THE ANALYST

PROCEEDINGS OF THE SOCIETY OF PUBLIC ANALYSTS AND OTHER ANALYTICAL CHEMISTS

A JOINT Meeting of the Society with the Food Group of the Society of Chemical Industry was held on Wednesday, February 1st, at 8 p.m., in the Chemical Society's Rooms, Burlington House. At the invitation of Dr. E. B. Hughes, Vice-Chairman of the Food Group, Professor W. H. Roberts, President of the Society, occupied the chair.

The following were elected members of the Society:—G. Carter, B.Sc., A.I.C.; O. B. Darbishire, B.Sc., A.R.C.S., D.I.C., A.I.C.; F. M. Dyke, B.Sc., F.I.C.; A. A. Eldridge, B.Sc., F.I.C.; Professor F. Feigl, Dr.Ing.; G. H. Fraser; M. B. Ichaporia, M.Sc., Ph.D., A.I.C.; R. Porter; A. C. Ratcliff, B.Sc.; W. H. Templeton, B.Sc., F.I.C.

Certificates were read in favour of the following Candidates for Membership:— F. C. Collins; R. Crosbie-Oates, B.Sc., F.I.C.; Prof. H. E. Fierz-David; J. G. Fife, M.Sc., F.I.C.; W. C. Hughes; N. E. G. Narayan Iyengar; W. E. Kemp; H. C. Lockwood, B.Sc., F.I.C.; W. Marsden; F. Michel, Dr.Chem.; A. H. Rheinlander, M.Sc., F.I.C.; L. W. Ruddle; J. Straub, Chem.Ing.; R. G. Thin, B.Sc., F.I.C.; H. Wilkinson, B.Sc., Ph.D.; E. G. Williams, M.A., A.I.C.; F. R. Williams, Ph.D., B.Sc., A.I.C.

The subject dealt with at the meeting was:—"The Analysis and Differentiation of the Composition of Iron, Phosphorus and Calcium Compounds in respect of Nutritional Requirements." The subject was introduced by Professor J. C. Drummond, M.C., D.Sc., F.I.C.; Iron Compounds were dealt with by Dr. R. A. McCance, M.A., M.D., M.R.C.P., M.R.C.S.; Phosphorus Compounds by Professor H. D. Kay, O.B.E., D.Sc.; Calcium Compounds by Professor E. C. Dodds, M.V.O., D.Sc., M.D., F.R.C.P., and Dr. J. D. Robertson, M.D., D.P.H., Ch.B.

NORTH OF ENGLAND SECTION

THE Fourteenth Annual General Meeting of the Section was held at Manchester on January 28th, 1939. The Chairman, Professor T. P. Hilditch, presided over an attendance of thirty-seven.

An appreciation of the late Dr. J. T. Dunn was given by Mr. W. G. Carey, F.I.C., and afterwards, at the request of the Chairman, the members stood in silence as a mark of respect.

The Secretary presented the Report and Financial Statement, which were adopted.

The following appointments for the coming year were made:—*Chairman*, Professor T. P. Hilditch; *Vice-Chairman*, J. R. Stubbs; *Committee*, W. G. Carey, E. G. Jones, A. Lees, H. M. Mason, C. J. H. Stock, A. R. Tankard; *Honorary Auditors*, U. A. Coates, J. W. H. Johnson; *Honorary Secretary and Treasurer*, J. R. Stubbs.

The Chairman delivered the Annual Address entitled "Speed Limits in Analytical Matters."

The following paper was read and discussed:—"On the Composition and Analysis of Hair Dyes," by H. E. Cox, D.Sc., Ph.D., F.I.C.

SCOTTISH SECTION

The Fourth Annual General Meeting of the Section was held in the Ca'doro Restaurant, Union Street, Glasgow, on January 26th, 1939.

The Secretary read the Report and Financial Statement for 1938, which were adopted.

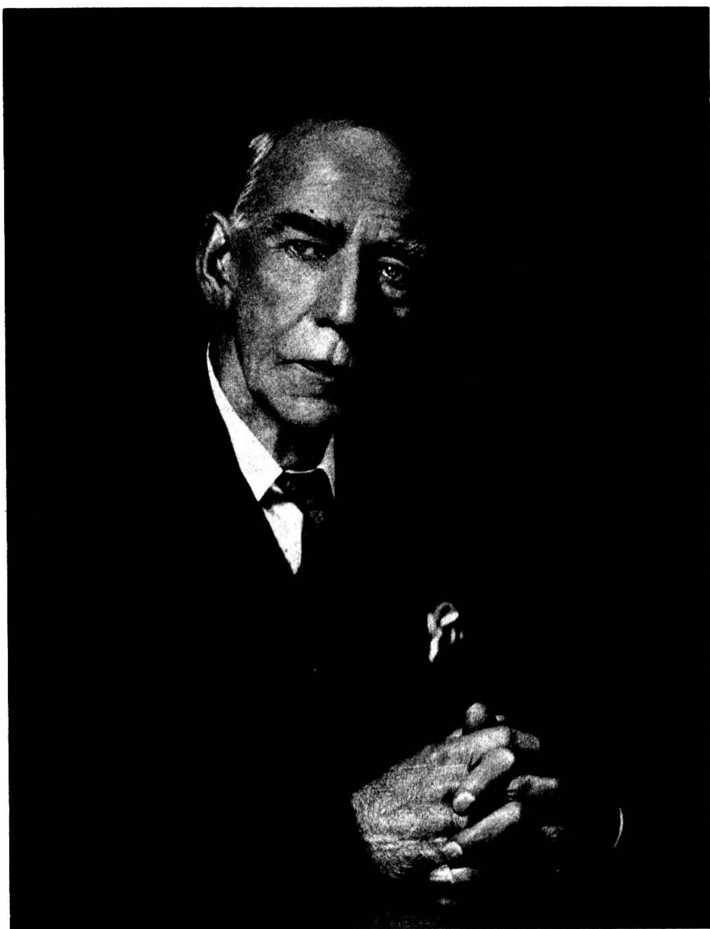
The following office-bearers were elected:—*Chairman*, T. Cockburn; *Vice-Chairman*, J. W. Hawley; *Committee*, J. Brown, H. Dryerre, A. R. Jamieson, H. C. Moir, A. Scott Dodd, J. F. Tocher; *Honorary Secretary and Treasurer*, J. B. McKean; *Honorary Auditors*, M. Herd and R. S. Watson.

The following papers were then read and discussed:—"Sampling," by W. M. Cameron; "Seasoning Materials," by R. H. McKinlay, F.I.C.

After some discussion, the meeting unanimously decided that a Food and Drugs Act applicable to Scotland, on similar lines to the Food and Drugs Act, 1938, was desirable. The Chairman and Secretary were instructed to draft a Memorandum on this subject and to submit it, if approved by the Council, to the Local Government and Public Health Consolidation (Scotland) Committee.

Death

With great regret we record the death of Mr. D. Lloyd Howard, former Member of Council.



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J. T. Dunn

Obituary

JOHN THOMAS DUNN

DR. J. T. DUNN died, in his 81st year, in Newcastle upon Tyne, on January 3rd.

The service at the West End Crematorium, Newcastle, on January 6th, was attended by a numerous congregation, representative of his scientific, public, social, cultural and other activities. It was conducted by the Rev. Herbert Barnes of the Church of the Divine Unity, who gave a short address of consolation and an appreciation of Dr. Dunn's attainments and services and of his many-sided character.

This Society was represented by Mr. John Evans, Past President, and other members attending were Messrs. W. Gordon Carey (Sunderland), A. Scholes (representing Pattinson & Stead, Middlesbrough), C. J. H. Stock (Darlington), and H. C. L. Bloxam (Newcastle).

A memorial service was held on Sunday, January 8th, in the Church of the Divine Unity, Newcastle, where Dr. Dunn attended after he came to live in Newcastle. An eloquent address was delivered by Lord Eustace Percy, Rector of King's College, Newcastle, in which he referred to the long and valuable services of Dr. Dunn to the Council of the College.

Dunn was educated at Dr. Bruce's School and at Durham College of Science, later Armstrong College (now King's College), Newcastle. He graduated B.Sc. in 1877 and proceeded to M.Sc. and D.Sc. At Armstrong College he was Demonstrator in Chemistry and Physics and, later, Acting Professor of Chemistry (1882). He then turned to scholastic work, becoming science master at Gateshead School in 1884, and Head Master in 1887. In 1894 he became Head of the Technical School, Plymouth, and in 1895 Principal of the Northern Polytechnic Institute, London, where he was also Head of the Chemistry Department. In 1901 he joined John Pattinson in partnership in the firm of J. & H. S. Pattinson, Analytical and Consulting Chemists, Newcastle upon Tyne, of which he was the senior partner at the time of his death. On Pattinson's death Dunn became Public Analyst for the City and County of Newcastle upon Tyne, the County of Northumberland, the County Boroughs of Gateshead, South Shields, Sunderland, and Tynemouth, and the Borough of Berwick-upon-Tweed; these appointments he held until 1937, when, owing to his illness, his partner was appointed to them.

Dunn was elected a Member of this Society in 1905, served three periods as Member of Council, was Vice-President in 1917–18, and was elected President in 1930. He was President of the Society of Chemical Industry in 1933–4, and was a Member of Council of the Institute of Chemistry, 1918–21, and Examiner from 1921 to 1925 and from 1927 to 1932.

An eminent man has gone from us, but his example remains, and fortunately the many who came in contact with him during his early scholastic and university life, and later in his numerous public and professional activities, have had the opportunity to profit by it. A cultured and scholarly man, of a quiet and kindly disposition, tolerant of the views of others, yet ever ready stoutly to defend his own opinions with a vigour that surprised those who were unacquainted with the

depths of his character. He was a fluent extempore speaker, but nevertheless he wrote all important speeches, which he committed to memory and delivered with scarcely a reference to his notes. Of his own language he was a perfect master, and he derived much entertainment from journalese; he possessed an intimate knowledge of French and German, a working acquaintance with Italian, and for business purposes he even made a study of Russian.

As might be expected, he was a scholarly writer, and his manuscript and letters will long be remembered for his choice of words, beauty of handwriting and neat arrangement. Dunn accomplished so much because of his great powers of endurance (he was only once known to admit feeling tired) and because he permitted himself no idle moments; having a few minutes to spare before keeping an appointment or catching a train, he would employ them by writing a short letter, or in making a microscope examination, arriving without hurry, to catch the train almost at the due time for departure. He was never bored, nor did he feel the need of hobbies. Habitually he acted upon the injunction "do it now," rarely delaying or postponing, and no sooner was he asked to do something than he at once set about doing it, if he approved of the request. This characteristic sometimes led to wild searches in the waste-paper basket for a document or letter which, having been at once attended to, had been thrown away, but which, on second thoughts, he wished to file or to refer to again.

Dunn took a deep interest in Rotary, regularly attending the Friday lunches of the Newcastle Club, of which he was a Past President, and he often visited the Rotary Clubs in the neighbourhood of Newcastle; in 1928 he represented Newcastle Rotarians at the International Conference at Minneapolis, U.S.A. He never used the much-hackneyed word "service," yet he was ever ready to do a service for those who needed it; though charitable, he preferred to act through some organisation whose business it was to investigate cases of need. He had a great love of animals, particularly of dogs, and was Secretary and Treasurer of the Newcastle Dog and Cat Shelter, and a member of the Committee of the Newcastle Branch of the R.S.P.C.A. A lover of music, and no mean performer on the pianoforte, he was an active supporter of local music societies, and acted as Local Representative of the Associated Board of the Royal Schools of Music, London.

Dunn was a Justice of the Peace for Gateshead, a Freeman of the City of Newcastle, a member of the Freemen's Guild, and a member of the Ancient Company of House Carpenters, of which he was Senior Steward.

His was a crowded life, yet nothing appeared too great or too trivial to merit his attention, and in all he undertook he gave of his best. Although he passed the normal span of life, he never went through the period of old age, and thus we shall continue to remember him—a man of great activity and usefulness.

He leaves a widow and an only child, Mrs. V. A. Mundella.

H. Charles L. Bloxam

Selective Oxidation of Animal and Vegetable Fats: A New Constant

By W. A. ALEXANDER, B.Sc., A.I.C.

(*Read at the Meeting, December* 7, 1938)

INTRODUCTION.—In this work an attempt is made to utilise selective oxidation as a means of determining the purity of an animal or vegetable fat. Conditions were established under which the amount of oxidation of the more highly unsaturated acids was far greater than that of the less unsaturated, and the difference between them constant and measurable. Thus a "constant" was found which depended largely on the relative proportions of the unsaturated acid components of the fat, as well as on their total amount. The only other method achieving this end would seem to be the thiocyanogen value determination which, while giving valuable results, is rather troublesome for use as a routine method in an analyst's laboratory.

The conditions that were chosen as giving a sufficiently selective action while being conveniently attained are embodied in the following definition of the constant (termed subsequently the "oxidation value"):—the amount of oxidation, expressed as g. of iodine per 100 g. of fat, which takes place at 60° C. in one hour, when the fat is dissolved in an excess of a mixture of 10 vols. of a glacial acetic acid solution of crystallised sodium dichromate ($Na_2Cr_2O_7.2H_2O$) of normal oxidising strength, with 2 vols. of carbon tetrachloride.

Previous experiments with different oxidising agents were unsatisfactory. With a mixture of chromic and acetic acids the results were more in line with the iodine value, the total consumption of oxygen being considerably more than when sodium dichromate was used. It was also found difficult to avoid oxidation of the saturated glycerides with chromic acid or potassium permanganate in acid solution, and an acid solution seemed necessary for the development of a volumetric method with sharp end-point.

Although the products of oxidation have not been fully investigated (this not being essential for the purpose to which the results are put) the oxidation value has been calculated on the assumption that 4 oxygen atoms per double bond are taken up; *i.e.* that 1 ml. $N/10$ = 0·008 g. of oxygen or 0·003175 g. of iodine.

PROCEDURE.—After one or two variations in the titre of the sodium dichromate the following procedure was adopted.

Solutions required.—(*a*) $N/1$ sodium dichromate: 49·7 g. of pure crystalline sodium dichromate ($Na_2Cr_2O_7.2H_2O$) are dissolved in about 900 ml. of glacial acetic acid (not necessarily of analytical reagent quality) and, after being heated to 80° C. for 5 minutes, the solution is cooled and made up to 1 litre. As thus prepared, the strength is about 0·9 N, which is satisfactory. This solution can be kept in a well-stoppered bottle for as long as the Wijs reagent.

(*b*) $N/2{\cdot}5$ ferrous sulphate: Ferrous sulphate crystals 112 g.; conc. sulphuric

acid 30 ml.; syrupy phosphoric acid 100 ml.; in 1 litre. This need not be standardised, but 25 ml. should be slightly stronger than 10 ml. of (*a*).

(*c*) $N/10$ potassium permanganate, recently standardised.

(*d*) Diphenylamine oxidation/reduction indicator: 1 g. in 100 ml. of conc. sulphuric acid.

Method.—Two flasks (150 to 200 ml.) are fitted with loose glass stoppers, then cleaned with sulphuric acid and chromium trioxide, washed and dried thoroughly.

A quantity of the oil or fat, varying according to unsaturation (see Table I), is weighed into one flask and both flasks are placed in the thermostat at 60° C., accurate temperature control being maintained. Two ml. of carbon tetrachloride are pipetted into each, and, after solution is effected, 10 ml. of the sodium dichromate solution are added, the liquids are mixed, and the flasks are stoppered and left at 60° C. for one hour. A little water is then introduced into each flask (this practically stops the reaction) and, with a pipette, 25 ml. of $N/2{\cdot}5$ ferrous sulphate solution, after which the contents of the flasks are back-titrated with $N/10$ potassium permanganate solution, the same amount of indicator being used for each test. Oxidation value =

$$\frac{\text{ml. of } N/10 \text{ } KMnO_4 \text{ equivalent to oxidising solution used by oil} \times 0{\cdot}3175}{\text{weight of oil taken in g.}}.$$

A rough guide to the amount of oil to be weighed out is given below:

Iodine value	Oil required g.
0 to 30	0·6 to 0·5
30 ,, 60	0·5 ,, 0·3
60 ,, 90	0·3 ,, 0·2
90 ,, 120	0·2 ,, 0·1
120 ,, 180	0·1 ,, 0·05

Results.—The following results were thus obtained:

TABLE I

Oil	Oxidation value		Iodine value (Wijs)	Number of samples
	Average	Range		
Linseed	97·5	94·1–99·4	184	3
Walnut	69·5	—	147	1
Tung	158·8	—	165	1
Soya bean	58·0	—	130	1
Maize	34·9	—	111	1
Cottonseed	41·3	—	105	1
Apricot kernel	21·9	—	105	1
Sesame	39·1	—	104	1
Rape	27·9	27·3–28·5	100	2
Almond	22·0	22·4–21·9	96	3
Arachis	24·4	—	91	1
Castor	51·2	50·0–52·4	85	2
Olive	15·5	14·5–16·2	84	5
Palm	14·6	—	55	1
Cocoa butter	4·1	3·9 – 4·4	37	2
Coconut	1·6	1·6 – 1·6	9	2

TABLE I—*continued*

Oil	Oxidation value Average	Oxidation value Range	Iodine value (Wijs)	Number of samples
Cod liver	94·5	92·7–95·5	164	3
Seal (old)	63·2	—	114	1
Whale (old)	30·2	—	120	1
Sperm	17·8	—	86	1
Lard	12·1	12·0–13·5	65	4
Beef-fat	4·9	4·3 – 5·5	40	4
Mutton-tallow	4·7	4·4 – 5·1	39	4
Butter-fat	7·6	6·0 – 8·9	37	5
Hardened oils, m.p. °C.				
Whale 32/34 ..	4·1	4·8 – 3·4	62	2
„ (D) 34 ..	3·3	—	62	1
„ 40/42 ..	1·9	—	47	1
„ (D) 46 ..	1·6	—	29	1
„ 50/52 ..	1·4	1·6 – 1·3	12	3
Arachis (D) 33 ..	3·2	—	61	1
„ (D) 40/42 ..	3·1	—	56	1
„ „ ..	4·5	—	58	1
„ (D) 50/52 ..	1·6	—	37	1
Cottonseed (D) 41 ..	13·1	13·3–13·0	62	2
Coconut	0·6	—	0·6	1

Fats marked (D) were deodorised.

Certain mixtures were made up (involving little change in the iodine value from that of the main ingredient) and from the determined oxidation value the corresponding proportions were calculated (Table II):

TABLE II

Proportions taken	Per Cent.	Oxidation value	Iodine value (Wijs)	Proportions estimated Per Cent.
Lard	60	8·4	64	58
Hardened arachis oil, m.p. 33° C.	40			42
Olive oil	90	18·2	86	89
Cottonseed oil	10			11
Cottonseed oil	80	40·3	106	84
Maize oil	20			16
Cottonseed oil	20	36·0	110	17
Maize oil	80			83
Almond oil..	90	22·1	96	96
Arachis oil	10			4
Linseed oil..	95	100·2	183	95·6
Tung oil	5			4·4

INTERPRETATION OF RESULTS.—(*a*) It will be observed from these tables that the oxidation values are far from being proportional to the total unsaturation as measured by the iodine values. Fats with similar iodine values may have very different oxidation values; the greater these differences, of course, the more easy becomes the detection of adulteration.

The hydrogenated fats, as a class, seem to give very low results, which make their detection in, say, lard fairly certain. Hydrogenated cottonseed oil proved to be an exception—at least for the two samples examined. Presumably the low figures are partly due to the decrease in the proportions of the more highly unsaturated components as a result of selective hydrogenation.

The position occupied by castor oil in the table is interesting in view of the fact that ricinoleic glycerides are very resistant to atmospheric oxidation.

(*b*) Much work has recently been done on the atmospheric oxidation of animal and vegetable oils, but no data appear to exist with regard to oxidation in a one-phase system, such as we have here. The oxidation value, depending as it does on uncompleted, presumably irreversible reactions, should involve at least as many velocity constants as there are different kinds of unsaturated glyceride molecules to be oxidised. That is, we might expect a measurable difference in the rate of oxidation of oils of widely different glyceride structures. In order to have further evidence on this point the well-known examples of Hilditch's mixed glyceride rule—cacao butter and mutton-tallow—were subjected to a series of tests involving different times, temperatures, and concentrations of acetic acid, carbon tetrachloride and chromic acid without showing any greater difference between them than that slight, constant one due to different degrees of unsaturation.

With atmospheric oxidation, however, the glyceride structure would seem to be involved. Lea[1] gives a series of curves showing the rate of oxidation by molecular oxygen at 100° C., from which, in decreasing order of susceptibility, we have:—cod-liver oil, lard, olive oil, cottonseed oil, beef-fat, butter and castor oil. In decreasing order of oxidation values these stand:—cod-liver, castor oil, cottonseed oil, olive oil, lard, beef-fat and butter-fat.

The position of the double bond in the molecule, which is believed to affect the rate of oxidation,[2] may also influence the results for hydrogenated fats. Hilditch[3] gives the component acids of selectively hydrogenated cottonseed oil as:

Iodine value	Component acids, per cent. Oleic	Linolic	Saturated
72·1	62·0	11·0	27·0
65·2	68·5	3·5	28·0
57·5	67·0	0·0	33·0

from which one of iodine value 62 might contain 68 per cent. of oleic and 2 per cent. of linolic acid. If the "oleic" acid present consisted entirely of the $\Delta 9 : 10$ acid, the oxidation value would be [*vide* (*c*), *infra*] $68 \times 0{\cdot}135 + 2 \times 0{\cdot}87 = 10{\cdot}9$ on the fatty acid basis, whereas, the value found was $13{\cdot}1/0{\cdot}956 = 13{\cdot}7$. With hardened arachis oil of iodine value 61, if $\Delta 9 : 10$ oleic were the only unsaturated acid present, the value would be $72 \times 0{\cdot}135 = 9{\cdot}7$, as against $3{\cdot}2/0{\cdot}956 = 3{\cdot}3$ found. Hence, the low results cannot be fully accounted for by the diminution in the content of more highly unsaturated acids. This seems to point to the presence of *iso*-oleic glycerides as a contributory factor.

(*c*) *Relation between oxidation values and component fatty acids.*—If we write oxidation value $= a \times$ oleic $+ b \times$ linolic $+ c \times$ linolenic fractions of the fat,

and consider only those fats having negligible quantities of the very highly unsaturated acids, we can approximately evaluate *a*, *b* and *c*.

Fat	Oxidation value (O)	Sapon. value (S)	$1-\frac{3\cdot8S}{16800}$ (V)	Oxidation value on fatty acid basis (O/V)	Per cent. on fatty acid basis		
					Oleic	Linolic	Linolenic
Palm oil ..	14·6	198	0·955	15·3	44	10	—
Lard	12·1	195	0·956	12·7	57	5	—
Olive oil ..	15·5	193	0·956	16·2	81	7	—
Arachis oil ..	24·4	193	0·956	25·5	61	21	—
Sesame oil ..	39·1	191	0·957	40·9	49	38	—
Cottonseed oil	41·3	194	0·956	43·2	30	46	—
Soya-bean oil ..	58·0	193	0·956	60·6	28	54	5
Walnut oil ..	69·5	195	0·956	72·7	18	65	8
Linseed oil ..	97·5	193	0·956	102·0	10	43	38

As the unsaturated fatty acids listed above (mostly as given by Hilditch and his collaborators) are expressed on the total fatty acid basis, the oxidation values were re-calculated on that basis. By substituting these corrected values in the above equation, and solving graphically for those oils having no linolenic acid, we get $a = 0{\cdot}135$ and $b = 0{\cdot}87$ approximately. The value of c (1·67) may then be found from the equations for the remaining oils. The average numerical difference between the determined oxidation values and those obtained by calculating back from the component fatty acids and these factors is only 0·8 unit. The less unsaturated fats do not quite come into line with the others:

Fat	Oxidation value on fatty acid basis	Oxidation value calculated from factors and compositions
Mutton-tallow ..	5·0	8·0
Beef-fat	5·1	8·6
Cocoa butter ..	4·3	7·0
Coconut oil ..	1·7	2·7

In the first two on the list the small percentages of palmitoleic acid present have been added to those of the oleic acid.

The sample of maize oil examined also differed—the calculated value being 42·7 and the found 36·5—and it is noteworthy that these differences are all in one direction. Further work is being done on this point.

ORDER OF REACTION.—Determinations of the rate of oxidation of olive oil, under the conditions set forth above, show that the reaction (or rather sum of the reactions) is apparently similar to a unimolecular one. Three different quantities of oil with the same volume of solution were kept at 60° C., and equal volumes were withdrawn from each for titration at definite intervals. "T∞" (∞ equal to 3 days) corresponded with a consumption of 15 ml. of $N/1$ per g., while the times for the completion of any particular fraction of the reaction were approximately the same in all three instances, the time chosen for the "oxidation value" test corresponding with roughly one-third of the completed reaction. Since 1 g. of olive oil should require 26 ml. of $N/1$ for complete rupture, only 60 per cent. of this amount was

used. The determination of the order of the reaction was, however, not very satisfactory, as there was no definite evidence of any true end-point being reached.

SUMMARY.—A test has been devised for the detection of adulteration of animal and vegetable fats, which, while giving good and reproducible results, is rapid and suitable for use in routine analyses.

Taken in conjunction with the iodine value, it enables the proportions in a mixture to be calculated with greater accuracy, while for certain purposes, *e.g.* the detection of hardened fats, the test should prove of special value.

The results obtained show the very close relationship (linear) which may be brought about between the component unsaturated fatty acids of a fat and the amount of oxidation in solution.

The number of oils so far tested is admittedly rather limited, but they have all been from reliable sources. Oxidation values of samples received for analysis in the ordinary way have not been included in the list given above, even when the oils were found genuine by the customary tests.

I am indebted to Mr. Tankard, City Analyst, Hull; Mr. Appleyard, The British Creameries, Ltd., Hull; Mr. J. L. Bennet, Messrs. Alder & Mackay, Edinburgh; and Dr. Stokoe, The Craigmillar Creamery Co., Ltd., Edinburgh, for their kind help in procuring samples.

REFERENCES

1. C. H. Lea, *J. Soc. Chem. Ind.*, 1934, **53**, 388T.
2. ——, "Rancidity in Edible Fats." Dept. of Scientific and Industrial Research Food Investigation Board. Special Report No. 46, p. 86 (*cf.* ANALYST, 1938, **63**, 660).
3. T. P. Hilditch, *Chemistry and Industry*, 1935, **13**, 139, 163, 184.

CHEMICAL LABORATORY
4, LINDSAY PLACE
EDINBURGH

October 1st, 1938

DISCUSSION

Professor T. P. HILDITCH said that he wished in the first place to congratulate Mr. Alexander on the work he had done. It seemed that he had produced a definitely new characteristic for fatty oils or mixtures, which should prove of considerable service. It was, perhaps, remarkable that, with all the attempts that had been made previously, such a simple method of oxidation had not been brought forward before, and the Society was fortunate in having such a method presented to it that evening. He would like to ask Mr. Alexander how far he thought that the ordinary analyst could obtain with ease reproducible results such as had been shown in the paper. It seemed to him, for example, that possibly special attention would be required in thermostatic control, and for the exact maintenance of temperature, time, etc. He would like Mr. Alexander to elaborate in more detail the pros and cons of the oxidation method as compared with the thiocyanogen method, because although the latter was perhaps subject to certain difficulties, he thought that general experience was that, with sufficient attention to detail, it worked quite well; and it seemed to be no more difficult than the method now propounded. With regard to Mr. Alexander's explanation of the varying oxidation values obtained, he thought that glyceride structure would hardly enter into the matter; differences in the nature of the unsaturated acids present were more likely to be the cause. For instance, possible differences in the reactivity to oxidation of *iso*-oleic acids in hydrogenated oils, or of unsaturated

acids of varying molecular weight (chain length), as in whale oil, etc., might play a part.

Referring to "inhibitols," Professor Hilditch said that these were not resistant to oxidation. In the case of atmospheric oxidation they could protect a fat, but in this reaction, with a large excess of active oxygen present in the reacting system, they would be almost instantaneously destroyed by oxidation.

Mr. K. A. WILLIAMS offered his congratulations to the author on a very stimulating paper. He thought it a pity that 60° C. had been chosen for the reaction temperature. It was obvious that the temperature must be very closely regulated; and since 60° was not one of the temperatures usually maintained in the laboratory it would mean keeping special apparatus for this test alone if it were adopted as a routine test. He enquired whether it would be possible to use an ordinary incubator temperature or even 100° C. with corresponding alteration to the duration of the reaction. He had noticed with great interest the curious abnormality obtained with hardened oils and wondered if this was in any way due to a protective influence of the minor constituents of the oils concerned. In the case of cottonseed oil in particular he referred to a new waxy constituent of the unsaponifiable matter recently isolated in Mr. Bolton's laboratory, and suggested that this might not be without effect. He very much admired the regularity of Mr. Alexander's formula. He (Mr. Williams) suggested that further valuable information might be obtained by a study of the early stages of the reaction, since it seemed possible that greater differences would occur in the behaviour of different oils at early than at later stages.

Mr. H. E. MONK, referring to the question of temperature, said that the objection was that every author introduced a new temperature which had to be kept constant. The ordinary practising analyst would prefer a lower temperature. Perhaps the author could tell them something about the temperature coefficient. Had he considered the effect of the unsaponifiable matter, especially where this had a high iodine value, or examined the unsaponifiable matter separately by his test?

Mr. ALEXANDER, replying, said that it was of course necessary that the temperature be accurately controlled. It must not change more than plus or minus 0·5°; a constant temperature of 61° C. would make an appreciable difference; the result would be 0·2 unit higher with olive oil. He, personally, had found no difficulty in keeping to this temperature for one hour, even without thermostatic control. He had tested oils at temperatures other than 60° C., but at about that temperature the most selective results were obtained with *N* sodium dichromate. Using chromic acid, he found that 15° to 20° C. was more satisfactory, although results in general with the latter reagent were more in line with the iodine value. With regard to the rate of oxidation, the systems appeared to behave as though monomolecular reactions were taking place. This was indicated by the empirical constants *a*, *b* and *c*, which were derived, but for olive oil a more complete test, using three different concentrations (0·3 to 1·2 g. of oil per 10 ml. *N*/1), showed that in a definite time a definite fraction of the apparently completed reaction had taken place in all three instances.

He was very interested in Mr. Williams' observations regarding minor constituents; he did not know positively if they had any effect, but considered it unlikely, as the figures under consideration were too great (representing roughly one-third of the total oxidation) to be influenced by traces of unsaturated compounds. The cottonseed oil variation, commented on by Mr. Williams, was shown only by the hardened oil; unhydrogenated oil agreed very well.

The point about unsaponifiable matter was rather similar to that raised by Mr. Williams; he did not see how a quantity present only to the extent of about 1 per cent. at most could affect the results very much. He had not tested the unsaponifiable matter separately.

It had not seemed to him that this wet oxidation was comparable to atmospheric oxidation at all. He thought that the linear relationship found between the component unsaturated acid content and the oxidation values of the different oils showed that this value could not be influenced by the existence of pro- or anti-oxidants.

Mr. Williams had suggested that figures obtained in the initial stages of the reaction might give valuable results. That might be so (*i.e.* "*a*," "*b*" and "*c*" might show even greater differences), but such figures were more difficult to obtain, since the velocity in the early stages was so much higher. The time taken in manipulation was liable to introduce considerable error. Sixty minutes was found to be long enough to give reasonably accurate results in this respect, and yet not be inconveniently long.

In reply to Dr. Hamence, the author said that the figures given for lard showed that the oxidation range was not wide.

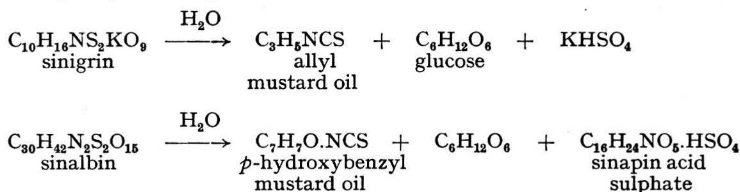
Determination of the Essential Oils of White and Brown Mustards

By R. C. TERRY, M.Sc., A.I.C., and J. W. CORRAN, Ph.D., F.I.C.

(*Read at the Meeting, December* 7, 1938)

THE mustard flour of commerce is a mixture of the flours of two seeds: brown or black, mustard (*Brassica nigra*) and white mustard (*Sinapis alba*). Its condimental properties are largely due to the essential principles of these two seeds.

The essential principle, or volatile oil, of brown mustard is allyl *iso*thiocyanate, a fairly stable liquid which lends itself to ready determination owing to its volatility in steam. The essential principles of brown and white mustards are not present as such in the seeds, but are produced from their respective glycosides, sinigrin (potassium myronate) and sinalbin, by the agency of the enzyme myrosin. These reactions, which are known as the resolution of the glycosides, take place when water is added to mustard flour, and consist in hydrolysis of the glycosides according to the following empirical equations:



$$\underset{\text{sinigrin}}{C_{10}H_{16}NS_2KO_9} \xrightarrow{H_2O} \underset{\text{allyl mustard oil}}{C_3H_5NCS} + \underset{\text{glucose}}{C_6H_{12}O_6} + KHSO_4$$

$$\underset{\text{sinalbin}}{C_{30}H_{42}N_2S_2O_{15}} \xrightarrow{H_2O} \underset{p\text{-hydroxybenzyl mustard oil}}{C_7H_7O.NCS} + C_6H_{12}O_6 + \underset{\text{sinapin acid sulphate}}{C_{16}H_{24}NO_5.HSO_4}$$

It would appear in the light of recent evidence that the mechanism of formation of the essential principles is inadequately expressed by the above stoichiometrical equations. This view is supported by the considerable variation in results obtained in ordinary determinations of volatile oil, when made under slightly differing conditions. This is discussed later.

The essential principle of white mustard is of similar condimental importance to that of brown, but there is up to the present no accepted method for its determination. This is due to the fact that it is more complex chemically than the

allyl compound, being the *p*-hydroxybenzyl ester of *iso*thiocyanic acid; it is a rather unstable substance, destroyed at a relatively low temperature, and, moreover, not volatile in steam. The method of determination that we have adopted is accordingly an indirect one, based on the resolution reaction shown above. It will be seen from this reaction that each molecule of sinalbin produces one molecule each of hydroxybenzyl mustard oil, glucose and the acid sulphate of an aliphatic base, sinapin.

If, therefore, the sulphate-content of a white mustard flour be determined both before and after resolution by myrosin, the difference will be a measure of the sinalbin (or of the essential principle) contained in the flour. Of these two sulphate determinations made on the flour, the first will be referred to as the "blank" determination, and the second as the determination of the "resolved value."

We have found the determination of this sulphate in the usual manner (*i.e.* with barium chloride) to be an operation of some difficulty. This is due to the presence of interfering substances (such as proteins) in the mustard, and therefore precipitation with benzidine under controlled conditions has been employed in the present method.*

For the determination of the "blank value" it is necessary to destroy the myrosin in order to prevent the resolution reaction; this is done by mixing the mustard with a saturated solution of mercuric chloride, followed by water. Any sulphate naturally present is thus extracted from the flour, and this extract is then treated with benzidine in acid solution, acetone being added to ensure complete precipitation of the benzidine sulphate. The precipitate is filtered off, washed and determined titrimetrically.

For determination of the "resolved value" the mustard is mixed with water and allowed to stand for a time, and the aqueous extract is then treated with benzidine as before. In both determinations a preliminary treatment of the aqueous extract to remove phosphate is necessary.

The following is a description of the method in full. It is applicable to all commercial mustard flour mixtures, as is explained below.

METHOD.—*Reagents.*—The following reagents are required:—(i) ammonia solution: approximately 6 *N* (33 per cent. of ammonia solution of analytical reagent quality); (ii) hydrochloric acid: approximately 2 *N* (20 per cent. solution of hydrochloric acid of analytical reagent quality); (iii) benzidine solution: 4 g. of purest benzidine are dissolved in 200 ml. of water plus 50 ml. of 2 *N* hydrochloric acid and filtered (moulds develop on this solution and it should therefore be filtered occasionally); (iv) acetone: of analytical reagent quality, diluted to 90 per cent., neutral; (v) saturated mercuric chloride solution; (vi) *N*/10 sodium hydroxide solution; (vii) *N*/10 sulphuric acid.

Method.—(*a*) *Determination of the "Resolved Value."*—Four g. of the mustard are weighed into a dry 100-ml. beaker and carefully mixed with 70 ml. of cold water, care being taken to avoid the formation of lumps. The mixture is allowed

* Since this paper was written a method for the determination of white mustard glycosides has been published by K. H. Bauer and A. Holle (*Pharm. Zentralhalle*, **1937**, **78**, 545). These authors determine the sulphate in mustard extracts, before and after resolution, by means of barium chloride. The solutions are freed from interfering substances by means of potassium ferrocyanide, zinc acetate and acetic acid (Carrez, *Ann. Chim. anal.*, 1909, **14**, 187).

to stand for 1½ hours at room temperature (16–18° C.) in order to resolve the glycosides, and then filtered cold into a 100-ml. graduated flask, and the residue is washed with sufficient cold water to bring the volume up to about 90 ml.

One ml. of ammonia solution is added, followed by about 0·2 g. of light magnesium carbonate, and the mixture is made up to the mark and well shaken. It is then left to stand for ½ to 1 hour, during which time a copious precipitate settles; this contains the phosphates. The mixture is filtered through a dry paper, and aliquot portions of 25 ml. are taken for precipitation of the sulphate.

Twenty-five ml. of the filtrate are placed in a 100-ml. beaker, acidified with 1 ml. of 2 *N* hydrochloric acid, treated with 20 ml. of benzidine solution very slowly, a few ml. at a time, with thorough stirring, and left for two minutes to ensure complete precipitation, after which 40 ml. of acetone are added and the liquid is left for 30 minutes.

The precipitate of benzidine sulphate is filtered off on a mat of asbestos, in a glass filter-tube provided with a perforated disc, with the aid of suction and washed well with 25 ml. of acetone; it is then washed together with the asbestos and disc into a flask with 50 ml. of water. Ten ml. of *N*/10 sodium hydroxide solution are added and the whole is heated to boiling and titrated at boiling-point with *N*/10 sulphuric acid, methyl red being used as indicator.

(*b*) *Determination of the Blank Value.*—Four g. of the mustard flour are weighed into a dry 100-ml. beaker, and stirred up with 10 ml. of mercuric chloride solution, added all at once. When all lumps are broken up, 60 ml. of water are added, and the mixture is allowed to stand for 5 minutes. It is then filtered cold into a 100-ml. graduated flask, and the residue is washed with sufficient cold water to bring the volume up to about 90 ml.

Two ml. of ammonia solution are added, followed by about 0·2 g. of light magnesium carbonate, and the mixture is made up to the mark and well shaken. It is left for ½ to 1 hour, for the copious precipitate to settle. The liquid is filtered through a dry paper. Twenty-five-ml. portions of the filtrate are treated with benzidine exactly as in the determination of the "resolved" value, and the precipitate is titrated with *N*/10 sulphuric acid as described.

The amount of sodium hydroxide used in the "blank" titration is deducted from that used in the "resolved" determination. These amounts are, of course, given by the difference between the alkali added and the acid titre in each instance.

Provided that exactly 4 g. of flour are taken in the first place, each ml. of *N*/10 sodium hydroxide solution used is equivalent to 4·8 mg. of sulphate (SO_4) produced in the reaction, and, hence to 0·495 per cent. of allyl mustard oil in a brown flour, or to 0·825 per cent. of sinalbin mustard oil in a white flour.

The glycoside equivalents are:—1·98 per cent. of sinigrin in a brown flour, or 3·67 per cent. of sinalbin (anhydrous) in a white flour, or 4·12 per cent. sinalbin (+5 H_2O) in a white flour.

NOTES ON THE METHOD.—(1) The ammonia and magnesium carbonate are added for the purpose of removing phosphate, which would otherwise interfere with the determination. (2) A fixed small amount of hydrochloric acid is used for re-acidification of the extract before precipitation. Too much produces a precipitate of protein nature, which renders the benzidine filtration very slow.

(3) All the manipulations are carried out in the cold, as some commercial compound mustards contain starch; in this way the starch is removed at the beginning of the determination. If the starch is gelatinised, subsequent filtrations are rendered very difficult. (4) The "blank" value on an ordinary mustard is very low, and will in general be zero. It may thus often be omitted, at the discretion of the analyst. (5) The sulphate is precipitated with benzidine slowly in order to obtain a granular precipitate which can be easily filtered off. (6) It is essential to use acetone of analytical reagent quality, as commercial grades frequently appear to yield a precipitate with mercuric chloride in the "blank" determination. (7) The times and temperatures of resolution must be closely adhered to. Prolonged resolution at high temperatures causes an apparent increase in sulphate, presumably owing to decomposition of essential oils. (8) The entire process must be carried through in a day. On standing overnight the mustard extracts (at any stage in the process) turn green and much of their sulphate is precipitated. Only the benzidine precipitate can be left overnight.

MIXTURES OF BROWN AND WHITE FLOURS.—The method, as detailed, can be used to determine the content of essential oil or glycoside in brown or white mustard alone. However, by combining it with the ordinary volatile oil determination, mixtures of brown and white flours, with or without the admixture of additional materials (such as starch, etc.), can be completely analysed. Such mixtures include the majority of commercial mustard flours. The total sulphate resulting from the resolution of the mixed glycosides is obtained by the present method. The volatile oil distillation method gives that portion of the sulphate due to the brown mustard flour present. The difference is a measure of the essential oil of white mustard.

The method employed for the determination of volatile oil is the usual laboratory method due to Raquet[1] in which mustard is resolved with water and alcohol for 2 hours at 37° C.; the volatile oil is then distilled into dilute ammonia. The distillate is treated with silver nitrate, the sulphide is made to coagulate, and the residual silver is titrated with thiocyanate.

CALCULATION FOR MIXTURES OF BROWN AND WHITE FLOURS.—The following results are typical of those given by an ordinary English brown mustard flour:

Volatile oil, by distillation	1·5 per cent.
Volatile oil, by sulphate method	1·75 ,, ,,

Thus results by the sulphate method are 15 to 20 per cent. higher than those obtained by distillation. This may for the moment be taken as an empirical fact; the matter is further discussed later (p. 171). Hence it is necessary to apply a correction factor of 1·2 in order to make distillation results comparable with those obtained by the sulphate method.

The present method gives a value, S, for the total sulphate (SO_4) evolved by the resolution of the mixed glycosides. If 4 g. of mustard are taken for the determination, each ml. of $N/10$ sodium hydroxide solution $\equiv$ 0·48 per cent. of sulphate produced from resolution of the glycosides.

From an ordinary volatile oil determination, S_b, that part of the total sulphate

corresponding with the brown mustard glycoside, is calculated by applying the above-mentioned factor of 1·2:

$$S_b = 0{\cdot}97\ (1{\cdot}2 \times V)$$

where V = volatile oil content.

The sulphate, S_w, due to the white mustard glycoside, is then given by the difference between these two values:

$$S_w = S - S_b,$$

and from this the percentages of essential oils of white and brown mustards in the sample can be obtained by using the appropriate factors:

Essential oil of white mustard	=	$1{\cdot}72 \times S_w$
Sinalbin ($5H_2O$)	=	$8{\cdot}59 \times S_w$
Essential oil of brown mustard	=	Volatile oil result, V
Sinigrin	=	$4{\cdot}01 \times V$

Typical values obtained for the essential oil contents of English mustard flours are as follows:

Essential oil of brown mustard, distillation method = 1·5 per cent.

" " " " " sulphate " = 1·75 " "

" " " white " " " = 3·2 " "

By using these figures as a basis, the constituents of any mixed mustard flour can be determined with fair accuracy. It must, however, be noted that the essential oil content, even of English flours, is subject to some variation and, therefore, determinations on the original components of the mixture, if possible, will lead to considerably increased accuracy.

Some values obtained by the present method on certain foreign flours are shown below:*

Flour from	Essential oil of white mustard Per Cent.	Essential oil of brown mustard Per Cent.
Dutch yellow seed	2·99	—
Roumanian yellow seed ..	3·08	—
German yellow cake	3·66	—
Montana yellow seed	3·32	—
Montana Oriental seed ..	—	1·36
Cochin seed	—	1·11

Optimum Conditions for the Sulphate Method.—During the investigation of the most favourable conditions for the sulphate determination, the method was examined step by step to ascertain the optimum conditions for each operation.

* These results were obtained through the courtesy of Dr. M. C. Albrech and Mr. A. Gendreau of the R.T. French Company, Rochester, N.Y., using the present sulphate method.

1. *Reaction of Resolution.*—The following results were obtained:

Temperature at which resolved ..	Brown flour 16° C.	Brown flour 37° C.	White flour 16° C.	White flour 37° C.
Hours resolved	Essential oil Per Cent.	Essential oil Per Cent.	Essential oil Per Cent.	Essential oil Per Cent.
1	1·69	1·76	3·17	3·27
2	1·74	1·81	3·21	3·31
4	1·77	1·89	3·25	3·39
8	1·75	1·99	3·22	3·54
16	1·81	1·94	3·41	3·82
32	1·88	2·07	3·57	4·06

These results clearly show that prolonged resolution, especially at high temperatures, causes a marked increase in sulphate; this may be due to an oxidation type of decomposition of the essential oil, and is specially noticeable with the unstable oil of white mustard. Resolution at 16° to 18° C. for about 1½ hours, however, produces constant results.

2. *Removal of Phosphate.*—Additions of 0·5 ml. up to 5 ml. of ammonia solution, and of 0·1 g. up to 5 g. of magnesium carbonate to the extract, have no effect on the result.

3. *Acidification of the Phosphate-free Extract.*—Additions of 0·5 to 2 ml. of hydrochloric acid here have little effect; quantities greater than this cause precipitation in the liquid. This precipitate has no effect on the final result, but causes great difficulty in filtration. It is believed to be of protein nature.

4. *Precipitation with Benzidine.*—The following results were obtained with white flour:

Benzidine added, ml.	5	10	20	40	20
Acetone added, ml.	40	40	40	40	0
Essential oil, on 4 g. of mustard flour, per cent.	3·12	3·15	3·22	3·24	3·14
„ „ „ 1 g. „ „ „ „ „	—	3·26	3·23	3·27	1·95

These figures show that, to ensure complete precipitation, a considerable excess of benzidine (10 to 30 ml.), together with 40 ml. of acetone, is necessary. The acetone is particularly essential when only small quantities of sulphate are being precipitated, as can be seen from the last column.

Production of Sulphate from Glycosides.—Eight g. of white mustard flour were resolved for 1½ hours in 100 ml. of water, and divided into two portions. The first portion was resolved in the usual manner, and after removal of phosphate was made up to 100 ml. Portions of 25 ml. were treated with benzidine and titrated as previously described.

The second portion was treated similarly, after having been resolved for a further hour with the addition of 0·100 g. of purest sinalbin ($5H_2O$) dissolved in 10 ml. of water. The following results were obtained:

	Sinalbin ($5H_2O$) I g.	Sinalbin ($5H_2O$) II g.
4 g. of mustard contained	0·5912	0·5925
4 g. of mustard plus 0·100 g. of sinalbin contained	0·6916	0·6919
Difference	0·1004	0·0994

These results therefore establish the theoretical basis of the determination and indicate that the method gives a fairly accurate quantitative assessment of sinalbin and its resolution products.

ANALYSIS OF MUSTARD FLOUR MIXTURES.—The following results were obtained, by various workers, on mustard flour mixtures made up for the purpose:

Mixture	Brown mustard flour		White mustard flour		Cereal addition	
	Present Per Cent.	Found Per Cent.	Present Per Cent.	Found Per Cent.	Present Per Cent.	Found Per Cent.
A	nil	nil	70	69	30	31
B	nil	nil	40	39	60	61
C	32	33	68	65	nil	nil
D	45	47	25	22	30	31
E	48	46	32	32	20	22

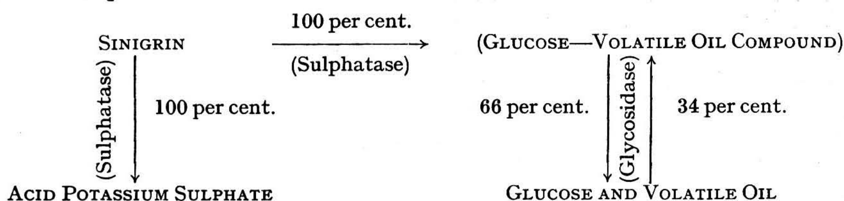
NATURE OF THE REACTION OF RESOLUTION OF MUSTARD GLYCOSIDES.—It has already been noted that when results obtained by the sulphate method and by the volatile oil distillation method are compared, it is necessary to apply a correction factor of 1·2 to the latter in order to make the two comparable. This presumes that the present method gives a true result, and that ordinary volatile oil results are low by about 15 to 20 per cent.

The determination of volatile oil has occupied the attention of a large number of chemists at different times, and consequently a great variety of different methods has been put forward. These chemists have disagreed mainly about the times and temperatures of resolution of the mustard. Thus the original method of Roeser[2] prescribes maceration for 2 hours at room temperature, whilst the official French Codex method gives only one hour at 17° C. In Raquet's[3] modification of the Codex method the maceration is for an hour at 30° to 35°, whereas Carles,[4] and Luce and Doucet[5] recommend only 15 minutes at 40° C. The optimum temperature for liberation of the volatile oil has even been placed as high as 68° to 70° C. by Astruc and Mousseron,[6] though it is known that the enzyme is destroyed at about 80° C.

Additional light has been shed on this problem by the work of Sandberg and Holly,[7] who have examined the resolution reaction of brown mustard under controlled conditions, determining only the sulphate and glucose split off. In the first place they have confirmed the conclusion of von Euler and Erikson,[8] that myrosin consists of two separate entities, a glycosidase and a sulphatase; the sulphatase is responsible for liberating the sulphate group from the glycoside, which is further split by the action of the glycosidase. They further find that only 66 per cent. of the theoretical glucose is split off, whereas the amount of sulphate liberated is as much as 100 per cent. of theory.

If, then, it is assumed that a solution of sinigrin has been resolved by myrosin, 100 per cent. of sulphate has been split off, together with 66 per cent. of glucose. The remaining 34 per cent. of glucose must remain combined with the volatile oil in some intermediate compound; the net effect is thus that only 66 per cent.

of the theoretical volatile oil **is** produced in this reaction; it is suggested that there may be an equilibrium as follows:



The 100 per cent. reaction takes place by action of sulphatase; the 34 to 66 per cent. equilibrium is catalysed by the glycosidase. Let us then assume that the mixture is distilled. As the temperature rises volatile oil is removed, and the equilibrium changes; the intermediate compound may also be decomposed to some extent. The net result is a yield of volatile oil, varying with conditions.

This hypothesis is put forward as an explanation of the lower results obtained by volatile oil distillation, compared with those given by the sulphate method. Some confirmation is yielded by recent work by Gros and Pichon[9] on the volatile oil estimation. These authors distil off volatile oil *in vacuo* at a temperature not exceeding 70° C.; at this temperature the enzymes are not destroyed. The yields are about 15 per cent. higher than those obtained by the Codex method. If the above explanation is correct, this would enable the equilibrium reaction to go to 100 per cent. completion, owing to the continuous removal of volatile oil.

SUMMARY.—A method has been developed for the determination of the glycosides of white (and brown) mustard. This depends upon determining the sulphate produced from these glycosides by the resolution reaction.

It has been found most expedient to determine this sulphate by precipitation with benzidine, filtration and titration of the precipitated benzidine sulphate.

By the use of this method, in combination with the usual volatile oil method, the essential oils in a mustard flour mixture, such as a table mustard, can be determined and the constituents of the mixture accordingly calculated with a fair degree of accuracy.

Certain discrepancies between the present method and the volatile oil distillation method are noted, and a possible explanation for this is put forward, together with the evidence in its favour.

The authors wish to thank the Directors of Messrs. Reckitt & Colman, Ltd., for permission to publish these results.

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RESEARCH DEPARTMENT
MESSRS. RECKITT & COLMAN, LTD.
CARROW WORKS, NORWICH

September 25th, 1938

DISCUSSION

Dr. H. E. COX asked why the authors had determined the sulphate when at first sight it would be thought simple to hydrolyse the hydroxybenzyl ester and then determine the hydroxybenzoic acid which would be more specifically characteristic of mustard than was sulphate.

Mr. H. S. REDGROVE remarked that the authors' work had thrown much light on the hydrolysis of sinalbin, and the facts they brought forward certainly seemed to indicate that their theory was correct. One point he wished to criticise was the authors' assertion that the statement that white mustard contained no essential oil was incorrect. What was the definition of "essential oil"? Surely volatility was an essential characteristic.

Dr. CORRAN, replying to Dr. Cox, said that, as analysts, they had aimed at getting the simplest method. Their experience showed that the essential principle of white mustard underwent chemical change very easily on the application of heat. They felt that in such circumstances an attempt to discover a method in the direction indicated by Dr. Cox would have been followed by less success than that claimed for the sulphate method.

Referring to the definition of essential oil, Dr. Corran said that of course there was no essential oil actually in mustard flour. It could hardly be denied, however, that the two pungent oils were regarded as the essential features of mustard. Perhaps the use of the term "essential principles" for these oils and their glycosides might be less controversial.

Electrolytic Determination and Separation of Bismuth

Part I. Determination and Separation in Chloride Solution

BY F. G. KNY-JONES, M.Sc.

(*Read at the Meeting, December* 7, 1938)

THE electrolytic determination of bismuth by deposition from a chloride solution has hitherto presented some difficulty.

Schoch and Brown[1] carried out determinations in solutions containing approximately 5 per cent. of conc. hydrochloric acid with hydroxylamine as depolariser and obtained good results. Engelenburg,[2] however, using similar conditions, found that, while bismuth was deposited quantitatively, the deposit was of a spongy nature and not weighable. Lassieur[3] found that he was unable to obtain deposits suitable for weighing and, after a series of experiments, finally abandoned the problem.[4]

At the beginning of the present work depositions were carried out under conditions similar to those recommended by Schoch and Brown (*loc. cit.*)[1] and the modified conditions previously tried by Lassieur (*op. cit.*).[3] By neither procedure was it possible to control the auxiliary potential at a voltage sufficiently low for the production of a good deposit. After a few minutes' electrolysis the auxiliary potential rose sharply, the deposit became spongy and in some experiments was detached from the cathode. This sharp elevation of the auxiliary potential is possibly connected

with the formation of oxychloride in the cathode layer. In order to circumvent this hypothetical cause of trouble, experiments were made to find a substance that would increase the solubility of the oxychloride without at the same time considerably affecting the *p*H. Oxalic acid was tried as a solvent for bismuth oxychloride and found satisfactory.

Depositions were carried out under conditions similar to those employed by the previous workers cited, but with the addition of 5 g. of oxalic acid to 100 ml. of electrolyte. With this modification the auxiliary potential could be satisfactorily controlled and good, adherent deposits were obtained.

EXPERIMENTAL

1. DETERMINATION OF BISMUTH.—The determinations were carried out with the use of the platinum gauze electrodes, saturated calomel auxiliary electrode vessel and voltmeter described by Lindsey and Sand.[5] Measured amounts of bismuth solution were taken. The stock solution was prepared by dissolving pure bismuth in conc. hydrochloric acid (sp.gr. 1·16) containing a small amount of nitric acid and was made up to contain about 10 per cent. of conc. hydrochloric acid.

To the solution taken for each experiment were added a further 10 ml. of conc. hydrochloric acid, 5 g. of oxalic acid, 0·5 g. of hydrazine hydrochloride[6] as depolariser and water to make up 100 ml. The electrolyses were carried out at a temperature of 80–85° C., the current being regulated to give an initial auxiliary potential of 0·15 to 0·17 volt; with higher initial potentials—up to 0·20 volt—deposits tended to be non-adherent. When the current fell to zero or a small residual value the auxiliary potential was raised in the usual manner in steps of about 0·02 volt to a final value of 0·25 to 0·30 volt. Results are given in Table I.

TABLE I

Expt. No.	Bismuth taken g.	Bismuth found g.	Time Minutes
1	0·2501	0·2509	15
2	0·2501	0·2497	20
3	0·2525	0·2525	18
4	0·1010	0·1011	17
5	0·2020	0·2021	22
6	0·2001	0·2002	15

2. USE OF BROWN'S AUXILIARY ELECTRODE.—Some determinations were carried out in which, instead of the glass auxiliary electrode, an electrode of platinum wire covered with bismuth as described by Brown[7] was used. The wire was 0·3 mm. in diameter and was held suspended in the solution by means of a clamp having silver jaws. The clamp was connected with the positive terminal of a 50,000 ohm 1·2 volt voltmeter, the other terminal being connected with the outer electrode. At the beginning of a determination the platinum wire and the outer electrode were placed in parallel by short-circuiting the leads to the voltmeter through a switch. A current of 0·2 amp. was then allowed to flow for 30 seconds, this time sufficing to give the wire a coating of bismuth. The short circuiting

switch was then opened and the P.D. (ΔE) between the wire and the electrode was adjusted to a suitable value by regulating the current.

It was found that a value of ΔE of 0·02 to 0·03V. gave good results; with a higher value the deposit obtained was non-adherent and became detached during the washing process. The wire was weighed with the cathode before and after the deposition. Results obtained are given in Table II.

TABLE II

Expt. No.	Bismuth taken g.	Bismuth found g.	ΔE volt	Initial current in amp.	Time Minutes
7	0·2525	0·2525	0·03	0·4	19
8	0·2313	0·2311	0·02	0·25	27
9	0·1156	0·1156	0·025	0·25	15
10	0·1010	0·1001	0·05	0·4	13

In experiment No. 10 the deposit was non-adherent.

3. SEPARATION OF BISMUTH FROM LEAD AND TIN.—While bismuth may be separated from lead in nitric acid solution, the separation from tin has not hitherto proved satisfactory. Lassieur[8] attempted the separation in nitric acid solution, and also in the same medium with the addition of either hydrofluoric acid or aluminium nitrate. In no instance was there complete deposition of bismuth.

By the use of oxalic acid it has been found possible to deposit bismuth completely in the presence of lead or tin from hydrochloric acid solution. The results in Table III were obtained by using the conditions given in Section 1 and in each determination controlling the auxiliary potential so that its final value did not exceed 0·3 volt. The values in Table IV were obtained by using Brown's auxiliary electrode and following the procedure described in Section 2.

TABLE III

Expt. No.	Bismuth taken g.	Bismuth found g.	Time Minutes	In presence of:
11	0·1000	0·0998	12	0·08 g. of Pb
12	0·1000	0·1004	20	0·10 ,, ,, Sn
13	0·2500	0·2500	29	0·25 ,, ,, Sn
14	0·2891	0·2887	25	{0·10 ,, ,, Sn 0·20 ,, ,, Pb
15	0·2891	0·2899	30	{0·10 ,, ,, Sn 0·20 ,, ,, Pb

TABLE IV

Expt. No.	Bismuth taken g.	Bismuth found g.	ΔE volt	Time Minutes	In presence of:
16	0·2274	0·2273	0·025	30	0·10 g. of Sn
17	0·1983	0·1983	0·025	30	0·25 ,, ,, Sn
18	0·2004	0·2009	0·02	20	0·10 ,, ,, Pb

4. DETERMINATION OF BISMUTH IN BISMUTH-TIN-LEAD ALLOYS.—Ternary alloys of bismuth, tin and lead are used in the manufacture of sprinkler heads and safety plugs for boilers and for making delicate castings.[9,10]

The application of the electrolytic method to these alloys gives a more rapid method of determining the bismuth constituent than the normal chemical oxychloride separation.[11]

Samples of alloys of known bismuth-content were available through the courtesy of the Chemical Laboratory of Messrs. Siemens Bros. & Co., Ltd.

The determinations, results of which are given in Table V, were carried out as follows:

About 0·4 to 0·45 g. of drillings taken from the alloy were attacked with 1 to 2 ml. of conc. nitric acid. After the reaction had subsided in violence, 10 ml. of conc. hydrochloric acid were added, and the resulting solution was boiled. After the addition of a further 5 ml. of conc. hydrochloric acid, the solution was diluted to 100 ml. with water; 5 g. of oxalic acid and 0·5 g. of hydrazine hydrochloride were then added, and the solution was heated to 80–85° C. The electrolysis was then carried out as described above for the separation from tin and lead.

TABLE V

Alloy	Bismuth in alloy Per Cent.	Wt. of alloy taken g.	Bismuth calc. g.	Bismuth found g.	Time Minutes
A	21·0	0·4005	0·0841	0·0844	18
B	33·3	0·4002	0·1334	0·1332	28
L	51	0·4003	0·2042	0·2046	25
X	49·75	0·4005	0·1993	0·1990	20
L	51	0·4507	0·2299	0·2291	25
B	33·3	0·4523	0·1508	0·1513	35
L	51	0·4245	0·2165	0·2170	26
S	37·6	0·4001	0·1504	0·1507	28

SUMMARY.—By the addition of oxalic acid to the electrolyte it has been found possible to effect a rapid electro-determination of bismuth in chloride solutions under conditions of controlled potential, the auxiliary electrode used being either the saturated calomel electrode of Lindsey and Sand or the wire electrode of Brown.

By means of this method it has been possible to determine bismuth in the presence of lead and tin and hence in ternary alloys of the three metals.

In conclusion I wish to express my thanks to Dr. H. J. S. Sand for his interest and help in this work.

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THE SIR JOHN CASS TECHNICAL INSTITUTE
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Nigerian Ginger

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The production of ginger in Nigeria, which was started on a commercial scale about ten years ago as the outcome of the pioneer work carried out by the Department of Agriculture in that colony, has developed rapidly during this period, with the result that Nigerian ginger has won a place among the recognised grades of ginger on the world's market. Large quantities of this ginger were consumed in the United Kingdom up to 1936, and proved quite satisfactory, but certain lots from the 1935 and 1936 crops and the bulk of the consignments of the 1937 harvest, on being submitted to chemical examination, were found not to fulfil the requirements expected for peeled ginger, and in one or two cases legal proceedings followed.

The particulars in which these consignments failed were the percentages of water-soluble extractive and water-soluble ash, for which figures as low as 7·1 and 0·2 per cent. respectively were obtained. The figures used as the criterion of quality are the same as those laid down in the British Pharmacopoeia for peeled Jamaican ginger, *viz.*:

Alcohol-soluble extractive	Not less than 4·5 per cent.
Water-soluble extractive	Not less than 10·0 per cent.
(both expressed on the air-dried material) and	
Total ash	Not more than 6·0 per cent. and
Water-soluble ash	Not less than 1·7 per cent.
(both figures expressed on the material dried at 100° C.).	

On account of this failure to reach the desired standard and the fear of consequent prosecution, the market for Nigerian ginger in the United Kingdom was seriously affected. The lack of demand caused large stocks of this ginger on hand in this country to remain unsold and severely restricted the importing of fresh supplies from Nigeria into the United Kingdom. Although the sale of Nigerian ginger to the Continent was not affected by the lower quality of the product, the difficulty experienced in disposing of supplies in the United Kingdom was of great concern to the authorities and producers in Nigeria. Accordingly, the Department of Agriculture in that colony in collaboration with the Imperial Institute undertook a series of tests to determine the cause of the deficiency in water-soluble constituents of recent crops, that is to say, to ascertain whether it was an inherent feature of the ginger now produced in Nigeria, or whether it was merely due to an unsuitable method of preparation of the ginger.

Various views had previously been put forward to account for this deficiency, but the opinion generally held was that in the preparation of the ginger the rhizomes had been soaked in water for too extended a period, the object of the

prolonged immersion being to obtain a pale-coloured product, a pale colour being one of the desiderata of the trade. It was also suggested that the falling off in quality was due to soil exhaustion, but, although this may be true of the ginger produced by a few growers, the natives as a rule move their plantations after two or three years to fresh ground where the crop is often grown on virgin soil. This question, however, is being investigated by the Nigerian Department of Agriculture.

For the purpose of our investigation a large batch of ginger rhizomes was obtained by the Department of Agriculture, Nigeria, and divided into smaller lots, and each lot was treated by one of the six methods (Trials 1, 2, 4, 6, 9, and 11) described in this paper. Samples of the products thus prepared (24 in all) were forwarded to the Imperial Institute, where they were submitted to chemical examination by the British Pharmacopoeia methods. The results obtained are recorded below.

Trial 1.—The rhizomes as harvested, after removal of the roots, were washed free from soil and dried in the sun.

The sample was examined with the following results:

Sample No.	Alcohol-soluble extractive Per Cent.	Water-soluble extractive Per Cent.	Total ash Per Cent.	Water-soluble ash Per Cent.
1	6·4	16·3	7·7	5·8

Trial 2.—Dried peeled ginger was prepared from the rhizomes by the method previously advocated by the Department of Agriculture, which yielded a product of a quality hitherto freely accepted by the trade.

The rhizomes were freed from roots and washed free from soil on the same day as they were dug. The next morning they were scraped. While the scraping was in progress the unpeeled rhizomes were stored in water, and as each hand was peeled it was placed in clean water. When scraping was completed the ginger was washed* by gently rubbing and stirring it in six changes of water. As it was necessary to wash the ginger in batches, some of it was soaking* for a considerable time. The average time of soaking between scraping and washing was 1 hour. After being washed the ginger was spread on mat-covered platforms and turned three or four times a day until thoroughly dry.

The dried ginger was soaked for 10 minutes, washed once in water for 5 minutes, then spread out on platforms and dried. This process was repeated eight times, *i.e.* until the required colour was obtained. The products were sampled at the end of each stage of the process, and the samples were examined.

Sample 1 was of light greyish-brown colour and hard. The colour of the hands improved from sample to sample throughout the whole series, No. 10 being very pale buff. As the colour improved, so the ginger became less hard, the hands of Sample 10 being fairly soft.

* "Washing" means stirring and gently rubbing the ginger in water. "Soaking" is used to denote leaving the ginger undisturbed in water.

The samples were examined, with the following results:

Sample No.		Alcohol-soluble extractive Per Cent.	Water-soluble extractive Per Cent.	Total ash Per Cent.	Water-soluble ash Per Cent.
1	1st washing ..	6·1	16·6	6·3	5·2
2	2nd ,, ..	5·9	14·8	6·0	4·9
3	3rd ,, ..	5·7	13·7	5·2	4·2
4	4th ,, ..	5·8	13·2	5·3	4·2
5	5th ,, ..	5·6	12·5	5·0	3·9
6	6th ,, ..	5·6	12·5	4·4	3·2
7	7th ,, ..	5·5	12·0	4·4	3·4
9	9th ,, ..	5·4	11·8	4·1	3·2
10	10th ,, ..	5·8	12·0	3·9	3·0

Trial 4.—In order to determine the effect of varying the period of soaking after scraping and prior to the first washing of the hands, separate batches of dried ginger were prepared by the same method as that used in Trial 2, *i.e.* by the Department of Agriculture method, but employing, instead of an initial soaking of 1 hour, a period of 2, 6, 12, 24, or 48 hours.

The five samples were examined, with the following results:

Sample No.		Alcohol-soluble extractive Per Cent.	Water-soluble extractive Per Cent.	Total ash Per Cent.	Water-soluble ash Per Cent.
4A	2 hrs. soaking ..	6·0	13·3	3·9	3·0
4B	6 hrs. ,, ..	6·0	12·9	4·3	3·3
4C	12 hrs. ,, ..	6·0	12·6	3·9	3·0
4D	24 hrs. ,, ..	5·9	12·8	4·2	3·2
4E	48 hrs. ,, ..	5·9	12·0	3·8	2·8

Trial 6.—*Native-prepared Ginger.*—In this experiment, the method used to prepare the ginger exported early in 1937 and condemned by the market was followed as closely as possible. The ginger was washed in a basket immersed in a stream, the basket being lifted periodically to allow the water to drain and then re-immersed. After being scraped the ginger was washed for 8 minutes and then dried. It was soaked overnight for 13 hours 40 minutes and washed for 10 minutes; dried; soaked for 6 minutes; washed for 5 minutes; dried; soaked for 5 minutes; washed for 5 minutes; dried; washed for 5 minutes without previous soaking; dried; washed for 5 minutes; dried; washed for 5 minutes; dried. The total number of washings given was thus 7.

The ginger thus prepared was very similar in appearance to Sample 10 of Trial 2, being perhaps slightly better in colour. On examination it gave the following results:

Sample	Alcohol-soluble extractive Per Cent.	Water-soluble extractive Per Cent.	Total ash Per Cent.	Water-soluble ash Per Cent.
Native-prepared ..	5·4	8·4	2·4	1·6

Trial 9.—The ginger was prepared as in Trial 2, but the first washing consisted of 3 changes of water instead of 6 changes. The total number of washings was 10.

The resulting ginger was very similar in appearance to Sample 10, Trial 2. It gave the following results:

Sample No.	Alcohol-soluble extractive Per Cent.	Water-soluble extractive Per Cent.	Total ash Per Cent.	Water-soluble ash Per Cent.
9	5·2	12·9	4·0	3·3

Trial 11.—The object of this trial was to determine the leaching effect of soaking the dried ginger overnight for 12 hours prior to each of the second and subsequent washings. Seven samples were prepared.

The seven samples were examined with the following results, which are shown in comparison with those obtained for corresponding samples in Trial 2:

Sample No.		Alcohol-soluble extractive Per Cent.	Water-soluble extractive Per Cent.	Total ash Per Cent.	Water-soluble ash Per Cent.
2 (1)	1st washing ..	6·1	16·6	6·3	5·2
11 (1)	2nd ,, ..	5·2	10·2	3·4	2·5
2 (2)	2nd ,, ..	5·9	14·8	6·0	4·9
11 (2)	3rd ,, ..	4·7	7·7	2·3	1·2
2 (3)	3rd ,, ..	5·7	13·7	5·2	4·2
11 (3)	4th ,, ..	4·7	6·9	1·9	0·6
2 (4)	4th ,, ..	5·8	13·2	5·3	4·2
11 (4)	5th ,, ..	4·3	6·5	1·7	0·6
2 (5)	5th ,, ..	5·6	12·5	5·0	3·9
11 (5)	6th ,, ..	4·2	6·5	1·5	0·5
2 (6)	6th ,, ..	5·6	12·5	4·4	3·2
11 (6)	7th ,, ..	4·4	6·0	1·5	0·2
2 (7)	7th ,, ..	5·5	12·0	4·4	3·4
11 (7)	8th ,, ..	4·4	6·2	1·5	0·3
2 (8)	8th ,, ..	(no sample received)			

The following comments may be made on the foregoing results:

Trial 1.—This unpeeled ginger is of satisfactory composition. Its figures meet the standard requirements, with the exception of the amount of total ash; in this connection it may be stated that the British Pharmacopoeia figures are intended to apply to *peeled* and not to *unpeeled* ginger.

Trial 2.—The results show that even after being washed and dried ten times the ginger produced still fulfils the requirements of the British Pharmacopoeia, provided that no prolonged soaking of the dried hands has taken place. It will be noticed that the effect of successive washings is a distinct gradual lowering of the percentages of water-soluble extractive, total ash and water-soluble ash, while the effect on the alcohol-soluble extractive is only slight. The figures also indicate

that the ginger does not suffer from any inherent deficiency of water-soluble constituents.

Trial 4.—This experiment shows that soaking the hands for periods up to 24 hours after scraping, and prior to their first washing, has no marked effect on the composition of the ginger. When the duration of the soaking was 48 hours a slight loss of water-soluble constituents took place, but the resulting product was still up to standard.

Trial 6.—The main differences in the methods of preparation of the native-produced ginger of Trial 6 and of Sample 7 of Trial 2 are that the former had prolonged immersion in a stream and a long soaking immediately prior to the second washing. The main differences in the preparation of this native-produced ginger and of Sample 1 of Trial 11 are that the former had a prolonged immersion in a stream and had been washed and dried five times more. Both Sample 7 of Trial 2 and Sample 1 of Trial 11 are up to standard, but the native-prepared ginger of Trial 6 is deficient in water-soluble constituents, and this deficiency would appear to be due to the ginger being kept in water too long.

Trial 9.—The results of this experiment show that reducing the number of changes of water used in the first washing has had the effect of slightly increasing the percentages of water-soluble constituents.

Trial 11.—The results of this trial indicate that soaking the dried ginger for long periods of time reduces very considerably the percentages of water-soluble constituents. The soaking for 12 hours prior to the second washing was sufficient to reduce the percentage of water-soluble extractive by 4·6 below the figure given by the corresponding sample in Trial 2 and the further soaking of 12 hours given prior to the third washing brought the product well below the standard.

The results of the investigation have shown that:

(1) There is no inherent deficiency of water-soluble constituents in Nigerian ginger as represented by the samples examined.

(2) A product of satisfactory quality can be readily prepared, even when the ginger is washed and dried ten times, provided that excessive soaking of the dried, peeled ginger is avoided.

(3) The method of preparation advocated by the Department of Agriculture in Nigeria, which in the past has given a product acceptable to the trade, is satisfactory and can be recommended.

(4) The ginger can be stored in water, after being scraped and prior to being washed for the first time, for a period up to at least 48 hours without serious loss of water-soluble constituents.

(5) The method of preparation as used by the natives in 1937 is unsatisfactory and should not be employed, as the prolonged immersion of the *dried*, peeled ginger in water reduces considerably the percentage of water-soluble constituents.

(6) Reducing the number of changes of water in the first washing increases slightly the percentage of water-soluble constituents.

(7) Prolonged soaking of peeled ginger, once it has been dried, has a serious adverse effect on the percentage of water-soluble constituents and should therefore be avoided.

With a view to getting the producers to conform more closely to the recommended method of preparation, the Department of Agriculture have adopted a scheme of instruction and educational propaganda. Already, improved results have been obtained, and ginger containing higher percentages of water-soluble constituents has been prepared by the native producers in Nigeria during the past year, but, although the content of water-soluble extractive has been raised to a satisfactory figure, there is still a strong tendency for the water-soluble ash percentage to be below the standard. Nevertheless, it is expected that before long the bulk of the Nigerian ginger prepared will fulfil the requirements of the consumers in the United Kingdom.

SUMMARY.—The present investigation has provided abundant evidence that Nigerian ginger, if properly prepared for the market, conforms to the standards of the British Pharmacopoeia. It shows that the deficiencies in regard to water-soluble constituents, observed in certain samples during **1935–1937**, are similar to those produced in these experiments by excessive soaking of the peeled ginger during preparation, and from the information received by the Imperial Institute regarding the manner in which the natives have been preparing the material there is no doubt that the deficiencies referred to are attributable to this cause.

The authors desire to express their thanks to the Director of Agriculture, Nigeria, and the Director of the Imperial Institute, for permission to publish the results of this investigation.

IMPERIAL INSTITUTE
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A Volumetric Method for the Determination of Sulphur and Sulphate Ion

BY BERTIL JOSEPHSON

MANY of the methods proposed for the volumetric determination of sulphur have been tested in this laboratory, but none has proved entirely satisfactory for amounts varying within wide limits. The value of the numerous benzidine methods is restricted by the solubility of benzidine sulphate, which is by no means negligible, and in the acidimetric[1] and alkalimetric[2] methods it is difficult to avoid the influence of either acid or alkaline products of the oxidation. The same source of error is inherent in the methods of titration with lead salt.[3,4] I have found that the most reliable method is to precipitate the sulphate with barium, precipitate the excess of barium with chromate, and titrate the excess of chromate with sodium thiosulphate, as proposed by Andrews.[5]

REAGENTS.—The following reagents are required:

(1) Barium chloride solution: 0·025 molar (6·11 g. per litre).
(2) Hydrochloric acid: 0·2 N.
(3) Acetate buffer solution: 530 g. of sodium acetate and 6 g. of acetic acid per litre.

(4) Potassium chromate solution: 0·1622 molar (3·15 g. per litre).
(5) Sodium thiosulphate solution: 0·0075 *N* (11·16 g. per litre); prepared in concentrated stock solution.
(6) Potassium iodate solution: 0·02 *N* (0·7134 g. per litre). This is used as the standard for the thiosulphate solution.
(7) Potassium iodide solution: 10 per cent.
(8) Starch solution: 0·5 per cent. containing a trace of mercuric iodide.
(9) Sulphuric acid: 2 *N*.
(10) Magnesia mixture: 220 g. of magnesium chloride, 110 g. of ammonium chloride per litre.
(11) *N* ammonia solution.

All the chemicals must be of reagent quality and the water used for the solutions should be boiled for 10 minutes.

METHOD.—Pipette into each of three 25-ml. graduated flasks 10 ml. of water or blank oxidation solution (*vide infra*), 2 ml. of 0·2 *N* hydrochloric acid and 2 ml. of the barium chloride solution. These represent the "blanks." Introduce the samples for analysis (not more than 10 ml.) into similar flasks, add 2 ml. of 0·2 *N* hydrochloric acid and 2 ml. of the barium chloride solution (if not already added during oxidation, *vide infra*). After some minutes add 3 ml. of the buffer solution (3) and, after a few more minutes, introduce 5 ml. of chromate solution (4) into each of the flasks, make the contents up to the mark with water and mix thoroughly. After 15 minutes filter through a fine filter-paper (Munktell 00) and pipette exactly 20 ml. of the filtrates into 50-ml. Erlenmeyer flasks. Add 10 ml. of 2 *N* sulphuric acid and 1 ml. of potassium iodide solution (7), leave the flasks in darkness for 15 minutes, and then titrate their contents with sodium thiosulphate solution (5), added from a 30-ml. burette graduated in 0·05 ml., until there is no colour with starch solution (8). The thiosulphate solution should be standardised on the same day against the potassium iodate solution (6).

Modification for Minute Quantities.—A modification for quantities between 0·05 and 0·30 mg. has also been devised. In this modification 5-ml. graduated flasks are used, with one-fifth of the above-mentioned quantity of each reagent, and the titration is carried out with a micro-burette graduated in 0·005 ml. Phosphates, if present, must be precipitated separately, as otherwise they will precipitate the barium. If only very small quantities (less than 0·1 mg.) are present, their influence can be almost completely prevented by the use of magnesia mixture and ammonia, as proposed by Köszegi.[6] Two ml. of magnesia mixture (10) and 1 ml. of *N* ammonia solution are added to the test and blank solutions, and the flasks are allowed to stand for 15 minutes, after which the buffer and chromate solutions are introduced as before. In this procedure the precipitated magnesium ammonium phosphate is dissolved only very slowly by the buffer solution. The effect of larger amounts of phosphates, however, cannot be avoided in this way. Heavy metals, which precipitate the chromate, must also be removed before the analysis. Strong acids and bases, oxidising and reducing substances must also not be present. Hence the method cannot be applied directly to biological liquids, such as urine, unless their reducing power is known. This can be ascertained by making a further blank determination with the same quantity of the sample, which

is treated in exactly the same way as in the original test, but without the addition of barium chloride. The titration value thus obtained represents the reducing power and should be subtracted from the value found in the usual way. This method, however, did not give such exact values as were obtained with oxidised samples.

CALCULATION.—The concentrations and amounts of the different solutions have been so chosen that each ml. of the sodium thiosulphate solution in excess of 10 ml. (the volume consumed by the chromate present, *vide infra*) corresponds with 0·1 mg. of sulphur in the original sample. Hence the difference in the titration values for the test and blank solutions corresponds with 0·1 mg. of sulphur for each ml., provided that the concentration of the thiosulphate solution (5) is calculated to that given above.

NOTES ON THE PROCESS.—The solubility of barium sulphate in water, which has been shown by Kohlrausch[7] to be about 1 in 430,000, has no significant influence on the results. Stockholm and Koch[8] found that in very dilute solution precipitation of barium sulphate was incomplete, but their solutions were relatively rich in nitrates, which are known to have a solvent action on barium sulphate. Co-precipitation of barium with the barium sulphate has no influence when the precipitation occurs slowly as in dilute solutions, such as those used in the present method (Treadwell[9]). Moreover, when the precipitate is extracted with hot hydrochloric acid, as in the oxidation process described later, any co-precipitated barium must be redissolved.

In alkaline solution barium tends to form complex chromates or adsorb CrO_3. This is a disadvantage of all methods (Callegari,[10] Norgulis and Hemphill[11]) in which barium chromate is precipitated from an acid solution by an alkaline reagent, such as ammonia or calcium hydroxide. On the other hand, in acid solution barium chromate is soluble, and for this reason Köszegi[6] and Photiadis[12] use sodium acetate as a buffer. The composition of the buffer chosen for this method is that by which the values obtained were most nearly theoretical. The solubility is also diminished by having an excess of $Ba^{\cdot\cdot}$ or CrO_4'' ions (Photiadis[12]). In previous methods (*e.g.* that of Klinke[13]), in which the chromate concentration is constant and the barium concentration varies between equivalence with the chromate and lower values, the titration curve will never follow a straight line. It has therefore proved preferable to have a considerable excess of chromate, and this has been arranged to correspond with 10 ml. of the thiosulphate solution in the titration.

Possible sources of error due to the liberation of iodine from the potassium iodide during the titration (Friedrich and Bauer,[14] Manov and Kirk[15]) are negligible with the concentrations and amounts of reagents prescribed in the present method.

OXIDATION OF SULPHUR IN ORGANIC MATERIAL.—As Friedrich and Watzlavek's[1] modification of the Pregl process is tedious, I have adapted the method of E. and M. Kahane[16] to the titration process.

The samples are introduced into small (about 25-ml.) Kjeldahl flasks of Pyrex or Duran glass. Two ml. of the barium chloride solution (1), 1 ml. of pure perchloric acid and 1·5 ml. of pure fuming nitric acid are added, and the flasks are heated gradually to 240° C. in a covered air-bath which is so constructed that the necks of the flasks project outside. After 3 to 5 hours the contents of the flasks will

have become a dry white powder (if not, the combustion with 1 ml. of perchloric acid and 1·5 ml. of nitric acid is repeated). The flasks are removed from the bath and allowed to cool; 2 ml. of alcohol saturated with ammonia are introduced, the flasks are replaced in the air-bath, and their contents are evaporated to dryness at 100° C. This treatment is repeated, and the residues are then each extracted with 2 ml. of 0·2 *N* hydrochloric acid on a boiling water-bath for 30 minutes, after which the contents of the flasks are transferred to 25-ml. flasks. The Kjeldahl flasks are rinsed out several times with water, the washings being added to the extracts. The remainder of the procedure is as described above. As in the Pregl method, blank oxidations must also be made, and the resulting solutions used as blanks for the titration.

The wet ashing process is of limited value, since with substances containing phosphorus, heavy metals or volatile sulphur erroneous results are obtained. With the refrigeration method of E. and M. Kahane,[16] also, some volatile sulphur is usually lost. Thus variable results were obtained with cysteine and ichthyol. When the perchloric and nitric acid oxidation was carried out in the absence of barium there was invariably a loss of sulphur trioxide during the evaporation of the perchloric acid.

TABLE I

WET ASHING METHOD

Substance	Concentration	Quantity	Sulphur		
			Calculated Per Cent.	Found Per Cent.	Error Per Cent.
Sulphuric acid ..	0·02008 *N*	0·5 ml.	0·161	0·165	+2·5
				0·163	+1·2
				0·163	+1·2
Sulphuric acid ..	0·02008 *N*	1·0 ml.	0·322	0·319	−1·0
				0·320	−0·6
				0·322	± 0
Sulphuric acid ..	0·02008 *N*	4·0 ml.	1·288	1·286	−0·2
				1·288	± 0
				1·281	−0·5
Taurine	0·0095 mol.	1·0 ml.	0·307	0·310	+1·0
				0·309	+0·7
				0·313	+2·0
Sodium sulphanilate	0·0114 mol.	1·0 ml.	0·365	0·361	−1·1
				0·369	−0·3
				0·365	± 0
Naphthionic acid ..	(14·08 per cent. S)	5·573 mg.	0·784	0·783	−0·1
		3·807 mg.	0·536	0·535	−0·2
		4·384 mg.	0·617	0·616	−0·2
Thiourea	(40·17 per cent. S)	3·000 mg.	1·238	1·276	+3·1
		1·500 mg.	0·619	0·620	+0·2
		1·094 mg.	0·452	0·454	+0·4
		2·189	0·904	0·930	+2·8

RESULTS.—Table I gives a selection of results obtained by the wet ashing method and Table II results by Pregl's method. In the latter method the calculated sulphur-contents were checked by macro analysis, the barium sulphate obtained after oxidation of the substance in a Parr's oxidation bomb being weighed.

TABLE II

PREGL ASHING METHOD

Substance	Sulphur-content Per Cent.	Amount mg.	Sulphur Calculated Per Cent.	Sulphur Found Per Cent.	Sulphur Error Per Cent.
Crystalline sulphur ..	100	0·861	0·861	0·853	−0·9
		1·145	1·145	1·157	+1·1
		0·436	0·436	0·453	+3·9
Cystine	26·5	2·435	0·645	0·640	−0·8
		2·979	0·789	0·762	−3·4
		2·439	0·646	0·657	+1·7
		2·928	0·776	0·775	−0·1
		1·016	0·269	0·274	+1·9
		0·837	0·222	0·227	+2·3
Taurine	25·60	1·812	0·465	0·467	+0·4
		3·310	0·849	0·854	+0·6
		4·550	1·166	1·157	−0·8
Naphthionic acid ..	14·08	3·979	0·560	0·564	+0·7
		6·577	0·926	0·931	+0·5
		3·968	0·559	0·557	−0·4
Thiourea	41·25	0·987	0·407	0·404	−0·7
		3·052	1·260	1·274	+1·1
		3·329	1·375	1·385	+0·7
Ichthyol	4·83	22·777	1·102	1·114	+1·1
		25·240	1·223	1·138	+1·2

The method has been used in this laboratory for several years with satisfactory results. The sulphur determinations of Ågren and Hammarsten and of Josephson and Jungner were made by the wet ashing procedure and those of Theorell by the dry procedure.

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CHEMICAL DEPARTMENT
KAROLINSKA INSTITUTET
STOCKHOLM

November 18*th*, 1938

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.

A PROPERTY OF RICE BRANS

(*Read at the Meeting of the North of England Section, December* 10, 1938)

THE milling of rice gives either rice bran or parboiled rice bran, according to whether the grain is milled raw or is previously soaked in hot water, treated with steam and dried. Since the prices of the two products differ, and parboiled rice bran is at times appreciably cheaper than rice bran, it is sometimes necessary to decide whether a sample of rice bran contains an admixture of parboiled rice bran.

Rice bran has an average "oil" (petroleum spirit extract) content of about 14·5 per cent., the corresponding figure for parboiled rice bran being about 22·5 per cent.; but the variations are very great, and the amount of "oil" in rice bran may be anything between 10 and 18 per cent., while that in parboiled rice bran may lie anywhere between 16 and 28 per cent.

Microscopic examination of the starch grains is an aid in distinguishing between the two products, and staining reactions help; some evidence can also be obtained from a determination of the percentage of free fatty acids in the "oil"; but these differences are not sufficiently sharp, in our experience, to serve as criteria, and they are of little use for detecting adulteration.

In May, 1936, one of us (F. L. K.) found that when rice bran is sprinkled on the surface of water it floats, unwetted, for a good many hours, whereas parboiled rice bran, or a mixture of the two, begins to sink very soon. The phenomenon is apparently due to differences in wetting properties. Sweat-damaged rice bran also sinks (rice bran has poor keeping qualities, and is liable to suffer from heating and sweating on shipboard or in store); but there is evidence that the sedimentation of parboiled rice bran (liquid cloudy) is different from that of sweated rice bran (liquid clear).

We have obtained and tested rice offals from as many sources as possible (we have had them from every Continent except Australia), and in spite of a number of unproved results (where this "sinking test" indicates the presence of parboiled or of sweated rice bran, and no clear confirmation can be obtained by other tests), we suggest that the method is valuable as affording at least corroborative evidence of admixture or of sweating.

The test can be applied by simply sprinkling the meal on water; but our routine method of applying it is as follows: In the course of the "oil" determination 2 g. of the meal are ground in a mortar with 2 g. of silver sand; when the extraction process is finished, the residue from the Soxhlet thimble is freed from petroleum spirit and poured on to the surface of some water in a beaker; if any appreciable amount of bran sinks within 3 hours, we take it as an indication that the rice bran may contain an admixture of parboiled rice bran, or alternatively, may have been sweat-damaged.

By testing mixtures made up for the purpose we find that addition of 5 parts of parboiled rice bran to 95 parts of rice bran is generally enough to cause sinking within 3 hours. Of the 167 samples of parboiled rice bran examined, all sank in much less than 3 hours.

In tests on 685 samples of rice bran 81 sank in less than 3 hours. Of these 81, we decided that a number were sweat-damaged, while others yielded confirmatory

evidence of adulteration with parboiled rice bran; but we know of no satisfactory means of proving our conclusions.

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C. LOUDEN
F. L. KINSELLA

SILCOCK'S LABORATORIES
LIVERPOOL

January, 1939

CLASSIFICATION OF RICE BRANS

THERE has been an attempt, for commercial purposes, to differentiate the two types of rice bran by calling every sample yielding under 16 per cent. of oil, "Rice Bran," and all above that figure, "Parboiled Rice Bran."

To this end a very large number of figures relating to the oil-contents of deliveries extending over 12 months were collected by the Liverpool Seed Oil Cake and General Produce Association, but that arbitrary classification has not been adopted.

I have used the method described in the previous note, and communicated to me by the authors two years ago, and can corroborate their findings.

In addition, I have found that since parboiled rice bran, after the usual grinding and sieving, is bulkier than ordinary rice bran, the weight of a given bulk is a valuable confirmatory test.

If a 10-ml. tube, such as is used for lozenges, is half filled with the sample and tapped on the bench to consolidate the mass, further small portions being added and the tube tapped after each addition until full and it is certain that it can hold no more, the weights of rice brans from various sources vary from 4·44 g. to 5·0 g. (the average being 4·64 g. for 10 ml.), whilst parboiled rice bran seldom varies in weight from 4·02 g. for 10 ml.

A standard Burma rice bran, accepted as such by the trade, when weighed thus by different operators, yielded 4·50 g., 4·56 g. and 4·62 g. per 10 ml.

When mixed with 25 per cent. of parboiled rice bran the weight of 10 ml. was 4·39 g.

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January, 1939

A CONVENIENT METHOD FOR ESTIMATING THE HYDROCYANIC ACID GENERATED BY LINSEED CAKE

(*Read at the Meeting of the North of England Section, December* 10, 1938)

MOST linseed cakes, when kept warm and wet, produce prussic acid by the action of an enzyme on a glycoside, and deaths of animals, particularly calves, have at times been attributed to this action. The picrate test for prussic acid has long been known and used, and was studied as a quantitative method by Chapman (ANALYST, 1910, **35**, 469), who found that it is not specific for prussic acid and therefore not to be trusted, especially in the analysis of decomposing viscera, where all sorts of volatile compounds might be present, some of which might give the red colour with sodium picrate. Confining our remarks to linseed cake, however, our experience is that:

(1) with many scores of samples, the picrate method has given consistently good results, agreeing with those obtained by other methods;

(2) when carried out as described below, the picrate method is very much more convenient than the usual methods involving distillation with steam; and

(3) the modification of the picrate method suggested here gives important information about the rate of development of prussic acid.

Apparatus.—About six glass containers are needed, about 3 inches in diameter, and fitted with air-tight lids. Inside each stands a crucible, basin, or flat dish.

Reagent.—Four g. of picric acid and 40 g. of anhydrous sodium carbonate are dissolved separately in water, mixed and made up to 1 litre.

Method.—One g. or 0·5 g. of ground linseed cake (or as much as will give 0·1 to 0·5 mg. of prussic acid) and 10 ml. of water are mixed in a glass container, and 10 ml. of sodium picrate solution are placed in the small basin, which is put into the container. The lid having been put on, the vessel is left in an incubator at blood heat until next day. Standards are made up in similar vessels, a very dilute, slightly acidified solution of potassium cyanide being used in place of linseed cake; these are put into the same incubator. Next morning the picrate solutions are washed into Nessler cylinders and compared.

It is possible to match the test solution with the standards without removing them from the small basins, and even to assess the prussic acid approximately without opening the containing vessels, thus getting some idea of the speed with which the prussic acid is being generated.

Results.—In the Nessler cylinders, differences due to 0·05 mg. of prussic acid (which we consider sufficiently close for our purpose) are very easily seen, and the method will detect 0·005 mg. of prussic acid. We append a table showing some of our results.

	Prussic acid produced per 100 g. of meal			
Sample No.	Picrate method mg.	Silver nitrate method mg.	Prussian blue method mg.	Iodine method mg.
1	45	45	40	45
2	40	42	40	—
3	60	62	60	—
4	65	65	—	—
5	45	45	43	—
6	45	45	42·5	—
7	42·5	42	40	—
8	42·5	—	42	—
9	80	80	80	—
10	60	60	60	—
11	65	65·6	—	—
12	10	12	—	—
13	75	—	75	—
14	100	—	100	—
15	65	—	65	—
16	35	35·1	—	—
17	40	40	40	—
18	42·5	42·1	—	—
19	40	39	—	—
20	37·5	39	—	—

Notes.—(1) Permanent standards could, no doubt, be prepared, but we have not found it worth while. We are now getting good results by measuring the colour in a photo-electric colorimeter.

(2) If the standard potassium cyanide solution is pipetted directly into the sodium picrate solution in the small basin, instead of into the outer container, the colours obtained are not quite the same; there appears to be a difference in the quality of the colour.

(3) Good results were obtained even when we allowed decomposition of the linseed cake to take place, rendering other methods of determination difficult or impossible to apply.

(4) Messrs. Towers of Widnes have made a convenient and cheap apparatus for us by grinding the flanges of Pyrex pie-dishes, so that two of them fit together to make a desiccator-like vessel; for the inner basins to hold the picrate solution, they have made flat dishes of glass, with three legs and a central handle.

RATE OF DEVELOPMENT OF PRUSSIC ACID.—We have observed great differences in the speed with which the red colour appears after the apparatus has been put in the incubator; with some of our worst samples (those that produced most prussic acid) the colour reached its maximum in four hours, all the prussic acid having been generated and having reacted with the picrate, while other samples required much longer incubation. The red colour was sometimes visible in 20 minutes, sometimes not for several hours. We suggest that this factor (the rate at which the linseed cake produces prussic acid), which appears to have been ignored hitherto, may be of great importance in any assessment of the toxicity of linseed, particularly with ruminants.

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January, 1939

STANDARDISATION OF HYDROCHLORIC ACID

IT has been pointed out by Bjerrum (*Die Theorie der alkalimetrischen und azidimetrischen Titrierungen. Sammlung chemischer und chemischtechnischer Vorträge*, Band XXI, Stuttgart, 1914) that in exact work it is necessary to titrate to some particular hydrogen ion concentration. The object of the few experiments quoted below was to ascertain the difference between the titration value of approximately $N/10$ hydrochloric acid as determined with silver nitrate using adsorption indicators and as determined when titrated to a pH value of 3·8.

The experiments were carried out in a manner similar to those already described with potassium iodide (ANALYST, 1939, **64**, 112). The same Stas pipette as before was used to deliver 63·73 ml. of hydrochloric acid ($N/10$). The titrations were completed by using $N/100$ hydrochloric acid with tartrazine as the adsorption indicator, and $N/100$ silver nitrate solution with phenosafranine as indicator. The quantity of silver nitrate taken for the titrations in which tartrazine was used was slightly greater than that for quantitative reaction with 63·73 ml. of $N/10$ hydrochloric acid, whereas when phenosafranine was used the weight of silver nitrate taken was slightly less than this amount. The magnitude of the experimental errors in the work was of the same order as in the titration experiments with potassium iodide.

The neutralisation experiments were carried out in a similar manner, but in every instance the quantity of potassium bicarbonate weighed was such as to be slightly in excess of that required for quantitative reaction with 63·73 ml. of $N/10$ acid. The titrations were therefore always completed by running $N/100$ hydrochloric acid into the alkaline liquid. The change of colour with screened methyl orange from the green alkaline colour to the magenta acid colour through the grey tint at which the pH of the solution is 3·8 could be determined to $\pm 0{\cdot}5$ ml. of $N/100$ acid. It was less precise than the colour change with an adsorption indicator. However, the total working error in a determination would probably not exceed one part in one thousand.

1. *Silver nitrate determinations.*

Silver nitrate taken g.	$N/100$ $AgNO_3$ or $N/100$ HCl required* ml.	Indicator	Determined vol. of $N/10$ HCl equiv. to 1 litre of $N/10$ $AgNO_3$ ml.
1·1017	12·5 HCl	Tartrazine	1003
1·0804	1·0 HCl	Tartrazine	1004
1·0164	38·0 $AgNO_3$	Phenosafranine	1002
1·0543	15·5 $AgNO_3$	Phenosafranine	1002

2. *Potassium bicarbonate determinations.*

Potassium bicarbonate taken g.	$N/100$ HCl required* ml.	Indicator	Determined vol. of $N/10$ HCl equiv. to 1 litre of $N/10$ $KHCO_3$ ml.
0·6559	22·0	Screened methyl orange	1005
0·6785	44·5	,, ,, ,,	1005

* After the addition of 63·73 ml. of $N/10$ HCl.

From these experiments it will be seen that there is a small but appreciable difference between the titration values of the acid according to whether the titration is completed with hydrochloric acid using tartrazine or with silver nitrate using phenosafranine as indicator. Taking the average value of 1003 ml. of $N/10$ acid from the adsorption indicator experiments as the most reliable figure, the value of 1005 ml. obtained from the neutralisation experiments in which a final pH of 3·8 was attained is what might be expected. Further experiments on the titration of acids to definite pH values are being carried out.

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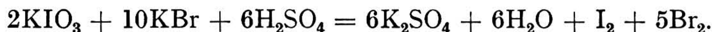
DETERMINATION OF BROMIDE IN PRESENCE OF CHLORIDE

It is not possible to determine bromide in the presence of either chloride or iodide by titration with silver nitrate using an adsorption indicator, because the solubility and adsorptive capacity of silver bromide is intermediate between that of the two other silver halides. The success of the method for titrating iodide in the presence of chloride previously described (Analyst, 1939, **64**, 112) is due to the considerable difference between silver iodide and silver chloride in these respects. Various methods for determining bromide in presence of chloride depending upon differential oxidation have been devised. The oxidising agent must be employed under carefully regulated conditions, as otherwise a certain amount of oxidation of the chloride will take place. One of the best methods of effecting the desired result, originally due to Bugarsky (*Z. anorg. Chem.*, 1895, **10**, p. 387) and modified by Andrews (*J. Amer. Chem. Soc.*, 1907, **29**, p. 275), consists in oxidising the hydrobromic acid to bromine with iodic acid thus:



$$2KIO_3 + 10KBr + 6H_2SO_4 = 6K_2SO_4 + 6H_2O + I_2 + 5Br_2.$$

The bromine and iodine are removed by distillation, and the excess of potassium iodate is determined by adding excess of potassium iodide and titrating the resulting iodine with sodium thiosulphate solution.

In my experiments the oxidation of the bromide was effected in the usual way, but a modification of the procedure was introduced for determining the unused potassium iodate. The solution was reduced by shaking with a 2 per cent. zinc amalgam to convert the iodate into iodide. The iodide thus obtained was then titrated directly with a fresh quantity of the original potassium iodate solution. In this way the iodate which had been used in the oxidation of the bromide was determined directly.

It was found that approximately correct results were obtained when the amount of bromide was present in relatively high proportion to the chloride. When, however, the proportion of chloride was considerable, high results were invariably obtained, doubtless owing to oxidation of some of the hydrochloric acid.

The following results were obtained with a standard solution of 3·42 g. of potassium iodate per litre.

(1) A solution consisting of 20 ml. of $N/10$ hydrochloric acid, 20 ml. of potassium bromide solution (11·25 g. of KBr per litre), 50 ml. of standard potassium iodate and about 3 ml. of dilute sulphuric acid was boiled for five minutes and left overnight. The solution was then boiled gently for over two hours, fresh water being added from time to time to replace loss by evaporation. The boiling was continued for some time after the absence of bromine in the steam had been proved by the use of filter-paper impregnated with fluorescein. The residual liquid was then reduced with zinc amalgam and titrated directly with the standard solution of potassium iodate in presence of a high concentration of hydrochloric acid in the usual manner; 13·4 ml. of potassium iodate solution were required.

(2) This experiment was a duplicate of (1). The quantity of standard potassium iodate solution required for the reduced solution was 13·2 ml. If the average titration value of 13·3 ml. is taken, the concentration of potassium bromide is 11·1 g. per litre.

(3) This experiment was carried out to investigate the effect of a high concentration of hydrochloric acid. Twenty ml. of N hydrochloric acid were present, otherwise the same quantities of the other constituents were used, and the boiling was prolonged for nearly three hours. The final quantity of potassium iodate solution required for the reduced solution was 12·2 ml.

(4) This experiment was an exact duplicate of (3). The final volume of potassium iodate solution required was 12·1 ml.

By taking the average titration value of the last two experiments, the concentration of potassium bromide is found to be 12·2 g. per litre.

High results in presence of much chloride have been noted by other investigators (see Sutton's *Volumetric Analysis*, 12th Ed., p. 197).

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Official Appointments

The Minister of Health has approved the following appointments:

Hugh Childs as a Public Analyst for the Borough of Chesterfield, in addition to John Evans (December 9th, 1938).

Hugh Childs as a Public Analyst for the County Borough of Lincoln, in addition to John Evans (December 9th, 1938).

Hugh Childs as a Public Analyst for the County Borough of York, in addition to John Evans (December 14th, 1938).

Albert Lester Williams as a Public Analyst for the County Borough of Birmingham, in addition to H. H. Bagnall.

The Minister of Agriculture has approved the following appointment since November 25th, 1938:

ERIC VOELCKER as Agricultural Analyst for the County Borough of Oxford.

H. CHILDS as Deputy Agricultural Analyst for the County Boroughs of Lincoln and York.

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.

METROPOLITAN BOROUGH OF DEPTFORD

ANNUAL REPORT FOR THE YEAR 1937

OF the 505 samples submitted under the Food and Drugs Act, 323 were taken formally.

PORK AND BEANS.—A prosecution was instituted against the vendors of a brand of canned beans in tomato sauce which was labelled and being sold as "Pork and Beans with delicious Tomato Sauce." The three cans examined contained no pork or pork-fat. Similar preparations have been on sale for many years as "Baked Beans with Pork and Tomato Sauce," and have contained only a very small piece of pork or pork-fat. This latter description might be held to mean simply "Beans with pork- and tomato-flavoured sauce," but the one objected to seems to imply that pork is a major constituent and constitutes a definite departure from the trade practice that has become established. The case was strongly defended, and it was stated that the pork had been introduced, not in its solid state, since customers sometimes objected to finding a piece of "greasy fat" in their beans, but in the form of pork stock, which, being perfectly distributed throughout the sauce, was free from such objections.

The analysis showed none of the normal constituents of pork stock, such as pork-fat, flesh fibres, creatine and other nitrogenous extractives, non-nitrogenous organic and inorganic extractives, which were evident in "control" analyses on samples prepared in the laboratory, and I was therefore able to state that no material quantity of pork stock could have been introduced into the tins which were sampled. This statement was disputed by experts on behalf of the defendants, who claimed that they had proved the presence of pork stock by means of the Gold Number test, and the third portion of the sample was sent to the Government Chemist, whose report confirmed the absence of pork and of any material quantity of pork stock; he was, however, unable to ascertain whether or not a very small proportion of pork stock was present, on account of the decomposition the sample had undergone on keeping since it was bought.

The magistrate took the view that, in any event, pork stock, made from the bones and bellies of pigs should not be described as pork, and, on the defendants indicating that they had already had different labels printed and their real desire for guidance in the matter, dismissed the case under the Probation Act on payment of 25 guineas costs.

H. AMPHLETT WILLIAMS.

Legal Notes

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

LIABILITY FOR TYPHOID FEVER CONTRACTED FROM MILK

Court of Appeal

Square v. *Model Farm Dairies (Bournemouth), Ltd.*

On January 24th an appeal was heard before Lord Justice Slesser, Lord Justice Clauson and Lord Justice du Parcq, against the judgment of Mr. Justice Lewis, in the second of two consolidated actions in favour of the plaintiffs.

Sir Walter Monkton, K.C., and Mr. Graham Dixon appeared for the appellant company, and Mr. H. J. Wallington, K.C., and Mr. H. Maddocks for the respondents.

The case for the plaintiffs in the original action was that in August, 1936, the defendant company had agreed to supply milk daily for the use of Mr. Square, his family and household. The plaintiffs said that it was a term of the contract, and that the defendants by a letter and a brochure expressly and implicitly warranted that the milk supplied should be clean and free from contamination or infection by disease or otherwise, and should be fit for human consumption. The milk was delivered to the plaintiffs daily from August 1st to 21st, 1936, and the complaint was that the milk, or some of it, was not pure, but was infected with the germs of typhoid fever. Alternatively, the plaintiffs complained that the defendants were negligent in that they had failed to take adequate precautions to ensure that the milk was pure. It was alleged that, in consequence of the defendants' default, Mrs. Square, her two infant children, the governess and Mr. Square's niece contracted typhoid fever and became seriously ill.

The defendants denied negligence or breach of any duty. They did not admit that the letter and brochure sent by them to Mr. Square contained any warranty or representation. Alternatively, they pleaded that, if they made the alleged warranties or representations, these were true, or were made innocently and in the honest belief that they were true. They also denied that they were guilty of any breach of statutory duty.

At the hearing before Mr. Justice Lewis it was conceded that it was from milk sold by the defendants that the infection arose. The Judge ruled that there was no evidence fit to leave to a jury on the charges of fraud, as there was no evidence that the statements in the letter or brochure which had been sent to Mr. and Mrs. Square were untrue, much less untrue to the defendants' knowledge. He also decided that there was no evidence of negligence on the part of the defendants. He held, however, that the defendants were liable in damages to Mr. Square, and also to those of his household who became infected with typhoid fever, by virtue of Section 2 (1) of the Food and Drugs Adulteration Act, 1928, on the ground that that section did not preclude the bringing of a civil action by a person injured through a breach of its provisions, and that the right of action for its breach extended not only to the purchaser but to anyone who suffered damage as a result of it.

It was conceded that Mr. Square was entitled to damages under Section 14 of the Sale of Goods Act, 1893, and these were assessed at £400. The plaintiffs (Mrs. Square, the children, the governess and the niece) in the second action, which had been consolidated with the first action, were awarded sums amounting in all to £525.

The defendants appealed against the award of damages in the second action, and the plaintiffs in the second action appealed against the ruling of the Judge on the question of fraud.

Lord Justice Slesser, giving judgment, said that where there was a statutory duty any person injured through a breach of that duty would normally, in the absence of any remedy in the statute creating the duty, have an action. Where the statute had itself provided penalties different considerations might arise. He proposed therefore to consider the purview of the Act of 1928 in order to see if any remedy was available other than the remedy or penalty specifically provided in the Act itself. In his opinion, the present case was fundamentally different from cases such as *Groves* v. *Lord Wimborne* (14, *The Times L.R.*, p. 493; [1898], 2 Q.B., 402), which dealt with the position where the legislature had imposed a new duty which did not exist at common law. The duties imposed by the Factory and Workshop Act, 1901, were an example, obligations being imposed on employers with regard to those in their employ which in many cases would not in fact arise out of any implied term of the contract between master and servant.

The difference between the case where a new duty had been imposed and the present case was that, in substance, the duty or right contemplated in Section 2 (1) of the Food and Drugs (Adulteration) Act, 1928, was to be found in the provisions of Sections 13 and 14 of the Sale of Goods Act, 1893, which were no more than a consolidation of the existing common law. Although there might be exceptional cases not covered by the Sale of Goods Act, in his opinion Section 2 of the 1928 Act did no more than impose penalties and other consequences in circumstances where there would be a right under the earlier Act to bring a civil action. The protection of the public and the relationship of vendor and purchaser were preserved by the Act of 1928. In other words, that Act was dealing solely with the question of sale of goods as between purchaser and vendor, and was imposing a penalty for doing what might otherwise give rise to a civil action.

It had been argued that, on the facts of the present case, it had not been shown that the defendants had sold an article which was "not of the nature, or not of the substance, or not of the quality demanded." He did not wish to express a concluded opinion on that matter, as the case could be decided on the assumption that the milk sold had in fact not complied with the requirements of the section.

There was yet another reason why the plaintiffs could not succeed in their action under the Act of 1928. It was pointed out in *Monk* v. *Warbey* (51 *The Times L.R.*, p. 79; [1935], 1 K.B., p. 84) that the duty imposed by the statute must be to one whom the legislature wished to protect. He was quite unable to see how it could be said on any view that there was any person to be protected in Section 2 other than the purchaser. The appeal must therefore be allowed.

With regard to the cross-appeals by the plaintiffs against the decision of Mr. Justice Lewis, who held that there was no evidence on the charges of fraud, his Lordship said that, on the interpretation placed by the Court on the letter and brochure sent by the defendants, there was not a shadow of evidence that the defendants did not have a reasonable belief in the truth of what they were saying. The action, so far as based on fraud, had necessarily failed, and the cross-appeals must therefore be dismissed.

Lord Justice Clauson and Lord Justice du Parcq agreed.

Leave to appeal to the House of Lords on the question arising under Section 2 (1) of the Food and Drugs (Adulteration) Act, 1928, was granted.

Imperial Institute

ANNUAL REPORT FOR THE YEAR 1938

IN his foreword to the Report the Director, Sir Harry Lindsay, surveys the developments during 1938. The two Advisory Councils and fifteen Consultative Committees (*cf.* ANALYST, 1938, **63**, 271) have continued to give the greatest assistance in the work of the Institute. On the Plant and Animal Products side, the two new Committees—on Gums and Resins and on Insecticide Materials of Vegetable Origin—have sketched out the lines of prospective monographs, one on gum arabic and the other on the chief classes of vegetable insecticides. Other monographs in preparation are a treatise on Flax Production and Research in the United Kingdom and a Report on Empire Flax Supplies. On the Mineral Resources side, monographs on Coal, Tin, Magnesium, Bauxite and Aluminium, Chromite and Vanadium are in preparation. A monograph on Manganese was completed and published during the year.

Details of the technical work of the Institute are given in Chapters I and II of the Report. Chapter III describes the Exhibition Galleries, and Chapter IV gives an account of progress made during the year in the Cinema and Empire Film Library and with the lecture programmes.

The investigations to which special reference is made include the following:

PREPARATION OF TUNG OIL.—In the course of a study on the preparation of tung oil from the fruits trials were made on the de-husking of *Aleurites montana* fruits by the Chinese fermentation method. The results showed that this method, although suitable for *A. fordii* fruits, did not work satisfactorily with fruits of *A. montana*, which have thick and resistant husks. A better method for *A. montana* consisted in removing by hand the nuts from the fruits, which readily split into segments on drying.

PERILLA SEED FROM BURMA.—The sample yielded 44·7 of oil as against the usual 35 to 45 per cent. The oil was of good quality and complied with the requirements of the British and American standard specifications. Perilla seed is not at present marketed in the United Kingdom, but a small amount is occasionally crushed in the United States.

CROTON SEED FROM NYASALAND.—The seed submitted was identified at Kew as *Croton megalobotrys* Muell. Arg. (=*C. gubouga* S. Moore). The seeds contained 37·4 per cent. of shell and 62·6 per cent. of kernel. The kernels yielded 47·3 per cent. of a clear, golden-yellow oil having constants that showed it to be of the "semi-drying" type. The seeds of *C. megalobotrys* have been recorded as showing purgative properties, but it is unlikely that the seeds or oil would find a market for medicinal use.

GINGER FROM TANGANYIKA.—A sample of native-produced and untreated material was very moist inside, and was therefore dried and sliced before examination. It then contained 17 per cent. of moisture and yielded 10·8 per cent. of alcohol-soluble, and 17·1 per cent. of water-soluble extractive. The yield of ash, expressed on the moisture-free material, was 5·5 per cent. Brokers in the London market considered that, if properly prepared, the ginger would be comparable in appearance with Sierra Leone ginger.

MINT OIL FROM SEYCHELLES.—This oil was distilled from specimens identified as *M. arvensis* L. It contained a high percentage of menthol—nearly 82 per cent. as compared with a range of 70 to 90 per cent. in natural Japanese mint oil. The demand for such oil might be limited, as the usual mint oil of commerce is dementholised.

TOXIC ROOTS FROM TRINIDAD.—Samples of roots of two plants, employed locally in Trinidad as fish poisons, were submitted in order to ascertain if they were of interest as sources of insecticides. Botanical specimens were also sent and were

identified at Kew as *Paullinia leiocarpa* Griseb. and *Serjania paucidentata* DC. Tests showed that the *Paullinia* roots contained no rotenone, saponin or alkaloids, and that the *Serjania* roots were also free from rotenone and alkaloids, but contained a little saponin. It was suggested that the strength of the insecticidal action, if any, might first be ascertained by biological methods and it was proposed to refer the question of the current demand for insecticides in the United Kingdom to the Consultative Committee on Insecticide Materials of Vegetable Origin.

VERMICULITE.—Firms have been requiring Empire supplies of this mineral to replace the American and Russian materials on the market. At present the only known potential sources in the Empire are in the Union of South Africa, Tanganyika and Western Australia. The question of using vermiculite for the same purpose as ground mica in wallpaper printing is under investigation, and samples have been supplied to a firm grinding this class of mica (*cf.* ANALYST, 1938, **63**, 272).

QUARTZ CRYSTALS.—The demand for high-grade quartz crystals both for piezo-electric and optical purposes has become increasingly urgent. The small amount of quartz of good quality received as a result of efforts made by the Institute and others to discover new sources of supply within the Empire has done little towards satisfying the demand for this mineral, which is an essential component of modern devices for telegraphic and telephonic communication.

VANADIUM FROM FUEL-OIL ASH.—Crude petroleum from certain localities, notably South America and Mexico, contains vanadium and, after combustion, the bulk of this remains as oxide in the ash. Since oil-fired boilers are largely used in steamships, it is usual to collect the ash from the furnaces and sell it for its vanadium-content.

SALT FROM BRITISH SOMALILAND.—Two samples of crude salt were sent for analysis and to ascertain if their iodine-contents were adequate for human and animal nutrition. The samples consisted of crude sodium chloride, the chief impurity being calcium sulphate. A trace of iodine was present in one sample, but none was detected in the other. The senders were told that an Institution specialising in nutrition problems had informed the Imperial Institute that, although the provision of a small quantity of iodine is essential to maintain health in man and animals, definite information is not available regarding the minimum amount necessary.

All-India Institute of Hygiene and Public Health

ANNUAL REPORT FOR THE YEAR 1937

THE Report of the Director and Professor of Public Health Administration deals with the activities in the various branches of the Institute during the year (*cf.* ANALYST, 1938, **63**, 116). Among the investigations in progress are the following:

EPIDEMIC DROPSY AND MUSTARD OIL.—Researches of the last three years have indicated that the disease is caused by some deleterious substance introduced through mustard oil, which is almost universally used as a cooking oil in North-Eastern India. Suspicion fell first on allyl *iso*thiocyanate, the pungent principle of the oil, but it was found that the whole of this substance disappeared during the process of cooking. It was also non-poisonous to rats, even when given in large doses. Moreover, the allyl *iso*thiocyanate content of samples of oil obtained from certain families who had been using it for a fortnight or more without ill effects was greater than that of the sample from Jamshedpur, which had given positive results in human experiments. Hence it would appear that the toxic element

is probably not allyl *iso*thiocyanate. Since the disease, as thus manifested, is confined to mustard oil consumers only, it is possible that the toxic seeds may be infected with specific moulds or that the toxic substance may be derived from some other seeds or oils which might be added to mustard oil in the course of preparation or sale. Work on these lines is in progress (*cf.* ANALYST, *loc. cit.*)

ASSAY OF FLAVINE AND VITAMIN B_6 IN INDIAN FOODS.—The flavine-contents of about 90, and the (antidermatitic) vitamin B_6 contents of 30 Indian foodstuffs were estimated. Pulses and leafy vegetables were found to be relatively rich, and fruits poor sources of flavine, whilst pulses, cereals and fish gave the best results for vitamin B_6.

EXCRETION OF VITAMIN B_1 IN HUMAN URINE.—The daily excretion of vitamin B_1 was studied in five normal healthy subjects fed on different diets. It varied between 6·4 and 12 per cent. and, having regard to the previous dietetic history of the subjects, it varied in general in proportion to the amount ingested. There was, however, some evidence to show that the excretion of B_1 (total caloric and B_1 intake being kept constant) was much lower in those taking a carbohydrate diet than in those on a diet rich in fat. This would suggest the possibility that the metabolism of carbohydrates demands a greater amount of vitamin B_1 than that of fats.

RÔLE OF ASCORBIC ACID IN MALARIA.—It has been found that during the malarial paroxysm the body excretes vitamin C in considerable amounts, and that as a result of repeated attacks of malaria a condition of sub-clinical hypovitaminosis develops. In view of the important relationship between vitamin C and the reticulo-endothelial system, it is possible that the results of this investigation may throw some light on the rôle of vitamin C in the cure of malaria and in prevention of relapses.

BIOCHEMICAL CHANGES IN THE BLOOD IN ANAEMIAS.—The investigation of the two types of haemoglobin in connection with anaemias has been continued, and systematic estimations of glutathione have also been made. The evidence so far obtained indicates that the percentage of alkali-resistant haemoglobin varies between 8 and 17 per cent. in normal subjects, and that little or no variation occurs in the anaemia of either hyperchromic or hypochromic type. In this respect these anaemias differ from pernicious anaemia, in which reduction and disappearance of the alkali-resistant type has been recorded. The glutathione-content of the blood appeared to vary with the percentage of haemoglobin.

Bengal

ANNUAL REPORT OF THE CHEMICAL EXAMINER'S DEPARTMENT FOR THE YEAR 1936

WITH the creation of the separate province of Orissa the medico-legal and other analytical work of the province is being carried out in the same way and on the same terms as were in force for the combined province of Bihar and Orissa. The Department is controlled by Dr. K. N. Bagchi.

During the year under review 7747 articles were examined as compared with 7422 in the previous year. In the medico-legal section 5784 articles connected with 2347 cases were examined as compared with 6158 articles in connection with 2470 cases in the previous year.

The following were some of the important investigations that were carried out:

COCAINE.—Of 459 samples examined, only 29 consisted of pure cocaine hydrochloride, the remainder being novocaine or cocaine adulterated with novocaine, aspirin, soda, lime, etc.

OPIUM.—Only 38 of the 160 samples examined were genuine in accordance with the Excise Act.

ARROW POISON FROM ASSAM.— A sample of a dark gummy substance used by the hill tribes of the Naga Hills, Assam, for poisoning arrow-heads was submitted by the Deputy Commissioner, Naga Hills. A preliminary investigation was made with the help of the Pharmacological Department of the Tropical School of Medicine, Calcutta. To some extent the action of the poison is similar to that of *Antiaris toxicaria*, but it is ten times more toxic to cats. Its most peculiar characteristic is that it has no ill effect upon toads. Further work on the identification of the poison and the discovery, if possible, of a suitable antidote is in progress.

HUMAN POISONING CASES.—Of the 878 human viscera examined, 40 were received in connection with abortion cases. Poison was detected in 336 specimens, *i.e.* 38·2 per cent. as compared with 27·9 per cent. in 1935 and 25·8 per cent. in 1934. As usual, the poison most frequently detected in human poisoning cases (*i.e.* in viscera, vomit and excreta) was opium. It was detected in 171 specimens—35·4 per cent. of the detected poisons. Alcohol, arsenic, atropine, aconite, oleander and hydrocyanic acid came next in the order given.

ANIMAL POISONING CASES.—Poison was detected in 58 of the viscera of 94 horned cattle received. Arsenic was found in 52, oleander in 3, strychnine and brucine in 1, aconite in 1, and a cyanogenetic compound in 1. Thirty-two articles suspected to be cattle poisons were received. In 21 poison was detected—white arsenic in 13, arsenic sulphide in 3, cyanogenetic compounds in 3, copper sulphate in 1, and oleander in 1.

BAHERA (*TERMINALIA BELLERICA*) POISONING.—A Hindu male child, about 4 years old, swallowed some bahera fruit about 24 hours before his death, and was brought to the hospital in a comatose condition. At the post-mortem examination the stomach was found to be healthy. Both sides of the heart contained blood. The membranes were congested and there was a slight meningeal haemorrhage. No poisonous substance could be detected in the viscera, and attempts to isolate a toxic principle from extracts of the fruit (pericarp and kernels) were unsuccessful. Similar cases, in all of which the victims were little children who developed symptoms almost identical with those described, were reported elsewhere. Further investigation to discover the poisonous principle is urgently required.

AKANDA (MADAR JUICE) POISONING.—A boy of about 8 was given some *Akanda* juice (*Calotropis gigantea*) with milk for "treatment of dog bite." Immediately afterwards he became restless and there was vomiting and purging, followed by convulsions and death in about half-an-hour. Madar juice was detected in the vomit, in a piece of cloth with brownish stains said to be faecal stains, and in the viscera (*cf.* ANALYST, 1930, **55**, 683; 1931, **56**, 665; 1932, **57**, 717; 1934, **59**, 37, 542; 1935, **60**, 759). The stomach wall was found congested and oedematous.

SUSPECTED *BONDUCELLA* POISONING.—The viscera of two women said to have died about 3 hours after taking the pulp of *nata* seeds (*Bonducella caesalpina*) were received. The pulp of *nata* seeds is usually taken as a cure for fever and is not known to have been used for criminal purposes. In post-mortem examination the organs did not show characteristic features of poisoning except a congested patch of mucous membrane in one of the stomachs. No poisonous substance could be detected in the viscera.

About 1 lb. of *nata* seeds were obtained and analysed. The glycoside bonducin (*J. Indian Acad. Science, U.P.*, 1934, Vol. **4**, Part 2) was isolated and administered to experimental animals, but produced no ill effects. The extracts of the pulp with various solvents were also non-poisonous. The actual cause of the death of the two women remained unexplained.

NITRIC ACID POISONING WITHOUT CHARACTERISTIC STAINING.—The viscera of a man who died from swallowing nitric acid were examined. There was a black

mark extending from the lower lip to the chin, and nitric acid was detected in the stomach and in the empty bottle found by the man's side. The post-mortem examination showed ulceration and a large perforation (2 in. by 2½ in.) in the posterior wall of the stomach. There were also patches of blackening of the mucous membrane of the stomach, but there was no yellow staining anywhere. No trace of sulphuric or acid other than nitric was detected.

ACONITE IN COUNTRY LIQUOR.—The viscera of a Hindu, about 20 years of age, were submitted. He and two other men had drunk *pachai* sold by an unlicensed vendor. All felt sick and had pains in the head and a burning sensation in the stomach. There was vomiting and all became unconscious in about 3½ hours. One died next morning and the others recovered. Aconite was detected in the viscera and in the sample of *pachai*. Sometimes aconite along with datura seeds or *kuchila* (nux-vomica) is mixed with *bakhar*—the fermenting agent containing crude yeasts—with the idea of increasing the intoxicating properties of the liquor. In these cases poisoning is usually accidental.

CHOPPED HAIR AND NAILS AS POISON.—A tuft of chopped hair and small fragments of human nails, together with a vegetable root, were detected in the vomit of a Muhammadan. They had been given with rice and vegetables by his wife. No poison was detected in the root. Chopped hair is frequently used by cattle poisoners, but it is the first time that it is known to have been given to a man. The object of giving these substances is unknown, but they were probably intended as love-philtres. The woman was prosecuted under Sec. 328, I.P.C. (Poisoning).

Home Office

HYDROGEN CYANIDE (FUMIGATION)

STATUTORY RULES AND ORDERS, 1938. NO. 1578*

THESE Rules, which came into force on February 1st, prescribe that notice of any forthcoming fumigation shall be given to the officer in charge of the nearest police station and to the Medical Officer of Health not less than 48 hours before the fumigation. No fumigation shall be carried out except by an adequate fumigating staff, and effective means shall be taken to ensure that persons other than the staff are not present and to prevent contamination of liquids and foods. After the fumigation tests shall be applied to ensure that the area is free from danger, and any adjoining buildings shall be kept under observation to discover any penetration of the fumigant and to safeguard the occupants of such buildings. All bedding, clothing, etc. which have been exposed to the fumigant shall be treated as prescribed, and tests shall be applied to ascertain that they are free from danger. The particulars relating to each fumigation shall be entered in a register which shall be kept by the person who undertakes the carrying out of the fumigation.

* THE HYDROGEN CYANIDE (FUMIGATION OF BUILDINGS) REGULATIONS, 1938, DATED DECEMBER 19, 1938, MADE BY THE SECRETARY OF STATE UNDER SECTION 1 OF THE HYDROGEN CYANIDE (FUMIGATION) ACT, 1937 (1 EDW. 8 & 1 GEO. 6, c. 45). 1939. H.M. Stationery Office, York House, Kingsway, London, W.C.2. Price 2d. net.

Dominion of South Africa

ANNUAL REPORT OF THE DIVISION OF CHEMICAL SERVICES FOR THE YEAR 1938

THE Division of Chemical Services is a branch of the Department of Agriculture and Forestry, but also supplies chemical services to other State Departments that require them, notably to the Departments of Public Health, Justice, Customs and Excise, Lands, Defence and Interior. It is under the direction of Dr. J. P. van Zyl.

ARSENIC ON FRUIT.—One of the regulations under the Fruit Export Act (No. 17 of 1914) is that "no fruit showing visible spray stains or fruit which may contain more than 1/100th grain per pound of arsenious oxide shall be allowed to be exported." Of the total samples examined at Capetown and Port Elizabeth during the season January to April, 1938, 204 contained arsenic in excess of 1/100 grain per lb. However, only 5 samples gave results exceeding the 1/50 grain per lb. permissible under the regulations of the Food, Drugs and Disinfectants Act of South Africa. Some growers have difficulties in complying with the regulations, but certain larger growers who have introduced scientific washing machines experience no difficulty. This may in some measure account for the fact that the percentage of samples exceeding the 1/100 grain limit was only 4·6 at Capetown as against 7·2 at Port Elizabeth.

PUBLIC HEALTH.—Of the 4476 samples examined under the Food, Drugs and Disinfectants Act, 509 were adulterated or below standard. Of this total, 3129 were milks, of which 292 were adulterated or below standard. These figures are much the same as for the previous year and reflect a somewhat high percentage of adulteration.

Ice-Cream.—According to the regulations ice-creams must contain at least 10 per cent. of milk-fat. Of the 215 samples examined, 56 (26 per cent.) were adulterated. Although most of the large and reputable firms supply ice-creams containing more than the required percentage of milk-fat, there must be a number of dealers producing ice-creams of inferior value.

FLUORINE IN CITRUS FRUITS.—Experiments to ascertain the extent to which fluorine is absorbed by citrus fruits are being continued, and the fluorine-content of oranges sent to the London market from various parts of the world is being determined. The results so far obtained indicate that traces of fluorine are frequently present.

TRIORTHOCRESYL PHOSPHATE IN SOYA-BEAN OIL.—In the early part of the year a so-called "mystery disease" occurred in Durban and an investigation was carried out in the Johannesburg laboratory. The Government Pathologist at Durban formed the opinion that the problem was probably a chemical one and invoked the assistance of the division to investigate the possibility of a consignment of soya-bean oil having been the cause of the alleged disease. After an investigation lasting some months it was established that the oil contained triorthocresyl phosphate, a substance which some years ago had produced an epidemic of a similar nature in U.S.A. (*cf.* ANALYST, 1938, **63**, 813).

ABSORPTION OF FLUORINE BY CROPS.—The uptake of fluorine by various crops and grasses is being studied by pot culture. The different materials used comprise not only those rich in fluorine, but also commercial phosphatic fertilisers such as rock phosphate, which sometimes contains as much as 3 per cent. of fluorine, and superphosphate. Judging by appearance alone, the plants receiving a high dressing of sodium fluosilicate show very marked symptoms of poisoning. Analyses to show the amounts of fluorine taken up by the plants themselves are in progress.

"SOOTY BLOTCH" ON CITRUS AND ORANGE.—Until recently a mixture of chloride of lime and boric acid has been used to remove "sooty blotch" from

oranges, but it has been found to lose its bleaching properties rapidly. For citrus better results were obtained by the use of a mixture of sodium carbonate, chloride of lime and boric acid. The fruit is readily bleached and keeps well subsequently, but it is essential to coat metallic parts of the containers with a suitable paint or enamel. For the removal of "sooty mould" from oranges a mixture of sodium metasilicate and borax was found to be the most suitable.

EFFECT OF MOISTURE ON THE INTAKE OF PHOSPHORUS AND NITROGEN.—In the cultural experiments in pots the findings of the previous year were confirmed. Under dry-land conditions phosphate response alone is noted, whilst under irrigation or heavy rainfall response to nitrogen as well as phosphate is shown. The depressing effect of large quantities of potash on the grain yield were again noted.

ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

Food and Drugs

Effect of Exterior Temperature on Cooking Losses of Meat. A. M. Child. (*J. Agric. Res.*, 1938, **57**, 865–871.)—The cooking losses of (1) semitendinosus beef muscle, of (2) longissimus dorsi beef muscle, heated to 58° C. internally, and of (3) longissimus dorsi pork muscle heated to an internal temperature of 84° C. by roasting at various oven temperatures were studied. With (1), twelve samples were cooked at each of the following temperatures:—125°, 150°, 175° and 200° C. The cooking losses increased with the exterior oven temperature owing to losses from evaporation. Standing beef ribs (2) cooked at constant oven temperatures of 150° and 200° C. and at 150° C. following searing at 260° C., showed greater losses at the two latter temperatures than at 150° C. constant temperature, the loss at 200° C. being the greatest. With pork loin (3) cooked at 125°, 150° and 175°, and at 150° C. after searing for 20 minutes at 260° C. the cooking losses were not affected by oven temperature. Thus cooking losses are increased with the exterior temperature for meat cooked to 58° C. internally, but not in meat cooked at 84° C. internally. The press-fluid from the roasted meat is not affected by exterior oven temperatures, and the force required to shear the meat is only affected at 125° C. and lower (low shear force), and at 200° C. and higher (high shear force). The Warner–Bratzler apparatus, which was used for the shear test, is described. E. B. D.

Detection of Sucrose, Hydrogen Peroxide, Formaldehyde and Nitrates in Milk by means of Sulphuric Acid. E. Bohm. (*Z. Unters. Lebensm.*, 1939, **77**, 18–32.)—The adulteration of milk with sugar is limited to about 1 per cent. owing to the fact that concentrations approaching 2 per cent. are detected by taste. When 9 parts of milk are mixed with 1 part of a 10 per cent. sucrose solution the figures for the density, refraction (calcium chloride serum) and depression of freezing-point are slightly greater than the corresponding figures for the original milk. The majority of methods for the detection of sucrose in milk depend upon the preliminary preparation of a serum, but the method to be described is free from this disadvantage, a definite amount of conc. sulphuric acid being

added directly to the milk. The milk (2 ml.) is mixed either with 2 ml. or with 4 ml. of concentrated sulphuric acid and shaken until all curd is broken up, and the colour of the mixture is observed after 10 minutes. With milk containing no sugar the 2 : 2 mixture shows no change in colour, but in the 2 : 4 mixture a faint rose colour is developed. With milk containing 0·1 to 0·5 per cent. of sucrose the 2 : 2 mixture is coloured a light to deep rose and the 2 : 4 mixture a deep raspberry or red. Limitation of the time of reaction to 10 minutes precludes participation of lactose in the reaction. The temperature of the mixture should not rise above 50° C. and, since the reaction does not take place even with 0·5 per cent. of sucrose at 15° C., unnecessary cooling must also be avoided. Examination of the course of the reaction shows that the colour is produced by condensation of hydroxymethylfurfural derived from fructose with tryptophane derived from casein and albumin. It is prevented by the presence of 0·4 per cent. of hydrogen peroxide, a yellow colour being formed. With lower concentrations (0·4 to 0·2 per cent.) of hydrogen peroxide the colours are modified to beige, brown and reddish-violet and with concentrations less than 0·02 per cent. the colours due to sucrose are merely weakened. The test may therefore be used to detect the presence of hydrogen peroxide. In the absence of formaldehyde and nitrates any deviation from white in the 2 : 2 mixture and red in the 2 : 4 mixture to brown or yellowish-brown indicates the presence of hydrogen peroxide. The presence of formaldehyde also interferes with the test. With the 2 : 2 mixture a deep yellow colour is formed. With a formaldehyde concentration of under 0·004 per cent. a rose-red colour is formed in the 2 : 2 mixture and a pale violet colour in the 2 : 4 mixture. Very low concentrations of formaldehyde may simulate small amounts of sucrose. If in both mixtures no deviation from the colour reactions of unadulterated milk occurs, the milk may be assumed to be free from formaldehyde. Amounts of nitrate up to 11 mg. of nitric anhydride per litre, such as may occur in watered milk, do not interfere with the test for sucrose, but amounts of 50 mg. or more per litre, which are not to be expected in milk, prevent the reaction even when the sucrose concentration is 0·5 per cent. By the following procedure the test is adapted to the detection of nitrates:—The milk (2 ml.) is mixed with 0·1 ml. of 0·8 per cent. formaldehyde solution (1 ml. of commercial 35 per cent. formalin diluted with water to 450 to 500 ml.), and sulphuric acid is added in the ratio 2 : 4. In the presence of nitrates a blue-violet colour is formed and 3 mg. per litre of nitric anhydride are thus detectable. The applicability of these tests to cream has not yet been investigated. A. O. J.

Presence of Glycerol in Juice of Ripe Olives. H. Marcelet. (*Compt. rend.*, 1938, **207**, 869–870.)—The oil obtained by expression from the ripe fruit is accompanied by an aqueous brown juice. In one test this was found to contain 0·19 g. of free glycerol and 0·47 g. of combined glycerol per litre together with small amounts of various hexoses. S. G. C.

Pectin from Various Sources. Determination of the Strength of Gels. L. H. Lampitt and R. W. Money. (*J. Soc. Chem. Ind.*, 1939, **58**, 29–32.)—The pectin from various fruits, ripe or unripe, is prepared by submitting the chopped or pulped fruit directly to autoclaving and filtration, and the juice or extract is

partly purified by three precipitations from water with 70 per cent. alcohol. The precipitated pectin is separated, washed with acetone and finally air-dried, and a 5 per cent. solution in water of the powder used. If the "leaching" method is used in the preparation, a great deal of the pectin of ripe fruits is lost. Neither method produces an ash-free pectin, for which recourse must be had to dialysis. The alcohol precipitation is fairly effective in removing organic impurities, *i.e.* acids and sugars. The gel strength was measured by the torsion apparatus previously described (*J. Soc. Chem. Ind.*, 1936, **55**, 88T). There is a very great difference in strength of gel given by the various pectins; *e.g.* to give a strength equal to 100 angular degrees the equivalent concentrations of pectins were: apple, 100; gooseberry, 158; raspberry, 300; plum, 364; blackcurrant, 400. The linear relationship of strength to pectin concentration (*J. Soc. Chem. Ind.*, 1937, **56**, 290T) previously recorded for gelatin and for pectin was observed in every instance, but considerable variations in the slopes of the lines were noted, perhaps owing to the presence of compounds estimated as calcium pectate, but possessing differing powers of gel formation. Although for apple pectin the slope is independent of the *p*H value and of the sugar concentration, with raspberry, plum, and particularly blackcurrant, the slope is apparently dependent on the *p*H value. With apple pectin the gel strength increases to a maximum as acidity is increased, but with other pectins a continuous increase in strength with increasing acidity is observed over the range employed. When the sugar concentration in the gels varied from 55 to 80 per cent. with apple pectin the curve appeared to tend towards a maximum, owing to increased rate of setting, whereas with the other pectins no such tendency was found. It is to be expected that the compounds associated with the true pectin will vary owing to different degrees of ripeness of the fruit, and such compounds may also be the cause of anomalies in the experiments on effects of acidity and sugar concentrations, and differences in rate of setting of the pectins.

D. G. H.

Iodimetric Determination of Small Quantities of Glucose. E. C. Noyons. (*Rec. Trav. Chim. Pays-Bas.*, 1939, **58**, 17–22.)—The iodimetric method (*cf.* Kolthoff, Abst., ANALYST, 1923, **48**, 386; Hinton and Macara, ANALYST, 1924, **49**, 2) has been adapted to the determination of small amounts (0·2 to 2 mg.) of glucose. A definite quantity of the glucose solution (in the test experiments this was prepared by recrystallising glucose from absolute alcohol, drying it to constant weight and dissolving it in 500 ml. of twice-distilled water) is made up to 10 ml. with twice-distilled water in a 100-ml. stoppered Erlenmeyer flask, mixed with 1 ml. of $N/10$ iodine solution, and treated with 1 ml. of $N/5$ sodium hydroxide solution. The closed flask is allowed to stand at room temperature for 30 minutes, after which 6 ml. of 25 per cent. hydrochloric acid are introduced, and the excess of iodine is titrated with $N/200$ sodium thiosulphate solution. Increasing the alkalinity of the solution gives low results, and increasing the temperature gives high ones. To ascertain the effect of substances likely to have a disturbing effect determinations were made in presence of such quantities of acetone, urea, uric acid, lactose, hippuric acid, creatine, creatinine, galactose, alcohol, glycerol and lactic acid as occur in blood, and the results (which are tabulated for creatinine, alcohol,

glycerol, lactic acid, acetone, urea, uric acid, and hippuric acid) led the author to test the method for the determination of glucose in blood. The method of Fujita and Iwatake, who used precipitated cadmium hydroxide to de-albuminise the blood (*Biochem. Z.*, 1931, **242**, 48), was investigated, but was unsatisfactory in unbuffered solutions. The use of a buffer solution containing 50 ml. of *M*/potassium dihydrogen phosphate solution mixed with 75 ml. of *M*/sodium hydroxide solution was investigated, both with and without the de-albuminising agent. In absence of the de-albuminising agent the procedure was as described above, except that 1 ml. of the buffer solution was used instead of the sodium hydroxide solution and the time of oxidation was increased to 40 minutes; the results were more satisfactory than with sodium hydroxide solution. In presence of the de-albuminising agent, the method was as follows:—Two ml. of glucose solution were added to 16 ml. of a solution containing 13 g. of cadmium sulphate and 63·5 ml. of *N* sulphuric acid per litre, and 2 ml. of 1·1 *N* sodium hydroxide solution were added. The mixture was shaken for some time and then centrifuged. To 5 ml. of the centrifugate were added 1 ml. of *N*/10 iodine solution, 1 ml. of buffer solution, and 5 ml. of twice-distilled water, and the determination was carried out in the usual way, a blank determination being made. The accuracy was greatly increased by the addition of the buffer solution. E. M. P.

Differentiation of Natural and Artificial Orange Preparations by Formol Titration. A. Schrempf and E. Benk. (*Z. Unters. Lebensm.*, 1939, **77**, 39–46.)—The formol titration method used by Tillmans and Kiesgen (*Z. Unters. Lebensm.*, 1927, **53**, 132; Abst., ANALYST, 1927, **52**, 417) for the examination of natural and artificial citron juice was applied to the examination of products containing orange juice. To obtain the range for the natural product a number of samples of pure orange juice were prepared from peeled and unpeeled fruit. The juice (10 ml.) was nearly neutralised with aqueous sodium hydroxide solution and made faintly alkaline to phenolphthalein by titration with *N*/10 sodium hydroxide solution. A 40 per cent. solution of formaldehyde was neutralised to phenolphthalein, 10 ml. were added to the neutralised juice, and titration with *N*/10 sodium hydroxide was continued until the original faintly alkaline end-point was again reached. The number of ml. of *N*/10 alkali used in the final titration of 10 ml. of juice is the formol number. The range for the juice from peeled fruit was 2·05 to 2·9 and for juice from unpeeled fruit 1·7 to 2·15. Since many of the manufactured products are highly coloured with coal-tar dyes, which interfere with the formol titration both by obscuring the end-point and by reaction of their constituent amino groups, it is necessary to remove the dye by means of animal charcoal. The following procedure is recommended:—The beverage (100 ml.) or syrup or concentrate (100 ml. of a 10 per cent. dilution) is treated with animal charcoal and filtered. The clear colourless filtrate is almost neutralised to phenolphthalein with *N*/4 to *N*/1 sodium hydroxide solution according to its degree of acidity, and concentrated on the water-bath to 20 ml. When cold it is titrated to a faint pink colour with *N*/10 sodium hydroxide solution, 10 ml. of neutralised formalin are added, and the liquid is again titrated with *N*/10 sodium hydroxide solution. The final titration is calculated to 10 g. of syrup or 100 ml.

of diluted beverage. Since commercial orange syrup or concentrate contains about 35 per cent. of juice of sp.gr. 1·05, the formol value determined with 10 g. of syrup may be converted into the formol value for 10 ml. of juice by multiplying by 3. According to Tillmans, the range for citron juice is 2·1 to 2·6; the authors found a range of 1·7 to 2·9 for orange juice. The following examples indicate the value of the method as a means of distinguishing natural from artificial products. Orange syrups prepared from pure orange juice gave a formol number of 2·1 to 2·85; orange syrups prepared from the whole fruit including the peel gave 1·8 to 1·95; authentic commercial products gave 1·8 to 2·55; a number of commercial specimens, some admittedly not authentic, gave 0·3 to 1·2. All the above-mentioned figures are calculated to 10 ml. of juice, the actual experimental titration figures being one-third of the recorded figure. Aqueous and alcoholic extracts of orange peel (1 part of peel to 1 part of solvent) were prepared. The values for 10 ml. of extract were 0·8 to 0·9 (aqueous) and 0·7 to 0·85 (alcoholic). A syrup prepared with this extract gave a formol number of 0·25 ml. per 10 g. (0·75 ml. per 10 ml. of juice) and bore a close resemblance to certain commercial products which did not claim to be more than "orange flavoured." An artificial effervescing orangeade gave an average formol number of 0·1 ml. per 100 ml. A product prepared by tenfold dilution of genuine syrup gave a formol number of 0·6 ml. per 100 ml. Although the determination of the ash of the products was found to be of little value owing to variation in the mineral-content of local water supplies, and the determination of the phosphate-content was subject to large experimental errors, these determinations provided useful confirmation of opinions based on the formol number. A. O. J.

Determination of Stearic Acid in Fats. A. Heiduschka and W. Böhme. (*Z. Unters. Lebensm.*, 1939, **77**, 33–38.)—In the method of Hehner and Mitchell (ANALYST, 1896, **21**, 36) the stearic acid content of a mixture of fatty acids is determined by dissolving the acids in a warm alcoholic solution of pure stearic acid saturated at 0° C., cooling the solution to 0° C., and determining the amount of stearic acid which separates. Examination of the principle of this method revealed two difficulties: the stearic acid remaining in solution at 0° C. depends upon the initial concentration of the solution and its solubility is influenced by the presence of other acids. Varying amounts of a mixture of pure stearic and palmitic acids of known composition were subjected to this process and by comparison of the amounts of stearic acid found with those known to be present a correction table was constructed by means of which the stearic acid content of a mixture of fatty acids could be determined to within 1 per cent. If the mixture contains acids soluble only with difficulty (*e.g.* arachidic and behenic acids) these are precipitated with the stearic acid and their amount must be deducted. In the re-examination of the method it was found that most of the sources of error could be eliminated by the use of a thermostat controlling the temperatures of the solution to within 0·1° C., and the construction of such an instrument is described. The saturated solution of stearic acid was prepared by dissolving 4·500 g. of the pure acid in warm 99 per cent. alcohol and diluting the solution with alcohol to 1 litre at 20° C. The fatty acid mixture (0·5 g.) is dissolved in 100 ml. of this solution in a 200-ml.

flask immersed for a short time in boiling water. The flask is then connected with a stirring device and placed in the thermostat in which its temperature is maintained at 0° C. for 6 hours. The precipitated stearic acid is collected on a paper disc (No. 604, Schleicher and Schüll) in a Buchner funnel with gentle suction. On the filter-paper is a glass cylinder held against the paper by means of three hooked spiral springs, the object of which is to prevent the precipitate from settling on the sides of the funnel. The residue of stearic acid in the flask and on the stirrer is not removed, since both flask and stirrer form part of the apparatus subsequently used for extraction of the stearic acid. The filter-cake is sucked dry, 30 ml. of ether are placed in the empty flask, which is closed by a stopper carrying a glass "tulip" which in turn is connected with a reflux condenser. The filter with the stirrer and the glass cylinder are placed in the "tulip" and the ether in the flask is gently boiled. Extraction is usually complete in 5 minutes. After removal of the ether by evaporation, the flask is dried at 100° to 105° C. and weighed. The accuracy of the method is indicated by the agreement between parallel determinations. For exact determinations the final result is corrected by means of the table. The method was applied to the determination of the stearic acid content of pigs' fat and beef-fat. The percentage amounts of solid fatty acids found for the beef-fats by the lead salt and ether method were:—breast fat, 41·0; back fat, 40·0; kidney fat, 54·0. The amounts of stearic acid found were:—breast fat, 7·5; back fat, 11·0; kidney fat, 27·0 per cent. For pigs' fat the amounts of total solid acids were:—breast fat, 32·5; abdominal fat, 38·0; kidney fat, 53·0. The amounts of stearic acid found were:—breast fat, 12·0; abdominal fat, 13·5; kidney fat, 30·0. The differences between the amounts of stearic acid and of the total fatty acids were taken as palmitic acid. A. O. J.

Cycad Seed and Nigaki Fruit-coat Oils. M. Tsujimoto and H. Koyanagi. (*J. Soc. Chem. Ind. Japan*, 1938, **41**, 319B–320B.)—The shell of Cycad seed, obtained from the Okinawa Islands, contained 8·29 per cent. of moisture and 21·44 per cent. of oil, and the kernels 30·50 per cent. of moisture and 0·50 per cent. of oil. The oils (obtained by extraction with ether) had the following properties and constants: —(*a*) *Shell Oil.*—At 23° C. this was an orange-coloured viscous liquid containing fine crystals; it became clear at about 39°; d_4^{45}, 0·8919; n_D^{20} (determined in liquid state), 1·4653; acid value, 48·2; saponification value, 196·6; iodine value (Wijs), 59·0; unsaponifiable matter, 2·54 per cent. The fatty acids became soft at 23° C. and melted at 45–46° C.; neutralisation value, 203·6; iodine value, 59·8. The bromide test gave no precipitate in ether and only a slight one in petroleum spirit. The lead salt and alcohol method of separation yielded 42 per cent. (diff.) of solid acids (m.p. 57–58° C., neutralisation value 213·9, iodine value 12·1), and 58 per cent. of liquid acids (n_D^{20} 1·4640, neutralisation value 195·6, iodine value 101·2). The acids were chiefly palmitic and oleic, with a small amount of linolic. The unsaponifiable matter, a yellow-orange viscous substance, contained carotinoid compounds (*cf.* Nishida, *J. Soc. Agric. Chem.*, 1937, **13**, 89). (*b*) *Kernel Oil.*—At 23° C. this was an orange-yellow liquid, d_4^{30}, 0·9293; n_D^{20}, 1·4740; acid value, 8·7; saponification value, 173·2; iodine value (Wijs), 73·6.

Nigaki Fruit.—Oil from entire Nigaki fruit has previously been examined

(cf. *Rep. Tokyo Imp. Ind. Res. Lab.*, 1931, **26**, 45; *Bull. Chem. Soc. Japan*, 1933, **8**, 161); it contains petroselinic acid. The fruit-coats (excluding hard endocarp) of two specimens from Chôsen, Korea, contained about 50 to 60 per cent. of oil. The oils (1) and (2), extracted with ether from the corresponding specimens, differed considerably in properties, as follows:—*Oil* (1).—At ordinary temperatures this was a soft brownish yellow-orange mass with slight fluorescence; d_4^{30}, 0·9351; n_D^{30}, 1·4670; acid value, 52·3; saponification value, 206·9; iodine value (Wijs), 62·6; acetyl value, 33·5; unsaponifiable matter, 2·97 per cent.; petroleum-spirit-insoluble (resinous) 0·50 per cent. The fatty acids were brownish-yellow and crystalline; m.p., 47–48° C.; neutralisation value, 213·4; iodine value, 57·6; bromide test: no precipitate in ether, 7·6 per cent. in petroleum spirit (linolic tetrabromide, m.p. 113–114° C.). The lead salt and alcohol method of separation yielded 46 per cent. (diff.) of solid acids (neutralisation value 201·8, iodine value 14·5) and 54 per cent. of liquid acids (neutralisation value 187·1, iodine value 112·4). The acids were chiefly palmitic and oleic, with a little linolic, smaller amounts of C_{12} or C_{14} acids or both and of acids higher than C_{18}, and some acidic resinous substance. *Oil* (2).—This was a yellowish-orange liquid with a small deposit of solid matter; it was slightly fluorescent; d_4^{20}, 0·9287; n_D^{20}, 1·4712; acid value, 17·5; saponification value, 199·9; iodine value (Wijs), 81·7; acetyl value, 23·6; unsaponifiable matter, 2·60 per cent., petroleum-spirit-insoluble (resinous), 0·85 per cent. The fatty acids contained approximately 3 per cent. of petroleum-spirit-insoluble matter. Petroselinic acid appeared to be absent from the fruit-coat; the unsaponifiable matter, a yellow viscous solid, contained sterol.

E. B. D.

Fatty Acids and Glycerides of Solid Seed Fats. VII. Dika Fat. W. J. Bushell and T. P. Hilditch. (*J. Soc. Chem. Ind.*, 1939, **58**, 24–26.)—The kernels of *Irvingia gabonensis* (dika) used in this investigation were obtained from Sierra Leone and yielded 61 per cent. of fat with saponification equivalent 222·6; iodine value 1·8, and unsaponifiable matter 0·4 per cent. The component fatty acids (as per cent. mol.), calculated from the fractionation data, consisted of *n*-decoic, 3·8; lauric, 61·6; myristic, 30·8; palmitic, 1·6; stearic, 0·8; oleic, 1·4. Other recorded analytical data for *Irvingia gabonensis* show variations in iodine values from 2·1 to 9·8, and in saponification equivalents from 224·1 to 232·2. It is evident that there is more than one variety of *Irvingia gabonensis*, and the present sample was one of very low oleic acid content. Further, the seed fats of *Irvingia barteri* (Nigeria) and *Irvingia oliveri* (Cochin China) appear to be closely similar, but differ from the fat of *Irvingia gabonensis* in that their component acids include about 40 per cent. of lauric and 55 per cent. of myristic acid. The component glycerides of the dika fat were calculated after systematic crystallisation from acetone and oxidation of some of the fractions with potassium permanganate and determination of saponification equivalents. The calculation was only approximate owing to the presence of small proportions of mixed glycerides in which very small proportions of palmitic, stearic and oleic acids were combined as minor components. It may, however, be safely assumed that about two-thirds of the fat consist of dilauro-myristin, and also that this proportion and the probable

approximate proportions of laurodimyristin and of some of the minor components (including the mono-oleo-glycerides of fractions 4 and 7, corresponding with about 6 per cent. of the whole fat, or three times the molar proportion of oleic acid in the component acids) accord with the close operation of the "even distribution" rule in the component glycerides of this fat. D. G. H.

Active Principles of *Cannabis Indica* Resin. T. S. Work, F. Bergel and A. R. Todd. (*Biochem. J.*, 1939, **33**, 123–127.)—The resin obtained from a sample of Indian hashish was esterified with *p*-nitrobenzoyl chloride. Cannabinol *p*-nitrobenzoate crystallised out from the solution of the crude ester in petroleum spirit; it had m.p. 160° C. On being hydrolysed it gave an almost colourless oil that yielded a crystalline acetate, m.p. 75° C. The cannabinol was non-toxic to rabbits when injected in doses less than 2 mg. per kg. body-weight, but was highly toxic in doses in excess of this. It did not induce corneal anaesthesia (Gayer test). The resin obtained by hydrolysis of the non-crystalline *p*-nitrobenzoates was also tested on rabbits. It had no effect in doses less than 1 mg. per kg. body-weight, but in doses exceeding that amount it produced sleep and loss of the corneal reflex. Doses in excess of 5 mg. per kg. body-weight were lethal. By chromatographic adsorption of the non-crystalline *p*-nitrobenzoates a fraction was obtained giving a positive Gayer test in a dose of 0·25 mg. per kg. body-weight. F. A. R.

Constituents of the Seeds of *Cleome viscosa*. M. P. Gupta and S. Dutt. (*J. Indian Chem. Soc.*, 1938, **15**, 532–536.)—*Cleome viscosa*, Linn. N.O. *Capparidae*, is a common plant in tropical India and Ceylon, and is called Hurhur or Hulhul or Kampute in Hindustani and Hurhuriya in Bengali. *Cleone pentaphylla*, already examined (Misra and Dutt, *Proc. Nat. Inst. Sci. India*, 1937, **3**, 45), is known by the same name in Hindustani, and the two species are much confused. The seeds are said to be anthelmintic, rubefacient and vesicant, and are regarded as much superior to mustard seed and equal to European mustard. The whole plant, according to O'Shaughnessy (Kirtikar and Basu, *Indian Medicinal Plants*, 1918, **1**, 98), is used as a counter irritant and blistering agent in Cochin China, and in the United States the roots are said to be used as a vermifuge. A new acid, viscosic acid, and a new flavone, viscosin, have now been isolated from the seeds. Benzene extraction of the seeds yielded 36·59 per cent. of a reddish-yellow oil from which some waxy material separated. The oil deposited a crystalline material found to consist of myristic, palmitic and the new viscosic acid. This acid was obtained from the crystalline deposit by washing with benzene, drying and taking up with ether. The fraction insoluble in ether was crystallised twice from ethyl alcohol and then repeatedly from methyl alcohol and a yield of 0·1 per cent. of white crystalline needles, m.p. 97° C., was obtained. Viscosic acid does not reduce Fehling solution, dissolves in cold conc. sulphuric acid, gives no colour with ferric chloride solution, and does not form oximes or acetyl derivatives. Its composition corresponds with the formula $C_{27}H_{52}O_3$, and it appears to be an unsaturated fatty acid. On oxidation with dilute potassium permanganate it yields a dihydroxy acid, $C_{27}H_{54}O_5$, and has one double bond and an iodine value (Wijs) 22·5. On extracting the defatted seeds with boiling ethyl alcohol crystals were deposited which were found to be viscosic acid. The mother liquor was further

concentrated, freed from moisture and extracted with petroleum spirit. The insoluble portion was dissolved in alcohol, and the solution was treated with excess of lead acetate. The lead salt was collected and decomposed in alcoholic suspension with hydrogen sulphide. On dilution with water tannins and a yellow substance were precipitated. The colouring matter was freed from tannins by extraction with boiling water and recrystallised several times from water and then from aqueous methyl alcohol. Finally, the pale yellow hexagonal plates were crystallised from aqueous *iso*propyl alcohol. The viscosin obtained darkened at 285° C. and melted with decomposition at 294–295° C. Viscosin has a composition corresponding with the formula $C_{16}H_{12}O_6$ and contains no water of crystallisation. It gives a dark green colour with alcoholic ferric chloride solution, forms a triacetyl derivative and contains one methoxy group. It is, in fact, a monomethoxytrihydroxy flavone. A 1 per cent. alcoholic solution had a well-defined absorption band between 410 and $462m\mu$ with absorption maximum at $442{\cdot}5m\mu$. A study of the physiological action of viscosin is in progress.

D. G. H.

Microchemical Reactions for Acedicon. F. Amelink. (*Pharm. Weekblad,* 1939, **76**, 74–77.)—Acedicon is an isomer of acetylated codeine, differing from it in the position of the double bond, which is attached to the same carbon atom as the acetylated enolic hydroxyl group. It is prepared synthetically, has a m.p. of 152° to 153° C., and is sold in the form of the hydrochloride (by Boehringer). The following tests were evolved with the object of fitting it into a general scheme for the microchemical identification of alkaloids (*Schema zur Mikrochemischen Identifikation von Alkaloiden,* Amsterdam, D.B. Centen); most of the crystal-forms are illustrated by drawings in the paper abstracted. Platinic chloride produces a pale yellow precipitate, which in acid solutions forms doubly-refracting globules, 8 to 15μ in size, characteristic in appearance and grouping under the low powers of the microscope. In neutral solutions these are similar but larger (50μ), and form star-shaped clusters of radiating deformed needles. Sodium iodide is without effect on the reaction, which is definite with 0·1 per cent. solutions of acedicon. Gold chloride in neutral solution produces an abundant precipitate of strongly doubly-refracting, thin, flat prisms which appear pale green by transmitted light, and form star-shaped groups 90 to 270μ in size. They are dichroic (light yellow to colourless) and *d*-rotatory, and the reaction is unaffected by the presence of sodium bromide and is given by 0·1 per cent. solutions. Mercuric chloride in acid solution produces a white precipitate of droplets, which on warming, scratching and cooling form star-shaped groups of tree-like branched needles, 130 to 500μ in size. On adding a drop of water and scratching vigorously the crystals grow, forming strongly doubly-refracting, *d*-rotatory prisms (size 50 to 150μ), grouped in long, almost rectangular block-shaped aggregates; sensitiveness, 0·5 per cent. Potassium ferrocyanide gives no reaction in the presence of acid, but in a neutral medium a precipitate is formed (particularly if the liquid is warmed and the microscope slide scratched while it is cooling), consisting of groups in the form of stars and sheaves of thin, light-brown strongly doubly-refracting, *d*-rotatory needles, 120 to 700μ in size when produced from 1·0 per cent. solutions; sensitiveness,

0·2 per cent. Potassium ferricyanide gives a negative reaction in both acid and neutral solutions; Dragendorff's reagent (modified form, *loc. cit.*, p. 7) gives a brown precipitate which is useless as a means of identification. Potassium hydroxide solution produces a precipitate of white droplets. Ammonium dichromate forms star-shaped groups of strongly doubly-refracting brown crystals, 40 to 250μ in size, from cold neutral 1 per cent. solutions, but 0·5 per cent. solutions give indefinite results. Sodium anthraquinone-β-sulphonic acid produces abundant crystals from neutral 0·1 per cent. solutions. These, however, are not particularly characteristic, unless the solution is subsequently warmed, when fine, strongly doubly-refracting, *d*-rotatory needles are deposited (size, 70 to 130μ). Sodium carbonate may be used to "salt out" the base from neutral 0·1 per cent. solutions. In the cold, droplets result, which form star-shaped groups (size, 150μ) of crystals with brush-like ends; on scratching, groups in the form of stars and sheaves (size, 20 to 70μ) are obtained. Sodium bicarbonate behaves similarly, but only with solutions stronger than 0·5 per cent., and the crystals are star-shaped groups of prisms (size, 300 to 800μ). Sodium phosphate solution (Dutch Pharmacopoeia, Ed. V), however, is the best "salting out" agent; it produces sheaf-shaped groups of prisms (about 30μ in size at first, but growing to 130μ) from 0·2 per cent. solutions. In all three tests the crystals of the base are doubly-refracting and *d*-rotatory. Conc. sulphuric acid and Erdmann's reagent produce a pale yellow colour; Frohde's reagent, olive green to yellow; Marquis' reagent, yellow-brown, changing through red-violet to blue-purple. The platinic chloride, mercuric chloride, sodium anthraquinone-β-sulphonic acid and gold chloride reagents are specific for acedicon in dilute solutions, and the two last are considered the best. J. G.

Biochemical

Micro-determination of Oxyhaemoglobin, Methaemoglobin and Sulphaemoglobin in a Single Sample of Blood. K. A. Evelyn and H. G. Malloy. (*J. Biol. Chem.*, 1938, **126**, 655–662.)—When sodium cyanide is added to a solution of methaemoglobin, this is converted into cyano-methaemoglobin and the characteristic absorption band at 635$m\mu$ is almost completely eliminated; the resulting change in absorption at 635$m\mu$ is directly proportional to the concentration of methaemoglobin. The 620$m\mu$ absorption band of sulphaemoglobin is unchanged by the addition of cyanide, and thus the concentration of sulphaemoglobin is proportional to the absorption at 620$m\mu$ after the addition of cyanide, but a correction has to be applied for the small absorption of oxyhaemoglobin and cyano-methaemoglobin at 620$m\mu$. The concentration of oxyhaemoglobin can be calculated by difference. Fresh whole blood (0·1 ml.) is added to 10 ml. of $M/60$ phosphate buffer of *p*H 6·6 in a colorimeter tube. The solution is allowed to stand for 5 minutes, and a reading (L_1) is made with filter 635 after the galvanometer has been adjusted to 100 with a blank tube containing water only. One drop of a solution made by mixing equal parts of 10 per cent. sodium cyanide solution and 12 per cent. acetic acid is then added and after 2 minutes a second reading (L_2) is made with the same filter and blank tube. The difference (L_1–L_2) is proportional to the concentration of methaemoglobin. The solution is cleared by the addition

of one drop of conc. ammonium hydroxide and a third reading (L_3) is made with filter 620 and a blank tube containing water only. Finally, 2 ml. of the solution are pipetted into a second colorimeter tube containing 8 ml. of $M/15$ phosphate buffer of *p*H 6·6 and 1 drop of 20 per cent. potassium ferricyanide solution. The tube is allowed to stand for 2 minutes to allow all the oxyhaemoglobin to be converted into methaemoglobin. After the addition of 1 drop of 10 per cent. sodium cyanide solution, the mixture is allowed to stand for another 2 minutes to convert the methaemoglobin into cyano-methaemoglobin and a fourth reading (L_4) is then made with filter 540 and a blank tube containing 10 ml. of water and 1 drop each of 20 per cent. potassium ferricyanide solution and 10 per cent. sodium cyanide solution. Then

$$\text{concentration (g. per 100 ml.) of total haemoglobin (T)} = \frac{100 \times L_4}{2{\cdot}38}$$

$$\text{,, (,, ,,) ,, methaemoglobin (M)} = \frac{100(L_1 - L_2)}{2{\cdot}77}$$

$$\text{,, (,, ,,) ,, sulphaemoglobin (S)} = \frac{1000 \times L_3 - (8{\cdot}5 \times M + 4{\cdot}4 \times T)}{100}$$

$$\text{,, (,, ,,) ,, oxyhaemoglobin} = T - (M + S).$$

The numerical values of the calibration constants which appear in the above equations only hold good for the particular colorimeter used. The first equation is not strictly accurate when sulphaemoglobin is present, and a correction equal to $0{\cdot}22 \times S$ must be added to the value of T. The error in the determination of total haemoglobin is about 0·2 g. per 100 ml. in the absence of sulphaemoglobin and 0·4 g. when this is present. The error in estimating methaemoglobin is less than 0·2 g. per 100 ml. and that for sulphaemoglobin probably not much higher. Little interference was encountered from extraneous pigments present. F. A. R.

Colorimetric Method for the Determination of Inulin in Blood Plasma and Urine. K. Steinitz. (*J. Biol. Chem.*, 1938, **126**, 589–593.)—Inulin is one of the most satisfactory substances for the determination of glomerular filtration and an accurate method of estimating inulin in blood plasma and urine is therefore highly desirable. The method proposed is that used by Roe (*cf.* ANALYST, 1934, **59**, 835) for the estimation of fructose, as it was found that inulin was completely hydrolysed by conc. hydrochloric acid in the time taken for full development of the fructose colour (8 minutes).

The estimation was carried out according to the procedure adopted by Roe, except that with plasma determinations standard solutions of fructose were prepared containing 0·5, 1, 2 and 3 mg. per cent. in saturated benzoic acid solution. For the estimation in urine, dilutions of 5, 10, 20 and 30 mg. per cent. in 1 per cent. acetic acid with 1 per cent. sublimate was prepared. The colour of the unknown solution was compared in a colorimeter with that of the standard solution that most nearly matches it, a green filter transmitting at 510$m\mu$ being used. F. A. R.

Study of the Passage of Fatty Acids of Food into Lipins and Glycerides of the Body, using Deuterium as an Indicator. B. Cavanagh and H. S. Raper. (*Biochem. J.*, 1939, **33**, 17–21.)—A fat containing 4 to 5 atoms per cent. of deuterium was fed to rats, and the distribution of the deutero-fatty acids in the "lipin" (acetone-insoluble) and "glyceride" (acetone-soluble) fractions of liver, kidney, brain and blood was determined 6, 10 and 24 hours afterwards by combustion of the fats and measurement of the density of the water so formed. At the end of 6 hours about 26 per cent. of the plasma glyceride, 18 per cent. of the liver glyceride, 14 per cent. of the liver lipin fatty acids, and 2·5 per cent. of the kidney glyceride were derived from the deutero-fat administered, which indicates a selective intake of fat from the blood by the liver soon after its absorption, the lipins of the liver also participating in this phenomenon. The brain and adipose tissue contained only traces of deuterium. At the end of 24 hours, the amount of deuterium in the glyceride fraction of the liver and of the plasma had decreased markedly, but the deuterium-content of the lipoid fraction of the liver, the kidney and the brain showed comparatively little change. These results emphasise the important part played in fat metabolism by the liver lipins. F. A. R.

Researches on the Protoplasm of Green Plant-cells. I. Isolation of the Chloroplasts of Spinach-leaves. W. Menke. (*Z. physiol. Chem.*, 1938, **257**, 43–48.)—Fresh spinach-leaves were ground in a roller-mill so as to disintegrate the tissue without destroying all the chloroplasts, and the press-juice was centrifuged. The sediment, containing cell-fragments and unchanged chloroplasts, was suspended in a buffer-solution and again centrifuged, first at a low speed to remove coarse particles, and then at a high speed to separate the chloroplasts. These were purified by further fractional centrifuging, and finally dried *in vacuo* after being frozen. The lipoid-content of the material was estimated by extraction first with ether and then with a mixture of ether and alcohol; the protein-content was estimated by multiplying by 6·25 the nitrogen-content determined by the micro-Kjeldahl method; the ash was determined by ignition. The percentages of protein, lipoid and ash in the chloroplasts and in a "chloroplast-substance" previously prepared by extraction (*Z. Bot.*, 1937, **32**, 273) were respectively: 47·7, 56·4; 37·4, 31·9; 7·8 and 4·7. It is concluded from the lower protein-content of the chloroplasts that the extract previously made was contaminated with some 15 per cent. of "cytoplasm-substance." The protein of the chloroplast, unlike that of the cytoplasm, which is a glutelin, is soluble to the extent of only about 20 per cent. in water, dilute salt solutions or dilute acid or alkali. When the fat-free chloroplasts were extracted with water made very slightly alkaline, a protein containing phosphorus was extracted and could be precipitated by adding acid; this resembled a nucleo-protein. F. A. R.

Application of Schryver-Fosse Reaction to Tests for Oxalic Acid, Ascorbic Acid, Tartaric Acid and Uric Acid. M. Paget and R. Berger. (*Compt. rend.*, 1938, **207**, 800–802.)—*Detection and Determination of Small Amounts of Oxalic Acid.*—Oxalic acid is reduced by means of zinc and hydrochloric acid to glyoxylic acid, which is then determined colorimetrically by the Schryver–Fosse reaction with phenylhydrazine. To 2 ml. of the solution, containing 5 to 70 mg.

of oxalic acid per litre, is added 1 ml. of *N* hydrochloric acid and a piece of zinc foil. The liquid is stirred electrically at 275 r.p.m. for 30 minutes. A 2-ml. portion is transferred to a Pyrex tube, and 2 drops of a 1 per cent. solution of phenylhydrazine hydrochloride solution are added. The tube is heated in boiling water for 2 minutes and cooled, 1·8 ml. of conc. hydrochloric acid and 2 drops of hydrogen peroxide (10 vol.) are introduced, and the tube is kept in darkness for 10 minutes to allow the colour to develop. The colour is matched colorimetrically against a standard prepared by treating an oxalic acid solution of known strength in an exactly similar manner. Sensitiveness of test: 1/200th mg. of oxalic acid. The cations Na, K, NH_4, Li, Ba, Sr, Ca, Mn, Fe and Zn do not interfere. *Ascorbic Acid.*—This acid is oxidised, yielding a mixture of threonic acid and oxalic acid, as follows: To 1 ml. of the ascorbic acid solution are added 2 drops of conc. sulphuric acid and 2 drops of 3 per cent. potassium permanganate solution. After 2 minutes the solution is decolorised by the addition of hydrogen peroxide. The oxalic acid in the solution is then determined as described above. Sensitiveness: 1/100th–2/100th mg. of ascorbic acid. *Tartaric Acid.*—Oxidation of the solution as for ascorbic acid yields a mixture of glyoxylic acid (small amounts), oxalic acid and butane-dione-2·3-dioic acid. The oxalic acid may be tested for as described above. Other hydroxy-carboxylic acids may give similar reactions. *Uric Acid.*—Heating a solution of uric acid made alkaline with sodium carbonate yields allantoic acid, which is capable of being tested for by the Schryver–Fosse reaction. S. G. C.

Determination of Ascorbic Acid in Urine with the Photoelectric Colorimeter. K. A. Evelyn, H. T. Malloy and C. Rosen. (*J. Biol. Chem.*, 1938, **126**, 645–654.)—Estimation of the ascorbic acid content of urine by titration with 2 : 6-dichlorophenolindophenol, according to the usual procedure, gives high results because other substances that reduce the dye are present in urine. A somewhat empirical method has been devised for reducing the errors introduced by the presence of these interfering substances, based on the observation that whilst ascorbic acid reduces the dye almost instantaneously, other substances react much more slowly. Nine ml. of an aqueous solution of dichlorophenolindophenol are added from a specially prepared rapid delivery (1 sec.) pipette to 1 ml. of 5 per cent. acetic acid in a colorimeter tube and the colour is measured immediately in a photoelectric colorimeter (filter 520) after the galvanometer has been adjusted to 100 with a tube containing water only. The corresponding reading (L_1) is noted. The galvanometer is then adjusted to 100 with a blank tube containing 9 ml. of water and 1 ml. of urine acidified with 5 per cent. (by volume) of glacial acetic acid. Nine ml. of the dye solution are introduced into another tube containing 1 ml. of the acidified urine, from the special pipette, and the galvanometer reading is recorded 5, 10, 20 and 30 seconds after the addition of the dye began. These readings are plotted and the smooth curve obtained is produced backwards to cut the ordinate. The galvanometer reading so extrapolated (L_2) to zero time is noted. Then the concentration (X) of ascorbic acid in mg. per 100 ml. of acidified urine is calculated from the equation:

$$X = \frac{10{\cdot}8\,(L_1 - L_2)}{A}$$

where 10·8 is the calibration constant of the particular instrument used obtained by measurement of solutions of pure ascorbic acid and A is the volume (ml.) of acidified urine used in the test. The equation is accurate only when the proportion of the dye solution to urine is 9 : 1. Whereas by the usual method of titrating with the dye, the presence of sodium thiosulphate and cysteine often leads to too high a value for the ascorbic acid content of solutions, the photoelectric method gives results much more closely in agreement with the theoretical values. In a number of normal urines that were examined the ratio of titrimetric to photoelectric values varied from almost 1 : 1 to 10 : 1 and more, and considerable doubt is thrown upon the urinary excretion test used to detect vitamin C subnutrition. An attempt was also made to improve the accuracy of the method still further by a preliminary purification of the urine, and this was found to be most conveniently effected by precipitation of interfering substances with barium acetate (*cf.* P. J. Drumm, H. Scarborough and C. P. Stewart, *Biochem. J.*, 1937, **31**, 1874; ANALYST, 1938, **63**, 58). F. A. R.

Catatorulin Test for Vitamin B_1. R. A. Peters. (*Biochem. J.*, 1938, **32**, 2031–2036.)—An improved catatorulin test for estimating vitamin B_1 is described, the chief improvement being the use of sodium pyruvate instead of lactate as substrate. A small amount of the minced brain (cerebrum and optic lobes) of avitaminous pigeons is suspended in Ringer-phosphate solution (pH 7·3) containing 6 mg. of sodium pyruvate. Triplicate estimations, if possible, of the oxygen uptake of the minced brain are made after the addition of 0, 0·25 and 0·5γ of the vitamin. The tubes are allowed to stand at room temperature for about 10 minutes before filling them with oxygen, and the volume of oxygen absorbed by the three lots of tissue during the period 1 to 2 hours is measured and then plotted against the amount of vitamin added. The resulting curve usually approximates to a straight line, and from it the amount of vitamin B_1 present can be calculated, since the value of the oxygen uptake of the pure vitamin under the same conditions is known. The errors involved in the estimation are variable, the results from some brains having to be discarded. F. A. R.

Estimation of Vitamin B_1 in Blood. H. M. Sinclair. (*Biochem. J.*, 1938, **32**, 2185–2199.)—The claim made by Meiklejohn (*Biochem. J.*, 1937, **31**, 1441), *viz.* that the estimation of vitamin B_1 in blood by its effect on the growth of the mould *Phycomyces blakesleeanus* "provides a quantitative estimate of the true vitamin B_1 content of the blood," is subjected to examination, and his results are not confirmed. It has now been shown that hydrolysed casein is a more satisfactory source of nitrogen than the asparagine used by Meiklejohn, and that the marked adjuvant action of blood in the presence of asparagine is due to sources of nitrogen in the blood; Meiklejohn's medium was also deficient in salts. A further cause of the adjuvant action of blood is its buffering action, which allows the fungus to continue to grow for longer than it otherwise would; the addition of calcium carbonate invariably produces an increase in growth in solutions without blood. If consistent results are to be obtained, care must be taken to control such factors as the temperature of autoclaving and the length of time the blood is stored; the variations that occur are probably due to destruction of adjuvant factor in

the first case and to an increase as a result of autolysis in the second. Substances other than vitamin B_1 that act as growth factors for the fungus are: bios, breakdown products of vitamin B_1 (producing growths much greater than those obtained with excess of vitamin B_1) autoclaved marmite, autolysed yeast and "Peter's eluate." Indole, amongst other substances, inhibited growth. It is concluded that when the possible sources of error are borne in mind and controlled as far as possible, the method is valuable for comparing the apparent vitamin B_1 contents of different samples of blood. F. A. R.

Occurrence of Vitamin B_2 (Lactoflavin). Part I. Vitamin B_2 in Muscle. J. Schormüller. (*Z. Unters. Lebensm.*, 1939, **77**, 1–18.)—In order to determine lactoflavin in extracts of animal tissue it was converted by irradiation in alkaline solution into lumiflavin and the colour of this was measured photometrically. It is necessary to distinguish between the determination of total flavins and the determination of free flavins and the flavin combined with protein (yellow enzyme). The determination of total flavins was made by the procedure of v. Euler and Adler (*Z. physiol. Chem.*, 1934, **223**, 105) by repeated extraction with aqueous 60 to 80 per cent. acetone or with boiling water. The combined extracts were concentrated *in vacuo* and purified with ether, chloroform or petroleum spirit. Irradiation, chloroform extraction and colorimetric measurements followed the procedure of Kuhn and his collaborators (*Ber.*, 1934, **67**, 1455). Often, in spite of repeated purification, the chloroform solution was coloured brown. In these instances, before the photometric measurement, the extract was subjected to chromatographic separation by means of a layer of aluminium oxide, the intensely coloured lumiflavin being separated by a mixture of 100 parts of pyridine, 10 parts of methyl alcohol and 10 parts of chloroform. Complete separation of lumiflavin from non-fluorescent impurities was thus effected. Lumiflavin, purified by recrystallisation from absolute alcohol, appeared under the microscope as fine tufts of needle crystals. Its m.p. (uncorr.) was 237 to 238° C. and, by the method of mixed m.p., muscle lumiflavin was found to be identical with liver-lumiflavin. To separate the flavins of low molecular weight (lactoflavin and its phosphoric ester) from the flavin combined with colloidal carriers (yellow enzyme) the aqueous extracts were dialysed in cellophane tubes immersed in distilled water for 16 hours at 3° C. The two fractions were then converted into lumiflavin and analysed. Lactoflavin was separated from its phosphoric ester by extraction of the aqueous solution with benzyl alcohol in which the unesterified compound is soluble (Emmerie, *Nature*, 1938, **141**, 416). The lactoflavin-content (γ per 100 g. of dry material) of various kinds of muscle fibre is as follows:—lean beef, 155·6 to 222·7; lean veal, 280·8 to 291·0; lean pork, 348·6 to 374·6; lean mutton, 268·1; fat mutton, 399·2; lean horse flesh, 425·3 to 433·9; hen (total muscle), 199·8; dog (total muscle), 179·4; Liebig's meat extract (original undried substance), 1932 to 2071. Little variation in vitamin B_2 content was found in corresponding portions of animals of the same species. A comparison of the figures obtained by biological methods and by chemical analysis shows that, although the amounts found by the two methods may vary widely, they give similar ratios for the distribution of the vitamin among different organs. Liver has from 10 to 15 times as much vitamin B_2 as muscle,

and cow's heart-muscle has 7 times as much as lean beef. A considerable amount of man's daily requirement of vitamin B_2 (2 to 3 mg. of lactoflavin) is supplied by meat, especially by such organs as heart, liver and kidneys. An examination of the smooth muscle and mucous membrane of the cow's intestines showed that smooth muscle contains about 3 times and the mucous membrane of the intestine about 6 times as much lactoflavin as striped muscle. The muscle fibre of fresh-water and sea-water fish was found to be comparable with lean beef in vitamin-content, whilst turbot roe contains 16 times as much as cod-muscle. An experiment designed to imitate the ordinary boiling of meat showed that 70 per cent. of the flavin appears in the broth, and this accounts for the high vitamin B_2 content of meat extracts. An inverse relationship appears to exist between the lactoflavin-content and the glycogen-content of muscle. Tonic muscle with slow movement (*e.g.* smooth viscera-muscle and red leg-muscle) contains much lactoflavin and little glycogen. Non-tonic, quick-moving muscle (*e.g.* pale breast-muscle) is rich in glycogen and poor in lactoflavin.

A. O. J.

New Method for the Isolation of α- and β-Tocopherols. A. R. Moss and J. C. Drummond. (*Biochem. J.*, 1938, **32**, 1953–1956.)—The saponification of wheat-germ oil and extraction of the unsaponifiable matter apparently effects a fourteen-fold concentration of the vitamin E present, but evidence has recently been accumulating to show that the concentration effected is in fact much smaller than this because of the sensitivity of the vitamin to alkali. Accordingly, an attempt was made to prepare a vitamin E concentrate by an alternative method. This was successfully done by chromatography. A 10 per cent. solution of wheat-germ oil in petroleum spirit was poured on to a column of alumina, but instead of the chromatogram being developed with fresh solvent, more of the solution was added until the band containing vitamin E reached almost to the bottom of the column. Three bands were formed: a pinkish-yellow zone which eventually passed through the column into the filtrate, a yellow zone which passed slowly down the column and tended to separate into two bands, and a deep orange yellow band which was adsorbed at the top of the column. After the column had been washed with petroleum spirit, the two bands of the yellow zone were separately eluted with a mixture of ether, benzene and methyl alcohol. They contained the vitamin E, and the concentrates so made were (together) some 14 times richer than the original oil. Both had absorption maxima at about $295m\mu$, but yielded only oily allophanates. After saponification, however, crystalline allophanates of β- and α-tocopherol were obtained from the upper and lower bands respectively. Two kg. of wheat-germ oil yielded 1 g. of α- and 0·75 g. of β-tocopherol allophanate.

F. A. R.

Colorimetric Determination of α-Tocopherol (Vitamin E). A. Emmerie and C. Engel. (*Rec. Trav. Chim. Pays-Bas*, 1938, **57**, 1351–1358.)—The method of estimating vitamin E proposed by Karrer and Keller (*cf.* ANALYST, 1938, **63**, 835) was based on the reduction of gold chloride solution by the vitamin. It has now been found that, although tocopherol only slowly reduces ferric chloride alone, the reduction is much more rapid in the presence of αα′-dipyridyl, which immediately removes the ferrous ions formed. One ml. of a 0·2 per cent. alcoholic

solution of ferric chloride (freshly prepared) is added to about 1 ml. of an alcoholic solution (containing 0·1 to 0·4 mg.) of tocopherol. The two solutions are mixed, 1 ml. of a 0·5 per cent. alcoholic solution of $\alpha\alpha'$-dipyridyl is added, and the solution is diluted to 25 ml. The red colour of the ferrous-dipyridyl complex is measured after 10 to 15 minutes by means of the Zeiss–Pulfrich photometer, screen 50 and a 1 cm. cell being used. A blank solution is prepared with ferric chloride solution and $\alpha\alpha'$-dipyridyl and placed in the other cell of the photometer. A standard curve is prepared by using α-tocopherol. Since carotene also reduces ferric chloride, allowance must be made for this in calculating the vitamin E contents of oils or concentrates that also contain carotene. A standard curve is constructed for carotene, and the conversion factor for carotene into tocopherol is calculated from it. In estimating the amount of vitamin E in oils, these are first saponified by heating with twice their volume of 2 *N* methyl alcoholic potassium hydroxide solution for 10 minutes in an atmosphere of nitrogen. The unsaponifiable matter is extracted with ether from the soap solution after dilution with water and then dissolved in alcohol. The results were in close agreement with those obtained by Karrer's method. F. A. R.

Quantitative Photometric Estimation of Vitamin E. M. Furter and R. E. Meyer. (*Helv. Chim. Acta*, 1939, **32**, 240–250.)—Both natural β-tocopherol and synthetic *d,l.*-α-tocopherol in alcoholic solution give deep red solutions on being treated with nitric acid, but the nature of the reaction is not known. The colour is not given by any substance with which synthetic or natural tocopherol is likely to be associated. Thus hydroquinone, xyloquinone, trimethyl hydroquinone, durohydroquinone and naphthoquinone, equilin and equilenin, tyrosine and β-carotene gave yellow solutions; phytol and phytyl bromide, ascorbic acid, androsterone and oestrone, phenol, α- and β-resorcylic acids and salicylic acid, glucose, starch and lactose gave colourless solutions. Wheat-germ oil was the only oil tested that gave a red colour, though castor oil gave a pale rose colour; linseed, sesame and cod-liver oils gave yellow solutions and arachis and olive oils colourless solutions. The reaction has been made the basis of a method of estimating vitamin E. The absorption spectrum of the coloured solution has a well-defined maximum at 467$m\mu$, and the intensity of the absorption at this wavelength is strictly proportional to the concentration of the vitamin and to the thickness of the layer of solution. Moreover, the intensity did not decrease on standing for 24 hours. The estimation of tocopherol can be carried out with fair accuracy, the error involved seldom being greater than 3 per cent. The minimum quantity of material that can be estimated is 0·05 per cent. One to 5 mg. of material, containing not less than 0·3 mg. of tocopherol are weighed into a 25-ml. flask and dissolved in about 5 ml. of absolute alcohol. Alternatively a 5-ml. aliquot portion of an alcoholic solution of known strength is transferred to the flask. One ml. of pure conc. (65 per cent.) nitric acid is added from a burette, the flask being gently rotated during the addition. A small piece of porous pot is put into the flask, which is then fitted with a reflux condenser, and the contents are heated gently under reflux for 3 minutes, after which the flask is cooled and allowed to stand for 15 minutes. A little of the solution is transferred by means

of a pipette to the 1-cm. cell of a Pulfrich photometer fitted with filter S47. A mixture of 83·5 per cent. (by volume) of absolute alcohol and 16·5 per cent. of conc. nitric acid is put into the other cell of the photometer. From the extinction value of the solution the vitamin E content is calculated by reference to a standard curve made with pure α-tocopherol. The two tocopherols give identical values.

One great advantage of the method over those previously described is that the estimation of vitamin E in oils can be done without saponifying them. A quantity of the oil (0·2 to 0·3 g.) is weighed into a 25-ml. flask and dissolved or suspended in 5 ml. of absolute alcohol. After the addition of 1 ml. of conc. nitric acid, the mixture is boiled for 3 minutes. On cooling, two layers form, the red colour being partitioned between them. The upper alcoholic layer is separated and filtered through a micro-filter funnel and the filtrate is made up to a volume of 6 ml. The lower oily layer is dissolved in 3 ml. of pure acetone, and the solution is poured through the filter used for clarifying the alcohol layer. The flask and filter are washed with 3 small portions of acetone, and the volume of the acetone solution is made up to 6 ml. The colour of each solution is measured in the photometer, the compensating cell being filled with a solution of the untreated oil in acetone when the colour of the acetone solution is measured. On reference to the standard curves, the sum of the extinction values gives the vitamin E content of the oil.

F. A. R.

Investigations into the Method of Estimating Vitamin E. III. Relation between Dosage and Response to Vitamin E. A. L. Bacharach. (*Biochem. J.*, 1938, **32**, 2017–2023.)—Six groups of virgin rats reared on a vitamin E-free diet were given respectively 1, 2, 4, 8, 16 and 32 "units" of a vitamin E concentrate that had been stored in an atmosphere of nitrogen in sealed tubes. The number of rats in each group varied from 20 to 64. When the percentage of fertile rats ("fertility rate") in each group was plotted against the dose, a smooth curve resulted which flattened out very markedly at a fertility rate greater than 90 per cent. The curve affords a means of expressing the vitamin E content of oils and concentrates in terms of the "standard" concentrate, and if a fertility rate near to 50 per cent. results from the administration of a particular dose, the activity can be assessed with fair accuracy.

The amount of α-tocopherol required to produce 50 per cent. fertility ("mean fertility dose") was found to be 1·5 mg., and that of β-tocopherol rather over half this value; this latter estimate, however, is subject to a high degree of error. The mean fertility doses of three samples of wheat germ oil were found to be 280, 700 and 250 mg., and that of a concentrate prepared from the last-mentioned oil 15 mg.

F. A. R.

Specificity of Vitamin E Activity. F. v. Werder, T. Moll and F. Jung. (*Z. physiol. Chem.*, 1939, **257**, 129–139.)—About 20 compounds more or less closely related to the tocopherols were prepared and tested for vitamin E activity. Durohydroquinone, pseudocumoquinone and their simple derivatives were either inactive or only slightly active when given in a 100 mg. dose. The compounds 2, 5, 7, 8-tetramethyl chroman, 2, 5, 7, 8-tetramethyl-6-hydroxy-chroman, 2, 4, 6, 7-tetramethyl-5-hydroxy-coumarone and 2, 4, 6, 7-tetramethyl-5-hydroxy-coumaran

were inactive, as was also 6-desoxy-*d.l.*-α-tocopherol prepared by condensing phytyl bromide with 1, 2, 4-trimethyl-6-hydroxy-benzene in presence of zinc chloride. *d.l.*-α-Tocopherol was found to be active in a dose of 3 mg. in 22 out of 28 animals. F. A. R.

Bacteriological

Dark-ground Studies of Flagellar and Somatic Agglutination of *B. typhosus*. A. Pijper. (*J. Path. and Bact.*, 1938, **47**, 1–17.)—An account is given of dark-ground methods, in which the sun is used as the source of light, so that the flagella of *B. typhosus* and similar bacteria swimming in broth can be seen and photographed. *B. typhosus* and similar bacteria swim by means of a long tail. At rest, the tail unwinds itself into two somewhat broadly coiled spiral flagella which are attached approximately near the middle of the bacterium and take up a position at an angle to its long axis. When resuming activity the two flagella begin to revolve round their own axis, stretch and become twisted round one another at the rear end of the bacterium where they form the tail. The two flagella consist of a number of extremely thin threads as can be seen when they finally disintegrate. The traditional picture of peritrichous flagella is shown to be due to artifacts resulting from the drying of films. In "H" agglutination the tails and flagella become covered with a granular deposit, which finally completely covers and ensheathes them. The resulting stiff and thickened spiral structures become entangled with those of the other cells. In "O" agglutination the cells exert a real mutual attraction, the force acting in the direction of the long axis, so that they join up end to end and build up clumps which exhibit a regular pattern. Neither tails nor flagella take part in this process. D. R. W.

Destruction of Bacterial Spores. Low Temperature Sterilisation. C. E. Coulthard. (*Pharm. J.*, 1939, **142**, 79.)—Several previous papers on this subject from the bacteriological laboratory of Boots' Pure Drug Co. have been published (*cf.* ANALYST, 1937, **62**, 217). This paper records more recent work, the investigation coming under three principal headings: (1) The use of germicides to increase the bactericidal value of intermittent heating at 80° C.; (2) the use of germicides to increase the bactericidal value of (*a*) a short period of heating at 80° C., and (*b*) a relatively long period of heating at this temperature; (3) the use of germicides to increase the bactericidal value of heating at 100° C. Spore suspensions in dilute glycerin were made from soil and laboratory cultures of *B. mesentericus* and related organisms. These suspensions were distributed into bottles, heated to 80° C. to destroy vegetative cells and stored just above freezing-point. Five-ml. volumes of the various test solutions were distributed into vials, sown with spore suspensions, heated in water to the desired temperature and subsequently tested. The results of the experiments show that: (1) a large number of spores are viable after intermittent heating at 80° C. without antiseptics, a greatly reduced number are viable when 0·5 per cent. phenol or 0·3 per cent. tricresol is present, and usually none (but sometimes a few) remain viable when 0·25 per cent. of *p*-chloro-*m*-cresol is present; (2) after 1 hour's heating at 80° C. the addition

of sufficient mineral acid to give a *p*H of about 2·25 is necessary for the destruction of spores; this is also effected by the addition of phenyl mercuric nitrate in concentration of 1: 5000 and nearly effected with concentration of 1:10,000 to 1:20,000. Experiments with *p*-chloro-*m*-cresol suggest that heating for 4 hours at 80° C., or for 15 minutes at 100° C., in presence of 1–400 *p*-chloro-*m*-cresol would suffice for sterilisation; this reagent is more effective in acid solution; at *p*H 5, 2 hours' heating at 80° C. are sufficient for sterilisation. The results of the various experiments are set out in tabular form, and tentative outlines for the process of sterilisation of medicaments are given. A table showing the aerobic colony counts in nutrient agar upon spore-infected preparations after they had been heated at 100° C. for the periods stated, with and without the addition of germicidal agents, is given below. The + sign after a number signifies that it is an under-estimate, owing to spreading growths.

Preparation	Test 1		Test 2		Test 3	
	15 mins.	30 mins.	15 mins.	30 mins.	15 mins.	30 mins.
Physiological saline alone	66+	16+	98+	19	110+	25
Physiological saline with						
p-Chloro-*m*-cresol 1 in 1000 ..	16	5	11	2	53	14
,, ,, 1 in 500 ..	3	0	3	0	21	3
,, ,, 1 in 400 ..	2	1	4	0	17	2
Hexyl-resorcinol 1 in 3000 ..	4	1	7	1	—	—
Phenol 1 in 200 ..	—	—	—	—	26	20
Phenol 1 in 100 ..	—	—	—	—	17	8
Tricresol 1 in 300 ..	—	—	—	—	22	19

The author has previously drawn attention to the high lethal efficiency at 80° C. of hexyl-resorcinol in oils (*Pharm. J.*, 1934, 133, 447). This substance is unstable in aqueous solution. D. R. W.

Isolation of Water Leptospirae in Pure Culture and their Biology. H. Sugimoto. (*Japanese J. Exper. Med.*, 1938, **16**, 143–159; *Bull. Hygiene*, 1939, **14**, 75.)—A new method for the isolation of water leptospirae is described. The number of leptospirae is first increased by cultivation in the following enrichment medium:—(1) A 20 per cent. suspension of faeces in tap water is boiled for one hour and then filtered. (2) A 3 per cent. suspension of agar in water is sterilised at 120° C. for twenty minutes and then run into test tubes in 7-ml. quantities without filtering. The water to be tested (83 ml.) is mixed with 10 ml. of the faeces filtrate (1) and 7 ml. of melted agar (2) and the resulting mixture is poured into four Petri dishes and cultivated for two weeks at 30° C. The presence of agar was found to increase the percentage of positives from 38 when faeces only was used to 77·4. The mixed culture is next grown in a chloral hydrate medium prepared by mixing a 10 per cent. solution of chloral hydrate with 0·5 per cent. agar in water in such proportions that the resulting mixtures contain 0·1, 0·07 and 0·05 per cent. of the former. These mixtures are transferred to tubes in 10-ml. quantities and, after seeding, the cultures are incubated at 25° C. for ten days. The tubes containing 0·1 per cent. of chloral hydrate gave the best results

for primary mixed cultures. Pure cultures were obtained on a medium consisting of 2 per cent. of agar, 0·06 per cent. of chloral hydrate and 10 per cent. of inactivated rabbit serum; the colonies of leptospirae formed were surrounded by a white ring. In semi-solid agar media two rings are formed round the colonies. Water leptospirae have a haemolytic action on the erythrocytes of various animals including those of man, the horse, guinea-pig, rabbit and rat; they also reduce oxyhaemoglobin. The optimum conditions for the growth of pure cultures appear to be a temperature of 30° C., a serum-content of 20 per cent. and a *p*H of 7·4 to 7·6. These organisms are aerobic. D. R. W.

Agricultural

Determination of Organic Carbon in Soils. Modification of the Chromic Acid Reduction Method. E. R. Purvis and G. E. Higson, Jr. (*Ind. Eng. Chem., Anal. Ed.*, 1939, **11**, 19–20.)—W. T. Degtjareff (*Soil Sci.*, 1930, **29**, 239) and L. E. Allison (*id.*, 1935, **40**, 311) have found that, although Schollenberger's chromic acid reduction method (*id.*, 1927, **24**, 65; 1931, **31**, 483) for the determination of organic carbon in soils does not give quantitative results as compared with the combustion method, the results are constant and reproducible, and the correct value may be obtained by the use of the factor 1·15. In the method 0·2 to 0·5 g. of soil is oxidised with a measured quantity of a standard solution of chromic acid in sulphuric acid, the extent of reduction of the reagent being determined by titration with 0·2 *N* ferrous ammonium sulphate solution, with diphenylamine as indicator. Various devices have been suggested for accelerating the oxidation reaction (*cf.* Degtjareff, *loc. cit.*, and Walkley and Black, *id.*, 1934, **37**, 29), but they are shown to impair the accuracy of the method. In particular, this is now proved to apply to Degtjareff's proposal (*loc. cit.*) that the mixture should be heated for 10 minutes at 165° C. in a bath of sulphuric acid, the titre of the chromic acid being reduced by 0·008 and 0·072 *N* when temperatures of 140° and 180° C. are used. This is due to both evaporation and reduction of the chromic acid in contact with the glass container, parts of which may attain a temperature of 800° C. over an open flame. The authors therefore suggest balancing the temperature and the period of heating, so as to render negligible the change in strength of the reagent. An electric oven suitable for this purpose is described; it accommodates 10 test-tubes (25 × 150 mm.) and is capable of operating at 400° C., and of raising the temperature of the 10 ml. of solution in each test-tube to 175° C. in about 3 minutes. If the top two-thirds of the test-tubes project out of the oven, and so act as a condenser, this and the fact that the actual glass does not attain a temperature over 400° C. minimise the loss in strength of the solution. Tests on 5 soils containing 0·65 to 5·22 per cent. of organic matter, showed that the results after oxidation for 3 minutes were the same as those obtained by heating over the open flame for 90 seconds; thus 100 samples may be oxidised in an hour. The reagent is best prepared by heating for 1 hour on the water-bath, with occasional stirring, a mixture of 10 g. of powdered potassium dichromate (which has previously been dried at 100° C. for 1 hour) and 500 ml. of a mixture of equal volumes of phosphoric acid and sulphuric acid. This produces

a reagent approximately 0·4 *N* in (oxidising) strength and of maximum stability; there is no necessity to add phosphoric acid subsequently to activate the diphenylamine indicator; the stated proportions of acids ensure the sharpest end-point.

J. G.

Colorimetric Determination of Traces of Nitrates in Form of Ammonia. H. Sallinger and Y. Hwang. (*Z. anal. Chem.*, 1939, **115**, 174–177.)—For the determination of ammonia in filtered soil solutions strongly discoloured by humic substances not removable by precipitation with ferric hydroxide, etc., the authors convert the nitrates into ammonia, which is distilled. Twenty ml. of the solution are diluted with 120 ml. of water in a conical 300-ml. flask. One g. of Devarda's alloy and 0·5 g. of magnesia are added, and 20 to 40 ml. are distilled into a small flask containing a few ml. of water. The distillate or an aliquot part is Nesslerised. Another portion of solution is distilled as before but without the alloy, and the ammonia thus found is subtracted from the first result. A blank determination must be made with each assay. The process is sensitive to 0·01 mg. of nitrogen, and quantities of 0·02 to 0·5 mg. can be accurately determined. W. R. S.

Organic

Determination of Small Amounts of Alcohol by means of Sulphuric Acid—Chromate Mixture. A. Rapin. (*Helv. Chim. Acta*, 1939, **22**, 72–75.)—When ethyl alcohol is oxidised by means of a mixture of potassium dichromate and sulphuric acid, variable quantities of acetaldehyde are formed, and this leads to erratic results. The trouble can be overcome by carrying out the oxidation in a pressure-flask. Exactly 1 ml. of *N* potassium dichromate solution, 1 ml. of 20 per cent. sulphuric acid and 1 ml. of the alcohol solution to be tested, are measured into a 50-ml. pressure-flask having a long neck fitted with a stopper kept in place by a wire clip. After being stoppered the flask is immersed in a boiling water-bath for 10 minutes. A blank experiment without alcohol is made at the same time. The two flasks are allowed to cool and the contents are carefully rinsed out and treated with a strong solution of potassium iodide, the liberated iodine being titrated with 0·1 *N* sodium thiosulphate solution. One ml. of *N* solution is equivalent to 0·0115 g. of ethyl alcohol. Almost exact agreement with the theoretical value was obtained by the method, which can also be used to estimate methyl alcohol. F. A. R.

***m*-Bromobenzazide as a Reagent for the Identification of Amines. P. P. T. Sah and L.-H. Chang.** (*Rec. Trav. Chim. Pays-Bas*, 1939, **58**, 8–11.)—Organic azides decompose on heating in an anhydrous solvent to form gaseous nitrogen and an isocyanate; in the presence of an amine, the isocyanate reacts with it, giving an insoluble substituted urea, the properties of which can be used to characterise the amine. Secondary amines react with more difficulty than primary amines; amides, amino-acids, and amino-phenols also react, but the yield of the product is not as high as with primary amines. The following experimental procedure has been adopted.

Preparation of m-*Bromobenzazide.*—Ethyl *m*-bromobenzoate was condensed with hydrazine hydrate to form *m*-bromobenzhydrazide (*m*-bromobenzoylhydrazine), which separated from dilute ethyl alcohol in white needles, melting at 152° C. Ten g. of the *m*-bromobenzhydrazide were dissolved in 100 ml. of glacial acetic acid, and the solution was cooled. An ice-cold solution of sodium nitrite (10 g. in 20 ml. of water) was slowly dropped into the hydrazine solution, with continuous stirring and efficient cooling. The reaction mixture was stirred for 10 minutes and then diluted with 300 ml. of cold water, which precipitated the azide as a heavy yellowish oil. The product was extracted with three portions of toluene (50, 25, and 25 ml.), and the toluene layer was separated, washed with cold dilute sodium carbonate solution and then with water, and immediately dried over fused calcium chloride. After standing overnight the solution was filtered and stored in a flask containing fused calcium chloride. Ten ml. of the solution were found to contain about 1 g. of the azide.

Identification of Amines.—To 5 ml. of the azide solution in a large test-tube was added a clear solution of an equivalent weight of amine in dry toluene. The test-tube was fitted with a cork carrying a long glass tube to act as a condenser, and the mixture was heated in an oil-bath to 120° C. and kept at that temperature for at least 15 minutes. Some condensation products separated immediately from the hot solution, and others after standing and cooling. The crystals of the *m*-bromophenylureas were filtered off with suction, washed with toluene, and recrystallised from a suitable solvent (ethyl alcohol, benzene, acetone or a mixture of ethyl alcohol and benzene). Tables give the physical properties and the analyses of the *m*-bromophenylureas prepared from 23 amino compounds. The following are typical melting points of the *m*-bromophenylureas:—Aniline, 196–197° C.; *o*-toluidine, 212–213° C.; *m*-toluidine, 248–249° C.; *p*-toluidine, 222–223° C.; α-naphthylamine, 259–260° C.; β-naphthylamine, 240–241° C.; diphenylamine, 141–142° C.; acetamide, 201–202° C.; acetanilide, 118–119° C. E. M. P.

p-Bromobenzazide as a Reagent for the Identification of Alcohols. P. P. T. Sah and K.-Y. Tao. (*Rec. Trav. Chim. Pays-Bas*, 1939, **58**, 12–16.)—Azides of organic acids react with alcohols to give crystalline urethanes, the properties of which can be used to characterise the alcohol. Primary alcohols give the best results; with secondary and tertiary alcohols either the yield of urethane is poor or no reaction occurs. The following experimental procedure has been adopted.

Preparation of p-*Bromobenzazide.*—*p*-Toluidine was converted into *p*-bromotoluene by the Sandmeyer–Gattermann reaction (Bigelow, *Organic Syntheses*, 1925, V, p. 21), and the purified product was oxidised with potassium permanganate to *p*-bromobenzoic acid (Adams and Marvel, *Organic Chemical Reagents*, University of Illinois Bulletin, 1921, III, 31), which was esterified to ethyl *p*-bromobenzoate (Errera, *Gazz. Chim. Ital.*, 1887, **17**, 211). Condensation of the ester with hydrazine hydrate gave *p*-bromobenzhydrazide (Curtius and Portner, *J. prakt. Chem.*, [2], 1898, **58**, 201; Kahl, *Chem. Zentr.*, 1904, II, 1493; Kendall and Sherman, *J. Am. Chem. Soc.*, 1908, **30**, 1451; Wang, Kao, and Sah, *Science Reports, National-Tsing Hua University*, 1935, A III, 279–284). From 10 g. of the hydrazide,

dissolved in 80 ml. of glacial acetic acid, after treatment in the cold with 10 g. of sodium nitrite in 25 ml. of water, a 90 per cent. yield of *p*-bromobenzazide (Sah, Kao, and Wang, *J. Chinese Chem. Soc.*, 1936, **4**, 193–197) was obtained. The product was filtered off, washed thoroughly in cold water, sucked dry, and finally dried in a vacuum desiccator over phosphorus pentoxide; it must be absolutely dry before being condensed with the alcohols.

Identification of Alcohols as p-*Bromophenylurethanes.*—0·5 g. of the reagent is treated, in a test-tube previously dried by heat, with the calculated quantity of the alcohol dissolved in petroleum spirit (ligroin, b.p. 90–120° C.), and the mixture is heated under reflux in an oil-bath first at 80° C. and finally at 120° C. for half-an-hour. The boiling solution is filtered hot and the residue is washed once with boiling ligroin. With alcohols the urethanes of which have to be recrystallised from dilute alcohol (ethylene glycol, glycerol, and borneol), benzene solutions of the reactants are used. After the solution has been heated under reflux at 80° C. for half-an-hour, the solution is evaporated to dryness and the residue is extracted with dilute alcohol. Usually the urethanes crystallise out quickly, but standing overnight in an ice-box gives better results. Tables give the physical properties and the analyses of the *p*-bromophenylurethanes from 27 alcohols. E. M. P.

Fats and Fatty Acids with an Odd Number of Carbon Atoms. W. Keil, H. Appel and G. Berger. (*Z. physiol. Chem.*, 1939, **257**, I–III.)—The chain length of coconut oil fatty acids was increased by one carbon atom by means of the series of transformations: fatty acid → alcohol → bromide → nitrile → fatty acid. Certain properties of the glyceride of the product, which contained an odd number of carbon atoms, were compared with those of the natural coconut oil. The two glycerides, when administered to rats, reduced the respiratory quotient to the same degree, were hydrolysed at the same rate by pancreas lipase, gave rise to body fats that had identical iodine values in rats receiving them in their diets, and when fed in large quantities to goats resulted in the excretion of urine having the same carbon : nitrogen ratio. F. A. R.

Oxidation Products of the Unsaturated Acids of Linseed Oil. L. C. A. Nunn and I. Smedley-Maclean. (*Biochem. J.*, 1938, **32**, 1974–1981.)—The oxidation of the mixed acids of linseed oil by means of cold alkaline potassium permanganate solution was studied. A 96 per cent. yield of dihydroxystearic acid was obtained from the oleic acid present and the quantitative nature of the oxidation was unaffected by the proportions of linolic or linolenic acids. The method can therefore be employed to estimate oleic acid. The yields of tetra-hydroxy-acids from the linolic acid present was only 28 to 32 per cent., whereas yields of 52 to 54 per cent. were obtained from the pure acid. The yields of hexa-hydroxy-acids from the linolenic acid were 40 to 52 per cent., but only 22 to 25 per cent. from the pure acid. Thus in mixtures linolenic acid is more readily degraded than linolic acid and exercises a protective action on the oxidation of the latter. An acid, $C_{12}H_{18}O_4$, was isolated from the oxidation mixture and identified as the γ-lactone of hydroxy-Δ1 : 2-decene-1 : 10-dicarboxylic acid; this was also identified as a product of the oxidation of pure linolic acid. The γ-lactone of 3: 4-dihydroxy-decane-dicarboxylic acid was also identified together with a small amount (1·2 per cent.) of azelaic acid. F. A. R.

Some Aquatic Animal Oils. M. Tsujimoto and H. Koyanagi. (*J. Soc. Chem. Ind. Japan*, **1938**, **41**, 320B–321B.)—*Eel-liver Oil.*—Successive extractions of eel livers with alcohol and ether yielded approximately 8 per cent. of oil. After removal of the acetone-insoluble substances (approximately 4 per cent. of the extract) a brownish-yellow oil, semi-solid (*i.e.* liquid containing crystals) at 12° C. was obtained. It had the following constants:—d_4^{15}, 0·9318; n_D^{20}, 1·4810; acid value, 68·1; saponification value, 181·6; iodine value, 184·4; unsaponifiable matter, 5·50 per cent. The unsaponifiable matter was deep orange-yellow and crystalline. It melted for the most part above 100° C. and contained 71·8 per cent. of sterol and a small amount of methanol-insoluble substance (probably hydrocarbon). The oil yielded brownish-orange-yellow crystalline fatty acids having m.p. 33–34° C., neutralisation value 191·5, iodine value 192·6, and yielding 65·5 per cent. of ether-insoluble bromides.

Eel-bone Oil.—This was a yellow liquid with a little solid deposit. It gave d_4^{15}, 0·9247; n_D^{20}, 1·4755; acid value, 3·6; saponification value, 194·0; iodine value, 129·7; unsaponifiable matter, 0·87 per cent. In the antimony trichloride test it gave a faint blue colour. The unsaponifiable matter was orange-yellow and crystalline, partly melting at 100° C. The fatty acids were orange-yellow and crystalline and gave m.p., 35–36° C.; neutralisation value, 201·6; iodine value, 133·9; yield of ether-insoluble bromides, 27·4 per cent.

Carp-liver Oil.—This was an orange-yellow liquid, depositing solid matter at 12° C. It gave d_4^{15}, 0·9288; n_D^{20}, 1·4795; acid value, 0·82; saponification value, 192·6; iodine value, 169·7; unsaponifiable matter, 0·59 per cent. The unsaponifiable matter was orange-yellow and crystalline and melted above 100° C. The fatty acids were orange-yellow and crystalline, and gave m.p., 30–31° C.; neutralisation value, 198·6; iodine value, 176·7; yield of ether-soluble bromides, 47·9 per cent.

Dolphin-liver Oil.—Part of the liver of a dolphin, treated in the same way as eel-liver, yielded 4·7 per cent. of crude oil, containing 29 per cent. of acetone-insoluble substances. The refined (acetone-soluble) portion was a brownish red-yellow liquid. The oil gave the following constants:—d_4^{20}, 0·9171; n_D^{20}, 1·4784; acid value, 59·7; saponification value, 154·6; iodine value, 163·3; unsaponifiable matter, 18·91 per cent. The unsaponifiable matter was yellow-orange and crystalline, with m.p. usually above 100° C., iodine value 147·0 and sterol 70·2 per cent. The fatty acids were dirty yellowish-orange and crystalline, and gave m.p., 35–36° C.; neutralisation value, 191·8; iodine value, 169·7; yield of ether-insoluble bromides, 56·1 per cent.

The crude extract from the rest of the liver (2616 g.) was saponified and the unsaponifiable matter was extracted with ether; yield, 19 g. This contained approximately 2 g. of methanol-insoluble matter. After removal of vitamin A (by filtration of a petroleum spirit solution through a column of Japanese acid clay) and of sterol (by means of digitonin) and subsequent treatment in ether with animal charcoal, about 1 g. of an orange-yellow, very viscous liquid hydrocarbon, insoluble in acetic anhydride, was obtained. Its constants and properties were: d_4^{25}, 0·9084; n_D^{20}, 1·5134; iodine value, 330·1; molecular weight (cryoscopic method), 1089; carbon 85·65 per cent., hydrogen 11·93 per cent. The additive bromine compound (containing 66·97 per cent. of bromine), was a white ether-insoluble

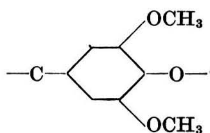
powder sintering and blackening at 165–170° C. The hydrochloride (recrystallised from acetone) was a nearly white powder, sintering at about 116° C. and melting (with decomposition) at 125–127° C. (chlorine 31·23 per cent.). The cause of the high molecular weight of the hydrocarbon, which is nearly double that of the hydrocarbon of the liver oils of certain whales, etc., which resemble it in other constants and properties, is unknown. Polymerisation of the original hydrocarbon by the acid earth is suggested, but the other constants appear to contradict this. When squalene was similarly filtered through the earth the molecular weight and carbon and hydrogen contents were not altered but the other constants were considerably changed. E. B. D.

Wool Wax. T. Kuwata and M. Katuno. (*J. Soc. Chem. Ind. Japan*, **1938, 41,** 227–229B). *Two New Alcohols.*—The unsaponifiable matter of merino wool wax was dissolved in five times its weight of methanol and separated into fractions according to solubilities. Fraction 4 was dissolved in acetone, the solution was cooled to —10° C., and the soluble semi-solid reddish-brown material (23 per cent. of the total unsaponifiable matter) was acetylated and distilled under reduced pressure. The first two fractions, distilling at 120–190° C. and 190–210° C., were repeatedly re-distilled, and the fraction obtained between 210–225° C. (10 mm. mercury) with saponification value 178, was collected. The free alcohol from this fraction, separating as a bulky mass from organic solvents, was converted into phenylurethane and recrystallised from petroleum spirit, the crystals finally obtained melting at 79·5 to 80·0° C. Lano-octadecyl alcohol of m.p. 42–43° C. was then obtained from the urethane by reaction with sodium ethylate, and its composition agreed with the formula $C_{18}H_{38}O$. The original fraction (6), amounting to about 6 per cent. of the total unsaponifiable matter, soluble in methanol at —10° C., was converted into its acetyl ester and distilled under reduced pressure. The fraction of highest b.p. was a yellow liquid with saponification value 156. The free alcohol of this fraction, when recrystallised from acetone, petroleum spirit or ethyl acetate, was a white crystalline powder with m.p. 78–78·5° C.; its composition agreed with the formula $C_{21}H_{40}(OH)_2$, and it was termed lanyl alcohol. After it had been dried for a month in a vacuum desiccator its m.p. rose to 79·5–80·0° C. Lanyl alcohol does not absorb bromine at room temperature and gives a phenyl urethane with m.p. 97·97° C. *Isolation of Cholesterol from Wool Wax.*—The unsaponifiable matter of wool wax is extracted with hot methanol, in which isocholesterol is only sparingly soluble, and the crude cholesterol is precipitated by cooling the solution to room temperature. Acyclic alcohols separate first as a gelatinous precipitate, followed by cholesterol in fine long needles. When recrystallised once from alcohol or methyl acetate these yield crystals of pure cholesterol with m.p. 145–146° C. The acetyl ester has m.p. 113–114° C.

D. G. H.

Chlorine-Sodium Sulphite Colour Reaction of Woody Tissues. II. Bearing of the Colour Reaction on the Constitution of Hardwood Lignin. W. G. Campbell, J. C. McGowan and S. A. Bryant. (*Biochem. J.*, **1938, 32,** 2138–2141.)—The well-known colour reaction given by woody tissues with sodium sulphite following chlorination is not characteristic of lignin only, but is also given

by tannins. It was concluded from this that the reaction was probably specific for phenolic compounds containing the 1 : 2 : 3-trihydroxybenzene nucleus, though this was not known to occur in native lignin until recently; but it is now established (Harris, D'Ianni and Adkins, *J. Amer. Chem. Soc.*, 1938, **60**, 1467) beyond doubt that lignin is aromatic in structure. In the present work various phenols and phenolic derivatives were exposed to gaseous chlorine and then treated with sodium sulphite, and of these, gallic acid, trimethylgallic acid and syringic acid gave colours similar to that given by lignin; 1 : 2 : 3-trimethoxy-benzene gave only a faint reaction, but this may have been due to the insolubility of the compound in water. Gallic acid and syringic acid also gave the colour on treatment with other oxidising agents in presence of sodium sulphite, but trimethylgallic acid gave the colour only after preliminary chlorination, and this was found to be due to partial demethylation by the chlorine, the resulting hydroxy-compound being responsible for the colour reaction. Since, so far as is known, chlorination must also be applied to hardwood lignin before the characteristic colour is produced by sodium sulphite, there exists a close analogy between the behaviours of lignin and trimethylgallic acid, and it is suggested that lignin contains the modified pyrogallol nucleus



as a recurring unit. F. A. R.

Action of Ethanolamine on Woody Tissue. L. E. Wise, F. C. Peterson and W. M. Harlow. (*Ind. Eng. Chem., Anal. Ed.*, 1939, **11**, 18–19.)—W. G. Van Beckum and G. J. Ritter (*Paper Trade J., Tech. Sect.*, 1937, **104**, 19, 49) have described a method for the determination of holocellulose, which gives almost the whole of the total carbohydrate fraction of the cell-wall, as well as part of the methoxyl compounds and most of the acetyl compounds present in the wood. It depends on alternate treatment with chlorine and a 3 per cent. solution of mono-ethanolamine in 95 per cent. alcohol, and the following modified method described by the present authors is based on this work:—The sample (*e.g.* 1 g. of oven-dried, beech heart-wood sawdust) was extracted for 5 hours in an oil-bath at approximately 170° C. with 50 ml. of monoethanolamine, the solvent in contact with the wood being just below the boiling-point. An Erlenmeyer flask fitted with a funnel covered with a watch-glass was found to be suitable; cork or rubber stoppers must be avoided. The mixture was then diluted to 50 ml. with water, and the residue was separated and washed on a sintered glass crucible and then bleached for 20 minutes in the crucible, which was placed in a covered beaker in saturated chlorine water. The liquid was removed by suction and replaced by sulphurous acid which, after 3 minutes, was itself removed, and the residue was washed. Treatment in succession with a hot 3 per cent. solution of sodium sulphite for 30 minutes, chlorine water, water, sulphurous acid, water and dilute ammonia then followed, and the residue was finally dried and weighed. The two results for the wood in question were 60·4 and 60·5, the average Cross and Bevan cellulose

value of the sample being 60·7 (per cent. ?). If this concordance between the two methods is confirmed for other woods, the ethanolamine method offers the simpler and more convenient procedure. Harlow and Wise (*Amer. J. Botany*, 1938, **25**, 217) obtained results of 30·0 and 29·4 per cent. for the evaluation of the woody portions of rhizomes of brake fern by a similar ethanolamine method and by the Cross and Bevan method, respectively. Blocks of the dry sapwoods of red pine, sitka spruce, red elder and catalpa, which had been aspirated under cold water and stored in 15 per cent. alcohol, were cut into transverse sections (10μ thick), immersed in cold mono-ethanolamine, and examined, at intervals, after washing in water and treatment of separate portions with the phloroglucinol reagent for lignin and with 72 per cent. sulphuric acid. After 3 days the phloroglucinol produced an orange-red colour, and the acid showed no signs of disintegration of the walls. After 2 weeks the former reaction was negative, and there were indications of partial disintegration in acid (especially with the conifers), whilst after 3·5 months disintegration of the secondary walls was almost complete. In every instance the "compound middle lamella" of Kerr and Bailey remained, showing that the reagent was unable to attack the lignin of the central layers. Further experiments with a wider range of woods at 28° C. and with boiling ethanolamine gave similar results, the rate of delignification being greater as the temperature rose. The middle lamella was attacked and removed almost completely after 2 to 6 hours in the boiling solvent. The fact that the appearance of the section resembles closely that obtained as the result of chlorination or bromination followed by the action of dilute sodium sulphite or 10 per cent. ammonium hydroxide solution, adds support to the opinion that ethanolamine cellulose and Cross and Bevan cellulose are very similar, if not identical. J. G.

Inorganic

Indicator for Saturation, Oxidation, and Precipitation Methods. E. Schulek and P. Rózsa. (*Z. anal. Chem.*, 1939, **115**, 185–195.)—*p*-Ethoxychrysoidin hydrochloride, $C_2H_5O{\cdot}C_5H_4{\cdot}N_2{\cdot}C_6H_3(NH_2)_2{\cdot}HCl$, is blackish-red, and soluble in water or alcohol to produce a deep red colour which turns yellow in the interval *p*H 3·5 to 5·5, thus being intermediate between methyl red and methyl orange. A drop of a 0·2 per cent. solution in water or 96 per cent. alcohol is used in saturation analysis, the end-point being sharp and easily observed. Methyl orange must be used for the cold titration of carbonates and bicarbonates, but the new indicator is more suitable for borates; when 0·1 *N* solutions are used the removal of carbon dioxide by boiling is not essential, but with 0·02 or 0·01 *N* solutions this should be done before the end-point is reached. The aqueous 0·2 per cent. solution is a reversible indicator for oxidimetric work, the red tint being discharged by a drop of 0·1 *N* ceric sulphate or potassium permanganate solution, and restored by a trace of sodium hyposulphite, and on slight warming by a ferrous salt, arsenite, or antimonite. In an acidified bromide solution, a drop of 0·01 *N* bromate deepens the red tint, but a second drop discharges it. The indicator was successfully applied in the titration of ferrous salt solutions with 0·1 and 0·01 *N* ceric sulphate

and 0·01 *N* permanganate solutions. The iron solutions were acidified with 10 ml. of 50 per cent. sulphuric acid and 5 ml. of 20 per cent. phosphoric acid, and diluted to 50 or 100 ml. Arsenite and antimonite solutions were titrated with 0·1 and 0·01 *N* bromate solutions after addition of 1 g. of potassium bromide, 5 ml. of strong sulphuric acid, and for antimony, 0·5 to 1 g. of tartaric acid. The indicator acts in 5 per cent. hydrochloric acid solutions, but a higher acidity is prejudicial to its sensitiveness, hence the solution must be suitably diluted. Neutral 0·1 and 0·01 *N* iodide and 0·1 *N* thiocyanate solutions may be titrated with silver nitrate in presence of 0·5 ml. of a 0·02 per cent. alcoholic solution of the indicator. The originally yellow tint changes to rose-red upon addition of silver solution; the tint gradually deepens, but changes at the end-point to pale lemon-yellow with flocculation of the silver precipitate in the manner of adsorption indicators. A small correction is required for silver solution consumed by the indicator. W. R. S.

Quinaldinic Acid as an Analytical Reagent. P. Rây and N. K. Dutt. (*Z. anal. Chem.*, 1939, **115**, 265–268; *cf.* ANALYST, 1934, **59**, 199; 1935, **60**, 494.)—Zinc can be determined as quinaldinate in presence of silver, mercuric or cuprous salts after addition of thiourea, which forms stable complex cations with these metals. The neutral solution is treated with 8 g. of potassium iodide, which converts mercury into the complex iodide and precipitates silver iodide. A freshly-prepared solution of 4 to 8 g. of sodium bisulphite and a solution of 4 to 8 g. of thiourea are added, copper being reduced to cuprous salt and silver iodide dissolving. The solution is acidified with 2 to 5 ml. of dilute acetic acid, diluted to 200 ml., heated on the water-bath, stirred, and treated drop by drop with excess of a neutral 5 per cent. solution of sodium quinaldinate. The precipitate is left to settle, collected in a Gooch crucible, and washed with hot water—first by decantation—until free from precipitant. The crucible is dried at 125° C. and weighed. Zinc factor, 0·1529. W. R. S.

Colorimetric Determination of Small Quantities of Iron Oxide in Glass and Glass-making Sand. R. C. Chirnside. (*J. Soc. Glass Tech.*, 1938, **22**, 41–44.)—The thioglycollic acid method has been applied to glass. The method is unaffected by most of the basic or acid radicals which interfere with the thiocyanate test. A 0·2-g. sample is treated in a platinum crucible with 15 ml. of hydrofluoric acid and 2 ml. of dilute sulphuric acid (1: 6), and the acids are evaporated to dryness. The residue is strongly ignited for 15 minutes and fused with 2 g. of sodium carbonate, the mass is dissolved in 10 ml. of hydrochloric acid and the solution is diluted to 100 ml. Twenty ml. are transferred to a 50-ml. Nessler tube and 10 ml. of 10 per cent. tartaric acid solution, 1 ml. of thioglycollic acid and 10 ml. of dilute (1: 1) ammonia are added. The purplish-red colour produced is matched by introducing into another Nessler tube, containing similar amounts of reagents, a standard iron solution (1 ml. = 0·00001 g. of Fe_2O_3). Lovibond colour-glasses are available for the iron-thioglycollic acid determination. A blank test on the reagents is advised. Owing to the readiness with which platinum apparatus in general use becomes contaminated with iron a platinum crucible should preferably be reserved solely for this determination. S. G. C.

Determination of Thallium by means of Thiourea. C. Mahr and H. Ohle. (*Z. anal. Chem.*, 1939, **115**, 254–257.)—The solution containing 2 per cent. of free perchloric acid is treated with an equal volume of 10 per cent. thiourea, which precipitates $TlClO_4 \cdot 4CS(NH_2)_2$. The beaker is cooled for half-an-hour in running water, and the precipitate is collected in a porous glass crucible and washed with cold 5 per cent. thiourea solution slightly acidified with perchloric acid. The precipitate is dissolved in hot water and, if lead is present, the aqueous solution is again acidified with perchloric acid and the precipitation is repeated. Thallium is recovered as chromate, the solution of the purified precipitate being treated with 5 ml. of 10 per cent. ammonia and enough potassium chromate to yield a 2 per cent. solution. After standing for 12 hours the precipitate is collected in a porous porcelain crucible and washed with 1 per cent. potassium chromate solution followed by 50 per cent. alcohol until the washings are colourless. The precipitate is dried at 120° C. and weighed. W. R. S.

Detection of Carbon Disulphide. A. Castiglioni. (*Z. anal. Chem.*, 1939, **115**, 257–259.)—On adding 2 or 3 drops of a 2 per cent. solution of piperazine in 95 per cent. alcohol to 1 ml. of the solution to be tested, a yellowish-white precipitate is formed if carbon disulphide is present. The reaction is given by solutions in various solvents, *viz.* benzene, toluene, xylene, acetone, deca- or tetra-hydronaphthalene. Neither hydrogen sulphide nor thiophene reacts with piperazine under these conditions. The reaction will detect 0·0005 g. of carbon disulphide, this quantity giving a cloudiness after some minutes' standing.

W. R. S.

Colour Reaction for Sulphur. L. Van Itallie. (*Pharm. Weekblad*, 1938, **75**, 1445–1448.)—The author's reaction (ANALYST, 1938, **63**, 624) is best carried out by shaking the substance to be tested with 1 to 2 ml. of 4 *N* sodium hydroxide solution, and then adding carefully 1 to 2 ml. of pyridine. If the liquids are mixed in such a way that the subsequent formation of separate layers is not affected, 4 zones are eventually apparent, *viz.* the lowest (sodium hydroxide) layer, which is colourless, a yellow zone at the interface between this and the pyridine, and a green and a blue zone in the pyridine layer. If the blue zone is not apparent, the green zone being present, dilution with pyridine is recommended. The minimum quantity of sulphur detectable is 5μ, but the blue colour fades fairly rapidly and eventually disappears, and this is attributed to the formation of a sulphur compound of different composition. The green zone is due to admixture of the colour of the adjacent (yellow) zone. With homeopathic triturates, 50 mg. of sample should be shaken with 1 ml. of pyridine, and on the addition of 2 drops of the 4 *N* alkali, the blue colour appears if sulphur is present. Positive results are obtained with both precipitated sulphur and colloidal sulphur in a highly-dispersed state. The reaction is unaffected by the presence of chloroform, ether or benzene, but carbon disulphide prevents the formation or, if added subsequently, destroys the blue colour. The mechanism of the reaction is discussed. Thus, J. C. Gil (*Z. anal. Chem.*, 1894, **33**, 54) showed that when a weak solution of an alkaline polysulphide (but not a monosulphide) was added to

a flask containing warm 96 per cent. alcohol (the air having been previously displaced by the alcohol vapour) a faint sky-blue colour was obtained which subsequently turned green, or which, on cooling the flask in air, disappeared, leaving a turbidity due to the formation of sodium thiosulphate. The blue colour did not return on warming again, and selenium and tellurium gave negative results. Paternòen and Mazucchelli (*Atti R. Accad. dei Lincei*, 1907, **16**, 1, 465) showed that acetone may be used in place of alcohol, and that a high temperature is essential. The function of the organic solvent was believed to be the limitation of the degree of ionisation of the substance produced by the reaction, and this appeared to be confirmed by the stabler colours obtained when pyridine or ethylamine was used, and the absence of a blue colour in a medium containing only water. Van Itallie found that powdered metallic selenium dissolves in the mixture of pyridine and alkali to give a yellow-brown solution, which on dilution with pyridine deposits red selenium. The reaction is favoured by heating, and under these conditions alcohol may replace pyridine. When acetone was used a red solution was obtained which had a narrow and a wide absorption band in the yellow-green and blue-green regions, respectively. In this connection Espil (*Bull. Soc. Chim.*, 1910, **7**, 155) has shown that the action of alkali on selenium is to produce a selenide, which deposits red selenium on exposure to air. When metallic tellurium is warmed with alcohol and solid potassium hydroxide, a red-violet colour is produced, and on dilution with water a fine dark grey powder separates. J. G.

Microchemical

Identification of very small Quantities of Copper and Mercury by means of Urobilin. C. A. Sagastume and V. Oliva. (*Revista de Ciencias Quim. la Plata*, 1938, **12**, 43–45.)—The method is based on the reaction of urobilin with salts of copper or mercury to form complexes which give pink colours in dilute solution and purple colours in stronger solutions. The reaction has already been applied to the micro-determination of copper (Emmerie, *Chem. Weekblad*, 1930, **27**, 552; Abst., ANALYST, 1930, **55**, 718). The present authors used the following method for mercury. To 0·5 ml. of a dilute aqueous solution of a mercury salt is added 0·15 ml. of a 1/10,000 alcoholic solution of urobilin; in a control tube 0·5 ml. of water is treated with the urobilin solution. A pink colour develops but is obscured by a yellow tint. The pink complex is extracted by introducing 0·1 ml. of chloroform into the tube, and allowing it to stand; the pink colour can then be detected in the chloroform layer. In absence of copper this reaction can be used to detect 0·001 mg. of mercury, which can be identified in solutions as dilute as 1 in 10,000. E. M. P.

Micro-Colorimetric Determination of Aluminium in Plant Ash. O. Schams. (*Mikrochem.,** 1938, **25** 16–46.)—A number of different colorimetric methods for the determination of aluminium were tested, a Leitz colorimeter and a Lange photo-electric colorimeter being used. Methods tried included those using

*Now united with *Mikrochimica Acta.*

as reagent (1) haematoxylin, (2) the ammonium salt of aurintricarboxylic acid, otherwise known as aluminon or aurin; (3) 1-2-5-8-oxyanthraquinone or quinalizarine, (4) the ammonium salt of nitroso-phenylhydroxylamine or cupferron, (5) alizarine S, (6) eriochromcyanin R conc. G, (7) morin, (8) precipitation with 8-hydroxyquinoline followed by colorimetric determination of the oxine with Folin's reagent, (9) colorimetric determination of the oxine by coupling with sulphanilic acid, (10) colorimetric determination of the oxine by coupling with naphthionic acid. The last-named method is recommended as the most suitable for amounts of aluminium from 10–50γ in plant ash. *Method.*—The hydrochloric acid solution of the ash is evaporated to 3 or 4 ml. in a centrifuge tube. Iron and aluminium are separated as phosphate from the other constituents by the addition of at least 20 mg. of iron as ferric chloride, 0·5 ml. of saturated ammonium phosphate solution and 0·5 ml. of saturated ammonium chloride solution; the mixture is rendered just alkaline to phenolphthalein with dilute ammonia. The tubes are heated for 2 minutes in a glycerin bath and then cooled, 3 ml. of glacial acetic acid are added and the mixture is diluted with water to 8 ml., stirred for 3 minutes and centrifuged for 10 minutes at 2000 r.p.m. The precipitate is washed with 8 ml. of 10 per cent. acetic acid and, after removal of supernatant liquid, is dissolved in 2 ml. of water and 1 to 2 drops of hydrochloric acid, and reprecipitated with 0·25 ml. of saturated ammonium phosphate solution and 0·25 ml. of saturated ammonium chloride solution. The rest of the procedure is the same as before except that 1 ml., instead of 3 ml. of glacial acetic acid is added before centrifuging and the precipitate is washed finally with water only, and dissolved in 0·5 to 2 ml. of 10 per cent. hydrochloric acid. To separate the aluminium from the iron, the ferric iron is converted into ferrocyanide ion by means of 6 to 15 drops of freshly-prepared, saturated solution of potassium cyanide to which has been added 0·2 ml. of saturated ammonium tartrate solution and 3 to 8 drops of conc. ammonia. After 2 minutes' heating the reduction is completed with 1 drop of saturated potassium metabisulphite solution. The liquid is then treated with 0·5 ml. of saturated ammonium chloride solution and heated to 60° C., and the aluminium is precipitated by means of 2 to 8 drops of oxine solution (prepared according to Berg). The volume of the solution should be so adjusted that 10γ of aluminium are precipitated from 0·5 to 1 ml. and 20 to 50γ from 2 ml. The precipitate is left for 30 minutes at 60° C. and for 4 hours at 30° C. It is then washed either with 3 ml. of cold water, in 3 portions and with benzene, or with hot 50 per cent. ammonium chloride solution. For filtration, a B 2 porcelain filter-crucible is used, with suction. Finally the precipitate is dissolved (if less than 50γ by means of warm 30 per cent. acetic acid, but larger quantities by means of 10 drops of glacial acid or 3 drops of conc. hydrochloric acid) and transferred with the aid of suction to a 200-ml. measuring cylinder; warm water is used for washing. Coupling and diazotisation are carried out by the addition of 1 ml. of 0·345 per cent. sodium nitrite solution and 1 ml. of naphthionic acid solution (1·17 g. in 100 ml. of 0·01 *N*-sodium hydroxide solution) to 150 ml. of the liquid. After 2 minutes, 20 ml. of 8 *N*-sodium hydroxide solution are added and the liquid is made up to the mark with water. The resulting permanganate-coloured solution may be compared with the colour obtained with a standard oxine solution (1 ml. $\equiv$ 4γ Al) either at once or after an hour (kept in dark). For

values ranging from 10γ to 40γ the error does not exceed 2 per cent. The method was tested exhaustively on artificial salt mixtures and on plant ash to which known amounts of aluminium had been added. Fifty references are given.

J. W. M.

Apparatus for Micro Steam Distillation. J. Erdös and B. Lárzló. (*Mikrochim. Acta,* 1938, **3**, 304–305.)—The apparatus may be constructed in two forms (Fig. 1 and Fig. 2), the second for the distillation of substances that volatilise with difficulty. The flask A is filled with water, through C to such a height

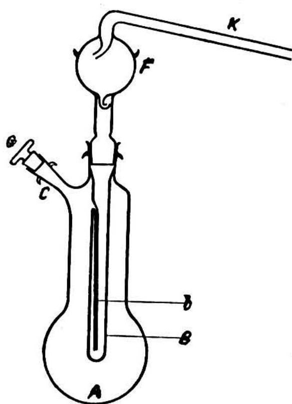


Fig. 1

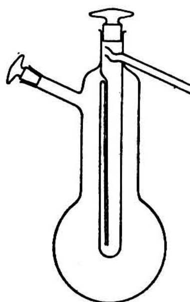


Fig. 2

that the bottom of B is submerged. A few pieces of pumice are added to prevent bumping. The substance to be distilled is placed in the bottom of the tube B, either by means of a capillary pipette, if liquid, or by forceps, if solid. The stopper F, with attached delivery tube K, is then filled. For very volatile substances K is fitted with a water-condenser. When the water boils in A the steam passes into B through the narrow tube *b*.

J. W. M.

References to Current Microchemical Literature. (*Mikrochem.*, 1938, **25**, 349–382.)—References are given with complete titles and, where necessary, a further explanation of contents, not exceeding 1 line. In each instance the address of the author's laboratory is included. The references are arranged in alphabetical order of the authors' names under the following headings:—Inorganic: preparative and analytical, 53 references; physico-chemical and physical methods, 42 references; organic: preparative and analytical, 84 references; biochemical, 100 references; medico-chemical methods, 37 references; pharmacology, toxicology and forensic chemistry, 20 references; plant chemistry, agricultural chemistry and food chemistry, 36 references; applied chemistry (technical, mineralogical, etc.), 10 references; apparatus, 27 references.

J. W. M.

Collected References. Micro-Halogen Determinations : Fluorine and Chlorine. F. Hernler and R. Pfeningberger. (*Mikrochem.*, 1938, **25**, 267–348.)—References are given with brief descriptions of the methods as follows:—

Fluorine (*qualitative*), 54 references; (*quantitative*) (*a*) gas-volumetric, 3 references; (*b*) gravimetric, direct, when precipitation with lanthanum acetate or as the triphenyl tin fluoride are most recommended, 10 references; indirect, by precipitation as calcium fluoride and volatilisation in various ways, 7 references; (*c*) etching methods, 3 references; (*d*) volumetric, 18 references; (*e*) colorimetric and potentiometric, 36 references; (*f*) nephelometric, 1 reference; (*g*) spectrographic, 3 references. *Chlorine* (*qualitative*), 36 references; (*quantitative*) (*a*) gas-volumetric, not recommended; (*b*) volumetric, especially applied to drinking water, 8 references; (*c*) colorimetric, 42 references. *Chloride ion* (*quantitative*) (*a*) gravimetric, 10 references; (*b*) volumetric, 33 references; (*c*) determination in biological material, especially in blood and urine, 120 references. *Chlorine* in organic liquids, 10 references. *Chloride*, colorimetric and nephelometric, 22 references; spectrographic, 1 reference; *Chlorine*: determination of the element in organic compounds, 8 references. The total number of references cited exceeds 400. J. W. M.

Physical Methods, Apparatus, etc.

Viscometer for the Routine Determination of Proteolytic Activity of Malts. J. R. Koch, O. Nelson and L. Ehrnst. (*Ind. Eng. Chem., Anal. Ed.*, 1939, **11**, 35–41.)—The viscometer consists of two Erlenmeyer flasks, the top one inverted and connected in a vertical position at the neck with the lower one by means of a capillary tube, which enables the solution under examination to flow from one to the other. An air-tube, passing through the two stoppers, is provided, so that no air-pressure will be built up in either flask when the viscometer is inverted for subsequent determinations to be made. In one of the two forms illustrated the necks are fitted with rubber stoppers through which pass the capillary viscosity tube (bore 1·5 mm., length 2·5 to 3·7 cm.), and the open air-vent tube, which terminates about half-way down each flask. The ends of the capillary tube should project beyond the rubber stoppers, so that the solution may run freely from them without touching the sides of the flask; allowance for this should be made when measuring out the 50 ml. of solution used for the determination (see below). In the other (all-glass) type, the capillary tube is sealed into the closed necks of the flask, the air-vent (which is also used for filling and emptying the apparatus) being a T-tube, each arm of which is bent at the end and sealed into the respective flask near the base. At room temperature 50 ml. of water should pass through the capillary in 35 to 36 seconds. A fresh solution of a pure gelatin is prepared by stirring 25 g. into 125 ml. of water, allowing the vessel to stand for 30 minutes, and then placing it in a water-bath at 60° to 65° C.; the solution is diluted to 250 ml. in a graduated flask. The malt is ground in a Miag–Seck mill, and 20 g. are mixed with 100 ml. of water at 40° C. and maintained at 40° C. for 30 minutes. The mixture is filtered, and 35 to 40 ml. of filtrate are placed in a water-bath at 40° C. for 8 minutes, 25 ml. being then mixed with 50 ml. of the gelatin solution at 40° C., and the time noted ("zero-time"). The viscometer is warmed to 40° C., and viscosity determinations are made on the same 50 ml. of the mixture at intervals of 15 minutes for 3 hours, by repeatedly inverting the apparatus, an extra predetermined volume being used if the capillary tube projects

into the flask (see above). Typical curves are given for various malts, to illustrate the effects of differences in processing. It is convenient to indicate the proteolytic strength in terms of the number of minutes required to produce a fall in viscosity of 20 per cent. The choice of this time is justified by the shape of the curves shown, because (especially with papain and trypsin, see below), the curves may fall sharply at first and flatten out to a horizontal line almost parallel with the time-axis as the quantity of enzyme is increased. If the curves are extrapolated back to the time-axis (the "zero-time"), the time of flow is the same as that obtained in a blank experiment with a mixture of gelatin and an infusion of malt which has been boiled to destroy the enzymes. It is therefore unnecessary to standardise the instrument when it is used in this way, and purchasers of malts are enabled to arrange any number of samples in order of their proteolytic activities. Viscometers prepared with equal lengths of capillary tubing cut from the same piece give the same proteolytic strengths for the same malt. Curves are also given for the evaluation of the activities of trypsin and papain, and reliable results were obtained with 6 to 500 and 50 to 1500 parts of enzyme per million of substrate, respectively. The effect of the *p*H at which the determination is made is also considered, and it is concluded that 5 to 6 is a suitable range, the variation in activity between these values being within the order of experimental error. Moreover, the *p*H varied very little during the run, showing that the gelatin is adequately buffered, so that the addition of buffer salts (which might interfere with the reaction) is avoided. If it is desired to compare results obtained in different laboratories, the respective viscometers must be standardised with two liquids, *e.g.* water and a 40 per cent. solution of sucrose, which are used to determine the constants A and B in the formula for the kinematic viscosity (U/V), *viz.* ($AT-B/T$). U is the absolute viscosity in centipoises, V the density of the liquid in g. per ml., and T the time of flow in seconds. A calibration-curve is thus constructed by plotting U/V against T; values of U/V for water and the sucrose solution at 40° C. are 0·662 and 2·94, respectively. Universal values for the proteolytic activity of malts (P) may then be obtained from the expression ($250-t$), where t is the time in seconds to produce a fall of 20 per cent. in the value of U/V; each minute of this time corresponds with a difference of 1 unit in P. The theoretical justification for the expression of activities in this way is discussed, and analyses of malts, which include values of P, are tabulated. J. G.

Determination of Density Differences by the Flotation Temperature Method. M. Randall and B. Longtin. (*Ind. Eng. Chem., Anal. Ed.*, 1939, **11**, 44–46.)—The method, which is based on the work of Richards and his co-workers (*J. Amer. Chem. Soc.*, 1912, **34**, 599; 1914, **36**, 1; and 1916, **38**, 1000) and of Briscoe and his co-workers (*J. Chem. Soc.*, 1934, 1207), depends on the variation in density of a liquid with temperature and the dependence on this of the behaviour of a float in the liquid. An apparatus is described which enables the flotation temperature of 0·1 ml. of sample to be determined to within 0·005° C., and its applications in connection with the separation of the isotopic forms of water are outlined. The sample was purified by distillation, first over alkaline permanganate solution and then (if ammonia was present) from phosphoric acid, carbon dioxide

and other dissolved gases being removed by heating it, with agitation, at 50° C. in a vacuum. It was then placed in a small Pyrex tube containing a float and immersed in a thermostatically-controlled water-bath, the temperature of which was alternately raised and lowered (by addition of hotter or colder water, respectively) until the temperature limits above which the float sank and below which it rose were brought to within 0·005° C. as shown by a Beckmann thermometer. The movement of the float was followed by observing, through a telescope provided with a cross-hair, the extent to which it intercepted a beam of light. It was found necessary to agitate both the float in the tube and the mercury column of the Beckmann thermometer to prevent sticking, and this was conveniently achieved by means of an electric buzzer placed in contact with the respective supports. The micro-floats were made from the thin-walled glass tubing obtained by drawing out the central part of a Pyrex test-tube, this tube being sealed at one end, whilst the other was drawn out to a fine long capillary about 4 mm. away from the sealed end. The float was adjusted to ride in the liquid in question by cutting off pieces of the open end of the capillary until a mark on the capillary was on a level with the surface of the liquid. The end of the capillary was then melted gradually until the volume of the float was so decreased that it would just barely sink. Adjustments in the other direction were made by heating the float until the glass just softened, when the internal expansion of the air produced the necessary increase in volume. The drop of glass at the end of the capillary served to weight the float, so that it could ride in the tube in an upright position without sticking. Floats about the size of a grain of wheat may be made in this way, and the diameter of the sample tube in which they are immersed must be adjusted so as to be the smallest in which the float will move freely; the smaller the clearance, however, the longer the time required (usually 10 to 15 minutes) for the float to react to changes in density. The flotation temperature is then given by the point of intersection of the curves representing temperature plotted against the density of the sample and that of the float; at this temperature the float and sample have the same density. Richards (*loc. cit.*) has shown that if the flotation temperatures of the float in 2 standard liquids (whose densities are known over a wide range of temperature) are determined, the coefficient of expansion of the glass may be calculated and allowance made for the variation in the density of the float at different temperatures. It is thence shown how the density of a sample may be calculated from its flotation temperature and from that of a substance of known density. Application to the calculation of densities and compositions (*e.g.* of heavy water) are discussed, consideration being given to the thermal expansion of the float, changes in the temperature of flotation of the reference sample, and deviations from the laws of ideal solutions. A slide rule or nomogram for calculating the percentage composition (*e.g.* of heavy water) from the flotation temperature is described.

J. G.

Calculation of the Area Basis Metal Exposed at Discontinuities in the Tin Coating of Tinplate. W. E. Hoare. (*Phil. Mag.*, **1938**, **26**, 1077–1084; *International Tin Research Dev. Council, Publication No.* 86.)—The calculation is based on the experimentally determined results of the effect of thickness of the tin

coating on porosity, and on theoretical work on the size of non-reactive areas on the steel surface necessary to produce normal pores. The area of basis metal exposed, A, is found to decrease regularly with increase of thickness of coating and is given by the expression $A = 1{\cdot}1 \left\{ \frac{Y}{Y^{1{\cdot}785}} - 0{\cdot}119 \right\}$ sq. mm. per sq. metre, where Y is the thickness of coating expressed in pounds per basis box. Selected results are as follows:

A	1	0·5	0·25	0·1
Y	1	2	4	7

S. G. C.

Reviews

LIBRARY GUIDE FOR THE CHEMIST. By BYRON A. SOULE. Pp. xiii + 302. New York: McGraw-Hill Book Co., Inc.; London: McGraw-Hill Publishing Co., Ltd. 1938. Price 15s. net.

This book is intended to provide training in the use of bibliographical tools, especially those peculiar to the field of chemistry.

The first two chapters deal with the arrangement of a library and catalogue from the aspect of the reader rather than of the professional librarian. Sources of biographies are next referred to in detail. The succeeding sections refer to periodicals and the evaluation of original papers and the use of summaries appearing in abstract journals, reviews, reports and encyclopaedias. Then follow sections devoted to specialised reference books including textbooks and tables of constants. These sections are divided into the four major divisions of chemistry—inorganic, organic, analytical and physical. Remaining sections relate to patents and patent literature, and Government publications, and finally there is an informative section on the presentation of oral and written reports.

Primarily the book is intended as a guide to the use of keys and summaries, and in this respect it is excellent. In particular the explanation of the arrangement and instructions as to the use of extensive compilations and summaries such as "Beilstein" and the "International Critical Tables" should be of considerable value to students.

It is regrettable, however, that the book is marred by errors in historical facts. On page 53 the impression is given that The Chemical Society did not publish an abstract section until 1862, thirteen years after the French, whereas 82 pages of foreign abstracts appeared in the very first volume of the Quarterly Journal published in 1848. The first French abstracts were included not in the "Bulletin," at that time a publication of 84 pages, but in two substantial volumes entitled "Répertoire de Chimie pure (et appliquée)." The forerunner of the "Zeitschrift für angewandte Chemie" was entitled "Zeitschrift für die chemische Industrie," and not "Zentralblatt für technische Chemie."

In numerous instances the bibliographical information is out of date, but limitation of space only permits mention of a few examples. The 1924 edition

of "Sutton" is stated to be "the standard English text"; the revised edition of 1935 is not mentioned. Britton's "Hydrogen Ions," 1929, is referred to, but not the edition published in 1932, whilst Findlay's "Phase Rule," 1923, is quoted, but not that of 1931.

The index has at least three wrong alphabetical sequences and seven errors which may be attributed to the printer.

Notwithstanding these blemishes the book is a useful one, though it cannot be so strongly recommended as it would have been if these defects were not present.

F. W. Clifford

Standard Methods for Testing Tar and its Products. Standardisation of Tar Products Tests Committee. Second Edition. Pp. xiii + 434. London: S.T.P.T.C., 166, Piccadilly, W.1. 1938. Price 21s. net.

It is generally recognised that standard tests cannot be rigidly permanent but need periodic revision in order to keep pace with new developments both in manufacturing processes and analytical technique, and also to meet the changing requirements of users of the basic materials and their products. Accordingly, during the nine years that have elapsed since the issue of the First Edition of this publication, the Tar Products Tests Committee has been engaged in collating the experience of users of the 1929 tests and, with the assistance of a Revisionary Panel and eight Panel Groups, has now published a second edition which renders the first edition both inadequate and obsolete.

The general lay-out of the new edition follows closely that of the first. Despite a saving of space achieved, without loss of clarity, by cutting down the size of many of the diagrams, the new volume has grown from 296 to 434 pages. Certain tests which experience has shown were not in general demand have been omitted, many have been modified or replaced by others of greater accuracy, while a large number of additional tests called for by increasing stringency of modern requirements have been introduced. By way of examples, Section I on General Principles provides for the determination of density as well as specific gravity both by the bottle and hydrometer methods. The Committee also recommends that, as an indication of the presence of low-boiling constituents, the "drip" point be replaced by the temperature reading when 5 per cent. of the sample has distilled. Under Refined Tar is included a description of the method recently published by the Committee for the determination of the Equi-viscous Temperature. Lower Boiling Fractions are now dealt with under Crude Benzole, Light and Middle Oils and Refined Lower Boiling Products. The Section on Coal Tar Phenols—the term preferred to Tar Acids as tending to dispel the idea that tar products contain corrosive acids—includes methods for the determination of phenol in phenol-cresols mixtures and of phenol and the cresol isomers in cresylic acid, together with a facsimile reproduction of the U.S. Customs distillation method for cresylic acid. The extended field of use for creosote oil is reflected by the inclusion of tests for fluidity, calorific value, flash-point, viscosity and coke residue. It is noted that Gooch crucibles prepared with asbestos have been substituted for sintered glass crucibles, which have proved unsatisfactory for filtration of matter insoluble in toluole. Softening-point methods for pitch have been supplemented

by inclusion of the Ring and Ball and the Half-Inch Cube tests. British Standard Apparatus has been adopted so far as possible, and in such cases only summaries of the relevant British Standard Specifications are given in the Apparatus schedules composing Appendix I. In Appendix II is to be found a very useful table showing the physical constants of the more common constituents of coal tar. Graphs have now been included, more conveniently, in the text instead of at the end of the book, but copies printed on special paper suitable for mounting are still obtainable from the office of the Committee.

The high standard of printing and material used in the first edition is maintained in the present volume. The price, however, has increased nearly threefold, and it can only be assumed that the first edition was published at an uneconomic figure. The Committee is to be congratulated on the excellence of the second edition of Standard Tests, and there is no doubt that it will be universally welcomed by all who are concerned in the production, marketing and use of coal tar and its products.

F. R. Ennos

THE PHYSICAL PROPERTIES OF COLLOIDAL SOLUTIONS. By E. F. BURTON, B.A., Ph.D. Third Edition, prepared with the assistance of MAY ANNETTS SMITH, M.A., Ph.D. Pp. viii + 235. London: Longmans, Green & Co. 1938. Price 15s.

The first edition of this well-known work appeared in 1916, and the second in 1921. Very considerable research has been published during the ensuing 17 years, but the volume under review has only been increased by some 45 pages.

The Preface by Professor Burton states that over one-half has been completely re-written. "Our aim has been to accentuate the contribution of the study of colloidal solutions to the confirmation of the basic principles of the kinetic theory of matter. For example, more prominence has been given to the treatment of the Brownian movement and its immediate consequences and less to the abstruse optical properties of suspensions than in former editions. One cannot hope to treat fully all phases of the large field, but we have aimed at some degree of completeness in those phases which have been selected for treatment." This admission prepares the student for the very specialised character of the volume and for the absence of much which he would normally expect to find.

The thirteen chapters deal successively with:—Colloidal Solutions Defined; Historical Background; Classification and Preparation of Colloidal Solutions; Forces Regulating the Size of Colloidal Particles; The Ultramicroscope; The Brownian Movement; The Distribution of Particles in a Colloidal Solution—Macroscopic Equilibrium and Microscopic Fluctuations; Determination of Avogadro's Number; The Optical Properties of Colloidal Solutions; The Size of Colloidal Particles; Electrokinetic Phenomena; The Coagulation of Colloidal Solutions; Conclusion.

To the reviewer this book is a real disappointment. It is agreed that the earlier editions, especially the first, filled a gap in the text-book literature on colloids. To-day, however, the subject is so extended that Burton's treatment is quite inadequate. The references to original sources are too few. Again, even admitting the specialised treatment in limited fields of inquiry, there is inadequate

account of the more modern work. The chapter dealing with electrokinetic phenomena may be cited. Herein is given an excellent account of early work and a detailed discussion of the Helmholtz Double Layer and the related formulae for electro-endosmosis. Modern views on the structure of the double layer are ignored, and the names of Gouy, Stern, Lens and others well known in this connection do not appear. Other chapters have similar deficiencies as regards modern investigations.

The book can be read with profit as a statement of the classical aspect of colloid chemistry. In this respect the chapters dealing with the ultramicroscope and with the Brownian movement deserve special mention.

The reviewer would assess this volume as a sound introduction to the study of colloidal systems, typical of the earlier schools, but to be read with the understanding that modern developments must be studied in larger works or special monographs. The book is well bound and printed and reasonably priced.

WILLIAM CLAYTON

MODERN ASPECTS OF INORGANIC CHEMISTRY. By H. J. EMELÉUS, D.Sc., A.R.C.S., and J. S. ANDERSON, Ph.D., A.R.C.S. "Twentieth Century Chemistry" Series, edited by J. C. PHILIP, F.R.S. Pp. xi + 536. London: George Routledge & Sons, Ltd. 1938. Price 25s.

Where inorganic chemistry is concerned much water has flowed under the bridges since the opening of this century, and it is amazing to realise how much of the foundations of chemistry has been dug up without seriously dislocating the enormous superstructure which had been raised thereupon. Dislocation of text-books is, however, inevitable; hence the many attempts to produce books that will fill in the gaps that have appeared. To this category belongs the book under review, although it is one which is strongly individual and is difficult to classify at all. The preface states that "this volume has been designed to give a review of some of the important developments which have taken place in Inorganic Chemistry during the last two decades or so, and to relate those developments to the science as a whole"; this may be taken as a very fair account also of what the book has achieved. The form adopted is that of a series of more or less disconnected essays, each dealing very thoroughly with some field of modern research and giving, in the authors' words, "a cross-section of the subject" as ordinarily presented. There are fifteen such essays headed as follows:—Atomic Structure and the Periodic System; Atomic Weights and Isotopes; Structure of Molecules of Inorganic Compounds; Co-ordination Compounds and Inorganic Stereochemistry; Poly-acids and Silicates; Hydrogen and the Hydrides; Free Radicals of Short Life; Non-metallic Oxides and Related Substances; Recent Chemistry of the Non-metals; Peroxides and Per-acids; Recent Chemistry of the Metals; Metallic Carbonyls, Nitrosyls and Related Compounds; Intermetallic and Interstitial Compounds; Reactions in Liquid Ammonia and Liquid Sulphur Dioxide; Radio-activity and Atomic Disintegration. The various essays are well, concisely and clearly written; this does not, however, imply that they are easy reading; the time is probably yet distant when all these modern theories shall be welded into one clearly comprehensible system, and this book is addressed to

"teachers, honours students and research workers." To get a similar amount of information on some of this modern work one would need to have recourse to a text-book very large and very recent indeed, and, apart from the labour of extracting that information from thence, it is very doubtful if any existing text-book contains more than a fraction of it.

Two minor criticisms that may be advanced refer not so much to this book in particular as to certain tendencies which are becoming wide-spread and are in need of regulation. One of these tendencies is the more or less arbitrary assignation of symbols to organic radicles; a specially glaring example of this occurs on p. 142, where the symbol allotted to propyl is indistinguishable, in type or otherwise, from that accepted for praseodymium. The other criticism refers to a matter in which the authors, presumably, had no choice, the fault lying with the original investigators; the use of the word "co-ordination" both in a chemical and a crystallographic sense does not tend towards the ready comprehension of an argument in which it is being used in both senses.

There is a certain resemblance in plan between this book and Morgan and Burstall's *Inorganic Chemistry: A Survey of Modern Developments* (*cf.* ANALYST, 1937, **62**, 422), but the two books differ widely in content and treatment and there is no serious competition between them.

The volume is attractively bound and the printing is excellent. Proof reading, too, seems to have been well carried out, for only one slip has been noted (p. 449), unless the statement (p. 489) that the number of helium atoms produced by 1 g. of radium in a year ($4{\cdot}53 \times 10^{18}$) is "in good agreement" with the number of α particles emitted ($4{\cdot}53 \times 10^{18}$) is another slip and not merely a rather quaint mode of expression. An index of 8 pages (less than twice the length of the list of contents) seems very inadequate in a book of this type.

A significant sentence occurs in the preface—"If this practice (their method of reference) has led to an apparent preponderance of references to foreign—especially German—investigations, this must be taken as reflecting the comparative neglect of Inorganic Chemistry in Britain to-day." A glance at many recent indexes of the *Journal of the Chemical Society* will amply confirm this statement. The authors' "main objective" of "presenting the subject as one which still ranks as an absorbing experimental science and which offers unlimited opportunities for the development of new experimental methods and the exploration of little known fields" has been fully achieved, and the book is warmly to be recommended to all who wish to keep in touch with the more recent work on the subject.

B. S. EVANS

QUALITATIVE INORGANIC ANALYSIS. By A. J. BERRY, M.A. Pp. 147. Cambridge: University Press. 1938. Price 6s.

While much in the traditional system of qualitative chemical analysis is likely to remain of value for some time to come, rapid progress has been made in recent years with new developments—new reagents, spot tests and so on. The author has evidently given careful thought to the difficulties thus created in the presentation of the subject to students, for he has very ably selected from the old and the

new to give in a book of modest size, and, consequently, of moderate price, a balanced treatment well suited to modern needs.

The book is in two subdivisions: General (28 pp.) and Special (remainder). The first gives a brief non-mathematical account of relevant chemical theories followed by a description of methods and reagents. The second gives reactions of the metals (49 pp.), reactions of the acid radicals (36 pp.), systematic analyses for metals (12 pp.) and examination for acid radicals (11 pp.). It will thus be seen that most of the space is devoted to reactions, a good knowledge of which is of the first importance to the student. A number of elements formerly classified as "rare," *e.g.* vanadium and molybdenum, are included. There are one or two pages on dry tests; these are too useful on occasion to merit complete exclusion. Among the newer reagents described are ammonium mercurithiocyanate (for cobalt, cadmium and zinc), diphenylcarbazide (the value of this reagent in testing for traces of chromic acid is not well emphasised), salicylaldoxime (for copper) and quinalizarine (for magnesium and beryllium). The value of zinc uranyl acetate as a means of testing for sodium, however, is not mentioned. The group separations are clearly described and a tabular summary of these is given at the end of the book.

The style throughout is clear and concise. The author states that the book was written primarily for his own pupils. It deserves to be widely adopted for teaching purposes.

S. G. CLARKE

MIKROMETHODIK. QUANTITATIVE BESTIMMUNG DER HARN-, BLUT- UND ORGANBESTANDTEILE IN KLEINEN MENGEN FÜR KLINISCHE UND EXPERIMENTELLE ZWECKE. By LUDWIG PINCUSSEN. Sixth Edition. Pp. vii + 193, 31 illustrations. Leipzig and Vienna: Franz Deuticke. 1937. Price RM.5.

The fifth edition of this laboratory handbook was published seven years ago, and having regard to the recent developments of micro methods applied to biology, a new edition was required to bring it up to date. New matter includes the determination of carbon in urine, the sulphur of glutathione, indican in blood and a procedure for the determination of the fractions of blood protein. A new method is substituted for the method previously given for the determination of sodium in blood. The methods described are mainly nephelometric, volumetric and colorimetric rather than gravimetric. There is also a chapter on gas analysis.

As the book has been brought up to date in many respects, it is a pity that it is still incomplete. Among the descriptions of burettes, the 10-ml. Pregl burette with automatic filling is not mentioned, the modern Parnas and Wagner Kjeldahl method for nitrogen is not described, nor yet the use of selenium as a catalyst, or even of perhydrol to accelerate the decomposition of organic matter. No methods are given for the determination of silica in urine or blood, of arsenic and lead in urine or of alcohol in blood. The book retains its original format, and is of a very convenient size for laboratory reference. The index, as in so many German publications, is very incomplete.

J. W. MATTHEWS