

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 at Burlington House, November 2nd, 1938. The President, Professor W. H. Roberts, and later Mr. J. R. Nicholls, Vice-President, took the chair.

The following were elected members of the Society:—W. J. Blackie, M.Sc., A.I.C., E. Collins, M.A., F.I.C., R. F. Corran, Ph.D., M.Sc., A.I.C., L. A. Dauncey, B.Sc., A. V. Delaporte, B.A.Sc., Chem.E., E. C. Dodds, M.V.O., D.Sc., Ph.D., M.D., F.R.C.P., S. Druce, B.Sc., J. W. E. Harrisson, P.D., Ph.M., S. H. Jenkins, M.Sc.Tech., Ph.D., F.I.C., S. Rajagopal Naidu, M.B.E., B.A., M.B., B.S., M.Sc., D.I.C., F.I.C., S. C. Ray, B.Sc., M.B., B.S., D.P.H., F. B. Richardson, M.A., R. W. Richardson, F.I.C., J. W. Stillman, B.S., M.A., Ph.D., E. T. Terry, A.I.C., A. L. Vale, B.Sc., F.I.C., A. G. R. Whitehouse, M.Sc., Ph.D., F.I.C.

Certificates were read in favour of the following candidates for membership:—W. A. Alexander, B.Sc., A.I.C., D. R. A. Davies, B.Sc., E. R. Jones, B.Sc., Ph.D., F.I.C., M.D., Ch.B., D.P.H., H. A. Jones, A.I.C., B. A. Macola, J. M. Malcolm, A.I.C., Mrs. S. M. L. Tritton, M.P.S., F.I.C., S. A. Ullah, B.Sc., Ph.D., A.I.C.

The following papers were presented and discussed:—"The Determination of Nitrogen in Mixed Fertilisers containing Nitrates and Chlorides," by Bernard Dyer, D.Sc., F.I.C., and J. Hubert Hamence, Ph.D., M.Sc., F.I.C.*; "The Determination of Acid in Wool," by J. Barritt, B.Sc., A.I.C., H. H. Bowen, F. L. Goodall and A. Whitehead†; "The Vitamin-A and -D Contents of Butter: II, Seasonal Variation," by H. Wilkinson, B.Sc., Ph.D.; "Cacao Shell in Cocoa and Cocoa Products," by H. C. Lockwood, B.Sc., F.I.C.

Obituary

PATRICK HENRY KIRKALDY

PATRICK HENRY KIRKALDY was the son of John Givens Kirkaldy, manufacturer of paint and anti-fouling compositions, of Poplar, and was born at 73, Stoke Newington Road, London, N. He was educated at the Brewers' Company's School and at George Green's School, Poplar, of which his father was one of the Governors.

After having spent three years in the office of the New Oriental Bank, Threadneedle Street, from 1887 to 1891, he turned to science, and studied at King's

* See ANALYST, 1938, 866.

† See ANALYST, 1938, 782.

College, London, under Professor John Millar Thomson, F.R.S. He gained the Daniell Scholarship for original research in 1894, and, in the same year, was appointed a demonstrator in the college.

Professors and staff alike were devoted to the conscientious training of their students, many of whom have shown, by their success in their subsequent careers, the soundness of the foundation laid at King's. Kirkaldy had his full share of the work. He was in charge of the practical work of the second- and final-year candidates for B.Sc., Lond., and the examinations of the Institute of Chemistry, and he lectured to the second-year students in both inorganic and organic chemistry. He was painstaking in the preparation of his lectures, and popular in the laboratory with the students. To them he was "Pat," though he did not know it until the war, when letters arrived from the front in which old students enquired after or sent messages to him.

He was also for several years Secretary of the Senior Common Room, and in that capacity he and his colleagues organised the Old Students' Dinners and Smoking Concerts, to which he himself contributed songs from the Savoy Operas and violin solos.

In 1909 he became Assistant Professor, and held that position until 1919, when he retired and was elected a Fellow of the College. In the course of this period he co-operated wholeheartedly in the work in connection with the production of benzol and toluol, which the College undertook during the war, and for these services he declined to accept any reward or recognition from the State.

After his retirement he was an Examiner to the Pharmaceutical Society from 1920 almost continuously until 1936, but his main interest became centred in the Institute of Chemistry. He had passed the Examination for the Associateship under the Regulations in force prior to 1893, and had been elected a Fellow in 1897, but he was not elected to the Council until 1916. Thereafter, he served—as a Member of Council, Vice-President and Honorary Treasurer—for nearly 20 years in the aggregate, and actively participated in the work, not only regularly attending meetings, but frequently calling for friendly conference at other times.

As Honorary Treasurer he was Chairman of the Finance and House Committee and of the Benevolent Fund Committee; he was also Vice-Chairman—virtually Chairman for about 12 years—of the Nominations, Examinations and Institutions Committee. He was Chairman for two years of the Lectures and Library Committee, and first Chairman of the London and South-Eastern Counties Section. Finally, he represented the Institute on the Chemical Council in 1935 and 1936.

He was a Fellow of the Chemical Society from 1895, and a Member of this Society since 1907, serving on the Council in 1917 and 1918. The Society includes on its roll many of his former students, to whom his downright straightforwardness, his friendly sympathy, loyalty and sense of humour will ever remain a cherished memory.

His was a retiring and sensitive nature, but the comparatively few who were privileged to count themselves among his intimate friends could not fail to be aware of a charm of personality which manifested itself in many ways, quite unknown to those less intimate with him. He had a great love of the country, the open air and gardening. In early life he was devoted to walking and cycling,

and from about 1902 was a keen motorist, his first car being a Panhard with a back entrance. He was a keen tennis player and golfer, and to share with him either of these pastimes was a delightful experience. His knowledge of bird life was profound; no matter how concentrated he appeared to be on the road or in the game, he never failed to remark on the presence or song of a rare bird, or to draw attention to some feature of the countryside. Those who knew him best will feel that they have lost not only a kindly and sympathetic friend, but one whose quiet unobtrusive nature was responsible for many generous and unrecorded acts which he would have been the last to wish to be made known.

He died at Cricklewood on September 14th, 1938, in his 68th year. At his funeral at Chipperfield, Hertfordshire, the Society was represented by Mr. E. Richards Bolton, Past President, and a tribute of flowers was sent in the name of the President, Council and Members.

RICHARD B. PILCHER

HAROLD DOUGLAS ELKINGTON

By the sudden death of Harold Douglas Elkington on July 1st, at the comparatively early age of 48, the Society has lost a member of over twenty-five years' standing.

He was the youngest son of George Elkington, J.P., F.R.I.B.A., and was educated at King's College School, Wimbledon, and King's College, London, where he graduated B.Sc. with 1st Class Honours in Chemistry in 1911, and in the same year was elected to the Associateship of the College, and passed the examination for election to the Associateship of the Institute of Chemistry in Branch E. He proceeded to the Fellowship in 1914, and was appointed a Demonstrator at King's College. After six months' experience as Assistant to Dr. John Muter he became Demonstrator at the Royal Agricultural College, Cirencester, and conducted research on the attack of weevils on grain, for which he was awarded the degree of M.Sc.

Owing to an injury sustained before the war he was unable to proceed on active service, and in 1918 joined the firm of Dicker & Pollak, Chartered Patent Agents.

Soon after qualifying as a Patent Agent, in 1927, he started practice on his own account as a consulting chemist and chartered patent agent, and this practice (in which he was engaged at the time of his death) increased rapidly, owing to the high appreciation of his services as a patent agent specialising in chemical matters.

To Elkington the profession of chemistry was something more than a name, as was shown by his service on the Council of the Institute of Chemistry for three years as an ordinary member and for two years as a Vice-President, and even more by the generous help and encouragement which he gave to more than one fellow chemist who had met with misfortune.

His many good qualities will long be remembered by his friends, particularly the painstaking nature of his professional work, the uncomplaining way in which he bore the severe and almost continual pain from his injured leg, and his extreme generosity.

At the funeral the Society was represented by Mr. K. A. Williams.

JAMES G. FIFE

The Determination of Nitrogen in Mixed Fertilisers containing Nitrates and Chlorides

BY BERNARD DYER, D.Sc., F.I.C., AND
J. HUBERT HAMENCE, Ph.D., M.Sc., F.I.C.

(Read at the Meeting, November 2, 1938)

THE Jodlbauer modification of the Kjeldahl method (using phenol or salicylic acid) laid down in the Statutory Rules and Orders (1932) of the Fertilisers and Feeding Stuffs Act for the determination of the total nitrogen in mixed fertilisers containing organic, ammoniacal and nitric nitrogen, is satisfactory in the absence of chlorides.

When, however, the fertiliser contains a substantial quantity of potassium chloride or sodium chloride, as is often the case, this method is apt to give low results, owing apparently to the loss of some of the nitric nitrogen in the form of nitrosyl chloride, which is not trapped by the phenol or salicylic acid, even though the operation is carried on in an ice-bath.

The following experiments illustrate this somewhat drastically:

TABLE I

Composition of mixture Per Cent.		Nitrogen present Per Cent.	Nitrogen found Per Cent.
Potassium nitrate	100·0	13·85	13·75
Sodium chloride	nil		
Potassium nitrate	87·5	12·12	11·95
Sodium chloride	12·5		
Potassium nitrate	75·0	10·38	9·91
Sodium chloride	25·0		
Potassium nitrate	50	6·92	5·07
Sodium chloride	50		

In the old pre-Kjeldahl days of the soda-lime combustion method of determining nitrogen in fertilisers J. Ruffle, in 1881, devised a means of dealing with fertilisers containing nitrate by the addition of a liberal quantity of sodium thio-sulphate, sulphur and powdered charcoal to the soda lime. This gave correct results, even in presence of chlorides, but it entailed the use of a large iron combustion tube and, though satisfactory as far as it went, was cumbersome and tedious. The soda-lime combustion method for determining nitrogen has long become a method of antiquity, and there are probably few surviving analysts who can look back to it (as one of the authors can) as a matter of daily routine.

The need for a modified "wet" method has been realised for some time. The problem resolves itself into a search for a reducing agent capable of converting the nitrate present into ammonia before the digestion of the organic matter with sulphuric acid.

Combinations of the Ulsch method of nitrate reduction with the Kjeldahl method have been suggested, but have not given satisfactory results owing to the lack of uniformity in reduced iron, which (in our somewhat extensive experience) always contains enough nitrogen to necessitate a considerable correction, a correction that, moreover, cannot be relied on for any one portion of the iron.

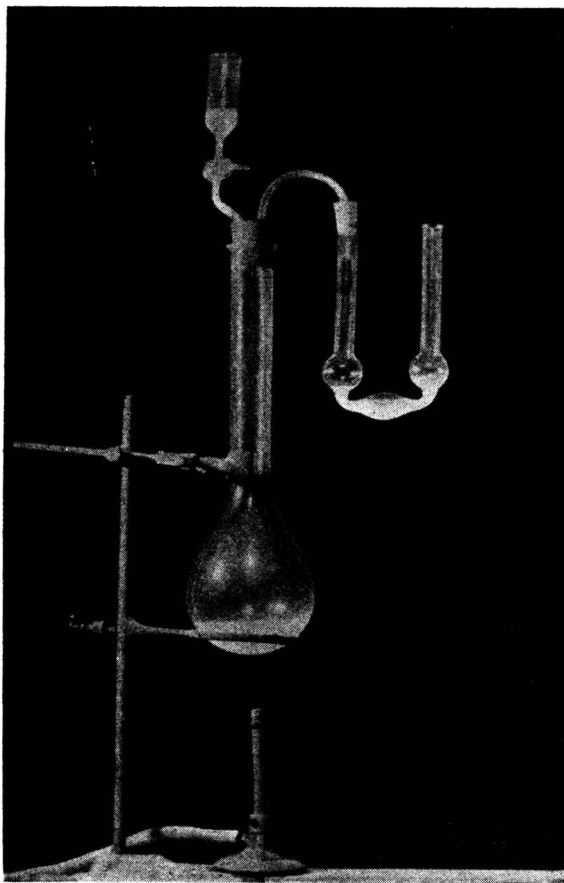


Fig. 1

Preliminary alkaline reduction with Devarda metal followed by distillation of the ammonia formed and subsequent Kjeldahling of the residue gives accurate results; but the manipulation involved is cumbrous and breaks down when much organic matter is present, owing to frothing in the initial distillation.

After a good deal of experimental work a combination of the Devarda and Kjeldahl methods in one operation was arrived at and found to be satisfactory.

The apparatus employed consists of a 500-ml. Kjeldahl flask having a doubly perforated rubber bung fitted with a tap-funnel and a U-tube with bulbs (see Fig. 1). Before commencing a determination the U-tube is charged with 10 ml. of 10 per cent. v/v sulphuric acid, and a small plug of filter-paper is placed in the top

of the tube in order to catch any spray that might be produced during the bubbling of gas through the dilute sulphuric acid.

Two g. of the sample and 3 g. of finely powdered Devarda metal are transferred to the Kjeldahl flask, and the sides of the flask are washed down with 50 ml. of water. The flask is closed with the rubber bung (with its tap-funnel and absorption tube), and 5 ml. of sodium hydroxide solution of sp.gr. 1.4 (about 500 g. of caustic soda per litre) are added through the tap-funnel.

TABLE II

Composition of mixture Per Cent.		Nitrogen present Per Cent.		Nitrogen found	
				Jodlbauer method Per Cent.	New method Per Cent.
Mixture A:					
Potassium nitrate	33.33	Nitric	4.61	} 11.68	10.78
Ammonium sulphate	33.33	Ammoniacal	7.07		
Sodium chloride	33.33				
Mixture A	50.00	Nitric	2.31	} 12.94	12.65
Dried blood	50.00	Ammoniacal	3.54		
		Organic	7.09		
Mixture A	50.00	Nitric	2.31	} 5.85	5.37
Superphosphate	50.00	Ammoniacal	3.54		
Mixture A	33.33	Nitric	1.54	} 8.61	8.47
Dried blood	33.33	Ammoniacal	2.35		
Superphosphate	33.33	Organic	4.72		
Mixture A	50.00	Nitric	2.31	} 9.39	9.27
Dried blood	25.00	Ammoniacal	3.54		
Superphosphate	25.00	Organic	3.54		
Potassium nitrate	25.00			} 7.00	6.33
Dried blood	25.00	Nitric	3.46		
Superphosphate	25.00	Organic	3.54		
Sodium chloride	25.00				7.01
Dried blood	40.00			} 7.75	7.63
Potassium nitrate	15.00	Nitric	2.08		
Superphosphate	25.00	Organic	5.67		
Sodium chloride	20.00				7.82

The flask is allowed to stand for 30 minutes, during which time a vigorous reaction is produced if nitrate is present in substantial quantity. The flask is then heated with a small flame to just short of the boiling-point for a further 1 hour, being gently shaken at intervals. It is then allowed to cool, and 20 ml. of 50 per cent. v/v sulphuric acid are added through the tap-funnel in such a manner that the sides of the Kjeldahl flask are well washed by the acid. The flask is again allowed to cool, after which the rubber bung is removed and rinsed into the flask, into which the contents of the U-tube are washed. Twenty-five ml. of conc. sulphuric acid are now added, the water is boiled off, and the residue is Kjeldahled

in the usual way, with a drop of mercury as accelerator, and the amount of nitrogen is arrived at in one distillation.

The total nitrogen in mixtures prepared in the laboratory was determined by the procedure which has just been described and also by the official Jodlbauer process, and the results are given in Table II.

These results demonstrate that the total nitrogen may be determined accurately by the new procedure; they also indicate the type of low result that may be obtained by the official Jodlbauer process.

CALCIUM CYANAMIDE.—Calcium cyanamide is sometimes put on the market with a small admixture of nitrate which is added in order to convert it into a satisfactory granular form. The total nitrogen in such mixtures may be determined satisfactorily by the new method, as is shown by the following results (Table III):

TABLE III

Composition of mixture Per Cent.		Nitrogen present Per Cent.		Nitrogen found Per Cent.	
Potassium nitrate	15.13	Nitric	2.00	19.53	19.50
Calcium cyanamide	84.87	Organic	17.53		
Potassium nitrate	9.67	Nitric	1.34	19.89	19.85
Calcium cyanamide	90.33	Organic	18.55		

It is important that the quantities of reagents given in the description of the method should be adhered to. These quantities have been found to produce the best conditions for the reduction of nitrate to ammonia by Devarda metal in alkaline solution. One interesting observation was made during this investigation of the action of Devarda metal. If insufficient Devarda metal is present to convert all the nitrate into ammonia, no initial heat of reaction is experienced and the nitrate is converted mainly into nitrite, although on the addition of more Devarda alloy the reaction becomes exothermic and all the nitrate is reduced to ammonia.

The nitrogen in sodium and potassium nitrates may be determined satisfactorily by this method, but the quantity of Devarda alloy should be proportionally larger than with a mixed fertiliser. Operating on 1 g. of nitrate, 4 g. of Devarda alloy should be used.

The Devarda alloy powder used in our experiments had a fineness of 90 per cent. through a 60-mesh wire sieve.

17, GREAT TOWER STREET
LONDON, E.C.3

November, 1938

DISCUSSION

Mr. W. S. CLARK observed that the present-day fertilisers contained very little chloride. He was grateful for having been invited to listen to such an excellent paper, which was of great interest both to his firm and to the fertiliser industry.

Mr. BAILEY said that, in his experience, it was not a very frequent occurrence for fertilisers to contain chlorides as well as nitrates. After hearing the authors' method of dealing with the difficulty he realised how frequently a very simple method proved to be the correct one.

Mr. J. R. NICHOLLS said that it was true that the fertiliser and feeding stuffs regulations did not make provision for determining total nitrogen in those infrequent cases in which nitrates and chlorides were present in organic matter. The official methods for the determination of nitrogen present as nitrates were satisfactory in the presence of chlorides, and Dr. Dyer had coupled one of these very neatly with the Kjeldahl process in order to obtain the total nitrogen. There was no reason why the other official method—that using reduced iron—should not be followed by the Kjeldahl treatment. As a matter of fact, some years ago, having a sample in which chlorides were present, he had used such a method and found it quite satisfactory. Reduced iron had, in the past, caused a certain amount of difficulty, being irregular in action and containing variable amounts of nitrogen. Nowadays it was possible to obtain satisfactory reduced iron which was practically nitrogen-free. This being so, he thought it preferable to use a method whereby reduction was carried out in acid solution, thus avoiding the necessity of trapping the ammonia evolved during the alkaline reduction as proposed.

Mr. F. L. OKELL said that, apart from nitrogen, reduced iron contained many other impurities; if, however, one prepared it oneself it was possible to obtain reduced iron that was quite pure.

Mr. G. TAYLOR said that a further objection to the use of reduced iron was that it was difficult to dissolve the large amount of ferric sulphate that it formed in the Kjeldahl process.

A new Volumetric Process for Vanadium*

By B. S. EVANS, M.C., M.B.E., D.Sc., F.I.C.

THE ordinary volumetric method of titrating from quadrivalent to quinquevalent vanadium with $N/10$ permanganate solution suffers from one serious disadvantage: owing to the high factor (1.0 ml. of $\text{KMnO}_4 \equiv 0.0051$ g. V), one cannot titrate quantities of less than, say, 0.003 g. of vanadium with sufficient accuracy. The strength of the permanganate can be reduced a little perhaps without sacrificing sharpness, but this process cannot be carried far, because what one gains by dilution one loses by blurring of the end-point; indicators such as disulphine blue cannot be used because the permanganate acts on them faster than it does on the vanadium. It has long been known that plain iodimetric methods cannot be used with vanadium¹; quinquevalent vanadium certainly oxidises hydriodic acid, setting free iodine, but the reaction, whilst going beyond the V^{IV} stage, does not progress as far as a quantitative reduction to V^{III} , though the statement has been made that this can be effected by distilling from phosphoric acid.¹ Another method advocated is to effect the reduction with bromide, instead of iodide, and distil the bromine produced into potassium iodide solution, on the assumption that the bromide reduction does not proceed beyond V^{IV} .¹ This method was tested at some length and had to be discarded for the reason that the extent of the reduction seemed to depend upon the concentration of acid present, and it was quite easy to get results varying from incomplete reduction to V^{IV} to a reduction considerably beyond V^{IV} by simply altering the acid concentration, either

* Communication from the Research Department, Woolwich.

in presence or absence of phosphoric acid. It seemed probable that if the quadrivalent vanadium could be removed as fast as it was formed the iodide reduction would not pass beyond this stage; it has been shown that vanadium is very completely precipitated by ferrocyanide,² and tests soon brought to light the fact that it is the quadrivalent, not the quinquevalent, vanadium that is precipitated. True, if an acid solution of quinquevalent vanadium is treated with potassium ferrocyanide, vanadium ferrocyanide is precipitated, but there is a simultaneous production of ferricyanide in proportion to the V^v originally present. Ferricyanide in the presence of a zinc catalyst will oxidise potassium iodide quantitatively to iodine; consequently, we have the required conditions, *viz.* the oxidising power of the $V^v \rightarrow V^{IV}$ reaction is manifested as liberated iodine, and the quadrivalent vanadium itself is removed from solution. Experiment showed that the above assumptions and reasoning are correct, but that various minor details require attention.

- (a) Fairly strong hydrochloric acid has to be used as the acidifying agent. This prevents the precipitation of Prussian blue, which is otherwise liable to occur.
- (b) Air has to be excluded, otherwise some oxidation takes place.
- (c) A freshly-prepared solution of ferrocyanide must be used and the blank determined immediately after the vanadium has been precipitated, as freshly-prepared ferrocyanide solution has an appreciable oxidising power, and this steadily mounts on exposure to the air. Blank determinations, made at intervals on a solution of potassium ferrocyanide which had been allowed to stand in the air, gave the following results:

			N/100 thiosulphate required ml.
Fresh solution	0.30
After 3 hours	0.50
,, 20	,,	..	0.90

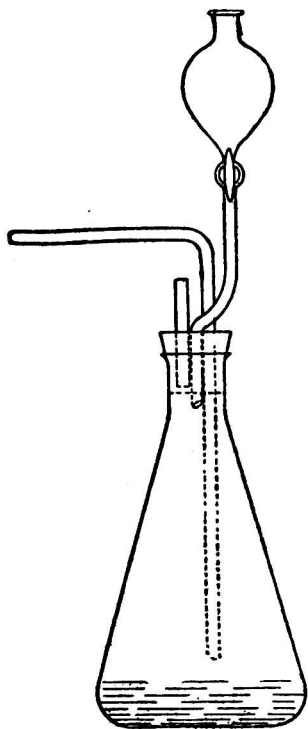
METHOD.—The following process was worked out:

Apparatus.—The same apparatus is used as that described for the titration of tin³; for convenience of reference the description is repeated here. The apparatus consists of a 750-ml. Erlenmeyer flask, the mouth of which is closed by a three-holed rubber stopper carrying the following attachments:

- (a) A leading tube connected by about 2 feet of rubber tubing with a Kipp's apparatus delivering carbon dioxide and passing down to within about $\frac{1}{2}$ inch above the liquid in the flask.
- (b) A small tapped funnel with its stem bent twice, so that the bulb of the funnel clears the burette, which is subsequently inserted in the third hole.
- (c) A removable glass plug.

After the requisite solution has been placed in the flask the air is removed by a stream of carbon dioxide, which passes in through the leading tube and out

through the open tap of the funnel, the glass plug being left in position. When it is necessary to add reagents this can be done through the funnel, the glass plug being removed while the liquid is running in and the carbon dioxide turned down to a slow stream. To carry out the titration the glass plug is removed and the



jet of a burette is inserted in the hole; if the tap of the funnel is opened and a stream of carbon dioxide is left running, the liquid can readily be titrated from the burette without admitting air.

Procedure.—The vanadium in the form of vanadic acid, contained in a few ml. of solution, is placed in the titrating flask, and the liquid is rendered slightly alkaline with sodium hydroxide. Five ml. of potassium ferrocyanide solution (10 per cent., freshly prepared) are added, followed by 50 ml. of dilute (1:1) hydrochloric acid; the stopper with attachments is inserted, and the air is swept out for 5 minutes with a rapid stream of carbon dioxide. The glass plug having been withdrawn, 20 ml. of potassium iodide solution (4 per cent.) and 1 ml. of zinc sulphate solution (10 per cent.) are introduced into the tap funnel and run into the flask, the level of the liquid not being allowed to fall below the tap, after which the tap funnel is rinsed in 30 ml. of water, which is run into the flask completely; the tap having been closed and the plug re-inserted, the flask is shaken and allowed to stand for 5 minutes. At the end of this time the plug is replaced by a burette charged with $N/100$ thiosulphate solution, the tap of the funnel is opened, and the iodine colour which has developed in the liquid, proportional to the amount of vanadic acid present, is titrated nearly away (it must be remembered during this titration that the colour of the vanadium ferrocyanide precipitate is yellow); starch solution is then added through the funnel, and the titration is finished in the usual manner. During the sweeping out of the apparatus with carbon dioxide the blank of the ferrocyanide solution should be determined in the following way:—The same amounts of all the reagents, *i.e.* 5 ml. of potassium ferrocyanide solution made slightly alkaline with sodium hydroxide, followed by 50 ml. of dilute hydrochloric acid (1:1) and then by a mixture of 1 ml. of zinc sulphate solution (10 per cent.) and 20 ml. of potassium iodide solution (4 per cent.), are placed in a flask which is allowed to stand for 2 minutes, starch solution is then added, and the blue colour is titrated away with $N/100$ thiosulphate solution. Air need not be excluded in making the blank determination, as it appears to be vanadium which catalyses the air oxidation. The blank figure is deducted from the titration figure, and the difference is a measure of the amount of vanadium present.

1 ml. of $N/100$ thiosulphate solution \equiv 0.0005095 g. of vanadium.

The process was tested on known amounts of vanadium, with the following results:

Vanadium taken g.	Titration $N/100 \text{ Na}_2\text{S}_2\text{O}_3$ ml.	Theoretical titration $N/100 \text{ Na}_2\text{S}_2\text{O}_3$ ml.	Vanadium found g.
0.0100	$\frac{21.60-0.60}{1.075} = 19.55$	19.63	0.00996
0.0090	$\frac{19.65-0.60}{1.075} = 17.72$	17.66	0.00903
0.0080	$\frac{17.05-0.30}{1.076} = 15.58$	15.70	0.00795
0.0070	$\frac{15.10-0.30}{1.076} = 13.75$	13.74	0.00701
0.0060	$\frac{13.15-0.30}{1.076} = 11.85$	11.78	0.00604
0.0050	$\frac{10.85-0.30}{1.075} = 9.82$	9.82	0.00500
0.0040	$\frac{9.00-0.50}{1.087} = 7.82$	7.85	0.00399
0.0030	$\frac{6.60-0.30}{1.075} = 5.87$	5.89	0.00299
0.0020	$\frac{4.50-0.30}{1.075} = 3.91$	3.93	0.00199
0.0010	$\frac{2.40-0.30}{1.075} = 1.95$	1.96	0.00100

The divisor in the titration figure represents the factor of the thiosulphate solution used.

Attempts were made to eliminate the blank altogether by keeping the ferrocyanide solution over an insoluble reducing agent (*e.g.* magnesium amalgam), but so far without success.

REFERENCES

1. Treadwell and Hall, *Analytical Chemistry*, Vol. II, 8th Ed., 1935, p. 613.
2. B. S. Evans and S. G. Clarke, *ANALYST*, 1928, **53**, 475.
3. B. S. Evans, *Id.*, 1927, **52**, 570.

RESEARCH DEPARTMENT
WOOLWICH

September, 1938

A new Volumetric Process for Tellurium*

By B. S. EVANS, M.C., M.B.E., D.Sc., F.I.C.

THE advantages of being able to use $N/100$ iodine solution in the titration of an element of so high an atomic weight, and used in such small proportions, as tellurium are manifest. In a former paper¹ I have described a method for such a titration carried out on precipitated tellurium and involving its solution in an excess of iodine solution, which excess is subsequently titrated away. Apart from the fact that a back titration is to be avoided if possible, the fact that it is the precipitate which is being titrated makes it necessary to carry this out in presence of the filter (always an objectionable procedure) and, worst feature of all, there is some slight doubt as to the exact state of oxidation of the tellurium when dissolved in an excess of iodine. The process gives good results, but requires careful handling. In the following method these objections are all eliminated and titration is as rapid, clean and simple as can be desired. A feature of hypophosphorous acid as a reducing agent is the rapidity with which its reducing activity falls off as the temperature and the acid strength of the solution are diminished; I have shown earlier² that it is possible to reduce a tin solution with hypophosphite, and then by mere cooling and dilution bring it to a condition in which it can be titrated back to the stannic condition with iodine. Tellurium is easily reduced to metal by hypophosphorous acid, and the precipitated metal readily dissolves in iodine solution in the presence of dilute acid; it would seem, therefore, that it should be possible to titrate tellurium in much the same way as tin if suitable acid conditions could be found. Tellurium, as ordinarily precipitated, is too dense to allow of ready solubility in iodine solution unless a distinct excess of the latter is present; if, however, gum arabic is added before precipitation the tellurium does not separate at all but remains in colloidal suspension, which is practically clear and dark brown in colour; in this form the tellurium is immediately attacked by iodine in presence of dilute acid. The acid conditions have to be such that the tellurium is promptly oxidised before there has been any measurable attack on the large excess of hypophosphorous acid present; if the acid is too strong, results are high; if it is too weak, it seems impossible to oxidise all the tellurium. Hydrochloric acid, which was first tried, proved unmanageable, but with phosphoric acid excellent results are readily obtained. The full process is as follows:

PROCESS.—The tellurate solution is neutralised with sodium hydroxide or hydrochloric acid as the case may be; 5 ml. of gum arabic solution (1 per cent.) and 5 ml. of syrupy phosphoric acid are added, and the solution is diluted to 60 ml.; about 2 to 3 g. of sodium hypophosphite are added, and the solution is then heated to boiling-point and cooled. Just below the boiling-point the liquid suddenly darkens, and with any considerable amount of tellurium (*e.g.* 0.01 g.) becomes almost black. When completely cool the solution is diluted with 150 ml. of water and titrated with $N/100$ iodine solution; the brown colour rapidly lightens as the tellurium is oxidised and gives a very good indication of the progress of the titration; it is not, however, desirable to try to titrate it entirely away, as this

* Communication from the Research Department, Woolwich.

seems invariably to lead to over-titration. When the brown colour is only very pale 4 ml. of carbon tetrachloride are added and the liquid is well shaken; this should result in the complete decolorisation of the aqueous layer, while the carbon tetrachloride should be coloured brown by the remaining tellurium. The titration is then proceeded with, cautiously and with vigorous shaking after each addition; the brown colour of the tetrachloride gets paler and paler and finally disappears, then after a further drop or two the tetrachloride is tinged a very faint pink, indicating an excess of iodine; this is the end-point.

1.0 ml. of *N*/100 iodine \equiv 0.000319 g. of tellurium.

The process was tried on a solution of sodium tellurate prepared from purified tellurium, with the following results:

Tellurium taken g.	Titration (ml. of <i>N</i> /100 iodine)	Tellurium found g.
0.0005	1.65	0.00052
0.0010	3.3	0.00105
0.0020	6.3	0.00201
0.0030	9.4	0.00300
0.0040	12.6	0.00402
0.0050	15.7	0.00501
0.0100	31.4	0.01002

It seemed likely that a similar titration could be performed with arsenic, and if so a great saving of time and trouble would be effected in the determination of arsenic by the hypophosphite method.³ On trial, however, only a very limited success was achieved; the principal trouble appeared to be the slow rate of solution of the arsenic giving time for the iodine to attack the hypophosphite.

Most of the methods for volumetric determination of tellurium depend on the $\text{TeO}_2 \rightarrow \text{TeO}_3$ reaction⁴ or its converse⁵; it is an obvious advantage to use the $\text{Te} \rightarrow \text{TeO}_2$ reaction, inasmuch as it provides twice the amount of reduction. The recently published method of Vignoli and Khaled⁶ seems to differ in no important detail from that which I published in 1933.

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RESEARCH DEPARTMENT
WOOLWICH

September, 1938

A New Iodine Method for the Determination of Starch. Part V

Starch in Leaf Material

BY J. J. CHINOY, M.Sc., Ph.D., D.I.C.

(Read at the Meeting, October 5, 1938)

THERE is a consensus of opinion among workers on starch that the most accurate method of determining this substance in plant material should involve the almost specific reaction with iodine. The nature and composition of the starch-iodine complex has been partly dealt with in a previous communication¹; even as early as 1887 Mylius² regarded starch iodide as a definite addition product, and it also appears to be the experience of other workers (*e.g.* Denny,³ Sullivan,⁴ and Pucher and Vickery⁵), that under standardised conditions a definite quantity of iodine enters into combination with starch. It has been shown in Parts I and II of this series that under certain standard conditions the amount of iodine present in the starch iodide complex is constant, and that if these conditions are observed it is possible to determine starch accurately and rapidly.

Regarding the hydrolytic methods of starch determination there is complete unanimity of opinion among all the workers cited that the enzyme preparations available on the market are not specific for starch, and hence that the results obtained by these methods do not represent the values of "true" starch in the material. It is generally found that diastase methods give higher results than the methods involving the starch-iodine reaction.

The *pros* and *cons* of various methods involving in some measure the starch-iodine reaction have already been dealt with in a previous communication in this series,¹ and the need of a method applicable to biochemical work has been duly stressed. In the course of work on plant physiology it was thought desirable to investigate the applicability of the starch iodide method to various plant materials.

EXTRACTION OF STARCH FROM PLANT MATERIAL.—The accuracy with which starch can be determined in plant material depends upon its complete extraction. Sullivan⁴ has brought out this point well for woody plants, showing that the extraction is greatly facilitated and the accuracy of the determination increased if the material is finely ground. In the present investigations similar difficulties in the extraction of starch were encountered, but after a few preliminary trials the following technique was developed and by its means complete extraction of starch from the plant material was achieved.

The material, after repeated extraction with 80 per cent. alcohol, followed by several extractions with ether to remove soluble carbohydrates, glucosides, alkaloids, tannins and colouring matters, is dried in the oven at 100° C. and finely powdered so as to pass through a 100-mesh sieve. Suitable aliquot parts (1 to 5 g.) from such dry powder are weighed accurately and placed in centrifuge tubes* together with 2 to 3 g.

* Centrifuge tubes with a spout can be made from thick-walled Pyrex tubes (35 mm. in diameter). These tubes serve for the extraction of the material.

of fine purified sand; a strong glass rod with a flattened end is also placed in the tube. The mixture is wetted with 1 or 2 ml. of 0.7 per cent. potassium hydroxide solution and crushed for about 5 minutes against the bottom of the tube with the glass rod. If required, 1 or 2 ml. more of the alkali are added to make the thoroughly crushed mixture into a paste. Fifteen to 20 ml. of the alkali are added, and the tubes are placed in a vigorously boiling water-bath for one hour. It is convenient to start 8 to 12 extractions at a time, and for this purpose a small copper bath provided with a metal plate with twelve perforations to receive the extraction tubes will be found very useful. With a slight up-and-down movement of the stirring rod the plant material is crushed against the bottom of the tube. This ensures proper stirring of the material and facilitates extraction of starch. All the tubes in the bath are similarly treated in turn, the grinding operation in each being continued for half-a-minute at a time. The volumes in the tubes which are depleted by evaporation are made good by addition of distilled water. After about one hour's heating the tubes are removed from the bath and centrifuged. The extract is decanted (an easy operation because the insoluble matter, with the sand, sticks at the bottom; the extract is often pale yellow or sometimes dark yellow) and the tip of the spout is washed with 1 or 2 ml. of water. A few ml. of the alkali are put into the tube, and the grinding is repeated. After about 5 minutes the tubes are again put into the water-bath, 15 to 20 ml. of the alkali are introduced into each, and the extraction is carried out as before, the heating being continued for 40 to 45 minutes instead of 1 hour. Four such extractions are usually sufficient to remove all the starch. After the centrifuging and decantation of the fourth extract about 10 ml. of water are added, and the material is shaken and again centrifuged. In order to test if extraction of starch is complete, a few drops from the last extract are acidified and a drop of iodine solution is added. If a blue colour is obtained a fifth extraction is made.

DETERMINATION OF STARCH.—All the extracts are combined, concentrated to a suitable volume on the water-bath, and filtered into a 50-ml. graduated flask, the beaker and filter-paper are washed, and the solution is made up to volume. Sometimes a flocculent precipitate separates on concentration of the solution. This is not starch, as can be proved by the addition of a drop of iodine reagent to the washed precipitate. As a preliminary test, 1 ml. of the starch solution is taken in a test-tube and after neutralisation of the alkali with 10 per cent. acetic acid a few drops of iodine solution are added. Thus the aliquot part of the solution to be taken for analysis, as well as the amount of acid required for neutralisation of the alkali, can be determined; from the intensity of the blue colour developed or the amount of precipitate formed it is easy, with a little practice, to ascertain within the requisite limits the volume to be taken.

The required amount of the solution (15 to 20 ml. or more), as ascertained by the preliminary test, is pipetted into a centrifuge tube and neutralised with the necessary quantity of 10 per cent. acetic acid, and 2 ml. of 0.1 *N* iodine solution and 5 ml. of 10 per cent. potassium acetate solution are added. It is not necessary to have the solution exactly neutral; a slightly acidic solution is quite suitable for the precipitation of the starch iodide. The precipitate thus obtained is kept overnight (if necessary) for proper coagulation.

The washing of the starch iodide precipitate is carried out conveniently and expeditiously as follows:—The solution, together with the precipitate, is centrifuged and the supernatant clear liquid is removed by introducing a capillary tube into the solution and applying gentle suction at the other end. About 20 ml. of 30 per cent. alcohol are added to the precipitate, and, after thorough mixing, the mixture is again centrifuged. This solution is also removed and the precipitate is washed with a fresh quantity of 30 per cent. alcohol and centrifuged for the third time. After removal of the major portion of the liquid the precipitate is then transferred to a tared alundum crucible (medium grade), filtered, washed with rectified spirit dried and weighed, in accordance with the technique previously described.¹

DETERMINATION OF STARCH IN LEAVES.—Starch was determined by the method above in the leaves of three varieties of common vegetables, *viz.* *Solanum tuberosum*, *Lycopersicum esculentum* and *Brassica rapa*. Two pickings were made of all three, the first at 6 a.m. and the second at 6 p.m. The morning and the evening samples are designated Sample I and Sample II respectively in all three instances. After preliminary extractions with alcohol and ether to remove soluble substances, the samples were dried at 100° C. and powdered to pass through a 100-mesh sieve. Starch was then extracted and determined according to the technique outlined above, in two separate aliquot parts of each sample and in duplicate for each portion. The results were as follows:

TABLE I
STARCH CONTENT OF LEAVES

Plant material	Sample analysed g.	Starch iodide found in 20 ml. aliquot part g.	Starch (dry weight) Per Cent.
Potato leaf, No. I	4.4166	0.0554	2.78
" " " " " " "	" "	0.0572	2.87
" " " " " "	5.3300	0.0633	2.63
" " " " " "	" "	0.0642	2.67
Potato leaf, No. II	2.5834	0.0682	5.85
" " " " " "	" "	0.0690	5.92
" " " " " "	2.2401	0.0564	5.58
" " " " " "	" "	0.0548	5.48
Tomato leaf, No. I	4.2178	0.0550	2.89
" " " " " "	" "	0.0562	2.95
" " " " " "	4.2967	0.0506	2.61
" " " " " "	" "	0.0510	2.63
Tomato leaf, No. II	2.3893	0.0534	4.95
" " " " " "	" "	0.0524	4.86
" " " " " "	1.8790	0.0452	5.33
" " " " " "	" "	0.0460	5.43
Radish leaf, No. I	3.3693	0.0488	3.21
" " " " " "	" "	0.0476	3.13
" " " " " "	3.3469	0.0521	3.45
" " " " " "	" "	0.0514	3.40
Radish leaf, No. II	2.3605	0.0720	6.76
" " " " " "	" "	0.0728	6.84
" " " " " "	1.7811	0.0573	7.13
" " " " " "	" "	0.0583	7.25

From a study of the data in the last column it appears that the duplicates of the same extract vary within 1 to 2 per cent. A close agreement is also noticed for the figures obtained in separate extractions; in every instance except one the differences are within 5 to 8 per cent. This clearly shows that the plant material was homogeneous and also that there was complete extraction of starch. The evening samples all show increases in the starch-content over the corresponding morning samples. This is evidently the result of the photosynthetic activity of the leaves during the day.

RECOVERY OF PURE STARCH.—Recovery of pure starch was studied in the following two ways: (1) A known quantity of pure rice starch was added to a sample of the above-mentioned materials and starch was determined as before. (2) A known quantity of pure rice starch was added to starch-free material (leaves of *Allium cepa*) after the usual preliminary extraction of soluble matter with 80 per cent. alcohol and ether, drying and powdering, and the extraction and subsequent determination were carried out as described. The results are given in Table II.

TABLE II
RECOVERY OF PURE RICE STARCH

Plant material	Sample analysed	Rice starch added	Starch found per g. (dry weight)	Percentage recovery
	g.	g.	g.	
Potato leaf, No. II ..	2·3024	nil	0·0563	—
" " " " ..	2·0941	0·2315	0·1594	93·24
Tomato leaf, No. II ..	2·8432	nil	0·0509	—
" " " " ..	2·3315	0·2568	0·1675	105·84
Radish leaf, No. II ..	1·8163	nil	0·0716	—
" " " " ..	1·8010	0·2195	0·1890	96·30
Onion leaf ..	3·2300	nil	—	—
" " " " ..	2·8590	0·2313	0·0779	96·28
" " " " ..	3·1015	0·1109	0·0349	97·51

It will be noted that the recovery of pure starch fluctuates within wider limits with plant material containing starch. This is probably due to the slight variations in the starch-content of different aliquot portions, as seen in Table I. With starch-free material the recovery is 96 to 98 per cent. of the starch added.

EFFECT OF PECTIN.—When pectic material, which is a normal plant constituent, is treated with 0·7 per cent. potassium hydroxide solution for the extraction of starch some of it will be converted into potassium pectate, which is readily soluble. In order to ascertain if potassium pectate is precipitated along with starch on treatment with iodine and potassium acetate, various quantities of pectin were added to starch-free material and extracted with 0·7 per cent. potassium hydroxide solution, as already described. In some experiments pure rice starch was also added. The solutions were made as before, and to a neutralised aliquot (20 ml.), 0·1 N iodine solution and 10 per cent. potassium acetate solution were added to

precipitate starch iodide. Starch-free solutions were also treated in a similar manner. The results are given in Table III.

TABLE III

EFFECT OF PECTIN

Onion leaf g.	Pectin added g.	Starch added g.	Starch iodide g.	10 per cent. potassium acetate solution	Percentage recovery
2.7895	0.2500	nil	—	25.0	—
2.5917	0.3000	nil	—	25.0	—
2.9534	0.5000	nil	—	20.0	—
2.1580	0.1500	0.1635	0.0715	5.0	96.92
1.9356	0.3000	0.2013	0.0880	5.0	96.89
2.0152	0.4500	0.1312	0.0578	5.0	97.64

In the preceding table the amounts of 10 per cent. potassium acetate solution required to obtain a slight precipitate in the first three experiments (starch-free) are noted in the fifth column. Addition of the usual quantity of potassium acetate for coagulation, or even a quantity four times that amount, did not bring about any coagulation of potassium pectate. In the last three experiments addition of the usual quantities of potassium acetate brought about precipitation of starch iodide. Thus it is established beyond doubt that pectic material does not interfere with the precipitation of the starch iodide by the present method. The figures for starch recovery also clearly demonstrate that fact. Another important observation made in these experiments was that when pectin was heated with 0.7 per cent. potassium hydroxide solution the liquid turned yellow. It therefore appears that the yellow colour of the alkali extracts of plants is probably due to the presence of pectin. It is also quite likely that, to a certain extent, dextrans may be responsible for the colour.

COMPARISON WITH THE TAKA-DIASTASE METHOD.—In order further to ascertain if substances other than starch interfere with the method, determinations of starch in the same samples were carried out by the taka-diastrase method. Leaf samples, after the preliminary treatment to remove sugars and other substances, were gelatinised for an hour or two with 0.7 per cent. potassium hydroxide solution by the method described in a previous communication,⁶ and neutralised with acetic acid to the right pH (about 5), 0.1 g. of taka-diastrase was added, and the mixture was incubated for 24 hours at 37° C. The solution was heated to boiling, cleared with the requisite quantity of neutral lead acetate, de-leaded with 10 per cent. disodium hydrogen phosphate solution ($\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$), filtered and made up to volume. The reducing power of the sugar produced was determined in an aliquot part of the solution by means of the ferricyanide method of Widdowson.⁷

The values obtained by the hydrolysis method are higher than those given by the starch iodide method. This result bears out the contention of former workers, *e.g.* Denny,³ Sullivan⁴ and others, that hydrolytic methods of starch determination do not give the values of "true" starch. The fluctuations are also greater. The

figures obtained by the starch-iodide method are usually much more consistent (Table I) and appear to represent "true" starch.

TABLE IV

Plant material		Starch* (by starch-iodide method) Per Cent.	Starch (by taka-dia- stase method) Per Cent.
Potato leaf, No. I	..	2.74	3.11
" " No. II	..	5.69	5.75
Tomato leaf, No. I	..	2.77	3.08
" " No. II	..	5.14	5.56
Radish leaf, No. I	..	3.30	3.48
" " No. II	..	7.00	7.38

* These values are the averages of four determinations for each plant material in Table I.

DISCUSSION OF THE RESULTS.—Potassium hydroxide solution (0.7 per cent.) has been found to be a very suitable reagent for the extraction of starch from plant material. It greatly facilitates extraction by its macerating action and is to be preferred above all other reagents so far used, owing to the stability of starch in alkaline solution. Rask⁸ used 21 per cent. hydrochloric acid in the cold for the extraction of starch from plants. The choice of this reagent was not a happy one, owing to the fact that appreciable losses of starch take place by hydrolysis within half-an-hour, even if the solution is kept at a low temperature. We have indicated in a previous communication¹ that low values are obtained if hydrochloric acid is used as a coagulant for starch iodide. This is probably due to the fact that hydrolysis of starch proceeds even when the starch is present as an iodide.

Some previous workers have used concentrated calcium chloride solution for extracting starch from plant material. This has also been found by Pucher and Vickery⁵ to be a very unsatisfactory reagent, as serious losses of starch may occur during the course of extraction if the *p*H of the solution is not properly adjusted.

The starch-iodide method gives very concordant results, slightly lower than those obtained by the hydrolytic method. The discrepancy is probably due to the fact that taka-dia-*stase* is not as selective in its action as is generally believed. The starch iodide reaction being the most specific, values obtained by the present method may be safely taken as representing "true" starch. All the substances that are likely to interfere with the accurate determination of starch are removed by preliminary treatment of the material with 80 per cent. alcohol and ether. Consequently, starch iodide is probably the only compound that separates by coagulation on the addition of potassium acetate. It is clearly indicated (Table III) that impurities such as potassium pectate are not precipitated by iodine and potassium acetate. Alkali as weak as 0.7 per cent. has no appreciable action on cellulose, hemicelluloses or lignin.

It is hoped that, owing to the ease of manipulation and the accuracy of the results obtainable, this method will find ready application for the determination of starch in various investigations. It may be mentioned, in passing, that I have developed a micro-technique on similar lines for the determination of starch in

leaves, roots, stems and reproductive organs of plants, in which amounts of starch iodide weighing 2 to 3 mg., and even less, are determined on a micro-balance. It is intended to present this subject in a subsequent communication.

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5, CONVENT STREET
BOMBAY

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DISCUSSION

Mr. A. L. BACHARACH said that he was very interested in the difference between the figures obtained by Dr. Chinoy's and the taka-diastrase method. R. A. Peters had described certain experiments which pointed to the presence of phosphatase in samples of taka-diastrase. Possibly the products obtained by the action of taka-diastrase contained phosphates which might be present in leaves as phosphorylated carbohydrates.

Mr. L. EYNON thought it was very significant that the starch iodide obtained by the Edwards-Nanji-Chinoy method had practically the same composition independently of the kind of starch. If it was only an adsorption product, this was very extraordinary.

Mr. E. HINKS said that he understood Dr. Nanji to say that on applying this method to pure starch added to leaves there was a recovery of 95 per cent. He noticed that the difference between the figures obtained by Dr. Chinoy and those obtained by the taka-diastrase method was in the region of 5 per cent. Would it not seem from this that the taka-diastrase method was the more correct?

Mr. NICHOLLS was glad that Dr. Nanji had stressed the grade of crucible to be used. He himself had tried the method and found the filtration unsatisfactory with either fine or coarse grade alundum, but that the medium grade was just right. One point which required examination was the nature of the starch iodide compound. In a number of tests the colour of the product varied considerably, being sometimes black, dark brown, yellow, or even almost pure white. Mr. Edwards had not been able to explain this, as he had not obtained such variations. It did not appear to be a fault in the washing. Could such differences in colour occur with a substance of constant composition? Regarding the figures shown, he was not quite satisfied that the precipitate could be regarded solely as starch iodide. It might include substances closely allied to, but not ordinary starch. It was clear that the increase of starch from 2 per cent. in the morning to 5 per cent. in the evening was due to photosynthesis. It was probable, however, that the leaves next morning would again show about 2 per cent., owing to the fact that the freshly formed products had been used during the night for plant metabolism. It was difficult to imagine that 3 per cent. of starch could be degraded in one night without many of the remaining starch grains showing lack of regular form when examined microscopically.

Mrs. S. TRITTON remarked that she had worked in the laboratories of Messrs. Parke Davis for some time; they always standardised taka-diastrase against starch and she could not see how it could fail to be accurate.

The PRESIDENT asked if in this standardisation one particular starch was used, and she replied—"Yes, potato starch."

Mr. D. M. FREELAND asked if the author had considered the determination of starch in presence of its degradation products. He had found that it was not possible to get total recovery of starch by this method when some of it had been broken down by heat processes.

Dr. NANJI, replying to the point raised by Mr. Bacharach about the presence of other enzymes such as carboxylase in taka-diastrase, said that he had worked with several taka-diastrase preparations and quite often had found the enzyme pectase which would break down pectic material. It was also known that most taka-diastrase samples had some action on hemicelluloses, and he felt sure that other enzymes were present also. In reply to Mr. Eynon's question about the starch-iodine complex, he said that it was true that there was still some controversy as to whether it was really a chemical compound or merely an adsorption product, but the important relevant point was that under the standardised conditions the weight of starch iodide from a given quantity of different pure starches was found to be constant. Replying to Mr. Hinks, he said that 95 per cent. was mentioned as an average, but the recovery of added starch actually varied from 92 to 99 per cent. Mr. Nicholls had raised an important point about the difference in colour of the starch iodide. It was true that the residue of starch iodide left on drying at 100° C. was almost colourless in some cases—they had recently observed this with maize starch in particular. Nevertheless, the increase in weight under the conditions of the test was found to be constant with all the starches they had tried. In reply to Mr. Freeland's question, Dr. Nanji said that a method had already been worked out for the determination of starch in presence of dextrin and sugars (*cf.* ANALYST, 1938, 697). With regard to the remarks about the taka-diastrase method, it was true that this gave satisfactory results with pure starches, but when other polysaccharides such as pectins and hemicelluloses were present the amount of starch indicated was usually higher than by the iodine method.

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 DETERMINATION OF TIN IN BRONZE

THE following method has been worked out to meet the difficulty of determining tin in bronze* containing elements—arsenic, antimony, phosphorus and iron—occluded in the precipitate obtained by hydrolysis. It is based on the fact that, under certain conditions, stannic tin in oxalic acid solution forms a fairly stable compound with sulphur, which can be titrated with iodine. Under those conditions antimony sulphide reacts slowly, if at all, with iodine; phosphorus and iron have no effect. Arsenic is removed in the preliminary hydrolysis by addition of an excess of phosphoric acid, arsenic acid remaining in solution.

METHOD.—To 2 g. of bronze, in a tall 400-ml. beaker, add 8 to 10 ml. of 5 N hydrochloric acid, followed by 10 ml. of conc. nitric acid. Allow the reaction to proceed in the cold. When solution is complete, boil for a few moments until dense brown fumes are expelled, remove from the plate, and add 8 to 10 ml. of 10 per cent. ammonium phosphate solution. Dilute at once with *boiling* water to 250 ml. and boil vigorously for a few minutes. The introduction of a piece of fine shingle will ensure even boiling. Wash down the sides of the beaker, allow the precipitate to settle and filter through a Whatman filter (541P). These

* In general, copper alloys high in tin.

are made of hardened paper, from which the precipitate can subsequently be readily washed off, and their shape enables the precipitate to be washed free from the mother-liquor without difficulty.

Wash thoroughly with hot 0.3 *N* nitric acid and with hot water. Wash the precipitate back into the beaker. Add 25 ml. of 10 *N* sulphuric acid, and neutralise to methyl orange with 8 *N* ammonia. Add 6 or 7 g. of oxalic acid, and boil till clear. Then, having a volume of about 100 ml., concentrating if necessary, saturate the hot solution with hydrogen sulphide, cooling being prevented.

Dilute with cold water to 250 ml., cool to room temperature, and pass a stream of air through the solution, at the rate of 1.5 to 2 litres a minute, for from 15 to 20 minutes, to remove free hydrogen sulphide. Run in standard iodine solution from a burette, adding not more than 1 ml. in excess. Titrate the excess at once with standard thiosulphate solution.

The solution contains one atom of combined sulphur for each atom of tin present. Hence 1 ml. of *N*/10 iodine solution is equivalent to 0.005935 g. of tin.

In test experiments mixtures of 2 g. each of the metals shown below were made. Arsenic, antimony and iron were introduced as solutions of their chlorides, nickel in a nitric acid solution, and lead and zinc copper and tin as metals. The thiosulphate solution was standardised with electrolytic copper. The iodine solution was also separately standardised by means of "Chempur" tin. Results with both standards are shown.

The method of standardising with pure tin is as follows:

To 0.2 g. of tin in a tall 400-ml. beaker, add 13 ml. of a mixture of equal parts of conc. sulphuric acid and 6 *N* nitric acid (sp.gr. 1.2). Heat gently till solution is complete, then more strongly to expel nitric acid. Cool thoroughly, dilute somewhat, neutralise with 8 *N* ammonia, add oxalic acid, and proceed as described.

The following results are typical of those obtained:

	Per Cent.	Per Cent.	Per Cent.	Per Cent.
Tin	9.66	8.90	12.30	7.00
Arsenic	—	0.5	0.3	—
Antimony	—	0.8	0.1	—
Iron	—	0.5	0.2	—
Lead	—	—	—	6.0
Nickel	—	—	—	5.0
Zinc	—	—	—	5.0
Copper to	100	100	100	100
Tin found—				
"Copper standard" ..	9.67	8.97	12.33	7.00
"Tin standard"	9.64	8.94	12.29	6.97

I have to thank the Admiralty for permission to publish this paper.

W. C. G. WHEELER

THE CHEMICAL LABORATORY
H.M. DOCKYARD, PORTSMOUTH
September, 1938

A NEW METHOD FOR THE WET ASHING OF URINE

WET ashing is generally considered a convenient method of dealing with biological material, but ordinary wet ashing of urine is not very satisfactory because of frothing and charring. Francis, Harvey and Buchan¹ find that if urea is first removed by treating the urine with nitrosylsulphuric acid in the cold, the subsequent wet ashing is practically free from the objectionable frothing and charring. In the method to be described, removal of the urea is based on the fact—used by Folin²

as long ago as 1901 and since by several other authors³—that urea is rapidly decomposed by dilute mineral acids at about 150° C. In addition, use is made of the observation that at this temperature nitric acid is much superior to other acids in effecting considerable decomposition of other constituents of the urine. The evaporation and digestion of the specimen are concluded in a semi-closed system, in a rapid current of air.

METHOD.—Place 500 ml. of urine in a 700-ml. round-bottomed flask which has a standard ground neck and add 30 ml. of conc. nitric acid (sp.gr. 1.4). Cover the flask with a beaker and place it on a small metal stand in a high-pressure autoclave which contains, in place of water, 250 ml. of 10 per cent. sodium hydroxide solution. Heat the autoclave for five minutes after the contents boil, close the blow-off valve, and continue the heating until the temperature reaches 310–320° F. (as indicated by the autoclave thermometer), then allow the autoclave to cool. At this stage the mixture should be an almost clear yellow solution, nearly free from suspended matter. Add to the contents of the flask 15 ml. of conc. sulphuric acid (sp.gr. 1.84), 10 ml. of 60 per cent. perchloric acid (sp.gr. 1.54), and a few chips of silica, and then close the neck of the flask with a suction adapter. Place the flask on a sand-bath and connect the side arm of the suction adapter with a filter-pump by way of two Pyrex filter-flasks arranged as wash-bottles. The first flask has a capacity of two litres and contains 60 ml. of 40 per cent. sodium hydroxide solution, whilst the second flask has a capacity of 1 litre and contains 100 ml. of 5 N hydrochloric acid. Heat the autoclaved material to boiling and maintain a fairly rapid current of air through the apparatus during the evaporation and digestion. When all the water has been evaporated, the yellow mixture becomes rather viscid and froths a little until the nitric acid has passed off, after which it rapidly darkens to a deep brown. Rarely is there any separation of free carbon. From this stage the digestion proceeds rapidly and smoothly, and is usually complete within 5 to 10 minutes.

After cooling, dilute the digest and proceed with the determination of the metal.

A few notes in explanation and extension of the procedure may be of value: The autoclave used in this laboratory is a Fisher Urea Nitrogen Autoclave 6–378 and measures internally 8 in. high by 4½ in. diameter.

The method is independent of the age and condition of the specimen, being as effective with normal urine as with urine containing large quantities of blood, pus, albumen, bacteria or precipitated salts. Many other acids were tried in place of nitric acid, but in every instance charring or some other objectionable action occurred. A temperature not lower than 150° C. (302° F.) is essential for satisfactory working, for at lower temperatures the urea is not completely decomposed in the time, and in place of a clear yellow hydrolysate one may obtain a dark brown solution containing much charred material and undecomposed urea. Very rarely is the quantity of nitric acid used (30 ml. per 500 ml. of urine) insufficient; in that event the hydrolysate is not acid to Congo red and has the same appearance as after autoclaving at too low a temperature. The addition of more nitric acid and repetition of autoclaving will produce a satisfactory preparation. The sodium hydroxide in the autoclave is intended to prevent action of the nitric acid on the copper autoclave either by fuming or in event of breaking of the container, and also to absorb the carbon dioxide from the decomposed urea. It is dangerous to omit from the autoclave either the sodium hydroxide or the metal stand on which rests the flask of acidified urine.

As there is little frothing during the process, the digestion of 500 ml. of urine can easily be done in the 700-ml. flask. The quantity of perchloric acid used (10 ml.) has been selected as a convenient amount for routine use, even though it is more than enough for most specimens of urine. Occasionally it is necessary to use a larger volume of perchloric acid.

The first filter flask acts as a condenser for the water vapour from the digestion

flask and contains alkali to neutralise the acid evolved during the digestion. The dilute acid in the second flask prevents the passage of free ammonia from the first flask into the pump and thence into the laboratory atmosphere. The least possible length of rubber tubing should be exposed to the acid vapours where the suction adapter is connected with the filter flasks.

The procedure outlined may be carried out in a general laboratory without the need of a fume cupboard. The actual working time for the hydrolysis, evaporation and digestion is surprisingly small. The autoclave needs close attention while being heated to the requisite temperature, but during the rest of the process the apparatus needs only an occasional glance.

The method has been developed in this laboratory chiefly for the determination of lead in urine, but it has also been used satisfactorily as a preliminary process in the determination of other metals. The slight excess of perchloric acid which normally remains is usually of no consequence, even in the determination of arsenic by the Gutzeit or similar method, for, as Allcroft and Green⁴ state, perchloric acid does not interfere with the liberation of arsine.

ROBERT J. BARTHOLOMEW

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DEPARTMENT OF PATHOLOGY
THE PRINCE HENRY HOSPITAL
SYDNEY, AUSTRALIA

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

ANNUAL REPORT OF THE CITY ANALYST FOR THE YEAR 1937

OF the 5613 samples taken in connection with the Food and Drugs Act, 5422 were bought informally.

RUBBING LOTION.—This lotion was advertised for use in cases of rheumatism, arthritis, etc., and the formula was printed on the label. It was stated to contain 94.8 per cent. of industrial alcohol, with small amounts of ammonia, castor oil and essential oils, but analysis showed it to contain only 77 per cent. of alcohol. The vendor said that he was under the impression that it was legal to break down alcohol with water and still call it alcohol, but it was pointed out to him that no mention was made of water as an ingredient. He agreed that future mixings should correspond with the formula.

SODAMINT TABLETS.—In the B.P. Codex "sodamint tablets" is a synonym for "compound sodium bicarbonate tablets," and each tablet should contain 0.324 g. of sodium bicarbonate and 0.008 g. of ammonium bicarbonate with small amounts of saccharin and peppermint oil. There has been much controversy as to the composition of commercial sodamint tablets. Some authorities deprecate the use of ammonium bicarbonate, others object to the use of saccharin, and

others, again, apply the name to a simple sodium bicarbonate and oil of peppermint tablet. Of the various samples examined, a number contained the correct amount of sodium bicarbonate but no ammonium bicarbonate. One firm stated that they supplied the Codex article only when ordered in a prescription or when asked for as "Sodamint tablets B.P.C." They suggested that they might label the unofficial tablets as "Sodamint tablets (old style)," since they were made from a formula in existence before the Codex adopted the name as a synonym. They were advised that this would be a contravention of the labelling section of the Food and Drugs Act. For the present the shop concerned has been instructed to supply the Codex article when sodamint tablets are asked for.

H. H. BAGNALL

Report of the Government Chemist upon the Work of the Laboratory

FOR THE YEAR ENDING 31ST MARCH, 1938*

THE chemical work for the various Government departments has been carried out as before (*cf.* ANALYST, 1938, 63, 43-45) mainly at Clement's Inn and also at Custom House, the Chemical Stations, Geological Survey Museum, Park Royal and Deptford. The total number of samples examined was 562,549, compared with 545,233 in the previous year. The increase was chiefly in the tobacco samples, Air Ministry samples, imported spirituous preparations and silk samples, whilst wines, cocoa and chocolate samples decreased. In many instances samples have to be examined under more than one class for several dutiable ingredients, but are only recorded as one sample.

MINISTRY OF AGRICULTURE AND FISHERIES.—Butter.—The water-content of the 848 samples of butter varied from 11·34 (1 sample) to 17·0 per cent. in 2 samples; 38 samples contained over 16 per cent. of water, 235 between 14 and 15 and 500 between 15 and 16. The Reichert–Meissl value was between 24 and 26 in 37 samples; 26 to 28 in 149; 28 to 30 in 292; 30 to 32 in 263; 32 to 34 in 107 samples, whilst the Polenske value varied between 1·0 and 3·35.

Margarine.—One sample of 74 contained boric acid, and the percentage of water varied between—under 7 in 2 samples; between 7 and 10 in 9; 10 and 14 in 3 samples; 14 and 15 in 12; 15 and 16 in 44; 16·0 and 16·1 in 4 samples.

Cheese.—No sample of the 127 examined contained foreign fat; the water-content varied from 11·5 to 60·3 per cent. and fat from 9·1 to 40·2 on the cheese, or 15·9 to 80·8 on the dry matter.

Cream.—The percentage of fat in the 87 samples of cream varied greatly. Two contained 54 to 56 per cent., 4 samples 30 to 35, and the remainder between 20 and 29 per cent.

Sheep Dips.—Five of 57 samples examined would not have satisfied the Ministry's requirements when prepared as directed.

Sea Water.—The salinity of 6323 samples was determined and results were sent to Copenhagen in accord with the Oceanic Research Scheme.

Water Pollution of Rivers.—To ascertain the condition of fishing streams, 107 samples of river water were examined, and 10 samples of proprietary road dressings were tested for harmful or toxic bodies.

Fertilisers and Feeding Stuffs Act, 1926.—Seven fertilisers (3 compound manures, 1 superphosphate, 1 shoddy, 1 hoof and horn meal and 1 basic slag) were alleged not to conform to the statutory statement supplied with the article. Eleven feeding stuffs (3 feeding meals, 2 fattening nuts, 2 ground oats and 4 wheat

* Obtainable at H.M. Stationery Office, York House, Kingsway, W.C.2. Price 9d. net.

offals) were examined. The feeding meals and fattening nuts were found not to conform to their statutory statements; one ground oats contained foreign cereal, and the other was alleged to contain an excess of oat husk; one sample of wheat offals contained rice husk, and the remaining three, two of which were sold under the local name of Dan, contained tapioca. The results obtained in the Government laboratory confirmed those of the agricultural analysts who had originally examined the samples in all cases except that the basic slag was found to agree with the statutory statement, the hoof and horn meal, although deficient in nitrogen, was within the limits of variation allowed, and the proportion of oat husk in ground oats was not regarded as excessive.

Agricultural Produce (Grading and Marking) Acts. National Mark Schemes.—The 312 samples of flour included 105 marked Yeoman. Malt extract and extract with cod-liver oil samples numbered 17; cider 300; perry 15; honey 130. The National Mark regulations for cheeses remain as before (ANALYST, 1938, 63, 44) and under these 491 samples were examined, including 148 Cheshire, 112 Stilton, 11 Cheddar, 20 cream, 91 Caerphilly, 102 Lancashire, 1 Wensleydale, 4 Leicester and 2 Derby cheeses. In order to market watercress under the National Mark scheme the water from the intakes has to be examined chemically and bacteriologically, and for this purpose 123 samples were received. Of miscellaneous samples examined, 2 were in connection with sprays against Colorado beetle and 6 were derris preparations for warble fly dressings.

CUSTOMS AND EXCISE.—Beer.—Of the total 47,291 samples examined, 455 were of materials used for brewing, 3951 of wort or beer as a check on brewers' declarations (of which 89 samples were found to be under-declared); 3066 of spoilt beer; 520 of beer as retailed (of which 11 samples gave evidence of dilution—in 5 instances to an equivalent of over 3 gallons of water per barrel)—and 40 of non-alcoholic beer, the alcohol in 5 of which ranged from 2 to 3 per cent. of proof spirit. Of beer exported on drawback, 16,039 samples were examined, and of these only 37 were found to be over-declared. Of imported beer, 19,543 samples were examined, mostly from Eire. Eighteen samples of brewing materials were found to contain arsenic in slight excess of the recommended limit.

Cocoa and Chocolate.—The total number of samples examined was 12,384, a decrease of about 2000 on those of the previous year, nearly all accounted for by the fall in import samples to the 1935 level.

Coffee and Chicory.—Eleven samples were taken from importations described as dandelion root, and 28 from preparations such as roasted cereals, whilst drawback was found to be incorrectly declared on 5 samples of 1902 examined.

Dangerous Drugs Act.—Of 22 samples received of suspected goods, one was heroin, one cigarettes containing Indian hemp and 3 were Indian hemp.

Hydrocarbon Oils Duty.—The Finance Acts affecting these duties remain as before, as do the standard cone penetration and needle penetration tests, the material under test not being regarded as solid or semi-solid unless it has a penetration number of less than 200 units at 60° F. Imported goods are examined to determine duty, and exported goods to check drawback, and fixed rates have been established for goods regularly imported or exported. The total number of samples examined was 14,447, of which 8616 were from imported and 5831 from exported goods. Hydrocarbon oils accounted for 8153 samples and the remainder were from miscellaneous and composite goods such as enamels, insecticides, etc.

Hydrometers and Graduated Vessels.—The use of the new Tate saccharometer has been extended, and about 800 have now been issued.

Silk and Artificial Silk.—In connection with the duties in force 19,049 samples were examined, of which 6070 were from imported goods concerning which it was required to know the proportion, if any, of real or artificial silk present.

Spirits.—Samples examined in connection with duty-free spirits (4355

samples); spirits deposited on drawback (3664); exported spirituous preparations (15,619); imported spirits (13,299); imported spirituous preparations (8867); imported methyl alcohol (49); fusel oil (55); illicit distillation and uncustomed spirits and grogging 63 samples, of which most afforded evidence of revenue offences; wash, yeast pressings, etc. (205) and miscellaneous samples (503).

Sugar, Glucose and Saccharin.—Samples of sugar, of which 868 were from beet sugar factories, comprised 22,016; of preparations containing sweetening material there were 52,996 (45,689 of which were from imported goods); of glucose 1208; saccharin was searched for in 102 samples of imported substances, while 581 samples of saccharin and articles containing it were examined for assessment of drawback.

Teas.—Of 13,059 samples of imported tea, 129 (representing 346 packages) were reported against, 2 on account of foreign substances and 127 as unfit for human consumption.

Tobacco.—The increase in samples continues, and 201,751 in all were examined. Adulteration of tobacco is almost non-existent in this country, but 4 samples of cigarettes reconditioned from waste were found to be contaminated with foreign matter. Most of the samples examined were in connection with customs duty on raw imported tobacco, drawback on manufactured tobaccos, offal tobacco and manufactured tobacco for home consumption. Under this last heading examination has frequently to be made for addition of such substances as glycerin or diethylene glycol added by foreign manufacturers to improve flavour or retain moisture, but which, except for "Cavendish" tobacco, are illegal in this country.

FOOD AND DRUGS ACTS.—Twenty samples of food were examined during the year, consisting of eleven milks alleged to be deficient in fat or non-fatty solids, four jams alleged to be deficient in fruit, one sample of pork and beans alleged to contain no pork, two biscuits alleged to contain added boric acid, one mint sauce incorrectly described as containing malt vinegar, and one sample of mince alleged to contain excess sulphur dioxide. The results were in agreement with those put forward by the prosecution in every case except the following:—One milk was not found to be deficient in fat; one jam was not found to be deficient in fruit; the amounts of boric acid found in the biscuits were consistent with their having been naturally present in the cocoa used for making the chocolate filling; the amount of sulphur dioxide present in the mince, when examined, was within the permitted limits, but it was not possible to say how much had been lost since the sample was taken.

MINISTRY OF HEALTH.—*Condensed Milk.*—Of 131 samples of condensed milk taken from imports, 18 were reported against, 1 as deficient in fat and 17 for labelling offences.

Preservatives.—Of 1262 samples of imported dairy produce and 668 of other foods, 49 were reported as contravening regulations. These included 37 containing sulphur dioxide, one sample of margarine containing boric acid, and 11 of soda fountain preparations containing benzoic acid either contrary to regulations or in excess of the quantity allowed.

D. G. H.

Western Australia

ANNUAL REPORT OF THE CHEMICAL BRANCH, MINES DEPARTMENT, FOR THE YEAR 1937

THE Government Analyst (Dr. E. S. Simpson) gives an account of the work done for the various Government Departments, including those of Health and Police. The section concerned with food, drugs and toxicology is under the direction of Mr. H. E. Hill, A.I.C.

REGULATION AS TO FREEZING-POINT OF MILK.—As the result of some years' experience of the freezing-point test, Regulation 27 (1) under the Health Act was amended on the recommendation of the Advisory Committee to read "Its freezing-point shall not lie between zero Centigrade and 0.540° C. below zero as determined by the Winter method."

REFEREE'S FUNCTION IN COURT.—In a disputed case the milk vendor's chemist submitted figures indicating a normal unadulterated milk. The Government Analyst was called in as referee, and submitted figures for the third portion of the original sample identical with those submitted by the prosecution; yet because the whole three reports did not agree, the vendor was given "the benefit of the doubt" and the case was dismissed. In such circumstances the true function of a referee is disregarded, and a vendor need only submit a substituted sample to his chemist or employ a venal or unreliable analyst to escape punishment every time. Owing to imperfect sealing, substitution of samples is not impossible.

YELLOW DYE APPROVED FOR FOOD.—Requests were made for several additional dyes to be added to the list of those permitted to be used in foodstuffs. On the recommendation of the Advisory Committee only one, "Edicol F.C.S. (Sunset Yellow)" was added to the approved list.

ADOPTION OF JAM STANDARD.—Of 36 tendered samples examined, 17 were rejected. This large proportion of rejections was due to the adoption, for the purpose of Government supplies, of the standard of not less than 68 per cent. of total soluble solids, which is substantially that agreed upon in England by the Food Manufacturers' Federation and Society of Public Analysts. In addition, a number of mixed jams did not comply with the Food and Drugs Regulation, that of the fruits named on the label, at least 50 per cent. must consist of that named first. Samples of loganberry and gooseberry jams contained added colouring matter.

WHOLEMEAL BREAD.—Analyses, made for the Commissioner of Public Health, of "Wholemeal" breads on the local market gave the following percentage results :

		"WHOLEMEAL"						
		No.	24	25	28	33	34	35
Water	38.50	39.10	37.70	41.40	38.50	42.10
Fat	1.67	1.60	2.49	1.57	3.25	1.73
Protein (N × 5.7)	8.72	8.06	8.70	8.46	7.24	8.22
Fibre	0.94	0.91	0.86	0.81	0.94	1.12
Mineral matter	2.18	2.37	1.91	2.05	2.25	1.83
Carbohydrate (by difference)	47.99	47.96	48.34	45.71	47.82	45.00
			100.00	100.00	100.00	100.00	100.00	100.00
Digestible calories per 100 grams	..		242	238	251	231	249	228

A further five samples were variously described as follows:—No. 26.—“Wholemeal with small quantity (plain white) flour.” No. 30.—“Half wholemeal, half ordinary flour, coloured with molasses.” Nos. 27, 32.—“White flour 150 lbs., gristed wheatmeal 35 lbs., pollard 25 lbs.” No. 31.—“Special wheatmeal plus extract of malt.” These gave the following percentage results:

	No.	26	30	27	32	31
Water	37.70	38.90	35.00	37.80	37.50
Fat	1.30	1.26	1.66	1.61	2.15
Protein (N × 5.7)	..	8.56	7.79	8.60	7.79	7.82
Fibre	0.26	0.38	0.82	1.03	0.74
Mineral matter	1.81	1.83	2.27	2.29	2.13
Carbohydrate (by difference)	..	50.37	49.84	51.65	49.48	49.66
		<u>100.00</u>	<u>100.00</u>	<u>100.00</u>	<u>100.00</u>	<u>100.00</u>
Digestible calories per 100 grams		247	242	256	244	249

The percentage of whole-wheat flour used in the baking of these breads was calculated on the assumption that 100 per cent. of whole-wheat bread contains 2.50 per cent. of fibre calculated on a water-free basis (West Australian, 1937, F.A.Q. wheat, 2.75 per cent.). The following percentage results were obtained:

Description	No.	Crude fibre calculated on water-free basis	Whole-wheat flour present	Deficiency
“Wholemeal” ..	24	1.53	61.2	38.8
	25	1.49	59.6	40.4
	28	1.38	55.2	44.8
	33	1.38	55.2	44.8
	34	1.53	61.2	38.8
	35	1.93	77.2	22.8
Various (see above) ..	26	0.42	16.8	83.2
	30	0.62	24.8	75.2
	27	1.27	50.8	49.2
	32	1.66	66.4	33.6
	31	1.18	47.2	52.8

If the fibre figure in the West Australian, 1937, F.A.Q. wheat had been taken, the deficiency would have appeared greater. Since white or “patent” flour contains only about 0.1 per cent. of fibre, the deficiencies shown in the table represent the approximate amounts of white flour used in making these “wholemeal” breads. Whole-wheat or wholemeal bread is defined in the Food and Drug Regulations of the West Australian Health Act as bread made from whole-wheat flour (itself the product of grinding wheat without any process of sieving), with the addition of not more than 10 per cent. of added (white) flour.

The introduction of standards for wholemeal and modified wholemeal breads seems highly desirable, since the public desirous of eating such breads is entitled to receive what it asks for, and to know what it is getting.

COPPER DEFICIENCY IN GINGIN SHEEP DISEASE.—By means of the spectrograph the cause of gingin sheep disease (enzootic ataxia), locally known as rickets, was shown to be due to a serious deficiency of copper in the affected animals. The

actual amounts of copper were then determined microchemically by the method of Sylvester and Lampitt (ANALYST, 1935, 60, 376), with the following results:

	Normal animals (sheep and lambs)	Affected animals (sheep and lambs)
Copper in livers (p.p.m.)		
Range	120-360	3-8
Average	222	6
Copper in whole livers (mg.) ..	2.7-41.0	0.05-1.10

A similar condition was found as regards the blood and milk. It was proved by the field officers of the Agricultural Department that a small supplement of copper prevented and checked the disease. A remarkable fact recorded was that chemically pure ammonium chloride, given to the sheep with the idea of curing what was at first supposed to be lead poisoning, had no curative effect, but that commercial ammonium chloride, later shown to be appreciably contaminated with copper, was distinctly beneficial.

ZINC DEFICIENCY IN PINE TREES.—As the result of spectrographic tests to determine the cause of the irregular growth of exotic pines (*P. radiata* and *pinaster*), it was found that spraying the young trees with weak solutions of zinc sulphate (and to a less extent of certain other salts) gave a good response as to growth and colour of the needles. Analyses are in progress to determine the amounts of zinc normally present in healthy and affected trees.

VITAMIN C IN WEST AUSTRALIAN FRUITS.—The fruits examined included citrus fruits (lemon, orange, mandarin, lime, kumquat and citron), Cape gooseberry, large yellow guava, English mulberry and passion fruit. Up to date it has been found that local fruits are within the range and up to the ascorbic acid-content of the same kinds grown and examined elsewhere. Loquats contained no ascorbic acid.

WATER SALINITY TOLERATION.—Ground waters reached by wells up to 150 feet in depth continue to be the main source of individual supplies throughout the interior of the State. These are rarely quite fresh and the salinity may reach that of sea water or higher. The problem that is constantly presented to the Chemical Branch is what can be safely done with a more or less saline water prior to permanent well sinking. The standards of total salinity safe for various kinds of stock, when on dry feed, have stood the test of time; they are as follows:

For horses and pigs ..	maximum	450 grains per gallon			
For cattle	"	700	"	"	"
For sheep	"	900	"	"	"

It is much more difficult to decide what is the limit for continuous or occasional consumption by man and for the irrigation of various commercial crops. The decision with regard to man is a serious problem, especially in the Murchison region, where the equivalent of several grains of sodium nitrate per gallon is present in many of the well waters. In some places hardened miners are drinking regularly water containing up to 300 grains of total salts and 5 grains of sodium nitrate per gallon. H. St. J. Philbey, in *The Empty Quarter*, records camping and travelling in Southern Arabia for two weeks and having to drink a well water with the following composition: sodium chloride, 238.7; sodium sulphate, 79.8; magnesium sulphate, 70.7; calcium sulphate, 115.5; calcium carbonate, 22.4; total salts, 527.1 grains per gallon. This water caused serious discomfort, however, even after partly correcting the osmotic and inflammatory effects by making an infusion in it of tannin from tea, coffee and a local herb.

With regard to crops it is apparent that a high degree of salinity (up to at least 250 grains per gallon) will be tolerated under local conditions by tomatoes,

lucerne, cauliflowers, celery, silver beet, and several other plants of economic importance.

CHEMICAL DIAGNOSIS OF DRUNKENNESS.—Work done in the Laboratory during 1937 confirmed the accuracy of methods of determining alcohol in the body fluids. In one local case in which a person, known to be considerably under the influence of liquor, was run over and killed after lying on the road, the concentration of alcohol in the blood was found to be 170 mg. per 100 ml., and in the urine 290 mg. per ml., indicating a considerable degree of intoxication.

Milk and Nutrition. Part III*

THE Milk Nutrition Committee have already issued two reports on the effect of pasteurisation on milk; the first (*cf.* ANALYST, 1937, 62, 463) described the results of investigations on small laboratory animals, and in the second (*cf.* ANALYST, 1938, 434) observations on the effect of milk on the growth and development of school children were recorded. The present report deals with the results of three experiments in which the calf was the test animal. The tests were made by S. Bartlett, A. G. Cotton and J. Mackintosh, of the National Institute at Shinfield, and J. A. Crichton and W. A. Biggar of the Rowett Institute, Aberdeen. The calves were divided into two comparable groups, one of which received pasteurised and the other raw milk.

At Reading 11 pairs of calves from tubercle-free herds received the milk for a period of six months. At the beginning and end of the experiment the live weights of the calves reared on each kind of milk were almost identical, and the rate of progress was remarkably uniform. Small supplements of solid food were given in the later stages of the course, but there was no difference in the appearance of the calves in the two groups, in the amount of food they took, or in the chemical analysis of their blood. Those taking raw milk, however, showed a slight but statistically insignificant advantage in depth of chest, height at withers and birth depth. At the end of the course tuberculin tests were applied to all the calves; 8 of those fed on raw milk and 1 of those on pasteurised milk gave a positive reaction.

At Aberdeen two separate experiments were made. In the first each group contained 6 heifer and 10 bull calves, and in the second 10 bull calves were used in each group. All the calves came from herds not tuberculin tested. In each experiment one group was given raw milk and the other commercially pasteurised milk, with equal supplements of solid food in the later stages. In the first experiment the groups were given as much milk as they wanted, up to 30 lb. per day. Both groups did well, with an advantage, not significant, in favour of raw milk. In the second experiment 15 lb. of milk per day were given, with solid food as soon as the calves would eat it. The calves taking pasteurised milk took less supplementary food than those receiving raw milk, and their rate of growth was slightly but, again not significantly, slower. When the results of the Reading experiment are included with those of the Aberdeen experiments, no statistical difference is found between the effects of raw and pasteurised milk on growth.

Summarising all the information available on this subject since 1926, Bartlett notes that of 75 calves fed on raw milk, 41 (55 per cent.) gave a positive reaction in the tuberculin test, whilst of 77 fed on pasteurised milk, only 6 (8 per cent.) gave a positive reaction. In the three experiments described in this report the figures were less striking—46 and 13 per cent. respectively; even so, the difference

* National Institute for Research in Dairying, Shinfield, Reading. Pp. 27. Price 2s. post free.

was noteworthy, especially when considered in conjunction with the fact that pneumonia appeared to be commoner among the calves fed on raw milk.

The conclusions drawn from these experiments are that raw and pasteurised milk have an almost identical nutritive value for calves, and that there is a considerable risk of spreading tuberculosis among calves given raw milk. The consideration of the bearing of these results on human nutrition is reserved for a later report in which the complete investigations on school children will be described.

Commonwealth of Massachusetts

ANNUAL REPORT OF THE DEPARTMENT OF PUBLIC HEALTH FOR 1937

THE Food and Drug Division of the Department, under the direction of Mr. H. C. Lythgoe, is entrusted with the enforcement of laws relating to food and drugs, etc., the mattress law, and certain phases of the narcotic law and with the examination of samples submitted by the Police departments.

VITAMIN D MILK.—Samples of metabolised vitamin D milk produced by feeding the cows with yeast, of irradiated milk and of milk containing added vitamin D concentrate were examined. The last-named is sold, not as milk, but as a proprietary drug. One sample of this type was highly deficient in vitamin D. Two samples of metabolised vitamin D milk were also deficient in the vitamin.

ORANGEADE.—A number of samples were wrongly labelled "Orangeade." They consisted of carbonated water, sugar, citric acid, flavour and colour, whereas orangeade is orange juice, sugar and water. The use of the term "Orange Soda" for this type of beverage is legal (according to a ruling of the U.S.A. Department of Agriculture). The use of artificial colour in orange preparations constitutes an adulteration, whether or not there is a declaration on the label. The inter-shippers of material of this type made shipments without added colour, and sent the colour in a separate package. This left the enforcement of the ruling to the States concerned. Attempts to enforce the ruling were unsatisfactory, and most of the cases were dismissed. In one instance the U.S.A. Department of Agriculture made a seizure in Louisiana, and the State court held that the coloured orange concentrate was not adulterated within the meaning of the law.

CREAM.—The standard for "light cream" is 16 per cent., for "medium cream" 25 per cent., for "heavy cream" 34 per cent., and for "extra-heavy cream" 38 per cent. of fat. Only two samples contained less fat than required by the specific grade by which the cream was sold.

ICE-CREAM.—There were 684 bacteriological examinations made of ice-cream, the bacterial count varying from 500 up to 1,200,000, the standard set by regulation being not more than 100,000. There were 604 samples conforming to that standard. The lower quartile was 3666. The median was 11,643, which corresponded very closely with the geometric mean of 12,549. The upper quartile was 40,000, which means that 75 per cent. of the samples contained less than half the maximum amount of bacteria permitted by the regulations.

CLEANSING OF MINERAL WATER BOTTLES.—The regulations require that the bottles be washed in a 2 per cent. caustic solution. There were 59 samples of this solution obtained. The lowest caustic strength found was 0; 27 per cent. of the samples varied between 0.1 and 0.5 per cent. of caustic alkali; 44 per cent. of the samples varied between 0.6 and 1.9 per cent.; and only 27 per cent. of the samples showed full caustic strength, varying from 2.0 to 4.7 per cent. The average of the samples containing less than 1 per cent. of caustic alkali was 0.46 per cent.;

and the average of the samples containing from 1 to 2 per cent. was 1.53 per cent. of caustic alkali, calculated as sodium hydroxide. Among the excuses for these conditions was one that is fast becoming stereotyped, namely, that the bottles were being washed for storage purposes and were not intended to be filled until they were washed again.

LAW RELATING TO BEDDING AND UPHOLSTERY.—During the year the usual examinations of mattress and upholstery filling were made, the material being examined under ultra-violet light, and its urea-content and (occasionally) starch-content determined. The amount of urea in samples found to be new material ranged from 0 to 0.86 mg. per 100 g. One sample contained as much as 1.18 mg. This is within the limits of what may be expected in new material, but ultra-violet light indicated that there might have been an extremely slight admixture of secondhand material.

One sample of kapok was found by ultra-violet light examination to be new and contained no urea. Three samples of shredded cloth, free from urea, were found to contain starch. This material is secondhand within the meaning of the law. The urea-content of a number of secondhand samples of cotton varied from 2.35 to 26.5 mg. per 100 g. One of the materials not properly labelled was a mixture of wool, jute, hair and cotton, which was labelled "white cotton." Under the law passed in 1937 the so-called "garnetted" clippings may be made into felt, used in upholstered furniture and bear a "new" label. They cannot, however, be used as a mattress filling unless it bears the "secondhand" label.

Secondhand upholstery material camouflaged as new is definitely of the past in the Commonwealth. It is only in the furniture stores catering for the poorer class of purchasers that the red-tagged secondhand mattresses are to be purchased. The law requires all used secondhand material to be sterilised. Hydrocyanic acid has been used in one plant, formaldehyde in others, and sulphur dioxide in a few plants. In factories where air-tight chambers have been installed good results have been obtained. Some manufacturers have tried to circumvent the law by using "sterilised" tags on mattresses never submitted to chemical treatment. A sample taken from one mattress showed a bacterial count of 1,780,000 colonies per gram. Samples of sterilised material showed counts of 2000, 72,000, 4000, 24,000, 8000 and 80,000. The enactment of the law requiring sterilisation has caused many manufacturers to use mill waste and low grade cotton by-products instead of actually used secondhand material.

British Standards Institution

THE following British Standard has been issued*:

No. 818—1938. CHEMICAL SYMBOLS AND ABBREVIATIONS.

This British Standard list is based on the Report of a Joint Committee of the Chemical Society, the Faraday Society, and the Physical Society†—a report which follows general principles adopted after considering the views of representative bodies, including the International Conference on Physics (1934) and the American Standards Association. The Report embodies the agreed views of British physicists and chemists.

The British Standards Institution acknowledges its indebtedness to the Joint Committee for the use of this Report and draws attention to certain general recommendations of the Joint Committee. These were put forward in order to make the best use of the limited number of

* Obtainable from the Publications Department, 28, Victoria Street, London, S.W.1. Price 3s. 6d. net; post free, 3s. 8d.

† Cf. ANALYST, 1937, 62, 800.

alphabets and founts commonly available and thus minimise the number of uses to which each letter in a given fount and case is put. The recommendations are substantially as follows:—

- (a) Symbols for quantities (except purely mathematical constants) should be printed in *italic* (*not* Roman) type when they are not Greek.

It is recommended that symbols for certain important physical constants in common use should be printed in *black italic* type. This will relieve the pressure on certain overworked letters and have the advantage that symbols for these quantities having known constant values will stand out against symbols for variables in equations. In writing, as distinct from printing, it is suggested that where possible the symbols for these physical constants be represented by cursive letters, *e.g.* \mathcal{F} as distinct from F, or, especially in blackboard writing, by means of a different colour. In typescript, these symbols may be underlined in red to distinguish them from those to be printed in ordinary italic (underlined in black).

- (b) Symbols for purely mathematical constants and symbols denoting mathematical operations should be printed in Roman (*not* italic) type when they are not Greek.
 (c) Symbols for the chemical elements should always be printed in Roman type.
 (d) Abbreviations for words—as distinct from symbols for quantities—should be printed in Roman type and followed by a full stop.

Single capital letters used as abbreviations should be printed in small Roman type, so that they stand out in the text and are readily distinguished from symbols for the chemical elements.

Some engineering symbols and abbreviations used in the chemical industry are included in the list; in the selection of these, the Joint Committee's Report, the British Standard Engineering Symbols and Abbreviations (B.S. 560—1934), and current practice in the chemical industry have been taken into account.

It is hoped that teachers of chemistry and authors of chemical publications will adopt the standard symbols. Whilst abbreviations are convenient in technical publications, it is hoped that they will be used only where strictly necessary.

The Report includes the following sections:—The Greek Alphabet; Mathematics; Certain Important Physical Constants; Subscripts and other Modifying Signs; General Physics and Chemistry; Heat and Thermodynamics; Electricity and Magnetism; Optics; Units; Elements; Organic Radicals; General; Index.

Erratum: November issue, p. 825, l. 11, for "CE(C) 9509" read "CE(C) 9505."

ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

Food and Drugs

Evaluation of the Degree of Freshness of Hens' Eggs from the Fluorescence of the Egg-Shell, and from the Phosphate-Content of the Egg-White. J. Straub and W. J. Kabos. (*Chem. Weekblad*, 1938, 35, 739–741.)

—Egg-shells have normally a dull red fluorescence in filtered ultra-violet light, but on storage this gradually disappears and is eventually replaced by a blue colour. The initial intensity of the fluorescence varies considerably from one hen to another, and is associated with differences in the oöporphyrin-content of the shell; eggs from white Leghorns and crosses between these and Minorcas were used in the present experiments. The fluorescence of the eggs from individual hens is also liable to regular changes. The rapidity of the change on storage depends on the initial intensity of the fluorescence, but it is considered that an appreciable percentage of eggs having a blue fluorescence in a batch of eggs of similar age and origin indicate that the eggs are at least one month old; on the other hand, the absence of blue eggs does not necessarily indicate that the batch is fresh (*cf.* ANALYST, 1938, 51). The phosphate-content of egg-white is best determined by matching the blue

phosphomolybdenum compound in a Pulfrich photometer, after removal of protein turbidity by treatment with a carbonate and sulphite reagent (*cf.* A. Janke and L. Jirak, *Biochem. Z.*, 1934, **271**, 309). A series of experiments analogous to those described above showed that the inorganic phosphate-content of the egg-white increases regularly with the period of storage after the date of laying, the rate of increase being similar for all eggs from the same hen, although it differs for different hens. It is considered that a phosphate-content equivalent to more than 10 γ of P₂O₅ in 2 ml. of egg-white indicates that the egg is at least a month old. J. G.

Examination of Tomato Purées. E. L. Krugers Dagneaux. (*Chem. Weekblad*, 1938, **35**, 703-707).—Samples (25) of Dutch, Italian and Hungarian origin have been examined according to the following methods, and the results are presented in tabular form. Comments are made regarding the method of packing, taste, odour, colour, consistence, and the presence of moulds (as indicated by examination under the microscope), starch (iodine test), and artificial colouring matters. The dry solids were determined from the refractive index (n) at 20° C. (*cf.* K. Taxner, *Z. Unters. Lebensm.*, 1933, **65**, 220), the result being calculated (presumably as a percentage) from the formula $(n - 1.3300)/0.001875$. The ash-content was determined by drying and subsequently igniting a mixture of 2 g. of sample and 1 ml. of conc. sulphuric acid; the factor 0.9 was used to obtain the final result according to the Dutch official method. The dry solids were also obtained by drying a mixture of 5 g. of purée with a little alcohol and 25 g. of sand, and weighing the residue after 4 to 6 hours at 102° C., care being taken that no lumps were formed during the drying process. A more rapid, although less accurate, method is that of C. Grimme (*Pharm. Zentralhalle*, 1932, **75**, 483), in which a mixture of 10 g. of sample and 2 g. of potassium hydroxide is distilled with xylene and the volume of water in the distillate is measured. The American method, in which a thin layer of sample is dried in a dish for 4 hours at 70° C., in a vacuum (*cf. Methods of Analysis, Assoc. Off. Agric. Chem.*, 1930) is also recommended. The chloride-content was determined on the filtrate obtained from a mixture of 25 g. of sample and 250 ml. of water, by titration with silver nitrate according to a modified Volhard-Drechsel method; the same solution was used for a determination of the sp.gr. and thence of extract. The dry solids (a) obtained by the method of direct drying (see above) may be corrected for the total chloride (as sodium chloride) content (b), and the true tomato-solids content (d) obtained according to the expression, $d = 1.0169 (a - b)$. The natural salt-content (as distinct from added salt) is given by $0.1 (a - b)/5.9$. The sugar-content is determined by the copper-reduction method, before and after inversion, a suitable dilution of the filtrate from the extract determination being used. Since invert sugar is formed during the ripening process, the amount present in the purée is an indication of the quality of the fruit used; this is expressed in terms of the amount of invert sugar present in 100 parts of dry true tomato-solids, and this figure should exceed 50. The total acidity is determined by titration of 20 ml. of the above filtrate to phenolphthalein with 0.1 *N* sodium hydroxide solution (after removal of carbon dioxide by boiling), and the result is calculated as citric acid (see below); the volatile acidity is expressed as acetic acid. The main conclusions drawn from the

results are as follows:—(1) A product should be regarded as a true tomato purée only when it is prepared by the concentration of tomato pulp by evaporation. (2) The true, dry tomato-solids present should normally be at least 16 per cent. (or at least 28 and 36 per cent. for “double” and “triple” concentrated purées, respectively). (3) The sugar “safety factor” (see above) should exceed 50. (4) There should be between 7 and 11 parts of citric acid (obtained from the acidity determination, see above) in 100 parts of true, dry tomato-solids. (5) The percentage of volatile acid, calculated as acetic acid, multiplied by $d/100$ should exceed 1.

J. G.

Colorimetric Method for the Determination of Dissolved Oxygen in Beer. H. Rothchild and I. M. Stone. (*J. Inst. Brewing*, 1938, **44**, 425–431.)—For this determination the following reagents are used:—(a) Indigo disulphonate solution: The concentration of this is such that 1 ml. of dye solution in the volume used is equivalent to the colour produced by 1 p.p.m. of oxygen. The calculated weight of the purified assayed dye is triturated in a mortar with water, and the solution is made up to 100 ml. It must be freshly prepared every few days. (b) Sodium hydrosulphite solution: Approximately 2.5 g. of sodium hydrosulphite are dissolved in 50 ml. of water immediately before use. (c) Reduced indigo disulphonate solution: A portion of (a) in a small flask is covered with a one-inch layer of paraffin oil, and (b) is run in from a pipette with the tip below the oil until the solution becomes yellow; approximately 0.8 to 1.0 ml. of hydrosulphite solution per 50 ml. of dye are required. Immediately before use the reduced dye solution is titrated to a faint pale green colour with the unreduced dye. The apparatus consists of a glass bottle or large test-tube, on to which is wired a two-holed rubber stopper. Through the holes pass a short glass tube, flush with the under surface of the stopper and extending about 1 cm. above it, and a longer glass tube, which passes to the bottom of the bottle and is bent at right angles above the stopper. Rubber tubes are wired on to the glass tubing and held with screw clamps. The capacity of the bottle used by the authors was 135 ± 1 ml. In making a determination the bottle, from which the air has been displaced by carbon dioxide, is completely filled with beer, which is passed through it slowly for some time. The two clamps on the rubber tubes are successively closed, and the sample is kept cold until it is tested. A portion of the oil on (c) and then 5 ml. of the solution (c) are drawn up into a 5 ml. pipette. The clamp controlling the short tube is opened and one drop of dye is allowed to fall from the pipette, which is immediately inserted through the tubing. The second clamp (controlling the longer tube) is then opened and the dye solution (c) is forced into the beer, the flow being stopped before the paraffin oil reaches the tip (exactly 5 ml. are not required, as excess of dye is present if the beer contains less than 5 p.p.m. of oxygen). The clamps are then closed, the pipette is withdrawn, the beer and reagent are mixed by inverting the bottle several times, and the bottle is placed for an hour in a water-bath maintained at 25° C. After 10 minutes the clamp controlling the longer tube is opened and closed to release the pressure. The dissolved oxygen is determined by comparison with standards, the sample being viewed through water and the standards through untreated beer. The standards are prepared

to give 10 per cent. intervals (*e.g.* 2.0 p.p.m., 2.2 p.p.m., etc.) near the approximate oxygen-content of the sample and consist of varying quantities of the reagent (*a*) in water in a bottle similar to the test bottle. Stress is laid upon the point that daylight is necessary as a light source, and a yellow filter (*e.g.* yellow-tinted cellophane) aids the comparison. Results are concordant with those of the Winkler method on water and of an evolution method on beer. The dye used, indigo carmine, is neither oxidised nor reduced by beer within the limits of the test, and *pH* variations, sulphur dioxide, and yeast within the limits likely to be met have no effect on the determination. Typical results obtained in several breweries are tabulated, and variations possible in the oxygen-content of beer at different levels of a tank (*e.g.* top, middle and bottom) are shown. The method is simple, accurate, and may also be applicable to water; it is particularly suitable for waters of low oxygen-content.

E. B. D.

Progressive Hydrogenation of Groundnut and Sesame Oils. T. P. Hilditch, M. B. Ichaporia and H. Jaspersen. (*J. Soc. Chem. Ind.*, 1938, 57, 363–368.)—Detailed analyses of the fully saturated glycerides of arachis and sesame oils produced by hydrogenation to different stages have now been carried out as described for olive, tea-seed and cotton-seed oils (*ANALYST*, 1932, 57, 661; 1938, 63, 119). The “agitation” process was used in the presence of a small quantity of nickel-kieselguhr catalyst, and each oil was hydrogenated first to the stage at which almost all linolic glycerides had been selectively reduced to oleic and *iso*-oleic glycerides, and then to several further stages towards complete saturation. The data obtained show that groundnut and sesame oils conform to the “even distribution” rule, and the amount of tri- C_{18} glycerides was in each instance close to the possible minimum. The two oils were also hydrogenated by the “drip” continuous process, and similar data to those recorded for cotton-seed oil were obtained. The differences found in this method of hydrogenation, namely, the greater proportion of fully saturated glycerides at similar iodine values, and the smaller proportion of *iso*-oleic acid, together with the less selective hydrogenation of linolic glycerides, are regarded as entirely due to the mechanical conditions of flow which operate in the “drip” process. The investigation is illustrated by 10 tables of figures, including comparative tables for the two hydrogenation processes.

D. G. H.

Component Fatty Acids and Glycerides of the Body Fat of He-Goats. D. R. Dhingra and D. N. Sharma. (*J. Soc. Chem. Ind.*, 1938, 57, 369–370.)—The tallow used in the investigation was obtained from the back tissues of he-goats of Lahore by steaming and pressing. The fat had the following characteristics:— n_D^{40} , 1.4564; setting *p.*, 45.9° C.; saponification equiv., 282.0; iodine value (Wijs), 33.5; acid value, 2.1; unsaponifiable matter, 0.18 per cent. Fatty acids:—saponification equiv., 265.7; iodine value, 34.0; titre, 46.2° C.; solid acids, 58.1 per cent.; *iso*-oleic acid, 8.9 per cent. These figures closely resemble those for Swiss goat tallows (Pritzker and Jungkunz, *Pharm. Acta Helv.*, 1932, 594). The component fatty acids of the tallow were determined by ester fractionation as lauric, 4.7; myristic, 2.5; palmitic, 27.0; stearic, 26.8; arachidic, 2.1; oleic, 36.9 per cent. molar, showing that the goat tallow resembles mutton more closely than beef

tallow; similar differences were found in the milk-fats of goats, cows and sheep. The composition of the glycerides was studied by oxidation with potassium permanganate in acetone and subsequent separation of the fully saturated glycerides. The association ratio in the non-fully saturated glycerides was 0.92, and the limiting values for the molar percentages of mixed glycerides were deduced as (mol. per cent.) fully saturated 29.2; mono-unsaturated disaturated, 30.9 to 50.9; di-unsaturated mono-saturated 39.9 to 0 and tri-unsaturated 0 to 19.9. Fully-saturated glycerides were separated into simple glycerides by fractional crystallisation from dilute alcohol and acetone; and the m.p. and saponification equivalents of the fractions were determined. The fully-saturated glycerides (29.2 molar per cent.) are regarded as consisting of myristic, 2.9; palmitic, 48.5; stearic, 45.6; arachidic, 3.0 per cent.; the saturated unsaturated glycerides (70.8 molar per cent.) as lauric, 6.7; myristic, 2.4; palmitic, 18.1; stearic, 19.1; arachidic, 1.6; oleic, 52.1 per cent. While the percentages of the component fatty acids of the whole fat of goat tallow resemble those of the acids of mutton and beef tallows, the corresponding acids in the two fractionated parts of the glycerides differ to some extent. D. G. H.

Active Principle of Kurjan Seed (*Myrsine africana*). (*Bull. Imp. Inst.*, 1938, **36**, 319-322.)—"Kurjan seeds" obtained from Jigjiga, Abyssinia, have been identified at Kew Gardens as the fruit of *Myrsine africana*. From a sample received at the Imperial Institute, 4.8 per cent. of reddish-orange crystals and 1 per cent. of quercitol were isolated by the method previously used for an Indian sample (*cf.* Krishna and Varma, *J. Indian Chem. Soc.*, 1936, **13**, 115). The properties and reactions of these crystals were those of the embelic acid obtained by Kaul, Ray and Dutt (*cf.* *J. Indian Chem. Soc.*, 1929, **6**, 577). Comparison of the melting-points of the purified crystals (145-146° C.) and of a re-crystallised benzoyl derivative (97.5° C.) with those of embelic acid and its di-benzoyl derivative obtained by various chemists confirm the conclusion that the crystals are embelic acid. This acid occurs in the berries of *Embelia ribes* (Warden, *Pharm. J. Trans.*, 1888, [3], **19**, 305), and is considered responsible for their anthelmintic properties (*cf.* Hasan and Stedman, *J. Chem. Soc.*, 1931, 2112). The anthelmintic properties of "Kurjan seeds" are evidently due to embelic acid. The Krishna and Varma method of isolation of embelic acid and quercitol is described.

E. B. D.

Colour Test for *Cannabis sativa*. J. C. Munch. (*Amer. J. Pharm.*, 1938, **110**, 423.)—For the detection of *Cannabis sativa* in pharmaceutical preparations or tobacco, the sample is extracted with petroleum spirit of low b.p. or with acetone, the extract is evaporated to dryness, and the residue is treated first with a solution of *p*-dimethylaminobenzaldehyde and then with water. In the presence of *Cannabis sativa* a characteristic indigo-violet colour is obtained; this may be readily distinguished from the colour produced by certain ergot alkaloids. No other substance giving a similar reaction has been found.

Active Principles of Curare. P. de B. Carneiro. (*Compt. rend.*, 1938, **206**, 1202-1204.)—Samples of curare and of the extract of the bark of *Strychnos lethalis* were investigated by fractional precipitation with Bertrand's silicotungstic acid reagent (*cf.* ANALYST, 1899, **24**, 187). Two main silicotungstic fractions were

obtained by precipitation at pH 9.3 and pH 5.0, respectively. The corresponding bases isolated from the silicotungstic precipitates had the formulae $C_{22}H_{27}O_4N$ and $C_{25}H_{30}O_7N$. The first, named Strychnoethaline, is red, and the second, named Curaethaline, yellow. Each compound has a characteristic fluorescent spectrum. It is stated that the proportions of the bases varied in the different samples, but yields are not given. S. G. C.

The British Pharmacopoeia Test for Readily Carbonisable Substances.

I.—Phenacetin. J. L. Pinder and R. F. R. Venables. (*Pharm. J.*, 1938, **141**, 299.)—Fractional crystallisation of commercial phenacetin with alcohol yielded an impurity which was isolated as an orange-yellow liquid, $C_{11}H_{16}O_2N$, the merest trace of which gave an intense orange colour with conc. sulphuric acid. The British Pharmacopoeia test for readily carbonisable materials by admixture with conc. sulphuric acid detects the impurity at great dilution, as no measurable quantity is obtainable from phenacetin complying with the Pharmacopoeial limit; there is probably very much less than 0.04 per cent. in commercial phenacetin. It is possible for phenacetin to be contaminated with tarry matter without containing the specific impurity, and such contamination would not be detected by the Pharmacopoeial test for readily carbonisable impurities, so that the validity of the test is very considerably qualified. It is therefore suggested that the limit test "for readily carbonisable substances" be replaced by a more comprehensive limit for tarry matter, for which there would suffice a simple solubility test, such as dissolving 2.5 g. in 50 ml. of 95 per cent. alcohol by heating, cooling to $15^{\circ}C.$, and viewing in a Nessler cylinder; the solution should be colourless. E. M. P.

Detection of Small Amounts of Phenolphthalein in the Presence of Emodin and Chrysophanic Acid. E. H. Maechling. (*Ind. Eng. Chem., Anal. Ed.*, 1938, **10**, 586.)—The methods usually employed for the detection of phenolphthalein (*Assoc. Off. Agric. Chem., Methods of Analysis*, 1935, 4th Ed., p. 569; Palkin, *Ind. Eng. Chem.*, 1920, **12**, 766; Abst., *ANALYST*, 1920, **45**, 372) are not suitable for its detection in the presence of plant products containing polyhydroxyanthraquinone derivatives (Beal and Okey, *J. Amer. Chem. Soc.*, 1917, **39**, 716; Abst., *ANALYST*, 1917, **42**, 203; Hubbard, *Ind. Eng. Chem.*, 1917, **9**, 518; Abst., *ANALYST*, 1917, **42**, 277). These anthraquinones, *viz.* frangula-emodin (also present in *Cascara sagrada* and rhubarb), aloe-emodin (also present in senna) and particularly chrysophanic acid (Bailey, *Ind. Eng. Chem.*, 1914, **6**, 320; Abst., *ANALYST*, 1914, **39**, 353) exhibit, in alkaline solution, a variety of stable colours ranging from cherry-red to purple red and indistinguishable from the colour given by phenolphthalein (Warren, *Amer. J. Pharm.*, 1914, **86**, 444; Westman and Rowat, *J. Amer. Pharm. Assoc.*, 1918, **7**, 759). A solution of phenolphthalein in aqueous potassium hydroxide solution, when treated with excess of hydrogen peroxide, undergoes gradual oxidation, with formation of phthalic acid which may be extracted with ether from the acidified solution and identified by its m.p. and its reaction with resorcinol. Since some other constituents of plant material, as well as some organic acids, such as malic, citric, oxalic and succinic acids, respond to the resorcinol test under certain conditions, all material to be

tested must first be treated with excess of sodium bicarbonate solution and the phenolphthalein separated by extraction with ether. A suspension of 0.5 to 1 g. of the powder in 20 ml. of sodium bicarbonate solution is extracted three times with ether. The residue left after evaporation of the ether is dissolved in 20 ml. of 10 per cent. potassium hydroxide solution and oxidised by successive additions of 30 per cent. hydrogen peroxide with gentle heating. The purplish sediment of the alkaline salts of the anthraquinone derivatives is removed by filtration, and the filtrate is acidified with dilute sulphuric acid and extracted three times with ether. After evaporation of the ether the residue is heated with resorcinol, no condensing agent being necessary. The presence of 5 to 10% of phenolphthalein can be detected.

A. O. J.

Polarimetric Determination of Calcium Gluconate. I. Vintilescu, C. N. Ionesco and N. Stanciu. (*J. Pharm. Chim.*, 1938, **28**, 283-293.)—The specific rotatory power of calcium gluconate is very low, making its determination in dilute solution almost impossible by the polarimetric method. De Carli (*Annali di Chim. appl.*, 1931, **21**, 465) found that addition of bismuth nitrate increased the rotation, and Fisher and Bailey (*J. Assoc. Off. Agric. Chem.*, 1932, **15**, 461) observed a similar increase with uranyl acetate. Similar increases have now been found to occur on addition of sodium phosphotungstate, sodium tungstate, ammonium vanadate, sodium molybdate or ammonium molybdate. The biggest increase of all occurred with ammonium molybdate and acetic acid, and this is made the basis of a method of determination. Ten ml. of the solution of calcium gluconate are treated with 0.5 ml. of glacial acetic acid and 4.5 ml. of a saturated solution of ammonium molybdate. The rotation of this solution is measured in a 2-dm. tube at 20° C., the sodium D line being used. For concentrations less than 4 per cent., the rotation is strictly proportional to the concentration, which can be calculated from the expression:—

$$C = \frac{\alpha 100}{226.67 \times l}$$

where C is the concentration (in g. per 100 ml.) of the original calcium gluconate solution, the volume of acetic acid and molybdate reagent being ignored, and *l* is the length of the polarimeter tube in dm. The specific rotatory power of calcium gluconate under these conditions is 226.67°. When the concentration of calcium gluconate is greater than 4 per cent., the solution must be diluted. The method is very sensitive, for the angle of rotation obtained under the conditions specified is comparatively large. Thus a 2 per cent. solution gives a rotation of + 8.98°; without the molybdate the rotation is only 0.36°. F. A. R.

Biochemical

Rapid Procedure for Estimating the Tryptophane-Content of Casein. M. X. Sullivan, H. S. Milone and E. L. Everitt. (*J. Biol. Chem.*, 1938, **125**, 471-474.)—A method of estimating the tryptophane-content of casein has been based on the formation of a blue colour with *p*-dimethylaminobenzaldehyde in the presence of relatively strong hydrochloric acid. The time required for full

development of the colour, according to Holm and Greenbank (*J. Amer. Chem. Soc.*, 1923, **45**, 1788), is 7 to 9 days, and this is a serious disadvantage. It has now been found that with addition of dilute hydrogen peroxide solution the colour reaches its maximum within 30 minutes, and that the intensity of the colour is the same as in the longer method. The casein sample, weighing 0.1 g., is placed in a 250-ml. Erlenmeyer flask with a mixture of 99 ml. of 17.5 per cent. hydrochloric acid and 1 ml. of a 5 per cent. solution of *p*-dimethylaminobenzaldehyde solution in 10 per cent. sulphuric acid. The mixture is heated to 85° C. and maintained at that temperature for 15 minutes. Then 0.3 ml. of a 0.3 per cent. hydrogen peroxide solution is added, and the contents of the flask are well shaken, cooled and diluted to 100 ml. with water. The colour is measured after 30 minutes; it is stable for about 48 hours. Tryptophane could not be employed as a standard for this method owing to its instability in hot acid. Better results were obtained by using tryptophane dissolved with a mixture of other amino-acids; even so, some loss of tryptophane occurred. Several different varieties of casein were found to contain 2.4 per cent. of tryptophane.

F. A. R.

Simplified Estimation of Lactate in Normal Human Blood. H. T. Edwards. (*J. Biol. Chem.*, 1938, **125**, 571–583.)—Lactate in blood has hitherto been estimated by haemolysis in water, removal of the proteins by the method of Folin and Wu (*J. Biol. Chem.*, 1919, **38**, 81), treatment of the filtrate with copper sulphate and calcium hydroxide according to Van Slyke (*J. Biol. Chem.*, 1917, **32**, 455) and estimation of the lactate in the centrifugate by the aeration method of Friedemann, Cotonio and Shaffer (*J. Biol. Chem.*, 1927, **73**, 335; ANALYST, 1927, **52**, 418). Frequently a preliminary aeration is given prior to oxidation. A method has now been devised which normally renders unnecessary the treatment with copper sulphate and lime, the preliminary aeration, and the cleaning of the apparatus between runs. With some types of pathological blood, however, these precautions were found to be necessary.

The apparatus employed is a modification of that of Wendel (*J. Biol. Chem.*, 1933, **102**, 47; ANALYST, 1933, **58**, 712). It comprises a two-necked (ground-glass) 275-ml. reaction flask, a short condenser which fits into the larger of the two necks, and a tap-funnel which fits into the smaller neck. The condenser is made by fusing a test-tube into a slightly larger tube having a side-arm near the top, cold water being circulated through the inner tube. The side-arm is bent at right angles and passes through a rubber bung into a test-tube. The rubber bung also carries an absorption tower, 30 cm. long, which reaches to the bottom of the test-tube. The tap funnel is cylindrical and has a long stem which reaches to the bottom of the reaction flask. To the top of the reservoir of the funnel is sealed one end of a glass tube, the other end being sealed to the stem of the funnel just below the tap. This serves as a by-pass for air, which is drawn in through the top of the tap-funnel into the liquid in the reaction flask by applying suction at the top of the absorption tower. When whole blood is used, 1 ml. is pipetted into 45 ml. of water, and, after mixing, 2 ml. of $\frac{2}{3}$ N sulphuric acid are added, followed by 2 ml. of 10 per cent. sodium tungstate solution. With plasma, 1 ml. is pipetted into 17 ml. of water, and 1 ml. of $\frac{2}{3}$ N sulphuric acid and 1 ml. of 10 per cent. tungstate solu-

tion are added. With the fraction containing blood cells, 1 ml. is pipetted into 53 ml. of water, and 3 ml. of $\frac{2}{3} N$ sulphuric acid and 3 ml. of 10 per cent. tungstate solution are added. The solution is then centrifuged for 5 to 10 minutes at 3000 r.p.m. Five ml. of $10 N$ sulphuric acid, 10 ml. of 10 per cent. manganese sulphate solution and 50 ml. of water are introduced into the reaction flask, and an aliquot portion of the centrifugate is added from a pipette. About ten estimations can be made with one lot of reagents, it being unnecessary to clean out the apparatus before each one. One ml. of 5 per cent. sodium bisulphite solution is put into the test-tube. The reservoir of the tap-funnel is filled with $0.005 N$ potassium permanganate solution. Air is drawn at a brisk rate through the solution, which is heated to boiling by means of a micro-burner. The permanganate solution is added at the rate of 1 drop every 10 to 15 seconds, the addition being continued for 15 minutes. The burner is then removed, and the flow of air is stopped. The absorption tower is washed with four 2-ml. portions of water, and the washings are collected in the test-tube. The contents of the test-tube are titrated with $0.005 N$ iodine solution, starch solution being used as indicator. The bound bisulphite is then liberated by adding 5 g. of disodium phosphate (hydrate) ground to a powder, and the titration is continued. One ml. of $0.005 N$ iodine solution \equiv 0.225 mg. of lactic acid. One estimation can be completed in 25 minutes. The results obtained were in close agreement with those given by the method of Fletcher and Hopkins (*J. Physiol.*, 1907, **35**, 247), and the recovery of added lithium lactate was 96 to 102 per cent. of the theoretical value.

F. A. R.

Colorimetric Determination of Blood Glucose. M. S. Mihaéloff. (*J. Pharm. Chim.*, 1938, **28**, 293–296.)—The method is a modification of that of Folin and Wu (*J. Biol. Chem.*, 1919, **38**, 81). Exactly 0.5 ml. of the freshly-drawn blood is treated with a few mg. of sodium fluoride and 2 ml. of water to prevent haemolysis. The solution is treated with 1.25 ml. of potassium ferrocyanide solution (1.5 per cent. in a physiological saline) followed, after stirring, by 1.25 ml. of zinc sulphate solution (2 per cent. of the hydrate in saline). The mixture is placed on the steam-bath for 3 minutes and stirred. A precipitate of zinc ferrocyanide is formed and this adsorbs the proteins. The solution is filtered through a plug of asbestos, and the precipitate is washed with a little warm saline. The filtrate, which is clear and colourless, is cooled and diluted to 10 ml. with saline. Two ml. of this solution are transferred to a graduated 10-ml. tube containing 0.2 ml. of Fehling's solution. In a similar tube 2 ml. of a solution, made by diluting 0.5 ml. of 0.1 per cent. glucose solution to 10 ml., are added to 0.2 ml. of Fehling's solution. The two tubes are then placed in a boiling water-bath for 5 minutes, and, to prevent possible oxidation of the glucose by air, they are stoppered during the first minute of heating. The tubes are then immersed in cold water, and after cooling for 3 minutes, the contents of each tube are diluted to 5 ml. with phospho-molybdo-tungstate reagent. This is made by dissolving 1 g. of sodium tungstate and 7 g. of molybdic acid in 40 ml. of 5 per cent. sodium hydroxide solution, boiling for 30 minutes to expel ammonia and, after cooling, adding 25 ml. of 85 per cent. phosphoric acid; the solution is then diluted to 100 ml. with water. The cuprous oxide formed by reduction of the Fehling's solution

reduces molybdcic oxide, giving a blue colour which is proportional to the amount of glucose originally present. The colours of the solutions are compared in a Duboscq colorimeter.

F. A. R.

Nature of Vitamin A in Cod-Liver Oil. A. O. Tischer. (*J. Biol. Chem.*, 1938, **125**, 475-477.)—A concentrate of vitamin A, prepared by the molecular distillation of Norwegian medicinal cod-liver oil, was allowed to stand at room temperature for 10 days with maleic anhydride in benzene solution. From the reaction mixture a compound $C_{44}H_{64}O_8$ (m.p. 219-220° C.) was isolated. This was the maleic anhydride adduct of vitamin A palmitate, since the compound obtained in the same way from maleic anhydride and vitamin A alcohol esterified with palmityl chloride gave no depression of the melting-point when mixed with it. Vitamin A palmitate therefore exists in cod-liver oil.

F. A. R.

Estimation of the Vitamins B₁ and B₂ Contents of Foodstuffs, particularly of Fish and Fish Products. G. Lunde, H. Kringstad and A. Olsen. (*Skrifter Norske Videnskap-Akademi, Oslo*, 1938, No. 7.)—Vitamin B₁ was estimated biologically by the bradycardia method (*cf.* Birch and Harris, *Biochem. J.*, 1934, **28**, 602) and physico-chemically by the fluorometric method (*cf.* Jansen, *Rec. trav. chim. Pays-Bas*, 1936, **55**, 1046). Both gave identical results when synthetic aneurin was compared with the international standard adsorbate, 1 international unit being equivalent to 3 γ of aneurin. There was also close agreement between the results obtained by both methods for the vitamin B₁ contents of foodstuffs. To determine chemically the vitamin B₁ content of a foodstuff, 10 to 15 g. of the substance were heated on the water-bath for 15 minutes with 50 to 70 ml. of 96 per cent. alcohol, and the mixture was filtered. The residue was extracted twice more with 50 per cent. alcohol, and the combined extracts were concentrated to about 100 ml., acidified to pH 3 with hydrochloric acid, and extracted with ether. The aqueous solution was shaken for 1 to 2 hours with "frankonite" (a German bleaching earth) and the resulting adsorbate was filtered off and dried in a desiccator. The adsorbate was oxidised to thiochrome in the usual way. Lactoflavin was estimated biologically by a rat-growth method similar to that of Bourquin and Sherman (*J. Amer. Chem. Soc.*, 1931, **53**, 3501), and physico-chemically by direct measurement of the absorption of a purified aqueous solution. These two methods gave results in good agreement. Twenty to 50 g. of the substance to be tested, containing 50 to 60 γ of lactoflavin, were digested for 15 minutes with 5 volumes of boiling 96 per cent. alcohol, the extract was filtered, and the residue was extracted twice more with 70 per cent. alcohol. The combined extracts were concentrated to 100 ml., acidified with hydrochloric acid to pH 3 and extracted with ether. The aqueous solution was shaken for 1 hour with "phlorodin XX" or "frankonite," and the adsorbate was filtered off, washed with water and eluted with 50 ml. of a mixture of pyridine, methyl alcohol and water (1:2:3) by heating on the water-bath for 2 hours. The suspension was filtered, and the adsorbent was washed with 15 ml. of the pyridine mixture. Twenty ml. of the eluate were mixed with 2 ml. of glacial acetic acid and 2 ml. of saturated (6 per cent.) potassium permanganate solution and allowed to stand for 10 minutes. One ml. of 6 per cent. hydrogen peroxide solution was then added to decompose the remaining potassium

permanganate, and the solution was filtered. The absorption of this solution was measured in a Pulfrich photometer in a 3-cm. cell and with the use of a filter VS 45, which has a maximum transmission at $448m\mu$ (lactoflavin has an absorption maximum at $445m\mu$). With this filter pure lactoflavin gave $E \frac{100\gamma/\text{ml.}}{1 \text{ cm.}} = 3.03$

to 3.08. Some of the more important results obtained are shown in the following table:

Substance	Vitamin B ₁		Substance	Vitamin B ₁	
	I.U. per 100 g.	Lactoflavin γ per 100 g.		I.U. per 100 g.	Lactoflavin γ per 100 g.
Flesh of fish	10 to 50	150 to 660	Fish milt	40 to 70	300 to 600
Flesh of warm-blooded animals (except pig) ..	12	} 240 to 350	Ox- and pig-liver ..	100	3000
Pig flesh	180		Colostrum	20	275
Fish roe (except herring and brisling)	300 to 700	} 1000 to 1400	Potatoes	27	480
Roe of herring and brisling	Trace		Wheat germ	40	—
Fish liver (except herring)	60 to 130	600 to 1200	Bakers' yeast, moist ..	800	—
Herring liver	Trace	—	Bakers' yeast, moist ..	300 to 400	1480
			Preserved cod roes ..	200	650
			Fish liver paste ..	100	—
			Preserved beans, spinach, etc.	200 to 280	10 to 25

F. A. R.

Bacteriological

Use of Orange Juice in Cultivation of Anaerobic Bacilli. V. Cianci. (*Boll. de Soc. Ital. di Biol. Sperim.*, 1937, 12, 110-112; *Bull. Hyg.*, 1938, 13, 824.)—It is known that ascorbic acid aids in the promotion of anaerobic growth. The author demonstrates the favourable effect of orange juice; this is neutralised to $pH = 7.0$ with sodium hydroxide, buffered with disodium and monopotassium phosphates, and sterilised by filtration through a porcelain filter. The treated juice was added to broth tubes which had been boiled and cooled, and the tubes were inoculated at the bottom with a Pasteur pipette and incubated *aerobically*. It was found that by using 0.5 ml. of fresh orange juice in 5 to 6 ml. of broth satisfactory growth of one of the gas gangrene anaerobes and even of the tetanus bacillus was obtained. The orange juice became less effective after incubation at $37^\circ C.$ for 3 to 4 days and after autoclaving at a quarter of an atmosphere for 15 minutes.

D. R. W.

Stabilisation and Sterilisation of Biological Media by Supercentrifuging. M. Piettre. (*Compt. rend.*, 1938, 207, 97-99.)—Cultures prepared in liquid media of bacteria occurring in food and milk could be rendered perfectly clear when centrifuged at 45,000 r.p.m.; subsequent filtration through a Berkfeld filter effected sterilisation. Separation of microbic toxins could be obtained by simple supercentrifuging, but filtration was necessary to eliminate all micro-organisms. Yeasts proved difficult to separate from vegetable plasma and fruit juice. After being centrifuged at 23,000 r.p.m. the liquids remained slightly turbid, but when subsequently kept at $5^\circ C.$ rapid clarification occurred, owing to flocculation of colloidal material which carried down the remaining micro-organisms. Tables are given showing the composition of tomato and apple juice prepared by the centrifugal method.

S. G. C.

Method of Using Coal Gas in the Brown, Fildes and McIntosh or Other Anaerobe Jars on the Laidlow Principle. J. H. Brewer and J. H. Brown. (*J. Lab. and Clin. Med.*, 1938, 23, 870-874; *Bull. Hyg.*, 1938, 13, 824.)—Ordinary coal gas may be used for the McIntosh and Fildes anaerobic jar, provided that two precautions are taken. First, means must be taken to allow for the low pressure of gas usually obtainable in the laboratory, which is insufficient to introduce enough hydrogen to start its catalytic combustion; this is easily achieved by reducing the pressure in the jar to about 50 cm. of mercury. Secondly, the excessive amount of carbon dioxide that will result from the combustion of carbon monoxide, ethane and methane must be removed, since it may inhibit bacterial growth; this is easily done by placing a tube of soda-lime in the anaerobic jar.

D. R. W.

Simple Staining Method for the Detection of Ascospores in Yeasts. J. L. Shimwell. (*J. Inst. Brewing*, 1938, 44, 472.)—In the systematic study of yeasts it is necessary to determine whether ascospores are formed or not. Some species sporulate readily and some only with difficulty; it is with the latter kind that one needs to stain for spores because of their sparsity in the culture and lack of visual contrast. Prolonged staining in hot carbol fuchsin with subsequent decolorisation in dilute acid has been largely superseded by staining in malachite green (5 per cent.) with gentle warming and subsequent washing with tap water; usually the spores appear emerald green, whilst the remainder of the cell and vegetative cells remain colourless. In some instances, however (notably with the *S. cerevisiae* strains), the vegetative cells, as well as the spores, tend to retain the stain. If, however, after being washed under the tap the film is rinsed with rectified spirit for 30 seconds, only spores retain the stain, and a single sporulating cell may be instantly picked out in a crowded microscope field. The following procedure is recommended:—“Prepare a cover-glass film in the usual way, fix gently in a flame and flood with 5 per cent. aqueous malachite green for about one to three minutes, warming gently to steaming several times; (2) Wash off the stain under the tap and flood again with rectified spirit for about 30 seconds. Wash again, mount and examine under the microscope.”

D. R. W.

Oxalic Acid Fermentation : Mechanism of Oxalicogenesis by Moulds.

R. Jacquot. (*Ann. des Ferm.*, 1938, 4, 284-294; *J. Inst. Brewing*, 1938, 44, 444.)—This paper records an investigation to discover the responsible factor or factors determining the formation of oxalic acid by certain moulds. The moulds studied were *Sterigmatocystis nigra*, *Penicillium solitum* (Westing) and *Aspergillus oryzae* (Ahlb.), and the medium employed was Czapek's mineral salt solution with the addition of glucose and a single source of nitrogen. It was found that oxalic acid is not a normal and obligatory product of metabolism but occurs under certain conditions of culture only. Neither the nature, nor the amount, nor the lack of balance of the ternary nutriment, nor the ageing of cultures has any effect; the production of oxalic acid is entirely due to the pH of the substrate. When potassium nitrate is used as the source of nitrogen a base is liberated, the medium becomes alkaline, and oxalic acid is produced to neutralise the alkalinity; conversely, when the source of nitrogen is ammonium sulphate acid is formed and no oxalic acid is

produced. Oxalicogenesis can be provoked by the addition of alkali or prevented by the addition of acid and can be regarded as a natural reaction against a toxic alkalinity. Different organisms are affected at different pH values and have limiting values above which production of oxalic acid will occur. For *Sterigmatacystis* this is 5.8 to 6.5, for *Penicillium* 7.5 to 7.8, and for *Aspergillus* 8 to 8.5. The production of oxalic acid is accompanied by marked diminution of growth and increase of production of carbon dioxide. The carbon dioxide is derived from the transformation of the sugar into oxalic acid in accordance with Schoen's hypothesis postulating the intermediate formation of glycollic acid together with two molecules of carbon dioxide.

D. R. W.

Agricultural

Further Examples of Rotenone Determinations on Derris, Timbo and Barbasco. W. M. Seaber. (*J. Soc. Chem. Ind.*, 1938, 57, 372.)—The cold chloroform process (*J. Soc. Chem. Ind.*, 1937, 56, 168T; Abst., ANALYST, 1937, 62, 630) and the 6-hour carbon tetrachloride extraction process (Seil, Putt and Rusby) were compared by making series of determinations on derris, timbo, barbasco and cubé. The latter method was carried out by extracting 50 g. of the fine powder (passing 60 mesh at least) with carbon tetrachloride for 6 hours in a Soxhlet apparatus after adding 0.34 g. of rotenone carbon tetrachloride complex. The carbon tetrachloride was distilled off until 40 ml. remained; this residue was dealt with as usual. The chloroform process almost invariably gave higher results for derris, and generally lower results for timbo and barbasco, whilst with cubé results tended to be a little higher. Where results were lower it appeared to be due to extraction of larger quantities of resins which prevent crystallisation. With timbo higher results are obtained with short extractions than with longer ones, apparently owing to actual decomposition of rotenone, and in this respect timbo seems more sensitive than derris. Probably the highest results are always nearer the truth, so that for commercial purposes derris is assayed by the cold chloroform process and other roots by both processes.

D. G. H.

Organic

Separation, by Distillation with Acetic Acid, of the Aromatic Hydrocarbons from the Fraction of a Petroleum Boiling between 154° and 162° C. F. W. Rose, Junr. and J. D. White. (*U.S. Dept. Commerce Research Paper*, R. P. 1123, pp. 167–184.)—Petroleum fractions boiling within narrow limits, are separated by distillation with an azeotropic agent, acetic acid, into concentrates containing (1) a mixture of paraffins and naphthenes and (2) the aromatic hydrocarbons. This procedure simplifies the subsequent separation of the individual components, and can be applied to petroleum fractions boiling in the range 130° to 175° C. Data are given on the behaviour of synthetic mixtures of *n*-nonane and isopropylbenzene with acetic acid. Except for about 10 per cent. of intermediate material, the separation of these mixtures in a 30-plate glass column is roughly quantitative. The systematic distillation with acetic acid of the fraction of an Oklahoma

petroleum boiling normally between 154° and 162° C. resulted in a paraffin-naphthene mixture and an aromatic concentrate. Traces of aromatic hydrocarbons were removed from the paraffin-naphthene mixture by adsorption on silica gel. Re-distillation of the aromatic concentrate with acetic acid removed all but the aromatic hydrocarbons. By proper re-cycling of the intermediate fractions, only about 1 per cent. of the entire fraction remained not allocated to the concentrates. Distillation of the paraffin-naphthene mixture as oil at 215 mm. of mercury showed (1) paraffinic constituents boiling near 157° C., and (2) naphthenic constituents boiling near 161° C. On distillation of the aromatic concentrate, the major portion of the distillate had a boiling range of 158.5° to 164.5° C., about 5° higher than that of the material from which it came. Apparently this material contains *n*-propylbenzene and the methylethylbenzenes as well as higher boiling trimethylbenzenes. The detailed results obtained with the petroleum fraction are given in a table.

E. M. P.

Characteristics of Some Antarctic Whale Oils Obtained from Various Parts of the Animals. S. Ueno and M. Iwai. (*J. Soc. Chem. Japan*, 1938, 41, 297–298B.)—Analytical data are recorded for oils obtained from various parts of (a) a male blue whale 45 feet long, and (b) a male humpback whale 80 feet long. (a) *Blue whale*.—The oils examined were obtained from the fatty layer, back skin, flesh, mount skin, ridge, head skin, lip skin and entrails. The acid values varied only between 0.31 and 0.68; the saponification values between 195.2 and 198.3; the iodine value (except for the oil from the fatty layer, which had a value of 148.9) between 122.6 and 134.2; the n_D^{40} , between 1.4650 and 1.4690 (flesh oil 1.4690), and the unsaponifiable matter between 0.68 and 1.32 per cent. (b) *Humpback whale*.—The oils were obtained from the ridge, bone of body, tail, tail flesh, tongue, mount skin, belly skin, back skin, head skin, entrails fat, bone, head bone, neck skin, lip skin and “meka.” The iodine value (Wijs) diminished in the order named from 129.5 for ridge fat to 94.9 for lip skin fat, and that of the “meka” fat was 64.6. The n_D^{40} diminished from 1.4660 to 1.4619, and the value for the “meka” fat was 1.4575. Only with the tail fat, lip skin and “meka” fat did the saponification value reach 200. Acid values were between 0.17 and 0.65.

D. G. H.

Separation of Hard Lac Resin by Cold Polymerisation and Fractional Precipitation. M. Venugopalan and H. K. Sen. (*J. Soc. Chem. Ind.*, 1938, 57, 371.)—(a) *Urea Polymerisation Process*.—Seven to eight per cent. of finely powdered urea (on the weight of lac) are added to a 40 per cent. solution of shellac or seed lac in acetone, made at room temperature, or with slight warming, and the mixture is stirred until the urea is dissolved, after which the vessel is closed airtight and kept at room temperature (25–30° C.) for 4 days. The hard jelly-like mass is then broken up in a porcelain mortar and extracted with acetone until the extracting liquid is practically colourless. The soft resin portion of shellac, not polymerised by urea, is extracted by the acetone in this process, and the hard polymerised resin is left in the extractor. In order to render the hard resin soluble in alcohol it is boiled with water several times for 30 minutes each time, with frequent changes of water, after which it will have become completely depolymerised, and practically all the urea will have dissolved and can be recovered.

The hard resin is then dried at room temperature or in a vacuum oven; it has all the properties of a pure hard resin prepared by other methods.

(b) *Fractional Precipitation Method*.—A weighed quantity of shellac or lac is dissolved in acetone or in acetone-benzene, and diluted with acetone until the concentration of lac is about 15 to 20 per cent. The vessel is kept cooled with ice, and the solution is stirred for about 30 minutes between 0° and 5° C. The gelled mass is squeezed through fine muslin to separate the hard and soft resins, and a second washing of the gelled hard resin is made with cold acetone to obtain the maximum separation. The hard resin is boiled with water, separated and dried. The colour and original properties of the hard resin are superior to those of resins obtained by the hot extraction process, and samples stored for 2 years showed no deterioration or alteration.

D. G. H.

Inorganic

Relative Value of Certain Azo Derivatives of 8-Hydroxyquinoline as Analytical Reagents. T. Boyd, E. F. Degering and R. N. Shreeve. (*Ind. Eng. Chem., Anal. Ed.*, 1938, 10, 606-608.)—An investigation begun by Gutzeit and Monnier (*Helv. Chim. Acta.*, 1933, 16, 233, 478) on the development of specific spot-test reagents has been continued, the reagents investigated being 18 azo derivatives of 8-hydroxyquinoline. The test solution contained 3 mg. of metallic radical per ml., the solvent was 20 per cent. nitric acid (chlorine free) or hydrochloric acid or *aqua-regia*, and the reagent was a saturated solution of the dye in 95 per cent. alcohol. In the paper test one drop of the alcoholic solution of the reagent is placed on filter-paper and treated with a drop of the test solution. The spot-plate test is made by adding a drop of the test solution to two drops of the reagent in the depression of a spot plate. The following new tests for mercury were developed. With 5-(3-hydroxyphenylazo)-8-hydroxyquinoline, mercury gives a violet-red paper stain destroyed by hydrochloric acid. On a spot-plate a "red-violet, shade two" precipitate soluble in hydrochloric acid forms immediately. Palladium gives similar reactions, but the stain and precipitate are insoluble in hydrochloric acid. (The colour descriptions refer to Mulliken's *Identification of Pure Organic Compounds* (Colour Charts), New York, John Wiley & Sons, 1916.) With 5-(3-chlorophenylazo)-8-hydroxyquinoline, mercury gives a "red-violet, tint two" on paper and an "orange-red, tint one" colour and a "violet-red, tint two" precipitate on the plate, all distinguished from the palladium reactions by solubility in hydrochloric acid. With 5-(4-chlorophenylazo)-8-hydroxyquinoline mercury gives on paper an "orange-red, tint two" spot with a fugitive ring of the same colour and on the plate an "orange-red, tint one" shade destroyed by hydrochloric acid. The reactions with palladium are easily distinguished. With 5-(3-tolylazo)-8-hydroxyquinoline a "violet-red" spot forms on paper and, on the plate, a "red, shade two" colour and a precipitate settles out. Solubility in hydrochloric acid distinguishes these reactions from those of palladium. With 5-(4-arsonophenylazo)-8-hydroxyquinoline, mercury forms an "orange, tint one" ring destroyed by hydrochloric acid on paper, and an "orange-red" precipitate soluble in hydrochloric acid slowly settles from the plate test. This test is specific

for mercury. Copper may interfere with it. With 5-(8-hydroxy-3,6-disulpho-1-naphthylazo)-8-hydroxyquinoline a "red-violet, tint one" spot forms on paper and a "violet-red" colour and precipitate on the plate, all destroyed by hydrochloric acid. Palladium gives the following reactions:—With 5-(2-hydroxyphenylazo)-8-hydroxyquinoline, an "olive-green" spot changing to an olive-green ring encircling a pink spot on paper. On standing, the ring becomes purple. On the plate, a brownish-grey precipitate settles out from a "violet-red, tint one" liquid. Hydrochloric acid dissolves some of the precipitate and the mother liquor becomes orange. With 5-(2-chlorophenylazo)-8-hydroxyquinoline, an "orange, shade one" spot forms on the plate with a brown precipitate which becomes violet or purple on standing and is somewhat soluble in hydrochloric acid. With 5-(3-chlorophenylazo)-8-hydroxyquinoline, a "red, tint one" spot from which a purple precipitate settles forms on the plate. Hydrochloric acid causes the colour of the precipitate to change to brown. The presence of tartrate ion does not affect the reaction. With 5-(benzidinemonoazo)-8-hydroxyquinoline, a "yellow-orange, shade one" spot forms immediately on the plate and a brown precipitate deposits on standing. Tartrate ion retards the test. This reagent serves also as a test for vanadium pentoxide, the vanadyl ion and the metavanadate ion, which give a very brilliant "yellow-green, tint one" spot on paper and a distinctive "yellow, shade one" colour on the plate. In practically all the reactions investigated the presence of chloride ion in the test solution obscures the results, but tartrate ion has little effect. The statement of Gutzeit and Monnier (*loc. cit.*), *viz.* that polar groupings para to the point of coupling in the aromatic nucleus cause a more intense reaction, should be modified so as to include only those groupings (*e.g.* hydroxyl, amino, sulpho, carboxyl) which tend to increase solubility in a semipolar or polar solvent.

A. O. J.

Determination of Mercury with *s*-Diphenylcarbazide. F. W. Laird and S. A. Smith (*Ind. Eng. Chem., Anal. Ed.*, 1938, **10**, 576–578.)—The conditions influencing the accuracy of the colorimetric determination of mercury by means of *s*-diphenylcarbazide have been investigated. The colour was measured by interposing the solution in a 10-inch cell between the source of light and one of two electrically opposed Weston photronic cells balanced by means of a variable resistance. The alteration in the resistance necessary to restore the balance, as indicated by a galvanometer, is a measure of the light absorption. Measurements of *pH* were made with a glass electrode. Standard solutions were prepared by dissolving pure mercuric chloride, sulphate or nitrate in triple-distilled water, and the reagent was a 1 per cent. (approximately saturated) solution of diphenylcarbazide in absolute alcohol freshly prepared each day. The colour reaction was found to be independent of the amount of reagent used, provided that the ratio of reagent to mercury was not less than 2 to 1. This suggests that the coloured product is the inner metal complex (*i.e.* a carbazide) of Feigl and Lederer (*Monatsh.* 1924, **45**, 115) and not a carbazone as suggested by Cazeneuve (*Bull. Soc. Chim.*, 1900, **23**, 492, 701). The order of mixing and the stirring have no effect upon the rate of formation of the colour, which attains its maximum intensity in 15 minutes, and, unless unduly exposed to atmospheric oxidation, is stable for several hours.

Stock and Pohland (*Z. angew. Chem.*, 1926, **39**, 791) report that zinc, iron, cobalt, nickel, lead, copper, silver, gold, cyanide, bromide and iodide ions (the last three may be present in small amount) interfere with the reaction, and that sodium, potassium, ammonium, magnesium, calcium, strontium, barium, aluminium, manganese, fluoride and chloride ions (the last two in low concentration) do not interfere. These statements were confirmed, with some exceptions. Zinc interferes only when its concentration is not less than five times that of the mercury. Cadmium does not interfere. Chloride ions have a very destructive effect, a concentration greater than 0.0001 *N* causing decomposition of the coloured compound into a colourless soluble product. The conclusion of Majer (*Z. anal. Chem.*, 1932, **87**, 352), *viz.* that ammonium ions have a destructive effect, was confirmed, but since he used ammonium chloride, the great effect he attributed to the ammonium ion was due partly to the chloride ion. The chromate ion interferes with the reaction by forming a violet compound with the reagent. High concentration of electrolyte tends to cause precipitation of the coloured complex, the sulphates and nitrates of the alkalis causing precipitation at concentrations of 0.003 to 0.004 *N*. Precipitation tends to occur when the *pH* is high. When the *pH* exceeds 6 a precipitate frequently appears even in the absence of electrolyte. The higher the concentration of mercury, the smaller the concentration of electrolyte necessary to induce flocculation. Above *pH* 7 the reagent acts as an indicator, and below *pH* 2.6 the solution is decolorised. Attempts to buffer the solution with urea, sodium acetate or acid potassium phthalate failed. The optimum *pH* range was found to be 3.5 to 4.5, and this may be secured by titration of an aliquot portion of the solution with dilute acetic acid or sodium acetate, with bromophenol blue as indicator, or with a glass electrode. The addition of these substances does not interfere with the colour reaction unless the total electrolyte concentration exceeds the salting-out value. Under the stated experimental conditions with a *pH* of 3.5 to 4.5 (the value being maintained within 0.3 *pH* unit for a series of comparisons) the reagent will indicate as little as 0.4 mg. of mercury in a litre of solution with a precision of about 5 per cent. For quantities of 5 to 50 γ per 10 ml. the average precision is about 3 per cent.

A. O. J.

Determination of Antimony in High-Lead Mixtures by the Permanganate Method. R. G. Myers. (*Philippine J. Sci.*, 1938, **66**, 75-92.)—Tests were carried out with a process closely similar to that previously described (*id.*, 1937, **64**, 365; *Abst.*, *ANALYST*, 1938, 623) to determine the effect of foreign metals in amounts up to 1 per cent. of the antimony present. Arsenic produces high results; each mg. of arsenic corresponds with 1.66 mg. of antimony, giving a correction factor which may be deducted from the apparent weight of antimony when the amount of arsenic present is known. Vanadium tends to cause low results, the percentage error in the antimony result varying from 0.6 to 6.4 in the presence of up to 1 per cent. of vanadium. Iron also produced slightly low results; the effect was not proportional to the amount of iron present, the error being fairly constant at about 2 per cent.; it is therefore considered that, with small amounts of iron present, the antimony result should be multiplied by 1.02. The effects of arsenic and iron are additive. In presence of tin the error due to iron was reduced,

a correction for iron being then unnecessary. Although the influence of bismuth, copper, cadmium and zinc were not investigated, it appeared probable that these metals, when present in small amounts, would have no effect on the antimony determination.

S. G. C.

Gravimetric Determination of Manganese as Sulphate. **G. Brinkmann and W. Schmedding.** (*Z. anal. Chem.*, 1938, **114**, 161–170.)—On account of the hygroscopicity of anhydrous manganous sulphate, the authors recommend weighing the monohydrate. The manganese solution is evaporated with a few drops of sulphuric acid on a sand-bath, and the excess of acid is expelled at 250° C. The residue is heated for 20 to 30 minutes at 500° C. in an electric oven, cooled, moistened with water, dried at about 100° C., and heated in an air-oven at 110° C. to constant weight (about 30 minutes). The manganese factor of $\text{MnSO}_4 \cdot \text{H}_2\text{O}$ is 0.3251.

W. R. S.

Determination of Iron in Metallic Mercury. **A. Castiglioni.** (*Z. anal. Chem.*, 1938, **114**, 257–260.)—Fifty g. of mercury are dissolved in 80 ml. of cold nitric acid (2:1 water). When solution is complete, the beaker is kept on a steam-bath for 30 minutes, the solution is brought to incipient boiling, removed from the flame, and treated with excess of pyridine. If the amount of iron is very small, no immediate precipitation takes place, but the liquid assumes a yellow colour. It is allowed to stand until the precipitate settles and the liquid becomes clear and colourless. The precipitate is collected and dissolved in nitric acid, and the iron is determined either gravimetrically or colorimetrically.

W. R. S.

Oxidation of Tervalent Chromium by Alkaline Mercuric Oxide. **P. Raychaudhury.** (*J. Indian Chem. Soc.*, 1938, **15**, 317.)—An excess of mercuric chloride is added to a solution of a chromic salt, and the liquid is rendered alkaline with sodium hydroxide solution and boiled for about 13 minutes. Chromium is thus completely converted into chromate, mercuric oxide being reduced to metallic mercury. The chromate may be determined in the filtrate from the precipitate. Only the freshly precipitated mercuric oxide actively oxidises the chromium; commercial red mercuric oxide was found to be non-reactive in this respect.

S. G. C.

Photometric Determination of Tungsten in Steel. **G. Bogatzki.** (*Z. anal. Chem.*, 1938, **114**, 170–181.)—The colour reaction of tungsten with hydroquinone in strong sulphuric acid forms the basis of the process. Iron and molybdenum do not interfere after reduction with stannous chloride, but the process cannot be applied to steels containing titanium, as this reacts like tungsten. The steel (0.5 g.) is dissolved in a graduated 100-ml. flask in 25 ml. of a mixture of sulphuric and phosphoric acids by warming (40 per cent. of H_3PO_4 and 12 per cent. of H_2SO_4 by volume). The solution is oxidised by dropwise addition of nitric acid, and evaporated until white fumes appear. After cooling, 50 ml. of water are added, and the solution is allowed to cool completely. Five ml. of 20 per cent. stannous chloride are added, the volume is adjusted to 100 ml., and 2 ml. are pipetted into a small flask. Twenty ml. of a freshly prepared 10 per cent. solution of hydroquinone in strong sulphuric acid are pipetted into the flask during

agitation. After standing for a few minutes, the cold solution is matched in a Pulfrich photometer against water and filter S57. If the steel contains chromium, a deduction of 0.01 per cent. of tungsten for each 1 per cent. of chromium is made.

W. R. S.

Determination of Iodine in Drinking Water, Urine and Substances Containing only about 1000 Times as Much Organic Matter as Iodine.

A. C. Bratton, J. F. McClendon, W. Foster and R. White. (*Ind. Eng. Chem., Anal. Ed.*, 1938, **10**, 600–605).—The material is fused with sodium hydroxide in a nickel crucible, dissolved in water with a few mg. of sodium azide (to destroy nitrite and reduce iodate), transferred to a micro-still provided with a watertrap in the neck, and its volume reduced to 3 ml. by boiling. After the addition of 3 ml. of 21 *N* sulphuric acid and 0.2 ml. of 4 per cent. ferric sulphate solution the liquid is distilled into a receiver containing 2 ml. of bromine water. When fumes of sulphur trioxide appear in the flask the flame is reduced, and the spray-trap in the neck is heated with a large flame until all the water has been driven into the condenser. Distillation is then continued for 4 minutes, after which the condenser outlet is washed into the receiver. Bromine is removed from the distillate by aeration at 100° C. The iodate formed in the distillate by oxidation of iodine with bromine is determined by the addition of 1 ml. of 0.1 per cent. potassium iodide solution and electrometric titration of the liberated iodine with 0.001 *N* sodium thiosulphate solution. A blank determination is made with all the reagents used. The method is not applicable to ordinary tissues of land or fresh-water organisms, but is applicable to samples containing only mg. amounts of organic matter, chlorides or silicates and at least 0.2 γ of iodine. It is therefore applicable to nearly all organic compounds containing iodine as well as to thyroid gland, seaweed, sponge and other tissues containing relatively high percentages of organic iodine compounds. Although moderate amounts of chloride (or even bromide) do not interfere with the determination, amounts of more than 0.5 millimole of chloride ion cause too great a concentration of hydrochloric acid in the distillate. The method is not applicable to sea water, brine or even some mineral waters, but it is possible to determine the iodine in less than a litre of drinking water since the chloride-content of this is usually low. The presence of silica renders the method inapplicable to soil, but a soil solution may be used provided the chloride-content does not exceed 0.5 millimole. Thyroid gland or marine animal and plant tissues are fused with alkali and 2 mg. of mixed rare earth oxides, and are transferred to the still with a few ml. of sodium azide solution (500 mg. per 100 ml.) and water. Urine (5 ml.) containing 0.2 γ of iodine (*e.g.* from a subject taking iodised salt) may be analysed after destruction of urea by fusion with sodium hydroxide and rare earth oxides until ammonia ceases to be evolved. Natural water samples are made alkaline with sodium hydroxide, sodium azide is added, and the residue left after evaporation is fused with a trace of mixed rare earth oxides. Chlorinated water is evaporated and fused before addition of azide. Experiments with water and urine containing known amounts of iodine showed that the method is accurate to within 2 per cent.

A. O. J.

Microchemical

Centrifuging Apparatus for Micro-Gravimetric Analysis. A. Langer. (*Mikrochimica Acta*, 1938, 3, 247-252.)—An apparatus is described for centrifuging in gravimetric analysis; it may be constructed in different sizes for micro and for semi-micro work. It consists of three parts, the first a pear-shaped glass portion for the preliminary operations and precipitation. This reaction vessel has two openings: a narrow one underneath corresponding to the stalk of the pear, and another, rather wider, at one side of the top or widest portion of the pear. This apparatus will lie on its side so that when it is half full of liquid none escapes through the openings. The sample is weighed into this vessel, which normally has a capacity of 10 to 12 ml., from the usual micro weighing bottle, and solution and precipitation are carried out under the desired conditions. The vessel may be heated, if necessary, on a gas or electrically heated block which is bored with a hole of suitable shape to hold the vessel without its contents being spilled. When the precipitate is ready to be centrifuged the neck of the pear-shaped vessel is fitted into a platinum Neubauer crucible. The crucible in its turn is fitted into a glass receiver vessel by means of a rubber washer and ebonite stand, which also supports the reaction vessel. When assembled the three parts of the apparatus fit into an outer metal centrifuge tube and are held in position with rubber rings. The apparatus is so designed that the Neubauer crucible is never more than half full. To ensure complete transference of precipitate to the platinum Neubauer crucible, in which it is finally to be weighed, it is advisable to rub down the sides of the reaction vessel with a feather during each washing, and finally to wash them with a little alcohol. Centrifuging of a barium sulphate precipitate is complete in a few minutes at 2000-3000 r.p.m., and good results are quoted for the determination of sulphate on samples of copper sulphate weighing from 3 to 16 mg.

J. W. M.

Detection of o-Dioxo- and Oxo-Methylene Compounds. M. Ishidate. (*Mikrochimica Acta*, 1938, 3, 283-290.)—The test described reverses the well-known method of detecting nickel by means of dioximes. The 1,2-dioxo compounds are converted into the oxime which is identified by the addition of a nickel salt. A drop of the alcoholic test solution is sealed up in a capillary tube with a little selenious acid and heated for 20 minutes at 150° to 170° C. After cooling, the capillary tube is opened, and the contents are transferred to a micro-centrifuge tube and heated with 2 drops of hydroxylamine solution to reduce any excess of selenious acid to selenium; this is then centrifuged to the bottom with a little animal charcoal. A drop of the supernatant liquid is placed on filter-paper and treated with a drop of a 5 per cent. solution of nickel acetate, when a yellow or red fleck indicates that the test is positive. The limit of identification varies from 0.5 to 50 γ , and the limit of dilution from 1: 10,000 to 1: 500. The test is successful except for dioxo compounds of quinonoid structure, such as phenanthrene quinone. These substances are reduced by the hydroxylamine and consequently oxime is not formed.

J. W. M.

Microchemical Reaction for the Alkaloids of *Atropa belladonna*.
F. Amelink. (*Pharm. Weekblad*, 1938, **75**, 1196–1199.)—Since preparations of the root of *Atropa belladonna bulgarica* are now used for the treatment of post-cephalic Parkinson's disease, it is desirable to compare the reactions of the alkaloids present with those from the leaves. In the author's method (for details of the technique used, see *Schema zur Mikrochemischen Identifikation von Alkaloiden*, p. 3) the sample is made alkaline with ammonia and extracted with chloroform. The residue left on evaporation of a drop of the extract on a microscope slide, in the air, is dissolved in 1 drop of 4 *N* hydrochloric acid, and 1 drop of a solution of hydriodic acid (sp.gr. 1.7) is added. Fine crystals are formed around the edge of the drop, probably because precipitation of colloidal matter by the reagent results in the optimum concentration in this region. If an excess of reagent is avoided a colourless background should be obtained, but, if not, the drop is yellow and the crystals are very small (*e.g.* 1 to 3 μ), although very characteristic; these crystals are illustrated by photomicrographs. The normal range of dimensions of the crystals is between 2 and 25 μ , most of them being about 10 μ . The results obtained with Bouchardat's reagent (*i.e.* a solution containing 1 part of iodine and 2 parts of potassium iodide in 20 parts of water) are also described. With *Extr. Rad. Belladonnae*, the alkaloid-content of which is given as 0.2 per cent. for the Bulgarian grade and 0.08 per cent. for the W. European grade, 1 drop of reagent is added directly to a mixture of 1 drop each of the sample and *N* hydrochloric acid. If the mixture is then warmed rapidly sphenoids (*i.e.* crystals or half-crystals in a hemihedral form) are produced at the edges of a colourless drop; this is a characteristic reaction. In their simplest form the crystals are diamond-shaped, and as they frequently occur in pairs which may be in contact either at an angle or along one of the sides, these appear as "hour-glasses" or diamonds, respectively; they are illustrated photographically, under a magnification of $\times 300$, in the original paper. With *Radix Belladonnae*, a fragment of the sample, about the size of a pea, is warmed with 10 drops of *N* hydrochloric acid, and 1 drop of the extract is treated as described above; the crystals usually appear blue, owing to the action of the iodine (from the reagent) on the starch present. With *Folia Belladonnae*, 1 mg. of the powdered leaves is treated in a similar way, and the extract is tested after filtration, while *Tinctura Foliorum Belladonnae* or *Extr. Foliorum Belladonnae* may be mixed directly with the acid, and the above procedure is then followed. The results show that there is no appreciable difference either qualitative or quantitative (*i.e.* in sensitiveness? ABTRACTOR) between the reactions given by preparations from the Bulgarian and W. European roots; and that there is no difference between the reaction given by different portions of the same plant (*e.g.* the leaves and roots). The crystals are larger and better defined, however, when the reaction is applied to extracts of the root, probably because there is less of the interfering substances present.
J.G.

Micro-Gravimetric Determination of Cobalt with α -Nitroso- β -Naphthol. **F. Hecht and F. Korkisch.** (*Mikrochimica Acta*, 1938, **3**, 313–316.)—The precipitation of cobalt with α -nitroso- β -naphthol, as recommended by Meyr and Feigl for the macro scale (*Z. anal. Chem.*, 1932, **90**, 15; Abst., ANALYST,

1932, 57, 739), gives very accurate results on the micro scale with solutions of pure cobalt salts, but in the presence of nickel, zinc or aluminium errors varying from 1 to 5 per cent. occur. Where errors of this magnitude can be tolerated, as in the determination of minute amounts of cobalt in certain minerals, the micro method may be useful. For the determination of cobalt in the presence of other elements the cobalt and nickel are first isolated by the method of Harwood and Theobald (ANALYST, 1933, 58, 673). An aliquot portion of the solution of the chlorides of these two elements is evaporated to dryness in a small (10 to 20 ml.) porcelain crucible with completely glazed surface, which has been weighed together with a porcelain filter-stick. The residue is taken up in a few drops of water and treated with 2 drops of perhydrol. After the addition of 1 drop of *N* sodium hydroxide solution and waiting until no more oxygen is evolved, 4 ml. of glacial acetic acid are added to dissolve the precipitate, and the mixture is gently heated, diluted with 4 ml. of hot water and treated with about 0.5 ml. of the reagent (a 2 per cent. solution of α -nitroso- β -naphthol in 70 per cent. acetic acid). Each mg. of cobalt requires 1 ml. of the reagent for precipitation. The mixture is boiled very gently for 5 minutes and filtered through the porcelain filter-stick, and the precipitate is washed 3 times with 0.5 ml. of acetic acid and 4 times with 0.5 ml. of hot water, dried at 130° C. and weighed on the micro-balance together with the crucible and filter-stick.

J. W. M.

Microchemical Recognition of Pigments from Paintings. S. Augusti. (*Mikrochimica Acta*, 1938, 3, 239-246.)—The position on the picture from which the sample is taken is chosen with the aid of a low-power microscope (10 to 30 \times). The surface is moistened and the varnish is dissolved by means of a small wad of cotton wool soaked in alcohol held by forceps, or by a small brush with thin short bristles. A small fragment of paint film is then taken on a needle, under the microscope, and the sample is placed on a slide. In this way the position of sampling is scarcely detectable by the naked eye. An old oil painting may resist the alcohol treatment, and a stronger solvent be necessary. Both spot tests and crystal tests are used. The pigments are divided according to colour: white, blue, green, yellow, red, brown and black. The systematic identification is based on responses to general reagents (dilute nitric acid for white and yellow pigments; dilute hydrochloric acid for green and red pigments; sodium hydroxide for blue pigments) or on their behaviour when heated (brown and black pigments). The investigation of each pigment includes a microchemical study of the cation and anion components. A table is given of recommended tests for the cations of most frequent occurrence in pigments. There is also a tabular list of pigments classified under colours, with the chemical composition and the ions to be detected in each instance (*cf.* Laurie, ANALYST, 1930, 55, 162; 1933, 58, 468; 1934, 59, 657).

J. W. M.

Physical Methods, Apparatus, etc.

Direct-View Particle Counter and Portable Ultra-Microscope. S. C. Blacktin. (*J. Soc. Chem. Ind.*, 1938, 57, 361-363T.)—The apparatus consists of 2 portions, *viz.* the inductor, which maintains the flow of gas containing the fine

particles, and the cell in which particles are counted. The inductor is a cylinder (4 cm. in diameter) fitted with a piston and piston-rod through the centre of which, and free to rotate within it, is an archimedean screw, which is continued inside the cylinder in a shaft and terminates outside the piston in a turning-lever. Thus, when the lever is turned the piston and rod will turn also, and at the same time move away from the end of the cylinder at which the lever is situated, and so draw the sample in through an aperture at the turning-lever end of the cylinder. Each successive turn of the lever therefore, will cause successive suction of the sample into the piston. This device has been found more convenient for use in the field than the usual type of water-aspirator. The cell consists of a vertical metal rod (*e.g.* length 4 cm., diameter 2 cm.) bored longitudinally with (presumably 2) holes 1 to 3 mm. in diameter, the orifices of which at one face of the rod are suitably recessed and blackened. A cylindrical tube (2 cm. long) of thin clear glass, closed at one end with a microscope cover-slip (which is cemented on so as to make a gas-tight fit) is slipped over the recessed end of the rod, and is chosen so as to give a sliding fit without play. The cover-slip and the face of the end of the rod should not be in contact, and their distance apart is maintained by means of a rubber band which grips the glass cylinder at its lower end and forms an air-tight joint between it and the rod. In this way an observation-cell is formed into which the sample may be passed continuously or intermittently (*i.e.* in through one of the longitudinal holes, across the recessed face of the rod, and out through the other hole) when the lever of the inductor is turned. Particles in the sample are rendered visible by adjusting the position of the rod (which is threaded for the purpose) so that a pencil of light rays enters the side of the glass tube in the direction of flow of the particles as they pass from the inlet to the exit hole, the portion of the cell diametrically opposite the point of inlet of the light being silvered. By adjusting the depth of focus of the viewing-microscope (which, presumably, is placed vertically over the cover-slip), the particles brightly in focus may be counted against a dark background and in such a way that they are unaffected by the proximity of the cell-wall. On the other hand, a microscope combination having a focal depth greater than the depth of the cell cannot be used, whilst the cell walls obscure certain particles which should be counted; a coating of quinoline has been used to prevent adherent particles from confusing the foreground or background. A special ultra-microscope viewing system is also described, the purpose of which is to provide an intense pencil of light for illuminating the cell without sensible transmission of heat. The whole apparatus (including the inductor and cell, but excluding the illuminating source) occupies less than 0.50 cb.ft. The source of light was an ordinary 150-watt (200-volt filament lamp in a vertical tube which was closed at the top by a reflector and fitted with a double-convex lens at the base, so that a bright image of the lamp-filament was received on the mirror of the ultra-microscope; the latter reflected this image through a tube fitted with a 1.5-in. objective on to the centre of the observation cell. A table showing the depths of focus (between 0.17 and 0.72 mm.) corresponding with various combinations of objectives and eyepieces is given; thus, for average conditions, a focus depth (y) of 0.30 mm. is obtainable from an 8-mm. objective and a No. 3 eyepiece. Diaphragms of known area (as determined by means of a stage

micrometer) were fitted into the eyepiece used, and the volume of gas (z) containing the average number of particles counted (n) was obtained from the expression $\pi(x/2)^2y \times 10^{-3} \text{ ml.}$, where y is the diameter of the diaphragm. The number of particles present, therefore, was n/z . The range of the instrument was 2.8×10^{-7} to $8.3 \times 10^{-4} \text{ ml.}$, or 1200 to over 10^7 particles per ml. A set of 6 diaphragms cut from black paper and punched with a central circular hole was used, and these provided a choice of 36 different area values and 36 different volumes. The apparatus was tested by means of the stable smoke prepared by burning safety-match heads in a metal smoke-chamber fitted with a fan, and a table and curve show the quantitative variation of smoke, after time intervals of 0 to 210 minutes, in terms of the particle counts.

J. G.

Reviews

THE CHEMICAL ANALYSIS OF FOODS AND FOOD PRODUCTS. By M. B. JACOBS, Ph.D. Pp. xxii + 537. London: Macmillan & Co., Ltd. 1938. Price 25s.

In the preface the author states that he had certain aims in writing this book. To mention some of these will, perhaps, be the best way of indicating the scope of the volume. He says that he wished to deal with the salient facts of the chemical analysis of foods and food products, and to include certain of the newer aspects of food analysis, such as the chemical assay of vitamins, the detection of incompletely pasteurised milk and methods for revealing the sophistication of foods. His aim was to give information which it has hitherto been difficult to obtain, on such subjects as gums, jams, jellies, milk products in other foods and soya bean flour in meats.

He has endeavoured to present short practical methods which are usable and efficient. He has tried to make the book useful as a textbook and as a manual for manufacturers for control work. He hopes it will prove useful, too, in "regulatory laboratories both governmental and commercial."

It will be seen that the scheme is an ambitious one, but the book does, in a large measure, fulfil the above aspirations.

In seventeen chapters the author has covered extensively the ground of the analysis of foods. The first seventy-five pages deal with general methods and physical and chemical determinations, describing briefly the instruments used. Then follow, to page 143, the subjects of colouring matters, preservatives and metals in foods. Thus the processes and descriptions which are of general application are dealt with before the particular foods are considered in detail.

The processes are clearly described, instructions are definite, and—a great advantage—each process is generally preceded by one or two sentences stating the general underlying principles of the method. Reading the descriptions of processes gives the impression that the author must have personally carried out many of them, and sometimes one is recommended rather than another; there are few instances where such vague instructions occur as "add sulphuric acid" without any statement as to the strength. There is, too, occasionally the unusual experience of finding a method mentioned but not recommended.

Of course, it has to be taken into account that this work was not written specifically for Public Analysts or even for British analysts, but presumably for the Americans. No reference is made, therefore, to the food legislation of this country, and from internal evidence of the book itself it would appear that conditions vary a good deal between the two countries; for instance, the author considers that, in America, if a permitted preservative conceals damage or inferiority in a food it comes to be regarded as an adulterant.

Considerable difference, too, must exist in the type of adulteration, for on page 163 the following statement occurs:—"the older ways of adulterating milk by skimming, watering . . . have given place to much subtler methods." The experience of the reviewer suggests quite the opposite, so far as this country is concerned. Under the heading "added water" only five and a half lines are given, and they form a reference to the refractive index of the serum. In the writer's opinion no clear evidence has yet been brought forward to support the position that the refraction of the serum is a reliable criterion of the presence of added water. The freezing-point is relegated to the general section on physical methods.

One other point calls for mention. The admittedly difficult question of the recognition of the presence of hardened oils in edible fat receives no attention, and the lead-salt-ether method is disposed of in thirteen lines, which quaintly conclude: "this method is of limited value, time consuming and laborious—therefore the details are not given," and so the matter ends. But for all that, the analyst may still have the thorny question before him of the presence of hydrogenated fat supplied partly or wholly to a demand for lard.

With reservations on the two points just mentioned it can be said that the book will be found of great service in the analysis of foods.

It is printed in clear type, on good paper, and proof reading has been fairly well done. Only a few obvious misprints have been noticed. The American spelling is not so generally used as it is in some scientific works from across the water.

J. R. STUBBS

THE CHEMISTRY OF THE PROTEINS. By D. JORDAN LLOYD, D.Sc., F.I.C., and AGNES SHORE, B.Sc., A.I.C. 2nd edition. Pp. xi + 532. London: J. & A. Churchill, Ltd. 1938. Price 21s.

In its second edition this useful book has been much improved, the scope has been reduced somewhat by the omission of sections on the industrial aspects of protein chemistry, and the size has been increased. For these reasons it is possible to treat most subjects more adequately than in the previous edition. In places, however—for example, the chapters on the biochemistry of proteins and amino acids and on proteolytic enzymes—it still shows a certain capriciousness in the choice of material, which suggests that in any further editions it might be well to restrict the field still more. In places the book is marred by rather superficial passages on the general biological importance of particular proteins and by a tendency to treat contributions to the theory of protein structure, based on a knowledge of geometry and a vivid imagination, as if they had some experimental foundation.

The sections on the chemical and physical properties of the amino acids are

balanced and reasonably comprehensive and the literature citations are adequate. Two-thirds of the book is devoted to a treatment of the physical chemistry of the proteins, both in the solid state and in solution. This section, as might be expected, treats a complex subject in a lucid and admirable manner.

The printing and indexing are good, but it is a pity that Messrs. Churchill so often complicate the process of finding the index in their books by binding in a set of advertisements at the end.

N. W. PIRIE

INTRODUCTION TO PHYSIOLOGICAL CHEMISTRY. By M. BODANSKY, Ph.D. Pp. viii + 686. Fourth Edition. New York: John Wiley & Sons, Inc. London: Chapman & Hall, Ltd. 1938. Price 20s. net.

Although this book is described as the fourth edition, the particulars given inside show that it is actually the fifteenth printing; the first printing of the first edition appeared in January, 1927. The third edition was the subject of a review (*ANALYST*, 1934, 59, 851), which concluded with the following words: "Large though the number of textbooks on physiological and biological chemistry, it is impossible to resist the conclusion that this is one of the most valuable of all, both absolutely and relatively to its price."

The fourth edition contains 22 more pages than the third edition. Its cost is 5s. less, a remarkable and almost incredible change in these days of steadily mounting book prices. To say anything further about Professor Bodansky's achievement, other than to insist that this reviewer sees no reason whatever to modify the views expressed in 1934, apart from the observation that any doubt as to the superiority of Professor Bodansky's book over all others in the English language can now be eliminated, would be a work of supererogation. A. L. BACHARACH

AIDS TO FORENSIC PHARMACY. By A. W. LUPTON. 2nd Edition. Pp. viii + 260. London: Baillière, Tindall & Cox. 1938. Price 3s. 6d.

Ignoratio legis neminem excusat is a phrase well-known to business men, but to every analyst or pharmacist it is of especial significance, particularly when new laws and official regulations are so frequently made for the benefit of either the public or particular industries.

Now that the pharmaceutical examination syllabus requires an adequate acquaintance with many aspects of the law, this addition to the series of Students' Aids will be of great assistance, and not only to students, but to everyone affected by the frequent alterations, additions and enactments.

The author has summarised the Acts of Parliament and Official Regulations very plainly, and in the space of 229 pages has enumerated all the essentials of The Shops Act, Registration of Business Names Act, Medicine Stamp Act and the Regulations for the Use of Stills, Sale of Spirits, Methylated and Surgical Spirit; of particular service to practising analysts is the chapter dealing with the Food and Drugs (Adulteration) Act of 1928. The 1938 Act, which has received the Royal Assent, is too recent to be dealt with in this volume.

Under *Addenda*, the New Poisons Amendments, which were issued recently, and will come into force on January 1st, 1939, are given, and supplement a very complete explanation of the Pharmacy and Poisons Act, 1933.

Other chapters deal with Sale of Abortifacients, The Dangerous Drugs Act, 1920-1932, and the Regulations of 1937, The Therapeutic Substances Act, 1925, and Regulations of 1931 to 1937.

No reference is made to the regulations regarding the storage of inflammable liquids or the Petroleum Act, neither are the regulations for Preservatives in Food nor the use of Prohibited Colours in Food mentioned, but the inclusion of these would add to the general usefulness of the book.

The chapter dealing with the Classification of Poisons according to the different Schedules of the 1933 Act should be of particular use as a quick means of reference, and the book will fill the purpose of another useful "Aid."

C. EDWARD SAGE

TECHNOLOGY OF SOLVENTS. By O. JORDAN. English translation by A. D. WHITEHEAD. Pp. 351 and 14 (indexes). London: Leonard Hill, Ltd. 1938. Price 25s.

Many English chemists will be familiar with the original German work by Dr. Jordan, *Chemische Technologie der Lösungsmittel*, published in 1931, and will have learned to appreciate its quality; they will be all the more ready to look with pleasure upon this English translation, which has been extremely well done. Whilst the English text does not differ from the German in the more theoretical portions of the book, sufficient revision has been carried out in other parts, particularly by the inclusion of recent references, to justify the claim that the book is up-to-date.

The book is divided into:

- (1) A General Section, in which the physical properties (and other attributes, such as physiological action) of solvents and plasticisers, are considered according to type and projected use, and are considered in relation to current fundamental conceptions of molecular structure. In this section also there is a substantial chapter on the Manufacture of Solvents, but, as the author points out quite properly, care has been taken to eliminate such details—particularly regarding plant required for manufacture—as would not materially assist a clear presentation, and therefore might hinder a proper understanding of the chemical operations involved.
- (2) A Special Section, in which the properties, characteristics and special uses of individual solvents and plasticisers are considered separately.

Then follows an excellent series of Tables, an Index to Proprietary Names, which all technical workers in the field will find most acceptable, and, in addition to the usual Author and Subject Indexes, there is a Patent Index.

The Tables above mentioned comprise:—*A.* Physical Data of Solvents, which are unusually detailed and include such matters as Explosive Limits and Heats of Evaporation. *B.* Constants of Plasticisers—data are given for 34 of the more important plasticisers. *C, D* and *E.* Solubility Tables for Resins and Rubber, Oil-Soluble Dyestuffs, and Lacquer Dyestuffs. *F.* Reactions of Various Solvents to Dracorubin Paper. *G.* Various Weight and Percentage Composition Tables for

the Alcohols, Water, Ether and Mixtures thereof. *J.* Characteristics of the more important Azeotropic Solvent Mixtures.

Altogether this is a notable book; the subject-matter is essentially scientific, even profoundly so, yet it is treated in such a way that the practical man, who may not be too well versed in chemical fundamentals, will still find satisfaction therein.

L. A. JORDAN

BEVERAGE MANUFACTURE (NON-ALCOHOLIC). By R. HAROLD MORGAN, M.Sc.
Edited by ARTHUR T. E. BINSTED. Pp. 225. London: Atwood & Company, Ltd. 1938. Price 30s. net.

This book fills a much-felt want in the vast supply of technical literature. It is a veritable vade-mecum for the mineral water industry, and should find its place in every syrup room, where it will tend to eliminate the many abominations that have helped to bring the industry into disrepute.

The author discusses the various processes of manufacture—the *pros* and *cons* of the hot and cold methods of syrup making, and the “satisfactory, fair and unsatisfactory” metals which may be used in the lay-out of the plant. The reviewer would have preferred only two categories, which would have bracketed the fair and unsatisfactory together.

Chapter XIV describes the making of brewed and fermented goods. It may be hoped that in a future edition instructions for making beverages which are fermented and matured in the bottle, where the alcohol-content has to be kept below 2 per cent. of proof spirit, will be deleted. As the period during which they are palatable is very short, they soon become nasty, are always unsightly, and are one of the chief causes of discredit being brought on the industry. For the small maker, whose experience is bound to be limited, it is far better to procure the concentrated products from some essence firm of repute than to risk the practical certainty of turning out an unpleasant beverage.

On page 44 the chlorination of water is discussed. The amount of chlorine used is stated to be between 2 and 12.5 p.p.m.; this amount in a water would render it very unpalatable. In Chapter II, in the description of the use of saccharin, it is not stated that every beverage containing saccharin is so much the worse for its presence. In the section devoted to syphons and their filling it might be explained with advantage, in a future edition, why the water delivered from the syphon is usually less well aerated in the glass than that obtained from a bottle. Syphons play an important part in the mineral water industry, and the aeration of the water, when poured into the glass, varies tremendously. Often there is high pressure in the syphon and perfectly flat water in the glass. Supposing the water in the cylinder is well aerated and the supernatant gas free from air, then the quality of the aeration in the glass will be in inverse ratio to the pressure in the syphon. The flatness is due to the constriction of the water-way, and the friction of the water passing through this constriction drives most of the gas out of the water.

The author condemns the use of refrigerated water, but if the top pressure in the syphon is kept down to about 60 lbs. the best possible results will be obtained

from a syphon. Only about two-thirds of the contents will empty without shaking, but the aeration in the glass will be the maximum obtainable.

Part II is devoted to the description of machinery and its lay-out, filling machines, bottle-washing plant, detergents, and so forth. Many machines made by our foremost makers are fully described. In addition, the book contains detailed descriptive advertisements by most of the well-known British makers.

FRANK R. STEPHENS

OFFICIAL METHODS OF ANALYSIS OF THE INTERNATIONAL SOCIETY OF LEATHER TRADES CHEMISTS. Pp. 164. Published by A. Harvey, 17, Leathermarket Street, London, S.E.1. 1938. Price, members 5s.; non-members 8s. 6d.

This volume contains the Official and the Provisional Official Methods of Analysis and Sampling of the various materials and solutions used in the leather industry, and has been compiled by a sub-committee consisting of Messrs. W. R. Atkin, P. Chambard, R. Loos and F. C. Thompson. It gives the various amendments, adopted at the biennial International Conferences, including that held at Copenhagen in 1937, and published in English and French.

The methods cover tanning materials (vegetable and chrome); hide powder (the standard material used for estimating tannin); leather (vegetable and chrome); oils and fats, including sulphonated oils; sodium sulphide; lactic acid; used lime liquors; fresh and used chrome-tanning solutions; the determination of the *pH* value of vegetable-tanning extracts and solutions; the sulphur dioxide content of bleaching extracts.

The constituents of vegetable-tanned leather included are moisture, ash, fats, water-soluble matter, hide substance, glucose, Epsom salts and mineral acidity. In addition to the well-known Procter-Searle method, the modern methods of Innes and of Atkin and Thompson are given. The constituents of chrome leather for which Official Methods are now available are: moisture, ash, chromium, aluminium, grease, uncombined sulphur and alkaline salts.

Amendments and additions at future conferences will be circulated to members of the International Society on gummed, loose leaves, and blank pages have been bound into the book to accommodate these.

The publication of these official methods in book form supplies a long-felt want to the large numbers of leather users and analysts outside the leather industry who may be called upon to examine leathers or tanning materials.

The book is well printed, and the matter is clearly set out with table of contents and index.

R. F. INNES

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