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THE ANALYST

PROCEEDINGS OF THE SOCIETY OF PUBLIC ANALYSTS AND OTHER ANALYTICAL CHEMISTS

AN Ordinary Meeting of the Society was held on Wednesday, May 4th, at the Chemical Society's Rooms, Burlington House, the President, Professor W. H. Roberts, being in the chair.

Certificates were read in favour of Alexander Andrew, B.Sc., A.R.T.C., A.I.C., Nadhim Jaafar Chalabi, B.A., Cyril Charles Harris, B.Sc., A.R.C.S., Frederick Denison Maurice Hocking, M.B., B.S., M.Sc., F.I.C., A.C.G.F.C., Cecil Owen Tattersall, B.Sc., A.R.C.S., F.I.C.

The following were elected Members of the Society:—Daniel Ceiriog Evans Jenkins, M.Sc., F.I.C., Roland Gordon Minor, A.I.C., Rollo K. Newman, B.Sc., A.A.C.I., Reginald Wilfred Wallington, Ph.D., M.Sc., A.I.C.

The following papers were read and discussed:—"The Gravimetric Estimation of Germanium," by G. R. Davies, M.Sc., Ph.D., and Sir Gilbert Morgan, D.Sc., LL.D., F.R.S.; "Analysis of Vinegar. Part I—Spirit, Artificial, Malt and Distilled Malt Vinegars and their Differentiation," by F. W. Edwards, F.I.C., and H. R. Nanji, B.Sc., Ph.D., D.I.C., F.I.C. (*Work done under the Society's Analytical Investigation Scheme*); "The Detection and Determination of Ouabain and Strophanthin," by W. D. Raymond, B.Sc., A.I.C.

NORTH OF ENGLAND SECTION

ON the kind invitation of W. J. Rees, Esq., a Meeting of the Section was held at Sheffield University on May 7th. The Chairman (Professor T. P. Hilditch) presided over an attendance of forty-four.

The members inspected the equipment for the testing of refractories and moulding sands in the Department of Refractory Materials and also the new laboratories in the Department of Metallurgy.

A paper was read by W. J. Rees, M.Sc., F.I.C., on the "Chemical Analysis and Physical Testing of Refractories."

The Chairman expressed the congratulations of the meeting to Professor W. H. Roberts on his election as President of the Society. It was decided that a letter should be sent to Dr. Dunn conveying the pleasure of the members on hearing of his recovery from illness.

A resolution of thanks was unanimously passed to Mr. Rees for arranging the meeting.

The Gravimetric Determination of Germanium

By GLYN REES DAVIES, B.Sc., Ph.D., and
Sir GILBERT MORGAN, O.B.E., D.Sc., F.I.C., F.R.S.

(*Read at the Meeting, May* 4, 1938)

During the course of an investigation into the rare metal content of flue dusts[1] it became necessary to acquire fairly accurate methods of determining micro and macro quantities of germanium, and for this purpose an examination was made of existing procedures, which showed that some of them did not always give acceptable results. The limitations of these methods are now indicated while, in addition, two new ones are advocated. The first of these deals with the precipitation of germanium dioxide with tannin, whereas in the second germanium is determined as cinchonine germano-molybdate.

Precipitation of Germanium as Sulphide and Conversion to Oxide.—This, the oldest method of estimating germanium, is quite accurate when the procedure of Johnson and Dennis[2] is employed, according to which the sulphide is converted into oxide by means of ammonia and hydrogen peroxide, the solution being evaporated and the residue ignited at 900–1000° C. Platinum ware is necessary for this oxidation, for we have confirmed the observation of the above-mentioned workers that the use of glass dishes leads to high results. The only questionable part of this method was the best concentration of acid necessary to secure complete precipitation of germanium as sulphide. Johnson and Dennis, employing sulphuric acid, place this at 6 *N*, whilst Geilmann and Brünger,[3] who used hydrochloric acid, recommend 3 to 4 *N*. There seemed no reason why the optimum normality should differ so largely in the two instances, and so a series of determinations was carried out using hydrochloric and sulphuric acids respectively. Table I gives some of the results obtained; in the first series the sulphide was oxidised to germanate in the usual way and then converted into cinchonine germanomolybdate. The results confirm those of the foregoing workers, the optimum normality with hydrochloric and sulphuric acids being 3 and 5 *N* respectively. This difference may probably be due to the increasing risk of loss as germanium tetrachloride as the strength of the hydrochloric acid is increased. This would also account for the greater variation in the results when this acid is used than with sulphuric acid.

In the last series the peak value actually occurred at 5·5 *N*, when the weight of GeO_2 obtained was 151·4 mg.

DETERMINATION AS MAGNESIUM GERMANATE (Mg_2GeO_4).—In this method, due to Müller,[4] a neutral or faintly acid solution of germanium is treated with excess of *N* magnesium sulphate solution together with at least an equal volume of 2 *N* ammonium sulphate solution followed, with stirring, by conc. ammonia (sp. gr. 0·880). For quantities of 0·5 to 0·2 g. of germanium dioxide the quantities recommended are 25 to 20 ml. of ammonium sulphate solution, 25 to 15 ml. of magnesium sulphate solution and 20 to 15 ml. of ammonia for 100 ml. of the germanium solution. The mixture is boiled for a few minutes and left for 12 hours. The precipitate is filtered off on paper, washed with not more than 50 ml. of a solution of 10 ml. of ammonia and 90 ml. of water and ignited. Results are given of analyses covering a range of some 430 to 1·3 mg. of germanium dioxide.

TABLE I

GeO_2 taken mg.	Acid used	Wt. of GeO_2 found after precipitating as sulphide from acid of following normality:— 2*N*	3*N*	4*N*	5*N*	6*N*	7*N*
3·90	HCl	3·85	3·90	3·89	3·80	3·74	3·73
58·7	HCl	58·1	58·8	58·4	57·8	57·2	57·0
62·9	H_2SO_4	62·4	62·5	62·6	63·0	62·5	62·3
151·3	H_2SO_4	—	—	150·8	151·1	150·9	—

Three operators have carried out determinations according to the details given above and have been unable to obtain consistent results; these were invariably too high, although no trace of precipitate is given in the absence of germanium. Selected analyses are given in Table II; in the last example (No. 6) the germanium was first precipitated as sulphide and this was converted into germanate with ammonia and hydrogen peroxide. The germanium dioxide used was in the form of a solution of sodium germanate and was preserved in wax containers.

TABLE II

No.	GeO_2 taken g.	Vol. of solution ml.	Vol. of *N* $MgSO_4$ ml.	Vol. of 2 *N* $(NH_4)_2SO_4$ ml.	Wt. of precipitate g.	GeO_2 found g.
1	0·0145	25	4	6	0·0336	0·0190
2	0·0436	50	8	12	0·1121	0·0633
3	0·0884	100	15	22	0·2091	0·1181
4	0·1768	100	17	20	0·3658	0·2080
5	0·2270	100	16	25	0·4909	0·2772
6	0·2270	100	16	25	0·4897	0·2765

An analysis of one of the above precipitates gave 29·6 per cent. of germanium and 32·4 per cent. of magnesium, whereas Mg_2GeO_4 requires 39·2 per cent. and 26·2 per cent. respectively.

It is obvious that magnesia is co-precipitated and that an adjustment of the relative amounts of ammonium and magnesium sulphates is needed. Further experiments indicated that the method could be made to work under a limited set of conditions which, however, required a rough knowledge of the amount

of germanium to be determined. As already indicated by Müller, it is not feasible to prevent precipitation of magnesium hydroxide by adding too much ammonium sulphate—otherwise low results are obtained—while the addition of too little of this reagent gives high values. Where the volume of germanium solution is 100 ml. 24 to 30 ml. of 2 *N* ammonium sulphate solution must be added, while the amount of *N* magnesium sulphate must be such as to supply an excess of 2 to 3 ml. The governing factor seems to be the ratio of ammonium sulphate solution to excess of magnesium sulphate, and this should lie between 8 and 12. Results obtained under these conditions are given in Table III, which also shows in Nos. 6 to 8 the effect of using lesser and larger amounts of ammonium sulphate. In every instance the time of boiling the solution was 1 minute, and the precipitate was collected on a porcelain filter crucible (A 2) and washed with 25 to 30 ml. of the wash-solution.

TABLE III

No.	Vol. of Ge solution ml.	Vol. of 2 *N* $(NH_4)_2SO_4$ (*a*) ml.	Excess of $MgSO_4$ solution (*b*) ml.	Ratio $\frac{a}{b}$	GeO_2 taken g.	GeO_2 found g.
1	90	24	2	12	0·0290	0·0291
2	60	15	1·75	8·6	0·0145	0·0142
3	100	24	2·4	10	0·0727	0·0731
4	100	30	3	10	0·1710	0·1712
5	100	25	2·5	10	0·2270	0·2268
6	100	20	2·0	10	0·1710	0·1789
7	100	35	3·5	10	0·1710	0·1674
8	100	18	1·0	18	0·1710	0·1758

Analysis of any one of the precipitates (Nos. 1 to 5) which gave a correct result showed that they contained 39·15 per cent. of germanium.

Even when the amount of germanium to be analysed is unknown, fairly accurate results may generally be obtained by double precipitation, although high results are still sometimes experienced if the amount of magnesium hydroxide in the first precipitate is considerable. For this purpose the precipitate is dissolved in 7 to 10 ml. of warm 2 *N* sulphuric acid, and the solution is nearly neutralised with ammonia and diluted to 100 ml. Fifteen ml. of ammonium sulphate reagent and 1 ml. of magnesium sulphate solution are added followed by the addition of 15 to 20 ml. of ammonia and boiling. Incidentally, it is not necessary to wait 12 hours before filtering, as precipitation is complete in 3 to 4 hours.

DETERMINATION BY MEANS OF TANNIN.—The valuable researches of Schoeller and his collaborators[5] have shown that tannin is a useful agent for precipitating metallic hydroxides and hydrous oxides, and we have found it particularly serviceable in the determination of germanium. When suitable amounts of ammonium salts are present, germanium dioxide is readily precipitated by this reagent as a colourless tannin complex even from fairly acid solutions; indeed, with sulphuric acid the acidity may be allowed to rise to 1 *N*. With nitric acid the maximum acidity is much lower, and the precipitate is also somewhat finer and neither settles nor can be filtered off quite as readily as when the former acid is used. The method,

which is best adapted for amounts of dioxide up to 50 to 60 mg. (large quantities give too bulky a precipitate), is carried out as follows:

The neutral germanium solution (150 to 250 ml.) is treated with 5 to 15 ml. of 2 *N* sulphuric acid and 8 to 10 g. of ammonium sulphate and heated almost to boiling, after which 10 to 30 ml. of a freshly-prepared 5 per cent. tannin solution are slowly added, with stirring. The mixture is left in a warm place until the colourless flocculent precipitate has settled and is then filtered through "ashless" filter-paper with the aid of gentle suction. The precipitate is washed with 5 per cent. ammonium nitrate solution containing 5 ml. of 2 *N* nitric acid per 100 ml. and a little tannin. As the precipitate readily adsorbs foreign material, it is advisable to return it to the beaker with a jet of wash liquor, stir with 50 ml. of that liquor and refilter; the complex is further washed on the filter. The wet precipitate and filter-paper are transferred to a tared crucible, dried over a small flame and ignited to germanium dioxide. This ignition may be carried out directly if the carbonaceous matter is removed below 700° C., but it is preferable, in order to minimise the risk of reduction to the volatile germanous oxide, to oxidise the tannin and filter-paper by adding a few drops of sulphuric acid and successive small portions of nitric acid. When most of the carbon has been removed the residual acid is carefully evaporated, and the residue is ignited, cautiously at first, and finally at 1000° C. The following are typical examples of the results obtained:

Germanium dioxide taken, mg. ..	24·9	43·7	12·4
" " found, mg. ..	24·9	43·6	12·5

Since germanium is precipitated by tannin from acid solution, it should be possible to use this reagent for separating the element from most other metals excepting tungsten and probably niobium and tantalum. No exhaustive study has yet been made of such separations, but successful results have been achieved in one precipitation from binary mixtures with arsenic, gallium, zinc, copper, iron, manganese, vanadium, titanium and zirconium. For the first six metals the conditions already outlined suffice, but with the remainder it is necessary to increase the acidity in order to prevent co-precipitation. The method is then slightly modified as follows:

The neutral solution (250 to 300 ml.) is treated with the requisite amount of sulphuric acid (4 to 10 ml. of 18 *N* acid) and 8 to 10 g. of ammonium sulphate, then heated almost to boiling and treated, dropwise and with stirring, with 10 to 20 ml. of 10 per cent. tannin solution, followed by 2 to 3 g. of ammonium sulphate dissolved in a little water. The mixture is left until cold and then filtered, and the precipitate is washed and ignited as before. The material in the filtrate may be determined either by adding more tannin and reducing the acidity with ammonia until precipitation occurs, or by concentrating the filtrate, removing excess of tannin with nitric acid, and then determining the germanium by any suitable method. Some of the results obtained are given in Table IV.

Germanium and molybdenum could not be separated by this method since co-precipitation took place even from very dilute solutions. Molybdates alone gave no precipitate, there being only a deep red coloration, reminiscent of that developed with thiocyanate and reducing agents. A separation, not entirely

satisfactory, may be achieved if nitric acid is used, although double precipitation is necessary. In this procedure the molybdate-germanate solution (300 ml.) is treated with 30 ml. of 2 *N* nitric acid and 20 ml. of 25 per cent. ammonium nitrate solution and heated to 80° C., and 10 to 15 ml. of a 10 per cent. solution of tannin are added. The mixture is left until cold, and the supernatant liquor is then tested for complete precipitation by the addition of a little 20 per cent. ammonium acetate solution. If a colourless precipitate is formed, more of the reagent is added until precipitation is complete, and the mixture is again heated. After 12 hours the precipitate is collected and washed, as previously described. The

TABLE IV

Mixture taken					Found		
GeO_2 mg.	Other constituent	mg.	Vol. of solution ml.	Vol. of 18 *N* H_2SO_4	GeO_2 mg.	Other constituent	mg.
24·9	As_2O_3 ..	86·5	200	2	25·1	As_2O_3 ..	86·8
24·9	$ZnCl_2$ ..	257·1	250	3	25·0	$ZnCl_2$ ..	258·0
24·9	$CuSO_4$, $5H_2O$	155·4	250	3	24·9	$CuSO_4$, $5H_2O$	155·8
22·9	Ga_2O_3 ..	27·3	200	2	23·2	Ga_2O_3 ..	27·0
28·1	Mohr's salt	124·0	200	3	28·0	Mohr's salt	124·0
28·1	$MnCl_2$, $4H_2O$	66·0	200	2	28·2	$MnCl_2$, $4H_2O$	67·4
28·1	ZrO_2 ..	28·4	250	4	28·0	ZrO_2 ..	28·8
31·2	V_2O_5 ..	37·6	300	8	31·3	V_2O_5 ..	38·0
28·1	TiO_2 ..	43·4	300	10	28·1	TiO_2 ..	43·4

paper and precipitate are transferred to a 250-ml. beaker, where organic matter is destroyed by gently heating with 2 ml. of conc. sulphuric acid and 10 ml. of nitric acid, additional amounts of the latter being added if necessary. The clear liquid is evaporated until fumes of sulphuric acid appear, then cooled, diluted and transferred to a 500-ml. beaker. Any germanium dioxide which has separated out on the bottom of the 250-ml. beaker is dissolved in a little colourless ammonium sulphide solution, and the solution is oxidised with hydrogen peroxide, the excess of which is removed by boiling; the resulting solution is added to that in the 500-ml. beaker. The combined liquids are neutralised with ammonia and diluted to 300 ml., and the germanium is precipitated as before, filtered off, washed and ignited. The following is an example of the results:

Taken	Found
GeO_2 28·1 mg.; MoO_3 41·2 mg.	GeO_2 27·6 mg.; MoO_3 40·8 mg.

Precipitation with tannin enables germanium to be determined in the usual germaniferous materials, such as germanite, without preliminary distillation with hydrochloric acid and chlorine. With germanite the procedure is as follows:

The finely-powdered mineral (0·5 g.) is dissolved in 12 ml. of dilute (1 : 1) nitric acid and 1 ml. of conc. sulphuric acid, and the liquid is concentrated to about half its original volume and then diluted to 50 ml. It is filtered through a small filter-paper which, after washing, is returned to the beaker and treated with a little ammonium sulphide to dissolve any germanium dioxide deposited on the beaker or filter-paper.

The sulphide is filtered off, the residue is well washed with water, and the filtrate is oxidised with hydrogen peroxide. After excess of this reagent has been boiled off, the solution is added to the original filtrate, which is further diluted to 350 to 400 ml. Ten ml. of 18 *N* sulphuric acid and 10 g. of ammonium sulphate are added, the solution is heated almost to boiling, and the germanium is precipitated with 15 ml. of 10 per cent. tannin solution followed by 2 g. of ammonium sulphate. When cold, the mixture is filtered, and the precipitate is treated as previously described. Examples of the results are:

Germanite taken g.	Germanium dioxide found g.	Germanium Per Cent.
0·5050	0·0329	4·52
0·5028	0·0324	4·48

The percentage of germanium obtained by distillation of germanium chloride was 4·44.

DETERMINATION AS PYRIDINE GERMANOMOLYBDATE.—This procedure, due to Geilmann and Brünger,[3] consists in adding successively 10 ml. of 50 per cent. ammonium nitrate solution, 15 ml. of 3 per cent. ammonium molybdate solution and 10 ml. of nitric acid (sp.gr. 1·2) to the neutral germanate solution (50 ml.), warming on the water-bath until the mixture turns yellow, and then introducing 15 to 20 ml. of a saturated solution of pyridine nitrate. After settling completely the precipitate is filtered off and washed with 50 ml. of a wash-water made by dissolving 12 g. of ammonium nitrate, 0·5 g. pyridine nitrate and 10 ml. of nitric acid (sp.gr. 1·2) in 250 ml. of water; it is dried at 160° C. The germanomolybdate is stated by Geilmann and Brünger to have the formula $Py_4H_4[Ge(Mo_2O_7)_6]$ for which the factor for germanium would be 0·0326, but as this figure gives low results, they adopt an arbitrary factor of 0·0353.

According to Illingworth and Keggin[6] the formula of the precipitate should probably be $Py_4[GeMo_{12}O_{40}]$, for which the factor would be 0·0333.

TABLE V

Germanium taken mg.	Time of heating minutes	Temp. of solution ° C.	Wt. of precipitate g.	Factor
3·26	0	19	0·0566	0·0576
3·26	4	48	0·1025	0·0318
3·26	8	66	0·1061	0·0307

This method should be eminently suitable for the gravimetric determination of small quantities of germanium but, unfortunately, the procedure is somewhat unsatisfactory and the published details too meagre. Thus, no indication is given as to the time of heating on the water-bath or of the temperature attained; the statement "until the mixture turns yellow" is useless, for the solution becomes coloured immediately on addition of acid. Furthermore, the precipitate is appreciably soluble in the wash-liquor, and, although a definite quantity of the latter is employed, it is obvious that the losses from this source will vary with such factors as the method of washing, the rate of filtration during washing and the size of the crystals forming the precipitate. It is not practicable to heat the

solution too near the boiling-point, as molybdenum trioxide may also be deposited. Without any heating the precipitate is micro-crystalline and readily dissolves in the wash-liquor. The influence of temperature is shown in the following table (Table V).

The effect of altering the method of washing the precipitate is seen from the results in the two following experiments, in which other conditions were kept as nearly alike as possible:—(*a*) The precipitate was washed three times by decantation, 10 ml. of wash-liquor being used each time and the remaining 20 ml. employed to transfer the germanomolybdate to the filter and to wash it thereon. (*b*) There was no washing by decantation, but only on the filter.

	Germanium dioxide taken mg.	Wt. of precipitate g.	Factor
(*a*)	3·45	0·1091	0·0316
(*b*)	3·45	0·1125	0·0307

Except in the first example, it will be seen that the factors obtained are considerably less than the 0·0353 adopted by Geilmann and Brünger, and we have been able to obtain results consonant with this factor only by washing slowly and filtering the wash-liquor slowly. The effect of the rate of filtration is seen in the two following results, where in the first instance a porcelain filter-crucible of type A2 having a fast rate of filtration was employed, whilst in the second a Gooch crucible with a thick asbestos pad was used and slow filtration was adopted.

Germanium dioxide taken mg.	Wt. of precipitate g.	Factor
1·53	0·0480	0·0316
1·53	0·0436	0·0352

As there are so many variables, it is obvious that the gravimetric factor to be employed will depend on the precise working conditions of each analyst and must be determined by trial under those conditions. Carried out as follows, the method gives reasonably reliable results for a factor of about 0·0317.

Twenty ml. of 25 per cent. ammonium nitrate solution, 15 ml. of 3 per cent. ammonium molybdate solution and 10 ml. of nitric acid (sp.gr. 1·2) are added successively to the germanium solution (40 ml.), which is then heated on a water-bath until the temperature reaches 50° C. A saturated aqueous solution of pyridine nitrate (15 ml.) is stirred in, and the mixture is left for four hours. The supernatant liquor is decanted through a filter-crucible, and the precipitate is washed twice by decantation, 10 ml. of the wash-liquor being used each time and poured off as soon as most of the precipitate has settled. The remainder of the wash-liquor (30 ml.) is used to transfer the germanomolybdate to the filter and to wash it thereon: filtration is carried out as rapidly as possible. The precipitate is dried for 3 hours at 160° C.

Determination as Cinchonine Germanomolybdate.—As pyridine is not an entirely satisfactory precipitant for germanomolybdic acid, many other amines, particularly tertiary amines, have been tried in the hope of producing a more insoluble precipitate. Unfortunately, when this condition was fulfilled it invariably happened that a precipitate was also formed with molybdic acid.

The amount of this precipitate was often small and appeared only after the lapse of some hours. Matters were further complicated by the fact that these germanomolybdates were usually amorphous and required fairly long periods in which to settle out completely, unless considerable excess of the reactants was used; in that event the amount of adventitious precipitate was also increased. By carefully regulating the conditions, however, it was found that cinchonine could be used and that the results obtained were more constant than those given by pyridine. The cinchonine germanomolybdate forms a yellow amorphous precipitate which is not appreciably soluble in dilute ammonium nitrate solution; when dried at 160° C. it has the formula $(C_{19}H_{22}ON_2)_4H_4[GeMo_{12}O_{40}]$, which corresponds with 2·385 per cent. of germanium. The determination, which serves best for amounts up to 5 mg. of germanium, is carried out as follows:

To the neutral germanium solution, occupying a volume of 40 ml., are added 20 ml. of 25 per cent. ammonium nitrate solution, 16 ml. of 2 per cent. ammonium molybdate solution and, with stirring, 20 ml. of 2 *N* nitric acid, followed by 9 ml. of a 2·5 per cent. solution of cinchonine in 0·25 *N* nitric acid. The mixture is allowed to remain with occasional stirring, for 2 to 4 hours according to the amount of the precipitate. A little practice is required to judge when precipitation is complete, for the supernatant liquor never becomes quite clear but retains a faint opalescence. If, however, after stirring and allowing to settle for 10 minutes, the opalescence does not increase during the next 30 minutes, the precipitate may be filtered off. It is collected on a filter-crucible, washed with 2·5 per cent. ammonium nitrate solution containing 5 ml. of 2 *N* nitric acid per 100 ml. and dried at 160° C. for 2 hours.

TABLE VI

Germanium taken mg.	Vol. of ammonium molybdate ml.	Vol. of cinchonine solution ml.	Time of standing hours	Wt. of precipitate mg.	Germanium found mg.
0·59	16	8	2	25·1	0·60
0·78	16	9	2	33·5	0·80
1·17	16	9	2	49·4	1·18
1·39	16	9	2·5	59·2	1·41
1·56	16	9	2·5	65·9	1·57
1·63	16	9	2	67·6	1·61
2·34	16	9	3	99·6	2·37
2·75	16	9	2·5	104·2	2·72
3·12	16	9	3	130·8	3·12
4·06	16	9	3·5	165·5	3·95
4·31	16	9	12	176·0	4·20
4·66	16	9	3·5	189·4	4·52
5·17	17	9	4	208·8	4·98
4·66	18	10	12	194·5	4·64
5·17	18	10	12	212·8	5·08

The results are somewhat low when more than 4 mg. of germanium are present, but if it is known that this is so, somewhat more accurate values are obtained by increasing the amounts of ammonium molybdate and cinchonine solutions to 18 and 10 ml. respectively. It is also advantageous to allow the mixture to

remain overnight before filtering. Alternatively, the conditions may be left as originally and a correction applied by adding 0·1 mg. to the result when 4 mg. of germanium are present and 0·01 mg. for every succeeding 0·1 mg. of germanium. Thus a result of 4·5 mg. would require the addition of 0·15 mg. Table VI gives some of the results obtained, no correction having been applied.

We desire to express our thanks to Messrs. Ryan and Hancock for assistance with some of the foregoing analyses.

This work forms part of the programme of the Chemistry Research Board and is published by permission of the Department of Scientific and Industrial Research.

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Discussion

Dr. B. S. Evans said that certain points of resemblance between arsenic and germanium made it seem probable that germanium would give a Reinsch test. With regard to the difference noted in the behaviour of hydrochloric and sulphuric acids he had met this in other circumstances (*e.g.* with arsenic) and had put it down to formation of undissociated chloride.

Dr. Davies replied that germanium resembled arsenic in many ways; for instance, it formed volatile hydrides and could be determined by the Marsh test. It also gave a precipitate with silver nitrate. Germanium dioxide, like the sulphide, also showed a difference of behaviour towards hydrochloric acid, for whereas the solubility of the dioxide in acids usually decreased with increasing concentration, with hydrochloric acid a minimum was reached at 3 *N*.

Mr. F. L. Okell asked if any attempt had been made to determine germanium by oxidation from germanous to germanic compounds. In view of its possible effect on other determinations, what effect had germanic salts on the ordinary reducing agents used in volumetric analysis? The volatility of germanous sulphide was a further indication of that element's relationship to tin; stannous sulphide also was volatile.

Dr. Davies said that there was no method in which the oxides were used as suggested by Mr. Okell. It was not so easy to produce the lower oxide as in the case of tin.

Mr. Hamence suggested that the fact that germanium reacted in the Marsh test might explain some of the abnormal results obtained when testing for arsenic.

Dr. Davies said that germanium hydride was not produced in acid, but only in alkaline solutions, so that it would not affect the arsenic reaction.

Mr. L. Eynon asked whether germanium and gallium were likely to be present in the living plant as well as in the coal from which they had been derived.

Sir Gilbert Morgan said that they thought that this process was going on, and that living woody tissues were doing it. If one subjected coal to cleaning, it

did not take out the metals: they were in the coal substance. Northumbrian and Durham coal contained a considerable amount of germanium, but Kent coal none.

Mr. A. L. BACHARACH asked whether any work had been done on the physiological action of germanium on plants and animals.

Dr. DAVIES replied that a little work had been done on plants, but the results were not very definite. Continental workers had made a few experiments to see whether germanium was necessary to plant life, but it did not seem to be so. As regards animals, it appeared that germanium might be quite good for anaemia and possibly of some use for cancer.

Dr. G. ROCHE LYNCH asked if the authors could mention the practical uses of these elements.

Sir GILBERT MORGAN replied that gallium, the metal which melted in the hand, had already been used as an alternative to mercury for thermometry. Germanium had had very little use so far, although it had been suggested as a component in the material used for coating television viewing screens. His colleague could tell them more about that; it was a metal which induced phosphorescence. So far, very little germanium had been available. The work on it had come to a standstill after Winkler's discovery until some time in the present century, when Professor Dennis of Cornell University had been able to get some considerable quantity, and only since he had taken it up had there been any considerable advance made.

Mr. D. M. FREELAND enquired whether the spectroscope had proved useful for the analysis of these ashes.

Dr. DAVIES replied that the spectroscope had been very useful in the preliminary work on these ashes, and it showed coal to be usually a very complicated mixture; it had even revealed small quantities of osmium.

Hair Dyes. I. The Chemistry and Analysis of Henna

BY H. E. COX, D.Sc., PH.D., F.I.C.

(*Read at the Meeting, April* 6, 1938)

IN view of the importance of henna as a hair dye and, in the East, as a medicine, it is a little surprising that there is so little published information on its chemical properties. As a hair dye it produces a wide variety of shades in normal use, and when improperly applied sometimes gives rise to interesting but unwelcome colours. The plant (*Lawsonia alba*, N.O. *Lythraceae*) has many synonyms, such as camphire, cypress shrub, Egyptian privet, and according to Watt[1] has been used both as a cosmetic and as a drug from the earliest times. The commercial use of henna is now so extensive that very large tracts of land in Egypt and elsewhere are devoted to its cultivation. The shape and kind of leaf, the size of the plant and a plantation are shown in the accompanying illustrations which were obtained from Upper Egypt.

The constituents of the root from which, as is well known, the dyestuff alkanet used to be obtained, differ from those of the leaf, with which alone we are concerned as a hair dye, though according to Lal and Dutt[2] almost every part of the plant is used in some form or other for the treatment of ailments so diverse as liver or kidney diseases and leprosy. The botanical characteristics of the leaves have been

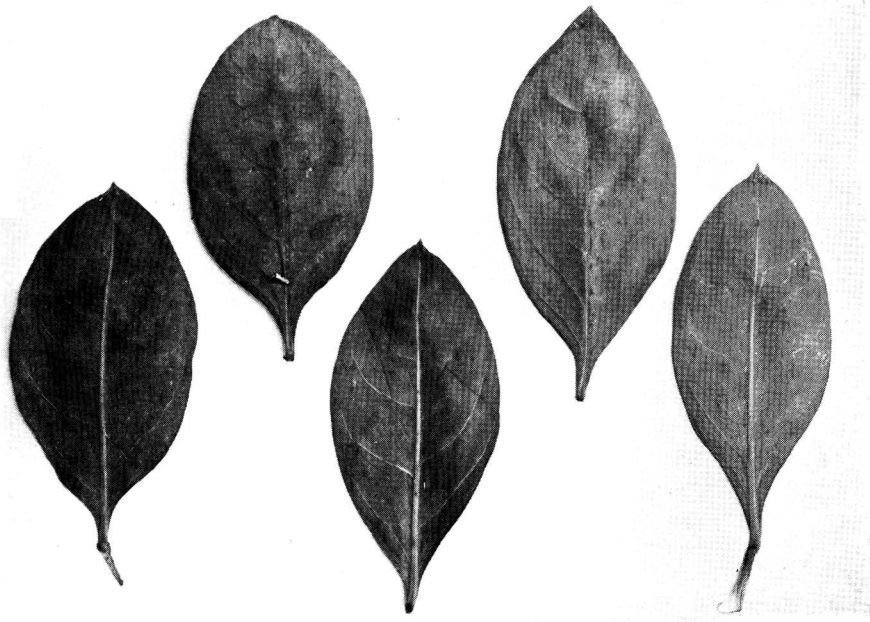
described by Holmes[3] and others, so need no repetition, but I would lay stress upon the absence of hairs and of prismatic crystals of calcium oxalate in the henna leaf, though star-shaped crystals and clusters of calcium oxalate are common. These points have practical importance in the microscopical examination of henna powders because various other leaves are sometimes mixed with them for special purposes.

CONSTITUENTS OF HENNA LEAF.—In considering the important constituents of henna one of the first points which arises is whether or not there is any tannin. Watt quotes an ancient author, Abd-el-aziz Herraory, who stated that the leaves contained a kind of tannin called *Hennotannic acid*, which Dymock (1863) showed to precipitate gelatin and to give the usual colour with ferric chloride. Watt also quotes a report to the Government of India by Wardle in which tannin is stated to be absent. On account of this divergence of statement, which is still current, I have examined henna leaves of various ages, applying all the well-known tests for tannin. It is true that extracts from the leaves will precipitate gelatin and give tannin-like colours with iron and copper salts, but the extracts show no tannin reaction with cinchonine or quinine, and above all they do not tan. The gold beaters' skin test, which is perhaps the only real proof of the presence or absence of a tannin, was quite negative with every specimen tried. The colour reactions obtainable are due to gallic acid, which is present to the extent of 5 to 6 per cent. Tannin and chlorogenic acid are definitely absent. As will be shown later, the presence of gallic acid accounts for some of the vagaries of henna when improperly applied. The leaves also contain glucose, but there is no starch and no pyrogallol. Mannitol has also been isolated, but there is some doubt if it is a normal constituent.[4] As has been shown by Lal and Dutt[2] and by Tommasi,[5] the active colouring matter in the henna leaf (not the root) is β-hydroxynaphthaquinone, sometimes called lawsone. This substance acts as an acid dyestuff and is present in the dry leaf to the extent of about 1 per cent. As henna is often admixed with other substances—vegetable, organic compounds and metallic salts—a record of the average constituents of known pure henna is useful in evaluating mixtures. A typical specimen of air-dried henna gave the following results:

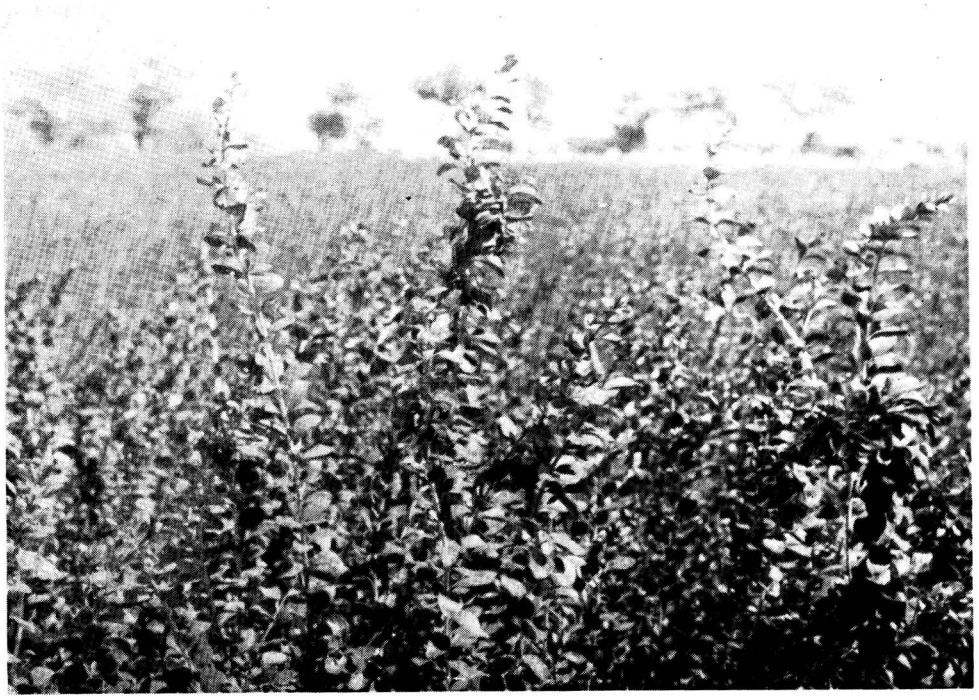
	Per Cent.		Per Cent.
Moisture	8·4	Crude fibre	6·8
Total ash	7·5	Starch	absent
Nitrogen	1·45	Emodin	,,
Cold-water extract	36·6	Sugars, as dextrose	11·3
Alcohol extract	28·9	Tannin	absent
Benzene extract	7·1	Gallic acid	6·0
Petroleum spirit extract	9·4	Hydroxynaphthaquinone	1·0

The occurrence of a hydroxynaphthaquinone in the henna leaf is by no means unique; it will be recalled that walnut leaves and skins contain juglone, which is 5-hydroxynaphthaquinone, and that alkanet, obtained now from the root of *Anchusa* but formerly from the henna root, is a complex derivative of 5:8-dihydroxynaphthaquinone.[6] When such a compound exists in the root it is not difficult to imagine how the pigment, and perhaps the gallic acid compound, arise in the leaves.

A Henna Field.

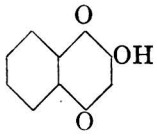


Henna Leaves. (Natural size.)



A Field in a Henna Plantation.

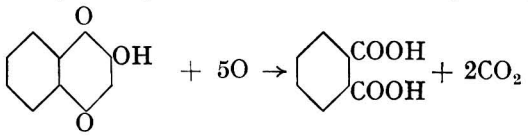
REACTIONS OF 2-HYDROXYNAPHTHAQUINONE.—Pure 2-hydroxy 1: 4 naphthaquinone has m.p. 192° C.



Its reactions are peculiar because the acidic character due to the hydroxy group modifies the quinone structure. The compound may be extracted from henna leaves or may be synthesised by acetylation of either α- or β-naphthaquinone, both of which produce the 1 : 2 : 4 acetate, and this upon hydrolysis with acid gives 1 : 2 : 4 trihydroxy naphthalene which oxidises in air to the 2-hydroxy-1: 4 naphthaquinone.[7] The trihydroxy compound is extraordinarily oxidisable, a fact which makes the quantitative determination of the quinone difficult.

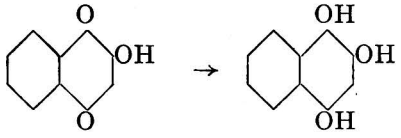
For the qualitative detection of hydroxynaphthaquinone the following derivatives and reactions may be found useful:—The quinone readily forms a monoacetyl derivative with m.p. 128° C., and with hydroxylamine a bright yellow monoxime, m.p. 180° C. The hydrazone melts at 229° C., and with 2 : 4 dinitro-phenylhydrazine there is formed a bright red crystalline dinitrophenylhydrazide, m.p. 225° C. An alkaline solution coupled with diazobenzene chloride produces a bright red dye, also with m.p. 225° C. Aqueous solutions of hydroxynaphthaquinone are of an orange colour; they show a greenish fluorescence in ultra-violet light; they liberate carbon dioxide from sodium bicarbonate but not from the normal carbonate. Solutions of the quinone give no colour or precipitate with silver nitrate, normal lead acetate, sodium nitrite or copper sulphate. Ferric chloride gives a brown colour and in alcoholic solution nickel acetate gives a bright red colour. Vigorous oxidation in solution gives rise to phthalic acid—a compound easily recognised by the fluorescein test. Most quinones liberate iodine from potassium iodide, hydroxynaphthaquinone does not. When allowed to stand in contact with 0·1 N iodine solution it slowly absorbs 1 molecule of iodine, and by using bromide-bromate mixtures further equivalents of iodine can be added, but the amount is much influenced by the precise conditions. The orange colour of solutions of hydroxynaphthaquinone is deepened by alkali, changed to pale yellow by acid, and discharged by strong solutions of stannous or titanous chlorides, but reappears on oxidation by the air.

QUANTITATIVE DETERMINATION OF β-HYDROXYNAPHTHAQUINONE.—The accurate determination of β-hydroxynaphthaquinone is beset with certain difficulties which arise from the effects of the hydroxy group. So far as I am aware, no methods have been published. Naphthaquinone itself, α- or β-, can be determined iodimetrically or by direct reduction by titanous chloride, but not so the hydroxy compound. It does not absorb bromine or iodine; when acetylated it forms a triacetyl derivative, but this is not amenable to quantitative hydrolysis. Upon oxidation with permanganate in acid solution it yields phthalic acid

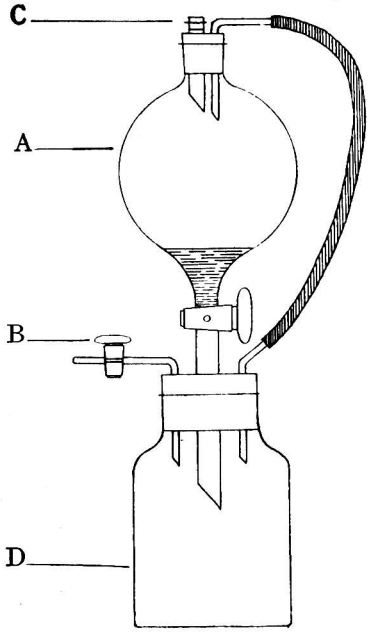


but this reaction requires a considerable excess of permanganate and is, of course, not specific or available in the presence of other organic matter.

Reduction with titanous chloride or sulphate proceeds readily, with the formation of trihydroxynaphthalene



but there arises a difficulty over the end-point which I have not been able to overcome. The colour of hydroxynaphthaquinone in acid solution is not strong enough to serve as its own indicator. Trihydroxynaphthalene is itself a most powerful reducing agent and oxidises almost instantaneously in air. Hence any indicator, such as methylene blue or chrysophenine or other added dye which might be used, is itself reduced almost instantaneously by the smallest quantity of trihydroxynaphthalene. The difficulty cannot be overcome by adding an excess of titanous chloride and titrating back, as ferric salts or other compounds which might be used to titrate the excess are themselves reduced by the trihydroxy compound, so bleaching of the indicator goes on continuously and the titration ends back at the starting point.



Zinc dust, zinc-copper couple, iron filings and the like do not reduce dilute solutions of the hydroxynaphthaquinone, but I find that a mercury zinc amalgam does. Provided that the operation be conducted in an inert atmosphere such as carbon dioxide the resultant trihydroxynaphthalene can be titrated with a mild oxidising agent. Dichromate or permanganate are unsuitable, owing to side reactions and their action on any other organic compounds which may be present; ferric sulphate may be used with methylene blue as internal indicator. I find the following method and apparatus gives satisfactory results:—Mercury zinc amalgam is prepared by heating 100 g. of clean mercury with 5 g. of zinc filings and 10 ml. of dilute sulphuric acid on the water-bath for about an hour. The amalgam, which should be quite fluid when cold, is washed with water, filtered through calico to remove any solid particles, and then poured into the separating funnel A (see diagram). To the solution of the hydroxynaphthaquinone are added 5 ml. of dilute sulphuric acid and 1 drop of a 1 per cent. solution of methylene blue, and the mixture is poured into A through the tube C. Carbon dioxide from a cylinder is passed in at B, and when all air is displaced, B and C are closed and the gas current stopped. The apparatus is then shaken vigorously for about three minutes or until all yellow colour has disappeared. The green colour vanishes almost

at once, but the complete reduction takes longer. Then the mercury amalgam is run through the tap into the lower vessel D; a gentle stream of carbon dioxide is passed, and the trihydroxynaphthalene in A is titrated with standard ferric sulphate solution until the green colour reappears. The tip of the burette is conveniently inserted through the tube C for purposes of titration. It will be observed that the orange-brown colour of hydroxynaphthaquinone reappears quite soon, but the end-point is not reached until the methylene blue is re-oxidised. As the dye itself requires a small amount of ferric solution, a "blank" should be deducted from the observed titration; using 50 ml. of water with acid and methylene blue it will amount to about 0·1 ml. The strength of the ferric solution may conveniently be about 2 g. of iron per litre. The reactions involved are simple:

$$C_{10}H_6O_3 \rightarrow C_{10}H_8O_3 \rightarrow C_{10}H_6O_3$$

so that 56 g. of ferric iron are equivalent to 87 g. of hydroxynaphthaquinone. The following are examples of the results obtained:

	Hydroxy-naphthaquinone taken g.	Hydroxynaphthaquinone: Ferric solution (1 ml. = 0·002 Fe^{III}) Vol. required ml.	Blank ml.	Hydroxynaphthaquinone found g.
1.	0·025	7·95	0·10	0·0247
2.	0·025	7·80	0·10	0·0243
3.	0·050	15·75	0·10	0·0493

ESTIMATION OF HYDROXYNAPHTHAQUINONE IN HENNA.—It is possible to estimate the hydroxynaphthaquinone in henna powder either colorimetrically or volumetrically; the former is but approximate, as the visual comparison of orange-coloured liquids is open to considerable personal error. Although the colour of hydroxynaphthaquinone is very sensitive to acids, it is stable towards alkali; so, for the direct estimation of henna it is only necessary to add excess of ammonia to a *clear* filtered alcoholic extract of the henna and compare the colour in any good colorimeter with that of a known solution of the quinone. As in determinations of creatine in meat extract, the errors are considerable unless the height of the standard colour is kept constant. Eight mm. of 0·05 per cent. hydroxynaphthaquinone form a convenient standard, but it is difficult to match the colour accurately.

The titration method described above works well with purified cold aqueous henna extracts; fortunately there are no other substances present that are appreciably affected either by mercury-zinc amalgam or by ferric sulphate. The extraction must be made with cold water and not with acid; if acid is used, substances are dissolved which rapidly reduce either methylene blue or ferric salts. Ten g. of the powdered henna are extracted overnight with about 950 ml. of cold water, then 5 ml. of zinc acetate* solution are added, and followed, after mixing, by 4·8 ml. of potassium ferrocyanide* solution. The whole is well mixed, made up to 1 litre and filtered. The filtrate should be quite clear; 50 or 100 ml.

* 21·9 g. of crystallised zinc acetate and 3 ml. of acetic acid in 100 ml.; 10·6 g. of potassium ferrocyanide crystals in 100 ml.

are titrated after reduction with mercury amalgam in the apparatus described above.

The results on three specimens of powdered henna (not containing added substances) were as follows:

	Volume of ferric solution required (less blank) for 1 g. ml.	Hydroxynaphthaquinone Per Cent.
1.	3·85	1·21
2.	3·90	1·23
3.	3·45	1·08

The method is not applicable without modification in the presence of added substances such as copper salts, pyrogallol or diamines.

HAIR DYEING WITH HENNA.—The acidic character of 2-hydroxynaphthaquinone has already been mentioned; from the dyeing point of view it is of great importance. There is no evidence that gallic acid, or indeed any added tannin, combines with the naphthaquinone, and my experiments show that with animal fibres the dyeing effect of henna is precisely the same as that of a corresponding quantity of the 2-hydroxynaphthaquinone; this substance alone is the tinctorial agent when henna is applied in the normal way. Aqueous extracts of henna have a slightly acid reaction, the *p*H is about 5·5, and hydroxynaphthaquinone only dyes when applied in acid solution. It has no affinity at all for cotton or other cellulose fibres, but provided that an acid liquid or paste be used, there is definite affinity between the protein of the hair or wool and the quinone. If alkali is added, the dye is stripped from the fibre. Thus there exists the curious fact that a solution of the quinone in dilute acid, which is almost colourless, dyes wool a bright orange colour, but a neutral or alkaline solution, which has a pronounced orange colour, leaves the fibres colourless. Failure to colour hair satisfactorily is usually due to making the paste too nearly neutral or even alkaline. Soap, borax or sodium carbonate, as may be found in some shampoos, has this effect. These facts, though easily demonstrated, do not appear to have been appreciated or recorded.

If a shampoo or hair dye preparation based on hydroxynaphthaquinone itself is applied in alkaline solution, no colouring of the hair results. But when henna itself is used there is the added complication of the combined gallic acid in the leaf. The application of henna powder plus 1 per cent. of ammonia solution to white hair produces a slight greenish colour—a result not obtainable with the quinone. If the hair or wool contains a little iron (or some other metals) either naturally or by reason of previous treatment, the result is not orange or green, but a dirty brown or greenish-black, due to the combination of the iron and gallic acid in neutral or alkaline solution. This result is reproducible by the use of a mixture of gallic acid and hydroxynaphthaquinone, whereas the slight green colour is not; the slight green is due to the chlorophyll of powdered henna leaves. Curious colours of this kind have, in my experience, sometimes led to litigation; their production necessitates an improper composition of the mixture or inappropriate application, and may be accentuated by previous doses of iron compounds which produce green-brown colours with gallic acid. It is unlikely that they would

produce visible results except with white or light grey hair, but clearly there is need for careful standardisation in the production and use of henna products.

Processes exist for the extraction of the hydroxynaphthaquinone from henna leaves[7] for the sake of the dye, and there are on the market henna preparations which have been so extracted. It is also well known that many commercial henna preparations contain additions either of vegetable powders or metallic salts, polyhydric phenols or amino compounds. Such products are usually sold under special names.

SUMMARY.—1. The constituents of the henna leaf are described; contrary to some early literature, henna does not contain any tannin.

2. The reactions available for the detection of 2-hydroxy-1 : 4 naphthaquinone are described and its dyeing properties are outlined.

3. Henna dyes only in acid solutions and by virtue of its content of hydroxynaphthaquinone. The reason why curious defects or colours sometimes appear in hair dyed with henna are considered.

4. Hydroxynaphthaquinone cannot be successfully titrated with titanous chloride or similar reducing agent, and oxidation to phthalic acid is not adaptable for quantitative purposes.

5. A method is described for the volumetric determination of hydroxynaphthaquinone by itself or in henna powder, together with an apparatus suitable for the purpose. The quinone is reduced to trihydroxynaphthalene by a mercury-zinc amalgam in an atmosphere of CO_2 and titrated with ferric sulphate in the presence of methylene blue.

It is a pleasure to acknowledge the kindness of the Director of the Horticultural Section of the Ministry of Agriculture, Cairo, who obtained for me the photographs of henna plantations from Upper Egypt. I am also indebted to Dr. A. D. Mitchell for a useful suggestion.

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11 BILLITER SQUARE,
LONDON, E.C.3
March 1, 1938

DISCUSSION

Mr. H. S. REDGROVE said that Dr. Cox had brought forward one or two points that were of great practical importance. He had one question to ask regarding lawsone: he had not been quite clear on reading the literature as to whether this was free or combined with sugar in the form of glycoside. The general state of ignorance concerning henna was only too true. Text-books written for hairdressers often showed that the authors apparently did not know what henna was. The question of the *p*H value was an extremely interesting and important one. It was a common practice to incorporate henna in shampoos, and, according to Dr. Cox, when the shampoos were of a strongly alkaline nature the addition of

henna would apparently be of no value. To-day the technique of shampoo manufacturers had been altered, and the *p*H of a shampoo could be adjusted; it ought to be possible to get a shampoo that would give the right *p*H value. So far as the ordinary dyeing of hair was concerned, the usual practice was to use plain henna and water made hot, and it would seem that that technique ought to be modified and acid added. In the course of his work he had come across very few instances of henna extracts being used: one difficulty was the trouble of getting a satisfactory extract. One thing that puzzled him was why synthetic lawsone had not entirely replaced the use of henna; surely it would be more simple to prepare this than to keep large tracts of land set aside for the growing of henna.

Mr. C. E. SAGE said that occasionally he had had large consignments of henna to examine. Dr. Cox might have come across consignments of henna which were called henna or sometimes "green henna." When some samples of "green henna" were sprinkled on water one could see specks of methylene blue standing out quite plainly. He could not help thinking that some of the substances put on the market as henna would succumb altogether to Dr. Cox's process and give negative results. If hennas were to be sold on Dr. Cox's test some shipments would certainly be condemned.

Mr. F. S. LAW, in expressing his appreciation of the invitation to be present, emphasised the importance of the scientific study of henna and of the mysterious mixtures which sometimes passed as henna. Problems, both chemical and dermatological, frequently arose in litigation, and while the dermatologist could study the patient, the scientific aspects were more in the province of the chemist, and he was much impressed by the test he had seen demonstrated.

Mr. N. EVERS asked if it was possible to get any indication of the amount of lawsone in henna powder from the colour. The colour varied from pale greenish-brown to dark brown. Was there any relation between the amount of lawsone and the depth of colour?

Mr. A. L. BACHARACH remarked on the great interest many chemists had in chemical compounds having practical use which occurred in natural products. He asked whether Dr. Cox thought that henna leaves never contained starch, as starch was often supposed to be necessary in a green leaf.

Dr. COX, replying, said that he had examined henna leaves, young and old, but had never found starch. He thought that in many leaves tannins took the place of starch, as, for example, in tea leaves. That was why he was so surprised at not finding tannin in the henna leaf. There was no doubt that complex hydroxyquinones could play an important part in the plant physiology. In reply to Mr. Evers's question he thought that the colour of the powder was dependent upon age, oxidation and chlorophyll content. Mr. Redgrove was quite right about the importance of adjusting the *p*H value in shampoos; if they were not suitably acid there would be no red colour imparted to the hair.

The Determination of Acidity in Knitted Woollen Goods

By S. R. TROTMAN, M.A., F.I.C., and A. BRAMLEY

The acids commonly present as impurities in both dyed and undyed knitted woollen goods include sulphurous acid (purposely left in stored goods to prevent "yellowing"), sulphuric acid (formed by oxidation of sulphurous acid during storing or introduced in carbonising or dyeing) and acetic and formic acids (used in dyeing). The difficulty of removing acid from dyed goods is wel known. Even after drastic washing with boiling water a large proportion of sulphuric acid is retained.[1,2] Only part of the acid is removed by alkali unless this is used in excess, when it strips the dyestuff and spoils the shade and "handle." The following table (Table I) shows the quantities of acid commonly present in stored and dyed knitted goods:

Table I

	Stored goods				Dyed goods				
	(1)	(2)	(3)	(4)	(1)	(2)	(3)	(4)	
Sulphur dioxide, per cent. ..	0·20	0·07	0·15	0·80	1·10	1·02	1·73	2·14	
					(5)	(6)	(7)	(8)	(9)
Sulphuric acid, per cent. ..	—	—	—	—	1·17	2·20	2·30	2·35	2·50

Methods of Determination.—Having regard to complaints that knitted woollen goods have caused dermatitis (*cf.* Trotman[2]), the exact determination of their acidity is important. The principal methods have been investigated by Hirst and King[3] and by Trotman and Gee[4]; they may be briefly summarised as follows:—(*a*) Extraction with water or alcohol[3]; (*b*) extraction with ammonia[3] or triethanolamine[4]; (*c*) extraction with sodium bicarbonate[5]; (*d*) digestion with magnesium carbonate and determination of resulting magnesium sulphate; (*e*) the terephthalic acid method[3]; (*f*) method based upon distribution of the acid between two immiscible solvents—wool and water[6]; (*g*) calcium carbonate method[6]; (*h*) distillation in presence of sodium acetate[4]; (*i*) extraction with an aqueous solution of pyridine.[7]

Of these methods, extraction with water is useless, whilst alcohol extracts substances other than acid. No method involving treatment with alkali gives accurate results because wool has a strong affinity for alkali. Triethanolamine is removed from wool more easily than ammonia or sodium carbonate,[4] and if the wool is washed by the method described below, accurate results can be obtained. The pyridine method[7] is simple, but, as will be shown later, does not always give the whole of the acid present. In our opinion, the only method applicable to both dyed and undyed goods is distillation with sodium acetate and titration of the acetic acid distilled. In the experiments described subsequently three methods were used: (1) extraction with pyridine; (2) distillation with sodium acetate; (3) a new method—extraction with borax. The distillation method has been fully dealt with previously.[4] In the pyridine method, as described

by Barritt,[7] a weighed quantity of the wool is digested with a cold 0·1 per cent. aqueous solution of pyridine, and an aliquot part of the liquid is filtered and titrated directly with 0·1 *N* sodium hydroxide solution in presence of phenolphthalein. Better results are obtained when the wool is washed 10 to 12 times with distilled water in the manner described below, but even under these conditions the whole of the acid present is not always recovered.

THE BORAX METHOD.—Borax can be removed without difficulty from wool by washing with water. It can be determined directly by titration with 0·1 *N* hydrochloric acid, using methyl red as indicator, and the presence of boric acid does not affect the result. A simple method for the determination of acidity can be based on these facts:—From 1 to 2 g. of the sample, cut into small pieces, are soaked for about 6 hours in 50 ml. of 0·2 *N* borax solution. The liquid is then decanted into a tapped funnel, the stem of which passes through an opening in the stopper of a large flask, while another tube, also passing through the stopper, is connected with a pump. A plug of glass wool is put at the bottom of the funnel. Finally, the wool itself is transferred to the funnel and washed ten times with water, the tap being closed for a minute after each addition and then opened, and the liquid drawn through by means of the pump. The united washings and filtrate are titrated with 0·2 *N* hydrochloric acid, methyl red being used as indicator. By saturating the borax solution with salt it can be used in many instances with dyed goods.

EXPERIMENTS WITH UNDYED WOOL.—One-gram portions of purified wool were treated with definite volumes of 0·1 *N* sulphuric acid, dried and tested by the distillation method. The results, shown in Table II, indicate that the method gives accurate results.

TABLE II

Sulphuric acid taken, ml. ..	0·0	1·0	2·0	3·0	4·0	5·0
» » found, ml. ..	0·0	1·0	2·0	3·1	4·1	5·1

In the next experiments, comparative tests were made by (1) distillation with sodium acetate; (2) borax extraction; (3) pyridine extraction. The results are seen in Table III.

TABLE III

N/10 H_2SO_4 added ml.	*N*/10 acid found		
	Distillation method ml.	Borax method ml.	Pyridine method ml.
1·0	1·0	1·0	1·0
2·0	2·1	2·2	1·9
2·0	2·1	2·2	1·9
3·0	3·0	3·0	3·0
5·0	5·0; 5·0	4·9; 5·0	4·3; 4·4
5·0	5·0	4·9	4·5
5·0	5·0	—	4·5; 4·5
5·0	5·0; 5·0	4·9; 5·0	4·3; 4·4
5·0	4·9; 5·0	5·0	4·3; 4·3

It will be observed that when the amount of acid present is large the whole of it is not extracted by pyridine. If, however, the pyridine-extracted residue is dried and distilled with sodium acetate the rest of the acid is recovered. It was thought that residual Glauber's salt might affect the action of pyridine, but samples treated with sulphuric acid and sodium bisulphate gave identical results. Since these experiments were made Eavenson and Creely[8] have published the results of similar experiments and state that the whole of the acid present in wool is not always removed by pyridine extraction, especially when it has been dried in.

THE EFFECT OF STORING.—Samples of purified wool were treated with sulphuric acid and dried at a low temperature. Portions were tested at once and the remainder of the samples were kept in air-tight tins for some days and again tested. It will be seen from Table IV that under these conditions practically no change takes place.

TABLE IV

Acid added	Acid found: Distillation method ml.	Borax method ml.	Pyridine method ml.
2·0 ml.	2·1	2·2	2·1
After 7 days	2·1	2·2	2·1
	Per Cent.	Per Cent.	Per Cent.
Yarn treated with H_2SO_4 ..	2·05	2·05	1·96
After 3 days	2·01	2·02	1·91
" 14 "	2·06	2·02	1·91
" 28 "	2·16	—	1·91
Yarn treated with H_2SO_4 ..	2·35	2·35	2·20
After 40 days	2·30	2·35	2·25

EXPERIMENTS WITH YARN TREATED WITH SULPHURIC ACID AS IN ACID DYEING. Five-gram lots of yarn were treated with sulphuric acid under the same conditions as in dyeing. The yarn was collected on a Buchner funnel, well pressed and dried. The unabsorbed acid was determined, and the absorbed acid was calculated. The results obtained were as follows (Table V):

TABLE V

Calculated acidity Per Cent.	Acid found: Distillation method Per Cent.	Borax method Per Cent.	Pyridine method Per Cent.
3·07	2·84	2·94	2·75
1·92	1·91	1·72	1·57
1·99	1·96	—	—

Table VI gives comparative results for undyed samples containing unknown amounts of acid.

THE EFFECT OF DRYING AT A HIGH TEMPERATURE.—Further experiments were made in which purified wool was treated with acid and dried at about 90° C. for a known time. As before, little difference was found when the percentage of

TABLE VI

Acid found		
Distillation method Per Cent.	Borax method Per Cent.	Pyridine method Per Cent.
4·31	4·36	4·36
2·05	1·86	2·05
4·75	4·65	4·65
4·20	4·20	4·00
2·84	2·94	2·55
5·04	—	4·59
3·91	4·01	3·91

acid was small, but when a considerable amount was present low results were obtained by the pyridine method (Table VII).

TABLE VII

Acid added ml.	Acid found: Distillation method ml.	Borax method ml.	Pyridine method ml.	
5·0	5·0	5·0	4·5	Dried 6 hours at 90° C.
5·0	5·0; 5·0	4·8	4·3; 4·4	" 12 " " "
5·0	5·0; 5·0	—	4·5; 4·5	" 6 " " "
5·0	5·0	—	4·4	" 6 " " "

It was found subsequently that when the pyridine extraction was repeated further quantities of acid were obtained, which brought the total acidity near to that given by the other methods. The following examples illustrate this:

	(1) Per Cent.	(2) Per Cent.
Acidity by distillation	4·80	4·01; 3·96
" " pyridine	4·70	3·53
" " " after 2 extractions	4·80	3·68
" " " " 3 "	—	3·68

A fabric was treated with sulphuric acid and dried at a low temperature. The acidity was tested, and the remainder of the sample was kept for a month in a tin. The acidity was then determined again, and portions were dried at 90° C. for 4 and 8 hours. The results obtained were as follows (Table VIII):

TABLE VIII

	Acid found: Distillation method Per Cent.	Borax method Per Cent.	Pyridine method Per Cent.
1. Original dry fabric ..	3·91	3·91	4·01
2. After 1 month	4·01	—	—
3. Dried 4 hours	4·11	—	3·63
2 extractions	—	—	3·77
4. Dried 8 hours	—	—	3·72
2 extractions	—	—	3·77
3 "	—	—	3·80

An interesting example of the difficulty of extracting the whole of the acid with pyridine was afforded by a garment alleged to have caused dermatitis. The acidity was found to be:—(1) By distillation, 1·45, 1·42; (2) by borax method, 1·45; (3) by pyridine method, 0·49, 0·49 per cent. A second extraction with pyridine raised the acid to 0·83 per cent. and three further extractions increased it to 1·20 per cent., after which no more could be removed.

DYED GOODS.—Many of the commercial samples which have to be examined in connection with complaints have been dyed with acid dyestuffs, and may contain sulphuric acid, formic acid or acetic acid. Both inorganic and organic acid may be present, because when a weak acid is used, sulphuric acid is often added towards the end of the dyeing to exhaust the dye-bath. It has been mentioned already that extraction methods can rarely be used for dyed goods, and it is not possible to give comparative figures for the different methods. Nevertheless, those given above for undyed goods establish the reliability of the distillation process. Experiments made by Trotman and Gee[4] to determine the effect of dyestuffs showed that their action is negligible.

Since dyed goods might contain free fatty acids, there is a possibility that these might distil over with acetic acid. It has been shown by Trotman and Gee[4] that the error thus produced is very small unless a large quantity of fatty acid is present. Wool would not contain more than 1 per cent. of fatty acid, and if 1 g. were used for the analysis, the error would not be more than about 0·08 per cent. Further experiments have been made which confirm this conclusion. One-gram samples of wool were treated with 0·02 g. of oleic acid (i.e. 2 per cent. by weight) and tested together with the same untreated wool. The results of these experiments were as follows (Table IX):

TABLE IX

	(1)	(2)	(3)	(4)
Acidity of untreated wool, per cent. ..	0·29	3·91	5·00	3·77
" " treated " " " ..	0·29	3·94	5·04	3·82

Some experiments have been made with four acid dyestuffs—Acid orange, Indigo carmine, Bordeaux B and Kiton red—in order to determine the effect of drying. It will be seen from the table below (Table X) that drying at 90° C. does not cause any alteration (*cf.* Table VII).

TABLE X

Dyestuff	Acid when dried Per Cent.	Acid after heating at 90° C. for 8 hours. Per Cent.
Acid orange	3·08	3·08
Bordeaux B	3·62	3·84
Indigo carmine ..	3·52	3·52
Kiton red	3·52	3·52

Portions of the sample dyed with acid orange were incubated for 3 days at 37° C., both dry and in presence of moisture. In neither sample was any alteration in the acidity found. Comparative figures for pyridine extraction could not be obtained.

THE DETERMINATION OF ORGANIC ACID.—Both acetic acid and formic acid can be removed by simple distillation with steam. In order to determine which is present the distillate, after neutralisation, is evaporated to a small volume, filtered, and carefully neutralised with acetic acid. A few drops of mercuric chloride solution are then added, and the mixture is heated. In presence of formic acid a turbidity or precipitate is formed. The absence of any reaction indicates acetic acid. The test will not detect formic acid in greater dilution than 1 in 1000.

THE DETERMINATION OF SULPHUROUS ACID.—When the sample has an acid reaction sulphurous acid may be determined by distilling into hydrogen peroxide, adding barium chloride to the cold distillate, and weighing the barium sulphate. Alternatively, the hydrogen peroxide may be made neutral to bromophenol blue and the sulphuric acid determined by titration. When the material is not acid a few drops of phosphoric acid are introduced into the distillation flask.

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Analysis of Vinegar

Part I. Spirit, Malt, Distilled Malt and Artificial Vinegars and their Differentiation

(WORK DONE UNDER THE SOCIETY'S ANALYTICAL INVESTIGATION SCHEME)

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(*Read at the Meeting, May* 4, 1938)

INTRODUCTION.—It is common knowledge that many complications have been introduced into vinegar analysis by the appearance, on the British market, of various new kinds of vinegar. It is also obvious that distilled malt vinegar cannot be identified by means of its total solids, ash, phosphates and nitrogen, as ordinary malt vinegar can. Again, it is a rather common practice to add deteriorated malt vinegar to artificial vinegar and to sell the mixture under the latter name, so that samples submitted as artificial vinegar which give curious values for nitrogen and phosphates are occasionally encountered; artificial vinegar which has been sophisticated by the addition of phosphates and nitrogenous compounds is no new

thing to the analyst; furthermore, this type of "vinegar" is no longer made exclusively from "wood acid," so that it may become possible to take exception to the sale as "wood vinegar" of those samples made by dilution of acetic acid prepared by modern synthetic processes, especially if traces of the mercury catalyst used are found therein.

In referring to the newer types of vinegar, it is well to state here that the definitions drawn up by the Society[1] are accepted without qualification, so that there should be no need further to define those discussed in this and any succeeding communications unless for some reason certain kinds which may be dealt with cannot be made to fall within those definitions.

Spirit vinegar is a relatively new product here, although it has been known on the Continent and in America for a number of years. It is a commodity having several advantages over some other kinds of vinegar, as, for instance, in the manufacture of pickles, particularly in the de-salting operation and in the preservation of vegetables. It is produced by brewing in two stages, the raw material used being cane molasses. A syrup of the required density is prepared, and this is fermented with a pure culture yeast. The alcohol produced is distilled from the "fermenters," and the distilled spirit is mixed with a proportion of vinegar, water and bacterial nutrient before being passed through the acetifiers, where the second fermentation takes place. The finished vinegar runs off the acetifiers at a strength of up to 12 to 13 per cent. in acetic acid. It is sold at this strength and at dilutions suitable for various purposes. Further details of the process of manufacture as well as of the nature of the product and its uses are given by Sarson.[2]

In the analysis of spirit vinegar the usual analytical data are of little use for distinguishing it from synthetic vinegar, as will be seen from the following results:

	Spirit vinegar	Artificial vinegar
Total solids, per cent. ..	0·16–0·3	0·3 –0·45
Ash " " ..	0·04–0·09	0·02–0·05
Nitrogen " " ..	0·03–0·04	Nil–0·04
P_2O_5 " " ..	0·02–0·03	Nil–0·03

Recently several samples were submitted to us under the Food and Drugs Act as spirit vinegar. In spite of a diligent search through the literature and testing the analytical methods suggested by different workers for characterising this type of vinegar, we found it extremely difficult to determine the exact nature of the samples. Other analysts who have consulted us also encountered the same difficulty.

It is therefore the purpose of this communication to show that certain analytical methods may, with modifications, be used in such a way as greatly to simplify differentiation. We found it impossible in the time at our disposal to extend these investigations to all types of vinegar, but it is our intention to do so and to present the results in subsequent papers. For the moment we limit our observations to three varieties—spirit, malt, distilled malt—and to the commodity known as artificial vinegar. Similarly, we confine our observations to those tests and analytical methods that we have found most useful for the identification of these articles.

METHODS FOR DISTINGUISHING VINEGARS.—The various newer methods and tests that have been suggested from time to time for characterising different vinegars may be classified into:

1. *Tests Depending upon the Detection of Methyl Acetol.*[3, 4] These have been found to be unreliable by us and by some other observers.

2. *Tests Depending upon the Detection of Furfuraldehyde.*—Lampitt, Hughes and Trace[5] have suggested a colorimetric test for distilled fermented vinegars depending upon the presence of the furfuraldehyde which they have shown is produced during distillation at a temperature above 80° C. by hydrolysis of the pentosans. The test was based on a modification of the method of Youngburg and Pucher.[6] We tried this test on spirit vinegars, but unfortunately the results were negative, the most probable explanation being that modern methods of spirit distillation render very unlikely the occurrence of furfuraldehyde in vinegar derived from such spirit.

3. *Tests for Alcohol.*—A positive reaction is said to be indicative of spirit vinegar, but in our experience the iodoform test was strongly positive even in the cold with all the "wood" vinegars we examined, this being due, no doubt, to the presence of acetone. We know of no test for alcohol that gives infallible indications of the origin of a vinegar about which the analyst has no more information than is usually supplied and of which no more than the usual small sample is submitted.

4. *Methods Depending upon Determination of the So-called Oxidation Value and Iodine Value* (*cf.* Pratolongo,[7] Schmidt,[8] Ruziczká,[9] Rudolp and Barsch,[10] and Bailey[11]).—Schmidt suggested that these values should be determined both before and after treatment with active carbon for two minutes at room temperature, because vinegar which contains caramel gives very high values.

In our preliminary work it soon became obvious that the determinations promising most success were these oxidation and iodine values, and, in addition, the ester value.

We have tried the methods of determining these values suggested by previous workers upon several samples of spirit vinegar and artificial vinegar, both before and after treatment with carbon, but the results were extremely erratic and depended presumably upon the composition and amount of the caramel present. In fact, we found that some samples of artificial vinegar gave considerably higher oxidation and iodine values than spirit vinegar, and, indeed, it is difficult to see how interference by caramel can be overcome by simple treatment with active carbon, which probably removes little else than the colour. We therefore came to the conclusion that it is absolutely necessary to remove all the caramel and other interfering substances, and that the easiest way to achieve this is by simple distillation.

The Oxidation Value.—Upon directing our attention to the already published methods of determining the oxidation and iodine values we found in the literature some confusion regarding the precise meaning of these terms and many different definitions suggested for each of them. The oxidation value is described as being determined by adding dilute sulphuric acid to a given quantity of vinegar and then titrating with standard permanganate until a permanent pink colour is obtained. We have tried this procedure, and in our experience the end-point is extremely

ill-defined. Bailey (*loc. cit.*) also came to the same conclusion. Indeed, it is shown later why it is impossible to get a sharp end-point. We therefore elaborated a new procedure for the determination of this value, which follows closely the method for determining "oxygen absorbed" in water. An excess of standard permanganate is added to an aliquot portion of the vinegar *distillate* acidified with dilute sulphuric acid, and the mixture is allowed to stand at room temperature for a specified period. A solution of potassium iodide is then added, and the liberated iodine is titrated with standard thiosulphate. The end-point is extremely sharp, and consistent results are readily obtained. Furthermore, the oxidation value obtained by this procedure is considerably higher than that given by the older method; this is an obvious advantage.

A series of experiments was carried out to find the optimum conditions, especially the concentration of permanganate, the temperature and the time. The most satisfactory strength for the permanganate was $N/10$; weaker solutions gave lower oxidation values. The temperature had a considerable effect on the reaction—an effect common to all permanganate oxidations—and a rise of even 5° C. caused a measurable increase. All our results were obtained at about 18° C. The effect of varying periods is illustrated by the three curves obtained with spirit and malt vinegars. The reaction slows down towards the end; on the whole, we consider that a period of 30 minutes is both expedient and generally satisfactory.

From the graph (Fig. 1) it will be obvious that the reaction is not completed even after 70 minutes, and therefore it is easy to understand the difficulty of obtaining a sharp end-point in direct titration with permanganate, as suggested by earlier workers.

The definition of oxidation value which we suggest, particularly for its simplicity, is:—*The number of millilitres of* 0·01 N *potassium permanganate used by* 100 *ml. of vinegar in* 30 *minutes under the standard conditions described in the experimental section.*

The Iodine Value.—The method for the determination of iodine value is essentially a modification of that described by Wüstenfeld.[12] We have studied in detail the effect of concentration of alkali, strength of iodine solution, and time. The optimum conditions are fully described in the experimental section. We define the iodine value as:—*The number of millilitres of* 0·01 N *iodine absorbed by* 100 *ml. of vinegar under the standard conditions described in the experimental section.*

The Ester Value.—The ester value, which we define as:—*The number of millilitres of* 0·01 N *potassium hydroxide required to saponify the esters contained in* 100 *ml. of vinegar under the standard conditions described in the experimental section* may also be helpful in some instances. In its determination the results obtained are much more precise if the sample is distilled and the ester value determined on the distillate instead of on the vinegar itself. If 100 ml. of vinegar are distilled, all the esters usually come over in the first 30 ml.

In work of this nature there must always be some element of empiricism; we have endeavoured to standardise the experimental conditions without introducing unnecessary complications, and we hope that we have succeeded in doing this so as to make it possible for other workers to reproduce our results.

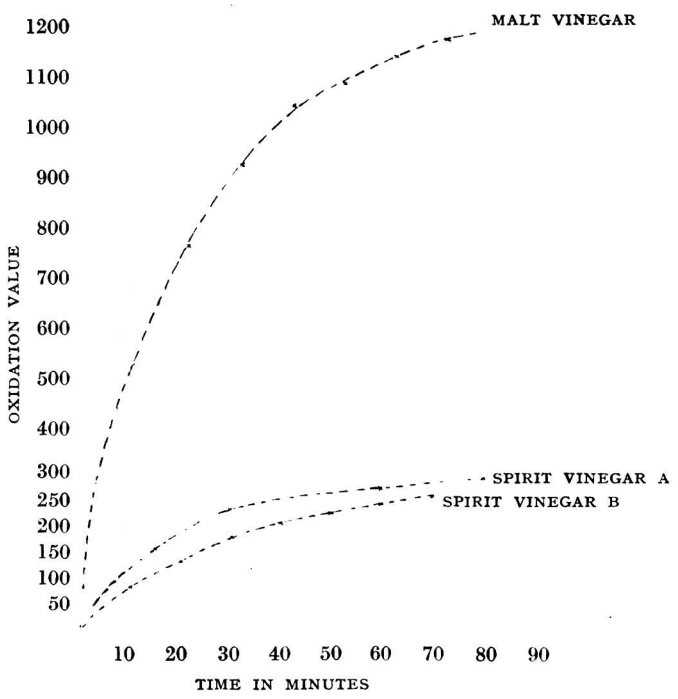

Fig. 1

Experimental

For the reason previously indicated it must be emphasised that it is essential to follow exactly the directions given below.

Distillation of the Sample.—Mix 60 ml. of the sample with 15 ml. of water in a 400-ml. distilling flask, add a little pumice and distil the mixture slowly until exactly 60 ml. of distillate are collected.

Oxidation Value.—Measure 25 ml. of the distillate into a glass-stoppered bottle of about 200-ml. capacity, add 10 ml. of dilute sulphuric acid (1 vol. of conc. acid mixed with 3 vols. of water) followed by 10 ml. of 0·1 *N* potassium permanganate solution, accurately measured. Allow the mixture to remain at a temperature of about 18° C. for exactly 30 minutes; then add 5 ml. of 10 per cent. potassium iodide solution and titrate the liberated iodine with 0·02 *N* thiosulphate, adding starch solution as an indicator at the end.

Carry out a blank determination at the same time, using 25 ml. of distilled water in place of the vinegar distillate.

Let the volume of 0·02 *N* thiosulphate used in the blank titration be A ml., and that in the test, B ml.; then

$$\text{Oxidation value} = 8\,(A-B).$$

Iodine Value.—Measure 25 ml. of the distillate into a glass-stoppered bottle of about 200-ml. capacity and just neutralise to litmus paper with 10 *N* potassium hydroxide solution. Add 10 ml. of *N* potassium hydroxide followed by 10 ml. of 0·1 *N* iodine solution, accurately measured. Allow the reaction mixture to stand

at room temperature in the dark for 15 minutes, then add 10 ml. of dilute sulphuric acid (1 vol. of conc. acid mixed with 3 vols. of water) and titrate the liberated iodine with 0·02 *N* thiosulphate solution as usual.

Make a blank test in an exactly similar manner, using the same quantity of all the reagents as in the first experiment, but substituting for the vinegar 25 ml. of distilled water.

Let the volume of 0·02 *N* thiosulphate used in the blank titration be A ml., and that in the test, B ml.; then

$$\text{Iodine value} = 8\,(A - B).$$

Ester Value.—Measure 100 ml. of vinegar into a 400-ml. distilling flask, add a little pumice and distil slowly until 30 ml. are collected. Add a few drops of phenolphthalein solution to the distillate and then *N* potassium hydroxide solution until the mixture is just pink. Add 0·02 *N* hydrochloric acid, drop by drop, from a burette until the colour is just discharged; then add 10 ml. of 0·1 *N* potassium hydroxide solution, accurately measured, and saponify by reheating the mixture under reflux on a boiling water-bath for 2 hours. Cool, add a few more drops of phenolphthalein solution, and titrate the excess alkali with 0·02 *N* hydrochloric acid.

For the blank test, take about 30 ml. of water and 10 ml. of 0·1 *N* potassium hydroxide solution, heat under reflux in a similar manner, and finally titrate with 0·02 *N* hydrochloric acid, using the same quantity of phenolphthalein as in the actual test.

Let the volume of 0·02 *N* hydrochloric acid used in the blank titration be A ml., and that in the test, B ml.; then

$$\text{Ester value} = 2\,(A - B).$$

Discussion of Results

Spirit and Artificial Vinegars.—From Table I it will be seen that the oxidation value for spirit vinegar is considerably higher than that for "wood" and other artificial vinegars. This value affords an easy method of distinguishing between these two types.

The iodine value divides them roughly into *three* classes, of which the artificial vinegars constitute two. Those samples submitted to us as artificial vinegars which were presumably prepared by dilution of pure acetic acid have a rather low iodine value; spirit vinegars belong to the intermediate class, and "wood" vinegars have sometimes extremely high values. There is admittedly some overlapping, but, nevertheless, the iodine value is definitely diagnostic. The reason for the division among the artificial vinegars is not for the present to be explained satisfactorily, but it is hoped that further work will provide a clue.

The ester value is also useful, as reference to the table will show. Artificial vinegars have extremely low ester values, but unfortunately there is the probability of spirit vinegars also giving low values, because on long storage (see p. 417) it seems that the esters are hydrolysed. A low ester value has therefore little significance by itself, although, taken together with the oxidation and iodine values

it has a definite use in diagnosis. On the other hand, an ester value above 8·0 definitely rules out the possibility of a particular sample being artificial vinegar not containing added esters.

TABLE I

ARTIFICAL AND SPIRIT VINEGARS

Type	Acetic acid Per Cent. w/v	Oxidation value	Iodine value	Ester value	Remarks
Spirit:					
No. 1	4·3	171·2	11·2	14·0	
" 2	4·3	224·8	12·0	13·0	
" 3	4·3	170·4	8·0	12·0	Prepared in the laboratory by dilution of 12 per cent. strength vinegar to 4 per cent.
" 4	4·3	176·0	8·0	12·0	
" 5	4·3	171·2	8·8	12·0	
" 6	4·3	88·0	12·8	6·0	
" 7	4·3	93·6	27·2	6·6	
" 8	4·3	124·8	12·0	8·6	
Artificial (not described as "wood" vinegar):					
No. 1	—	4·0	3·7	0·5	
" 2	5·9	4·0	4·0	2·0	
" 3	5·4	6·4	3·6	—	
" 4	4·2	4·0	9·6	0·4	
" 5	4·8	4·0	6·4	0·6	
" 6	4·56	1·0	2·4	—	
Artificial ("wood"):					
No. 1	5·52	3·2	188·0	0·6	
" 2	5·4	0·8	252·0	1·1	
" 3	5·8	6·4	121·6	0·6	
" 4	4·56	4·8	50·4	1·2	
" 5	4·8	3·2	26·4	—	
" 6	4·56	2·4	26·4	—	

FLUCTUATIONS IN THE OXIDATION VALUE OF SPIRIT VINEGAR.—It will be observed that the oxidation values of the different spirit vinegars examined, whilst entirely distinct from those of the synthetic vinegars, show a good deal of variation among themselves, although the samples originated from the same brewery. Samples Nos. 1 to 5 came direct from the brewery, whilst Nos. 6, 7 and 8 were obtained through various retailers. It is therefore feasible that these variations were natural, or were more probably due to the effect of storage. We have studied this important aspect of the fluctuations in oxidation value, and, from storage experiments conducted in the laboratory, we are convinced that whilst there is undoubtedly some natural variation, this is small compared with the effect of storage, to which, in our opinion, these relatively great fluctuations are due. The oxidation value slowly but steadily diminishes on keeping; as an extreme instance, four spirit vinegars that had been stored for about eighteen months gave the following results:

TABLE II

SPIRIT VINEGARS AFTER STORAGE

Number	Acetic acid Per Cent. w/v	Oxidation value	Iodine value	Ester value
9	4·4	65·2	8·0	3·2
10	4·32	82·4	16·0	3·6
11	4·32	72·8	12·0	2·0
12	4·32	64·0	14·4	2·0

We have also observed that this diminution in oxidation value takes place only if the vinegar is stored after dilution to the 4 per cent. strength, there being no change in the oxidation value on storing the strong 12 per cent. vinegar. This might possibly suggest that the changes taking place are due to biological and not chemical agencies.

We are at present unable to say what precise significance the diminution in this value has on the quality of the vinegar. There are many possibilities. It may be indicative of a kind of maturing process with an accompanying improvement in flavour and aroma; on the other hand, it might be an indication of partial loss of those elusive qualities.

MALT VINEGAR.—In extending the determination of oxidation value and iodine value to malt vinegars, the following slight modification was found to be necessary in the procedure:

Twenty ml. of the sample are diluted to 100 ml. with water and transferred to a distilling flask; 25 ml. more water are added, and the mixture is distilled as usual, 100 ml. of distillate being collected. It is necessary to distil very slowly at first to lessen the excessive frothing which occurs with some kinds of malt vinegar. For each of the determinations of oxidation and iodine values 25 ml. of the distillate —*i.e.* 5 ml. of the original sample—are taken. Both values will then be given by 40 (A—B), where A and B have the same meaning as on p. 414.

No change in the technique is necessary for the determination of ester value of malt vinegar, but usually a little reddish colour is produced during saponification, so that the end-point is not quite so sharp as with spirit or artificial vinegars. Some of the values obtained with different brands of malt vinegar are given in Table III, which represent a fair range. It will be observed that the values are very characteristic, and we would venture to suggest that, in general, they have as much diagnostic worth as, if not more than, other analytical data such as the proportions of nitrogen and phosphates. They are very useful for detecting any fictitious vinegar prepared from dilute acetic acid coloured with caramel and containing added phosphates and nitrogenous matter.

We have at present insufficient data to enable us to adduce reasons for the fluctuations observed in the oxidation, iodine and ester values of the different malt vinegars. It is possible that these values bear some relation to the kind of grain from which the vinegar was brewed, and it may ultimately prove to be possible to distinguish vinegar brewed entirely from malt from vinegar brewed partly or entirely from other grain; we consider this point worthy of investigation, for although it may appear to be somewhat academic, it may have some value

to manufacturers who for any particular reason may desire to have the vinegars they use evaluated in terms of their content of real malt vinegar. It is obvious, however, that research of this character can best be carried out with the help of the vinegar brewers, who alone possess the necessary knowledge of the origin and type of these many grades of "malt" vinegar.

TABLE III

MALT VINEGARS

Number	Acetic acid Per Cent. w/v	Oxidation value	Iodine value	Ester value
1	4·92	636	688	31·2
2	4·98	736	884	29·2
3	4·98	672	720	30·2
4	4·6	1068	1976	65·6
5	5·06	1192	1172	42·6
6	—	1320	860	31·6
7	5·04	772	844	35·2
8	5·1	552	712	57·0
9	4·32	880	1300	41·2
10	4·8	656	808	41·0
11	4·92	824	1108	40·2

DISTILLED MALT VINEGAR.—The distinction of distilled malt vinegar from diluted acetic acid by chemical means was heretofore very difficult, and an expert nose and palate were of greater value than chemical analysis. The method suggested by Lampitt, Hughes and Trace[5] has proved useful in some instances, but it should be remembered that this test is likely to fail entirely, or the indications may be inconclusive, if the vinegar is manufactured by distillation *in vacuo* at a low temperature, since no furfuraldehyde will be formed under these conditions.

For the determination of the oxidation and iodine values of distilled malt vinegar 20 ml. of the sample should be diluted to 100 ml. with water and 25 ml. taken for each test. No distillation is necessary, provided that the sample is practically colourless, as were all the brands we could obtain; a coloured sample, or one containing more than a trace of solid matter, should be treated exactly as an ordinary malt vinegar—see p. 417.

TABLE IV

DISTILLED MALT VINEGARS

Number	Acetic acid Per Cent. w/v	Oxidation value	Iodine value	Ester value
1	5·0	992	992	30·6
2	4·92	960	920	35·8
3	4·98	840	800	35·0
4	5·0	992	1040	33·6

Comparison of the figures given in Table IV with those in Table I shows that it is possible to distinguish conclusively distilled malt vinegar from "vinegar" prepared from diluted acetic acid.

As an interesting example of the application of these methods we may quote the analysis—No. 1, Table V—of the vinegar portion of a sample of mint sauce

which was claimed to be made from fresh mint and pure malt vinegar. When the results are compared with those of other samples which were prepared exclusively from malt vinegar (Nos. 2, 3, 4 and 5), or from distilled malt vinegar (No. 6), it will be readily seen that No. 1 was not prepared wholly from malt or distilled malt vinegar. The oxidation and iodine values indicate that the sample probably contained about 20 to 25 per cent. of malt or distilled malt vinegar. Furthermore, the vinegar portion contained only 0·028 per cent. of nitrogen and 0·019 per cent. of phosphates as P_2O_5, and after making allowances for the nitrogen and phosphates derived from the mint these results again indicate that it probably contained about 20 per cent. of malt vinegar.

TABLE V

MINT SAUCES

Number	Acetic acid Per Cent. w/v	Oxidation value	Iodine value	Ester value
1	7·08	196	96	10·7
2	4·92	1044	844	33·6
3	—	692	600	36·0
4	—	1132	780	29·4
5	—	612	800	24·8
6	5·0	760	820	27·6

In conclusion, we desire to record our thanks to Dr. C. A. Mitchell for much helpful advice, as well as for his aid in obtaining samples direct from vinegar manufacturers.

SUMMARY.—The complications introduced into vinegar analysis by certain types of the condiment are discussed, with special reference to spirit vinegar and distilled malt vinegar. Determinations of total solids, ash, phosphates and nitrogen, and some tests, are valueless in certain instances, but in these the oxidation, iodine and ester values are shown to be definitely useful.

We found it necessary to modify considerably the technique of earlier methods of determining these values, or to propose new ones, and experimental detail is given in full. The masking effect of the presence of caramel is overcome by distilling the sample and using the distillate for all the determinations; decolorisation by active carbon was found to be not in the least satisfactory.

Certain simple definitions are proposed for the oxidation, iodine and ester values in order to overcome the confusion which has arisen in the past by previous workers using different definitions having only a random relation, one with another.

It is demonstrated how the data derived by these new methods can be utilised in the distinction of spirit vinegar from artificial vinegar and of distilled malt vinegar from diluted acetic acid. The diagnostic value of the results obtained with ordinary malt vinegar is shown to be not less than that of the figures for phosphates and nitrogen.

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May 2, 1938

DISCUSSION

Mr. E. M. HAWKINS asked if all the figures for the three values were relative to one strength of acetic acid. Also, was the vinegar neutralised before distilling?

Mr. F. W. EDWARDS replied to the first question in the negative, saying that the figures were obtained on the actual samples. He agreed, however, that the expression of the results in terms of acetic acid strength might be helpful.

Dr. H. R. NANJI, replying to the second question, said that they had carried out some determinations of oxidation and iodine values after neutralising previous to distillation, but in most instances they did not find any appreciable difference.

Mr. W. A. DAVIS remarked that in the laboratory of the Distillers Co., Ltd., they had been doing work on the problem and had carried out a large number of tests, and they could fully confirm the authors' statements as to the insufficiency of the ordinary tests. They had found difficulty in working directly on vinegars because of the complications produced by the presence of caramel. He would like to congratulate the authors on the work done so far, which would be of great help for distinguishing between different types of vinegar, especially in discriminating between spirit vinegar and artificial vinegars.

Mr. F. A. ROBINSON asked whether the authors had any information concerning the nature of the substances responsible for these tests, and whether there had been assays of the rare aldehydes.

Dr. NANJI replied that they had not actually isolated all the secondary constituents which were responsible, but, generally speaking, there was evidence of the occurrence of alcohol, acetaldehyde, acetal, acetyl methyl carbinol, ethyl acetate and traces of other esters. Their difficulty had been to get sufficiently large samples to be able to isolate these secondary constituents.

Dr. C. AINSWORTH MITCHELL suggested that it might be possible to concentrate a number of the constituents which gave the different values by fractionation of the distillate. He thought that it would be of interest to examine a product called "spirit acid" which had been on the market some time ago; it was an 80 per cent. acid and was claimed to be a fermentation product concentrated by distillation.

Mr. F. W. EDWARDS said that they hoped, in co-operation with other chemists and by fractionating large quantities, to derive a good deal more information about the volatile constituents.

Mr. F. L. OKELL said that attention had been drawn to the drop in the oxidation value of diluted distilled vinegar. He would suggest that the authors might consider as a possible cause the effect of dissolved oxygen in the water used for dilution.

Dr. NANJI said that they were convinced that the effect of storage was mainly of the nature of an oxidative decomposition, and its rate depended on the amount of accessible oxygen in the container, the area of surface exposed, temperature, and possibly other factors besides dissolved oxygen in the water used for dilution.

The PRESIDENT remarked that the way in which the vinegar was stored—whether in glass or wood—might have some effect.

Dr. H. E. Cox observed that the ester value might be expected to diminish as a result of hydrolysis with age, and suggested that it might be possible to correlate the oxidation value with the alcohol-content or diminished ester value. He had tried estimations of ester value and iodine values, without distillation, but found the results unreliable when dealing with samples of unknown age or origin. He hoped the authors would continue their investigations and be able to put the problem on a really sound basis.

Mr. H. L. Monk remarked that it seemed possible that the nature and amount of the various constituents might be altered by the act of distillation, and that a standardisation of procedure was indicated.

Mr. A. L. Bacharach suggested that it might be better to distil under diminished pressure.

Mr. F. W. Edwards, in expressing his appreciation of the many helpful hints given by various speakers, replied that the remarks he had made on the empirical nature of these methods were intended to cover the points raised by Mr. Monk and some others.

A New Volumetric Iodide Method of Determining Starch—Addendum

By W. WHALE

A correction chart, which was omitted from the paper as published (Analyst, 1938, 328), may be constructed from the following figures:

$N/10$ iodine solution in excess in 50 ml. ml.		Starch contained in 50 ml. 0·025 g. Factor	0·050 g. Factor	0·100 g. Factor	0·200 g. Factor	
4·0	1 ml. of $N/100$ sodium thiosulphate solution =	0·00500	0·00518	0·00542	0·00552	g. of starch.
3·0		0·00522	0·00538	0·00558	0·00568	
2·0		0·00548	0·00564	0·00580	0·00590	
1·0		0·00594	0·00614	0·00622	0·00630	
0·5		0·00656	0·00676	0·00686	0·00696	
0·25		0·00716	0·00732	0·00752	0·00762	
0·10		0·00820	0·00820	0·00834	0·00840	

The above figures are for use when potassium acetate or sulphuric acid is used as coagulant.

The following figures give the approximate factor when alcohol is used as coagulant and the test solution contains 50 per cent. of alcohol by volume:

$N/10$ iodine solution in excess in 50 ml. ml.			
4·0	1 ml. of $N/100$ sodium thiosulphate solution =	0·0106	g. of starch.
3·0		0·0114	
2·0		0·0123	
1·5		0·0130	
1·0		0·0140	
0·5		0·0154	
0·25		0·0177	

Erratum.—Micro-tests for Elements in Organic Compounds. May issue, p. 334, line 5: "when excess of ferrous sulphate had *not* been added." Delete "*not.*"

Notes

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

THE DETECTION OF GLUCOSE SYRUP IN JAMS AND HONEY

(Read at the Meeting of the North of England Section, December 11, 1937)

GLUCOSE syrup, as is well known, is prepared by the action of acids on starch; it consists of a mixture of glucose, maltose, malto-dextrins and dextrin. It seemed possible that the freezing-point depression of an aqueous solution might be sufficiently different from that of an aqueous solution of honey to afford a convenient and rapid routine method of testing. Three commercial syrups of average composition prepared by different manufacturers were examined and found to give the following results:*

Sample	1	2	3
$[\alpha]_D^{20}$ (10 per cent. w/v solution) direct ..	117·7°	119·0°	120·2°
$[\alpha]_D^{20}$ (5 per cent. w/v solution) after fermentation	82·0°	85·2°	83·0°
Freezing-point depression (Hortvet) ° C. (10 per cent. w/v solution)	0·415	0·402	0·419
Dry solids, per cent. w/w	83·6	82·7	83·2

Fourteen samples of honey were found to have freezing-point depressions for 10 per cent. w/v solutions ranging from 0·883° C., to 0·919° C., with an average value of 0·899° C. These figures suggest that any appreciable proportion of glucose syrup present in a sample of honey will be indicated by a determination of the freezing-point depression of a 10 per cent. w/v aqueous solution.

The freezing-point depression of an aqueous solution of jam will obviously depend not only on the total soluble solids, but on the amount of invert sugar present, which will, in turn, depend upon the amount of invert sugar added, the acidity of the fruit, and the length of boiling to which the jam has been subjected. The figures found for seven commercial jams varied between 0·594° C. and 0·722° C., with an average value of 0·643° C. For jam, therefore, the figure is not so likely to be of use as for honey, but it may sometimes give useful indications, particularly if interpreted together with the optical activity. It is interesting to note that the freezing-point depression is the same whether the solution of the jam is made with cold water or with hot water, the hot solution being rapidly cooled immediately after preparation.

The detection and determination of glucose syrup in jams and honey by simple fermentation with yeast does not appear to be so widely known as it should be. For this examination a convenient volume, say, 250 ml. of the 5 per cent. w/v solution of the sample, is sterilised by boiling for a few minutes in a conical flask and then, after cooling, about 2 g. of washed and pressed brewers' yeast are added, and the mixture is incubated at 24° to 26° C. until all apparent action has ceased; usually from 48 to 72 hours. The mixture is then boiled to remove alcohol, made up to the original volume with alumina cream, and filtered. The optical rotation of the filtrate is observed. Jams and honey that are free from glucose syrup yield under the treatment a solution which is either optically inactive or which has a small negative rotation: $[\alpha_D^{20}]$ smaller than –0·5°. Glucose syrup, on the other hand, gives a strong positive rotation: $[\alpha_D^{20}]$, based on the original glucose syrup, about + 83°.

* In other commercial samples the value for $[\alpha]_D^{20}$ has been found to vary from 116° to 150°.
—EDITOR.

I am indebted to Mr. H. M. Mason, M.Sc., F.I.C., for samples of glucose syrup, and to my former colleagues, Mr. Arnold Lees, F.I.C., and Mr. R. J. Taylor, for most of the analytical determinations mentioned in this note.

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FURTHER NOTES ON A NEW COLORIMETRIC TECHNIQUE FOR THE ESTIMATION OF UREA IN URINE

THE technique for urinary urea estimation, originally published by us in 1934,[1] made use of the catalysing effect of urea on the reaction between stannous chloride and furfuraldehyde in the presence of a mineral acid.

An experience of four years with over 10,000 analyses has allowed us, not only to confirm the accuracy of the method, but to simplify it. The original technique called for a photoelectric densitometer, ice-baths and rather elaborate precautions. Our modified method is (*a*) more rapid as the conditions are less rigid; the reaction is allowed to develop at room temperature instead of 0° C.; (*b*) suitable for routine use in most laboratories, as it is now adapted to the Vernes-Bricq-Yvon[2] or Zeiss photometer or any of the usual colorimeters.

Notes on the Colour Reaction.—Stannous chloride dissolved in hydrochloric acid gives a purple colour with furfuraldehyde at boiling-point. The colour passes through a purplish opalescence to a black precipitate. The addition of a protective colloid inhibits both opalescence and precipitate and increases the depth of the blue component of the colour. In the presence of urea the colour develops at much lower temperatures, even at 0° C. Furthermore, the depth of colour is directly proportional to the amount of urea present, Beer's Law being followed.

The reaction appears continuous,[3] but it can be stopped at any given point by buffering the mineral acid present with excess of sodium acetate. This leaves a high concentration of acetic ions in solution, and the colour changes from an intense purple to a golden brown. This second colour change is not instantaneous, and the rate of change is again governed by temperature.

We have investigated the possible effect of other urinary constituents on the reaction, in concentrations much in excess of normal.

The following substances were tested alone, and in urea solutions, in the percentages mentioned:—ammonium salts, 15; creatinine, 0·5; creatine, 0·1; sodium phosphate, 1·5; glucose, 5; peptone (freshly prepared), 1·5; acetone, 1; calcium chloride, 2; magnesium chloride, 1; sodium chloride, 5; guanidine, 1; uric acid, 0·25; glycine, 10; cystine, tyrosine and tryptophane, saturated solutions in $N/10$ sodium hydroxide solution; bile salts, 0·1 per cent.

These substances, alone or in admixture with urea solutions, caused no change in the colour produced, with the exception of 1 per cent. of guanidine, which increased the colour to the same extent as 0·05 per cent. of urea. Since, however, guanidine is never present in urine in higher concentration than 0·1 per cent. and the experimental error of the technique (or any other urea method) is in the region of 0·01 per cent., this factor is negligible.

If much protein is present in the urine it must be removed, as a precipitate may form on adding the acid mixture. We suggest the following technique for deproteinisation.

Add 0·5 ml. of 5 per cent. basic lead acetate solution and 0·5 ml. of 10 per cent. acetic acid to 2 ml. of urine in a centrifuge tube. Leave for a few minutes and centrifuge. To remove excess of lead add 0·5 ml. of 10 per cent. sodium sulphate solution to 2 ml. of the supernatant fluid. Centrifuge again. Urea is then estimated in the protein-free supernatant fluid. The result must be corrected for a dilution factor of 15/8.

In theory, when hydrochloric acid is added to a highly pigmented urine, the resulting yellow colour might tend to make the result higher. We automatically eliminate this possible source of error by attaching a Wratten green filter (No. 74) to our photometer for this estimation. Alternatively, the urine should be treated with absorbent charcoal. It has been proved experimentally that treatment with small quantities of charcoal in slightly acid solution does not cause co-adsorption of urea.

Details of Modified Technique.—The following method is particularly suitable for the serial analysis of six or more urines. We find that two standard solutions of urea (1 and 3 per cent. respectively) are adequate for ordinary purposes. Although two periods of thirty minutes each are needed for colour development, the actual manipulative time is very short. Hence, the method compares favourably for speed, even with the usual rough hypobromite technique.

It is particularly suitable for work on animals when only small amounts of urine are available.

The urine (0·2 ml.) is pipetted into a small test-tube (4 in. × ½ in.), and 1 ml. of 10 per cent. solution of stannous chloride in conc. hydrochloric acid (this solution is not very stable and should be kept in ice when not in use) and 0·3 ml. of a mixture made by dissolving 0·3 ml. of freshly-distilled furfuraldehyde in 7 ml. of glacial acetic acid (made up to 21 ml. with 5 per cent. gum ghatti solution) are added. The contents of the tube are then shaken and allowed to stand at room temperature for thirty minutes. It is not essential to adhere rigidly to this period, as standard solutions of urea are under the same conditions as the unknown solution. If the room temperature is below 10° C., the time interval can be increased to 45 minutes, and if above 20° C. it may be decreased to 20 minutes. At the end of this time an intense purple colour develops. Four ml. of a mixture containing 3 parts of 30 per cent. sodium acetate solution and 1 part of 5 per cent. gum ghatti solution are then added, and the contents of the tube are mixed and allowed to stand for a further period of about 30 minutes. During this interval the colour changes from a deep purple through a brownish-green to a golden brown. The depth of colour is then read off in terms of extinction coefficient or optical density, or is matched with a standard solution subjected to the same technique, according to the type of instrument used—photometer or colorimeter.

The method has been checked against the gravimetric xanthydrol procedure and the results compare very favourably. The limits of accuracy of the methods depend entirely on the accuracy with which comparison can be made. With a colorimeter the accuracy is about ± 2 per cent. in normal cases, but ± 5 per cent. when the concentration of urea is of the order of 0·2 per cent. This compares favourably with the hypobromite technique, the accuracy with which is never better than ± 7 per cent.

E. Obermer
R. Milton

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THE USE OF ALTERNATING CURRENT MAINS IN ELECTROLYTIC ANALYSIS

THE low voltage direct current produced from alternating current mains by means of a transformer and a metal rectifier is unsuitable for the analytical deposition of metals by the method of controlled potential. Such direct current may be employed for other electrolytic processes (*e.g.* commercial electroplating) for which an unsmoothed current is not objectionable.

This note describes a method of obtaining from alternating current mains direct current sufficiently free from ripple for quantitative deposition and separation of metals.

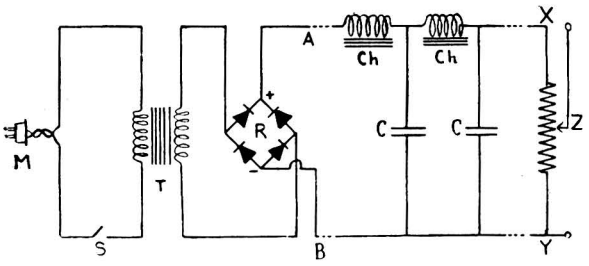

Fig. 1

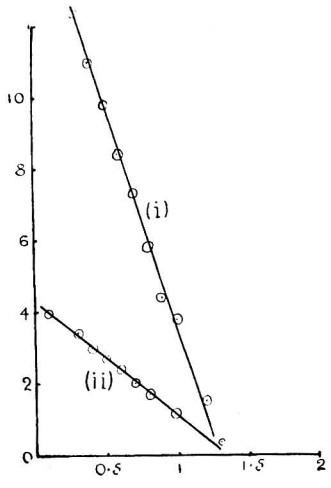

Fig. 2

The circuit is shown in Fig. 1. The rectifying circuit consists of a mains connection M, supplying the transformer T, through the switch S. The transformer has a primary wound to suit the mains voltage, and a secondary to deliver several ampères at 22 volts. This is connected with a full-wave Westinghouse rectifier, type L.T.5, producing at the points A B a direct current output of about one ampère at 12 volts.

This direct current is smoothed by the system of choke coils and condensers, Ch and C. These chokes are each wound on cores of laminated iron with air gaps, and have an inductance of 0·2 henry (with 1·5 ampères passing). The D.C. resistance of each coil is 2 to 3 ohms. The electrolytic condensers C (Dubilier Condenser Company, Ltd.), type A.D.6, have a capacity of 2000 microfarads and are capable of withstanding 12 volts.

A rheostat X Y, of 5 ohms resistance, is connected with the output terminals and, by means of the slider Z, a potential of from 0 to 4 volts can be selected for electrolyses.

The smoothed direct current obtainable with various load resistances at the points X Y was measured and a curve (i, Fig. 2) shows the relationship between voltage and current. The second curve (ii), shows the relationship between the potential selected between Z and Y and the current available for use at these points. This output is suitable for the micro-electrolytic method previously described.[1] The amount of smoothing effected by the filter-circuit was measured by means of a cathode ray oscillograph, and it was found that one choke coil

and one condenser were not sufficient to remove all the ripple. A low frequency hum could be detected in a pair of telephones connected after the first coil and condenser. With two choke coils and two condensers, however, no ripple could be detected either by the oscillograph or by the telephones. From other experiments with known alternating voltages applied to the telephones, it was calculated that the ripple remaining after two stages of smoothing was less than a few millivolts in amplitude. It was to be expected that this is a sufficiently small variation to have no effect on the separation of two metals the deposition potentials of which differ by about 0·3 volt, and this has been proved by actual analyses.

Bismuth has been separated from lead, and copper has been separated from lead, tin and other metals by the methods previously described.[2,3]

Quantitative separations of bismuth and lead could not be effected with current smoothed with one condenser and one choke coil.

The chief advantage which this apparatus has over accumulators is that it requires no maintenance. It is portable, it will provide current continuously for indefinitely long periods, and it may be left without attention for equally long periods. For the electrolytic separation of metals by graded potential, manual control of the applied voltage is always employed. Hence automatic stabilisation of the voltage from the mains is unnecessary.

I wish to thank Dr. H. J. S. Sand for his interest and criticism, and Mr. R. H. Humphry for the cathode ray oscillograph measurements.

A. J. Lindsey

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THE DETERMINATION OF COMBINED NITROGEN IN ALUMINIUM

Nitrogen combines with aluminium to form aluminium nitride, AlN. This compound in contact with water or acid is decomposed with formation of ammonia and ammonium salt respectively. Hence, to determine the combined nitrogen in aluminium, the metal may be dissolved in hydrochloric acid and the ammonia in the resulting ammonium chloride be determined in the usual way by distillation with alkali.

However, although the method is exceptionally simple in principle, in practice difficulties arise owing to the very small quantity of combined nitrogen present in aluminium. Tronstad[1] gives a figure of 0·0006 per cent. of nitrogen in 99·5 per cent. aluminium, and, in view of the smallness of this figure, it is necessary to free all reagents as far as possible from ammonium compounds and to determine the ammonia colorimetrically in preference to the normal volumetric procedure.

The apparatus described by Stanford[2] for the quantitative micro-determination of ammonia has been found suitable, with slight modifications, for the determination of nitrogen in aluminium. The method finally adopted is as follows:—Ten g. of the metal, in the form of freshly-prepared millings, are placed in a 600-ml. beaker containing 60 ml. of water and 10 ml. of redistilled hydrochloric acid. The mixture is heated until the reaction begins, and then more acid is added until a total of 160 ml. of 20·24 per cent. acid has been used. The solution is heated until all aluminium is dissolved, and is then evaporated down to about 100 ml. to remove part of the excess of acid.

Meanwhile a solution of 40 g. of potassium hydroxide in 100 ml. is made up and boiled for 10 minutes to remove any possible traces of ammonia. Three hundred ml. of this solution are transferred to the flask of the apparatus and the solution of the aluminium is gradually added from the dropping funnel, with occasional shaking. (It is better to add the solution of aluminium to the alkali, since very little precipitation of aluminium hydroxide then occurs, the alkali always being in excess.) The distillation is then started, and any ammonia is absorbed in the two receiving vessels, which contain 4 ml. and 3 ml. of 2 *N* sulphuric acid respectively. A guard-tube containing glass wool soaked in conc. sulphuric acid prevents any ingress of ammonia from the atmosphere. The distillation is continued until about 30 to 35 ml. of liquid have condensed in the first receiver. To prevent irregular boiling and back-suction the flask should contain a few pieces of broken glass, and a small, steady stream of air or oxygen, previously bubbled through conc. sulphuric acid, is passed through the solution.

The distillate and the solution in the receivers, which cannot be used directly for the colorimetric estimation owing to their high acidity, are then transferred to a flask similar to the boiling-flask of the apparatus, but having a capacity of 150 ml. Ten ml. of the potassium hydroxide solution are added from the dropping funnel and a second distillation is made, the two receivers now containing 4 ml. and 3 ml. of *N*/20 sulphuric acid respectively. The distillation is continued until about 25 ml. of liquid have condensed in the first receiver.

This liquid is allowed to cool, then transferred to a Nessler tube and diluted to 50 ml., and the ammonia is determined colorimetrically by means of Nessler's reagent. For the comparison a standard solution of 3·82 g. of ammonium chloride per litre is used.

To test the accuracy of the method, a number of determinations were carried out on solutions of aluminium to which known amounts of nitrogen (ammonium chloride solution) had been added. The results showed that the limits of error were approximately ± 0·00005 per cent. for nitrogen-contents not greater than 0·0010 per cent. Although no metal samples containing higher amounts of nitrogen were examined, experience suggests that the limits of error for nitrogen up to 0·0030 per cent. would not be greater than ± 0·0001 per cent. The results of actual determinations place the combined nitrogen-content of aluminium (99·5 per cent.) at about 0·0003 per cent.

For permission to publish this note we wish to thank The British Aluminium Co., Ltd., in whose laboratories the work was carried out. Our thanks are also due to Dr. A. G. C. Gwyer and Mr. G. B. Brook for their interest in the work.

A. L. DOYLE
W. H. HADLEY

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RESEARCH LABORATORIES
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November, 1937

USE OF MAMMALIAN (WHALE) LIVER OILS IN THE PREPARATION OF ARTIFICIAL OR "REINFORCED" HALIBUT-LIVER OILS

ERRATUM.—P. 336. First line below Fig. 1. *For* "factor A_2" *read* "discrepancy factor."

Notes from the Reports of Public Analysts

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

CITY AND COUNTY OF KINGSTON-UPON-HULL

Report of the City Analyst for the First Quarter, 1938

Of the 599 samples examined, 352 were taken formally and 247 informally.

Milk Curd.—Of the six samples of curd examined, four were passed as genuine, since they either contained no extraneous addition (2 samples) or were labelled as mixtures (2 samples). The two remaining samples contained 5·5 and 9·5 per cent. of moist wheat-flour and were returned as adulterated. The vendors were cautioned.

Ground Ginger.—One of the 6 samples of this product was similar in character to that reported on last quarter, and was deficient in valuable water-soluble constituents (adulterated). It is probable that this sample also was Nigerian ginger, and the vendor was cautioned, since the washing-treatment of this material abroad is being subjected to official investigation.

Arnold Tankard

CITY OF BIRMINGHAM

Report of the City Analyst for the First Quarter, 1938

Of the 1507 samples submitted by the food and drug sampling officers, 65 were bought formally and 1442 informally.

Prepared Honey.—Samples bought from two stores bore labels on which it was stated that the contents were mixtures of honey and glucose. Analysis indicated that actually they were mixtures of honey and commercial invert sugar, *i.e.* the same kind of sugar as is present in honey itself. The bottlers of one of the samples explained that in spite of orders to the contrary, their blender had not included glucose in certain batches of honey, because he was of the opinion that the mixture would set better if it were omitted. He had unfortunately overlooked the fact that in so doing he was departing from the wording of the label. In future, glucose would always be included in the product. The vendors of the other sample decided to withdraw their stocks from sale.

Kaolin Poultice.—This article should contain 52·7 per cent. of kaolin, 4·5 per cent. of boric acid and 42·5 per cent. of glycerin, together with small amounts of methyl salicylate, peppermint oil and thymol. Six samples in all were purchased during the quarter, and five of them agreed closely in composition with the formula. The sixth sample, however, contained an excess (15 per cent.) of boric acid. The manufacturers, when asked by the retailers for an explanation, said that during the heating at 120° C. for 1 hour of the mixture of kaolin, boric acid and glycerin (a process required by the B.P. to destroy bacteria), a reaction occurred between the glycerin and boric acid, resulting in the formation of glyceryl borate and water. This water, they stated, together with the small amount always present in the glycerin was lost during the heating, and therefore the final weight of the finished poultice would be less than the original quantity, and this loss of water would have the effect of increasing the boric acid content. The firm was informed that five other samples of the poultice bought on the same day were of correct composition, that if the boric acid on their theory should be high, so also should

the kaolin percentage, whereas actually it was lower than the B.P. requires, viz. 50·9 per cent. instead of 52·7 per cent.; and thirdly, that the water, even if lost in the way described, was present in so small an amount that the percentage of boric acid could only be increased very slightly indeed. The vendors were cautioned.

LIGHT MAGNESIA.—This article was asked for at six different retail pharmacists' shops, and in each instance carbonate of magnesia was supplied. Five of the samples were also labelled as carbonate. The British Pharmacopoeia, under the heading "Mag. Oxid. Lev." (light oxide of magnesium), gives as a synonym for this article, "light magnesia," so that one would expect that in response to a demand for the latter, the oxide would be supplied. It has, however, been a trade custom for many years, among a considerable number of pharmacists, to supply the carbonate, but this practice is not universal, so that it is possible to get the oxide at one shop and the carbonate at another. As it seems advisable that the practice in this respect should be uniform, a letter was addressed to the Secretary of the local branch of the Pharmaceutical Society, asking him to bring the matter forward at the next meeting, and invite the comments of the members on this point. If the general feeling seemed to be that the public expects to get the carbonate when they ask for light magnesia, there would seem a good case for the omission of the term in the B.P. as a synonym for the oxide. It seems only reasonable that the official description should correspond with trade practice in such a matter as this. The six pharmacists concerned all concurred in saying that the general public expect to get the carbonate, and that it was the trade custom to supply it, in spite of the fact that technically it was correct to supply the oxide.

H. H. BAGNALL

THE ROYAL BOROUGH OF KENSINGTON

REPORT OF THE PUBLIC ANALYST FOR THE FIRST QUARTER, 1938

LEAD IN CURRY POWDER.—Two samples of curry powder were found to be contaminated with lead; one contained 50 parts per million, and the other 14 p.p.m. As the latter amount is within a limit which gained acceptance in the past for certain articles, no action was taken. Emphasis must be laid upon the point, however, that there is no reason why curry powder should contain any lead at all.

F. W. EDWARDS

Ministry of Health

OUTBREAK OF FOOD POISONING DUE TO SALMONELLA, TYPE "DUBLIN" AND CONVEYED BY RAW MILK*

THE outbreak occurred in Wilton, Wiltshire, and affected over 100 persons, mostly children. It was limited to the consumers of a particular supply of raw milk on the 28th and 29th October, 1936, the onset of the symptoms occurring between 12 and 24 hours after its consumption. The symptoms were headache and nausea, severe vomiting but of short duration followed by diarrhoea persisting usually for from one to three days; there were no deaths. The importance of bacteriological examination was not realised until six days after the outbreak began, and by that time the examination of excreta yielded negative results, but from a specimen of milk supplied to one of the affected schools a *Salmonella* bacterium was isolated and afterwards identified as type "Dublin." The same organism was again

* Reports on Public Health and Medical Subjects, No. 38. By E. T. Conybeare and L. H. D. Thornton. H.M. Stationery Office, Adastral House, Kingsway, W.C.2, 1938. Price 2½d. post free.

isolated from the milk supplied by the same producer, and the blood of nine of the convalescents agglutinated this organism in titres of from 1 in 600 to 1 in 2500, indicating that it was the cause of the outbreak. Investigation of all those engaged in handling the milk showed that the blood of one only contained specific agglutinin for *Salmonella* "Dublin," but the circumstances suggested that this one was a victim and not the source of the infection. Investigation of the cows, of which there were 51, was undertaken and showed that the blood of about 50 per cent. gave agglutination in titre of 1–25 only, whilst the blood of three agglutinated in titres of 1–400, 1–800 and 1–800 respectively. Specimens of milk and dung from these three cows were examined on three occasions; in every instance the milk, and in the case of two cows the dung, showed no *Salmonella* bacteria, but the dung of the third was found to contain these bacteria in large numbers every time; a cow carrier had therefore been detected and inspection of the mechanical milking plant suggested that as the result of certain defects contamination of the milk with dung might easily occur. The blood of 71 cows from other herds was subsequently tested as a control and none agglutinated "Dublin" *Salmonella* in titre above 1–25. It is suggested that in the particular instance of food poisoning connected with milk or milk products a possible animal carrier should not be overlooked.

The report contains a prefatory note by the Chief Medical Officer, addressed to the Minister of Health, in which he says: " . . . This outbreak forms another addition to a long series of recorded outbreaks of disease which have resulted from the consumption of raw milk and is an example of the class of outbreak which is due to the infection of the milk by a diseased cow. No amount of care in milking and distribution of such raw milk can prevent it from being a danger to the consumer. In other words, a clean raw milk is not necessarily a safe milk. . . ."

D. R. W.

Department of Scientific and Industrial Research

WATER POLLUTION RESEARCH BOARD

REPORT FOR THE YEAR ENDED JUNE 30, 1937*

THIS, the Tenth Annual Report of the Board, includes the Report of the Director of Water Pollution Research (Dr. H. T. Calvert). Close touch is maintained with the work of the Joint Advisory Committee on River Pollution, which was set up in 1927 by the Ministers of Health and of Agriculture and Fisheries. In its second report† the Committee recommended that, subject to certain conditions, local sanitary authorities should be under a general obligation to receive and dispose of the industrial effluents of their districts, and that the traders should have a correlative right to discharge such effluents into the public sewers. Effect has now been given to this recommendation by the passing of the Public Health (Drainage of Trade Premises) Act, 1937, which comes into operation on July 1st, 1938.

WATER-SOFTENING MATERIALS.—Experiments have been continued on the preparation of materials for softening water by the base-exchange process. This process is used in household water-softeners and is also employed on a large scale at a number of waterworks. The investigations of the Board have shown that satisfactory water-softening materials can be prepared from fuller's earth which

* H.M. Stationery Office, Adastral House, Kingsway, London, W.C.2. May, 1938. Price 9d. net.

† H.M. Stationery Office (1930). Price 6d. net.

is found in parts of the British Isles. The base-exchange capacity of the final product depends on the type of fuller's earth used, yellow, weathered varieties being more satisfactory than blue varieties. In the method of treatment fuller's earth is mixed with dilute hydrochloric acid, then dried, baked at about 600° C, and treated with solutions of sodium silicate and sodium aluminate.

BASE-EXCHANGE AND ACID-EXCHANGE PROPERTIES OF SYNTHETIC RESINS.—Recent experiments have shown that the exchange values of the resins not only vary with the phenol, tannin or aromatic base from which they are derived, but are dependent also on the detailed conditions of preparation. For example, the base-exchange value of resin made from sulphited quebracho tannin is largely dependent on the proportion of sodium bisulphite allowed to react with the tannin before it is converted into a resin by addition of formaldehyde. Within limits, the larger the proportion of sulphite the greater the base-exchange value of the final resin. It has been found, however, that the quebracho tannin resins with the highest base-exchange values are to some extent soluble in water. Quebracho tannin resins with lower base-exchange values appear to be insoluble in water and in dilute solutions of acids and alkalis. In view of the possible use of the resins for the treatment of drinking water, experiments are in progress with the object of determining the possibility of the water being contaminated by substances derived from the resins. These experiments involve the determination of minute quantities of organic matter. The possibility of utilising the resins for the removal of boron and fluorine from natural waters is also under investigation.

CONTAMINATION OF WATER BY LEAD.—Further series of experiments have been carried out at the Chemical Research Laboratory on the conditions affecting the action of water on lead service pipes. The method devised for determining the average concentration of lead in drinking water withdrawn from household services over periods of several weeks has been tested in several towns in England and Scotland. Average concentrations of lead ranging from less than 0·1 to as much as 0·5 part per million have been obtained. By the method as first devised the water was passed through a meter and then through a filter containing a base-exchange zeolite to absorb the whole of the lead. Analysis of the zeolite for lead, however, was difficult and tedious. Later the filters were filled with powdered magnesia in which the lead could readily be determined, but the filters soon offered considerable resistance to the flow of water, particularly with hard water. This difficulty has been overcome by using a mixture of calcium carbonate and magnesia as the filtering medium; the proportions of calcium carbonate and magnesia in the mixture are varied according to the character of the water under examination.

MILK FACTORY EFFLUENTS.—Work on the purification of waste waters from dairies and milk products factories, which is being carried out in collaboration with the milk industry, has been continued. During the past year the industry, through the Milk Marketing Boards, has contributed a further £3250 towards the cost of the investigation. One of the most important results of the work has been to show that the loss of valuable products and by-products carried away with the waste waters from dairies and milk products factories can be considerably reduced by inexpensive modifications in the manufacturing processes. For example, when churns of milk are brought from farms to a central milk depot, they are inverted and emptied into a large receiving tank. If sufficient time is not given to allow the churns to drain, an appreciable quantity of milk remains in the churns and is later washed out and discharged with the waste waters. By a short increase in the time of drainage of the churns the average quantity of milk carried away with the waste waters can be reduced from more than 0·5 per cent. to less than 0·25 per cent. of the milk handled. For a depot receiving 10,000 gallons of milk daily, this represents a saving of over 9000 gallons of saleable milk annually. Besides the saving of valuable material, the polluting nature and thus the cost of treatment of the waste waters is greatly reduced. In many instances, the report

states, the value of the milk saved in two or three years is equal to the capital cost of the plant required for the treatment of waste waters. The work of the Board has shown that the unavoidable waste waters from dairies can be purified by processes similar to those used for the treatment of sewage. The most suitable method has been found to be biological oxidation in percolating filters operated under certain conditions. Several large-scale plants using this process have now been erected at various dairies. During the past year it has been shown that waste waters containing whey, from cheese factories, can be purified by methods similar to those which have been found suitable for waste waters from dairies and milk depots.

PURIFICATION OF SEWAGE.—The factors affecting the coagulation or flocculation of the organic matter and its adsorption by the sludge are under investigation under the supervision of Professor F. G. Donnan in the Department of Chemistry of University College, London, and the biology of the process is being studied in the Department of Biochemistry of the College, under the supervision of Professor J. C. Drummond.

During the past year a further series of experiments was made, in which bubbles of gas were passed for several hours through sewage liquor from which suspended particles had been removed by centrifuging. In the new experiments bubbles of air, oxygen, hydrogen and nitrogen, to which about 1 per cent. by volume of carbon dioxide was added to prevent precipitation of calcium carbonate, were passed through the liquid. The presence of carbon dioxide did not affect the quantity of organic matter coagulated by air and oxygen, but it caused some reduction in the quantity coagulated by hydrogen and nitrogen; this difference may be due to some biochemical change. Experiments have been continued on the effect of passing bubbles of gas through sewage liquor to which different proportions of activated sewage sludge had been added. The presence of activated sludge caused a great increase in the quantity of organic matter removed from the sewage liquor, though the increase was not so great in the experiments with bubbles of hydrogen and nitrogen as with bubbles of air and oxygen. One phase of the investigation has included a large number of experiments on the effect of additions of sewage and of proteins on the coagulation of suspensions of quartz and clays by means of electrolytes.

The experiments carried out in the Department of Biochemistry during the past year have been directed primarily towards obtaining information on the changes in the nitrogenous compounds of sewage and sewage sludge during the stage of biological oxidation in the activated sludge process. These changes appear to be the result of a series of concurrent reactions brought about mainly through the enzymic activity of bacterial cells. Organisms capable of liberating gaseous nitrogen from sewage have been isolated.

ESTUARY OF THE RIVER MERSEY

EFFECT OF THE DISCHARGE OF SEWAGE ON THE CONSERVANCY OF THE RIVER*

IN this report a detailed description is given of the results of a chemical, hydrographical and biological investigation of the effects of the discharge of crude sewage on the amount and nature of the deposits in the estuary of the river Mersey.

For many years the possible effects on the conservancy of the estuary of the direct discharge of sewage from a population of nearly one and a half million people has given rise to much controversy among the local interests concerned. To facilitate the passage of ocean-going ships the sea channels in Liverpool Bay have been deepened considerably by dredging, which has been carried out continuously

* "Water Pollution Research," Technical Paper No. 7. H.M. Stationery Office, Adastral House, Kingsway, London, W.C.2. Price 30s.

since 1890. It had been suggested that the nature of the material deposited in these channels was so altered by the presence of sewage in the water as to increase the difficulty of dredging. Above the Narrows, on which are situated the extensive systems of docks of Liverpool and Birkenhead, is a tidal pool. The upper part of this tidal pool is navigable only at high water, and contains large areas of banks of mud and sand. The maintenance of the tidal capacity of this pool is considered to be of great importance, since the water which flows into it on the flood tide and out on the ebb passes through the sea channels in Liverpool Bay and helps to maintain their depth by its scouring action. The capacity of the upper estuary is such that at high water of a spring tide the volume of water is about 1000 million cubic yards. During the period 1906 to 1931 the capacity decreased by approximately 52 million cubic yards, and it was suggested that this was due to the presence of sewage, which had caused the deposition of mud of so glutinous a nature that it was not again eroded by the tidal streams.

In 1932 the local sanitary authorities and the authorities and companies interested in the navigation of the estuary invited the Department of Scientific and Industrial Research to undertake an investigation into the effect of the sewage on the amount and hardness of the deposit in the estuary and agreed to pay the whole cost of the work. Accordingly, in 1933, a laboratory was set up in Liverpool and two boats, specially designed and equipped, were built for the purposes of the investigation. The investigation occupied four years and was made at a cost of about £26,000. The investigation was not concerned directly with such problems as the effect of sewage on the sanitary condition of the river and foreshores, nor on fisheries, but was directed solely to the study of the effect of sewage on the conservancy of the estuary.

Samples of mud and other solid matter for examination were collected from different parts of the upper estuary of the Mersey and from Liverpool Bay. The concentration of organic matter in mud from the Mersey was found to be approximately the same as that in similar samples of mud from the bed of the Irish Sea, from Liverpool Bay, and from the relatively unpolluted estuaries examined. Sewage, in the concentration in which it is present in the Mersey, has no appreciable effect on the composition of the intertidal deposits. Mud carried in suspension in the estuary water is in the form of comparatively large flocks, and in this condition its rate of sedimentation is not affected by sewage in the concentrations present.

Detailed examination and analysis of the records of the Mersey Docks and Harbour Board showed that there is no evidence of an increase during recent years in the difficulty of dredging in Liverpool Bay. The capacity of the upper estuary in 1936 was about 12 million cubic yards greater than in 1931; the capacity in 1936 was about the same as in 1871, although considerable fluctuations in capacity had occurred during this period. A reduction in capacity during the period 1906 (when the capacity was unusually high) to 1931 was due mainly to the deposition of sand in the deeper parts of the estuary and not to the deposition of mud.

In direct answer to the terms of reference, the report states "the crude sewage discharged into the estuary of the river Mersey has no appreciable effect on the amount and hardness of the deposits in the estuary."

Milk and Nutrition

New Experiments Reported to the Milk Nutrition Committee*

The steps which led to the formation of the Milk Nutrition Committee have been described in Part I (1937) of this series of reports (Analyst, 1937, **62**, 463). The Milk-in-Schools Scheme had been launched a few months before the Committee was set up, and it appeared desirable to ascertain the effects on growth and health of the amounts of milk usually consumed by children participating in the Scheme, namely $\frac{1}{3}$ pint and, less frequently, $\frac{2}{3}$ pint per head daily. A large-scale comparison of the nutritive values of milk supplements in these amounts would, in the view of the Committee, afford valuable information at a particularly opportune time.

The research was started in the last week of February, 1935, and lasted for an entire school year in each of the five areas in which it was conducted. It concluded in July, 1936. The areas of investigation in the order in which the studies were started and finished were Luton, Wolverhampton, Burton-on-Trent, Renfrewshire and Huddersfield. Analysis of the data is not yet complete, but it was considered desirable to report provisionally on the findings so as to indicate the general trend of the results so far obtained. A final report will be issued later.

Plan of Research.—After the consent of the local education authorities, the school medical officers and the teachers had been obtained and their co-operation assured, a letter was circulated to the parents requesting the participation of their children in the research. In all, 8435 children, distributed between the five areas, were enrolled. They were divided into four groups as follows:

Group I. 2000 children to act as "controls" who should not receive any supplement of milk at school but should get biscuits instead.
Group II. 2000 children to receive $\frac{1}{3}$ pint pasteurised milk.
Group III. 2000 children to receive $\frac{2}{3}$ pint pasteurised milk.
Group IV. 2000 children to receive $\frac{2}{3}$ pint raw milk.

The following conditions were laid down:—

(*a*) Children to be grouped at random from the roll of those taking part in each school, so that as nearly as possible an equal number of children of the same age and sex should be present in each group in each area.
(*b*) Each child to be medically examined, according to a fixed scheme, before the commencement of the supplementary feeeding, which should continue for a complete school year.
(*c*) The supplements for the groups receiving biscuits or $\frac{1}{3}$ pint of pasteurised milk to be given at the morning interval in the school curriculum, and the supplements for the groups receiving $\frac{2}{3}$ pint pasteurised or raw milk at the morning and afternoon intervals, $\frac{1}{3}$ pint milk at each interval.
(*d*) The milk for the investigation should come from the same source in each area.
(*e*) The supplements of milk and biscuits to be supplied free of cost to the children in each area.

Results.—It is well known that the rate of growth of children, whether measured by weight or height or both, varies with age, sex and season of the year. Variations in the first two respects have been revealed by the present experiments and they are pointed out in the Report, but as they are not strictly germane to the object of the investigation only brief reference is made to them. The examination of the data for this report was too limited to bring out evidence of seasonal variations, but that such exist is established beyond doubt. When

* Part II. The Effects of Dietary Supplements of Pasteurised and Raw Milk on the Growth and Health of School Children (Interim Report). To be purchased directly from the National Institute for Research in Dairying, Shinfield, Reading, 1938. Price 1s. 9d., post free.

the data have been completely analysed the influence of season on the results will probably be revealed and will be adequately discussed in the final report of this investigation.

The curve of growth for any single child may differ from that for any other, not only in the rate of growth at any stage, but also in the time at which changes in growth rate take place. It is not at present possible to reduce such a growth curve to a mathematical expression, and in consequence, no appropriate statistical method has yet been devised for dealing with masses of data of the kind collected. The simplest and only practicable mode of treatment is to average all the increases; but it is not improbable that this procedure applied to the present data may have reduced the apparent differences between the various groups of children. The percentage increment has little, if any, biological meaning, but is a convenient way to express rate of growth.

CONCLUSIONS.—The broad conclusions to which the various comparisons lead are illustrated by reference to the tables of results; they are as follows:

(1) Taking all ages and all areas together the average increase in height and weight over the year of inquiry rises on passing from the groups of children whose supplement was biscuits to the groups whose supplement was $\frac{1}{3}$ pint of milk, and again from the latter to the groups whose supplement was $\frac{2}{3}$ pint of milk.

(2) The absolute differences between the average increments of height and weight of the groups with milk as a supplement and the groups with biscuits as a supplement are not very large. Comparing the extreme groups—those with biscuits and those with $\frac{2}{3}$ pint of milk—they do not amount to more than 0·1 inch in height during the year or to more than $\frac{2}{3}$ lb. to 1 lb. in weight.

The increments produced by milk in this investigation are smaller than those obtained in former experiments of this type. The most probable reason is that the quantities of milk fed as supplements in the previous experiments varied from $\frac{3}{4}$ pint to $1\frac{1}{4}$ pints per head daily, whereas in this experiment they were $\frac{1}{3}$ and $\frac{2}{3}$ pint only. There is also evidence suggesting that the growth rate of the control children in the present investigation is higher than that of the control children in previous experiments, thus allowing a smaller margin for the supplements of milk to demonstrate their effects in the milk-fed groups.

(3) During the year boys with biscuits as a supplement increased their initial height by 4·16 per cent. and their initial weight by 10·87 per cent., while boys with $\frac{2}{3}$ pint of milk increased their height by about 4·4 per cent. and their weight by 12 per cent. Girls with biscuits as a supplement increased their initial height by 4·66 per cent. and their initial weight by 12·75 per cent.; girls with $\frac{2}{3}$ pint of milk increased their height by about 4·8 per cent. and their weight by about 14 per cent.

(4) The average increment for the year of those children whose supplement was $\frac{2}{3}$ pint of milk expressed as a percentage of the average increment of the children whose supplement was biscuits, shows that the groups with the milk supplement gained 4 to 6 per cent. more in height during the year and 9 to 10 per cent. more in weight than those with the biscuit supplement.

(5) Comparisons of the growth increments in three different age groups, 5–7 years, 8–10 years and 11–14 years, show that at each age children with $\frac{1}{3}$ pint of milk gained more in weight than those with biscuits, and probably—but rather less consistently—gained more in height. On the whole, children with $\frac{2}{3}$ pint gained at each age more than those with $\frac{1}{3}$ pint, again more consistently with weight than with height. The figures suggest that the excess gain of the group with milk over the gain of the group with biscuits was slightly greater at years 8–10 than at the higher or lower ages.

(6) Sub-division of the data into the five areas included in the investigation leads inevitably to a certain number of inconsistent results, due in all probability to small numbers. No systematic differences were observed between the five areas.

(7) Examination of the differential gains in height and weight of children clinically* assessed as (i) good or excellent, (ii) satisfactory or normal, (iii) poor or bad, leads to the conclusion that, on the whole, children of each clinical grade with milk supplements showed higher rates of growth than children in the corresponding clinical grades with the biscuits supplement. There was no clear difference between the relative advantages shown by the children of the three clinical grades. The absolute gains tended to be greater for each feeding group among the children initially defined as "well-nourished."

(8) Whether comparisons be made of the increments of growth in the total of children, or in sub-divisions by age, area, and clinical assessment, no consistent difference is apparent in these increments between children whose supplement was $\frac{2}{3}$ pint of pasteurised milk and children whose supplement was $\frac{2}{3}$ pint of raw milk.

The numerical value of the increments attributable to the larger supplements of milk were, however, so small—amounting to only about 0·1 of an inch for height, and $\frac{2}{3}$ to 1 lb. in weight during the year—that it is very doubtful whether any slight differences between the nutritive values of raw and pasteurised milk in supplements of this amount could be revealed by measurements of height and weight.

International Union of Chemistry

EIGHTH REPORT OF THE COMMISSION ON ATOMIC WEIGHTS OF THE INTERNATIONAL UNION OF CHEMISTRY†

THE report covers the twelve months ended September 30th, 1937. The following changes have been made:

H,	from	1·0078	to 1·0081.	Er,	from	167·64	to 167·2.
He,	»	4·002	» 4·003.	W,	»	184·0	» 183·92.
C,	»	12·01	» 12·010.	Os,	»	191·5	» 190·2.
Mo,	»	96·0	» 95·95.				

The atomic weights of *hydrogen* and *helium* have been derived from mass-spectroscopic data.

Carbon has been investigated by two methods: (i) combustion of chrysene, triphenylbenzene, and anthracene (Baxter and Hale), and (ii) comparison of benzoyl chloride with nearly equivalent weights of pure silver by the equal opalescence method (Scott and Hurley). The resultant figures are in excellent agreement. The densities of oxygen, carbon dioxide, and nitrous oxide were re-determined by Moles and Toral, giving C = 12·007 and N = 14·008.

Nitrogen.—The determination of the density of ammonia, obtained by heating nickel ammonia bromide, gave N = 14·009.

Aluminium.—The ratio $Al_2 : Al_2O_3$ was determined by Hoffmann and Lundell by solution of aluminium in hydrochloric acid, and precipitation with ammonia and ignition of the precipitate, or conversion of the chloride to sulphate and ignition of the latter to oxide. The final average (26·975) agrees very well with earlier analyses of the chloride (26·975 and 26·974).

Arsenic.—The atomic weight determined by the equal opalescence method in the reaction between arsenic tribromide and dissolved silver gave a mean value of 74·926.

* The methods of medical assessment are described in outline.

† By G. P. Baxter, O. Hönigschmid and P. Le Beau (*J. Amer. Chem. Soc.*, 1938, **60**, 737–745).

Molybdenum.—Carefully purified molybdenum pentachloride was compared with silver by Hönigschmid and Wittmann, using the equal opalescence method. The average result, 95·95, confirms Aston's isotopic analysis.

Europium.—(See ANALYST, 1938, 370.)

Erbium has been studied by Hönigschmid and Wittner, highly purified erbium chloride being compared with silver. The average figure (corrected for small quantities of yttrium and thulium determined by X-ray analysis) was 167·24.

Tungsten.—The hexachloride was compared with silver by Hönigschmid and Wenn, the silver nitrate being added to the ammoniacal solution of the hexachloride, which was then acidified with nitric and tartaric acids.

Osmium.—Nier has re-determined the isotopic abundance ratio, obtaining the figure 190·21, while Aston's measurements give 190·28. The figure 190·2 has been adopted for the Table.

Lead from several sources has been tested by Baxter, Faull, and Tuemmler, lead chloride being compared with silver. The following average results are reported:

Common lead	207·214
Samarskite	206·343
Beaverlodge Lake pitchblende	206·084
Katanga pitchblende (HCl extract)	206·051
" " (unaltered)	206·042
Galena from Great Bear Lake	207·205

The last figure was obtained by Marble, who used galena from a vein cutting one of the pitchblende veins of the Great Bear Lake deposit. W. R. S.

The Institution of Mining and Metallurgy

SILICOSIS RESEARCH

"The Consolidated Gold Fields of South Africa, Ltd." Gold Medal has been awarded to Professor H. V. A. Briscoe, A.R.C.S., D.Sc., F.I.C., "in recognition of his researches on the sampling and properties of industrial dusts," and their premium of forty guineas has been awarded conjointly to Dr. Janet W. Matthews, F.I.C., Mr. P. F. Holt, B.Sc., F.I.C., and Miss Phyllis M. Sanderson, B.Sc., "in recognition of their work, in association with Professor H. V. A. Briscoe, on the sampling and properties of industrial dusts."

British Standards Institution

THE following revised Standard Specification has been issued*:

No. 616—1938. SAMPLING OF COAL TAR AND ITS PRODUCTS.

This Specification, which was originally published in 1935, has been completely revised and brought up to date. Special attention has been given to the sampling of pitch and of those tar products, such as phenols and the benzols, which are marketed in the refined state.

The Specification provides for the representative sampling of viscous and partly solidified liquids, as well as liquid and solid products, and includes the sampling of heterogeneous liquids. It is emphasised that sampling should be carried out by an operator skilled in this type of work, and that the methods recommended are intended to supplement such experience.

* To be obtained from the Publications Department, British Standards Institution. Price 2/- net. Post free 2/2.

ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

Food and Drugs

Use of Different Sera for the Examination of Milk. R. Turnau. (*Chem. and Ind.*, 1938, **57**, 380–381.)—The following methods are available for the rapid preparation of sera for the routine examination of milk samples:—The milk (30 ml.) is heated on a boiling water-bath for 15 minutes with 0·25 ml. of an aqueous calcium chloride solution of sp.gr. at 20° C. 1·1375 ($n_D^{20} = 1{\cdot}35397$) and is then cooled and filtered (Ackermann, *Chem.-Ztg.*, 1904, **28**, 952, 1156; *Z. Unters. Nahr. Genussm.*, 1907, **13**, 186; 1908, **16**, 486; Abst., ANALYST, 1907, **32**, 117). By the method of Lythgoe (*Ind. Eng. Chem.*, 1914, **6**, 899), 40 ml. of milk are allowed to stand for 15 minutes with 10 ml. of copper sulphate solution (71·5 g. of $CuSO_4 \cdot 5H_2O$ per litre) and filtered. Beckel (*Z. Unters. Lebensm.*, 1931, **62**, 170) recommends the treatment of 20 ml. of milk with 1 ml. of copper sulphate solution (175 g. of $CuSO_4 \cdot 5H_2O$ per litre). For the serum of Vladescu (*Proc. XIth World Dairy Congress*, Berlin, 1937, **2**, 563) 20 ml. of milk are treated for 5 minutes with 1·2 ml. of copper sulphate solution (309·5 g. of $CuSO_4 \cdot 5H_2O$ per litre) and 0·8 ml. of potassium ferrocyanide solution (261·6 g. per litre). By the method of Ambuhl and Weiss (*Mitt. Lebensm. Unters. Hyg.*, 1913, **10**, 53) 30 ml. of milk are treated with 0·3 ml. of a saturated solution of mercuric chloride in conc. hydrochloric acid. The lead serum of Rothenfusser (*Z. Unters. Lebensm.*, 1931, **62**, 202) is prepared by the addition to 100 ml. of milk of 5 or 6 ml. of a 10 per cent. lead acetate solution with or without shaking the milk with carbon tetrachloride. Acid sera are prepared by precipitation with acetic acid and "sour sera" by precipitation with the lactic acid developed by natural souring. Sera obtained by cold precipitation contain albumin and globulin, and their refractive indices are not comparable with those of the calcium chloride serum. The cold methods of precipitation in which finely divided solid adsorbents and organic solvents are used in addition to salts of the heavy metals may be used for the preparation of sera free from soluble proteins. Turnau (*Z. Unters. Lebensm.*, 1934, **68**, 293) states that the refractive index of a copper-kaolin serum is identical with that of calcium chloride serum. Münchberg and Narbutas (*Milchw. Forschr.*, 1937, 19) recommend the copper-kaolin method for the determination of the protein-content of milk and for the preparation of sera for polarimetric work. The milk (10 ml.) is shaken with 0·5 ml. of Beckel's copper sulphate solution (*vide supra*), 4 ml. of benzene and 2·5 to 3 g. of kaolin for 2 minutes and centrifuged for 4 minutes. The aqueous and benzene layers are then separated for the determination of their refractive indices. Tetraserum I, an excellent serum for refractometric work, is prepared by shaking 50 ml. of milk with 5 ml. of carbon tetrachloride for a few minutes before adding 1 ml. of 20 per cent. acetic acid. Tetraserum II is prepared in the same way after the milk has been heated on a water-bath beneath a reflux condenser for 20 minutes. By comparing the refractive

indices of these two sera the amount of albumin and globulin in the milk may be determined, a difference of one degree on the dipping refractometer scale corresponding with 0·211 g. of soluble protein per 100 ml. of milk. By the "formol" titration of the original milk and of Tetraserum I, the casein-content can be determined. Refractometric measurements of Tetrasera I and II have served to detect high pasteurisation and sterilisation temperatures. Wedemann (*Z. Fleisch. Milch.-Hyg.*, 1924, **34**, 170), Kapeller (*Z. Unters. Nahr. Genussm.*, 1913, **25**, 285), Bengen and Bohm (*Z. Unters. Lebensm.*, 1933, **66**, 126; 1934, **67**, 379), and Schnetka (Abst., ANALYST, 1935, **60**, 478) have prepared sera by adding ammonium sulphate (the last-named with ammonia) to milk. A serum containing all the lactalbumin and suitable for serological purposes is that of Kluge (Abst., ANALYST, 1936, **61**, 709). For comparative work on a number of milk samples any serum may be used. To combine work on the serum with refractometric determination of the fat, copper-kaolin sera are the most suitable. For additional analyses, such as determinations of casein and soluble proteins, and for the detection of preservatives, the Tetrasera are recommended. The following are the values found for the refractivity (dipping refractometer degrees) of various sera prepared from the same milk:—milk, 50; Tetraserum I, 42·5; sour serum, 41·6; Tetraserum II, 40·4; copper serum (Beckel), 40·4; calcium chloride serum, 39·0; copper-kaolin serum, 39·0; copper serum (Lythgoe), 38·0. A. O. J.

Carob Bean Meal as a Thickening Agent in Fruit Preparations and other Foods. C. Griebel. (*Z. Unters. Lebensm.*, 1938, **75**, 35–37.)—A number of thickening agents intended for use in the domestic or commercial preparation of jam, etc. were found to consist of tragacanth, sugar, fruit acid and wheat or rice starch. Occasionally carob bean meal is used as a thickening agent. The tragacanth preparations appear to be of little value but, with carob bean preparations, thickening, as distinct from true jellying, does occur. The seeds of the carob bean (*Ceratonia siliqua* L.) have a brown seed-coat with the characteristic structure of the leguminous seed-coat. Since, in the preparation of thickening agents, the shelled, germ-free seeds are used, the characteristic palisade and bearer cells are not present. The meal consists largely of the mucilaginous endosperm, which has apparently been treated with steam during its preparation. The cells of the endosperm have strongly thickened walls and narrow, tube-like or stellate, lumina. The mucilaginous thickening on the walls is coloured yellow by iodine in potassium iodide solution, whilst the cell-contents acquire a yellowish-brown colour, thus rendering the lumen immediately visible. As a rule these features are easily recognised, although it may happen that, in small fragments, the cell-contents are absent and the structure of the cells confused by superimposed layers. In jam the fragments are often visible by direct microscopical examination without treatment with iodine because the cell-contents appear brownish against the mucilage deposits, which are colourless in the strong sugar solution. Frequently the cell-contents are coloured by the fruit pigments. Owing to the effect of heat and moisture in the preparation of the meal, the cell lumina often appear as highly refracting strands, and the swollen mucilaginous thickening may have a hollowed-out appearance resembling that of an independent cell. A. O. J.

Leafy and Non-leafy Vegetables of Ceylon. S. Kandiah and D. E. V. Koch. (*Analysis of Ceylon Foodstuffs*, 1938, 9–14.)—Some two dozen vegetables cultivated in Ceylon were analysed with the object of ascertaining their relative values in nutrition. Murunga leaf (*Moringa oleifera*) was the richest of the leafy vegetables examined in its organic constituents and was a good source of minerals. Agathi (*Sesbania grandiflora*) was of interest because of its high mineral content, particularly in calcium and iron. The amaranths (*Amarantus paniculatus* and *Alternanthera sessilia*) were also rich in iron. Locally-grown spinach (*Talinum sp.*), radish leaves (*Raphanus sativus*) and kankun (*Ipomea aquatica*) were deficient in minerals. Of the non-leafy vegetables, jak (*Artocarpus integra*), ash plantain (*Musa paradisiaca*) and bread-fruit (*Artocarpus communis*) had high-carbohydrate-contents, and jak was also rich in protein and minerals, ash plantain in minerals and bread-fruit in fat. Gourds and pumpkins were almost devoid of nutritional value. On the whole, the mineral and organic nutrient contents of local vegetables were lower than those of corresponding samples analysed in India. F. A. R.

Fatty Acids and Glycerides of Solid Seed Fats. T. P. Hilditch, M. B. Ichaporia, W. J. Bushell and T. G. Green. (*J. Soc. Chem. Ind.*, 1938, **57**, 44–53.)—*Seed Fat of Madhuca* (*Bassia*) *latifolia, Mowrah Fat.*—Mowrah fat is the seed fat of *Madhuca* (formerly *Bassia*) *latifolia* (N.O. *Sapotaceae*), a deciduous tree growing in northern India. The seeds are very similar to those of *Madhuca longifolia*, and the exported mowrah fat is often a mixture derived from both. The oil-cake contains saponin and is toxic, but is used as a fertiliser and in insecticide powders. The fat from seeds crushed in Baroda had saponification equivalent, 290·2; iodine value, 63·9; acid value, 20·1; and unsaponifiable matter, 2·1 per cent. The component fatty acids of the neutralised fat, as determined by ester fractionation, consisted of (per cent. mol.): palmitic, 25·5; stearic, 18·7; oleic, 42·3; linolic, 13·5. In round numbers the component glycerides were: di-palmito-stearins, 1; "oleo"-di-palmitins, 1; "oleo"-palmito-stearins, 27; palmito-di-"oleins," 41; stearo-di-"oleins," 30 per cent. (mol.). Thus the main components are about 40 per cent. of palmito-di-"oleins," and 25 to 30 per cent. each of stearo-di-"oleins" and "oleo"-palmito-stearins, these figures resembling those found for cacao butter (ANALYST, 1936, **61**, 422) in their general conformity to the rule of even distribution of the fatty acids among the glycerol molecules. As with cacao butter, the observed proportions of mono-palmito- and mono-stearo-glycerides accord closely with those that would result from independent glyceride formation from either saturated acid and the unsaturated acids, if the latter were divided between palmitic and stearic acids proportionally to the amounts in which they are present in the whole fat. *Seed Fat of Madhuca butyracea* (*Phulwara Butter*).—Phulwara butter is the solid fat in the seeds of *Madhuca* (formerly *Bassia*) *butyracea*, a shrub growing in north and north-west India. The nuts examined contained 76 per cent. of kernels yielding 61 per cent. of a cream-coloured solid fat with saponification equivalent, 285·4; iodine value, 40·6; acid value, 13, unsaponifiable matter, 2·1 per cent. The component acids determined by fractionation of the esters of the fatty acids separated by the lead salt and alcohol method, consisted of palmitic (with perhaps not more than 1 per cent. of myristic

acid), 59·0; stearic, 3·4; oleic, 34·0; linolic acid, 3·6 per cent. (mol.). The fat contained about 62 per cent. of "oleo"-di-palmitins and about 23 per cent. of palmito-di-"oleins" with subordinate proportions of tri-palmitin (about 8 per cent.) and probably "oleo"-palmito-stearins (about 7 per cent.). The very low content of stearic acid places this fat apart from other fats of the same family (*Sapotaceae*), and there is a greater proportion of more fully saturated glycerides than is usual in a seed fat with the observed proportions of saturated and unsaturated acids.

Shea Butter.—The component glycerides of this fat have been re-investigated by the methods made available since the previous work was carried out. It can now be stated definitely that the chief glycerides in shea butter are stearo-di-"oleins" (about 45 per cent.) and oleo-distearins (about 35 per cent.) together with palmito-di-"oleins" (about 10 per cent.). Palmito-stearins and tri "oleins" occur in small quantities, probably not more than 5 per cent., and similar small proportions of "oleo"-palmito-stearins are possibly present. This is in satisfactory agreement with deductions already made from previous work on shea butter (*cf.* ANALYST, 1932, **57**, 113). Apart from slight divergencies, the component glycerides conform to the usual rule of even distribution of the fatty acids among the glycerol molecules. As a necessary consequence of this rule of even distribution the linolic acid, as a minor constituent (oleic acid being a major one) is combined almost wholly in di-unsaturated glycerides and is almost absent from the mono-unsaturated glycerides. The same thing is seen in mowrah fat and phulwara butter. Again, as in mowrah fat, the deduced proportions of mono "oleo," di "oleo" palmitins and stearins are not widely removed from the figures obtained by experiment.

D. G. H.

Glycerides of Oils and Fats. XV. Babassu Oil. A. Bömer and H. Hüttig. (*Z. Unters. Lebensm.*, 1938, **75**, 1–33.)—The constants of the oil obtained by the extraction of babassu kernels with ether were as follows:—m.p., 25° C.; saponification value, 251·1; iodine value, 15·6; acid value, 2·0; Reichert–Meissl value, 5·9; Polenske value, 11·6; butyric acid value, 0·4; refractometer reading at 30° C., 40·5, at 40° C., 35·0; unsaponifiable matter, 0·3 per cent. A preliminary separation of the component glycerides of the fat into volatile and non-volatile fractions was made by distillation of the oil under greatly reduced pressure (cathode ray vacuum). The first fraction (5·2 per cent.), distilling at temperatures up to 150° C., was a bright yellow solid melting at 25·5° C., to a yellow liquid. The second fraction (77 per cent.) distilled between 150° and 295° C. and was an almost white solid melting at 26·8° C. to a yellow liquid. The non-volatile residue (17 per cent.) was a brown solid melting at 17·0° C. to a brown liquid. The first fraction consisted almost entirely of fatty acids formed by decomposition and was not investigated further. The second fraction was dissolved in acetone, and by repeated fractional crystallisation, combination of fractions of approximately equal melting-points, followed by further fractional crystallisations according to a definite scheme, principal fractions were obtained containing high concentrations of individual glycerides. From these the fatty acids were prepared and separated, by fractional precipitation as magnesium and barium salts, into mixtures of myristic, lauric, caprylic and oleic acids. By further fractionation, combined with

the determination of the constants of the acids and analyses of their silver, lead and copper salts, the identity and relative amounts of the acids were established. The residue from the vacuum-distillation was investigated in a similar manner. Contrary to the statement of Krafft, according to which only glycerides of fatty acids containing up to 14 carbon atoms should appear in the distillate, considerable amounts of the glycerides of oleic acid were found. The individual glycerides identified in the distillate were a myristo-dilaurin (m.p. 34·9° C.), which occurred in the largest amount, a considerable amount of a lauro-dimyristin (m.p. 36·1° C.) and a small amount of a palmito-dimyristin (m.p. 45·7° C.). The residue from the distillation contained the glycerides of oleic, stearic, myristic, lauric and caprylic acids and a small amount of stearo-dipalmitin (m.p. 55·9° C.). A. O. J.

Fat of the Green Turtle. T. G. Green and T. P. Hilditch. (*Biochem. J.*, 1938, **32**, 681–686.)—The component acids present in a specimen of the fat of the green turtle (*Chelone mydas* Linn.) from the Seychelle Islands have been examined. The fat had saponification equivalent, 268·9; iodine value, 66·1; acid value, 3·2; unsaponifiable matter, 1·5 per cent. Comparative tables are given for this and other green turtle fats, from both *Chelone mydas* and other species (Tsujimoto, samples from Japan, *J. Soc. Chem. Ind. Japan*, 1937, **40**, 185B; Abst., ANALYST, 1937, **62**, 619; Imperial Institute, samples from Ceylon and Panama; *Bull. Imp. Inst.*, 1937, **35**, 31; Abst., ANALYST, 1937, **62**, 882; see also Lee, ANALYST, 1935, **60**, 650). The acids from about 300 g. of fat were analysed by the esterification method after separation by the lead salt and alcohol method into 26·7 per cent. of "solid" and 73·3 per cent. of "liquid" acids. The liquid acids were fractionated through an electrically heated and packed column. The component acids of the fat (in per cent. mol.) were found to consist of: decanoic, 0·3; lauric, 16·9; myristic, 11·9; palmitic, 17·0; stearic, 3·7; as tetradecenoic, 1·5; hexadecenoic, 7·8; C_{18} unsaturated, 35·8 (—2·2 H); C_{20-22} unsaturated, 5·1 (—6·3 H). Generally speaking, the acids are qualitatively and quantitatively similar to those observed in depot fats of other amphibious animals, but they are unusual in including about 17 per cent. (mol.) of lauric acid and more myristic acid (12 per cent. mol.) than is customary. The combined molar percentage of saturated acids is about 50 per cent. of the total fatty acids. By successive crystallisations from acetone at 0° C. the glycerides of green turtle fat were separated into 9·3 per cent. (mol.) of less soluble and 90·7 per cent. of more soluble glycerides, and further analysis showed that for the most part the acidic components, including the unusual quantity of lauric acid, are distributed fairly evenly and widely in combination with the glycerol molecules. About 9·6 per cent. (mol.) of fully-saturated glycerides are present, chiefly mixed lauro-myristo-palmitins, and a somewhat similar quantity of tri-C_{18} glycerides, probably not wholly as triolein, but with a certain proportion of stearo-di-olein. At least one oleic group associated with one, or more frequently two, acyl groups is included in most of the glycerides. D. G. H.

Occurrence of Traces of Hexadecenoic (Palmitoleic) Acid in Vegetable Fats. T. P. Hilditch and H. Jasperson. (*J. Soc. Chem. Ind.*, 1938, **57**, 84–87.)—The unsaturated (or rather the "liquid") methyl esters of soya-bean, cottonseed and palm oils have been distilled in the special fractionating column previously

described, and in each instance definite evidence has been obtained of small proportions (of the order of 1 per cent.) of an unsaturated C_{16} acid, whilst in some of the fats there were indications of still smaller proportions of a tetradecenoic acid. Details of the experimental work are given. Hexadecenoic acid has already been shown to be present in amounts not exceeding 1 per cent. in arachis, olive and tea-seed oils, so that the acid may now be regarded as a usual constituent of vegetable seed and fruit-coat fats, although only present in traces, and is thus a component of all classes of natural fats. It is a major component only in fats from aquatic flora and fauna. In seed and fruit-coat fats the proportion appears to be approximately constant at about 1 per cent., and has no apparent relation either to the amount of palmitic, or to that of oleic or linolic acids, concurrently present. D. G. H.

Natural Caffeine-free Coffee. J. Pritzker and R. Jungkunz. (*Z. Unters. Lebensm.*, 1938, **75**, 34–35.)—Certain varieties of coffee containing little or no caffeine were encountered in a previous investigation (*Z. Unters. Lebensm.*, 1926, **51**, 97). The caffeine-content varied from 0·72 to 2·43 per cent., and it was thought that the caffeine was present in combination as a glucoside or tannin compound insoluble in the usual solvents (*cf.* König, *Chem. Menschl. Nahr. u. Genussm.*, 1903, **1**, 1508). Subsequent work by Rossi (Abst., *Z. Unters. Lebensm.*, 1937, **73**, 108) has revealed the existence of a number of species, e.g. *Coffea mogeneti*, *C. dubardi* and *C. perrieri*, growing wild in Madagascar, which are completely free from caffeine. Small samples of *C. dubardi* and *C. perrieri* were obtained, and the caffeine-content of the ground roasted bean was determined both by the method previously described (*loc. cit.*) and by the method of Hellberg (*Mitt. Geb. Lebensm. Unters. Hyg.*, 1933, **24**, 54). The following results were obtained for *C. perrieri*:—loss on roasting, 12·7 per cent.; aqueous extract, 26·1 per cent.; caffeine, nil; for *C. dubardi*:—loss on roasting, 17·0 per cent.; aqueous extract, 36·7 per cent.; caffeine, nil. The sample of *C. dubardi* had an exceptionally high aqueous extract not entirely attributable to the high loss on roasting. With the small amount of material available it was not possible to discover the reason for this high extract. A. O. J.

Biochemical

Fat Metabolism in Fishes. Seasonal Changes in the Composition of Herring Fat. J. A. Lovern. (*Biochem. J.*, 1938, **32**, 676–680.)—Representative samples of herring were obtained from sample batches of 20 herrings caught during April, when the fat is lowest (2 per cent. upwards); in June and July, during the period of intensive feeding and rapid rise in fat (to about 20 per cent.); in October from spent fish and from fish only about $\frac{1}{2}$ to $\frac{2}{3}$ ripe, and also from immature fish caught in April. Peculiar to all herring fats was the abnormally high content of C_{22} acids, unusual unsaturation of the C_{16} and C_{18} acids, and unusual saturation of the C_{20} and C_{22} groups. C_{20} acids ranged from 22·0 to 31·1 per cent. and C_{22} acids, from 10·5 to 29·1 per cent. The average degrees of unsaturation of the acids covered a wide range; C_{16} acids −2·6 to −3·4 H; C_{18} from −2·9 to −4·8 H; C_{20} from −3·9 to −5·5 H; and C_{22} from −4·1 to −5·7 H. Unsaturated acids are lowest

in starved, and highest in feeding fish, and the variations are regarded as brought about as follows:—Herring prefer a depot fat with comparatively saturated C_{20} and C_{22} acids, and thus ingested C_{20} and C_{22} acids are partially hydrogenated. In June the fish are feeding largely on the copepod *Calanus finmarchicus*, and an abruptly increased intake of highly unsaturated fat may temporarily be too much for the hydrogenation mechanism, and the unsaturation of all groups will then rise. Then hydrogenation would be accelerated and probably operate preferentially on the C_{22} acids, so that long before the intensive feeding period is over the rate of saturation of C_{22} acids is sufficient to cope with the ingested fat. C_{16} acids are dehydrogenated instead of hydrogenated and may provide part of the hydrogen required for the hydrogenation of the other acids. From analyses of the fats of spawned and normal herring caught long after intensive feeding had finished it was found that the acids of the above-mentioned partly starved fish were the more saturated, and preferential utilisation of the more unsaturated acids had apparently occurred. Immature herring had more highly unsaturated fat than adults caught at the same period. D. G. H.

Nature of Haemoglobin in the Red Blood Corpuscle. G. A. Adams. (*Biochem. J.*, 1938, **32**, 646–650.)—The ultra-violet adsorption band at $410m\mu$ is not shown by blood corpuscle suspensions (Adams, *Biochem. J.*, 1934, **28**, 482) and factors with a possible influence on this difference between haemoglobin inside and outside the corpuscle have been studied. Changes in the volume of the corpuscle brought about by solutions of osmotic strengths between 0·5 and 1·4 per cent. only accounted for small effects in the ultra-violet spectrum and probably for not more than 5 per cent. of the difference in the absorption. The possibility of chemical combinations between haemoglobin and cell constituents was then considered. Stromatin (stroma protein), prepared by a modification of Jorpes method (*Biochem. J.*, 1932, **26**, 1488), contained 10 per cent. of haemoglobin. It had a relatively flat spectrogram curve in the region of $410–420m\mu$. Little alteration was shown after the preparation had been freed from lipoids. When 10 ml. of 1 per cent. haemoglobin solution were added to 2 ml. of pure stromatin in alkaline solution (pH 9·8), the spectrogram taken after 5 minutes had the typical haemoglobin absorption band at $410–420m\mu$, but when the mixture was first heated at 37° C. for 30 minutes (pH 10·1) the colour changed to a slightly opalescent yellowish-red, and the typical haemoglobin absorption band disappeared. A control experiment in which the haemoglobin was heated with the same amount of alkali as used to dissolve the stromatin did not affect the spectrum. Stromatin and alkaline haematin did not combine on heating, nor did alkaline haematoporphyrin and stromatin, but reduced alkaline haematin and stromatin gave a substance with the spectrum of haemochromogen, so that the stromatin is presumed to combine with the globin portion of the haemoglobin corpuscle. The compound appears to be quite labile and is readily broken down by a variety of reagents. D. G. H.

Determination of Calcium in Blood and other Biological Material by Titration with Ceric Sulphate. C. E. Larson and D. M. Greenberg. (*J. Biol. Chem.*, 1938, **123**, 199–201.)—To 2 ml. of serum or an aliquot portion of liquid

containing 0·1 to 0·4 mg. of calcium and neutralised to *p*H 5 if necessary, 2 ml. of water and 1 ml. of saturated ammonium oxalate solution are added. The mixture is allowed to stand for at least two hours and then filtered through a micro filter and washed twice with 3-ml. portions of 2 per cent. ammonia solution. The precipitate of calcium oxalate is dissolved from the filter with three 1-ml. portions of hot 2 *N* sulphuric acid, the solution being allowed to run into a test-tube placed inside the filter-flask. The filter is washed with two 3-ml. portions of water, and 2 ml. of 0·01 *M* ceric sulphate solution (made by heating 13·2 g. of anhydrous ceric sulphate with 20 ml. of conc. sulphuric acid, adding water until dissolved, and then diluting to 1 litre) are added to the test-tube, and the mixture is allowed to stand for 30 minutes. Two drops of phenanthroline indicator are added, and the solution is titrated with 0·005 *M* ferrous ammonium sulphate solution. The colour-changes are purple through blue-green and blue to salmon-pink, the final change being very sharp. Each ml. of 0·01 *M* ceric sulphate solution is equivalent to 0·4 mg. of calcium. The phenanthroline indicator is made by dissolving 0·695 g. of ferrous sulphate ($FeSO_4.7H_2O$) in water, adding 1·485 g. of *o*-phenanthroline monohydrate, stirring until dissolved and making up to 100 ml. Immediately before use, 1 ml. of this solution is titrated to the neutral point (purple) with ceric sulphate solution. F. A. R.

Quantitative Precipitation of Citric Acid [from Urine]. A. C. Kuyper. (*J. Biol. Chem.*, 1938, **123**, 405–407.)—The precipitation of citric acid as the calcium salt is incomplete from aqueous solution, but is almost complete from urine. This difference is due to the presence of phosphate in urine. When a solution of citric acid is made alkaline, the citric acid is quantitatively precipitated if suitable amounts of calcium and phosphate are present in the solution. Calcium must be present in excess of the amount required to react with citrate and phosphate. Increasing amounts of phosphate are required to precipitate larger amounts of citrate. The nature of the reactions involved in the precipitation is not known. F. A. R.

Red Pigment of the Root of the Beet (*Beta vulgaris*). I. The Preparation of Betanin. II. A Method for the Determination of Betanin. G. W. Pucher, L. C. Curtis and H. B. Vickery. (*J. Biol. Chem.*, 1938, **123**, 61–70; 71–75.)—I. The pigment was extracted from dried beet-roots with acid alcohol, and isolated by neutralising the extract with aqueous lithium hydroxide solution. The precipitate of crude betanin was purified by reprecipitation with lead acetate solution and recrystallisation from water. The yield of pure pigment was 0·5 g. from 1 kilo of fresh beet-root, representing 40 per cent. of that originally present. The betanin obtained in this way was an indistinctly crystalline substance, nearly black in colour, with a green lustre. Its aqueous solution was purplish-red, and at a concentration of 0·005 mg. per ml. and at *p*H 5·2 had an extinction coefficient of 0·398 when measured in a 1-cm. cell with a Zeiss spectrophotometer with Filter S–53 (effective wave-length 530$m\mu$). The solution was but little affected by acids and weak bases, but with strong bases the colour was discharged to a pale yellow. The colour was also destroyed by permanganate solution, and the pigment can be titrated with permanganate to a colourless end-

point, 8 atoms of oxygen being required for 2 nitrogen atoms in the pigment. The substance is provisionally regarded as the glucoside of a nitrogenous anthocyanidin $C_{15}H_{13}N_2O_5Cl \cdot C_6H_{10}O_5 \cdot 3H_2O$, but it is not known in what state of combination the nitrogen occurs.

II. A sample of the dried, finely-ground beet-root, weighing 0·05 to 0·10 g., is transferred to a test-tube graduated at 50 ml., and moistened with a few drops of water. It is triturated with a rod, and 20 ml. of succinic acid-borate buffer of *p*H 5·2 are added. The solution is diluted to the mark with water and the tube is stoppered, shaken vigorously and then centrifuged. The extract is decanted through a plug of cotton wool and the transmission of the filtrate is measured within half an hour in a Zeiss spectrophotometer with Filter S–53, and the extinction coefficient K is calculated. Since the purified isolated pigment has an extinction coefficient of 0·398 at a concentration of 0·005 mg. per ml., the relation (K/0·398) × 0·005 gives the number of mg. of betanin in 1 ml. of the extract. In cases where a turbidity is encountered that cannot be removed by centrifuging, a preliminary alcohol extraction of the tissue is recommended, these extracts being discarded. The betanin-contents, expressed as per cent. of dry weight, of a number of varieties of beet are given in the following table:

Variety	Betanin
Detroit dark red (dark strain)	1·20
Improved blood turnip	0·34
Crosby's Egyptian	0·15
Good-for-all	0·47
Sutton's pineapple	0·46

F. A. R.

Vitamin C Contents of some Ceylon Fruits and Vegetables. A. W. R. Joachim and C. Charavanapavan. (*Analysis of Ceylon Foodstuffs*, 1938, 15–19.)—The ascorbic acid contents of about thirty fruits and ten vegetables were measured by titration of trichloroacetic acid extracts with 2 : 6-dichlorophenolindophenol. Of the fruits, guava contained the most ascorbic acid (127 mg. per 100 g.); papaw, orange, grapefruit, mandarin and mango contained 50 to 60 mg. per 100 g., the last-named being very variable and sometimes containing only small amounts; lemon, lime and tomato contained 20 to 40 mg. per 100 g.; tree tomato, custard apple and pineapple contained only 10 to 15 mg. per 100 g. Negligible amounts of the vitamin were found in coconuts, plantain, pomegranate and avocado pear. Of the vegetables tested, agathi (*Sesbania grandiflora*) leaves contained 181 mg., drumstick (*Moringa oleifera*) pods 80, spinach 66 and chillies up to 50 mg. per 100 g. Mukunuvenna (*Alternanthera triandra*) was also a rich source of the vitamin, but ash plantain (*Musa paradisiaca*) and gotu-kola (*Centella asiatica*) were poor sources. F. A. R.

Chromatographic Adsorption Analysis of Small Amounts of Carotenoids, with special reference to the Carotenoids of Milk and Serum. H. Willstaedt and T. K. With. (*Z. physiol. Chem.*, 1938, **253**, 40–46.)—Extracts of carotenoids from various sources were adsorbed on small columns of alumina ("reinst wasserfrei Merck"), and the columns were "developed" with mixtures of

petroleum spirit and benzene. The column usually employed was 100 mm. long and 10 mm. in diameter, and the proportion of benzine to benzene was usually 1 : 1. The zones resulting from the different carotenoids were separately eluted with a mixture of benzine and methyl alcohol, and the methyl alcohol was removed by washing with water. The benzine solutions were dried and distilled, and the carotenoid contents were measured in a Zeiss step-photometer, using Filter S–43. Losses of material invariably occurred, and the percentage loss was higher the smaller the scale of working. The greatest losses occurred with those carotenoids that were most strongly adsorbed on the column. These observations are illustrated by the following examples, in which the most strongly adsorbed constituent is recorded first.

Material tested	Carotenoids in crude material (γ)		Carotenoids found (γ)		Per Cent. loss	
Extract from lucerne	Total ..	1550	Phytoxanthin ..	550	Total ..	11
			β-Carotene ..	830		
" ..	Total ..	150	Phytoxanthin ..	48·5	Total ..	38
			β-Carotene ..	41		
" ..	Total ..	60	Phytoxanthin ..	16·5	Total ..	48
			β-Carotene ..	15		
Extract from ox-serum	Total ..	274	Oxidation products	23	Total ..	1
			β-Carotene ..	249		
Extract from human serum	Total ..	39·5	Phytoxanthin ..	2	Total ..	40
			Oxidation products	15		
			β-Carotene ..	7		
Mixture of phyto-xanthin and lycopene	Phytoxanthin	33	Phytoxanthin ..	15·8	Phytoxanthin	53
	Lycopene	36	Lycopene ..	28	Lycopene ..	22
Mixture of phyto-xanthin and β-carotene	Phytoxanthin	24·5	Phytoxanthin ..	13	Phytoxanthin	45
	β-Carotene	20·5	β-Carotene ..	13	β-Carotene ..	30
Mixture of lycopene and β-carotene	Lycopene	43·7	Lycopene ..	37·4	Lycopene ..	14
	β-Carotene	38·4	β-Carotene ..	36·2	β-Carotene ..	6

F. A. R.

Organic

Refractometric Determination of Oil in Aleurites Seeds. E. D. G. Frahm and D. R. Koolhaas. (*Rec. Trav. Chim. Pays Bas*, 1938, **57**, 395–398.)—Leithe's method (*cf.* ANALYST, 1936, **61**, 274) is modified as follows:—Two g. of the crushed sample are ground well with twice the volume of sand for 2 minutes, 4·5 g. of technical α-bromonaphthalene (weighed with an accuracy of 1 mg.) being then added. After grinding for a further 3 to 5 minutes, until a homogeneous mass is obtained, the mixture is filtered by suction and the n_D^{25} of the clear filtrate is determined in an Abbé refractometer. The above-mentioned solvent is chosen because it has a higher refractive index than that of the oil; it is a good solvent for the oil, and it is not appreciably volatile under tropical conditions. The commoner organic solvents fail in one or more of these respects. If the refractive index of the pure oil is determined on a small quantity expressed from the seeds by means of a micro-press, the proportion of oil in the seeds may be calculated by the method of mixtures, the temperature-coefficients of the refractive indices of the oil, solvent and solution being 0·00040, 0·00046 and 0·00045/1° C., respectively. In five determinations of the concentration of oil in seeds of *Aleurites montana* (*i.e.*

25·07 to 32·53 per cent. by vol.) the difference between the calculated and experimental refractive indices were 0 to 0·0002; in 10 similar determinations (concentrations of oil 44·8 to 60·0 per cent. by vol.) the differences between the values found by the refractometric method and the usual method of extraction with petroleum spirit in an atmosphere of carbon dioxide were 0 to 0·4 per cent. The oils had a mean $d_{27\cdot5}^{27\cdot5}$ of 0·933, and n_D^{25} 1·5151, and the n_D^{25} of the solvent was 1·6553. The oil from the seeds of *Aleurites moluccana*, however, has a different composition, little or no acids with conjugated double-bonds being present, and the refractive index is correspondingly lower (*e.g.* n_D^{25} 1·4754). For this oil the above value must be raised by 0·0005, and temperature-coefficients of 0·00037 and 0·00043/1° C. should be taken for the oil and the solution, respectively. In 5 experiments (concentrations of oil 24·17 to 39·09 per cent. by vol.) the differences between the observed and calculated (corrected) refractive indices were 0 to 0·0002; and in 4 further cases (56·6 to 66·6 per cent. of oil) the differences between the refractometric and extraction methods were 0 to 0·3 per cent. J. G.

Reactions Distinguishing Bourbonal from Vanillin. F. Hoeke. (*Chem. Weekblad.*, 1938, **35**, 316–319.)—Existing methods are first described and discussed. The m.p. of vanillin (80° to 82° C.) is too close to that of bourbonal (77° to 82° C.) to serve for distinguishing the two compounds, and the Pritzker and Jungkunz test (in which a solution of potassium hydroxide in alcohol is used to produce a yellow colour with bourbonal, the reaction being negative with vanillin) is difficult to operate in coloured (especially yellow) solutions. The test is not specific, and attempts to separate the bourbonal from any colouring matters have been unsuccessful. In connection with the Nickel-Kreis-Staudinger test (see ANALYST, 1928, **53**, 498) Dingemans has stated (*Chem. Weekblad*, 1930, **27**, 694) that bourbonal gives the same violet colour as vanillin, but the present author finds that if a mixture of a saturated solution of the sample and an equal volume of the reagent is allowed to stand at room temperature, vanillin produces a violet colour after 30 minutes, whilst with bourbonal a faint rose colour is obtained after 50 minutes, and this subsequently turns violet. Dingemans suggested the use of a saturated solution of hydrazine sulphate, 1 ml. of which is added to a solution of the sample in water; on addition of sufficient hydrochloric acid to bring the acidity of the mixture to about 4 *N* bourbonal gives a lemon-yellow precipitate, whilst that produced by vanillin is red to orange. This reaction is only satisfactory when applied to the pure substances, and piperonal (which is sometimes associated with vanillin in flavouring agents) behaves similarly to bourbonal, although if the strength of acid is 8 *N*, the precipitate from piperonal is first red to orange and turns yellow subsequently (see also Klotz, ANALYST, 1929, **54**, 752). Klotz (*Amer. J. Pharm.*, 1930, **102**, 274) made use of the m.p. of the phenyl hydrazones and dehydro dicompounds (see below), *i.e.* 124° to 126° C. and 232° to 235° C. for bourbonal, and 105° and 302° to 305° C. for vanillin, respectively (see also Wagenaar, ANALYST, 1932, **57**, 673). Klotz also investigated methods of identification depending on the nature of the crystalline substances formed on the addition of ferric chloride solution or of a solution of potassium hydroxide in alcohol (see below, and also N. Schoorl, *Organische Analyse*, 1937, II, 105). M. Krakowski (*Arch. Chem.*

Pharm., 1935, **2**, 164) used the m.p. of the condensation-products produced with barbituric acid. The use of Millon's reagent (*cf.* Thorpe and Williams, ANALYST, 1937, **62**, 398) has been found to be unsatisfactory, and this also applies to the other tests dealt with, which include that of Korenman (ANALYST, 1933, **58**, 371) and of Kolthoff (*Pharm. Weekblad*, 1918, **55**, 1021). The author recommends the following procedures:—(1) Vanillin dissolves in a 4 *N* solution of sodium hydroxide giving a colourless solution, whilst the presence of bourbonal produces a yellow-green shade. Since coumarin also gives this colour, the sample may be warmed with resorcinol and 80 per cent. sulphuric acid, when, on addition of an excess of sodium hydroxide and dilution, a yellow-green fluorescence is obtained from bourbonal and a green-blue fluorescence from coumarin (*cf.* Pavolini's reaction, Schoorl, *loc. cit.*, p. 265). (2) If a mixture of 3 ml. of a saturated aqueous solution of the sample, 3 drops of 4 *N* hydrochloric acid and 3 drops of a solution of ferric chloride is warmed, long, narrow, tapering crystal masses, frequently arranged in the form of large crosses, may be seen under the microscope on cooling; these are due to dehydro dibourbonal (see Klotz, above). Under similar conditions vanillin forms long and much more slender needles of dehydro divanillin, from which the cross-shaped structures are absent (*cf.* Schoorl, *loc. cit.*, p. 136). (3) If a drop of a solution of bourbonal in water (*e.g.* 1 : 2000) and a drop of a solution of phenyl hydrazine (after Denigès, see Schoorl, *loc. cit.*, p. 93) are mixed on a microscope slide, a milkiness develops, and the liquid when warmed is converted into a mass of small star-shaped clusters of fine small needles; if the amount of bourbonal present is too large, these are crowded together in such a way as to render their identification difficult. Illustrations are given in the original. This precipitate has the property of reversible phototropy, *i.e.* in the presence of daylight it turns from colourless to red, but becomes colourless again if left in the dark. The latter phenomenon requires 1 day at room temperature and 30 minutes at 80° C., and the time decreases with rise in temperature but only up to 126° C., because resinification occurs just below the m.p. (129·5° C.) of the crystals. When larger quantities of bourbonal are present the red precipitate should first be separated by filtration, and washed and dried for 30 minutes at 100° C. The original change (from colourless to red) does not occur above approximately 80° C., and it can take place when benzene or alcohol is used as the solvent. The milkiness is also produced with vanillin, but, on heating the liquid, oily droplets form, which set on cooling to a cream-coloured mass; this is not phototropic at room temperature, probably because this temperature is above the limiting value. The test is applied to vanillin sugar containing 0·25 per cent. of bourbonal by shaking 1 g. of the sample with two 10-ml. portions of ether, and testing a warm aqueous solution of the residue after evaporation of the extracts as described above. J. G.

Manometric Determination of Amino-acids. M. F. Mason. (*Biochem. J.*, 1938, **32**, 719–724.)—The method employed in the manometric measurement of the carbon dioxide split from the carboxyl groups of amino acids by heating with ninhydrin, proposed by Van Slyke and Dillon (*Proc. Soc. Exp. Biol.*, N.Y., 1936, **34**, 362) and elaborated by the author (*Proc. Soc. Exp. Biol.*, N.Y., 1937, **37**, 111), is now described, and results of tests are given. The technique recommended is very similar

to that described more recently by Van Slyke and Dillon (*C.R. Trav. Lab., Carlsberg*, 1938, **22**, 480). The solution to be analysed is made slightly acid and freed from dissolved carbon dioxide by shaking under reduced pressure or boiling, and an aliquot portion (1 to 5 ml.) yielding 0·05 to 2·0 mg. of available carboxyl carbon dioxide is placed in a dry combustion tube and followed by 1 or 2 ml. of saturated potassium dihydrogen phosphate solution (free from carbon dioxide) and 30 mg. of ninhydrin (triketohydrindene hydrate) and a glass bead. The tube is connected with a condenser, glycerin being used as a sealing fluid for the ground-glass joint, and the condenser is attached to the manometric gas pipette into which 2 ml. of the 0·5 *N* alkali, as described by Van Slyke *et. al.* (*J. Biol. Chem.*, 1933, **102**, 635) have been introduced. The gas chamber and combustion tube are connected by turning the stopcock, the mercury levelling bulb resting on the lower support, and the combustion tube is gently heated with a micro-burner. Before the b.p. is reached the mercury in the gas chamber is alternately raised and lowered several times, and after 3 minutes' boiling the flame is removed and the mercury is alternately raised and lowered (10 to 15 times) to draw the carbon dioxide into the alkali in the gas pipette, care being taken to avoid bumping, heating of the stopcock, and condensation of large amounts of vapour in the gas chamber with alteration of the volume of fluid over which the carbon dioxide is ultimately measured. The contents of the combustion tube are again gently boiled for 1 minute and the absorption is carried out as before. The unabsorbed gas is then ejected as described by Van Slyke (*id.*, 1933, **102**, 641), and the absorption of carbon dioxide is repeated at a greatly reduced pressure by altering the mercury another 10 times, the unabsorbed gas again being ejected and a final absorption made after altering the mercury level 10 times. The pressure during the final manipulation is about 50 mm. when the mercury level is at the 50-ml. mark. The condenser is disconnected, the stopcock capillary is sealed with mercury, and the last portion of unabsorbed gas is ejected. The carbon dioxide is then liberated with lactic acid, and the pressure is measured before and after absorption with 5 *N* alkali. Measurements are made at the 0·5-ml. or 2-ml. mark. Blank analyses are made omitting the ninhydrin, but one boiling period only is required unless the presence of substances decarboxylated by heat alone is suspected. Duplicate tests agree within 1 to 2 per cent., and the determination takes 15 to 20 minutes.

$Pco_2 = (p_1-p_2)-C$; $Pco_2 \times fco_2$ = mg. CO_2 in the sample analysed. The (p_1-p_2) reading in the blank analysis is the C correction. The factors fCO_2 are those derived by Van Slyke and Sendroy (*J. Biol. Chem.*, 1927, **73**, 141). Of 24 common amino acids thus analysed, all yielded 1 mol. of carbon dioxide per mol., except aspartic acid and cystine, which yielded 2 mols., and alanine, serine and tryptophane, which yielded slightly less than 1 mol. With peptides the reaction is not satisfactory unless they have free adjacent α-NH_2 and COOH groups. Various common organic acids (other than amino acids) were unreactive. The measurement of carboxyl carbon dioxide liberated by ninhydrin affords a highly specific means of determining amino acids in biological material, and its application to the analysis of blood filtrates is being studied. D. G. H.

Inorganic

Determination of Copper and of Cadmium by Internal Electrolysis. J. J. Lourié and M. I. Troitzkaia. (*Ann. chim. anal.*, 1938, **20**, 61–68.)—The electrode system consists of an ordinary cylindrical platinum foil cathode weighed before use, and a strip of the required anode metal placed vertically inside the cathode cylinder. This is immersed in the electrolyte, the anode and cathode being connected together above the level of the liquid (Fig. 1). No diaphragm is employed. To the solution containing the copper and cadmium (up to 50 mg. of each) as sulphates, 3 to 4 g. of ammonium sulphate are added; the solution is neutralised with ammonia to methyl red indicator, acidified with 2 drops of sulphuric acid (1 : 1), and diluted to 250 ml. For the deposition of copper an iron anode is employed. The electrode system is immersed for 40 minutes in the liquid heated to 50° to 60° C. It is then withdrawn and washed with water, and the cathode is detached, dipped in alcohol, and dried at 100° to 150° C. The increase in weight is the weight of copper. To the electrolyte containing the cadmium with the bivalent iron dissolved anodically in the preceding process, 15 drops of sulphuric acid (1 : 1) and 0·5 g. of ammonium persulphate are added, and the liquid is boiled for 15 minutes to oxidise the iron and destroy the excess of persulphate.

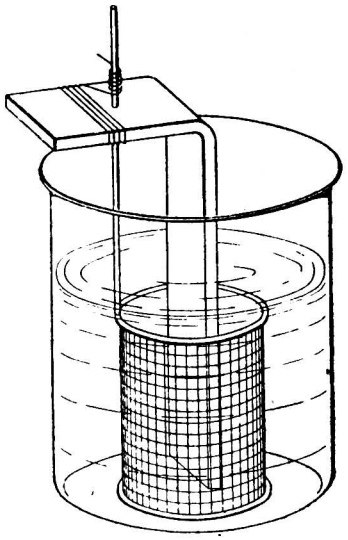

Fig. 1

The iron is then precipitated with ammonia as ferric hydroxide, which is filtered off and washed. The filtrate is concentrated to 150 ml. and neutralised with the aid of sulphuric acid; 1·65 ml. of 85 per cent. acetic acid and 5·9 g. of sodium acetate are added, and internal electrolysis with the use of a zinc anode is carried out for 30 to 40 minutes at a temperature of 75° to 80° C. The combined electrodes are withdrawn from the solution and placed for half-an-hour in 250 ml. of previously boiled water containing 2 drops of acetic acid and heated at 80° to 90° C. The electrodes are then rinsed in alcohol, and the cathode is dried and weighed, the increase in weight giving the weight of cadmium. (For further explanation of the method adopted for cadmium the reader is referred to *Z. anal. Chem.*, 1936, **107**, 34.) Almost theoretical results were obtained in test experiments. Zinc (up to 0·7 g.) may be present without effect, but lead and iron should be absent. Tests in which the copper and cadmium were simultaneously deposited from ammoniacal solution with the aid of a zinc anode gave low results, owing to the tendency of copper to deposit partially on the zinc anode. Difficulties from this cause were not experienced in the method described above when an iron anode was used. [*Abstractor's Note:*—The variety of iron employed is not specified; this may be of importance in regard to a possible tendency for copper to deposit on it.] The method has been applied to the determination of copper

and cadmium in commercial zinc, involving a sequence of preliminary chemical separations from iron, lead, arsenic, antimony, tin and the bulk of the zinc, for the details of which the original paper should be consulted. S. G. C.

Determination of Zirconium by Means of Methylarsinic Acid. R. Chandelle. (*Bull. Soc. Chim. Belg.*, 1938, **47**, 172–193.)—In the author's method for the determination of zirconium (ANALYST, 1937, **62**, 899) titanium and tin should not be present in the solution, as they are co-precipitated to a considerable extent. Copper does not interfere. W. R. S.

Volumetric Determination of Barium and Lead. H. N. Terem. (*Kimya Annali*, 1938, **3**, 10.)—Titration with a solution of sodium hexametaphosphate (Graham's salt) affords a rapid approximate method of comparing the strengths of solutions of barium and lead salts, respectively, in the absence of other metals. A solution of sodium hexametaphosphate containing 10·2 g. per litre ($N/10$) is used. A $N/5$ solution of barium chloride or lead nitrate, as the case may be, is employed for standardisation, as follows:—Ten ml. are titrated with the Graham's salt solution with constant shaking until the precipitate first formed dissolves, giving a clear or almost clear solution. It is convenient first to carry out a rapid preliminary titration, and then to do a second titration, adding, say, 0·5 ml. less than the required volume of titrating solution, the titration being completed drop by drop with efficient shaking. Titration of the sample solution is carried out in a similar manner. It is stated that some experience with the method is required for the ready detection of the end-point. S. G. C.

Separation of Magnesium from Potassium and Sodium. T. Krokowski. (*Z. anal. Chem.*, 1938, **112**, 183–186.)—Tetramethylammonium hydroxide ("Triton B") is now on the market at the price of 1 dollar per kg. (Suppliers: Röhm and Haas, Philadelphia). The base is recommended in systematic qualitative analysis for the separation of magnesium from the alkalis. In absence of ammonium salts, it precipitates magnesium hydroxide quantitatively at boiling heat when used in a 50 per cent. excess. The same effect is produced in boiling solutions by larger quantities of the base in presence of ammonium salts, as it displaces ammonia from ammonium compounds. For the subsequent identification of potassium and sodium, the excess of base must be destroyed by gentle ignition, as it gives insoluble compounds with potassium pyroantimonate and with perchloric acid. W. R. S.

Determination of Carbon Dioxide by a Conductometric Method. A. Lassieur. (*Compt. rend.*, 1938, **206**, 606–608.)—The method involves measurement of the conductivity of baryta solution before and after the absorption of the carbon dioxide. A simple, inexpensive and portable apparatus is used, consisting of a container to hold the gas sample connected by means of a tap with a conductivity cell containing baryta solution. A very weak direct current serves for the conductivity measurement, the electrical circuit comprising a "pocket flash-lamp" battery, a rheostat, and a sensitive galvanometer showing a full-scale reading of about 1 milliampere. The rheostat is first adjusted so that exactly 1 milliampere is passing. The baryta solution is then well shaken in the gas container for about 1 minute to absorb the carbon dioxide. It is then allowed

to flow back into the conductivity cell, and the reading on the milliammeter is noted. This reading is lower than the first owing to the consumption of baryta by the carbon dioxide with the formation of insoluble barium carbonate, and the difference between the readings is a measure of the carbon dioxide present in the gas sample. The method is standardised by trial with samples of known carbon dioxide content. By suitably varying the area of the electrodes or their distance apart, the strength of the baryta solution or the volume of gas taken, it is possible to determine amounts of carbon dioxide over a wide range. Since the determination depends on measuring the difference between current-readings it is independent of the actual voltage of the battery, provided that this remains constant during the test, and the temperature, being the same before and after the absorption process, is without effect. S. G. C.

Microchemical

Collected References. Determination of Iodine in Organic Substances. Th. Leipert. (*Mikrochimica Acta*, 1938, **3**, 73–91.)—The determination of iodine in organic material comprises: (1) Destruction of the organic matter and isolation of the iodine in the residue without loss; (2) determination of the amount of iodine, now present in the inorganic form. The Pregl method (burning the organic compound in oxygen, passing the vapours over platinum contacts, and absorbing the iodine in a sulphite solution containing sodium carbonate), and also the micro Carius method, are successful, except when only traces of iodine are present. In both these methods the iodine is finally determined either by weighing the silver compound or by titrating after oxidation to iodate. Both dry and wet methods of ashing are used to decompose the organic matter. Among the dry methods in an open vessel, the Fellenberg method or modifications of it appear to give the best results with traces of iodine, but the dry ashing methods in an open vessel are likely to be subject to large losses, so that a determination in duplicate is insufficient to obtain accurate results. Dry ashing in a closed system is capable of giving more accurate results but is very tedious. Methods of wet ashing are much more rapid. Methods for the determination of the inorganic iodine include colorimetric methods, and titration methods after oxidation with chlorine or bromine water and destruction of the oxidising agent by boiling, by phenol or by salicylic acid. Potentiometric titrations of various kinds are also described. The references total 131. J. W. M.

Identification of Organic Dyestuffs by Micro-sublimation. I, A. Kutzelnigg, and II, A. Kutzelnigg and E. Franke. (*Mikrochimica Acta*, 1938, **3**, 33–36 and 37–45.)—A large number of organic pigments will sublime when heated for 5 to 15 minutes at temperatures up to 210° C. on a microscope slide placed on an aluminium heating block heated by a micro-burner. The substance is best placed in a curved depression on the microscope slide and covered with a thin cover-glass which is examined under the microscope for the sublimate. When no sublimate is obtained at 210° C. the block is heated to 300° C. Dyestuffs containing sulpho

groups, or as metal lakes, or combined with tannin, katanol, tannol or phosphotungstate, do not usually sublime. As most water-soluble dyestuffs owe their solubility to a sulpho group, such dyestuffs rarely sublime; exceptions are Indian yellow G and dianil yellow G. Of the dyestuffs soluble in water, a large number of pigment dyes sublime readily; they are without exception red or yellow dyes, and include the following:—Hansa yellow 10 G, Hansa yellow 5 G, Hansa yellow 5 G transparent, Hansa yellow 3 G, Hansa yellow G (Hansa yellow G A, monolithol yellow G, pigment yellow 4 G L), Hansa yellow G transparent. Hansa yellow G G R extra, Hansa yellow G R, Hansa yellow 3 R. Lithol fast yellow R N (Helio fast yellow R). Helio fast yellow 6 G L (Helio fast yellow 3 G L). Lithol fast orange R N (permanent red G G, Hansa orange R). Permanent red R extra (Helio fast scarlet A R). Hansa red B (Lithol fast scarlet R N, lithol fast scarlet R G N, helio fast red R L, permanent red F 4 R, Sitara fast red). Lithol fast scarlet B. Permanent red F R L. Autol red B L (Pigment red B, Autol red B G L, Sitara red and other names). Autol red R L P. Of these, the Hansa yellows 10 G, 5 G, 5 G transparent, G R and 3 R all give clearly recognisable crystalline sublimates; the Hansa yellows 3 G, G, G transparent and G G R all give somewhat similar sublimates, and can only be identified by measurement of the crystal angles. Lithol fast yellow R N and helio fast yellow 6 G L are readily distinguished from the Hansa colours as they sublime only at 300° C. and are not appreciably pleochroic. The vat dyestuffs such as indigo, thio-indigo and tetrabromo-indigo are known to form sublimates; indanthrene dyestuffs appear to sublime at much higher temperatures and require longer time (such as ½ hour at 300° C. or 10 minutes at 300° C. under reduced pressure) when on the fibre than when tested in the form of powder. J. W. M.

Determination of the Melting-point of Porphyrins and other Dark Substances in Polarised Light. C. Rimington and P. Symons. (*Mikrochimica Acta*, 1938, **3**, 4–6.)—For determining the melting-point of dark-coloured crystals, and especially porphyrins, by means of a micro-heating block, such as that of Kofler, it is advantageous to illuminate with polarised light, and to observe the material through an analysing eyepiece. A birefringent solid will shine brightly when the surrounding field is at maximum extinction. At the melting-point the colour fades, but reappears on cooling, when re-solidification takes place. Double melting-points may be observed with ease upon the same preparation. Owing to the relatively great absorption of the nicol prisms it is advisable to use a carbon arc lamp as the source of light. J. W. M.

Detection of Cadmium with Brucine and Potassium Bromide. I. M. Korenman. (*Zav. Lab.*, 1937, **6**, 1461.)—Small amounts of cadmium may be detected in the presence of other metals by precipitation with a 5 per cent. solution of potassium bromide in sulphuric acid, which has been saturated while hot with brucine (*cf.* Meurice, *Ann. Chim. anal.*, 1926, **8**, 130; Abst., ANALYST, 1926, **51**, 367). On adding a drop of the reagent to a drop of the cadmium-salt solution on a microscope slide, a white finely-divided precipitate is formed; this rapidly becomes crystalline, the crystals consisting of many-rayed rosettes, the individual rays of which are parallelograms. By this method, 0·12γ of cadmium may be detected. Mercury and some other metals may be separated, if present, by

preliminary treatment with ammonia, after which one drop of the filtered solution is evaporated on the slide and heated to remove the last traces of mercury, as mercury also gives a reaction similar to cadmium. The residue is dissolved in a drop of water and treated as described above. If large amounts of zinc salt are present, several drops of sulphuric acid are added to prevent precipitation of a zinc compound. Silver bromide or sulphate, if precipitated, is readily distinguished microscopically from the cadmium compound. When alkaline earth metals are present, acetic acid must replace sulphuric acid in the preparation of the reagent. The minimum amounts of cadmium detectable range from 0·3 to 1·2γ according to what other metal is present. E. B. D.

Volumetric Determination of Bismuth as Bismuth Hydrogen Iodide Oxine. I. M. Kolthoff and F. S. Griffith. (*Mikrochimica Acta*, 1938, **3**, 46–51.)—The method of Berg and Wurm (*Ber.*, 1927, **60**, 1664) for the determination of bismuth as bismuth hydrogen iodide oxine, $C_9H_7ON \cdot HBiI_4$ is tested. Bismuth is separated in this way from other metals, except mercury, silver, lead and thallium. The indirect method, in which the excess of iodine is back-titrated, is preferred to the direct titration of the precipitate, particularly in the determination of very small amounts of bismuth. Low results are obtained when the chloride concentration is greater than about 0·1 *N* in the final mixture. J. W. M.

Detection of Indium with Morin and of some Heavy Metals with Cacotheline. G. Beck. (*Mikrochimica Acta*, 1937, **2**, 287–290.)—Morin gives yellow-green fluorescent compounds with aluminium, gallium, scandium and indium salts. These elements may be detected in the presence of one another by carrying out the test under the correct conditions. If the fluorescence disappears on the addition of sodium fluoride, either aluminium or scandium is present. If a second sample is treated with excess of ammonium carbonate, only scandium will give the yellow-green fluorescence with morin. Magnesium must not be present. For indium the reaction is strongest in weak solutions of mineral acids; sodium fluoride and sodium acetate reduce the intensity. Indium is precipitated quantitatively with hydrogen sulphide from solutions of the free mineral acids buffered with sodium acetate, and the fluorescence therefore disappears, whereas under the same conditions that of gallium, aluminium and scandium remains. In 1 ml. the limit of identification for indium is 1γ by daylight, 10γ in the presence of sodium fluoride, 0·02γ in ultra-violet light, and 0·5γ in the presence of sodium fluoride. Cacotheline reacts not only with tin salts but also with tervalent salts of titanium, uranium and rhenium, lower oxides of molybdenium and tungsten, and niobic chloride. The limit of identification is of the order of 1γ of the metal. J. W. M.

Analysis of Micro-samples of Steel. A. Portevin and A. Leroy. (*Compt. rend.*, 1938, **206**, 518–520.)—The following methods are indicated for the determination of certain constituents of steel samples of the order of 1 mg. *Manganese.*—The sample is dissolved in dilute nitric acid, and the manganese is converted into permanganic acid by oxidation with ammonium persulphate and silver nitrate,

added as solutions from micro-burettes in amounts appropriate to the sample-weight taken. The permanganic acid is titrated with arsenious acid solution added from a micro-burette giving drops of 1/125 ml. The result is obtainable in about 10 minutes with an accuracy of 0·5γ. *Phosphorus.*—The molybdenum-blue reaction is used. The solution of the sample in dilute nitric-sulphuric acid is oxidised with permanganate to convert phosphorous compounds into ortho-phosphoric acid; the iron is reduced with bisulphite, and the blue colour developed by the addition of molybdic acid and stannous chloride is matched colorimetrically. Silicon does not interfere (arsenic is not mentioned); the time required is about 30 minutes, and the absolute error is less than 0·05γ. *Chromium.*—The chromium in the solution of the samples in dilute nitric-sulphuric acid is oxidised to chromic acid by means of potassium permanganate, the iron is precipitated with sodium carbonate, and the chromium is determined colorimetrically with diphenyl-carbazide; only vanadium interferes. The error is of the order of 0·04γ. No practical details are given. The analysis of very small samples is of value in metallurgical work in connection with the study of heterogeneity and metallic diffusion, and the examination of surface layers in corrosion problems. S. G. C.

Microchemical References II. (Supplement to *Microchem.*, 1937–38, **23**, 171; for Part I, see ANALYST, 1937, **62**, 766.)—The references are given in alphabetical order of subjects, which range from fluorescence to heroin, from insulin to polarographic methods, from selenium to vitamin B. An index of authors is given at the end. This index comprises references to Parts I and II. J. W. M.

Physical Methods, Apparatus, etc.

Use of the Dilatometer in Measuring the Extent of Freezing in Ice-cream and Related Products. W. C. Cole. (*J. Agric. Res.*, 1938, **56**, 137–153.)—The principle of the method is the measurement of the changes in volume which occur during freezing in a special type of dilatometer. It is considered that this enables results to be obtained more accurately, more conveniently and over a wider range than existing methods which involve determinations of temperature changes, melting-points or the proportions of solid and liquid present (*cf.* W. C. Cole, *J. Dairy Sci.*, 1932, **15**, 254; A. Leighton, *ibid.*, 1927, **10**, 300). The dilatometer used consists of a narrow vertical tube which is filled to a certain mark with the sample, a portion of the latter then being transferred to a similar compartment below through a 3-way stop-cock; if the first compartment is again filled to the mark with the sample from a weight-burette, the amount transferred may be calculated. The top compartment is then emptied through the 3-way cock. Freezing takes place in the lower compartment, which with the upper compartment, forms the arm of a U-tube, the other arm being a vertical tube terminating at the top in a ground-glass joint, and having a 3-way cock at the same level as the similar cock separating the two compartments in the other arm. The joint is connected with a graduated horizontal capillary tube in which the expansion is measured, and the cock with a levelling-bulb, by means of which the whole apparatus may be filled with mercury. It is thus a simple matter to adjust the mercury reservoir

so that any change in the volume of the contents of the lower compartment is shown by a change in the position of the mercury in the capillary. Freezing is achieved by immersion of the sample in a brine-bath which is placed in a refrigerator, solid carbon dioxide being added to the bath when it is necessary to hasten cooling. The bath also contains a stirrer and a thermo-regulator which enable a temperature of 0° to –30° C. to be attained to within ± 0·01° C. The " mixes," which included 9 ice-creams, 3 ice-milks (*i.e.* a form of ice-cream containing not less than 4 per cent. of fat) and 4 water-ices were prepared according to commercial practice, the dairy products being pasteurised for 30 minutes at 65° C. and homogenised at the same temperature, and then cooled. The mixes were maintained at 2° C. for 1 to 2 days between the preparation and testing operations, most of the air being removed at the last moment by heating the sample in a vacuum of 25 to 28 inches until boiling started and then cooling immediately in the vacuum chamber. The graphs relating the temperature and dilatometer readings consist of 2 distinct portions, namely an inclined straight line representing data obtained before the sample had been frozen and after it had melted, and a curve intersecting it (at the f.p.) and representing readings obtained during the freezing and melting operations. The distance between the line and the curve in a direction parallel with the dilatometer reading ordinate represents the linear expansion of the mercury capillary for a given temperature, and this multiplied by the area of a cross-section of the capillary gives the change in volume which occurs in freezing. From this, the size of the sample and the expansion-coefficient for the conversion of water into ice (*i.e.* 0·0933 ml./g., as determined from standardisation experiments with a 20 per cent. solution of sucrose), the amount of ice formed at a given temperature may be calculated. The results obtained are compared with the f.p. (as determined in a Hortvet cryoscope) of portions of the same sample containing different proportions of water, and the agreement is satisfactory, especially in the temperature-range just below the original f.p. of the mixes; normally, the deviation is greatest at the lower temperatures (*e.g.* below –15° C.). Observations on ice-cream mixes indicated that samples having the same percentages of fat and total solids may have different freezing-points, because of differences in the amounts of soluble and ionisable constituents; in such cases the higher the f.p. of the mix the greater the amount of ice formed at a given temperature. The ice-milks behaved similarly to low-fat ice-creams, but the water-ice mixes (which contained approximately 23 per cent. of sucrose, 7 per cent. of glucose, 0·2 per cent. each of gelatin and agar, and had titratable acidity, as citric acid, 0·5 per cent.) differed to a pronounced extent in that less water was frozen at a given temperature, owing probably to the comparatively low f.p. of these mixes. It was found that the change in volume which occurs on freezing is given by the expression $(V_1 dn_1 + V_2 dn_2)$, where dn_1 and dn_2 are the changes in the number of mols. of solute and solvent (*i.e.* water), respectively, and V_1 and V_2 are the corresponding partial molal volumes (*i.e.* as given by dV/dn, where V is the volume of the solution). This expression is made the basis of a mathematical proof that the changes in volume which occur on freezing are due primarily to the separation of water as ice. The significance of the conclusions when applied to practical working conditions is indicated. J. G.

Reviews

ANNUAL REPORTS ON THE PROGRESS OF APPLIED CHEMISTRY, 1937. Vol. XXII. Issued by the Society of Chemical Industry. Pp. 816. Price to members, 7s. 6d.; to non-members, 12s. 6d.

These Reports have for many years past been reviewed in THE ANALYST and there surely cannot be many chemists who are not fairly familiar with their general structure. In this volume there are twenty-four sections, each dealing with special branches of applied chemistry and written by authorities in these branches. Each long chapter is in the nature of a progress report to which the several authors contribute critical remarks which themselves possess no little value and are calculated not only to quicken the interest of the general reader but also to be of value even to the expert.

Compared with last year's issue, this one is slightly smaller, there being fifty-three pages fewer; but in a volume of over eight hundred pages this difference is scarcely noticeable. The general excellence is well maintained, and so is the agreeable literary style which so tempts the reader to explore far beyond the confines of those chapters wherein his main interest lies. By the use of a pleasantly smooth paper, which is thin but reasonably opaque, the volume is kept within a manageable compass; it is thus a most grateful companion in the study. It is furnished with both a name index and a subject index, the former extending to thirty-eight pages and the latter to thirty pages. At a rough computation these must contain, respectively, some four thousand five hundred and two thousand seven hundred entries, which should afford some idea of the scope of the work. The number of journals and original papers consulted in the compilation is astonishing, and it would be safe to say that no work of real importance has been overlooked. Naturally, the authors have had to exercise their discretionary powers; some crave pardon of writers whose work receives scant mention or none at all, but it is evident that they have chosen well.

Although not a few sections open or close on an almost apologetic note because of the lack of anything outstanding in the way of research or discovery to record during the year under review, this lack does not necessarily detract from the interest of the volume as a whole. Progress in any science is not a series of epoch-making discoveries; sooner or later steady development must set in, and, like the middle game in chess, this is usually where the discerning mind finds most of interest.

That a volume of this size should be issued in the April following the close of the year with which it deals, seems to the reviewer no mean achievement, and one upon which the editor and the several authors, as well as the printers and the issuing body, are heartily to be congratulated. Praise must also be given for the remarkable freedom from typographical and other errors; the few discovered are innocuous. Such a work as this, so frequently consulted by research workers, can be made even more valuable to them by the printing of a list of corrections in the succeeding volume, even though the list be a small one.

Whoever reads this book to the end will derive from the last chapter something

he would not expect to find in such a serious work. In that chapter four American gentlemen are reminded that about a hundred years before them one Englishman, John Dalton, came to the same conclusion as they do in regard to gaseous partial pressures. F. W. EDWARDS

HANDBUCH DER EIERKUNDE. By Dr. J. GROSSFELD. Pp. vii + 375. Berlin: Verlag von Julius Springer, 1938. Price Rm.27, bound Rm.28·50.

It is difficult to overestimate the part which eggs have played in the chemical and biological investigation of foodstuffs and nutrition. Since each egg contains the complete ingredients for the formation of a living entity from relatively simple material, it is natural that it should play a large part in fundamental work on biochemical processes. The literature dealing with eggs is widespread and until now no comprehensive work on the subject has appeared in any language. The present volume is entitled to be called comprehensive since there appears to be no branch of the science and study of hens' eggs with which it does not deal.

The preface indicates that the collection and sorting of the data took some years; and since nearly every page contains several references, some to difficultly accessible journals, this can readily be understood. But the volume is not merely a collection of references. It is a unified survey of the work published in this field and admirably arranged. The principal headings in order of appearance are:— physiological and morphological structure and formation; factors affecting production, including the nutritive needs of the hen, and the occurrence of abnormal eggs; composition; chemical and physical properties of the constituents, including minor minerals, colouring matters, carbohydrates and ferments; changes with age and preservation; spoilage and its causes; digestibility and feeding values with notes on idiosyncracies; statistics and other information on the industry in Germany; legal standards for food products containing eggs; physical, chemical, mycological and industrial examination of eggs, both fresh and preserved, and of egg products.

The book provides a review of the history and the present theoretical and practical position of egg science which will be of great value to those directly concerned with eggs and also to many workers in branches of food chemistry dealing with such substances as proteins, fats, lecithins, vitamins, organic sulphur compounds, etc.

It is unfortunate that the publishers issue an important book, well produced in other respects, in paper covers and with the sheets uncut. It appears, however, that for a nominal increase in price a bound copy is available. J. R. NICHOLLS

METHODS OF QUANTITATIVE CHEMICAL ANALYSIS. By M. G. MELLON. Pp. ix + 456. London: Macmillan & Co., Ltd., 1937. Price 12s. 6d. net.

This introduction to the theoretical and practical basis of quantitative chemical analysis is chiefly of interest for its very complete and readable survey and classification of the principles upon which analytical chemistry rests. It was written to exemplify the ideas of the author with respect to chemical training; hence, it is intended to teach the teacher as well as the student. It represents an extension and application of the ideas presented in an essay by the author, in collaboration

with D. R. Mellon (*J. Chem. Educ.*, 1937, **14**, 365), and presents the subject from both the professorial and professional points of view.

It contains no original contribution to practical analytical chemistry. Nomenclature is very largely original; orthography and syntax are American.

F. L. OKELL

DIE BEHANDLUNG UND REINDARSTELLUNG VON GASEN. By ALFONS KLEMENC. Pp. x + 215, 80 illustrations. Leipzig: Akademische Verlagsgesellschaft m.b.H. Price Rm.14.80 (paper cover); Rm.16.80 (cloth cover).

This is an admirable little book, written with an economy which has allowed an enormous amount of valuable information to be given in a relatively small space. It covers the whole field of the technique of the laboratory manipulation of gases, and gives details of the preparation and purification of some seventy of them together with references relating to probably all known substances which are gaseous at ordinary temperatures. Among the gases the preparation of which is described are deuterium, parahydrogen, nitryl chloride, sulphur hexafluoride and xenon. The general planning of the book is shown by the space allotted to various subjects, as follows:—General technique of the preparation and manipulation of gases, 16 pages; analysis, 15 pages; purification methods without making use of condensation, 11 pages; condensation methods of purification, 22 pages. The last 109 pages deal with the preparation of individual gases.

Abundant references are given at the end of each short section, with a notable absence of any undue preponderance of Germanic authorities; few relevant papers appear to have been missed. On page 28 is a useful list of textbooks and monographs on gas analysis, with no important omissions.

Some very valuable tables are included: that on pages 23–25 gives methods for the determination of small quantities of various impurities in gases, giving the sensitiveness of each test, the gases which interfere, and the original references. On pages 90–92 is a comprehensive table of commercial cylinder gases, giving the methods by which they have been prepared, the approximate purity, the identity of the likely impurities and the names of the supplying firms; this list again is such as to be useful to chemists in all countries. Incidentally, cylinder nitrous oxide of a much higher purity than the 95 to 99 per cent. ascribed here to the I.G. product can be bought in this country. On page 191, the melting- and boiling-points of twenty-nine gaseous fluorine compounds are given, details of the preparation being left to the references; summarised properties are added.

Obvious care has been taken to make the diagrams as clear as possible to the eye, and also to the mind; most of them appear to have been drawn specially. Wherever any special piece of apparatus in mentioned, the manufacturer is cited.

The omission of magnesium perchlorate from the drying agents described (pages 38–39) prompts the question as to whether or not the omission is deliberate. On page 45 it is stated that potassium hydroxide used for making pyrogallate solution for oxygen absorption should not have been purified with alcohol. This statement, made by Hempel[1] (with no reasons given) in 1887 has been subsequently

1. *Ber.*, 1887, **20**, 1865.

repeated by several other writers. Anderson[2] gave evidence that the objection was not valid, and Haldane with his unrivalled experience does not appear to have mentioned it.[3] Professor Klemenc, however, repeats the ban, and extends it to alkali solution for the absorption of acid gases (page 42). It would have been valuable to know whether he has any direct information on the subject, or whether it is just a matter of what Hempel said in 1887.

The paper and print are good and the format attractive. In conclusion, one can say that the comprehensiveness, clarity and succinctness of the book, and the high degree of relevance of the information given, make it a handbook of exceptionally high value to any chemist who may be concerned with any gaseous substance.

H. R. AMBLER

CACAO FERMENTATION. By A. W. KNAPP, M.Sc., F.I.C., M.I.Chem.E. Pp. xii + 171. London: John Bale, Sons & Curnow, 1937. Price 10s. net.

In the preface to this book the author reminds us that the last complete survey of existing knowledge of cacao fermentation appeared in 1913, when a valuable collection of independent essays on the subject was published under the editorship of Mr. Hamel Smith. For twenty-five years this excellent work has been regarded as a standard book of reference by cacao planters and research workers, but knowledge of the scientific aspects of cacao fermentation has increased rapidly during recent years and the need for a full statement of the modern outlook on the subject is evident.

In writing this book Mr. Knapp has not only given us the benefit of his own wide practical and scientific knowledge of cacao fermentation, but has also assembled a mass of scientific material which had previously been scattered throughout the scientific journals of many countries. The treatment of the subject-matter, both scientific and practical, is excellent, and the book is easily readable—a feature which will be particularly appreciated by cacao planters and buyers. The chapters dealing with fermentation appeared originally in the *Bulletin of the Imperial Institute*, and to these have been added further matter to make a complete survey of the practical problems of cacao fermentation and the chemical changes occurring in the bean.

Cocoa and chocolate manufacturers have long recognised the desirability of using well-fermented beans, and the higher prices offered for cacao in this condition have encouraged planters and scientific workers to improve the methods used for fermentation. It is accordingly of interest to note that, in spite of the many alternative proposals suggested by scientists, the old-established custom of fermenting the beans in the pulp removed from the cacao pods is found to be without a serious rival in practice. Modifications have naturally been introduced, which make it possible to effect greater control and produce a more uniform product, while methods of fermenting small quantities of cacao have received special attention. The latter point is one of vital importance when considering improvements possible in the near future, for, as the author points out, 60 per cent. of the world's cacao is produced by farmers with small holdings, where elaborate

2. *J. Ind. Eng. Chem.*, 1915, **7**, 587.
3. *Methods of Air Analysis.*

scientific methods would be out of the question. The building of co-operative fermentaries may help to raise the general quality of the cacao in areas where most of the crop is grown by peasant proprietors, but it appears improbable that the stimulus to take such action is sufficiently strong at present. Much will depend on the results of researches in progress in various parts of the world and notably by Professor Hardy and his colleagues in Trinidad.

In his chapters on methods of fermentation the author describes the standard procedure practised in the chief cacao-producing countries, and his critical comments will be of special value to planters. Scientific workers will find his clear description of the chemical changes taking place in the pulp and bean during fermentation both interesting and stimulating—stimulating on account of the important problems still unsolved. Two of these outstanding problems are the establishment of a method of controlled fermentation which will produce a uniform product, and the discovery of the factor controlling the production of the characteristic chocolate aroma which develops in the roasted nib.

There can be little doubt that this book will become the standard work on cacao fermentation and will be welcomed by planters and research workers. It is well illustrated and is provided with a useful bibliography in which the references are arranged very conveniently in order of date. W. B. ADAM

BIOLOGICAL STANDARDISATION. By Professor J. H. BURN, M.D. Pp. xv + 288. Oxford Medical Publications, Oxford University Press, London, 1937. Price 21s. net.

THE BIOLOGICAL STANDARDISATION OF THE VITAMINS. By KATHERINE H. COWARD, D.Sc. Pp. viii + 228. Baillière, Tindall & Cox, London, 1938. Price 12s. 6d. net.

Until quite recently the analytical chemist considered that neither the methods nor the results of biological assay were of any direct concern to him, and he was almost certainly right. He may have had a few qualms when adrenalin first appeared in the Pharmacopoeia, but his misgivings had no further basis as soon as the pure synthetic chemical replaced "natural" preparations extracted from animal glands and possibly less pure.

History has, it seems, in some respects repeated itself; the inability of the public analyst to estimate biologically the vitamin C in food and drugs need worry him no longer, since a reasonably accurate chemical method for determining ascorbic acid, easily carried out by any qualified practitioner, has become available and is now official in the Pharmacopoeia.

Nevertheless, it is legitimate to ask how long this state of affairs may persist. There seems to be a tendency at the present time for the number of those physiologically active substances that can be assayed only by biological methods to increase in proportion more rapidly than the number of physiologically active substances present in or added to medicinal products. In Professor Burn's book we find set down with great clearness, and with an occasional sly dig that makes the reading even more attractive, the principles upon which these biological assays

must be carried out and the kind of conclusion that may legitimately be drawn from the assays.

Whether or not the analytical chemist in general, and the public analyst in particular, is concerned professionally with biological assays, it is quite certain that all would gain by reading Professor Burn's book and especially its introductory chapters. Analytical chemists are in the habit of using methods that have considerably more accuracy than is required for their purposes. There are, of course, exceptions to this rule, but it will be found to apply generally. This is equally true of the control work practised by most analytical chemists in industry. Indeed, it may be said that the object of the analytical chemist is to devise weapons rather sharper than he needs. The bio-assayist has a different problem; his difficulty is usually to devise procedures sufficiently acute to make such weapons worth using at all, and even when they have been devised, many people will succeed in blunting them by misapplication. The bio-assayist's instrument is not sharp enough for his requirements, and so he has been compelled to examine with great care exactly what degree of accuracy he may extract from his results, and in this work Dr. Coward has been in the very forefront. The examination has involved a pretty searching methodological analysis, by the application of certain accepted statistical principles. How many analytical chemists are there who precisely know the accuracy of their methods, at any rate in a statistical sense? If a careful reading of Professor Burn's introductory chapters causes some of us to pause and ask ourselves the question, "What are the probable limits of error of a series of acidimetric titrations?" or some similar question, the effect can hardly be anything but salutary. Moreover, in spite of the fact that it deals with the rather different problems already mentioned, Professor Burn's book points fairly clearly the way in which the same principles could be applied to problems of purely chemical analysis.

The great rate of expansion in this field is illustrated by the increase in the number of pages from the original edition of Professor Burn's work, published in 1928, which contained 126 pages, to the present revised edition which contains 288.

In the first three chapters of the book he has discussed units of measurement —a most valuable variation on the yard-stick theme—classification of methods, with an excellent exposition of what is meant by a lethal dose, and the mathematical treatment of results. Subsequent chapters are concerned with the assay procedures specific for substances from gland products, such as the active principles of the pituitary, the pancreas, the adrenals, the thyroid, the parathyroid, the ovary and the testes, alkaloids and glucosides not susceptible of quantitative chemical determination, and so to be estimated only by biological means. These include the active principles of digitalis, strophanthus, squill and ergot. Two further chapters discuss the comparison, whether for toxicity or therapeutic potency, of organic arsenic and antimony compounds and of anti-malarial substances. In addition to the subjects enumerated above, Professor Burn's book contains four chapters on the assay of those vitamins for which an International Unit and an International Standard Preparation exist. The same ground is covered, though at considerably greater length, in Dr. Coward's monograph, and it is perhaps inevitable that a certain amount of duplication has resulted. Dr.

Coward's book, however, is considerably more detailed in its discussion of technical methods and of the application to vitamin assay of statistical methods, described both in her book and in Professor Burn's. If, therefore, Dr. Coward's book has in some ways duplicated part of the contents of Professor Burn's, in other ways it acts as an admirable complement.

One might venture a slight criticism on a point that is rather a matter of opinion than of fact. It seems to me that both authors have tended to over-emphasise the procedure in which the same standard response-dosage curve is applied to all individual assays, at the expense of the method in which two doses of unknown are tested against two doses of standard, whereby in effect a response-curve is created for each individual assay. The relative advantages of these two methods is a matter about which there is still considerable difference of opinion among pharmacologists. The construction of a standard response-curve is made on the assumption that the group of animals used in any individual assay will show responses lying reasonably close to this curve. If their responses do not so lie, the particular assay will give a result correspondingly removed from the true value. On the other hand, if groups of animals are so liable to vary that the standard response-curve cannot be used, is the assayist justified in considering that animals used in any one assay are sufficiently comparable to give that assay any greater accuracy by using the "two against two" method than by using the standard response-curve? These are difficult matters and rather beyond the scope of the student, for whom, among others, Dr. Coward intended her book. Nevertheless, to omit reference to them may perhaps involve the danger of excessive confidence in one method as against the other.

Readers of Dr. Coward's book will note her repeated insistence that there can be no assay without units, and that there can be no units without agreed stable Standard Preparations. Disregard of this fundamental requirement has filled biochemical literature with many figures that are asymptotic to the worthless. In the next edition of Dr. Coward's book, it might be worth while to show how the principles of biochemical assay can be extended to meet a position like that of lactoflavin to-day. There is neither unit nor Standard Preparation of lactoflavin, but the pure crystalline substance, natural or synthetic, can be obtained. By using the pure compound in place of a Standard Preparation, comparative estimations of lactoflavin by weight, instead of in units, can be carried out on foods, using a technique strictly analogous with that required for the estimation of vitamins A and D, ascorbic acid and aneurin chloride as set forth by Dr. Coward. If a suitable animal test be found for nicotinic acid amide, the anti-pellagric factor, its estimation in foods will then become possible by biological means; otherwise we shall need to wait until the chemist discovers a specific and sensitive test for the compound.

To return to the general questions raised at the beginning of this review, we may ask whether these matters, apart from their inherent scientific interest, are really of any concern to the Public Analysts, or to any analysts other than those engaged in the pharmaceutical manufacture of products containing physiologically active compounds not susceptible of chemical determination. Presumably any one of the vitamin, hormone or bacterial products described in the British Pharma-

copoeia must legally comply with the standards laid down in these descriptions, from which it would follow that a sample may legitimately be taken by an inspector under the Food and Drugs Act and sent to the public analyst for analysis. The present requirement, that proceedings under the Act must be taken within 28 days, would rule out proceedings in respect of those vitamins of which the assay takes five or six weeks. Even if the new Act introduces modifications intended to meet this difficulty, there will still be other serious difficulties facing the Public Analyst who wishes to carry out such assays. The technique involved is so specialised, and the equipment so costly, that there actually exists in this country to-day only one laboratory—at least, only one non-industrial laboratory—where these tests can be carried out, for a suitable fee. Moreover, there are probably very few local authorities that would sanction the considerable expenditure involved over each sample submitted to this kind of examination outside the laboratory of its own Public Analyst.

It seems that advances in nutritional science, pharmacology and therapeutics have outstripped certain legal enactments. The Pharmacopoeia periodically recognises the value of certain new drugs and carries out frequent revisions to ensure that this recognition is maintained. The various monographs of the Pharmacopoeia are intended by the medical profession to protect not only its own members but the general public whom its members may be called upon to treat. The fact that the Pharmacopoeial monographs are taken as non-statutory standards by the courts, suggests that its attempts to safeguard doctors and patients has the sanction of the State. Yet such attempts may be completely frustrated because the provisions of the Food and Drugs Act are entirely unsuitable to ensure that certain Pharmacopoeial monographs should really have the force of law or to bring to book those who prevent their having it. The only safeguard for doctors and patients alike appears, at the present time, to be the honesty of those who manufacture such products. This honesty is perhaps the more dependable because many of them realise that honesty is indeed the best policy; they know that in the long run they will not succeed in selling drugs that fail to give the therapeutic results for which they are used.

A. L. BACHARACH

WEEDS, WEEDS, WEEDS. By SIR CHARLES V. BOYS, LL.D., F.R.S. Pp. 70. Published by Whiteman & Co., Ltd., Regency Street, London, S.W.1. Price 1s. net.

Weeds, weeds, weeds! How often has this been the despairing cry of the amateur gardener, and of his professional brother, too, for that matter. Here is a book which gives the solution to their heartrending problems.

Weeds are treated by Sir Charles in the same charming way as but a few years ago he treated soap bubbles. His book on the latter subject simply forced a reader to become a blower of soap bubbles—and what experts developed too!

After reading the book under review one almost feels induced to cultivate weeds for the sheer delight of trying the "cure." It places the possessor of an otherwise ineradicable weed in an enviable position. Some of the reassuring statements in the book are worthy of reproduction; for instance, "It is a simple matter to clear up the hawk weeds. It is merely necessary to go round with a can of

fine, dry sulphate of ammonia and sow it broadcast when the weather is hot and dry." And again, "I have not troubled about daisies. They are so easily destroyed by lawn sand or by sulphate of ammonia scattered lightly over them that nothing more need be said."

Some of the other treatments advocated are as follows:

Moss.—Dust lightly with sulphate of iron in fine powder.

Yarrow.—Use sulphate of ammonia or sodium chlorate solution.

Nettles.—Pull off shoots when young and as they appear for several weeks, or attack plant with a raspberry hook, swiping off the stalk close to the ground. Or use sodium chlorate solution.

Path Weeds.—Use sodium chlorate solution.

Thistles or Ragwort.—Treat with sulphate of ammonia or sodium chlorate solution or attack with raspberry hook.

Docks.—Use dock grubber or fork.

Horse Radish.—To eradicate is impossible. Never eat horse radish and then the cook cannot throw odd ends of the root on to the rubbish heap and so permit it to gain access to the garden. Eat horse radish only at the club.

The book is well worthy of careful study. F. W. F. ARNAUD

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AN INTRODUCTION TO MICROCHEMICAL METHODS. By C. WILSON. Pp. xi+196. London: Methuen & Co. 1938. Price 7s. 6d.

MODERN ASPECTS OF INORGANIC CHEMISTRY. By H. J. EMELÉUS and J. S. ANDERSON. Pp. xi + 536. London: G. Routledge & Sons, Ltd. 1938. Price 25s. net.

BRITISH CHEMICAL INDUSTRY: ITS RISE AND DEVELOPMENT. By SIR GILBERT T. MORGAN and D. DOIG PRATT. Pp. xii + 387. London: Edward Arnold & Co. 1938. Price 21s. net.

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