c.c.
nil	0·006	3·6
0·005	nil	3·55
0·005	0·006	7·15

Table XVI gives the results obtained with a mixture of morphine and strychnine.

TABLE XVI.

Morphine taken. Grm.	Strychnine taken. Grm.	$N/100$ thiosulphate solution required. c.c.
0·003	0·005	2·3
0·005	0·005	3·7

In the presence of strychnine, the morphine result will be a little on the high side (0·005 grm. being estimated as 0·0052 grm.). The same conditions must be observed with a morphine and strychnine mixture as were described for the quinine and strychnine mixture in regard to the end-point of the titration.

CODEINE.—To the dilute solution of codeine salt, excess of $N/5$ bromine is added, and the solution is aerated as described for morphine. Two c.c. of N hydrochloric acid and potassium iodide solution are then added, and the solution is set aside for 60 minutes. A slight excess of $N/100$ thiosulphate solution is added, and the solutions are allowed to stand for 3 minutes. The excess of thiosulphate is titrated with $N/100$ iodine (starch as indicator), the experiment being carried out at 28° C.

TABLE XVII.

Codeine taken. Grm.	$N/100$ thiosulphate solution required. c.c.
0·005	3·3
0·01	6·5
0·015	9·9

Codeine behaves similarly to morphine, i.e. 2 atoms of iodine are liberated after the addition of bromine, and aeration, and the addition of potassium iodide and N hydrochloric acid.

DIFFERENCES OBSERVED BETWEEN CODEINE AND MORPHINE.—At the dilution used codeine gives a precipitate with excess of bromine; morphine does not. On the addition of potassium iodide after aeration, codeine gives a precipitate; morphine does not. After aeration the colour of a codeine solution is pink in proportion to the quantity of alkaloid present. This colour disappears on the addition of acid, leaving the solution a very slight yellow. The pink coloration was confirmed with several codeine salts, e.g. the phosphate and hydrochloride. After passing the first 5 litres of air, no colour was noticed; with 10 litres passed, a slight pink coloration was obtained; with 15 litres, the intensity was much increased. The effect of oxidation observed on the codeine and bromine compound in this experiment led us to try the effect of other oxidising agents, and a new colorimetric determination for codeine was developed as follows:

The solution of codeine phosphate contained in 1 c.c. the equivalent of 0·001 grm. of codeine. Quantities of 0·5, 1·0, 1·5, 2 and 2·5 c.c. of this solution

were placed in matched 25 c.c. test tubes, and each made up to 3 c.c. with distilled water. A slight excess of $N/5$ bromine was added, drop by drop, until the yellow colour persisted, about 2 drops excess being added. One c.c. of 3 per cent. hydrogen peroxide was added to each tube, and they were then placed in a water bath at a temperature of 70° to 80° C. for 6 minutes, when the maximum pink colour developed. The solutions were cooled, transferred with wash water to a 50 c.c. Nessler glass, and made up to 50 c.c. with distilled water, and the colours were compared. The depths of colour were found to be strictly proportional to the amounts of codeine present. The limit of the test was found to be 1 part in 25,000 of alkaloid, when using 2 c.c. of this solution for the test, 2 drops of $N/5$ bromine and 1 c.c. of 3 per cent. hydrogen peroxide. The solution should be practically neutral before testing.

EFFECTS OF OTHER ALKALOIDS ON THE TEST.—Morphine and codeine, when mixed, give an orange colour due to the red of the codeine and the yellow of the morphine. Knowing the total concentration of the alkaloid in the solution as determined iodimetrically, it was found possible to estimate the percentages of morphine and codeine from this colour.

Strychnine in the proportion 0·0025 grm. to 0·005 grm. of codeine did not interfere.

Quinine interferes, as is shown by the following results:

Codeine	..	0·0005 grm.:	Quinine	..	0·0025:	No pink coloration.
,,	..	0·001 ,,	,,	..	0·001 :	Positive, but reduced reaction.
,,	..	0·001 ,,	,,	..	0·002 :	Faint coloration just noticeable.

The colorimetric determination of morphine and codeine in a mixture can be made, as described previously, by using the yellow colour developed on the addition of bromine and peroxide for the determination of the morphine, and the red colour on the addition of alkali for the determination of the total alkaloid.

NARCEINE.—The method of determination was similar to that for morphine; 0·015 grm. of the alkaloid in the form of hydrochloride required 10·9 c.c. of $N/100$ thiosulphate solution, and 0·010 grm. required 7·2 c.c. of $N/100$ thiosulphate. From these results it was concluded that the equivalent of 3 atoms of iodine is liberated on the addition of potassium iodide to the narceine and bromine compound after aeration.

THEBAINE.—On treating a solution of 0·005 grm. of the alkaloid in the form of hydrochloride in the same way as for morphine, 5·0 c.c. of $N/100$ thiosulphate were required; 0·010 grm. required 9·9 c.c. of $N/100$ thiosulphate. From these results it was concluded that the equivalent of 3 atoms of iodine is liberated on the addition of potassium iodide to the thebaine and bromine compound after aeration.

PAPAVERINE.—On the addition of bromine to a dilute aqueous solution of a salt of the alkaloid, a copious precipitate forms which does not clear on aeration. The method described is not satisfactory for the determination of this alkaloid.

NARCOTINE.—On the addition of bromine to a dilute aqueous solution of a salt of the alkaloid, a copious precipitate forms which does not clear on aeration. The method described is not satisfactory for the determination of this alkaloid. From the results of the experiment with papaverine and narcotine, it would appear that neither of these alkaloids reacts like morphine. Only a very small liberation of iodine from the bromine and alkaloid compound results on the addition of potassium iodide to either of these substances.

MECONIC ACID.—Although good end-points were obtained, on treating dilute solutions of meconic acid in the manner described for morphine, some irregularity was observed in the amount of $N/100$ thiosulphate solution required. It was concluded that in order to obtain concordant results with this substance, a period of more than one minute must elapse after the addition of $N/5$ bromine before the aeration. Very little increase in the quantity of thiosulphate solution used was observed after 5 minutes' standing, as Table XVIII shows, and, therefore, this time was adopted as the most suitable for the estimation.

TABLE XVIII.

Meconic acid taken. Grm.	Time of standing with excess of $N/5$ bromine before aeration. Minutes.	$N/100$ thiosulphate required. c.c.
0·005	1	3·5
0·005	5	4·1
0·005	10	4·2
0·005	30	4·3
0·010	5	8·3

Although within this time period there is no definite molecular liberation of iodine, consistent results are obtainable, and 1 c.c. of $N/100$ thiosulphate is equivalent to 0·00120 grm. of meconic acid. With this modification, the determination of meconic acid can be carried out as described for morphine.

MORPHINE DERIVATIVES AND SUBSTITUTES.—*Heroin*.—To an aqueous solution of heroin hydrochloride containing 0·015 grm. of anhydrous heroin, excess of $N/5$ bromine was added, and the test carried out as described for morphine, 2·8 c.c. of $N/100$ thiosulphate being required; 0·005 grm. of heroin required 0·9 c.c.

It was, therefore, decided that, although heroin does not give an exact molecular liberation of iodine under the conditions of the test, comparative results are obtainable. It was further observed that the bromine compound, after aeration, has not the yellow colour of the morphine compound. It was also found that, after the addition of slight excess of bromine, hydrogen peroxide and dilute alkali, as described in the determination of morphine, a red coloration was obtained.

Apomorphine.—The solution contained in 1 c.c. apomorphine hydrochloride equivalent to 0·001 grm. apomorphine. On the addition of $N/5$ bromine to a dilute aqueous solution, a strong red colour, or red precipitate, is given according

to the concentration of the solution. This precipitate does not dissolve on aeration, and, therefore, the iodo-volumetric method of determination is useless. The following procedure, however, gave a satisfactory colorimetric determination:

To a series of very dilute solutions containing, respectively, 0·0002, 0·0004, 0·0007, and 0·001 grm. of alkaloid in 3 c.c. of solution, $N/5$ bromine was added, drop by drop, until 2 or 3 drops in excess. This excess can be readily observed by the yellowing of the red colour produced. One c.c. of 3 per cent. hydrogen peroxide was then added to destroy the excess of bromine. It was found that the solutions were coloured red in proportion to the quantity of alkaloid present. This series of standards diluted to 50 c.c. with distilled water, was used to determine the quantity of alkaloid in an unknown solution of apomorphine treated in exactly the manner described. When the test was carried out in matched test tubes, using the same amounts of alkaloid as previously, and, after the addition of $N/5$ bromine and hydrogen peroxide, the tubes were heated to approximately 95° C. by immersion in a water-bath for exactly four minutes, it was found that the red colour was changed to a permanent green, the depth of which was proportional to the amount of alkaloid present. The solutions were then cooled and diluted to 50 c.c., and Nessler glasses were used for the comparison of colour. As the time of heating is important, both the standards and the sample under examination should be heated for exactly the same time in the same bath.

Ethyl morphine.—The volumetric method described for morphine is suitable for the determination of this derivative, 0·005 grm. requiring 3·1 c.c. of $N/100$ thiosulphate. The bromine derivative, after aeration, is not yellow, in this respect differing from morphine. On the addition of $N/5$ bromine, hydrogen peroxide and dilute alkali, a colour similar to that given by morphine develops. This substance was the only one encountered during this investigation which gave the same pink reaction as codeine on treatment with bromine and hydrogen peroxide and heating to 70° C. for six minutes. This test, if carried out as described for codeine, can be used for the quantitative colorimetric estimation of ethyl morphine.

EXPERIMENTS WITH OPIUM.—With the idea of using the volumetric method described as a means of determining the amount of morphine and codeine in opium, an artificial solution of opium was prepared, containing, in the form of salts, 0·10 grm. of morphine, 0·06 grm. of narcotine, 0·05 grm. of meconic acid, 0·01 grm. of papaverine, 0·005 grm. of codeine, and 0·005 grm. of thebaine. This solution was made up to 25 c.c. with distilled water, and made slightly acid to litmus with $N/10$ hydrochloric acid. The solution was then extracted with 10 c.c. of chloroform, and a further 5 c.c. of chloroform, each portion of chloroform being washed in succession with 5 c.c. of distilled water. The wash water was added to the original solution. The chloroform was evaporated to dryness, and the residue was weighed (0·0724 grm.). This represented narcotine, papaverine and thebaine. No reaction with formaldehyde and sulphuric acid could be obtained on the extract, showing the absence of morphine. A very slight reaction for meconic acid was obtained with ferric chloride. To the warm aqueous solution, after extraction

with chloroform, was added, drop by drop, 1·5 c.c. of 10 per cent. neutral lead acetate solution, this precipitating the meconic acid as lead meconate. The bulk of the liquid and precipitate was now made up to 45 c.c. with distilled water, and 40 c.c. of this were filtered, representing eight-ninths of the original solution. Hydrogen sulphide was passed through the warm filtrate for ten minutes. The precipitated lead sulphide was filtered off on a 7 cm. paper, and the filtrate was collected in a 100 c.c. graduated cylinder. The precipitate was washed three times with 10 c.c. of distilled water, and the total bulk of the solution, when cool, was made up to 89 c.c. with distilled water. Ten c.c. of this solution (equal to one-tenth of the original morphine and codeine added) after aeration with 10 litres of air to expel any hydrogen sulphide, were treated with excess of $N/5$ bromine and aerated as described for morphine. Potassium iodide and 2 c.c. of N hydrochloric acid were added, and the solution was set aside for one hour; 7·8 c.c. of $N/100$ thiosulphate solution were required for titration. This result is fairly good, as the quantities of morphine and codeine present should have required 7·5 c.c. Experiments with powdered opium, however, were not so satisfactory, the chief difficulty experienced being the solution of the alkaloids. Investigation is now proceeding on this point, and it is hoped shortly to describe a quick, reliable volumetric method for the determination of morphine and codeine in opium.

SUMMARY OF THE WORK ON OPIUM ALKALOIDS.—(1) A volumetric method is described, involving the use of the bromine and morphine compound obtained after aeration with excess bromine. From this compound the iodine liberated on the addition of potassium iodide is determined by means of thiosulphate solution. Codeine, narceine, thebaine and meconic acid can also be determined by this method. Narcotine and papaverine do not give satisfactory results, probably owing to the insolubility of the bromine compound formed. The method is applicable to the estimation of morphine in tablets, etc.

(2) A new colour reaction is described for the detection and determination of codeine, depending on the depth of the red colour formed by the oxidation of the bromine compound with hydrogen peroxide, and warming to 60° to 70° C. Ethylmorphine also gives this reaction.

(3) A method is described for the determination of morphine and codeine, depending on the yellow colour developed by morphine after the addition of bromine and hydrogen peroxide. On the addition of dilute alkali a red coloration, proportional to the amount of alkaloid present, is obtained.

PART III. THE IPECACUANHA ALKALOIDS.

EMETINE.—This alkaloid, tested in a manner similar to that described for the determination of morphine, behaved, under definite conditions, as if 2 atoms of iodine were liberated on the addition of potassium iodide to the aerated bromine and alkaloid compound, but the method, on further investigation, did not prove accurately quantitative. Much frothing was observed during the aeration, especially after the greater part of the excess of bromine had been removed. This

was apparently produced by the formation of a saponin-like substance during the course of the reaction. As the bromine is slowly removed by aeration the solution becomes colourless. After the decolorisation is practically complete, a deep yellow colour, very much more intense than that of the morphine and bromine compound, develops. This colour reaches its maximum after 20 to 25 litres of air have been passed. The intensity of the yellow colour was found to be proportional to the amount of alkaloid present, and could be used for its determination if the following conditions were observed:—

(1) After the addition of excess of $N/5$ bromine, the solution is set aside for complete absorption of the halogen before aeration.

(2) After aeration, further standing of one hour is required for development of the maximum intensity.

(3) The concentration of the solution under examination should not exceed 0·003 grm. of alkaloid in 15 c.c. of solution, *i.e.* 0·02 per cent.

(4) The temperature at which the experiment is carried out should be 25° C.

The details of the colorimetric method evolved for the determination of emetine are as follows:

Fifteen c.c. of the alkaloid, in the form of a solution of a salt, are placed in a 100 c.c. graduated measuring cylinder, and treated with excess of $N/5$ bromine. The solution is allowed to stand at a temperature of 25° C. for 20 minutes after the addition of the bromine; 25 litres of air are then passed through the solution at the rate of one-half litre per minute, the sides of the cylinder and the aerating tube being washed down after each 5 litres of air are passed, by sucking the liquid up into the bubbling tube and sluicing the cylinder and tube with it. After aeration is complete, the solution is set aside for 1 hour at 25° C. for complete development of the colour. Fifty c.c. of strong alcohol are then added to dissolve any slight precipitate, and the solution is made up to 100 c.c. with distilled water. It is then transferred to a Nessler glass. The colour obtained is compared with those of a series of standards prepared with varying amounts of pure emetine hydrochloride which have been treated with bromine in exactly the manner described, or it can be compared with a single standard in a Duboscq colorimeter. Fairly good results were obtained by using as a standard a $N/200$ solution of iodine to which 2·5 per cent. of sodium nitroprusside had been added (*i.e.* a mixture of equal parts of $N/100$ iodine solution and 5 per cent. solution of sodium nitroprusside). In making the comparison in this way 100 c.c. of 50 per cent. (by vol.) alcohol, containing approximately 0·5 grm. of potassium iodide, are put into a Nessler tube and the iodine and nitroprusside solution is added, drop by drop, from a pipette, the liquids being well mixed and compared after each addition, until the colour of the unknown solution is matched. Using this method it was found that:

0·001 grm. of emetine required 1·4 c.c. iodine and nitroprusside solution.
0·002 ,,   ,,   ,,   2·7 ,,   ,,   ,,   ,,   ,,
0·003 ,,   ,,   ,,   4·0 ,,   ,,   ,,   ,,   ,,

It was also found inadvisable to compare a greater depth of colour than that given by a 2 c.c. addition of iodine and nitroprusside solution, as, if this amount were exceeded, the colours of the standard and the solution diverged somewhat, making comparison difficult. A solution of stronger colour should be diluted with 50 per cent. alcohol until it is within this limit.

CEPHAELINE.—Cephaeline was found to behave in exactly the same manner as emetine, and by comparison with the iodine and nitroprusside standard it was found that the colours developed by the bromine cephaeline compound on aeration were as follows:

0·001 grm. of cephaeline required 1·4 c.c. iodine and nitroprusside solution.
0·002 ,,   ,,   ,,   2·7 ,,   ,,   ,,   ,,   ,,
0·003 ,,   ,,   ,,   5·2 ,,   ,,   ,,   ,,   ,,

PSYCHOTRINE.—Psychotrine was not investigated, but from its chemical structure it is likely that it would behave in a similar manner to emetine and cephaeline.

Based upon the results obtained from the investigation of emetine and cephaeline, the following colorimetric method is recommended for the determination of ipecacuanha alkaloids in such preparations as liquid extract of ipecacuanha, B.P.:

A suitable quantity (1 c.c.) is diluted to 100 c.c. by the addition of distilled water and thoroughly shaken. Ten c.c. are accurately pipetted into a 100 c.c. Nessler glass. This represents approximately 0·002 grm. of ipecacuanha alkaloid. Excess $N/5$ bromine (3 c.c.) is added, and the solution set aside for 20 minutes at a temperature of 25° C. Twenty-five litres of air are bubbled through at approximately one-half litre per minute, and the yellow solution is then set aside for 1 hour. The contents are made up to 50 c.c. with distilled water, and 50 c.c. of strong alcohol are then added, and the volume finally adjusted to 100 c.c. with distilled water (A SOLUTION).

Another 10 c.c. of the diluted ipecacuanha solution is transferred to a 100 c.c. Nessler glass and diluted to 50 c.c. with distilled water. Fifty c.c. of strong alcohol are added, and the solution is adjusted to 100 c.c. with distilled water (B SOLUTION).

Another Nessler glass is then taken containing 0·5 grm. of potassium iodide in 100 c.c. of distilled water.* To this solution is added, drop by drop, $N/200$ iodine solution containing 2·5 per cent. sodium nitroprusside, stirring well after each addition. The amount added to match B Solution accurately is noted, and the addition continued until A Solution is also matched. The number of c.c. of iodine and nitroprusside solution required to match A Solution, minus the number

* NOTE.—To avoid the formation of air bubbles caused by shaking the standard after each addition of iodine and nitroprusside solution it was found more suitable to use one-half per cent. of potassium iodide in water for the standard in place of 50 per cent. alcohol. For very accurate work, a known solution of emetine hydrochloride should be used as a standard, adding $N/5$ bromine and aerating in exactly the same manner as with the solution under investigation.

of c.c. required to match B Solution, divided by 1·4, equals the weight of ipecacuanha alkaloid in mgrms. in 0·1 c.c. of the original ipecacuanha, or:

$$\frac{A-B}{1\cdot4} = \text{percentage of ipecacuanha alkaloid in the original strong solution.}$$

SUMMARY OF WORK ON IPECACUANHA ALKALOIDS.—(1) Emetine and cephaeline cannot be accurately determined by the titration with sodium thiosulphate of the iodo compound formed by the addition of potassium iodide to the aerated bromine and alkaloid compound.

(2) A reliable means of determination is a modification of this method, using the depth of yellow colour developed by the aeration of the bromine-alkaloidal compound.

(3) Used as a qualitative test, this reaction is sensitive to 1 part of alkaloid in 25,000 parts of solution. In making the test it should be noted that the colour first disappears and then slowly attains a maximum. This reaction is characteristic of the ipecacuanha alkaloids.

(4) A colorimetric method is described for the determination of the ipecacuanha alkaloids in such preparations as liquid extract of ipecacuanha B.P.

PART IV. GENERAL APPLICATION OF THE TEST TO SUBSTANCES CONTAINING A PHENOLIC GROUPING.

PHENOL.—Pure phenol (6·97 grms.) was dissolved in 1 litre of distilled water. Ten c.c. of this solution were diluted to 100 c.c. with distilled water (1 c.c. = 0·000697 grm. of phenol).

To 1 c.c. of the dilute solution, placed in a 25 c.c. graduated cylinder, were added 12 c.c. of distilled water and 2 c.c. of $N/5$ bromine. After 1 minute it was aerated by the passage of 10 litres of air at the rate of 1 litre per minute, at a temperature of 20° C. The solution did not clear on aeration, but contained a flocculent white precipitate. This precipitate tended to creep up the cylinder, but this could be prevented by gently agitating the cylinder during aeration. After aeration was completed, the contents of the cylinder were transferred to a 100 c.c. separator, through a small glass funnel. The cylinder was washed with three 10 c.c. portions of distilled water, and then with 2 c.c. of chloroform, the latter being dropped from a pipette on to the funnel, and finally with 5 c.c. distilled water. Approximately 0·5 grm. of potassium iodide was then added to the solution in the separator and shaken. The phenol-bromine compound goes into solution in the chloroform, and the liberated iodine is titrated with $N/100$ thiosulphate, with starch as indicator. If chloroform is not used, the end-point is doubtful, owing to the presence of the flocculent precipitate mentioned. In this way the following results were obtained, the solution being made up to 15 c.c. with distilled water in each case before the bromine was added:

1 c.c. of phenol solution required 1·45 c.c. of $N/100$ thiosulphate.
2 ,, ,, ,, ,, 2·95 ,, ,, ,,
4 ,, ,, ,, ,, 5·9 ,, ,, ,,
10 ,, ,, ,, ,, 14·7 ,, ,, ,,

Therefore, phenol can be determined with accuracy by the method described, 1 c.c. of $N/100$ thiosulphate solution equalling 0·000474 grm. of phenol; *i.e.* 2 atoms of iodine are liberated on the addition of potassium iodide to the aerated bromine and phenol compound.

In order to ascertain if the bromo-phenol compound was volatile, 50 litres of air were passed through a solution containing 4 c.c. of the diluted phenol treated as described. This required 5·5 c.c. of $N/100$ thiosulphate solution. The compound is, therefore, slightly volatile, but, if the determination is carried out exactly as described, the loss is practically negligible. A correction can be made if desired.

META-CRESOL.—Meta-cresol behaves similarly to phenol, 1 c.c. of $N/100$ thiosulphate solution equalling 0·00054 grm. of meta-cresol. Ortho- and para-cresols were not investigated.

SALICYLIC ACID.—One grm. of the pure acid was dissolved in 1000 c.c. of distilled water.

On treating different quantities in exactly the manner described for phenol, the following results were obtained:

2 c.c. of salicylic acid solution required   2·8 c.c. of $N/100$ sodium thiosulphate solution.

| 5 | ,, | ,, | ,, | ,, | ,, | 7·1 | ,, | ,, | ,, | ,, | ,, |
| 25 | ,, | ,, | ,, | ,, | ,, | 35·8 | ,, | ,, | ,, | ,, | ,, |

(Where possible, all solutions were made up to 15 c.c. with distilled water before the addition of bromine.)

It was, therefore, concluded that the bromine compound, formed after the addition of excess of bromine and aeration, liberates 2 atoms of iodine from potassium iodide, which can be accurately titrated with thiosulphate solution.

THYMOL.—Thymol behaves similarly to phenol. The bromine compound is volatile, and for accurate results rigid conditions must be observed. The thymol (0·283 grm.) was dissolved in 255 c.c. of distilled water and alcohol, 15 c.c. of 90 per cent. alcohol being used (1 c.c. = 0·00111 grm. of thymol). Five c.c. of this solution were diluted to 15 c.c. with distilled water and 4 c.c. of $N/5$ bromine added. The solution was aerated by passing 10 litres of air; potassium iodide was then added, and the titration was carried out as for phenol, 7·15 c.c. of $N/100$ thiosulphate being required. Another 5 c.c. treated as above described, but with 50 litres of air used for aeration, required only 6·15 c.c. of $N/100$ thiosulphate solution. A correction of 0·2 c.c. should, therefore, be made for the volatility of the bromine compound, 7·15 c.c. thus becoming 7·35 c.c. With this correction, 2 atoms of iodine are liberated from 1 molecule of the aerated thymol and bromine compound on the addition of potassium iodide.

Other common substances, such as benzoic acid, tartaric acid, citric acid, etc., when tested gave negative results. It would, therefore, appear that the reaction
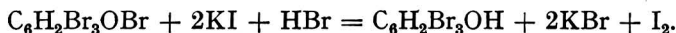
described is characteristic of a phenolic grouping, and, if conditions are modified to suit the particular substance under investigation, it should be possible to determine quantitatively most of these substances in this manner.

GENERAL CONCLUSIONS.—Taking phenol as the simplest substance investigated, the chemistry of the reaction would appear to be:
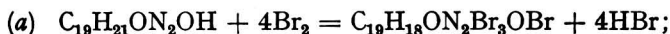
(a) On the addition of excess bromine and aeration

$$C_6H_5OH + 4Br_2 = C_6H_2Br_3OBr + 4HBr;$$

(b) On the addition of potassium iodide

$$C_6H_2Br_3OBr + 2KI + HBr = C_6H_2Br_3OH + 2KBr + I_2.$$

The liberated iodine is titrated with thiosulphate, with starch solution as indicator. Of the more complicated alkaloidal substances, cupreine is known to contain a phenolic grouping. It is, therefore, reasonable to suppose that the reaction would proceed as follows:

(a) $C_{19}H_{21}ON_2OH + 4Br_2 = C_{19}H_{18}ON_2Br_3OBr + 4HBr;$

(b) $C_{19}H_{18}ON_2Br_3OBr + 2KI + HBr = C_{19}H_{18}ON_2Br_3OH + 2KBr + I_2.$

Quinine, however, does not contain a phenolic grouping. A. Christensen (*Ber. Deut. Pharm. Ges.*, 1916, **25**, 256) describes the progressive action of chlorine on quinine. He gives a base, $C_{19}H_{21}O_3N_2Cl_3$, which is obtained by the action of 3 molecules of chlorine on 1 molecule of the alkaloid. This base contains one active chlorine atom capable of displacing iodine from potassium iodide and so probably containing the grouping—$CO.CCl_2$—in the quinoline nucleus. By analogy, the base formed on the addition of bromine, and aeration, to a quinine solution would be $C_{19}H_{21}O_3N_2Br_3$, which would contain the grouping—$CO.CBr_2$—also in the quinoline nucleus. This group by undergoing conversion into $C(OH):CBr$—would liberate two equivalents of iodine on the addition of potassium iodide, although one molecule of bromine only is active in the reaction. Thus in the case of quinine the reaction, as described, would proceed along phenolic lines, although no phenolic grouping is present originally in the alkaloid. Comanducci (*J. Chem. Soc.*, Abst., 1910, i, 581) also supports the hypothesis of a phenolic grouping being active in the thalleioquin reaction. With regard to the other alkaloids investigated, morphine is known to contain a phenolic group, and codeine, on treatment with a halogen, would probably undergo an intra-molecular rearrangement similar to that of quinine.

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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.

THE APPLICATION OF PIPERIDINIUM PIPERIDYL-DITHIOFORMATE TO THE COLORIMETRIC DETERMINATION OF COPPER.

HAVING found that, on the addition of an alcoholic solution of piperidinium piperidyl-dithioformate to a solution of a copper salt, a yellowish-brown coloration is produced, I made this the basis of a colorimetric method of determining copper in small amounts. This colour is very stable and is not affected by the presence of a slight excess of acid or alkali. Cadmium, mercury or bismuth cause no interference; iron, however, interferes to a pronounced extent, and must be removed. The maximum sensitivity of the test, when carried out in ordinary 50 c.c. colorimeter glasses, is of the order of 0·00001 grm. of copper. The following procedure is recommended:—Dissolve the sample in hydrochloric acid, dilute the solution with water so that the acid concentration does not exceed 15 per cent. by volume, heat to boiling, add a few drops of nitric acid, and pass a current of hydrogen sulphide through the solution until it is cold. Allow the precipitate to settle, filter by suction through a sintered glass crucible (Jena), wash with water acidified with acetic acid and saturated with hydrogen sulphide, until no more iron can be detected in the filtrate, place the crucible on a glass (or silica) triangle over a small crystallising dish standing on a glass water-bath, and add fuming nitric acid to the crucible. When no more liquid remains in the crucible, wash it with water and allow it to remain on the water-bath until the solution containing the copper has evaporated. Place the crystallising dish on a hot plate until no more acid fumes are given off, taking care not to decompose the nitrate. Take up the residue with water. This procedure has the following advantages:—(1) Complete separation from the metals of the 3rd group; (2) separation from mercury salts; (3) final solution practically neutral. Care must be taken, however, that the copper sulphide precipitate does not remain in contact with the air, and copper contamination must be excluded, *e.g.* by placing a glass plate over the crucible when filtering and dissolving. A very small amount of copper (a few thousanths of 1 mgrm.) can be precipitated and recovered by this method.

DETERMINATION OF THE COPPER.—The final solution, containing cupric ions, is transferred to a graduated glass-stoppered flask. If the colour of copper sulphide in the crucible has been noted previously, it is easy after several analyses to estimate roughly the copper present. This approximate estimation indicates the size of the flask which it is desirable to use. Aliquot portions of the neutral solution are compared with a standard solution of 0·393 grm. of pure (A.R.) copper sulphate in 1 litre (1 c.c. = 0·1 mgrm. of copper).

The colour comparison is made by adding 1 c.c. of 0·1 per cent. alcoholic solution of piperidinium piperidyl-dithioformate to the solution under examination and to the standard; it is most accurate with amounts of copper ranging from 0·00001 to 0·00008 grm. If desired, the piperidinium piperidyl-dithioformate may

be added first, and portions of the standard run in from a burette until the desired match is obtained. The colour is obtained immediately and is quite stable.

The following figures were obtained by using a Lovibond Tintometer (British Drug Houses) to match the colour.

Copper added mgrm.			Tintometer readings	
			Red	Yellow
0·002	..	..	0·2	0·5
0·003	..	..	0·2	0·7
0·004	..	..	0·4	0·7
0·005	..	..	0·7	0·8
0·006	..	..	0·2	1·8
0·007	..	..	0·4	1·9
0·008	..	.. .	0·7	1·7
0·009	..	..	0·8	2·4
0·010	..	..	1·3	4·0
0·020	..	..	1·8	7·0
0·030	..	..	2·6	8·9
0·040	..	..	3·6	11·0
0·050	..	..	4·0	15·0
0·060	..	..	5·0	18·0
0·070	..	..	5·9	19·0
0·080	..	..	6·6	19·1
0·090	..	..	5·9	21·0
0·100	..	..	7·8	24·0

North daylight was used for the comparison, and the solution was adjusted to 7 c.c. in a glass cell with internal dimensions (38×20×13 mm.).

The organic matter may be oxidised by ashing the substance in an electric muffle as follows: A large sample in a silica dish is placed on a hot plate until it is charred, and then in an electric muffle at a temperature not exceeding 400° C. After 30 minutes the dish is removed and allowed to cool, and its contents are treated with fuming nitric acid and evaporated to dryness on a hot plate. Sometimes the oxidation is complete after this treatment. If only a small quantity of organic matter remains, more nitric acid is added, and the mixture is again evaporated to dryness. With larger amounts the dish must be replaced for 30 minutes in the muffle and, after cooling, more nitric acid must be added. As a rule, all the organic matter is completely oxidised by this treatment, which should be repeated as often as necessary. Gebhardt and Sommer found that ashing in the muffle is liable to give low values if the temperature of the muffle is too high.

I wish to thank the Lovibond Tintometer Company for the loan of the instrument used in the above determination.

RALPH G. HARRY.

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Standing Committee on Uniformity of Analytical Methods.

UNSAPONIFIABLE MATTER IN FATS AND UNSAPONIFIED FAT IN SOAPS.

THE Standing Committee on Uniformity of Analytical Methods has appointed a Sub-Committee to formulate methods for the determination of these substances. The Members of the Sub-Committee are Prof. T. P. Hilditch, D.Sc., F.I.C. (Chairman), and Messrs. E. R. Bolton, F.I.C., L. V. Cocks, F.I.C. (Hon. Sec.), F. R. Ennos, B.Sc., F.I.C., N. Evers, B.Sc., F.I.C., L. A. Jordan, D.Sc., F.I.C., and W. H. Simmons, B.Sc., F.I.C.

Eighth Report of the Essential Oil Sub-Committee to the Standing Committee on Uniformity of Analytical Methods.

THE DETERMINATION OF CINEOLE IN ESSENTIAL OILS.

SECOND REPORT

THE Sub-Committee makes the following recommendations in regard to the determination of cineole in essential oils other than cajuput and eucalyptus oils.

(II) CAMPHOR OIL.

The ortho-cresol method, which was the subject of the Sub-Committee's first Report (ANALYST, 1927, **52**, 276–9), has been shown to be reasonably accurate when applied to camphor oil. As this oil usually contains less than 50 per cent. of cineole, the modification of the method mentioned on the second page (*ibid.*, p. 277) of the Report must be used.

This modification involves either enriching the oil by the addition of an equal weight of pure cineole before testing; or adding to the mixture of 3 grms. of the oil and 2·1 grms. of ortho-cresol an equal weight (5·1 grms.) of the pure recrystallised ortho-cresol compound, and then carrying out the test in the usual manner and making the necessary correction.

In the case of light camphor oil, *i.e.* one distilling below 200° C., the modified test is carried out directly on the oil. When a camphor oil contains high-boiling constituents, such as camphor and safrole, it is necessary to distil the oil through a fractionating column and carry out the test on the fraction boiling below 200° C. The cineole content of the original oil is then obtained by calculation.

The accuracy of the table of freezing points published in the first Report, when applied to camphor oil, has been confirmed by the members of this Sub-Committee. For this purpose mixtures of known cineole content were prepared by mixing pure cineole with a diluent prepared as follows:—A mixture of American turpentine, camphor and safrole was distilled through a fractionating column, and the fraction distilling up to 200° C. was collected. This was taken as representing the non-cineole portion of light camphor oil and was used for diluting the cineole.

Mixtures containing 50, 60, 66·7, 70, and 75 per cent. of cineole were prepared, such mixtures representing camphor oil containing, 0, 20, 33·3, 40 and 50 per cent. of cineole enriched with an equal weight of pure cineole.

The results obtained are shown in the following table:

SUMMARY OF RESULTS OF THE DETERMINATION OF CINEOLE IN ARTIFICIAL MIXTURES REPRESENTING CAMPHOR OIL ENRICHED WITH AN EQUAL WEIGHT OF CINEOLE.

ACTUAL CINEOLE CONTENT.

Sub-Committee Member.	50 Per cent.		60 Per cent.		66·7 Per cent.		70 Per cent.		75 Per cent.	
	F.Pt. °C.	= Per cent. cineole.	F.Pt. °C.	= Per cent. cineole.	F.Pt. °C.	= Per cent. cineole.	F.Pt. °C.	= Per cent. cineole.	F.Pt. °C.	= Per cent. cineole.
No. 1	27·0	49·5	35·4	60·4	39·8	66·5	42·1	70·7	44·8	75·7
No. 2	27·6	50·3	35·6	60·7	40·2	67·2	42·0	70·5	44·8	75·7
No. 3	27·5	50·2	35·7	60·8						
	27·9	50·7								
No. 4	28	50·8	36	61·2	40·6	67·8	42·3	71·1	45·2	76·5
No. 5	27·8	50·5	35·8	61·0	50·3	67·3	42·2	71·0	45·0	76·1
No. 6	27·1	49·6	35·2	60·2	39·8	66·5	41·8	70·1	44·8	75·7
	27·4	50·0	35·4	60·4	39·8	66·5	41·8	70·1	44·8	75·7
No. 7	27·2	49·8	35	60	40·05	67·0	41·8	70·2	44·7	75·6
No. 8	27·4	50	34·7	59·5	39·5	66·0	41·6	69·7	44·5	75·2
Mean		50·1		60·5		66·9		70·4		75·8
Variation from mean		−0·6 +0·7		−0·9 +0·4		−0·8 +1·0		−0·6 +0·8		−0·5 +0·8
Maximum variation	1·0°	1·3 per cent.	1·0°	1·3 per cent.	1·1°	1·8 per cent.	0·7°	1·4 per cent.	0·7°	1·3 per cent.

These results show that the experimental error between different analysts does not exceed ±1 per cent., but, as they require correcting for the added cineole, the limit of experimental error must be stated as ±2 per cent.

(III)   OTHER CINEOLE-CONTAINING OILS.

The presence of alcohols, esters, aldehydes and ketones in quantity in cineole-containing oils has been shown to raise the freezing-point of the ortho-cresol compound. The method, therefore, indicates a higher result than the actual content. No means has been found for carrying out accurate determination under these conditions, but the Sub-Committee is of opinion that the "apparent" cineole content shown by the ortho-cresol method has a considerable value. Fractions of eucalyptus oil are frequently used as adulterants in rosemary oil, and the apparent cineole content is a useful figure in the examination of such oils. The Sub-Committee recommends that the term "Apparent Cineole Content by Ortho-cresol" should be used in connection with the oils of rosemary, spike, and sage.

(*Signed*),

John Allan (Chairman), C. T. Bennett, S. W. Bradley, E. Theodore Brewis, L. E. Campbell, Thos. H. Durrans, T. W. Harrison, Ernest J. Parry, C. Edward Sage, W. H. Simmons.   T. Tusting Cocking (Hon. Sec.).

June 17th, 1931.

Notes from the Reports of Public Analysts.

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

CITY OF BIRMINGHAM.

REPORT OF THE CITY ANALYST FOR THE SECOND QUARTER, 1931.

THE number of food and drug samples examined during the quarter was 1230, of which 1181 were informal samples; of these, 142 were adulterated or incorrect.

VINEGAR.—Three samples were described as "Non-brewed Vinegar" on the label, and underneath in smaller type, "Suitable for Table Use." This appears to be a contradiction in terms, since table vinegar should consist of malt vinegar; actually, all three samples consisted of artificial vinegar.   No action, however, was taken.

CRYSTAL MINTS.—Two samples contained, respectively, 840 and 460 parts of sulphur dioxide per million.   The manufacturers of both were cautioned and agreed to take immediate steps to see that their products complied with the regulations.

Glucose syrup, according to the first Schedule of the Preservatives in Food Regulations, is allowed a maximum of 450 parts per million, and cane sugar a maximum of 70 parts of sulphur dioxide per million, and calculations from the composition of these mints showed that neither should have contained more than about 150 or 160 parts per million.

BEEF SUET.—The label of this sample stated that it would go half as far again as raw suet, and instructions were given for the use of one-third less than one would use of raw suet or lard.   As the sample contained only 84 per cent. of fat, as against about 95 per cent. for raw suet and 100 per cent. for lard, this was, by implication, a false label, and the makers were cautioned.

"FRUIT DRINKS."—The labels of these samples described them as "Fruit Drinks," and the advertisements connected with them implied that fruit juice was used in their manufacture.   As a matter of fact, the only connection with fruit was that oil of lemon or oil of orange and citric acid were present in very small quantities. No genuine fruit juice was present. The makers were approached and have agreed initially to withdraw the term "Fruit Drink," as applied to their product.   Correspondence is still in progress with the firm, and it is hoped that the position will, in due course, be entirely satisfactory.

DEVONSHIRE CREAM CHEESE.—This article was advertised in the vendor's shop under this name, but the sample contained only 56 per cent. of fat calculated on the dry solids, whereas genuine cream cheese should contain at least 80 per cent. calculated in this way.   The article was obviously ordinary whole-milk cheese. The vendor apologised, saying that he acted in ignorance and immediately withdrew the offending label.

H. H. BAGNALL.

METROPOLITAN BOROUGH OF STEPNEY.

Annual Report of the Borough Analyst for the year 1930.

Of the 1602 samples submitted, 1532 were foods and drugs, 948 being formal, 570 informal, and 14 "appeal to the cow" samples. Thirty-five samples were adulterated.

THE WARRANTY DEFENCE.—Proceedings were taken against the vendor of a sample of milk which was 8·3 per cent. deficient in fat. Three samples taken from the wholesaler were genuine, containing 3·50, 3·65 and 3·70 per cent. of fat, respectively, but it was stated for the defence that, although these samples were genuine, the results were of no importance, and that the samples were taken merely to comply with the regulations, so that the retailer might be entitled to plead a warranty defence. The warranty was produced, and the summons was dismissed, that being the sixth occasion upon which summonses against the vendor had been dismissed after the production of a warranty. If such a defence is to hold good in cases where the wholesaler's sample is genuine, there is no improvement on the old warranty conditions; neither party can be convicted, and the position remains as before.

Some defendants apparently do not bother to obtain a warranty. One wholesaler in the Borough refuses to give a warranty, but will pay the fines or costs should proceedings be taken against the vendor.

FACED PEARL BARLEY.—One sample of seven examined was adulterated with 0·65 per cent. of talc powder. It is highly important that pearl barley should be free from contamination of this kind, owing to its extensive use in the preparation of barley water for invalids and children.

PEPPER MIXTURE.—Fifteen samples of pepper were examined. One sample, which contained 50 per cent. of rice starch, bore the label: "This compound is sold as a mixed article, and is warranted to be of choice quality. Sale of Food and Drugs Act."

DIABETIC FLOUR.—A sample was taken formally, following the analysis of the same flour (Diabetic Flour, No. 1) for a private purchaser. It consisted of ordinary wheat flour in which the protein had been increased by about 10 per cent. (on the flour), the normal amount of starch being, in consequence, reduced to that extent. There could be no great difference in effect between taking this flour or a little less of an ordinary flour. It was sold in 6 lb. bags at 6s. 6d. per bag.

The sample was condemned as being "sold to the prejudice of the purchaser" and "not of the nature, substance and quality demanded." On the instruction of the Public Health Committee the vendor was cautioned. He stated that the flour was sold exactly as it was received from the millers, and that the millers accepted responsibility for the article. The millers stated that they published a booklet containing a warning that no diabetic patients should select their diet without first consulting a doctor (this booklet was not given with the flour), and that they sold three kinds of flour: No. 1, for mild cases; No. 2, for severe cases; and No. 3, for acute cases. They agreed to place an analysis of the contents in each bag.

ROOT GINGER.—A sample of root ginger contained 95 parts of sulphur dioxide per million. An article of food may contain preservative if it is to be used in the preparation of one of the foods in which preservative is permitted. If the root is used in the preparation of ginger wines (alcoholic and non-alcoholic), preservative

is permitted; but if the root is ground for use in cakes and puddings, preservative is not permitted.

METALS IN TINNED TOMATOES.—Two samples of tinned tomatoes were unsatisfactory owing to the presence of excessive amounts of tin and copper. The samples contained respectively 0·7 grain copper per pound and 2·7 grains tin per pound, and 0·6 grain copper per pound and 2·6 grains tin per pound.

Copper, when present as a metallic colouring matter, is prohibited; there is, however, no legal standard controlling the presence of copper otherwise in food.

METHYLENE BLUE IN TINNED PEAS.—A sample of tinned peas was examined for metallic contamination and for the presence of a dye. Complaints had been made of the effects produced after the peas had been eaten. Owing to the discontinuance of the use of copper salts for the production of a green colour in peas, other methods are now being used. In this case the peas were found to have been coloured with methylene blue. When this dye is taken internally it has the effect of colouring the urine green or blue according to the amount of dye present. This dye is not, however, included in the list of dyes prohibited for use in colouring food in this country, and its use, therefore, is permissible. Dyes, however, when combined with specified metals, are prohibited. Methylene blue occurs commercially in combination with zinc. As the zinc combined with the small amount of dye would be extremely small, it is not possible to say whether the methylene blue was originally combined with zinc or not, as appreciable amounts of zinc may occur naturally in tinned foods or accidentally from the soldering flux.

DOUGLAS HENVILLE.

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.

DISTINCTION BETWEEN POTTED SALMON AND SALMON PASTE.

ON May 5th a shop-keeper was summoned at Leeds for selling adulterated potted salmon.

The certificate of the Public Analyst (Mr. C. H. Manley) was to the effect that the sample contained 80 per cent. of salmon and 20 per cent. of a starchy "filler."

Mr. H. R. McDowell, prosecuting, said that the substance known as potted salmon should contain only pure salmon treated with melted butter, and a little seasoning. The substance which was widely sold should be known as salmon paste. It was only fair to the defendant, however, to say that only the commercial rate for salmon paste was charged and not that for potted salmon. The Health Authorities considered that there was a very big volume of meat food substances sold in Leeds under the designation of potted salmon, or potted meat, whereas, in fact, they contained a proportion of starchy substance and should be described as salmon paste or meat paste.

The summons was dismissed on payment of costs.

Ministry of Health.

TWELFTH ANNUAL REPORT, THE YEAR ENDING MARCH, 1931, WITH ABSTRACT OF REPORTS OF PUBLIC ANALYSTS.*

SALE OF FOOD AND DRUGS ACT.—Of a total of 136,515 samples examined (an increase of 2931 over 1929, *cf.* ANALYST, 1931, 110), 6496 (or 4·8 per cent.) were reported against, and in 519 instances the Public Health Preservatives Regulations were reported to have been contravened. One sample sold as preservative powder consisted of ground rice starch.

Spirits, Brandy, etc.—Two hundred and five samples of spirits were below the standard of 35 degrees under proof; traces of tin were present in three samples of rum; lead was present in two samples of beer, and a coal tar disinfectant in two samples of stout (probably due to insufficient washing of the bottle).

Coffee, Cocoa and Tea.—Most of the samples of coffee reported against contained chicory; arrowroot was present in one cocoa; five samples of tea contained iron filings, and four others extraneous mineral matter.

Non-Alcoholic Wines.—Two samples contained, respectively, 14·4 and 14·3 per cent. of alcohol.

Other Foods.—Of 179 samples of bread-and-butter sold as such, 35 were made with margarine or a mixture of margarine and butter. Arsenic was present in 18 samples of sweets reported against, and 38 other samples contained excess of sulphur dioxide. A "rum and butter" toffee contained no butter, and the "rum" was a synthetic flavour; other "butter" sweets contained little or no butter. Copper was present in two samples of peas and in one of cider, and in the "gold tips" of two samples of sweetmeat cigarettes, and lead was reported in five samples of cider, one of cake, one of beer and one of lemonade. Tin was present in 53 samples of tinned foodstuffs, including fruit, vegetables, fish, soup, and black treacle, and one sample of corned beef and one of a sauce contained zinc. Contamination of cheeses wrapped in tin foil is being dealt with by the manufacturers and others in order to reduce it to the lowest possible limit, but 19 samples were reported as unsatisfactory, owing to tin being present in varying amounts up to 8·33 grms. per lb.

MILK AND PARATYPHOID FEVER.—Inquiries into an outbreak of paratyphoid fever, involving some 300 persons and resulting in seven deaths, established strong *prima-facie* evidence against milk, as all the primary cases had consumed raw milk from a farm where an employee was found to be suffering from a mild attack of the fever. The milk supply was temporarily stopped, a new infection-free staff of milkers engaged, and, after thorough disinfection of all the utensils, the milk was pasteurised before distribution to retailers.

D. G. H.

REPORT OF THE CHIEF INSPECTOR OF FACTORIES AND WORKSHOPS FOR THE YEAR 1930. INDUSTRIAL DISEASES.†

AMONG the subjects dealt with in the Report are the following:

RESPIRATORS.—An investigation into the efficiency of various types of respirators is being made by the Department of Scientific and Industrial Research.

* Obtainable at Adastral House, Kingsway, W.C.2. Price 5s. net.
† Obtainable at Adastral House, Kingsway, W.C. 2. Price 2s. 6d. net.

LEAD POISONING.—The number of notified cases of lead poisoning was 265 (as compared with 244 for 1929) with 32 deaths, 10 for the pottery industry, and 13 in the painting of buildings. The Lead Paint (Protection Against Poisoning) Regulations of 1927 cannot be expected to be felt for some years.

ARSENICAL POISONING.—The one reported case was that of a man of 61, employed for 46 years in the manufacture of sheep dip, who, 10 years previously, had had an epithelioma removed from the right shoulder, and five years later another from the neck, and now had one on the left upper arm.

ANILINE POISONING.—The 24 (26 in 1929) cases of aniline poisoning reported (no deaths) included 10 due to inhalation of the vapour from 5-chlor-ortho-toluidine emitted from the paste which had been dried in a vacuum oven, with an abnormally high outside temperature. Headache, drowsiness and conjunctival or nasal irritation were followed by more serious effects, causing 10 of 13 men to cease work. Making intermediates accounted for 8 cases; aniline colours, etc. for 3; aniline black dyeing for 2, and handling aniline residue for one. An inquiry into ill-health in boot factories arising from the use of stains containing aniline oil has shown that nitro-benzene, in the proportion of 1: 50, has been used for three years as a solvent for certain dyes made in imitation of foreign preparations, and that more black spirit-stain than formerly is being used to cover defects of poor leather. Any cases of ill-health appear to be among wearers of recently dyed shoes and not among the operatives handling the dyes.

TETRACHLOROETHANE POISONING.—Several cases of jaundice associated with plant for degreasing wool were due to tetrachloroethane, but, if the concentration of this chemical in the air is below about 5 per cent., no permanent ill-effects appear to arise. Other less toxic solvents, producing, so far as is known, no chronic effects, may be used, but in sufficient concentration they have anaesthetic properties.

DERMATITIS.—Voluntarily reported cases of dermatitis numbered 789, and the increase is attributed to the greater importance assigned to the condition. Dermatitis cases may be reduced to a small proportion by strict adherence to the advice in the official pamphlets and placards. The method of cleansing the hands and arms after work was responsible for a large number of cases, and it is recommended that, if possible, a film of ointment should be applied to the skin before handling staining preparations; cleansing agents should be as weak as efficiency will permit, and thorough washing and a light application of ointment should follow the removal of stains from the skin. An alkaline antiseptic wash, such as liq. chlorinated soda with boric acid (B.P.C.), will prevent oil dermatitis.

OTHER GASEOUS POISONS.—Hydrogen sulphide poisoning, as the result of accidents, was reported in 5 cases. There were 5 cases of chlorine poisoning and 2 of ammonia poisoning. Benzole fumes poisoned 6 persons, one fatally.

NICOTINE POISONING.—Two oz. of concentrated nicotine were accidentally spilt on a girl's arm. The arm was washed with hot water, but in half-an-hour the girl was sick and collapsed. The clothing was removed and the skin was at once cleansed, and, after artificial respiration and cardiac stimulation, recovery took place.

CELLULOSE LACQUER RISKS.—Examination of the air of a room being decorated by spraying low-viscosity cellulose paints containing (1) 30 to 40 parts by weight of toluole, (2) 60 per cent. of xylole, with benzole as impurity, showed that 3 parts of xylole or toluole per 10,000 (in terms of benzole) is the maximum quantity which can be safely inhaled over long periods. In none of the 7 men engaged on the spraying could benzole poisoning be diagnosed.

D. G. H.

International Register of Spas and Medicinal Waters.

(See also the Review on p. 776.)

IN 1927 the Council of the International Society of Medical Hydrology appointed an International Standard Measurement Committee to formulate terms of expression to be known as "International Standard Measurements," and it is proposed that the letters "I.S.M." should henceforth be used to designate analytical and other data obtained in accordance with the prescribed methods.

I. INTERNATIONAL INFORMATION.

CLASSIFICATION OF WATERS.—A standard classification of waters according to their chemical, physical and medical characters is recommended, and examples to illustrate the style of reporting are given.

A. **The Chemical Classification** should be expressed as follows:

(a) Its composition in terms of its one or more dominant or most active ions; for example, chloride, sulphide, sulphate, calcium, ferrous iron, arsenic, etc. When desired, these may be followed by brackets enclosing symbols of other active ions in the order of their *medicinal* importance.

(b) Its *concentration* (ionic concentration) in terms of millinormality (N/1000).

(c) The *reaction* (hydrogen ion concentration) should be given in terms of pH at 20° C.; or at other temperature, if stated.

B. **The Physical Classification** according to

(i) *Temperature* (at the source):—(a) cold, below 20° C.; (b) thermal, 20° to 37° C.; (c) hyperthermal, above 37° C.

(ii) *Radioactivity*, (a) of the water itself, or (b) of the contained gas.

(iii) *Tonicity*, which may be expressed by the terms *hypotonic, isotonic, hypertonic*, in comparison with body fluids.

C. **The Medicinal Classification** should be based on analytical data and clinical experience, and be expressed in not more than ten words, using (a) *for their internal action*, aperient, diuretic, solvent, alterative, tonic, etc., and (b) *for their external action*, temperature effects, sedative, stimulant, counter-irritant, etc. *Disorders* for which the water is particularly indicated should then be named.

CALCULATION OF SALINES.—The analysis of a water gives the composition directly in terms of *Ions*, and from these data "Salines" may be derived by calculation. The letters "I.S.M." should be shown at the head of the saline table, whenever the salines have been calculated in accordance with the prescribed formula. *It is particularly requested that salines shall NOT be given if calculated according to any other formula ; or, if so, that the fact be very clearly indicated. Reporting salines is entirely optional ; it is not recommended.*

Formula for Calculating Salines.—Calculate (a) bromides, iodides, fluorides, sulphides (as hydrosulphide, NaHS), phosphates (Na_2HPO_4), arsenites (Na_2HAsO_3) and other anions present in only small amount as sodium salts; (b) Rubidium, caesium and, in non-alkaline waters, lithium and other cations present in only small amount, as chlorides; (c) in alkaline water, lithium, strontium, barium, zinc, lead, tin, manganese, aluminium and iron as hydrocarbonates; (d) in acid waters (pH <4), iron and aluminium as sulphates; (e) the major ions in the following order, after deducting from the total quantity of an ion present the sum of the quantities used for the foregoing ions.

Anions	NO_3'	Cl'	SO_4'	HCO_3'	CO_3'	OH'
Cations	$K^{\cdot}$	$Na^{\cdot}$	$Ca^{\cdot\cdot}$	$Mg^{\cdot\cdot}$		

(f) Silicon, titanium and boron as directed *infra*. In alkaline waters, the concentration of the anions of these weak acids should be calculated by means of the dissociation constant and the pH value.

Occasionally some slight departure from the formula is desirable, as when an ion is present in unusually large or unusually small proportion, or, for example, when sulphide occurs in a

normal sodium carbonate (Na_2CO_3) water it is better reported as "sulphide," Na_2S. When aluminium and phosphate ions are associated in appreciable amounts, they may be calculated in that form of aluminium phosphate which is in agreement with the pH value of the water.

NATIONAL REGISTERS OF WATERS.—At the annual meeting at Budapest, in 1929, the Society published specimen pages of a National Register, giving data relative to the spa, as well as those relative to the water, and, the style having been generally approved, it provides the model on which the Register now adopted is compiled.

II. DATA CONCERNING THE WATERS THEMSELVES.

(i)   The analysis, whether expressed in ions or salines, shall be in terms of parts per million; either as mgrms. per litre or mgrms. per kilo.

(ii)   The quantities of ions, also "salines," when given shall be given to one decimal place only, or, when the quantity is less than ten, to two significant figures, *e.g.* 357·6, 6·4, 0·18, 0·0027.

(iii)   The analysis of a water shall always be expressed in terms of ions, whether its interpretation in other terms be given or not.

(iv)   The analysis may also be expressed as "salines" provided these are computed by the arbitrary method of calculation which the Society has approved for international adoption.

(v)   The analysis, whether given as ions or as salines, shall be expressed in terms of "millinormality" ($N/1000$).

(vi)   The specific gravity shall be determined by comparison with distilled water at the same temperature, preferably between 15° and 20° C.

(vii)   Analytical tables shall be printed in four columns, in the following order:—(*a*) The name of the ion or saline. (*b*) Its concentration in the terms prescribed, marked "I.S.M." (*c*) The same, in terms customary in the country where the water originates, in those cases where these differ from the international terms, marked "National." (*d*) The same in terms of millinormality, marked "$N/1000$."

(viii)   *Gases* shall be determined in terms of the number of c.c. at normal temperature and pressure (0° C. and 760 mm.) contained in a litre of the water.

Spectroscopic Analyses under modern conditions, exploring both the visible and ultra-violet regions of the spectrum, should be made whenever possible. Elements recognised *and estimated* by spectroscopic methods should be indicated in the tables of ions by "Sp." against the quantity found; for example, HBO_2 trace (Sp.); Cr. 0·0015 (Sp.). *When necessary*, a chemical result may be distinguished by "Ch."

THE MINERAL CONSTITUENTS AND GASES should be reported in accordance with the foregoing resolutions and with the directions for printing the data. The following details should also be observed:—(i) The ions should be arranged in the following order, which is convenient and is an approximation to the periodic classification; the most commonly occurring are:

(*a*) *cations :* Li·, Na·, K·, Mg··, Ca··, Sr··, Ba··, Zn··, Cu··, Sn··, Pb··, Biv, Cr··, Mn···, Fe··, Fe···, Al···.

(*b*) *anions :* Cl′, Br′, I′, HS′, $NO_3′$, $IO_3′$, $SO_4″$, $PO_4‴$, $HCO_3′$, $CO_3″$, OH′.

(ii)   *Boron, Silicon and Titanium* should be given in terms of "Boric Acid—HBO_2"; "Silicic Acid—H_2SiO_3"; "Titanic Acid—H_2TiO_3" respectively, after the summation of the anions.

(iii)   *The total sum of the items determined* should then be given.

(iv)   *Total Solids* should, when the water after boiling, and cooling, gives an alkaline reaction with phenolphthalein, be determined by drying to constant weight at about 180° C. When the water does not so react, the total solids should be determined by the sodium carbonate method. This is followed by adding a small known quantity of sodium carbonate to the water before evaporating, then drying to constant weight at about 180° C., and deducting from the weight so found the weight of the sodium carbonate added. The quantity of water employed may conveniently be such as will yield about one grm. of total solids.

(v)   *Ions sought but not found* should be expressed by chemical symbols in small type in a single line; followed by "sp."=spectroscopically, "ch."=chemically. Such negative information is valuable *per se*, but also it is an indication of the completeness of the analysis.

PHYSICAL AND OTHER PROPERTIES OF THE WATER.—Under this heading may be included any suitable data which are satisfactory; for example:—(i) *Specific gravity.* (ii) *Turbidity or Colloidal properties* of the water as it issues from the source, or so many hours after collection.

(iii) *Degree of Ionisation*. The basis on which this is calculated should be given; for example, the *Cryoscopic Constant*, the *Ebullioscopic Constant*, the *Electrical Conductivity*, etc., giving the figures for each basis quoted. (iv) *Hydrogen-Ion concentration*. The means adopted for its determination should be indicated. (v) *Radioactivity*. This should be given in terms of radons or micromillicuries per litre. (vi) *Describe any changes* that take place in the physical and chemical condition of the water during a period of hours or days after it leaves the source.

III. DATA RELATIVE TO THE SPA.

Measurements should be given in the usual metric terms, but the same measurements in other terms may be added in brackets.

CLIMATE should be described with regard to (*a*) Character: whether "tonic," "sedative," "sheltered"; (*b*) *Temperature :* (i) during the year, (ii) during the season ; (*c*) *Rainfall :* (i) during the year in cm.; (ii) during the season in cm.; (*d*) *Atmospheric humidity*; (*e*) *Prevailing winds*.

Other data required include *Character of the surroundings* and the *Geology :* (*a*) of the district; (*b*) of the spring. "Description" of the spring. Details of other springs in the neighbourhood.

IV. STYLE AND COMPOSITION OF "THE REGISTER."

The sections comprise (i) "International Information"; (ii) "National Information"; (iii) The "data" relative to the spas; (iv) Appendix of unofficial information.

ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS.

Food and Drugs Analysis.

Detection of Certain Types of Heated Milk. S. Rothenfusser. (*Z. Unters. Lebensm.*, 1931, **62**, 210–214.)—Various processes for heating milk are discussed with special reference to the author's method of detecting heated milk (ANALYST, 1930, **55**, 758). The biological, bacteriological and chemical importance of processes which involve heating at about 79 to 85° C. is emphasised, and an oxidation-reduction reaction for such milk, suitable for routine work, is proposed. Di-*p*-diamino-diphenyl or (preferably) di-*p*-diamino-diphenylamine sulphate is used as a chromogen, since normally it is stable, but, on oxidation, gives a blue quinonoid compound (indamine). It is mixed with an organic acid (preferably tartaric acid) which has no water of crystallisation, slight acidity being required to promote the reaction. The oxygen carrier is magnesium perborate, which is stable and provides the correct degree of buffering in the milk. Since the chromogen is only sparingly soluble in water or organic solvents, the solid reagents are mixed and inserted in the top of a vertical glass tube containing an axial rod or plunger. The bottom of the tube dips into 5 c.c. of milk in a 10 c.c. tube, and the reagent is added at will by manipulating the central rod. Milk which has been heated at 79° to 80° C. retains its colour, but if a lower temperature has been used a blue colour results, whilst heating at 77° C. or for a brief period (*e.g.* 30 seconds) at 79° C. results in a slow reaction. The test should not take longer than 1 minute. J. G.

Suggested Method for the Determination of the Dextrinolytic Activity of Malt. L. Fletcher and J. B. Westwood. (*J. Inst. Brewing*, 1931, **37**, 470–478.)—The results of preliminary experiments indicate that J. L. Baker's

α-amylodextrin (*J. Chem. Soc.*, 1902, **81**, 1177) is a suitable substrate for determining the dextrinolytic activity of aqueous malt extracts. The early stages of the action of malt extract on this dextrin represent substantially the work of a single enzyme, dextrinase, or of two enzymes acting with equal velocities, since, up to the production of maltose corresponding with about R15, the velocity constant of the reaction remains practically constant. The procedure for the test is as follows: 30 grms. of the air-dried dextrin (9 to 10 per cent. of moisture) are dissolved in boiling water containing 10 grms. of mixed phosphate buffer (potassium dihydrogen phosphate and disodium hydrogen phosphate) of *p*H 4·9 to 5·0, and the liquid is cooled and made up to 1 litre. The malt extract is prepared by extracting 20 grms. of the ground malt with 500 c.c. of distilled water for 1 hour in a mechanical shaker. One c.c. of the extract is allowed to act at 40° C. on 70 c.c. of the α-amylodextrin solution for 1 hour, 3 c.c. of 2 *N* sodium hydroxide solution being then added to stop the action. The solution is made up to 100 c.c. at 15·6° C., and the cupric oxide reducing power is determined gravimetrically on 20 c.c. of the liquid. The result is calculated as percentage of maltose on the amylodextrin taken. Under these conditions 1 c.c. of malt extract from normal kiln-dried malt usually produces the equivalent of R5 to 10, the action lying well within the limits of the law of proportionality. Extremes in either direction may be corrected by repeating the determination with more or less of the malt extract.

The results obtained with a number of malts indicate that normal kiln-dried malts will always supply sufficient enzyme to liquefy starch paste in the mash tun and so provide soluble starch as substrate for the amylolytic enzymes. The values found for the dextrinolytic activity of certain malts suggest, however, that many mash tun troubles ascribed to shortage of liquefying enzymes might actually be due to a deficiency of dextrinase.   T. H. P.

Rancidity Changes and the Flavour of Fats. C. R. Barnicoat. (*J. Soc. Chem. Ind.*, 1931, **50**, 361–365T.)—The effect of free fatty acids on the flavour of fats in the absence of oxidation and rancidity was investigated with beef kidney and external fats, mutton kidney fat, and lard. Fatty acids were prepared from these fats with precautions against oxidation. These were mixed with the original fats, and it was found that the flavour of the fats was not impaired with additions up to 10 or 15 per cent. Under similar conditions of temperature and exposure to light the nature of the fat markedly influences the development of rancidity, and the active oxygen and Kreis values, corresponding with the point of first perceptible rancidity, increased from hard beef and mutton kidney fats, through beef external fat, to lard. Readiness to develop a rancid odour and flavour on oxidation appeared greater the smaller the proportion of linolic acid present, and was greatly increased by exposure of the fat to direct sunlight. The substances responsible for the odour and flavour of oxidised fats at ordinary temperatures are regarded as probably products of oxidation of oleic rather than of linolic or linolenic acids. Attempts to determine traces of aldehydes in rancid fats were not satisfactory, partly owing

to the formation of emulsions; a method giving promising results with animal fats but not with methyl oleate, consisted in shaking the fat at concentrations equivalent to about 50 mgrms. of heptaldehyde per litre, in benzene with dilute bisulphite for 12 hours, filtering, and titrating the bisulphite combined with the aldehyde with 0·001 N iodine.   D. G. H.

Susceptibility of Fats to Oxidative Rancidity.   D. P. Grettie and R. C. Newton.   (*Oil and Fat Ind.*, 1931, **8**, 291–294.)—The incubation test, in which a fat is kept in an oven at a high temperature and.inspected for odour and flavour at regular intervals, is long (2 to 40 days), and depends on personal judgment.   The method now described is a modification of the methods of Bailey and Issoglio, and consists in passing air over the heated fat and then into dilute permanganate solution, which is titrated with oxalic acid at definite intervals.   The air is first purified by passage through · a washing bottle containing acid permanganate solution and then passes (1) over the fat dispersed on filter paper in the middle of a tube (20 mm. inside diam.), heated in boiling water, (2) into a test tube (1 inch
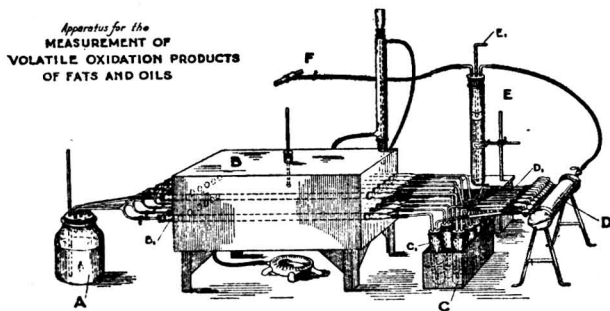

Apparatus for the
MEASUREMENT OF
VOLATILE OXIDATION PRODUCTS
OF FATS AND OILS

Figure 1

diam.) kept at 25° to 30° C. and containing 10 c.c. of 0·01 N potassium permanganate solution acidified with 1 c.c. of approximately 5 N sulphuric acid, (3) through a capillary tube, 10 cm. long and of 0·5 mm. bore, (4) through a device for maintaining a constant vacuum, and (5) through a tube, provided with a screw clip and connected with a vacuum pump.   A number of samples of fat may be examined simultaneously by using a number of tubes in the boiling water-bath, these tubes being connected at one end with the same acid permanganate air-purifier and at the other end with similar capillary tubes, each with its own acid permanganate condensing arrangement and all passing into a 2-inch iron pipe manifold connected with the vacuum pump.   This arrangement ensures the passage of the same volume of air over each sample of fat.

The tube to. contain the filter paper and fat is cleaned with acid dichromate and dried.   A piece of filter paper, 5 × 30 cm., is creased three times lengthwise to form pleats of four layers and placed across a clean watch glass on a balance, and 2 grms. of the fat are weighed on to it.   The fat is distributed so that, when melted, it will be soaked up by the entire paper.   This is then carefully introduced into the glass tube, about 8 inches from the end, and the tube placed in the .

boiling water oven.   After the connections have been made, the stream of air
(1 c.c. per second) is started by opening the screw clip on the vacuum tube.   At
the end of 20 minutes the test-tube containing the acid permanganate is replaced
by another.   The permanganate solution is then heated in a boiling water bath
with 11 c.c. of 0·01 N oxalic acid solution until decolorised and at once titrated
with standard permanganate.   This replacement and titration are repeated at
intervals of 20 minutes, until the rate of decomposition of the fat is sufficient to
use up 1 c.c. of the 0·01 N permanganate in one 20-minute period.   The total
time of the action before this occurs is taken as an indication of the stability of
the fat.   When the fat is exceptionally stable, intervals of 60 minutes are used.

 After use, the tube in which the fat is heated is left filled with strong acid
dichromate cleaning solution overnight, and then rinsed thoroughly with distilled
water.   In folding the filter paper, several sheets should be creased at once, the
outer ones, which the fingers have touched, being discarded.   The same type of
paper, of purest quality, should be used at all times.   The whole surface of the
filter paper should be exposed to the air current; if two of the folds adhere at one
end or if the flat surface of the paper is pressed against the glass tube, the result is
vitiated.   When several tubes are heated in the same oven, any one not being
used must be closed to the air by a pinchcock between the tube and the air-washing
bottle.   In case water condenses in one of the capillary tubes and thus prevents
the air from passing, this tube must be removed, washed with alcohol, and replaced.

<div align="right">T. H. P.</div>

**Determination of Butyric and Caproic Acids in Edible Fats.   J.
Grossfeld and F. Battay.**   (*Z. Unters. Lebensm.*, 1931, **62**, 99–126.)—The
difficulty of calculating the butyric acid value of a fat from its Reichert–Meissl
value is indicated.   It is shown that, for the edible fats, the former value is a
summation function of the butyric and caproic acid contents, and that a relation
of this type may be used, with the appropriate factors, for the purposes of calcu-
lation.   The ratios of the partition-coefficients (K) of these two acids between
water and various organic solvents were then determined, *viz.* ratio for butyric
to caproic acid: ether 10, benzene 20, toluene 20, xylene 21, petroleum spirit
(b.pt., 40°–60° C.) 26, petroleum spirit (b.pt. 120°–130° C.) 31, light petroleum
oil 34.   Determinations of K were also made for aqueous solutions of the acids
of various concentrations, and the law of mixtures was shown to hold for extraction
of them from mixed aqueous solutions; the results are tabulated and plotted as
curves.   On the basis of this work the following method is proposed:—Five grms.
of fat are heated in a 300 c.c. Reichert–Meissl flask with 2 c.c. of potassium
hydroxide solution (sp. gr. 1·5) and 10 c.c. of volatile acid-free glycerol until
saponified, and the soap solution is cooled to 20° C., decomposed and distilled
according to Grossfeld's procedure (*id.*, 1927, **53**, 382; see also ANALYST, 1931, 403).
Of the distillate (110 c.c.), (*a*) 50 c.c. are titrated to phenolphthalein with 0·02 N
sodium hydroxide solution (T c.c.), and 0·616T gives the butyric acid value;
 (*b*) 50 c.c. are shaken with 50 c.c. of neutral petroleum spirit (b.pt. 40°–60° C.) in a

200 c.c. flask for at least 1 minute, and the aqueous phase again titrated (T_1 c.c.). Then A (relative decrease in titration) = 100 $(T-T_1)/T$ per cent., allowance being made for blank titrations. If K_b and K_c are the decreases in concentration of the water layer when pure aqueous solutions of butyric and caproic acids, respectively, are extracted with petroleum spirit (b. pt. 40°–60° C.) under the above conditions, then the caproic acid (C), expressed as a percentage of the total titration $(T)=100(A-K_b)/(K_c-K_b)$. The percentages of caproic and butyric acids are thence obtained from the formulae P_2CT and $P_1(100-C)T$, respectively. P_2 is 0·00482 (P_1 0·00129) for butter fat, 0·00548 for coconut oil, 0·00396 (P_1 0·00127) for cocoa butter, and 0·00542 for lard. The data are plotted, and values of $100(K_c-K_b)$ and K_b are tabulated. Data provided by the examination of 32 samples of butter fat were examined by statistical methods, and a mean butyric acid content of 3·73 (caproic acid 1·72) per cent. was found; the mean ratio of the percentage of butyric acid to the butyric acid value was 0·186. The method of least squares gave mean deviations of a single value from the mean of all the values of 6·6 for butyric and 16·8 per cent. for caproic acid. Comparison of the contents of the two acids in the butter-fats by the correlation method gave a correlation-factor (r) of +0·70, the possible error (f) being ±0·061. Caproic acid occurs in edible coconut oil to the extent of 0·61 per cent., and has the lowest molecular weight of any fatty acid present. Examples of the applications of the method (*e.g.* the detection of fats containing butyric acid in other fats) are given.

<div align="right">J. G.</div>

The Reaction of Cocaine Hydrochloride Solutions. H. Rothlin. (*Farmaceutisk Revy*, 1931, 18; *Pharm. J.*, 1931, 127, 190.)—The reaction of aqueous solutions of cocaine hydrochloride to litmus varies from neutral to faintly acid, and the variable statements in the various Pharmacopoeias are regarded as due to the unsatisfactory nature of litmus as indicator. A litmus paper which was not up to the pharmacopoeia standard and was not reddened by 0·001 N hydrochloric acid, pH 3, yet showed a distinct red colour with a 2·5 per cent. cocaine hydrochloride solution of pH 5·5. The 5th edition of the Swiss Pharmacopoeia replaces the litmus test by the determination of the pH value, and allows a pH value of 4·6 to 6·4 for a 2·5 per cent. solution of the hydrochloride.

<div align="right">D. G. H.</div>

Determination of Emetine. F. C. Sinton. (*J. Assoc. Off. Agr. Chem.*, 1931, 14, 358–360.)—The following method is found to be satisfactory for the determination of emetine hydrochloride in tablets. All the unbroken tablets of the sample are weighed, and the average weight per tablet is calculated. A number of the tablets are powdered and mixed. A weighed amount, sufficient to represent about 0·1 grm. of the emetine salt, is dissolved in a small separating funnel in the minimum quantity of water, 5 c.c. of sodium hydroxide solution (4 grms. of NaOH per 100 c.c.) being then added. The solution is extracted with 30 c.c. of ether (previously washed, by shaking, with an equal bulk of water) and the aqueous solution is drawn off and the funnel swirled to remove water from its sides. The ether is washed with 1 c.c. of water, which is added to the aqueous solution. The

ether is decanted into a second separating funnel, the mouth of the funnel being washed with ether. The extractions are repeated with 25, 20, 15 and 10 c.c. portions of ether or until extraction is complete, washing with 1 c.c. of water each time, and combining the ethereal extracts in the third separating funnel. The total extract, together with ether used to rinse out the funnel, is filtered through cotton wool previously wetted with ether, and evaporated on a steam bath, the evaporation being completed at a low temperature. The residue is treated with 2 c.c. of neutral alcohol, which, with a watch-glass over the beaker, is allowed to reflux on the steam-bath for a few minutes. The liquid is then titrated to a faint pink colour with 0·02 N acid in presence of methyl red. The beaker is next covered and the solution is digested on a steam bath until all particles are completely dissolved, and cooled. After addition of 30 c.c. of recently boiled distilled water, the titration is completed to faint redness; 1 c.c. of 0·02 N acid $\equiv$ 5·6946 mgrms. of emetine hydrochloride ($C_{30}H_{44}O_4N_2$, 2HCl). The average of five results obtained by different analysts with a mixture of 1 part of emetine hydrochloride, U.S.P., with 3 parts of milk sugar differed from the theoretical value by not more than 0·2 per cent.   T. H. P.

Assay of Santonin. H. M. Burlage. (*J. Assoc. Off. Agr. Chem.*, 1931, **14**, 321–325.)—The following procedure is recommended for the assay of santonin in mixtures and tablets of non-fatty nature and, tentatively, in fatty mixtures. A weighed powdered sample, equivalent to approximately 0·15 grm. of santonin, is extracted with 10, 10, 10, 5, and 5 c.c. portions of petroleum spirit saturated with santonin (if the sample is fat-free this step may be omitted). Each portion of solvent is filtered, with the aid of suction, to complete dryness through a Gooch crucible provided with an asbestos mat, before following with another portion. The residue in the extraction flask and crucible is extracted with 15, 10, 5, and 5 c.c. of hot benzene, each portion being filtered as before. The benzene extract is transferred to a tared flask and, after evaporation of the solvent, the residue of santonin is dried to constant weight at 100° C. As a check, the residue is dissolved in 25 c.c. of warm aldehyde-free, neutral alcohol, the solution being neutralised and treated with 5 drops of phenolphthalein and 25 c.c. of 0·1 N potassium hydroxide solution. The liquid is digested on a water bath under a reflux condenser for 30 minutes, and then titrated, while hot, with 0·1 N hydrochloric acid, in presence of 5 drops of phenolphthalein solution. A blank test with 25 c.c. of aldehyde-free neutral alcohol and 25 c.c. of the alkali solution is made similarly.
                                                                        T. H. P.

Assay of Tablets of Resin of Podophyllum. L. E. Warren. (*J. Assoc. Off. Agr. Chem.*, 1931, **14**, 380–386.)—Tablets and pills of this resin usually contain starch, starch paste, talc, acacia, and liquid paraffin as binders, fillers, excipients, etc., and other laxatives, such as aloe, extract of colocynth, cascara or leptandra, resin of jalap or ipomoea, etc., are often present. The following procedure for extracting the resin and for determining the resin in the extract yields satisfactory results when other laxatives are absent :—A weighed quantity of the

powdered tablet material, representing about 0·75 grm. of resin of podophyllum, is mixed in a beaker with 10 grms. of fine washed sand, and the whole is transferred to a 30 c.c. Gooch crucible. Alcohol (25 c.c. in all) is poured in small portions through the crucible and is collected in a 100 c.c. measuring flask. After draining, the crucible is transferred to a Bailey or Soxhlet extractor, and extraction with 60 c.c. of alcohol is continued until the residue is completely exhausted of resin. This alcoholic solution is also placed in the 100 c.c. measuring flask and the whole is made up to volume with alcohol.

Ten c.c. of the tincture thus prepared are shaken in a separating funnel with 10 c.c. of chloroform and 10 c.c. of 0·6 per cent. hydrochloric acid (2 c.c. of the strong acid and 100 c.c. of water) and allowed to separate. The lower layer is transferred to another separating funnel, and the upper layer is extracted in the first funnel with three 15 c.c. portions of a mixture of 1 volume of alcohol and 2 volumes of chloroform, these extracts being added to the liquid in the second funnel. This liquid is now shaken with 10 c.c. of 0·6 per cent. hydrochloric acid and, after separation, the lower layer is drawn off into a weighed beaker or Erlenmeyer flask and the acid liquid is extracted with three 15 c.c. portions of fresh alcohol-chloroform mixture. The combined chloroform extractions are evaporated, the container being rotated in an inclined position as the last portions are dissipated. The residue is treated with 1 c.c. of dehydrated alcohol, which is then evaporated, the residue being finally dried to constant weight at 80° C.

By this procedure, nine brands of tablets of resin of podophyllum were found to contain from 83 to 124 per cent. of the resin contents claimed, the limits for seven of these being 90 and 112 per cent. T. H. P.

Amounts of Nicotine in Tobacco Smoke. G. Pyriki. (*Z. Unters. Lebensm.*, 1931, **62**, 95–98.)—Four oriental tobaccos, containing 0·06 to 1·28 per cent. of nicotine, were blended to give mixtures containing 0·06 to 1·08 per cent. of nicotine (moisture contents 11·3 to 12·2 per cent.), and from these cigarettes were made, 6·5 cm. long. These were "smoked" artificially in a stream of air (5 at a time, with mean total weight of 5·7 to 6·4 grms.) by the method of Pfyl and Schmitt (ANALYST, 1927, **52**, 728). The experiment was stopped when 5·2 cm. had been consumed, and pauses of 6 seconds were made every 4 seconds. For quantities up to 0·25 and 0·5 per cent. of nicotine in the original tobacco, 5 mgrms. per 100 grms. of dry tobacco were always found in the smoke obtained when the experiment was carried out in 5 and 8 minutes, respectively; a blank experiment on "nicotine-free" tobacco gave the same value. The corresponding (mean) values for tobaccos containing 0·3, 0·47, 0·6, and 1·1 per cent. of nicotine were 6, 25, 55, and 235 (for 5 minutes), and 5, 5, 40, and 174 (for 8 minutes), respectively. It is, therefore, considered that the proposed maximum limits for cigarette tobaccos described as "nicotine-free" (0·08 per cent.) and "poor in nicotine" (0·20 per cent.) are too low, and that 0·10 to 0·15 and 0·5 to 0·6 per cent., respectively, would be more suitable. J. G.

Biochemical.

Experimental Observations on Dermatitis due to Dyed Fur. G. H. Percival. (*Lancet*, 1931, **221**, 417–424.)—The experiments described were made (1) with dyed and undyed furs, and (2) by means of Jadassohn and Bloch's contact eczema procedure, in which the suspected irritant in low concentration is applied as solution, paste or ointment to a small area of the skin of the back. The application is covered with linen or oiled silk held in position with adhesive plaster, and is removed after 24 hours for examination of the skin. A positive test is indicated by a red and slightly oedematous condition of the skin, the surface of which is studded with minute vesicles. The individuals on whom the experiments were made were either normal, or subject to fur dermatitis itself or to skin disease of another type.

The results obtained lead to the following conclusions:—The potential irritant properties of a dyed fur are due to some substance used in the dyeing process and not to mechanical irritation by the fur. These irritant properties are enhanced by moistening the fur with water or saline solution, but such preliminary moistening is not essential for the production of a positive reaction. Normal skins do not, however, react to a dyed fur under conditions which would cause dermatitis in a fur dermatitis patient. Dermatitis is hence due to a hypersensitive skin, the reaction being allergic in nature. With furs dyed with a particular paraphenylenediamine compound (A) of the ursol type, it is either this compound itself or an early oxidation product of it which reacts with the allergic skin. Fur dermatitis cases react to concentrations of A varying from 0·005 to 0·5 per cent., there being considerable individual variation in the concentration necessary to produce dermatitis. Most normal skins fail to react with a 10 per cent. solution of A, although some react with a 5 per cent. solution. The degree of sensitivity of the skin of dermatitis cases to dyed fur ranges from at least 200 times to 2000 times that observed with non-fur dermatitis cases. It is possible for at least a 0·5 per cent. solution of A or its oxidation products to be formed in the fluid secretions of the skin when in contact with the dyed fur; such solution is without effect on non-fur dermatitis cases. The dermatitis resulting from a single application of a minute concentration of a chemical towards which the skin is allergic may persist for several weeks. The skin of a patient suffering from dermatitis caused otherwise than by fur is not specially apt to react to dyed fur, a specific capacity, bearing no relationship to the oiliness or humidity of the skin, being a necessary factor for reaction to occur. That positive reactions were obtained only when A was applied to the intact skin and that uniformly negative results were found by the scratch method, points to the allergy being localised in the epidermis.

By means of a cleansing process consisting in washing the dyed fur in water or benzene, and then brushing or drumming with sand, the harmful substance may be greatly reduced in amount, and, possibly, completely eliminated.

T. H. P.

Rôle of Copper in Haemoglobin Regeneration and in Reproduction.
H. L. Keil and V. E. Nelson. (*J. Biol. Chem.*, 1931, **93**, 49–57.)—The relation of copper to haemoglobin formation has attracted the attention of a number of investigators during the past few years, and only a superficial examination of the literature is necessary to reveal that there is considerable divergence of opinion regarding the rôle and necessity of copper in haemoglobin building. Experiments by the authors show the following results:—Nutritional anaemia is readily produced in rats on a whole milk diet. Pure iron as ferric chloride, when added to milk collected in glass, does not cause regeneration of haemoglobin. Salts of vanadium, titanium, manganese, nickel, arsenic, germanium, zinc, chromium, cobalt, tin and mercury also failed to stimulate regeneration of haemoglobin when added to milk collected in glass, and supplemented with pure iron as ferric chloride. Copper was the only element of those tested which had a positive effect on haemoglobin building. Reproduction was obtained on milk and iron as ferric chloride by the sole addition of copper sulphate (0·05 mgrm. daily). This was in contrast to the results of Krauss (*J. Dairy Sci.*, 1929, **12**, 74, 242), who did not obtain reproduction when rats were fed on a milk diet supplemented with copper and iron. Nutritional anaemia produces a distinct change in colour of the dark coated rats. Black or black-hooded animals replace the black colour with a silvery grey that is very striking. Grey-coated animals change to a buff or silvery grey with a yellowish tint. Animals on whole milk, plus pure iron as ferric chloride, also develop this change in the colour of the fur. The addition of 0·05 mgrm. of copper per day to the whole milk and iron as ferric chloride restores the original coat in about 2 months' time. P. H. P.

Colorimetric Determination of Calcium in Blood. S. Yoshimatsu. (*Tohoku J. Exp. Med.*, 1930, **15**, 355; *Mikrochem.*, 1931, **9**, 529.)—Calcium is determined in 0·1 c.c. of blood, diluted with 0·7 c.c. of water. The proteins are precipitated by adding 0·1 c.c. of 10 per cent. sodium tungstate and 0·1 c.c. of 2/3 N sulphuric acid, and, after 15 minutes, the mixture is filtered through a small glass filter, and the precipitate is washed three times with 0·3 c.c. of water. To the combined filtrates 0·3 c.c. of a 33 per cent. solution of Rochelle salt, 0·5 c.c. of 1 N sodium hydroxide solution, and 0·3 c.c. of a 5 per cent. alcoholic solution of oxyquinoline are added, drop by drop. The calcium oxyquinoline compound produced is separated by centrifuging and washed four times with an alkaline solution of Rochelle salt, 1 c.c. being used each time. The precipitate is then mixed with 1 c.c. of ammoniacal ammonium chloride solution (1 litre of 5 per cent. ammonium chloride mixed with 34 c.c. of ammonia solution of sp. gr. 0·9), and boiled for 5 minutes, one drop of ammonia solution being added twice during the heating. The mixture is then filtered through a glass filter, washed twice with 0·5 c.c. of water and then dissolved in 0·5 c.c. of 0·01 N hydrochloric acid, and the solution is treated with 1·2 c.c. of a 20 per cent. solution of sodium carbonate and 1 c.c. of Folin–Denis' phenol reagent (*J. Biol. Chem.*, 1915, **22**, 305). The mixture is then warmed for five minutes, and, after cooling, the colour is compared with that of a standard. J. W. B.

Michrochemical Determination of Magnesium in Blood without removing Calcium. S. Yoshimatsu. (*Tohoku J. Exper. Med.*, 1929, **14**, 29; *Mikrochem.*, 1931, **9**, 528.)—For each determination 1 c.c. of blood is diluted with 7 c.c. of water, and 1 c.c. of 1 per cent. sodium tungstate solution and 1 c.c. of 2/3 N sulphuric acid are added. The mixture is centrifuged, and 0·3 to 0·5 grm. of ammonium chloride is added to 5 c.c. of the supernatant liquid. The solution is then boiled, and 7 drops of ammonia solution (sp. gr. 0·96) and 7 drops of an alcoholic solution of oxyquinoline are added. The mixture is left on the water bath for 10 minutes, and after each 3 minutes one drop of ammonia solution is added. The precipitate is then centrifuged, while hot, washed 3 times with 2 c.c. of a 5 per cent. ammonium acetate solution, and then dissolved in N hydrochloric acid. The solution is mixed with 5 c.c. of a 25 per cent. sodium carbonate solution and 1 c.c. of Folin–Denis phenol reagent (*J. Biol. Chem.*, 1915, **22**, 305), and warmed for 30 seconds. The colour is compared, after cooling, with that given by a standard.   J. W. B.

Effect of Ultra-Violet Irradiation upon the Free Sterols of Lanolin. A. Bernhard and I. J. Drekter. (*J. Biol. Chem.*, 1931, **93**, 1–3.)—It has previously been shown that following lanolin inunctions and ultra-violet irradiation there is a rise in the blood sterol (cholesterol) of children and adults. The nature of this rise in sterol content after irradiation has been investigated further. Lanolin consists of different fatty acids, cholesterol, cholesterol esters, oxycholesterol, so-called isocholesterol, agnosterol and lanosterol. (The last two are not precipitated by digitonin.) Lanolin also contains other alcohols, such as lanolin alcohol, carnaubyl, ceryl, and undetermined higher alcohols, all of which make up about 50 per cent. of lanolin. The total sterol content found, after saponification, is about 20 per cent. The results show that the amount of free sterols in anhydrous lanolin which is precipitated by digitonin, expressed in terms of free cholesterol, is 0·93 per cent. of lanolin. When anhydrous lanolin was irradiated with ultra-violet light there was a progressive gradual rise in the amount of free sterols precipitated by digitonin; the maximum rise was reached at the end of 1 hour. The free sterols, expressed as free cholesterol, increased from 0·93 to 5·37 per cent. of lanolin. Three weeks after irradiation the amount of sterols precipitated by digitonin had not changed. Colour reactions for ergosterol in the lanolin, both before and after irradiation, were not specific, a brownish-green colour of the same intensity being obtained in both instances. The crystalline fractions obtained in attempts to isolate chlolesterol from lanolin which had been irradiated for 1 hour could not be identified as pure cholesterol. Therefore, digitonin precipitates substances other than cholesterol.   P. H. P.

Vitamin *A* and the Antimony Chloride Reaction. A. Emmerie, M. v. Eekelen and L. K. Wolff. (*Nature*, 1931, **128**, 495–496.)—The blue solution obtained with vitamin *A* and antimony trichloride shows two absorption bands at 572$\mu\mu$ and 610$\mu\mu$, respectively, and the blue colour depends on the intensity of the 610$\mu\mu$ band. Recently, however, Morton and Heilbron have stated that the strength of different vitamin *A* preparations corresponds better with the extinction

of the mixture in the $572\mu\mu$ region than in the $610\mu\mu$ region, and the following facts confirm this:—By treatment of a vitamin A preparation from saponified cod-liver oil or saponified extract of cow's liver with some drops of furan, methylfuran, pyrrole, indole, or skatole (all substances of related chemical structure), and the addition of antimony chloride, the mixture turns purple instead of blue, and in the spectroscope the $610\mu\mu$ band is no longer seen, whereas the $572\mu\mu$ band remains unaltered. The physiological activity is unimpaired and the preparation (without antimony chloride) shows the $328\mu\mu$ band just as the original substance did. It is known that liver may contain indole-like substances, and so it can be understood that in some vitamin A preparations the $572\mu\mu$ band is the stronger, and in others the $610\mu\mu$ band is the stronger. P. H. P.

Determination of Vitamin A in Butter. B. G. E. Morgan and K. H. Coward. (*Lancet*, 1931, **221**, 758–759.)—The method described by Coward, Key, Dyer and Morgan (*Biochem. J.*, 1930, **24**, 1952; 1931, **25**, 551), and Coward, Dyer, Morton, and Gaddum (*Biochem. J.*, 1931, **25**, 1102) for the determination of vitamin A in cod-liver oils has been shown to give consistent results also in the determination of vitamin A in butters and margarines. This is of value, for the testing of different amounts of butter involves the giving of different amounts of fat, in addition to the fat-free diet employed in this method, whereas the testing of different doses of cod-liver oils has always been carried out by diluting, so that the required dose is contained in the same amount (19 to 21 mgrms.) of olive oil. The manufacturers of the "Gold Chain" margarine used for the test claim for it a vitamin A potency equal to that of butter. The figures in a table giving the measurement of the vitamin A content of different doses of butter and margarine by reference to a standard sample of cod-liver oil, show that their claim is justified. A dose of 0·05 grm. of butter proved to be equivalent to a dose of 0·05 grm. of margarine (measured as being equal to 1·4 and 1·5 mgrms. respectively of the standard sample of cod-liver oil), and a dose of 0·1 grm. of butter proved to be equivalent to a dose of 0·1 grm. of margarine (measured as 3·5 and 3·4 mgrms. respectively of the standard sample of cod-liver oil). Incidentally it is of interest also to note that the vitamin A potency of the sample of butter, and also of the sample of margarine, is about one-thirtieth (1·4/50, 3·5/100, 1·5/50, 3·4/100) of the sample of cod-liver oil which was adopted by the authors as being an average medicinal cod-liver oil. P. H. P.

Application of the Uranyl Zinc Acetate Method of Determining Sodium in Biological Material.

Toxicological.

Determination of Carbon Monoxide produced from Painted Surfaces in Confined Spaces. F. H. Newington. (*J. Soc. Chem. Ind.*, 1931, **50**, 371–375T.)—A fatality which occurred when a workman entered a closed compartment of one of H.M. ships was found to be due to carbon monoxide poisoning. The compartment had been painted with paint composed of oxide of iron pigment, boiled linseed oil, and paste driers, and was closed while the paint was wet and kept

closed during the ensuing five years.   Investigation of the air in a number of such compartments and of the air in an iron boiled linseed oil drum which had been emptied and left closed for a few weeks, has shown that the carbon monoxide originates from the linseed oil, and that this gas may be formed from either the boiled or the raw oil during the process of drying.   The compartment in which the fatality occurred was afterwards repainted and closed (without vent) for six months, the air then giving the following results on analysis: Nitrogen, argon, etc., 86·47; oxygen, 8·72; carbon dioxide, 4·56; carbon monoxide, 0·131; hydrogen, 0·07; saturated and unsaturated hydrocarbons, below 0·05 per cent.   The air from a compartment on another ship which had been closed (with small vent) for 2 years contained: Nitrogen, argon, etc., 98·37; oxygen, 1·28; and carbon monoxide, 0·033 per cent.   The amount of carbon monoxide produced from linseed oil during drying in a closed space is controlled by the quantity of oxygen available for absorption by the oil.   Provided excess of the oil is present, as must be the case in a compartment coated internally with paint, the maximum concentration of the monoxide to be expected appears to be about 0·3 to 0·4 per cent.

As the amount of carbon monoxide to be expected in these atmospheres was very small, the method of determining it was based on the delicate reaction with iodine pentoxide, the iodine formed being absorbed in potassium iodide solution and subsequently titrated with $N/500$ thiosulphate solution in presence of starch. Owing to the large number of other gaseous constituents possibly present in the compartments, special precautions were necessary to ensure the removal by the purifying train of any gas or vapour other than carbon monoxide that might react with iodine pentoxide.   Contact of the gases with rubber joints or other organic matter must be avoided.   The apparatus, specially designed to be portable and to be usable on board ships in full commission, is described in detail; in essentials the method used is similar to that described by Davies and Hartley (*J. Soc. Chem. Ind.*, 1926, **45**, 164T).   T. H. P.

Toxicological Detection and Isolation of Barbital.   J. J. L. Zwikker. (*Pharm. Weekblad*, 1931, **68**, 975–983.)—*Detection.*—A pale red colour is given by barbital and its phenyl and allyl derivatives on addition of cobalt chloride in methyl alcohol solution free from water.   If a filtered saturated solution of barium methylate, prepared by shaking barium oxide with water-free methyl alcohol, is then added, a stable, deep indigo-blue colour results.   Urine (10 c.c.) should first be cleared with lead acetate, then extracted with 10 c.c. of ether, and the residue after evaporation dissolved in 1 c.c. of the methyl alcohol and tested. The test is sensitive to 0·5 mgrm. of barbital, and could probably be adapted to a colorimetric determination, though no details are given.   A blank test should give only a dirty yellow colour.   *Isolation.*—The reagent is 4 c.c. of 10 per cent. copper sulphate solution with 1 c.c. of pyridine and 5 c.c. of water, and 2 to 4 drops are added to 1 mgrm. or more of veronal or dial in 1 c.c. of water, when a sparingly-soluble, purple-red precipitate of $(C_8H_{12}O_3)_2$ $Cu(C_5H_5N)_2$ results.   This is washed with acid to liberate the barbital, which is removed, dried at 105° C., and weighed.

The m.pt. may then be found and the tests described by Van Itallie and Steenhauer (ANALYST, 1930, **55**, 717) applied. Urine (50 c.c.) is first cleared with 10 per cent. by volume of lead acetate solution, the filtrate shaken twice with 25 c.c. of ether, the extract is evaporated, and the residue is dissolved in water containing pyridine and precipitated as described. The reaction is specific and quantitative for 1 mgrm. of drug, and is given in the presence of caffeine, theobromine, santonin, and of tartaric and succinic acids. J. G.

Toxicity of Methyl Alcohol following Skin Absorption and Inhalation. C. P. McCord. (*Ind. Eng. Chem.*, 1931, **23**, 931.)—Thirty-one monkeys, 58 rabbits and 176 white rats were subjected to the action of various preparations of methyl alcohol, commercial and synthetic, by inhalation and by skin absorption. It was found that pure methyl alcohol is definitely toxic to animals most like men, and it is assumed that practical hazards for human beings may be produced under conditions of trivial exposure. Methyl alcohol (but no formaldehyde) was regularly recovered on distillation of all the organs tested from skin-treated animals, none being found in the controls. A quantity of 0·5 c.c. per kilo. of body weight, applied four times daily, will produce illness in monkeys followed by death. The threshold of danger by inhalation is well below 1000 parts of methyl alcohol vapour per million. R. F. I.

Bacteriological.

Chemical Changes in the Fat of Frozen and Chilled Meat. Part III, Frozen Bacon. C. H. Lea. (*J. Soc. Chem. Ind.*, 1931, **50**, 343–349T; *cf.* ANALYST, 1931, **56**, 538, 610).—Tank-cured bacon stored at −10° C. for periods up to 152 days, followed by hanging at 15° C. for 18 days, showed no evidence of attack of micro-organisms on the superficial layers of fat, and the rise in free acidity of the fat was of the same order as that previously observed for lamb fat. Oxidation has usually set in on the exposed fat at the end of curing, and at 15° C. it proceeds rapidly in the absence of light, in sharp distinction to the case of the fat of fresh beef and mutton. The fat at the same time turns yellow on the surface, and sometimes yellowing affects the interior of the fat. The oxidised fat has an unpleasant rancid flavour on cooking. The effect of smoking after storage at −10° C. was to arrest superficial oxidation for a time, and there is some evidence that interior oxidation may be retarded to some extent, presumably owing to the presence in the smoke of small quantities of anti-oxidants, possibly aromatic phenols. Rapid cooling of the carcases at 5° C. prior to curing appeared to have no effect on the fat. Addition of small quantities of cod-liver oil to the diet of the pigs did not affect the iodine value of the fat of the bacon, but the fat had a tendency to oxidise more readily at the inner centre, to develop a slightly deeper yellow colour on the exposed edge of the back fat, or gave an oily flavour on cooking. Probably such defects would not appear if the cod-liver oil were excluded from the diet some weeks prior to slaughter. D. G. H.

Yeasts found in Fermenting Honey. G. E. Marvin, W. H. Peterson, E. B. Fred, and H. F. Wilson. (*J. Agric. Res.*, 1931, **43**, 121–131.)—Crystallisation of honey and fermentation were found to be related, for, on granulation, the water formerly serving as solvent for the glucose crystals is retained in the liquid part, and increases the water content of the syrup until the syrup becomes dilute enough to permit of the growth of yeasts which could not grow in the uncrystallised honey. The fermentation process is slow (from six months to several years), and the chief fermentation products are about equal quantities of carbon dioxide and alcohol (not often over 5 per cent.) and small quantities of non-volatile acids. The morphological characters of the 5 yeasts isolated were studied, and what appear to be two new species are described. Fermentation may be prevented by keeping the honey at 100° F. for several months, or at 122° F. for 24 hours, or by heating to 160° F. In the latter case, if heating is rapid and the honey is poured into the containers and sealed while hot, and cooled soon, the colour and flavour will not be greatly changed. Unheated honey should be stored at a temperature below 52° F., as honey yeasts will not grow below this temperature.   D. G. H.

Organic Analysis.

New Colour Reaction for Soluble Organic Sulphur Compounds. I. W. Grote. (*J. Biol. Chem.*, 1931, **93**, 25–30.)—A new colour reaction has been found, apparently limited to compounds of divalent sulphur doubly linked to a single non-metallic element. The active reagent is prepared as follows: 0·5 grm. of sodium nitroprusside (sodium nitroferricyanide) is dissolved in 10 c.c. of water at room temperature, 0·5 grm. of hydroxylamine hydrochloride is added, followed by 1 grm. of sodium bicarbonate. After evolution of gas has ceased, 2 drops of bromine are added. Excess bromine is removed by aeration and the dark greenish or black-brown solution filtered and made up to 25 c.c. This solution behaves like that prepared by exposure of sodium nitroprusside to sunlight, and contains a mixture of several compounds, one of which reacts with the thiourea type and another like ordinary nitroprusside. The solution is stable for about 2 weeks. With the use of the new reagent a method is described to distinguish soluble organic compounds of the types C–S–H, C–S–S–C, and C=S from other types and from one another. The following is the method:—5 to 20 mgrms. of the compound to be tested are dissolved in 2 to 3 c.c. of water, and solid sodium bicarbonate is added to excess. About 0·5 c.c. of the general test reagent is then added. A purple-red coloration, produced instantly or within 10 minutes, indicates C–S–H, whilst an intense green or blue indicates C=S or E=S (where E is any single non-metallic element). Both colours may fade more or less rapidly, but often re-appear upon the addition of fresh reagent. If no colour appears within 10 minutes, an equal volume of 5 per cent. potassium cyanide solution is added. C–S–S–C compounds will give a pink to purple-red coloration within 30 minutes. Ring-linked sulphur compounds, both of the C–S–H and the C–S–S–C type, may fail to react, such as diparatolyl disulphide. Organo-metallic compounds, such as the type –C–S–Bi=R$_2$,

may require a preliminary warming with dilute ammonia before reacting. The new reagent may be used for quantitative colorimetric determination of thiosulphate, thiocyanate, thiourea, and other compounds of the C=S type. The colour with the C=S type is usually first green, then turquoise, and finally deep blue; it may stop at the deep green or turquoise stage, and, in a few cases, as with thiourea, it may go through the blue stage to a purple-red and finally crimson after several hours. Some compounds destroy the reagent, but many of these give transient colours of the correct type. No colour is given with sulphones, sulphonic acids, urea, barbituric acid, taurine, thiophene, or *iso*-thioureas. Among the compounds tested and found in the proper class are: (1) C–S–H: cysteine, glutathione, thioglycollic acid, thiosalicylic acid, butyl mercaptan, and bismuth sodium thioglycollate. Thiophenol and thiocresol give fugitive purple-red colours only, the reagent being rapidly destroyed. (2) C–S–S–C: cystine, diglycylcystine, dialanylcystine, dithioglycolic acid, dibutyl disulphide, dibenzyl disulphide, diformidine disulphide. (3) C=S and E=S: thiourea, allyl thiourea, tetramethyl thiourea, thiocarbanilide, thiobarbituric acid, thioacetic acid, potassium xanthate, sodium azido carbon disulphide, thioacetamide, potassium thiocyanate and sodium thiosulphate. Phenyl-isocyanate destroys the reagent in alcoholic solution giving a transitory blue coloration. Sodium diethyldithiocarbamate destroys the reagent, without coloration. P. H. P.

Examination of Commercial Egg-Yolk. R. F. Innes. (*J. Inter. Soc. Leather Trades Chem.*, 1931, **15**, 434.)—In addition to those constituents of eggyolk which are usually determined (moisture, fatty matter, mineral matter, and sodium chloride) the author suggests that determinations of nitrogen and phosphorus should be made, that the constants of the fatty matter be determined (unsaponifiable matter, free fatty acids, total fatty acids, including their m.pt. and iodine value) and that there should also be an emulsification test. The following figures were found for the yolk of fresh eggs expressed as percentages:— Moisture, 50·50; fatty matter, 26·20; mineral matter, 2·52; chlorides (as sodium chloride), 0·20; nitrogen, 2·61; and phosphorus, 0·58. The fatty matter contained 85·3 per cent. of total fatty acids, 0·90 per cent. of free fatty acids, and 6·43 per cent. of unsaponifiable matter. The total fatty acids had a m.pt. of 38° C. and iodine value (Wijs) 63·40. On adding one drop of the sample to 5 c.c. of water in a watch glass containing a trace of sodium chloride a spontaneous emulsion formed.

Four commercial egg-yolks were submitted to this scheme of examination. In two cases, which gave satisfactory results in dressing leather, the figures given were similar to the above. The remaining two gave greasy and unsatisfactory leathers, there was a deficiency of nitrogen, phosphorus and unsaponifiable matter, and the iodine value of the total fatty acids was much higher than with the others. No spontaneous emulsion was formed with water. R. F. I.

The Furfural Number of Tanning Extracts and their Mixtures with Sulphite Cellulose Extract. V. Němec. (*J. Inter. Soc. Leather Trades Chem.*, 1931, **15**, 440.)—The method of Laufmann, in which use is made of the

formation of condensation products of tannins with furfural is modified. Fifty c.c. of the unfiltered analytical tannin solution are placed in a conical flask together with 40 c.c. of a freshly prepared mixture of 7 per cent. aqueous furfural solution and 20 per cent. hydrochloric acid (equal volumes). The mixture is boiled over a naked flame, cooled and filtered through S and S 588 folded filter paper of 12·5 cm. diameter, 30 c.c. of the filtrate are evaporated, and the residue is dried and weighed "C". The total solids in 25 c.c. of the untreated tannin solution are designated "A", and the residue from 25 c.c. of the tannin solution and 20 c.c. of the furfural reagent is designated "B". The furfural number "F" equals

$$\frac{100 \times (B - 1·5 C.)}{A}$$

In the case of quebracho, mimosa bark, and gambier, the value of F lies between 94 and 105. For chestnut, sumac, valonia and myrobalans it lies between 2 and 9. Other values are:—Mangrove bark 62, pine bark 41, sulphited quebracho 93 to 96, sulphite cellulose −11 to −16. The test thus forms a means of quantitatively determining sulphite cellulose in pure tanning extracts. R. F. I.

Decay of Book-binding Leathers. R. W. Frey and I. D. Clarke. (*J. Amer. Leather Chem. Assoc.*, 1931, **26**, 461.)—A series of ten dummy books covered with various leathers was exposed in a rack near a west window, open during the day, for $8\frac{1}{4}$ years. At the end of this period all the bindings had become more or less deteriorated in the exposed (back) portion. An analysis was made of the backs, the protected sides, and the original leather preserved in sealed envelopes, for Procter–Searle acidity, total sulphur, total and fixed sulphates in the water extract, mineral matter, and fats and a determination was made of the class of tanning material used. The results of certain physical tests are also recorded— crackiness, "scuffiness," tensile strength, resistance to tear, porosity, and permeability. Nos. 1, 2 and 3 had suffered least, Nos. 5 to 10 the most, and No. 4 intermediate. The analyses may be summarised as follows:

Acidity (Procter-Searle), per cent. as sulphuric acid.	1 Sheep.	2 Goat.	3 Goat.	4 Goat.	5 Pig.	6 Sheep.	7 Goat.	8 Goat.	9 Cow.	10 Cow.
Back	2·57	2·01	2·54	4·09	6·13	8·62	7·73	7·27	5·26	9·38
Sides	0·36	0·00	0·51	0·88	2·14	2·31	2·49	1·16	1·78	2·32
Original	0·31	0·19	0·26	0·34	1·39	1·31	1·38	0·00	1·27	1·55
Original leathers—										
Ash	5·43	6·52	0·42	0·41	0·25	1·92	0·60	0·50	1·76	1·24
Fat	9·16	9·46	0·96	12·29	3·14	11·96	11·96	7·13	8·38	14·70

Tannage   Pyrogallol   Mixed Pyrogallol   Catechols

It is pointed out that decay runs parallel with sulphuric acid absorption from the outdoor atmosphere, and that the magnitude of this absorption in the comparatively short period of $8\frac{1}{4}$ years was quite remarkable. The Procter–Searle

values of the backs of Nos. 1 and 2 are much lower than the others. It is considered that this may be due to their high content of ash containing sodium chloride and calcium, since the fixed sulphates in the aqueous extracts of these are high. (The fixed sulphates are those left after evaporating and igniting the water extract and dissolving the residue in water.) Apart from these two, the only explanation for the variation in sulphuric acid absorption of Nos. 3 to 10 would appear to be the nature of the tanning material. Resistance to decay appears to be independent of the fat content, since leathers with both high and low fat content are found to have both high and low resistance. The use of a pyrogallol type of tanning material does not ensure resistance to rotting, since No. 5 rotted badly.

Determinations of nitrogen were made on the leathers before and after extraction with 0·1 N sodium carbonate solution. In a bad leather (No. 10) the percentage of total nitrogen in the original leather was 8·39, in the sides after extraction 8·33, and in the back after extraction only 2·19. Such loss becomes appreciable at 65 per cent. deterioration, as found by loss of tensile strength.

R. F. I.

Inorganic Analysis.

New Method for the Detection of Cobalt. T. Bersin. (*Z. anal. Chem.* 1931, **85**, 429–433.)—Cobalt may be detected in a solution, from which metals of Group II (the hydrogen sulphide group) are absent, by rendering it ammoniacal after the addition of ammonium chloride (the presence of precipitated hydroxide of iron, aluminium, etc., may be disregarded), boiling for about half-a-minute to oxidise the cobalt, and adding a solution of the anilide of thioglycollic acid in alcohol. The presence of cobalt is shown by the formation of a reddish-brown flocculent precipitate which does not dissolve when the liquid is subsequently acidified. The sensitiveness of the test may be enhanced by shaking the acidified solution with ether, chloroform, or benzene, when the precipitate collects at the junction of the solution and the organic liquid. The method is capable of detecting 0·5γ of cobalt in 5 c.c. of solution containing up to 0·1 grm. of nickel; in the presence of about 10 mgrms. of aluminium, zinc, chromium, ferrous iron, manganese and nickel in 5 c.c., the detection of less than 1 mgrm. of cobalt is uncertain. A simple method of preparing the reagent is described. S. G. C.

Quantitative Separation of Lead and Iron. H. Funk and O. v. Zur-Mühlen. (*Z. anal. Chem.*, 1931, **85**, 435–438.)—The separation depends on precipitating the lead as chromate, employing a somewhat higher concentration of acetic acid in the solution than is usual, in order to prevent the co-precipitation of iron as basic ferric acetate. To the slightly acid solution containing the lead and iron as chloride or nitrate, ammonium acetate is added, followed by 10 c.c. of glacial acetic acid; the liquid is diluted to 100 c.c. and heated almost to the boiling point. The lead is precipitated by slowly adding an excess of dilute ammonium dichromate solution (1 per cent.), with stirring. After cooling, the lead chromate is filtered off and washed with cold water containing a little acetic acid. The lead

can be determined either by weighing the precipitate or by the iodimetric titration of the chromate radicle in it; the iron in the solution can be precipitated as hydroxide and weighed as oxide or determined volumetrically.   Good results were obtained with 0·05 grm. to 0·2 grm. of lead mixed with 0·14 grm. to 0·05 grm. of iron.   S. G. C.

Volumetric Method of Determining Sodium. J. T. Dobbins and R. M. Byrd.   (*J. Amer. Chem. Soc.*, 1931, **53**, 3288–3291.)—The method is based on the precipitation of sodium as sodium zinc uranyl acetate in the usual way, and the titration of this with sodium hydroxide.   The precipitate is washed three or four times with 2 c.c. portions of ethyl alcohol (95 per cent. saturated with sodium zinc uranyl acetate) and dissolved in water (about 100 c.c.)   After the addition of five drops of phenolphthalein indicator, the liquid is titrated with 0·5 *N* sodium hydroxide solution until a red colour is produced which persists after heating the liquid to incipient boiling for five minutes; the excess of alkali which is then present is titrated with standardised dilute hydrochloric acid, the end-point being ascertained by allowing the yellow precipitate in the solution to settle after each addition of the acid, and viewing the supernatant liquid.   The amount of sodium present can be calculated from the equation

$$NaZn(UO_2)_3(C_2H_3O_2)_9 + 10NaOH \longrightarrow 9NaC_2H_3O_2 + Na_2U_2O_7 + ZnUO_4 + 5H_2O,$$
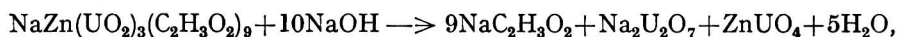
it having been found that 10 grm.-mol. of sodium hydroxide are required for each grm.-mol. of the triple salt.   It is recommended, however, to standardise the alkali solutions used by means of the solution of the precipitate of sodium zinc uranyl acetate given by a known amount (20 to 25 mgrm.) of sodium chloride, in order to allow for the personal error in determining the end-point of the titration.   The method is claimed to be superior to that in which the sodium zinc uranyl acetate is weighed, since it is independent of the somewhat uncertain degree of hydration of the triple salt.   S. G. C.

Application of the Uranyl Zinc Acetate Method of Determining Sodium in Biological Material.   A. M. Butler and E. Tuthill.   (*J. Biol. Chem.*, 1931, **93**, 171–180.)—Barber and Kolthoff (*J. Amer. Chem. Soc.*, 1928, **50**, 1625; ANALYST, 1928, **53**, 456) described a method for the quantitative precipitation of sodium with uranyl zinc acetate and the gravimetric determination of the sodium as uranyl zinc sodium acetate.   Samples with 2 mgrms. of sodium gave accurate results.   Phosphate was precipitated by the reagent, and thus had to be removed first, but its precipitation as uranyl phosphate has not been found satisfactory. Barber and Kolthoff reported the same difficulty, but obtained satisfactory results with known salt solutions after removal of phosphate by precipitation with magnesia mixture.   The determination of sodium in biological work is of importance, and the methods at hand are either laborious, expensive, or subject to considerable error, and, therefore, the applicability of the Barber–Kolthoff method to biological material has been studied.   The information obtained and modifications introduced have resulted in the development of definite procedures for the determination of

sodium in urine, serum and material high in phosphate and potassium such as whole blood, stool and tissue. These procedures are described in detail. Powdered calcium hydroxide is used for the precipitation of phosphate and found to be satisfactory, within the desired limits of error, and more convenient than the magnesia mixture. It simplifies the reagents required for the procedure. Its addition clarifies cloudy urines and produces no volume change, and thus eliminates the necessity of diluting to known volume the taking of aliquot parts and the quantitative washing of the precipitate. For urines containing protein solid mercuric chloride as precipitation reagent is used, and again volume changes are avoided. The triple sodium salt is precipitated in a stoppered glass filter, and thus the necessity of washing the precipitate on to the filter is eliminated, thereby saving time and reagent. The authors have studied the necessity of ashing urines, and have investigated conditions for the determination of sodium in urines containing relatively very small amounts of this element. Some results obtained with known solutions are given; they show small percentage errors.　　P. H. P.

Electrometric Analyses of Solutions of Hypochlorites. F. Abribat. (*Bull. Soc. Chim.*, 1931, **49–50**, 1119–1138.)—The usual procedure was followed; the platinised or polished platinum electrode and the end of the saturated calomel electrode were placed directly in the sample, a stable potential being obtained after 3 to 5 minutes, when an electrometric titration with (*e.g.* 0·25 N) sulphuric or hydrochloric acid could be carried out. The curves obtained (E.M.F. as ordinate) for commercial solutions of sodium hypochlorite show 5 distinct portions separated by well-defined points of inflexion, which enable the following determinations to be made:—*Total Hypochlorous Acid.*—This is given by the abscissa between the third and fourth points of inflexion, and corresponds with the reaction $HClO + HCl \rightleftharpoons Cl_2 + H_2O$ (E.M.F. 800 to 950 millivolts). The data obtained during the period of liberation of gaseous chlorine are in agreement with the accepted values for the reversible chlorine electrode. *Free Alkali.*—This is given in terms of the acid corresponding with the abscissa of the curve between the origin and the first point of inflexion. *Carbonate and Hypochlorite.*—The abscissa of the second region of the curve corresponds with the transformation of carbonate into bicarbonate, whilst the total abscissa of this and the adjacent (*i.e.* third) region represents the liberation by the acid of (*a*) carbon dioxide from the bicarbonate, and (*b*) hypochlorous acid from the hypochlorite. It is preferable, however, to take the sum of these abscissae as representing (hypochlorite+sodium carbonate), since the two regions concerned are not separated by a very sharp point of inflexion. *Chlorites* are also represented by the second region of the curve, and are, therefore, obtained by a second titration after removal of the carbonate by precipitation with lime. *Free hypochlorous acid* is obtained by difference from the hypochlorite and total hypochlorous acid values. *Chlorides* are determined by direct titration with silver nitrate solution by means of a silver electrode, since the silver salts of chlorous, hypochlorous and (in certain cases) chromic acids are precipitated only at the conclusion of this titration. Alternatively, this type of titration may be carried out,

before and after the reduction by the usual method, with arsenious oxide in the presence of sodium bicarbonate (*cf.* Penot, *J. prakt. Chem.*, 1851, **54**, 59). The method, which is rapid and unaffected by chlorates and perchlorates, was also applied to "chloride of lime," but in this case the results may be upset by the co-existence of two sets of reactions, *viz.* those taking place in the solution and those between the solid and acid liquid phases. It is pointed out that the term "active chlorine" has been applied to (*a*) the amount of hypochlorite chlorine; (*b*) the amount of free chlorine producing the same effect as a given weight or volume of a bleaching agent.   J. G..

Water Analysis.

"**Carbonate Number**" **in Water Analysis.** O. Mayer. (*Z. Unters. Lebensm.*, 1931, **62**, 271–291.)—The relation between degrees of carbonate hardness (x) due to magnesium and calcium, and the total solids (y) in grms. per 100 c.c., is given by the ratio $1000x/y$, and is known as the "carbonate number." This value serves a useful purpose for the characterisation of certain waters, and also for the detection of "infiltration" into the source of one water, of another having a different carbonate number. Its relation with the other analytical numbers, particularly with the total hardness, free carbon dioxide and total alkalinity, is discussed, and a table shows the relation between carbonate hardness and bound and free carbon dioxide. A rapid scheme of semi-quantitative analysis, based on these considerations, is outlined. Thus, an indication of the alkalinity is obtained by direct titration of 100 c.c. of the water with $0.1 N$ hydrochloric acid containing 5 c.c. of 0·05 per cent. methyl orange solution per litre, the end-point being the appearance of a brownish-red colour. The total hardness may then be found by bleaching the indicator with one drop of bromine water, the solution being boiled for a short time, diluted to 100 c.c. with distilled water, and 0·5 c.c. of a 0·4 per cent. alcoholic solution of phenolphthalein added. Titration with a clear $0.1 N$ solution of sodium palmitate by Blacher's method (Kolthoff, *Die Massanalyse*, 1928) may then be carried out. The gravimetric method should be used for more precise determinations, *viz.* the water is evaporated in a platinum dish, silica removed, the iron and aluminium precipitated with ammonia, and the calcium obtained as oxalate and determined as oxide, and the magnesium as pyrophosphate. "Chalk-aggressive" carbon dioxide is found by determination of the change in alkalinity of 250 c.c. of the filtered water after digestion with finely-powdered, washed marble for 24 hours to 3 days. Numerous examples of the method are given and discussed, and data for 91 waters (Bavaria and Danube valley) are tabulated and discussed with special reference to geological origin.

                                                                                             J. G.

Determination of Nitrates in Drinking Water. W. Mulder. (*Pharm. Weekblad*, 1931, **68**, 995–997.)—The conclusion of Scheringa (*id.*, 1930, **67**, 1362) that sodium salicylate gives a stronger colour than phenol-sulphonic acid in this determination, and is otherwise preferable, is confirmed. The correction factor for the colorimeter reading given by Scheringa to be used in the presence of chlorides

[namely $100/(100-10a)$, where a is the chloride present in mgrms.] is shown, however, to be valid only for 2 mgrms. or less of Cl' in the presence of 0·1 mgrm. of nitrate in 10 c.c. of water. Low results are obtained for 3–10 mgrms. of Cl' under these conditions, and the factor 1·25 should then be used. The author, therefore, takes a volume of sample (e.g. 10 c.c.) containing about 0·1 mgrm. of nitrate, adds 5 drops of 2 per cent. sodium chloride and 1 c.c. of 0·5 per cent. sodium salicylate solutions, evaporates to dryness on the water-bath, and adds 1 c.c. of sulphuric acid to the cool residue. After 10 minutes the mixture is washed into a colorimeter tube with 10 c.c. of ammonia or sodium hydroxide solution, and the colour is compared with that of a standard containing exactly 0·1 mgrm. of nitrate and 5 drops of 2 per cent. sodium chloride solution in 10 c.c., the colour being developed by a similar procedure. Accurate results are obtained for 0·1 mgrm. of nitrate in the presence of 0·01 to 0·1 mgrm. of nitrite, but for larger quantities (1 and 2 mgrms.) of the latter, high results are obtained. J. G.

Physical Methods, Apparatus, etc.

Chromium Steel Vessels for Analytical Purposes. A. Krüger. (*Chem. Ztg.*, 1931, **55**, 682–683.)—Vessels made from chromium steel (V4A steel) are claimed to be preferable to glass or porcelain ware for certain analytical work where freedom from contamination of solutions by substances, *e.g.* silica, alumina, etc., which can be dissolved from glass by alkaline liquids, is desired. Two examples, *viz.* the gravimetric determination of iron and of aluminium by precipitation as hydroxide, have been studied, and good results were obtained. The acid solution was kept in a glass vessel and poured into the stainless steel vessel containing an excess of ammonia, in which, in the determination of aluminium, the subsequent adjustment of the solution to feeble alkalinity for the precipitation was made. In tests of the resistance of chromium steel vessels to various solutions, hot dilute ammonia was found to be without action; but the vessels suffered slight attack by stronger alkaline solutions. The results are summarised in the subjoined table.

Liquid.	Temperature.	Time.	Test with sodium sulphide.
KOH (1 per cent.)	100° C.	20 minutes	Colourless
KOH (5 per cent.)	100° C.	20 ,,	Very slight yellow
KOH (10 per cent.)	100° C.	20 ,,	Slight yellow
KOH (1 per cent.) + H_2O_2	70° C.	20 ,,	Colourless
KOH (1 per cent.) + Na_2S	70° C.	20 ,,	Clear, colourless
KOH (5 per cent.) + H_2O_2	70° C.	20 ,,	Very slight yellow
K_2CO_3 (1 per cent.)	100° C.	20 ,,	Colourless
K_2CO_3 (5 per cent.)	100° C.	20 ,,	Very slight green
K_2CO_3 (10 per cent.)	100° C.	20 ,,	Slight green
NH_3 (5 per cent.)	70° C.	20 ,,	Colourless
NH_4Cl (3 per cent.) + Na_2S (2 per cent.)	70° C.	20 ,,	Clear, colourless
$Ba(OH)_2$ (0·5 per cent.)	100° C.	20 ,,	Colourless

(In these tests, 200 c.c. of liquid were used; this was tested for dissolved iron, after being in the vessel for the stated period, by adding sodium sulphide solution; "slight green" and "very slight green" was found from special experiments to correspond with the solution of 0·2 to 0·1 mgrm. of iron.) It is concluded that the

choice between a glass or stainless steel vessel for a particular analytical determination involving alkaline liquids will depend on which is the lesser of two evils: contamination by silica, etc., from the glass, or by iron from the chromium steel. (*Cf.* G. A. Stokes, ANALYST, 1929, **54**, 538.)   S. G. C.

Reviews.

CHEMISTRY, FLAVOURING AND MANUFACTURE OF CHOCOLATE, CONFECTIONERY AND COCOA. By R. H. JENSEN, M.Sc., F.I.C. Pp. x + 406. J. & A. Churchill, 1931. Price 27s.

The author of this book has been so singularly successful in condensing into the small space of one volume such a mass of information and experience that it is impossible adequately to review the work within the space allotted; all that can be done is to indicate the scope of the work and to refer in more detail to certain sections.

The raw material, cocoa, forms the subject of the first chapter, in which the types and commercial varieties are dealt with briefly and concisely. The nature of the changes which occur during the fermentation of the bean has been widely studied, and a résumé of this shows only too clearly that there is a long path to travel before we determine to what substances the cocoa aroma is due. The statistics of prices and production and the economics of the industry are useful additions not usually found in a work of this kind. The value of cocoa and cocoa products as a food, a fact which is more recognised abroad than in this country, has been given due prominence. It is doubtful whether sufficient notice has been taken of the work of R. O. Neumann on the digestion of the proteins, which, though now somewhat old, is still convincing.

Manufacturing processes are described minutely and a critical examination is made of a number of modern machines and appliances. Right through this section, as indeed through the whole book, the chemical and physical aspects are considered and explained, use being made of carefully selected data, in many cases hitherto unpublished. A criticism of some of the illustrations might be that, while they depict plant well known to those in the industry, they do not give much indication to the layman of their *modus operandi*.

A consideration of chocolate in the molten state shows it to be composed of finely divided particles of cellular and non-cellular structure, the solid phase suspended in a fatty fluid, the liquid phase. The behaviour of this magma during solidification determines the appearance, texture, and stability of the product. Particle size of the component parts of the solid phase requires measurement if conclusions are to be drawn as to the surface to be covered by the liquid phase. The methods for making these determinations are described. As regards the liquid phase, in recent years much work has been done on the behaviour of mixed glycerides during changes of state, and these the author has collated in an excellent chapter on confectionery fats. The significance of the solidification test

and of dilatometer measurements is rightly emphasised. The author has standardised an apparatus and minutely described the procedure for the former, a most essential factor omitted by other investigators, and, using the latter, gives interesting data from his own experiments, which go a long way towards explaining some of the abstruse phenomena occurring in manufacturing practice.

In the sections devoted to confectionery and the ingredients used therein, a mass of analytical data has been employed not only to evaluate these but to explain their properties and how to use them in order to obtain the desired results. Here, as elsewhere in the book, the author is handicapped in that he is not free to use and discuss much important and pertinent work which has been carried out by the British Association of Research for the Cocoa, Chocolate, Sugar Confectionery and Jam Trades.

A wide survey of flavouring materials and their properties fills a section, and is preceded by a chapter on the principles of flavouring, which, though somewhat dogmatic, indicates factors which must be taken into consideration in the successful use of flavouring materials. A few useful pages at the end are devoted to a summary of legal standards for cocoa and chocolate in various countries. The work shows the extent to which chemical and physical sciences, not to mention dietetics and physiology, enter into the manufacture of what is not only a delicacy, but a food stuff. The scope of the chemist's work is clearly indicated, and shows the way in which the value of his work may be most beneficial to the business.

The author is to be congratulated upon the production of a book which contains up-to-date knowledge on many points and is destined to take a very prominent place in the literature of this industry. B. G. McLELLAN.

LUNGE AND KEANE'S TECHNICAL METHODS OF CHEMICAL ANALYSIS. 2nd Edition, edited by the late CHAS. A. KEANE, D.Sc., Ph.D., and P. C. L. THORNE, M.A., M.Sc., Ph.D. Volume III. Pp. 698. London: Gurney & Jackson. 1931. Price £3 3s.

The progress of research in the different branches of chemical technology covered by the articles in this volume has been very different, so that some of the articles are new work rather than revision, whilst others show comparatively little alteration from those which they supersede.

The article on Clays, Ceramic Products, and Refractories, now divided into two portions—chemical examination and physical examination—comes under the first head. The chemical portion is written by Mr. H. V. Thompson, M.A., F.I.C., and the physical portion by Mr. S. R. Hind, B.Sc., A.R.C.Sc., F.I.C., both of them on the staff of the North Staffordshire Technical College, and each of them has carried out his task most satisfactorily. The methods of chemical analysis cover fireclays and silica rocks and their products, felspars, bauxites, limestones and dolomites, barytes, zirconia, chromite, fluorides and glazes; and though there is little that is new, yet all the methods are described with great clearness and conciseness, and details of manipulation, where attention to those details is necessary to success, are carefully and minutely given. The growth in importance of the

examination of physical characters is indicated by the greatly increased space devoted to this part of the subject. Specific gravity, true and apparent; grading and determination of particle sizes; adsorption and viscosity; plasticity, binding power, and shrinkage of raw clays; thermal expansion, refractoriness, and deformation under load; porosity; resistance to stresses and abrasion; dielectric strength: all these, and other related properties, are considered, and methods— many of which have been worked out in the Stoke College—are given for their determination or estimation. All are well and clearly described, and comprehension is aided by many diagrams and illustrations of apparatus. The whole forms a monograph of great merit, and will become almost indispensable to those who have to deal with these materials.

The same may be said of the Section on Glass, which has expanded from 37 pages in the old edition to 150 pages in this. Mr. J. D. Cauwood, M.Sc., of the Canning Town Glassworks, and Mr. J. H. Davidson, M.Sc., of Messrs. C. E. Ramsden & Co., have collaborated here, and have produced a work which everywhere bears the impress of minds and hands engaged in the industry of which they treat. Valuable features of this Section are, first, short accounts of raw materials and their impurities, the uses of the raw materials and the effects, on these uses, of the impurities; and second, tables giving the average composition of various types of glass. If works on analytical chemistry, especially of industrial materials and products, were not so strictly confined to the description of methods, but gave a little trustworthy information of this kind, it would be all to the good. It would be tedious to enumerate in detail the contents of this section; enough to say that methods are given for the analysis of all raw materials and of every variety of glass, and for the examination into the durability of glasses and their attack by chemical and other agencies; that the descriptions are almost uniformly good, concise, and clear; and that the authors have evidently tested even the most recent work, and have here embodied such of it as has met with their approval.

The Section on Calcareous Cements, by Dr. Geoffrey Martin and Mr. E. A. Bowes, covers the analyses of limestones, limes, lime-sand mortars, and natural and artificial hydraulic cements; and, like the previous article, it not only gives methods, but furnishes useful relevant information regarding hydraulic limes and natural cements, with typical analyses showing their composition. Not only the chemical analyses, but mechanical and physical tests are described. The analyst dealing with these substances will find all he wants here, though the descriptions are not all on the same high level as those in the first two sections: there is occasional loose phraseology, and there are slips which do not seem to be all "typographical." We find Warington misspelt on p. 287, "bulk" for "burette" on p. 297, and a wrong sign inside the brackets in the calculations near the top of p. 294; and a large part of the description of the Southard viscometer on pp. 312–314 is absolute and almost verbal repetition. We have also phrases like "Between each test" and "Between zero time to the point . . .", and the authors' fondness for that frequent trap "the latter," leads them to direct, on p. 322, that a glass plate should be placed on the trowel used, instead of on the mould. A little

more thought given to things like these might have greatly improved the article and helped the reader; but, in spite of these shortcomings, it is a valuable and useful contribution.

Drinking Water and Water Supplies, and also Sewage and Effluents, are treated in separate sections, by Dr. Gilbert Fowler. The physical, chemical, microscopical, and bacteriological examination of water are all dealt with, the chief part of the work being, naturally, concerned with the chemical examination. Dr. Fowler prefaces the chemical portion with the statement that the results are most conveniently expressed in parts per 100,000; but he himself apparently scorns convenience, for nearly all the figures given in the section are either "milligrams per litre" or "parts per million." The methods given cover the usual course, though modern developments have brought the need for detecting small quantities of free chlorine (for which the American Public Health Association's tolidine coloration, method is given), and of iodides—here Hunter and Brubaker's method might have been described. As the action of waters on lead is discussed, it seems a pity that Dr. Houston's very convenient method of estimating lead-solvency is not given. The methods for nitrates, and for determining gases in water, are fully and clearly described, and the whole section fulfils its purpose well.

The short article on Feed Water for Boilers, by Mr. L. O. Newton, of Sofnol Limited, confines itself to a description of methods of determining the mineral constituents of the water and its hardness, and is all very concise and clear.

The Section on Sewage and Effluents gives directions for sampling and preliminary examination, and for determination of dissolved solids, suspended and colloidal matters, oxygen absorption, alkalinity or acidity, nitrogen in its various forms, sulphides, chlorine, individual organic substances, coal gas products, and tests for putrescibility. These are followed by a short but valuable discussion on the criteria of contamination due to effluents, and their injurious effect.

Sections on Fertilisers and Feeding Stuffs follow, from the pen of Dr. Bernard Dyer. These form, to all intents and purposes, a new treatise. Much of the work is, of course, conditioned by the requirements of the Fertilisers and Feeding Stuffs Act, but the wealth of Dr. Dyer's experience crops out continually in the details of the methods of analysis given, and in the discussion of their applicability in various circumstances; such a description of the Kjeldahl process, for example, as is here given, is not to be found elsewhere. The account of calcium cyanamide, too, is very full and clear. Not only the official methods for Feeding Stuffs under the Act, but also those of the International Committee, are given, and the article ends with useful paragraphs on the detection of mustard, hydrocyanic acid, and castor oil seed in feeding stuffs.

"Soils" are dealt with by Sir Daniel Hall, who gives an account of the derivation and characters of different soils before going on to their sampling, mechanical examination, and chemical examination. Methods are given for nitrogen, soluble mineral constituents, carbonates, nitrates, pH value, lime requirement, and "exchangeable bases." The methods are not new, of course, but their collection here in one treatise is useful. There occur two or three slips (as it seems to me),

in description. On p. 592 we treat 20 grms. of soil and make the solution up to 250 c.c.; this is spoken of as a 10 per cent. solution. On the same page, in determining potassium, it is surely a strange direction to wash the platinum precipitate and excess platinum chloride on to a filter with ammonium chloride. And on p. 599 I do not follow the direction to deduct the results of the second litre of leachings from those given by the first.

The last Section, on Air, is written by Dr. J. S. Owens and Mr. J. H. Coste, and is entirely new. It describes methods for the determination, not only of the ordinary constituents of fresh air, but of impurities of various kinds which may occur locally in greater or smaller amount—usually in very small proportions—derived from the industrial and other operations of civilised life: carbon monoxide, nitrogen oxides, ozone, sulphur compounds, and various organic vapours; and it deals also with the suspended matter in the air and the various appliances in use—largely due to Dr. Owens—for its examination.

A review of a huge and detailed work like this, unless it is to be of the dimensions of a treatise, can be little more than a summary of the contents. Detailed criticism would swell it to intolerable dimensions, and, on the whole, little criticism is called for—the work worthily maintains the tradition of its predecessors, and yet represents the present-day position. There is, however, one general criticism, applicable in very differing degree to different sections in the volume, that I should like to offer. It is, perhaps, a matter of personal preference, and not to be decided absolutely, whether directions for analytical processes should be in the imperative mood or in the passive voice of the indicative. I go heartily for the imperative, but would blame no man for using the other. But we have, I think, a right to ask for consistency; it is to me, and I expect to others, very disturbing to read "Cool and add 150 c.c. of hydrochloric acid: the contents are now stirred and filtered: wash three times with water: the filter paper is now removed"; and so on. Many examples of this will be found throughout this volume, and I think one should protest against it.

Take it for all in all, this is a collection of excellent treatises, in which he who consults them will seldom, if ever, fail to find what he seeks, and which should be in the library of every analyst who has to do with any of the subjects of which it treats.

J. T. DUNN.

BACTERIOLOGICAL TECHNIQUE. By J. W. H. EYRE, M.D., M.S., Professor of Bacteriology in the University of London. Third Edition. Pp. xii+619, with 238 figures in the text. London: Baillière, Tindall & Cox. 1930. Price 21s.

A perfunctory survey of this book leaves the impression that little except hydrogen ions has disturbed the placidity of the routine in bacteriological laboratories since the publication of the second edition in 1913. However, the truth is that the book has been thoroughly revised, but so unobtrusive are the additions, numerous though they be, that only comparison, page by page, with the preceding volume reveals them. The Preface is useless as an indication of the progress of the book.

Great improvement in the illustrations is shown. Many of the old cuts have been replaced by diagrammatic sketches, and there are twenty-two new pictures of the microscope and accessories.

Newly described apparatus includes a reference to the electrical hot-air steriliser and the modern innovations in bacterial filters, namely, the Seitz asbestos, the plaster of Paris, and the collodion ultra-filters, are described, as also is the air-pressure method for testing porcelain candle filters.

So large a variety of stains was evolved at an early date in bacteriological history that no surprise is felt that only five additions are thought necessary. They are Czaplewski's carbol-fuchsin, Fontana's method, Murray's iron alum haematoxylin, van Giesen's Nile blue, and the Barnard Topley method for demonstrating flagella during life.

Additional media are more numerous: alkaline egg broth, alkaline egg agar, Robertson's cooked meat medium, five of the war-time "trypsin digests" media, Thornton's standardised agar, Wilson and Blair's G.I.B.S. agar, Russell's double sugar agar (coloured with phenol red as an alternative to litmus), Koser's citrate broth for *B. coli*, Douglas's tellurite serum for *B. diphtheriae*, two of the media devised by Flemming for the haemophilic bacilli, and five new media for protozoa.

Eight pages are devoted to new devices for achieving anaerobiosis and reduced oxygen atmosphere in cultures, and two methods for micro-selection are described.

It is only fair that it should be left to the deviser of Eyre's scale to publish coloured diagrams showing the very great difference between the pinkish phenol-phthalein end-point of the Eyre scale and the deep magenta to which the American Committee of Bacteriologists take their titration—a source of confusion all too little known.

Bacterial haemolysis and the bacteriophage are given attention, and acid production by bacteria now means another task for the comparator.

At a time when many are trying to classify bacteria, and there is an understandable over-eagerness to support something with plausible claims to notice, it is refreshing to find Professor Eyre summing-up that: "no really successful classification of the Schizomycetes has yet been drawn up, despite the elaborate system of families, tribes, and genera promulgated by the Society of American Bacteriologists in 1920, and the varying morphological characters of the Schizomycetes still continue to be utilised as a basis for classification, as they were by Baumgarten in 1890 and Sternberg a few years later."

Many processes of sanitary utility receive detailed notice, and alternative methods are often described. Two methods for the sanitary examination of shell-fish: that devised by Houston, and that of Klein which has been adopted by the Worshipful Company of Fishmongers for the control of retail trade, are mentioned. For disinfectants, the author describes at length the process he favours in which he now incorporates some of the details of *The Lancet* (1909) process. He also refers to the Hygienic Laboratory (U.S.A.) method. The Rideal–Walker method is dismissed in a few lines, which is scant justice to a device that, more than any other, had made the standardisation of disinfectants practical by recognising the

many circumstances that disturb the action of disinfectants on naked bacteria. It is still the method most frequently adopted, not only by commercial houses, b ⁞ by Government Departments. The Lister Institute method is not mentioned, in spite of the degree of popularity it has received. Much could be written in favour of Eyre's method, but the others are in equal demand and worth more than perfunctory notice.

Milk examination includes both Breed's "microscopical count" and Frost's "little plate method," but it ignores the starvation method of enumerating bacteria approved in "Memo. 139/Foods" for Graded Milk (ANALYST, 1929, **54**, 235).

Misprints include "nutose" for "nutrose" (inset p. 512), and "tinige" for "tinge" (p. 161), while a formula of $CaOS_4$ (p. 51) and the substance "piridin" (p. 18) are not serious. But for an author having an enviable acquaintance with his science and a profound knowledge of its applications to convey the impression that nothing is good unless German, can only signify the perpetuation of pre-war material. Witte peptone did good service for many years, but when, in 1930, it is prescribed (no alternative being allowed) for nutrient broth, lemco broth, peptone water, and even for the bile salt broths, it would appear that fair trial of modern English peptones has not been made, and that the disconcerting attributes of some former English preparations have unfairly prejudiced the entirely (I believe) satisfactory modern manufactures. English-made stains get little more appreciation: thus, on p. 102, "The stains employed should be those prepared" by two named German firms, and a paltry footnote mentions that an English firm prepares "a limited variety of reliable stains." The totally unnecessary exaltation of things German extends even to words; thus, while everywhere else a "loop" needle is called a "loop," we find here the information (p. 83) that it "is termed a loop or an oese." A footnote that points out with no evasion the advantage which chrome-nickel wire has over platinum will please all.

The modifications of the Romanowsky stains (those of Jenner aud Leishman are mentioned, whilst Giemsa stain, popular though it be, is not) are given a restricted sphere of blood-, and protozoa-staining, whereas they give illuminating information on pus films, milk sediments and many other preparations.

It is probably unintended, but may be seriously misunderstood when the routine scheme for the bacteriological examination of a water includes (p. 512) the following direction: "Determine the pathogenicity for mice (subcutaneous inoculation) and rabbits (intravenous inoculation) of the streptococci isolated." Only in a very exceptional case could the results have any significance.

All who have used this book appreciate it, but it is often involved in technique where simplicity is needed; and, though the little tips that lessen labour and smooth difficulties are well to the fore, these are largely original. There are hosts of others in books, English and American, that should be introduced if this book is to do the best service to bacteriology. At present, Professor Eyre has the field of bacteriological technique to himself, and did he but realise the worry his directions can cause, as well as the godsend he knows the book to be, he would have scrapped

much and re-written more. He would also have given us a less heavy book by remodelling binding, paper and type. WILLIAM PARTRIDGE.

DAIRY BACTERIOLOGY. By Prof. ORLA-JENSEN. Translated by P. S. Arup. Second Edition. Pp. x+198. London: J. & A. Churchill. 1931. Price 18s. net.

This book is a second and revised edition of Prof. Orla-Jensen's well-known work on a subject of which he is an acknowledged master.

The author has shown much wisdom in the treatment of the subject, in that he has not attempted to write a treatise on bacteriology and on the science of dairying, but has arrived at a happy blend of both. To secure the maximum benefit, the reader must possess a knowledge of both subjects, and the student will require the guidance of an expert. The book has been most admirably translated by Mr. Arup, and is well indexed.

The subject has been dealt with in two parts: Part I, which comprises about one-third of the book, deals with elementary bacteriology, and is splendidly illustrated. To the bacteriologist, parts of the classification and nomenclature are confusing, since they do not conform to any of the widely accepted standards.

The method detailed for milk examination appears likely to increase the inevitable errors of dilution and would not be applicable generally to English clean milk.

A serious error is found on p. 45, where organisms of the aerogenes-proteus type are said to form spores. They are, of course, not spore formers.

Part II, which comprises the greater part of the book, is devoted to the scientific control of milk and milk-products, the making and control of starters, and the ripening of cheese. The causes of the various faults encountered in raw and manufactured milk are adequately treated.

In the section devoted to clean milk production the English reader will find several statements with which he cannot agree. For example, on p. 64, it is stated that "Dry milking . . . is extremely difficult to carry out in practice: it is facilitated by smearing the teats with a little vaseline or fatty material." Apart from aesthetic consideration, this practice has been found to be unnecessary in England. It appears from the paragraph at the top of p. 65 that the author is not conversant with the progress which has been made in the efficiency and hygienic use of milking machines in England and America.

It is very astonishing to one accustomed to "clean milk," as understood in England, to learn, on p. 72, that it is advisable to freeze milk if it is to be kept longer than 24 hours; there is ample evidence that the numbers of bacteria in clean milk remain very small even after 24 hours at temperatures of 15° C. and over.

With the reservation that the reader will do well to recognise that the English and Danish ideas on clean milk are different, this book can be heartily recommended to the reader, who will profit by Prof. Orla-Jensen's unique knowledge of the application of the science of bacteriology to the dairy industry.

A. T. R. MATTICK.

INTERNATIONAL REGISTER OF SPAS AND MEDICINAL WATERS. Published by the International Society of Medical Hydrology, 55, Wellington Road, London, N.W.8.   Price 1s.

The object of the Register is to afford a full record of the characters, chemical, physical, biological, and therapeutical, of the medicinal waters of natural origin, such as occur in the well-known spas, and it is hoped later to deal with other media, such as sea-water, muds, peats, etc., which are now used in hydrological practice. It has been compiled by the Standard Measurements Committee of the International Society of Medical Hydrology, which was founded in 1921 to promote the study of Medical Hydrology and to form an International Union of Scientific Workers in this branch of medicine.

The immediate aim of the present publication is to aid the adoption, throughout the world, of uniform methods of examination (by analysis, etc.), and of recording the various data, which may enable medical practitioners and others to understand and compare the properties and therapeutic values of the waters throughout the world.

The adoption of standardised methods and of uniformity of expression is, of course, essential in an international publication, and the general plan of the Register should go far to ensure this, but the explanatory paragraphs can hardly be regarded as masterpieces of English prose.

Neither brevity nor clarity is their strong point. Some of the statements are too obvious to require publication, and in others the phraseology is, at its best, awkward, and at its worst barely intelligible.

What, for instance, is the meaning of the reference (p. 3, lines 2 and 3 of the last paragraph) to the biological properties of waters relative to their effect on "vegetable and animal life," or (on p. 7, lines 10 and 11), how does the method of combining the ions so as to form salts (or, as the Register calls them, "salines") "represent" the "physical state" of the water?   As a final example, on p. 9 (at the top of the page) are set forth some of the directions of the International Committee, as follows: "(a) To allow to appear in the Register only such data as are satisfactory," etc.   "(b) To reject all data which are not satisfactory."   Surely these paragraphs are identical in meaning, obvious and unnecessary.

These, and other eccentricities of diction are, no doubt, largely the product of a committee whose members are of different nationalities, but it is particularly unfortunate that the first edition of the Register should suffer in this way. However, the only aim of these criticisms is to be helpful, and it is fair to add that the specimen report on pp. 17–20 clears up most of the doubts and uncertainties in the general text.

A wide tolerance as regards methods of stating analytical results is natural, and perhaps unavoidable, at this early stage of the scheme, but it is hoped that before long one uniform plan can be adopted.   Unfortunately, even in this country, we are still far from unanimous, and this International Register may point the way.

The Society is to be congratulated on initiating a scheme which is as much needed as it will eventually be valuable to all concerned.   C. H. CRIBB.